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RAPD ANALYSIS OF THE GENETIC POLYMORPHISM IN EUROPEAN WHEAT GENOTYPES

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ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the main crops for human nutrition. The genetic variability of grown wheat has been reduced by modern agronomic practices, which inturn prompted the importance of search for species that could be useful as a genepool for the improving of flour quality for human consumption or for other industrial uses. Therefore, the aim of this study was to analyze the genetic diversity among 24 European wheat genotypes based on Random Amplified Polymorphism (RAPD) markers. A total of 29 DNA fragments were amplified with an average 4.83 polymorphic fragments per primer. The primer producing the most polymorphic fragments was SIGMA-D-P, where 7 polymorphic amplification products were detected. The lowest number of amplified fragments (3) was detected by using the primer OPB-08. The size of amplified products varied between 300 bp (OPE-07) to 3000 bp (SIGMA-D-P). The diversity index (DI) of the applied RAPD markers ranged from 0.528 (OPB-07) to 0.809 (SIGMA-D-P) with an average of 0.721. The polymorphism information content (PIC) of the markers varied from 0.469 (OPB-07) to 0.798 (SIGMA-D-P) with an average 0.692. Probability of identity (PI) was low ranged from 0.009 (SIGMA-D-P) to 0.165 (OPB-07) with an avarage 0.043. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. Within the dendrogram was separated the unique genotype Insegrain (FRA) from the rest of 23 genotypes which were further subdivided into two subclusters. In the first subclaster were grouped 13 genotypes and the second subcluster involved 10 genotypes. The first subcluster also included the genotype Bagou from France, in which were detected novel high – molecular – weight glutenin subunits using SDS-PAGE. Using 6 RAPD markers only two wheat genotypes have not been distinguished. Through that the information about genetic similarity and differences will be helpful to avoid any possibility of elite germplasm becoming genetically uniform.

Keywords: *Triticum aestivum* L.; PCR; RAPD marker; genetic diversity

INTRODUCTION

Wheat (*Triticum* spp.) is a self-pollinating annual plant, belonging to the family *Poaceae* (grasses), tribe *Triticeae*, genus *Triticum*. According to different classifications, number of species in the genus varies from 5 to 27. The two main groups of commercial wheats are the durums (*Triticum durum* L.) and bread wheats (*Triticum aestivum* L.) with 28 and 42 chromosomes respectively **(Šramková et al., 2009)**.

Bread wheat (*Triticum aestivum* L.) is one of the most important and widely cultivated crops used mainly for human consumption and support in the world.

The importance of wheat is mainly due to the fact that its seed can be ground into flour, which form the basic ingredients of bread and other bakery products, as well as pastas, and thus it presents the main source of nutrients such as proteins, carbohydrates, lipids, fibre and vitamins, to the most of the world population. Agronomical and nutritionally important status of wheat among the several other cereal crops has obtained because of its large genome size and multifaceted uses. Approximately 734.8 million tons of wheat is produced annually on 247 million hectare of the total cultivated land in the world and supports nearly 35% of the world´s population (http://www.fao.org/worldfoodsituation/csdb/en/).

Enormously growing population and the changing of life style have posed challenges to the wheat breeders to develop newer wheat varieties with high yielding performance, high quality seed and resistance to pests and stress conditions. Modern agronomic practices have reduced the genetic variability of cultivated wheats, which has given great importance in the search for could be useful in contributing genes for wheat improvement **(Jauhar, 1993)**.

Characterization of genetic diversity and genetic relatedness is a fundamental element in crop improvement strategies **(Zhu et al., 2000)**. Like any other crops, the first step of in wheat improvement is full assessment of the local materials, including collection, evaluation and molecular characterization of germplasm lines. Knowledge about morphological and agronomic traits and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies **(Abbas et al., 2008)**.

A number of methods are currently used for analysis of genetic diversity in germplasm accessions, breeding lines

and segregating populations. These methods were based on pedigree, morphological, agronomic performance, biochemical and molecular (DNA-based) data **(Mohammadi and Prasanna, 2003)**. The diversity patterns allow plant breeders to better understand the evolutionary relationships among accessions and to incorporate useful genotypes in the breeding programs **(Thompson et al., 1998)**.

However, diversity estimates based on pedigree analysis have generally been found inflated and unrealistic **(Fufa et al., 2005)**. Genetic diversity estimates based on morphological traits, on the other hand, suffer from the drawback that such traits are limited in number and are influenced by environment **(Maric et al., 2004)**. Molecular markers are useful tools for estimating genetic diversity as these are not influenced by environment, are abundant and do not require previous pedigree information. Among the biochemical markers, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS – PAGE) has been widely used due to its simplicity and effectiveness for estimating genetic diversity.

Among the various DNA – based markers, AFLP (Amplified Fragment Length Polymorphism) and RFLP (Restriction Fragment Length Polymorphism) have been used to study genetic diversity. These markers elucidate the phylogenetic relationships among various lines, for their efficient use in breeding and genetic resource management. These methods, however, involve the use of expensive enzymes, radioactive labeling, and are cumbersome and hence, appear unsuitable **(Gajera et al., 2010)**.

On the other hand, RAPD (Random Amplified Polymorphic DNA) markers have offered a valuable opportunity to characterize genetic variation and structure in plant population **(Ayana et al., 2000)** with requiring only small amounts of DNA sample without involving radioactive labels, are simplier as well as faster, and therefore they have been increasingly employed for analysis of genetic diversity **(Ebrahimi et al., 2011)**.

The use of RAPD molecular markers are routine methods for quickly and efficiently estimating relationships between lines and populations of many plant species. It is assumed that these markers are randomly spaced throughout the genome **(Mark et al., 1999)**. RAPD markers have proven to be quite efficient in detecting genetic variations and used for diversity assessment and for identifying germplasm in a number of plant species such as wheat **(Ahmed et al., 2010**; **Mahmood et al., 2011**; **Cifci and Yagdi, 2012**; **Rehman et al., 2013)**, rye **(Petrovičová et al., 2014)**, flax **(Bežo et al., 2005)**, castor **(Vivodík at al., 2015)**, amaranth **(Štefúnová et al., 2013)** or Echinacea **(Kapteyn et al., 2002)**, and also these markers have been used for identification and differentiation on other microbial or animal level, such as in the yeast microbiota grown on grapes **(Drozdz et al., 2015)** or in fishery food products **(Bajzík et al., 2010)**.

The present study is focused on estimation of genetic distance between 24 European wheat genotypes, included the genotype with probably novel high – molecular – weight glutenin subunits identified by SDS – PAGE **(Kuťka Hlozáková et al., 2015)** based on 6 RAPD markers. Although the information gathered here would be helpful in future for genomic mapping studies leading to development of wheat cultivars with broader genetic background to obtain improved crop productivity.

MATERIAL AND METHODOLOGY

Plant material: Twenty – four genotypes of hexaploid wheat (*Triticum aestivum* L.) grain originating from five different geographical areas (Slovakia, Czech Republic, Hungary, Germany and France) of Europe were obtained from the collection of genetic wheat resources of the Gene Bank of Slovak Republic in Piešťany.

Genomic DNA Isolation: DNA of 24 genotypes of wheat was extracted from the endosperm of intact, dry and mature single seeds using the Gene JET Plant Genomic DNA Purification Mini Kit (Thermo Scientific) supplemented with 2% polyvinylpyrrolidone (PVP) in lysis buffer.

RAPD Analysis: Amplification of RAPD fragments was performed according to **Cifci and Yagdi (2012)** using decamer arbitrary primers (Operon technologies Inc, USA; SIGMAD, USA). Amplifications were performed in a 25 µL reaction volume containing 5 µL DNA (100 ng), 12.5 µL Master Mix (Promega), and 1 µL of 10 pmol of primer. Amplification was performed in a programmed thermocycler (Biometra, Germany) with initial denaturation at 94 °C for 3 min., 40 cycles of denaturation at 94 °C for 30 sec., primer annealing at 38 °C for 1 min., extension at 72 °C for 2 min., and final extension at 72 °C for 10 min. Amplified products were separated on 1.2% agarose in 1 × TBE buffer. The gels were stained with ethidium bromide, visualised under ultraviolet (UV) light and documented using gel documentation system Grab-It 1D for Windows. The molecular weight of amplified fragments was estimated with the help of Thermo Scientific FastRuler Middle Range DNA Ladder (MBI, Fermentas).

Data analysis: The RAPD bands were scored as present (1) or absent (0), each of which was treated as an independent character regardless of its intensity. The binary data generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands and to prepare a dendrogram. A dendrogram based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) with the software package SPSS professional statistics version 17 was constructed. For the assessment of the polymorphism in the wheat genotypes using RAPD markers in their differentiation we used diversity index (DI) **(Weir, 1990)**, the probability of identity (PI) **(Paetkau et al., 1995)** and polymotphic information content (PIC) **(Weber, 1990)**, which were calculating according to formulas:

Diversity index (DI)

$$DI = 1 - \sum p_i^2$$

Probability of identity (PI)

$$PI = \sum p_i^4 + \sum_{i=1}^{i=n-1} \sum_{j=i+1}^{n} \left(2 p_i p_j\right)^2$$

information content (PIC)

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 \cdot p_j^2$$

(where p_i and p_j are frequencies of i^{th} and j^{th} fragment of given genotypes)

RESULTS AND DISCUSSION

Efficient and effective crop improvement program depends on the extent of genetic diversity either existing or created. The breeding of wheat has achieved hallmark progress that is able to feed ever increasing population in the world **(Rehman et al., 2013)**. Genetic diversity is one of the key factors for the improvement of many crop plants including wheat **(Ahmed et al., 2010)**. The efficiency of genetic gain by selection can be improved if the patterns of genetic diversity within a population of breeding lines are known. Genetic similarity or distance estimates among genotypes are helpful in the selection of parents to be used in the breeding program **(Van Becelaere et al., 2005)**.

In this work, 6 primers were screened for PCR amplification of DNA and RAPD analysis in 24 wheat genotypes. Table 1 shows codes and sequences of these primers, total number of amplified fragments from 24 wheat genotypes, the diversity index, the polymorphic information content and the probability of identity for each primer. All the primers produced 29 DNA fragments (Figure 1) with an average of 4.833 bands per primer. From these six primers, primer SIGMA-D-P was the most polymorphic, where 7 polymorphic amplification products were detected. The lowest number of different fragments (3) was detected in primer OPB-08. Of the 29 amplified bands, all 29 were polymorphic, with an average of 4.83 polymorphic bands per primer. The size of amplified products varied from 300 bp (OPE-07) to 3000 bp (SIGMA-D-P).

To determine the level of polymorphism in the analysed group of wheat genotypes, diversity index DI, probability of identity PI and polymorphic information content PIC were calculated. All three indicators were applied for all six RAPD primers and for their calculation, the individual frequences of fragments of each marker were used.

The diversity index (DI) of the applied RAPD markers ranged from 0.528 (OPB-07) to 0.809 (SIGMA-D-P) with

Table 1 List of RAPD primers, total number of bands and the statistical characteristics og the used RAPD markers.

Primers	Primer sequence (5´-3´)	Total number of bands	DI	PIC	PI
OPA-02	TGCCGAGCTG	5	0.761	0.736	0.016
OPA-03	AGTCAGCCAC	5	0.741	0.712	0.023
OPA-13	CAGCACCCAC	4	0.708	0.668	0.033
OPB-08	GTCCACACGG	3	0.528	0.469	0.165
OPE-07	AGATGCAGCC	5	0.779	0.768	0.014
SIGMA-D-P	TGGACCGGTG	7	0.809	0.798	0.009
Total	-	**29**	-	-	-
Avarage	-	**4.833**	**0.721**	**0.692**	**0.043**

Note: DI – diversity index, PIC – polymorphic information content, PI – probability of identity.

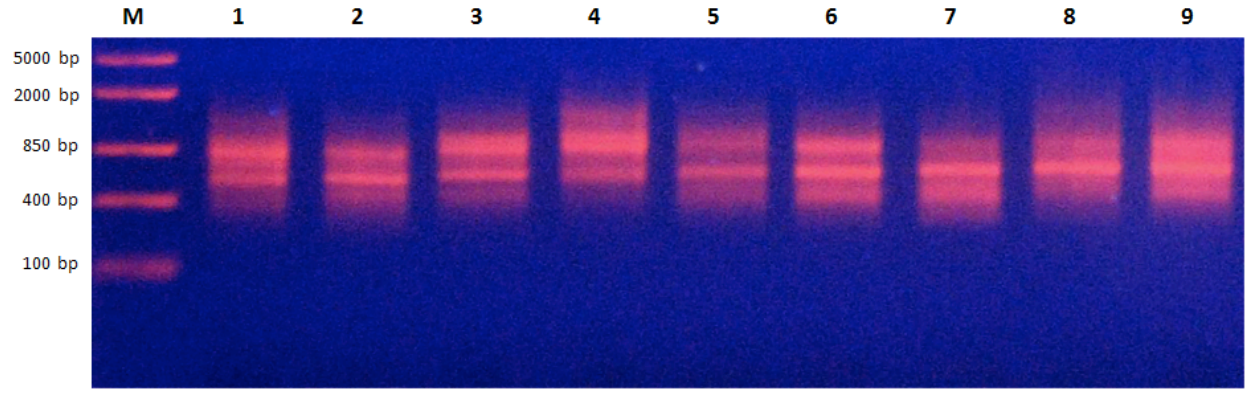

Figure 1 PCR amplification products of nine wheat genotypes with SIGMA-D-P primer: Lane M – Thermo Scientific FastRuler Middle Range DNA Ladder, 1 – Banquet (CZE), 2 – Kalif (FRA), 3 – Bonpain (FRA), 4 – Verita (SVK), 5 – Hana (CZE), 6 – MV Optima (HUN), 7 – Balthasar (FRA), 8 – Bagou (FRA), 9 – Ilona (SVK).

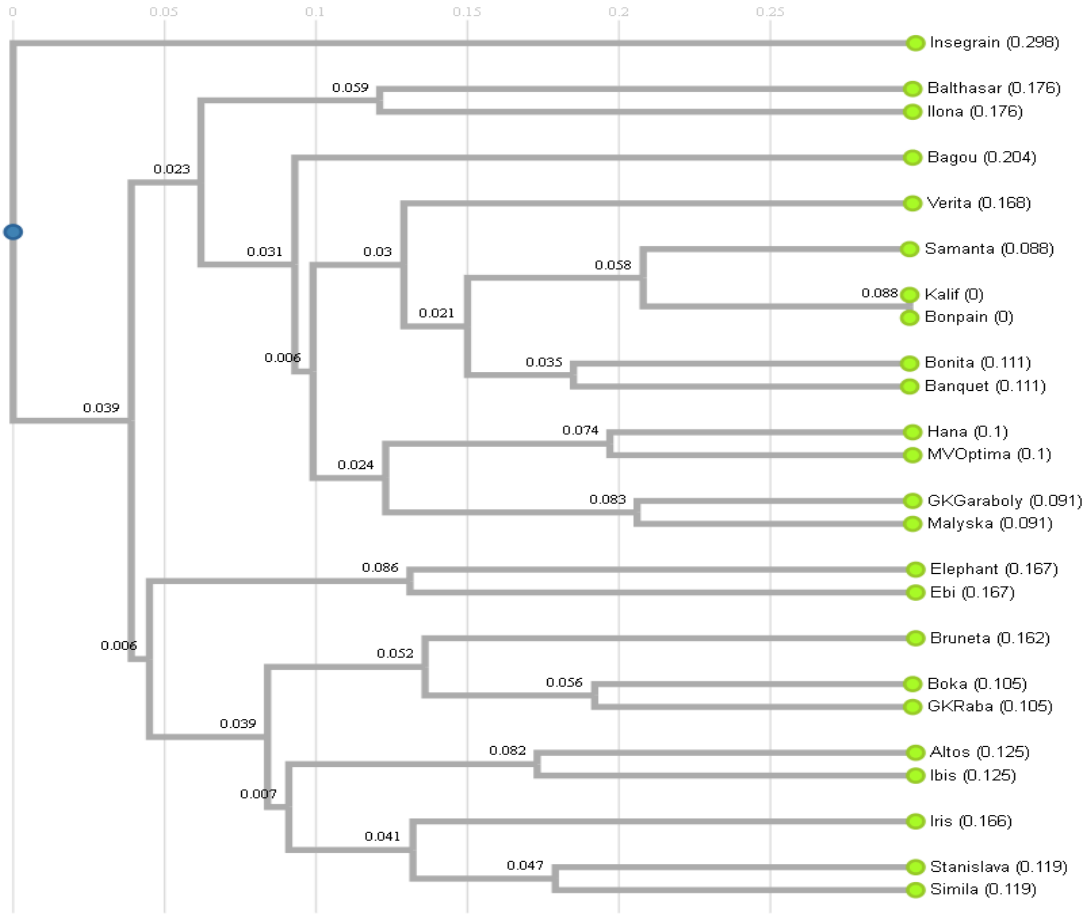

Figure 2 Dendrogram based on 29 RAPD fragments in 24 wheat genotypes.

an avarage of 0.721. The polymorphism information content (PIC) of the markers varied from 0.469 (OPB-07) to 0.798 (SIGMA-D-P) with an average 0.692. 83% of used RAPD markers had PIC and DI values higher than 0.6 that means high polymorphism of chosen markers used for analysis **(Vivodík et al., 2015)**. Probability of identity (PI) was ranged from 0.009 (SIGMA-D-P) to 0.165 (OPB-07) with an average 0.043. Cause of that, it is necessary to use a higher number of RAPD markers.

For the detection of genetic diversity, the dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared (Figure 2). This dendrogram separated unique genotype Insegrain (FRA, cluster I) from other 23 genotypes (cluster II) that were further subdivided into 2 subclusters. In the first subcluster were grouped 13 genotypes from Slovakia (38.5%), France (30.8%), Czech Republic (15.4%) and the same number (15.4%) from Hungary. Genotypes Kalif and Bonpain from France could not be distinguished because of their close genetic similarity. This subcluster also included the genotype Bagou from France, in which were detected novel high – molecular – weight glutenin subunits using SDS – PAGE. The second subcluster involved 10 genotypes from Slovakia (30%), Czech Republic (30%), Germany (20%), France (10%) and the same number (10%) from Hungary.

Lower polymorphism using RAPD analysis was detected by **Cifci and Yagdi (2012)** who used 17 markers to describe genetic similarity of 16 Turkish wheat genotypes. PIC values ranged from 0.11 to 0.92 with an average 0.59. **Ahmed et al., (2010)** also used 15 RAPD markers to analyse the genetic diversity of 32 wheat breeding lines and reached an average 4.1% polymorphism per primer.

On the other hand, high polymorphism was detected in set of amaranth **(Štefúnová et al., 2013)**, rye **(Petrovičová et al., 2014)** or castor genotypes **(Vivodík et al., 2015)**. Also higher polymorphism for RAPD was detected in Pakistan wheat landraces, where **Mahmood et al., (2011)** reached an average 7.8 % polymorphism per primer using 10 RAPD markers.

CONCLUSION

The present study was aimed to determine the genetic variation among wheat genotypes grown in Europe. Our results showed that RAPD markers are useful for exploring genetic diversity of raw material for developing new varieties. The dendrogram prepared based on UPGMA algorithm separated the unique genotype Insegrain (FRA) from the rest of 23 genotypes which were further subdivided into two subclusters. The first subcluster also included the genotype Bagou from France, in which were detected novel high – molecular – weight glutenin subunits using SDS-PAGE. Only two wheat genotypes have not been distinguished using these 6 RAPD markers. For better resolution of the analysed wheat genotypes, it is necessary to use a higher number of RAPD markers.

Despite that, the information gathered here would be helpful in genomic mapping studies and for the development of wheat cultivars with wider and diverse genetic background to obtain improved crop productivity.

REFERENCES

Abbas, S. J., Shah, S. R. U., Rasool, G., Iqbal, A. 2008. Analysis of genetic diversity in Pakistani wheat varieties by using randomly amplified polymorphic DNA (RAPD) primers. *American-Eurasian Journal of Sustainable Agriculture*, vol. 2, no. 1, p. 29-33.

Ahmed, F. A., Iqbal, M., Masood, M. S., Rabbani, M. A., Munir, M. 2010. Assessment of genetic diversity among Pakistani wheat (*Triticum aestivum* L.) advanced breeding lines using RAPD and SDS – PAGE. *Electronic Journal of Biotechnology*, vol. 13, no. 3 (online). http://dx.doi.org/10.2225/vol13-issue3-fulltext-2

Ayana, A., Bryngelsson, T., Bekele, E. 2000. Genetic Variation in Wild Sorghum (Sorghum Bicolor Ssp. Verticilliflorum (L.) Moench) Germplasm from Ethiopia Assessed by Random Amplified Polymorphic DNA (RAPD). *Genetic Resources and Crop Evolution*, vol. 47, no. 1, p. 471-482. http://dx.doi.org/10.1111/j.1601-5223.2000.t01-100249.x

Bajzík, P., Golian, J., Židek, R., Čapla, J., Belej, Ľ., Ondrejka, M., Mrázová, Ľ., Maršálková, L. 2010. Methods for fish species identification in food products. *Potravinarstvo*, vol. 4, no.3, p. 1-5. http://dx.doi.org/10.5219/25

Bežo, M., Candráková, A., Štefúnová, V., Žiarovská, J., Ražná, K., 2005. Identification of flax germplasm collection duplicates using randomly amplified polymorphism DNA markers (RAPD). *Acta fytotechnica et zootechnica*, vol. 8, no. 3, p. 62-66.

Cifci, E. A., Yagdi, K. 2012. Study of genetic diversity in wheat (*Triticum aestivum*) varieties using random amplified polymorphic DNA (RAPD) analysis. *Turkish Journal of Field Crop*, vol. 17, no. 1, p. 91-95.

Drozdz, I., Makarewicz, M., Sroka, P., Satora, O., Jankowski, P. 2015. Comparison of the yeast microbiota of different varietis of cool-climate grapes by PCR-RAPD. *Potravinarstvo*, vol. 9, no. 1, p. 293-298. http://dx.doi.org/10.5219/484

Ebrahimi, F., Mohammadi-Nejad, G., Baghizadeh, A., Abdolinejad, M. 2011. Genetic diversity evaluation of rapeseed genotypes (Brassica napus L.) based on phenotypic traits and random amplified polymorphic DNA (RAPD) markers. *African Journal of Biotechnology*, vol. 10, no. 76, p. 17391-17398. http://dx.doi.org/10.5897/ajb11.1786

Fufa, H., Baenziger, P. S., Beecher, B. S. M, Dweikat, I., Graybosh, R. A., Eskridge, K. M. 2005. Comparison of phenotipic and molecular marker – based classifications of hard red winter wheat cultivars. *Euphytica*, vol. 145, no. 1-2, p. 133-146. http://dx.doi.org/10.1007/s10681-005-0626-3

Gajera, B. B., Kumar, N., Singh, A. S., Punvar, B. S., Ravikiran, R., Subhash, N., Jadeja, G. C. 2010. Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. *Industrial Crops and Products*, vol. 32, no. 3, p. 491-498. http://dx.doi.org/10.1016/j.indcrop.2010.06.021

Jauhar. P. P. 1993. Alien gene transfer and genetic enrichment of bread wheat. Damania AB (ed) *Biodiversity and wheat improvement*, p. 103-119. ISBN 0-471-94137-9

Kapteyn, J., Simon, J. E. 2002. The use of RAPDs for assessment of identity, diversity and quality of Echinacea. *Janick, J., Whipkey, A. (Eds.), Trends in New Crops and New Uses.* ASHS Press, Alexandira, VA, p. 509-513.

Kuťka Hlozáková, T., Gregová, E., Gálová, Z. 2015. Genetic diversity of Glu – 1 in European wheat genetic resources and varieties. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 4, no. 2, p. 23-25. http://dx.doi.org/10.15414/jmbfs.2015.4.special2.23-25

Mahmood, T., Siddiqua, A., Rasheed, A., Nazar, N. 2011. Evaluation of genetic diversity in different Pakistani wheat land races. *Pakistan Journal of Bot*any, vol. 43, no. 2, p. 1233-1239. http://dx.doi.org/10.3923/pjbs.2006.2858.2861

Maric, S., Bolaric, S., Martincic, J., Pejic, I., Kozumplink, V. 2004. Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. *Plant Breeding*, vol. 123, no. 4, p. 366-369. http://dx.doi.org/10.1111/j.1439-0523.2004.00956.x

Mark, S., Adam, B., Lukaszewski, J., Whitkus, R. 1999. Development of arm specific RAPD markers for rye chromosome 2R in wheat. *Crop Science*, vol. 39, no. 6, p. 1702-1706. http://dx.doi.org/10.2135/cropsci1999.3961702x

Mohammadi, S. A., Prasanna, B. M. 2003. Analysis of genetic diversity in crop plant – salient statistical tools and consideration: Review and interpretation. *Crop Science*, vol. 43, no. 4, p. 1235-1248. http://dx.doi.org/10.2135/cropsci2003.1235

Paetkau, D., Calvert, W., Stirling I., Strobeck, C. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, vol. 4, no. 3, p. 347-354. http://dx.doi.org/10.1111/j.1365-294x.1995.tb00227.x

Petrovičová, L., Gálová, Z., Balážová, Ž., Vivodík, M., Wójcik-Jagla, M., Rapacz, M., 2014. *Genetic diversity of czechoslovak origin rye cultivars detected by RAPD markers. CECE 2014*. Brno: Institute of analytical chemistry AS CR, p. 353-356. ISBN 978-80-904959-2-0.

Rehman, R., Shah, K. N., Masood, M. S., Arshad, M., Ghafoor, A. 2013. Genetic divergence among Pakistani bread wheat varieties and advanced lines for randomly amplified polymorphic DNA (RAPD) markers. *Pakistan Journal of Botany*, vol. 45, no. S1, p. 327-332.

Šramková, Z., Gregová, E., Šturdík, E. 2009. Genetic improvement of wheat. *Nova Biotechnologica*, vol. 9, no. 1, p. 27-51.

Štefúnová, V., Bežo, M., Labajová, M. 2013. Molecular identification of amaranth (Amaranthus L.) species used in nutrition. *Potravinarstv*o, vol. 7, special no., p. 231-234. http://www.potravinarstvo.com/dokumenty/mc_march_2013/bezpecnost_potravin_rastlinneho_povodu/stefunova.pdf

Thompson, J. A., Nelson, R. L., Vodkin, L. O. 1998. Identification of diverse soybean germplasm using RAPD markers. *Crop Science*, vol. 38, no. 5, p. 1348-1355. http://dx.doi.org/10.2135/cropsci1998.0011183x003800050033x

Van Becelaere, G., Lumbbers, E. L., Paterson, A. H., Chee, P. W. 2005. Pedigree vs. DNA marker – based genetic similarity estimates in cotton. *Crop Science*, vol. 45, no. 6, p. 2281-2287. http://dx.doi.org/10.2135/cropsci2004.0715

Vivodík, M., Balážová, Ž., Gálová, Z., Kuťka Hlozáková, T. 2015. Differentiation of ricin using RAPD markers. *Pakistan Journal of Botany*, vol. 47, no. 4, p. 1341-1345.

Weber, J. L. 1990. Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics*, vol. 7, no. 4, p. 524-530. http://dx.doi.org/10.1016/0888-7543(90)90195-z

Weir, B. S. 1990. Genetic data analysis. Methods for discrete population genetic data. http://dx.doi.org/10.2307/2532683

Zhu, Y., Chen, H., Fan, W., Wanf, Y., Li, Y., Chen, J., Yang, S., Hu, L., Leung, H., Mew, T. W., Teng, P. S., Wang, Z., Mundit, C. C. 2000. Genetic diversity and disease control in rice. *Nature*, vol. 406, p. 718-722. http://dx.doi:10.1038/35021046

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COLONIZATION OF GRAPES BERRIES BY *ALTERNARIA* sp. AND THEIR ABILITY TO PRODUCE MYCOTOXINS

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ABSTRACT

Our research focused on identify the *Alternaria* species from grapes (surface sterilized berries and non-surface sterilized berries) of Slovak origin and characterize their toxinogenic potential in *in vitro* conditions. We analyzed 47 samples of grapes, harvested in years 2011, 2012 and 2013 from various wine-growing regions. For the isolation of species, the method of direct plating berries and surface-sterilized berries (using 1 % freshly pre-pared chlorine) on DRBC (Dichloran Rose Bengal Chloramphenicol agar) was used. For each analysis was used 50 berries. Only undamaged berries have been used for analysis. The cultivation was carried at 25 ±1°C, for 5 to 7 days in dark. After incubation, the colonies of *Alternaria* were transferred on PCA - potato-carrot agar and CYA - Czapek-yeast extract agar and cultured for 7 days at room temperature and natural light. A total 4 species-groups of the genus *Alternaria* were isolated from grapes berries: *Alternaria alternata* (1369 isolates), *Alternaria arborescens* (734 isolates), *Alternaria infectoria* (143 isolates), and *Alternaria tenuissima* (3579 isolates). According to European Union legislation mycotoxins produced by species genus *Alternaria* are not monitored in foods and food commodities. Mycotoxins such as alternariol and alternariol monomethylether are mutagenic and genotoxic in various *in vitro* systems. Selected strains were tested for production of altenuene, alternariol monomethylether and alternariol. In neither case of *A. infectoria* species-group isolates was confirmed the production of tested mycotoxins in *in vitro* conditions by TLC method. The ability to produce altenuene, alternariol monomethylether and alternariol in *in vitro* conditions was detected in isolates of *Alternaria alternata*, *Alternaria arborescens* and *Alternaria tenuissima* species-groups. Isolates of *Alternaria alternata* species-group (44 tested isolates) were able to produce altenuene (24 isolates), alternariol monomethyleter (42 isolates) and alternariol (43 isolates). Only one isolate did not produce any mycotoxins. Isolates of *Alternaria arborescens* species-group (38 tested isolates) were able to produce altenuene (24 isolates), alternariol monomethyleter (33 isolates) and alternariol (36 isolates). Only two isolates did not produce any mycotoxins. Isolates of *Alternaria tenuissima* species-group (87 tested isolates) were able to produce altenuene (42 isolates), alternariol monomethyleter (41 isolates) and alternariol (73 isolates). Thirteen isolates did not produce any mycotoxins.

Keywords: *Alternaria*; altenuene; alternariol; alternariol monomethylether; grape

INTRODUCTION

Grapes have a complex microbial ecology including filamentous fungi, yeasts and bacteria with different physiological characteristics and effects upon wine production **(Barata et al., 2012)**. The black mould genus *Alternaria* Ness is ubiquitously distributed and includes various saprophytic, endophytic and pathogenic species. Many of the genus *Alternaria* Ness commonly cause spoilage of various food crops in the field or post-harvest decay **(Ostrý, 2008**; **Logrieco et al., 2009)**. *Alternaria* species are pathogenic and saprophytic fungi widely distributed in soil. They are widespread in both humid and semiarid regions and can infect growing plants in the field. They are the principal contaminating fungi in wheat, sorgum and barley. In addition to cereal crops, *Alternaria* species have been reported to occur in oilseeds such as sunflower and rapeseed, tomato, apples, citrus fruits, olives and several other fruits and vegetables. *Alternaria* species grow at low temperature; hence they are generally associated with extensive spoilage during refrigerated transport and storage **(Ostrý, 2008)**. *Alternaria* genus is the main component of the wine grape mycobiota at harvest time **(Serra et al., 2005**; **Prendes et al., 2015**; **Tančinová et al., 2015)**. The most common fungi spoiling grapes were *Alternaria*, *Botrytis cinerea* and *Cladosporium* **(Tournas, et al., 2005)**. Moreover, several *Alternaria* species are known to produce toxic secondary metabolites, *Alternaria* mycotoxins **(Rotem, 1994**; **Prendes et al., 2015)**. Mycotoxins are secondary metabolites produced by filamentous fungi that have been detected in food commodities, including grapes and wine **(Serra et al., 2005)**. *Alternaria* species have the ability to produce a variety of secondary metabolites, which plays important roles in food safety **(Andresen, et al., 2015)**. The major *Alternaria* mycotoxins belong to three structural classes: tetramic acid derivate, tenuazonic acid; the dibenzopyrone derivates, alternariol, anternariol methylether and altenuene; and the perylene derivates, the altertoxins **(Andersen et al., 2002)**. Food relevant *Alternaria* species are able to produce many more metabolites **(Ostrý, 2008**; **Logrieco et al., 2009)**. *Alternaria* toxins occurred regularly in cereals, tomato sauces, figs, wine and sunflower seeds. Only incidental occurrence of the *Alternaria* toxins was

observed in fresh apples, fresh citrus, fresh tomatoes and olives **(López et al., 2016)**.

Our research focused on the identify the *Alternaria* species from grapes of Slovak origin and characterize their toxinogenic potential in *in vitro*.

MATERIAL AND METHODOLOGY

Samples

Forty-seven samples of grapes, harvested in years 2011, 2012 and 2013 from various wine-growing regions of Slovakia, from small and medium-sized vineyardswere analyzed. White and red grape variety were analyzed. *White grape*: Müller Thurgau (1), Velsch Riesling (4), Grüner Veltliner (5), Pálava (1), Pinot blanc (2), Pinot gris (2), Sauvignon (2), Tramin (1), Zala gyöngye (1). *Red grape*: Alibernet (1 sample), André (2 samples), Blaufrankise (8), Cabernet Sauvignon (2), Pinot noir (2), Saint Laurent (1). Samples (3 kg) were collected at the time of technological ripeness. Picked grapes were stored at 4 ±1 °C and analyzed within 24 h after harvest.

Mycological analysis

For the isolation of *Alternaria* sp. was used the method of direct plating berries: surface-sterilized berries and non-sterilized berries on DRBC (Dichloran Rose Bengal Chloramphenicol agar) **Samson et al., (2002)**.

The endogenous mycobiota was determined by the method of direct placing of superficially sterilized berries on agar plates **(Samson et al., 2002)**. More than 50 pieces of undamaged berries from each sample were superficially sterilized (using 1% freshly pre-pared chlorine). Sterilization was carried out for 2 minutes. Berries were rinsed 3 times with sterile distilled water and dried on sterile filter paper. Exactly 50 berries from each sample were placed on DRBC plates (agar with dichloran, rose bengal and chloramphenicol) **(Samson et al., 2002)**. Cultivation lasted from 5 to 7 days in darkness at 25 ±1 °C. For each analysis was used 50 berries. Only undamaged berries have been used for analysis. After incubation, the colonies of *Alternaria* were transferred onto appropriate identification media.

Identification of *Alternaria* species-groups. Grown micromycetes were classified into the genera and then isolated by re-inoculation on the identification nutrient media and identified by accepted mycological keys and publications. Isolates of the genus *Alternaria* were re-inoculated on PCA - potato-carrot agar and CYA - Czapek-yeast extract agar **(Samson et al., 2002)** and cultured for 7 days at room temperature and natural light.

In order to improve study of sporulation pattern we proceeded as follows. The colonized agar (piece of approx. size 0.5 x 1.0 cm) was cut and transferred to the agar surface, outside the colony. The growth was observed as early as one to two days of cultivation on the edge of the removed part. Main used identification keys were **Andersen et al., (2001)**; **Andersen et al., (2002)**; **Simmons, (1994)**; **Simmons, (2007) and Simmons and Roberts (1993)**.

Obtained results were evaluated and expressed in isolation frequency (Fr) at the species level. The isolation frequency (%) is defined as the percentage of samples within which the species occurred at least once **(Gautam et al., 2009)**. These values were calculated according to **González et al., (1996)** as follows:

$$Fr\ (\%) = (ns / N) \times 100$$

where ns = number of samples with a species; N = total number of samples.

Toxinogenity analysis

Toxinogenity of selected isolates was analysed by means of thin layer chromatography (TLC) by **Samson et al., (2002)**. This method was performed with modifications according to **Labuda and Tančinová (2006)**. Testing was focused on determination of the ability to produce mycotoxins altenuene (ALT), alternariol (AOH) and alternariol monomethylether (AME).

The colonies grown on yeast extract sucrose agar (YES) (7, respectively 14 days, in darkness at 25 ±1°C) were cut into squares of approximate size 2 cm x 2 cm and placed in an Eppendorf tube with 0.5 mL of extraction solution (chloroform: methanol - 2:1; Reachem, SR). The content of the tubes was stirred for 5 minutes by Vortex Genie ® 2 (MO BIO Laboratories, Inc. - Carlsbad, CA). The obtained extracts were applied to silica gel chromatography plate (Alugram ® SIL G, Macherey - Nagel, Germany) and plates were put into the TEF solvent (toluene: ethyl acetate: formic acid – 5 :4 :1; toluene - Mikrochem, SR; ethyl acetate and formic acid - Slavus, SR). After elution and drying, the mycotoxins identity was confirmed by visual comparison with the standards of mycotoxins (AME, ALT and AOH - Merck, Germany) under UV light with a wavelength of 254 nm and 366 nm.

RESULTS AND DISCUSSION

In the current study from all samples were isolated *Alternaria* species (from superficially sterilized berries and berries without sterilization, too). The cosmopolitan fungal genus *Alternaria* consists of multiple saprophytic and pathogenic species. Based on phylogenetic and morphological studies, the genus is currently divided into 26 sections. *Alternaria* section *Alternaria* contains most of the small-spored species with concatenated conidia, including important plant, human and postharvest pathogens **(Woundenberg et al., 2015)**. A total of 4 species-groups (Table 1) of the genus *Alternaria* (*Alternaria* section *Alternaria*) were isolated from grapes berries, namely *Alternaria alternata* group, *Alternaria arborescens* group, *Alternaria infectoria* group, and *Alternaria tenuissima* group. Isolates, which could not be closer specified or contaminated another species were specified as *Alternaria* sp., Sporulation patterns of *Alternaria* species-group are listed according to **Simmons, (2007).** The typical sporulation pattern of *Alternaria alternata* group (Figure 1) comprises a single suberect conidiophore and an apical cluster of branching chains of small conidia separated by short secondary donidiophores. Long, well-defined primary conidiophores of *Alternaria arborescens* group (Figure 2) characteristically bear a few terminal and subterminal branches. Each conidiophore branch bears a branching chain of conidia, giving a relatively tall, three-dimensionally arborescent apperarance to the suberect system.

Table 1 Species-groups of *Alternaria* isolated from berries of Slovak origin determinated by using plate direct method on DRBC agar from 47 samples.

Alternaria groups	**Superficially sterilized berries**		**Berries without sterilization**	
	Number of isolates	Isolation frequency (%)	Number of isolates	Isolation frequency (%)
Alternaria alternata	662	78.7	707	78.7
Alternaria arborescens	405	34.0	329	57.4
Alternaria infectoria	109	31.9	34	29.78
Alternaria tenuissima	1644	93.6	1935	89.36
Alternaria sp.	144	53.2	94	46.81

Note: DRBC - Dichloran Rose Bengal Chloramphenicol agar.

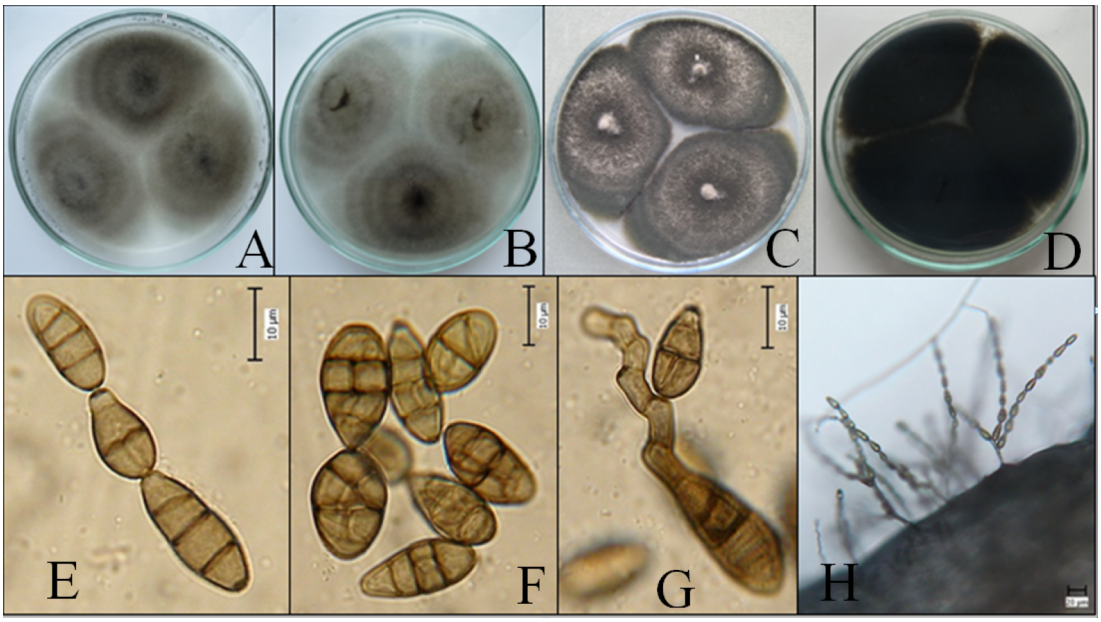

Figure 1 *Alternaria alternata* group 7 days of incubation. **A-B** colonies on PDA (A – top, B – reverse), **C-D** colonies on CYA (C – top, D – reverse), **E-F** conidia (scale bar = 10 µm), **H** – conidium sporulation pattern (scale bar = 20 µm) Photo: Mašková.

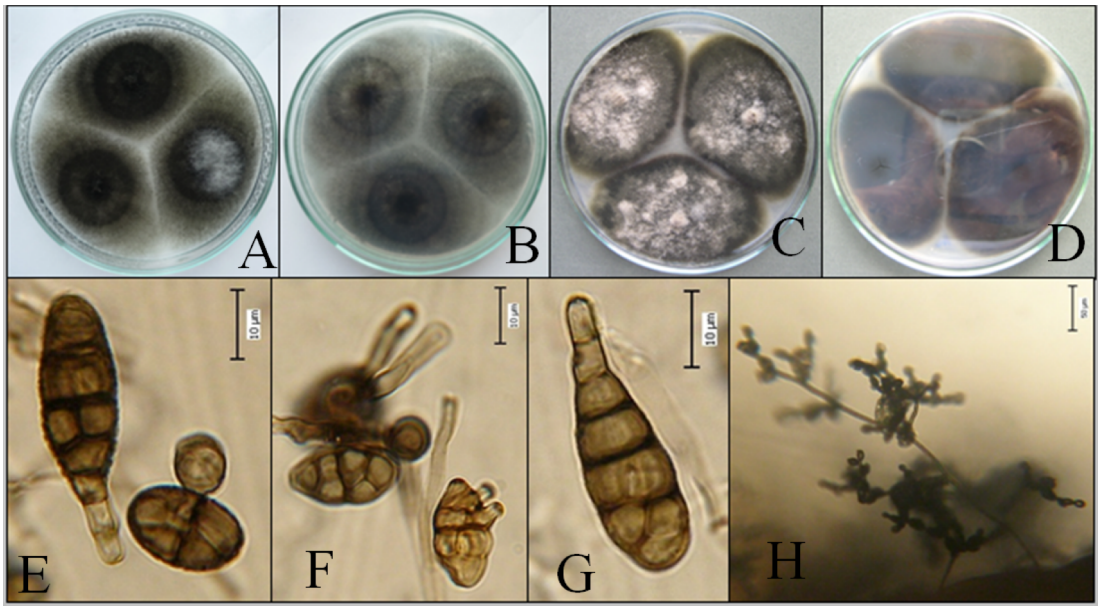

Figure 2 *Alternaria arborescens* group 7 days of incubation. **A-B** colonies on PDA (A – top, B – reverse), **C-D** colonies on CYA (C – top, D – reverse), **E-F** conidia (scale bar = 10 µm), **H** – conidium sporulation pattern (scale bar = 50 µm), Photo: Mašková.

Table 2 Potential ability of *Alternaria* species groups isolates to produce mycotoxins in *in vitro* conditions, tested by TLC method.

Species groups of *Alternaria*	Number of tested isolates	Number of isolates without the production of mycotoxins	Mycotoxins		
			Altenuene	Alternariol monomethylether	Alternariol
Al. alternata	44	1	24	42	43
Al. arborescens	38	2	24	33	36
Al. infectoria	15	15	0	0	0
Al. tenuissima	87	13	42	41	73

Note: TLC - thin layer chromatography, *Al. – Alternaria.*

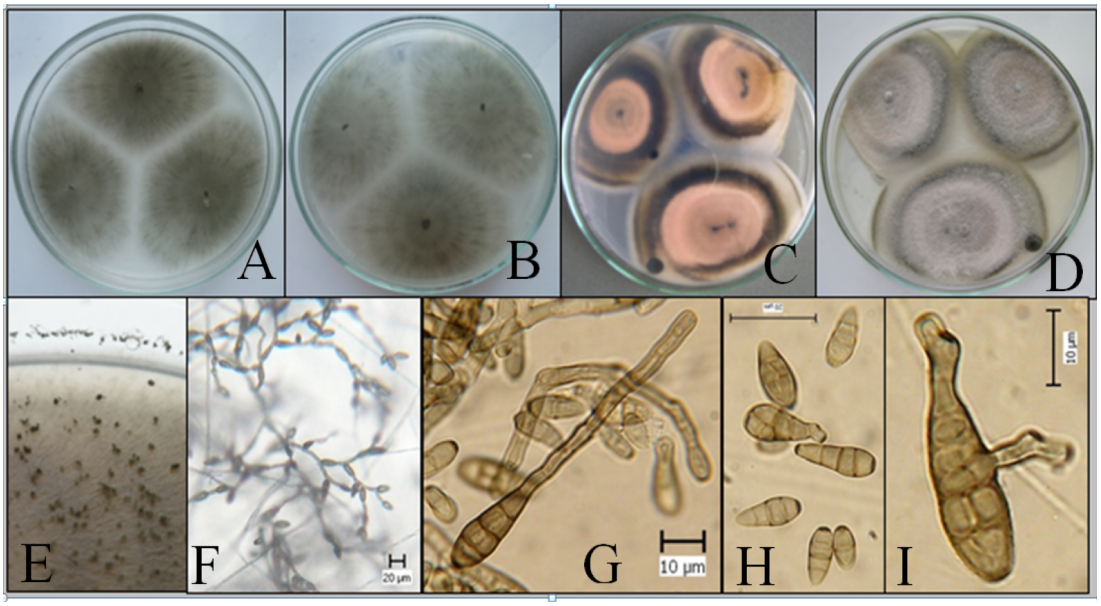

Figure 3 *Alternaria infectoria* group 7 days of incubation. **A-B** colonies on PDA (A – top, B – reverse), **C-D** colonies on CYA (C – top, D – reverse), **E** – granular look of colnies, **F** – conidium sporulation pattern (scale bar = 20 µm), **G-I** conidia (scale bar = 10, 20, 10 µm), Photo: Mašková.

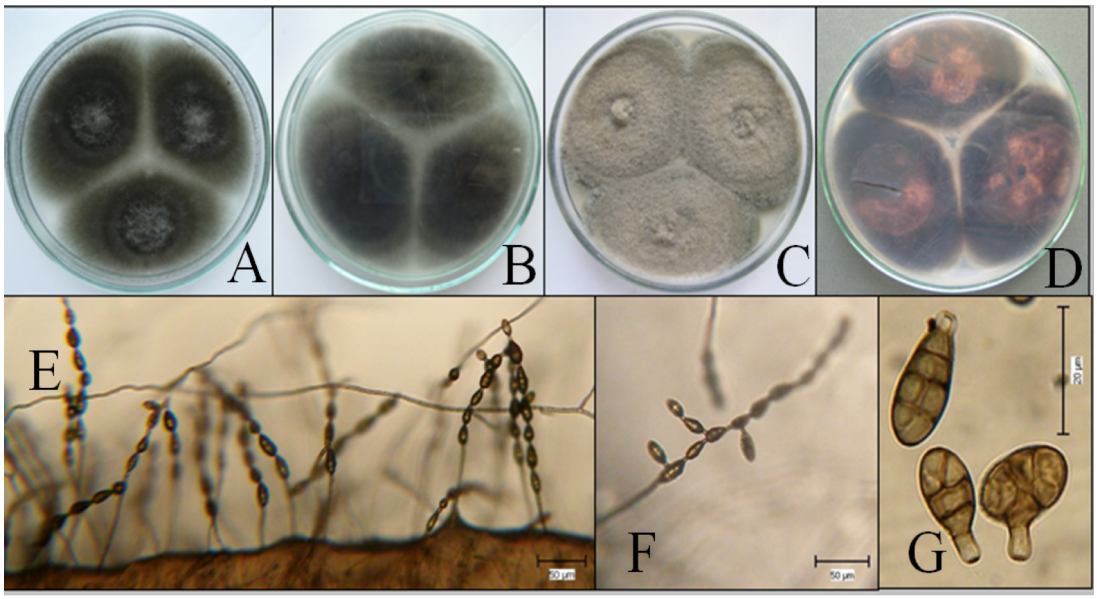

Figure 4 *Alternaria tenuissima* group 7 days of incubation. **A-B** colonies on PDA (A – top, B – reverse), **C-D** colonies on CYA (C – top, D – reverse), **E-F** – conidium sporulation pattern (scale bar = 50 µm), **G** – conidia (scale bar = 20 µm), Photo: Mašková.

Conidiophores of *Alternaria infectoria* group (Figure 3) that sporulate in the surface mass commonly are unbranched but have 1 – 3 geniculate extensions and conidiogenous loci incorporated in a total length of 50 – 100 µm. *Alternaria tenuissima* group (Figure 4) – produce uncrowded chains of up to 12 conidia on branching hyphae. The intial 1 – 2 and sometimes even 4 –5 lowest conidia of a chain usually have only transverse septa; only one or two mature conidia in a chain have the helpfully diagnostic median, subconstricting transverse septum that is such a striking feature of field conidia. The occurrence of the genus *Alternaria* in grape berries reported: **Serra et al., (2005, 2006)**; **Tournas et al., (2005)**; **Ostrý et al., (2007)**; **Polizzotto et al., (2012)**; **Chunmei et al., (2013)**; **Mašková et al., (2013)**; **Lorenzini and Zapparoli (2014)**; **Roseusseaux et al., (2014)**; **Prendes et al., (2015)**; **Tančinová et al., (2015)** and other authors. According **Bau et al., (2005)** predominant mycobiota of grape berries belonged to *Alternaria* spp., *Cladosporium* spp. and *Aspergillus* spp. These three genera were isolated from 75.6%, 22.5% and 17.3% of plated berries, respectively. **Magnoli et al., (2003)** reported that *Alternaria* genus was the most frequent (80% positive samples) from the surface-disinfected berries from Argentina. *Alternaria alternata* was the only species identified from this genus. **Ostrý et al., (2007)**; **Diguta et al., (2011)**; **Prendes et al., (2015**) recorded incidence of *Alternaria alternata*, also. Other authors mentioned the occurrence of *Alternaria* on the grape berries as follows: *Alternaria alternata* and *Alternaria tenuissima* **(Rousseaux et al., 2014)**; *Alternaria alternata* and *Alternaria arborescens* **(Lorenzini et al., 2014)**; *Alternaria arborescens* species-group and *Alternaria tenuissima* species-group **(Polizzotto et al., 2012)**. Isolation of *A. infectoria* species-group mentioned **Mašková et al., (2013)**, from Slovakian samples of grapes, too. In our sample was dominant *Alternaria tenuissima* group (1644 isolates – berries superficially sterilized and 1935 isolates – berries without sterilization), follow by *Alternaria alternata* group (662, respectively 707 isolates), *Alternaria arborescens* group (405 and 329 isolates) and Alternaria infectoria (109 and 34 isolates).

Mycotoxins are abiotic hazards produced by certain fungi that can grow on a variety of crops **(Marin et al., 20013).** According to European Union legislation mycotoxins produced by species genus *Alternaria* are not monitored in foods and food commodities. Mycotoxins such as alternariol and alternariol monomethylether are mutagenic and genotoxic in various *in vitro* systems. In addition, it has been suggested that in certain areas in China *Alternaria* toxins in grains might be responsible for oesophageal cancer. Hence, due to their possible harmful effects, *Alternaria* toxins are of concern for public health **(EFSA, 2011)**. According to **Prendes et al., (2015)**, *Alternaria*, one of the most mycotoxigenic genus commonly found in wine grapes, could represent a high risk for the wine consumer´s health. Representative isolates were selected for analysis to produce mycotoxins in *in vitro* conditions randomly from all obtained isolates. The results are presented in Table 2. A total of 184 isolates were tested. Production of selected secondary metabolites demonstrated the toxinogenity of isolates and on the other hand, it also served as an auxiliary indicator for identification (chemotaxonomy), mainly to distinguish the *Alternaria infectoria* species-group from the others **(Mašková et al., 2012)**. Production of mycotoxins by any of *Alternaria infectoria* strains still has not been demonstrated **(Andersen et al., 2002**; **Labuda et al., 2008**; **Piovarčiová et al., 2007)**. Conversely, *Alternaria alternata* and *Alternaria tenuissima* are known to produce several types of mycotoxins **(Andersen et al., 2002**; **Piovarčiová et al., 2007)**, which were confirmed in our study (Table 2). In neither case of the 15 tested isolates of *Alternaria infectoria* species-group we confirmed the production of mycotoxins ALT, AOH and AME. Although, the reputation of "nontoxigenic" strains of the *Alternaria infectoria* species-group has been undermined in recent years by isolation unknown metabolites **(Mašková et al., 2012)**. Conversely, isolates of other tested species-groups proved to be highly toxigenic (Table 2). Only one isolates of *Alternaria alternata* species-group and two isolates of *Alternaria arborescens* species-group did not produce tested mycotoxins in *in vitro* conditions detectable by TLC method. Robiglio and Lopez were tested eleven *Alternaria alternata* strains, isolated from Red Delicious apples in cold storage in Argentina, for alternariol and alternariol methyl ether production in laboratory media and in whole fresh fruits. Most of them were able to produce both toxins in all media. They were detected also in mycelium free filtrates from liquid cultures and in asymptomatic tissues from inoculated fruit. Thus, in the evaluation of mouldy core incidence in apples, the presence of *Alternaria alternata* toxins in tissues should be considered even in the absence of mycelia **(Robiglio and Lopez, 1995)**.

Small-spored *Alternaria*, such as *Alternaria alternata* group, *Alternaria arboresces* group, *Alternaria infectoria* group and *Alternaria tenuissima* group are important producers of mycotoxins, or other unknown metabolites but they were dominant fungal consortium in grapes berries in our samples. Considering that literature reported about the effectiveness of *Alternaria* endophytes against important grapevine pathogens, it should be interesting to elucidate the chemical structure of *Alternaria* unknown metabolites and to evaluate them as new biological method in the control of grapevine diseases **(Polizzotto et al., 2012)**.

CONCLUSION

From the 2350 surface-sterilized (47 samples) grape berries have been isolated 2964 strains of genus *Alternaria* and from the same number of non-sterilized berries 3099 isolates of this genus. Isolates were identified acording to sporulation patterns to four species groups: namely *Alternaria alternata* (1369 isolates), *Alternaria arborescens* (734), *Alternaria infectoria* (143), and *Alternaria tenuissima* (3579) and 238 isolates were not identified to species group. There were found out the ability to produce following mycotoxis: altenuene, alternariol and alternariol monomethylether in *in vitro* conditions by TLC method of chosen strains of genus *Alternaria*. In another research would be advisable to follow occurrence of these mycotoxins in grapes, must, wine and another grape products.

REFERENCES

Andersen, B., Kroger, E., Roberts, R. G. 2001. Chemical and morphological segregation of *Alternaria alternata*, *A. gaisen*

and *A. longipes*. *Mycological Research*, vol. 105, no. 3, p. 291-299. http://dx.doi.org/10.1017/s0953756201003446

Andersen, B., Kroger, E., Roberts, R. G. 2002. Chemical and morphological segregation of *Alternaria arborescens*, *A. infectoria* and *A. tenuissima* species-groups. *Mycological Research*, vol. 106, no. 2, p. 170-182. http://dx.doi.org/10.1017/s0953756201005263

Andersen, B., Nielsen, K. F., Fernández Pinto, V., Patriarca, A. 2015. Characterization of *Alternaria* strains from Argentinean blueberry, tomato, walnuts and wheat. International Journal of Food Microbiology, vol. 196, p. 1-10. http://dx.doi.org/10.1016/j.ijfoodmicro.2014.11.029

Barata, A., Malfeito-Ferreira, M., Loureiro, V. 2012. The microbial ecology of wine grape berries. *International Journal of Food Microbiology*, vol. 153, no. 3, p. 243-259. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.11.025

Bau, M., Bragulat, M. R., Abarca, M. L., Minguez, S., Cabañes, F. J. 2005. Ochratoxigenic species from Spanish wine grapes. *International Journal of Food Microbiology*, vol. 98, no. 2, p. 125-130. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.05.015

Chunmei, J., Junling, S., Qi´an, H., Yanlin, L. 2013. Occurrence of toxin-producing fungi in intact and rotten table and wine grapes and related influencing factors. *Food Control*, vol. 31, no. 1, p. 5-13. http://dx.doi.org/10.1016/j.foodcont.2012.09.015

Diguta, C. F., Vincent, B., Guilloux-Benatier, M., Alexandre, H., Rousseaux, S. 2011. PCR ITS-RFLP: A useful method for identifyting filamentous fungi isolates on grapes. *Food Microbiology*, vol. 28, no. 6, p. 1145-1154. http://dx.doi.org/10.1016/j.fm.2011.03.006

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2011. Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. *EFSA Journal 2011*, vol. 9, no. 10, 97 p. http://dx.doi.org/10.2903/j.efsa.2011.2407

Gautam, A., Sharma, S., Bhadauria, R. 2009. Detection of toxigenic fungi and mycotoxins in medicinally important powdered herbal drugs. *The Internet Journal of Microbiology*, vol. 7, no. 2, p. 1-8. http://dx.doi.org/10.5580/104b

González, H. H. L., Pacin, A., Resnik, S. L., Martinez, E. J. 1996. Deoxynivalenol and contaminant mycoflora in freshly harvested Argentinean wheat in 1993. *Mycopathologia*, vol. 135, no. 2, p. 129-134. http://dx.doi.org/10.1007/bf00436463

Labuda, R., Tančinová, D. 2006. Fungi recovered from slovakian poultry feed mixtures and their toxinogenity. *Annals of Agricultural and Environmental Medicine,* vol. 13, no. 2, 193-200. PMid:17195991

Labuda, R., Piovarčiová, Z., Tančinová, D. 2008. Sensitivity of the *Alternaria infectoria* species-group to cycloheximide. *Letters in Applied Microbiology*, vol. 46, no. 6, p. 673-675. http://dx.doi.org/10.1111/j.1472-765x.2008.02370.x

Logrieco, A., Moretti, A., Solfrizzo, M. 2009. Alternaria toxins and plant diseases: an overview of origin, occurrence and risks. *World Mycotoxin Journal*, vol. 2, no. 2, p. 129-140. http://dx.doi.org/10.3920/wmj2009.1145

López, P., Venema, D., de Rijk, T., de Kok, A., Scholten, J. M., Mol, H. G. J., de Nijs, M. 2016. Occurrence of Alternaria toxins in food products in the Netherlands. *Food Control*, vol. 60, p. 196-204. http://dx.doi.org/10.1016/j.foodcont.2015.07.032

Lorenzini, M., Zapparoli, G. 2014. Characterization and pathogenicity of Alternaria spp. strains associated with grape bunch rot during post-harvest withering. *International Journal of Food Microbiology*, vol. 186, p. 1-5. http://dx.doi.org/10.1016/j.ijfoodmicro.2014.06.008

Magnoli, C., Violante, M., Combina, M., Palacio, G., Dalcero, A. 2003. Mycoflora and ochratoxin-producing strains of Aspergillus section Nigri in wine grapes in Argentina. Letters in Applied Microbiology, vol. 37, no. 2, p. 179-184. http://dx.doi.org/10.1046/j.1472-765x.2003.01376.x

Marin, S., Ramos, A. J., Cano-Sancho, G., Sanchis, V. 2013. Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology*, vol. 60, p. 218-237. http://dx.doi.org/10.1016/j.fct.2013.07.047

Mašková, Z., Tančinová, D., Barboráková, Z., Felšöciová, S., Císarová, M. 2012. Comparison of occurrence and toxinogenity of *Alternaria* spp. isolated from samples of conventional and new crossbred wheat of slovak origin. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 1, p. 552-562.

Mašková, Z., Tančinová, D., Rybárik, Ľ., Felšöciová, S., Kačinová, J. 2013. Colonization of grape berries by representatives of the genus *Alternaria* and their occurence in the stum. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2, p. 1754-1764.

Ostrý, V., Škarková, J., Procházková, I., Kubátová, A., Malíř, F., Ruprich, J. 2007. Mycobiota of Czech wine grapes and occurrence of ochratoxin A and *Alternaria* mycotoxins in fresh grape juice, must and wine. *Czech Mycology*, vol. 59, p. 241-254.

Ostrý, V. 2008. Alternaria mycotoxins: an overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs. *World Mycotoxin Journal*, vol. 1, no. 2, p. 175-188. http://dx.doi.org/10.3920/wmj2008.x013

Piovarčiová, Z., Labuda, R., Tančinová, D. 2007. Výskyt *Alternaria* spp. na zrnách potravinárskej pšenice dopestovanej na Slovensku v sezóne 2006. In *Proceeding from workshop MICROMYCO 2007.* České Budějovice : Department of soil biology, Czech Republic, p. 124-132.

Prendes, L. P., Merín, M. G., Andreoni, M. A., Ramirez, M., Morata de Ambrosini, V. I. 2015. Mycobiota and toxicogenic *Alternaria* spp. strains in Malbec wine grapes from DOC San Rafael, Mendoza, Argentina. Food Control, vol. 57, p. 122-128. http://dx.doi.org/10.1016/j.foodcont.2015.03.041

Polizzotto, R., Andresen, B., Martini, M., Grisan, S., Assante, G., Musetti, R. 2012. A polyphasic approach for the characterization of endophytic *Alternaria* strains isolated from grapevines. *Journal of Microbiological Methods*, vol. 88, no. 1, p. 162-171. http://dx.doi.org/10.1016/j.mimet.2011.11.009

Robiglio, A. L, Lopez, S. E. 1995. Mycotoxin production by *Alternaria alternata* strains isolated from Red Delicious apples in Argentina. *International Journal of Food Microbiology,* vol. 24, no. 3, p. 413-417. http://dx.doi.org/10.1016/0168-1605(94)00035-5

Rotem, J. 1994. *The genus Alternaria: biology, epidemiology and pathogenicity*. St. Paul, MN: The American Phytopathological Society, 326 p.

Rousseaux, S., Diguta, C. F., Radoï-Matei. 2014. Non-*Botrytis* grape-rotting fungi responsible for earthy and moldy off-flavors and mycotoxins. *Food Microbiology*, vol. 38, p. 104-121. http://dx.doi.org/10.1016/j.fm.2013.08.013

Serra, R., Braga, A., Venâncio, A. 2005. Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. *Research in Microbiology*, vol. 156, no. 4, p. 515-521. http://dx.doi.org/10.1016/j.resmic.2004.12.005

Serra, R., Lourenço, A., Alípio, P., Venâncio, A. 2006. Influence of the region of origin on the mycobiota of grapes

with emphasis on *Aspergillus* and *Penicillium* species. *Mycological research*, vol. 110, no. 8, p. 971-978. http://dx.doi.org/10.1016/j.mycres.2006.05.010

Samson, R. A., Hoekstra, E. S., Frisvad, J. C., Filtenborg, O. 2002. *Introduction to food- and airborne fungi*. Utrecht: Centraalbureau voor Schimmelcultures, 2002. 389 p. ISBN 90-70351-42-0. http://dx.doi.org/10.5580/104b

Simmons, E. G. 1994. *Alternaria* themes and variations (106-111). Mycotaxon, 1994, vol. 50, p. 409-427.

Simmons, E. G. 2007. *Alternaria*, *An Identification Manual*. Utrecht : CBS Fungal Biodiversity Centre, 2007. 775 p. ISBN 978-90-70351-68-7.

Simmons, E. G., Roberts, R. G. 1993. *Alternaria* themes and variations. In *Mycotaxon*, vol. 73, p. 109-140.

Tančinová, D., Rybárik, Ľ., Mašková, Z., Felšöciová, S., Císarová, M. 2015. Endogenal colonization of grapes berries. *Journal of Microbiology, Biotechnology and Food Sciences* vol. 4, sp. no. 1, p. 69-73. http://dx.doi.org/10.15414/jmbfs.2015.4.special1.69-73

Tournas, V. H., Katsoudas, E. 2005. Mould and yeast flora in fresh berries, grapes and citrus fruits. *International Journal of Food Microbiology*, vol. 105, no. 1, p. 11-17. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.05.002

Woundenberg, J. H. C., Seidl, M. F., Groenewald, J. Z., de Vries, M., Stielow, J. B., Thomma, B. P. H. J., Crous, P. W. 2015. *Alternaria* section *Alternaria*: Species, formae speciales or pathotypes? *Studies in Mycology*, vol 82, p. 1-21. http://dx.doi.org/10.1016/j.simyco.2015.07.001

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RESEARCH AND PRACTICE: QUANTIFICATION OF RAW AND HEAT-TREATED COW MILK IN SHEEP MILK, CHEESE AND BRYNDZA BY ELISA METHOD

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ABSTRACT

The aim of this study was to test the reliability of commercial ELISA tests (RC-bovino) within raw and heat treated cow milk detection in sheep milk and cheese in order to obtain a high-quality, reliable and economically beneficial method suitable for routine application in practice. These tests were subsequently used for quantification of cow milk in commercial "Bryndza". Raw sheep milk, cow milk and heat-treated cow milk (pasteurisation at 72 °C for 15 sec or at 85 °C for 3 sec) were mixed in precisely defined proportions (0 – 100% cow milk in sheep milk). The milk mixtures were sampled to detect adulteration and subsequently cheese was made. By ELISA tests was possible to determine these amounts of raw cow milk in sheep milk: 0.5% (0.2%), 5 % (4.81%), 50% (42.08%) and 75% (56.52%). The pasteurized samples in different combinations gave lower optical density responses than those prepared from raw milk (by approximately 60%). In context with the above mentioned, the relationship between the real and detected amount of cow milk (%) in different production stages (milk, cheese) using a regression analysis was examined. However, a lower reliability of the detection was indicated by R^2 values, which ranged from 0.4058 (cheese) to 0.5175 (milk). In practice this means that although individual percentage (%) of cow milk in the sample can be detected, but in the unknown sample it can not be clearly confirm whether the cow milk was raw or heat-treated. In this context, the results can be inaccurate and may not correspond to the real situation. Within monitoring phase of this research, 9 samples of bryndza were analysed with the results of detected cow milk ranged from 11.56% to 14.3%. The obtained results confirm that the appropriate selection of ELISA tests can become an important factor in the setting of analytical capabilities for the detection of milk and cheese adulteration.

Keywords: ELISA; milk; cheese; adulteration; reliability

INTRODUCTION

Consumption of fresh dairy products is the important motive factor for their production in European Union **(Habánová et al., 2010)**.

The unknown mixture of milk from different species is a common fraud in dairy sector. Milk with high economic value is commonly adulterated with milk from species of lower cost. This adulteration is especially important for cheese makers, due to unknown milk mixtures produce changes in the final sensory properties and reduce the product quality. Sheep milk is more expensive than goat or cow milk and tends to be adulterated with those of lower cost **(Puchades and Maquieira, 2013; Mayer et al., 2012)**.

Fraudulent incorporation of nondeclared kind of milk during technological processing is a common practice that can cause a problem for reasons related to intolerance or allergy, religious, ethical or cultural objections, and legal requirements. Therefore, accurate evaluation of the milk species used in dairy products is needed, especially for high-grade cheeses made exclusively with sheep or goat milk, many of which are registered by European law with a Protected Designation of Origin (PDO) **(Zeleňáková et al., 2008)**.

Traditional bryndza is sharp, salty, grayish, grated and pin-rolled, crumbly, semi-spreadable 100% sheep cheese. There is no close equivalent in taste and texture among sheep, cow, or goat cheeses. Most modern commercially available bryndza is milder, bleached creamy white, and two of its three varieties can legally contain up to 49% cow cheese. The European Commission registered the latter as *Slovenská bryndza* on its food list of Protected Geographical Indications on 16 July 2008 **(Commission regulation (EC) No 676/2008)**.

For legal reasons and for consumer protection and confidence many analytical techniques for detecting mixtures of milks from different species have been developed in last decades **(Zachar et al., 2011; Zeleňáková et al., 2011)**.

The official EU reference method which is based on the IEF of γ-caseins **(Commission regulation (EC) No 273/2008)** is an appropriate tool to detect cow milk in products made from milk of other species (detection limit ≤0.5%). A high number of other analytical techniques (e.g. electrophoretic, chromatographic, immunological and molecular biological methods) have also been used for qualitative (and partly also quantitative) species

authentication in dairy products **(Bobková et al., 2009; Mayer et al., 2012; Pizzano et al., 2011; Asensio et al., 2008; Xue et al., 2010; Costa et al., 2008; Suhaj et al., 2010; Stanciuc and Rapeanu, 2010 etc.)**.

Zeleňáková et al., (2009) described current situation in adulteration of the sheep milk and sheep milk products in Slovakia as well as in some countries in the EU. The results were evaluated according to the requirements of the valid legal standards. From the total number 70 samples 20 were adulterated with nondeclared cow milk.

ELISA is the most widely used form of immunoassay in milk analysis and has advantages of high sensitivity, low cost and fast application. It is easy to use, reliable, rapid and readily automated **(Song et al., 2011; Costa et al., 2008)**.

The development of immunoenzymatic methods and their practical use depends mainly on the selection of the immunogenes, experimental animals, way of immunization, quality of used antiserum, or possibly used antibodies and specificity as well as sensitivity of the evidencing system **(Yeung, 2006)**.

An indirect enzyme-linked immunosorbent assay (ELISA) was developed for the detection and quantification of bovine milk adulteration in goat's milk. The polyclonal antibodies have been modified by mixing with goat's milk for the assay purposes. The absorbance at 450 nm in indirect ELISA revealed a linear relationship with the concentration of adulterated bovine milk at the range of 4% – 50% **(Xue et al., 2010)**.

Zarranz and Izco (2007) applied a protocol in order to validate a specific ELISA test for cow milk quantification in sheep milk, studying the main analytical properties displayed. The method was applied to analyze sheep milk samples collected from farms and it was found that 10% samples were adulterated with cow milk.

The aim of the study was to test the reliability of commercial ELISA tests for raw and heat-treated cow milk detection in the sheep milk and cheese and subsequently to quantify cow milk in commercial "Bryndza".

MATERIAL AND METHODOLOGY

1. Analysis of the samples in research part of the study:

Cow and sheep milk were obtained from a local dairy farm, refrigerated at 4 °C and tested for their quality. Both types of milk were mixed in the various alternatives, including heat treatment and subsequently cheese was made. In this research 32 samples were analysed what corresponded to 16 combinations of cow and sheep milk mixtures. At first, the intra assay and interassay were performed in terms of laboratory testing of results accuracy and repeatability. The sample extracts were pipetted into wells in duplicates.

Samples preparation:

Milk composition was performed at Lactoscan device. The working principle is based on measuring the speed of the *ultrasound* in milk. Observed parameters: Density ($kg.m^{-3}$), Fat content (g/100 g), Proteins (g/100 g), Lactose (g/100 g), Ash determined by calculation (g/100 g), Solids-non-fat (g/100 g), Freezing point of milk (°C). Other parameters: Calcium content (mg/100 g) by the complexometric titration method, Clotting activity (s), Titratable acidity of milk (°SH) by the method of Soxhlet-Henkel and Active acidity of milk by pH meter.

Raw sheep milk, cow milk and heat-treated cow milk (pasteurisation at 72 °C for 15 sec and at 85 °C for 3 sec) were mixed in precisely defined proportions (0, 0.5; 5; 50; 75; 100% cow milk in sheep milk). The milk mixtures were sampled to detect adulteration and subsequently cheese was made. At first the cheesiness test was performed and then 1 – 2.5 mL $CaCl_2$ per 1 liter was added to individual samples (depending on the level of heat treatment). The cheese production process included: cheesing of milk, processing of cheese curd, turning of cheese curd surface, its cutting, harping and mixing and finally formation of cloddish cheese. Subsequently the created clods were treated with 2% NaCl solution and left to mature at temperatures corresponding to the technological requirements (23, 19 and finally 8 °C). The temperature and pH in individual clods had been measured for 12 days. Subsequently they were processed and analysed according to the ELISA manufacturer instructions.

2. Analysis of the samples in monitoring and control part of the study:

The samples of bryndza (9 samples) were obtained in the grocery stores as well as from small sellers who product various sheep cheese. All the samples were refrigerated in the 30 mL boxes until the beginning of analysis. Subsequently they were processed and analysed according to the ELISA manufacturer instructions. The absorbance of the samples in research and monitoring part of the study was measured photometrically at 450 nm (STAT FAX 321/plus microwell reader - Awareness Technology, Palm City, FL). Comparisons of trends has been calculated with linear regression methods and visualized in graphs.

3. ELISA test characteristic:

ELISA tests RC-bovino (ZEU-INMUNOTEC, S.L, Spain) were used in our analysis. These tests are an enzyme immunoassay for the detection of cow milk in sheep or goat milk and their cheese. All reagents required for the enzyme immunoassay are contained in these test kits. The test kits are sufficient for 48 or 96 determinations (including standards). Detection limit is 0% cow milk. Assay time is approximately 90 minutes. The principle of the test is based upon the antigen-antibody reaction. The presence of cow milk in given sample is determined by the immunological detection of bovine IgG. The wells of the microtiter strips are coated with a specific antibody against bovine IgG. In the case of adulterated products, the antibodies contained in the cow milk will bind to the immobilized antibody. Any unbound components are removed in a washing step. By adding an antibody peroxidase-conjugate directed against bovine IgG, bound antigen is detected. Any unbound conjugate is removed in a washing step. Enzyme substrate and chromogen are added to the wells and incubated. The bound enzyme conjugate converts the colorless chromogen into a blue product. The addition of the stop reagent leads to a color change from blue to yellow. The measurement of the absorbance is made photometrically at 450 nm.

RESULTS AND DISCUSSION

In accordance with the ELISA instructions, within research part of the study, laboratory analysis of 32 samples of sheep milk and cheeses, adulterated with the addition of raw and heat-treated cow milk was performed. Prior to the analysis of these samples, quality control of ELISA tests was done. C.V. of results (n = 10) for inter and intra assay was 5.8% and 4.95%. As the basis for the evaluation, calibration curves were made by plotting % of cow milk in standard samples in a Y-axis and absorbance values in the X-axis. Values for the creation of calibration curves are shown in Table 1.

Table 1 The values for the creation of calibration curve for the detection of cow milk in samples by ELISA tests.

Standards	Concentration of cow milk in standards (%)	Absorbance at 450 nm	
		Analysis of milk samples	Analysis of cheese samples
1	0	0.369	0.401
2	1	0.492	0.526
3	5	0.973	1.036
4	10	1.483	1.528

The calibration curve should be linear in the range of 0 – 10% cow milk. It can then pass through the linear regression. These calibration curves were completed with trend lines of linear and polynomial function of the 2nd grade. Individual percentages of cow milk in the samples were calculated using regression equations or by interpolating the absorbance values obtained into the calibration curve. The obtained concentration data were the real values. They didn´t need any conversion factor. An example of calibration curve with regression equation for the detection of cow milk in mixed milk samples is shown in Figure 1. Numerous producers and sellers offer their own softwares for imunoanalysis data processing and these are also the part of fotometric analysers (four-parametric logistic model and spatial comparison method).

Czerwenka et al., (2010) have studied the calibration relationships in frame of chromatographic detection of buffallo milk adulteration by cow milk. β-Lg was the main marker and the results pointed that no effect was obtained in detection reliability when comparing the linear and the polynomic regression. The R^2 for linear regression was 0.9973 and for polynomic regression 0.9951.

García et al., (1994); Hurley et al., (2004 a, b); Zarranz and Izco (2007); Asensio et al., (2008) and many others also reported the very comparable calibration curves used for the detection of cow, goat and sheep milk and cheese adulteration. The degree of the variability calibration samples expressed R^2 was not less than 0.9 in all samples.

The above mentioned regression models were used in our data processing, too. The R^2 values ranged from 0.9981 up to 0.9956 for the linear regression and R^2 was 1 in two datasets for the polynomial regression models.

The important prerequisite for results evaluation was an adequate preparation of samples in which the series of dilutions was realized. The samples showing optical density over the valuef of highest standard were further diluted and tested again. The percentage of cow milk was calculated multiplying by diluting factor. The absorbancies that either exceeded the detection limit or were under it were not suitable for the quantitative analysis.

The lowest dilution amounts that possess the detectable absorbancies are summarized in the Table 2. All the absorbancies were analysed in the detection range of used ELISA kits. The absorbance values, that exceed the detection limit or were lower, were not possible to quantify. Based on the results, the dilution of samples in the range from 10^0 to 10^{-2} was used for the analysis 0 – 75% cow milk in sheep milk or cheese. The quatification was possible in the range from 10^0 to 10^{-1}. The only exceptions were the cow milk samples without

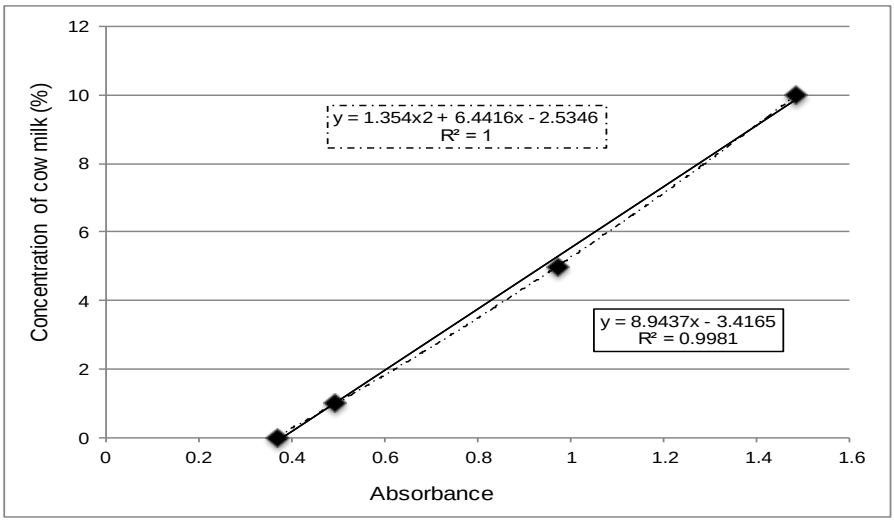
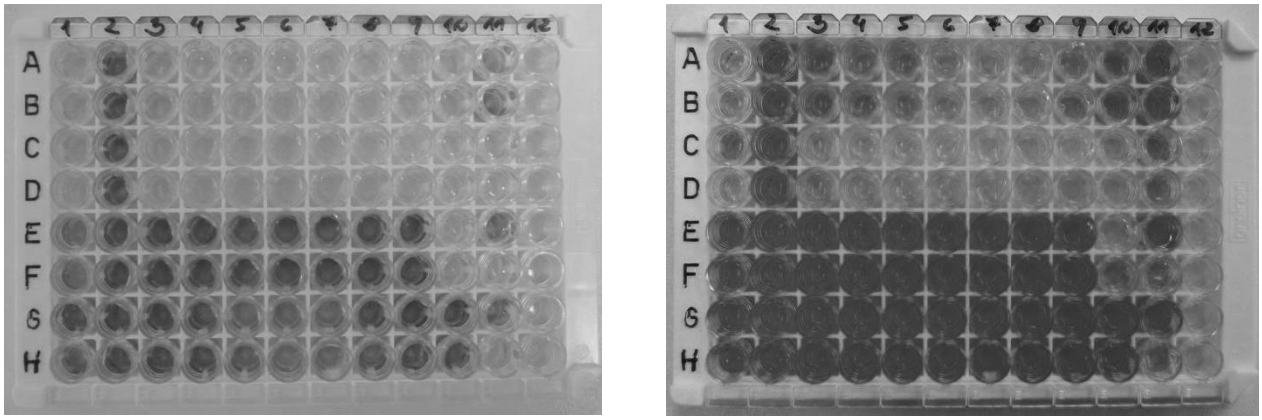


Figure 1 Calibration curve for the detection of cow milk in sheep milk.



A B

Picture 1 Visualization of ELISA test after addition of Substrate (A) and Stop solution (B).

sheep milk (14 M – 16 M and 14 CH – 16 CH). The absorbance values of these samples were similar instead of increased dilution what has influenced the final value of cow milk percentage. All these samples have no exact values in the Table 2 (!) and were not analysed further. For some samples (<75%), similar calculated concentrations were obtained, when two subsequently prepared decimal dilutions (from 10^0 to 10^{-1}) were used for the analysis. As an example is sample 9 CH. The absorbance 0.867 was detected for the dilution 10^0 what corresponds to the calculated concentration 3.95%. In the case of 10^{-1} dilution a lower absorbance was detected (0.452) what corresponds to the calculated concentration 3.98%. It was confirmed that the samples 1 M and 1 CH did not contain cow milk.

Regarding the choice of regression analysis model it can be said that with the increasing amount of cow milk, the higher values were calculated using linear regression equation. Nevertheless, producer of the used ELISA tests recommended analyze the obtained data by linear regression. The calculated values are reported in the Figures 2 – 4. ELISA tests of this producer are primarily designed to detect the adulteration of sheep and goat milk by raw cow milk. These amounts of raw cow milk in sheep milk it was possible to determine by ELISA tests: 0.5% (0.2%), 5% (4.81%), 50% (42.08%) and 75% (56.52%).

The amount of cow milk up to 10% (what is the detection range for these ELISA tests) can be analysed only by calibration curve including regression equations, without dilution of the samples. However, in a concentration range between 0 – 0.5%, quantification is more sensitive to imprecision. Therefore, it is important to prepare appropriate reagents, standards (especially in the concentration range from 0 to 1%) and keep a good laboratory practice. The producer also recommended creating a curve or curves with a specific detection range.

These curves were also used in analysis performed in study by **Zeleňáková et al., (2008)**. They found out that these types of curves can significantly affect the quality and accuracy of individual measurements. The same authors have reported that the results do not sometimes meet the quantitative criteria, especially at higher percentages. That can be caused by the saturation of the amount of specific antigens that are fixed in the microtitration plate and subsequently tight on the antigen surface.

ELISA is considered to be good quality when it can detect less than 1% foreign milk additives **(Song et al., 2011; Luis et al., 2009)**.

The next phase of the results analysis was focused on the evaluation of ELISA kits reliability within detection of different raw and heat-treated cow milk amounts in sheep milk and cheese. The results are reported in the Figure 2. The pasteurized samples in different combinations (including the cheese manufacturing) gave lower optical density responses than those prepared from raw milk. The detected amount of cow milk was in some samples (0.5 – 5%) under the detection range.

The main advantages are processing of a large number of samples, creation of calibration curve and measuring of blind samples simultaneously on one microtitration plate, which eliminates the impact of the changing conditions during the determination. ELISA has also disadvantages, for example in that it detects unimpaired proteins, but the protein hydrolysates need not react immunologically **(Hurley et al., 2006b; Taylor et al., 2009)**.

The caseins feature advantage in being more or less stable under high temperature conditions. Therefore, they can be successfully used as the main antigens in heat treatment (pasteurization, UHT) of milk and milk products. Their major disadvantage is weak immunogenicity and higher sensitivity to protheolytic degradation. The whey proteins are much better immunogens and they are protheolytically degradable only in minimal quantity. In respect of high temperatures the whey proteins are less resistant **(Lowe et al., 2004)**.

In context with the above mentioned, the relationship between the real and detected amount of cow milk (%) in different production stages (milk, cheese) using a regression analysis was examined. Four detection trends were setf for the analysed ranges from 0 to 75%. All of them were characterized by the linear functions with the appropriate regression equations.

Table 2 Comparison of assay sensitivity by two regression models.

Sample - cow milk in sheep milk (M) and cheese (CH)	Absorbance at 450 nm	Dilution	Detected amount of cow milk (%)	
			Linear function	Polynomial function
1 M (0% raw)	0.309	a	——	——
2 M (0.5% raw)	0.404	a	0.197	0.289
3 M (0.5% low pasteurized)	0.334	a	——	——
4 M (0.5% high pasteurized)	0.327	a	——	——
5 M (5% raw)	0.919	a	4.806	4.529
6 M (5% low pasteurized)	0.37	a	——	——
7 M (5% high pasteurized)	0.34	a	——	——
8 M (50% raw)	0.853	b	42.08	39.409
9 M (50% low pasteurized)	0.534	b	13.597	12.913
10 M (50% high pasteurized)	0.458	b	6.797	6.997
11 M (75% raw)	1.014	b	56.524	53.894
12 M (75% low pasteurized)	0.609	b	20.257	18.865
13 M (75% high pasteurized)	0.528	b	13.058	12.44
14 M (100% raw)	!	!	!	!
15 M (100% low pasteurized)	!	!	!	!
16 M (100% high pasteurized)	!	!	!	!
1 CH (0% raw cow)	0.204	a	——	——
2 CH (0.5% raw)	0.409	a	——	0.114
3 CH (0.5% low pasteurized)	0.407	a	——	0.101
4 CH (0.5% high pasteurized)	0.398	a	——	——
5 CH (5% raw)	0.634	a	1.98	1.768
6 CH (5% low pasteurized)	0.411	a	——	0.128
7 CH (5% high pasteurized)	0.405	a	——	0.088
8 CH (50% raw)	0.569	b	13.254	12.177
9 CH (50% low pasteurized)	0.867	a	3.945	3.55
10 CH (50% high pasteurized)	0.637	a	1.985	1.723
11 CH (75% raw)	0.646	b	20.023	17.866
12 CH (75% low pasteurized)	1.025	a	5.334	4.435
13 CH (75% high pasteurized)	0.648	a	2.024	1.805
14 CH (100% raw)	!	!	!	!
15 CH (100% low pasteurized)	!	!	!	!
16 CH (100% high pasteurized)	!	!	!	!

Dilution: 10^0 (a); 10^{-1} (b); differences within individual dilutions (!); outside the detection range (——).

In the Figure 3 it can be seen that individual curves are indeed increasing character that corresponds to the growing amount of cow milk. However, a lower reliability of the detection was indicated by R^2 values, which ranged from 0.4058 (cheese) to 0.5175 (milk). In practice this means that although individual percentage of cow milk in the sample can be detected (%), but in the unknown sample it can not be clearly confirm whether the cow milk was raw or heat-treated. In this context, the results can be inaccurate and may not correspond to the real situation.

Creating the specific regression curves for each way of cow milk heat treatment (Figure 4) was performed in order to asses the relationship between the real and detected amounts of cow milk in sheep milk. The values of determination coefficients (R^2) were higher than 0.82. Reliable detection of the real amount of cow milk can be performed in the praxis by both, interpolation as well as the regression analysis. The basic limitation for the precise detection is to know the way of cow milk heat treatment. Similar regression curves can be provided for the detection of cheese adulteration, too.

As the various processing of milk can negatively affect the reliability of adulteration detection, such type of the analysis has not been applied in the praxis yet and also there is not recommended for the use. Therefore, the use of these ELISA tests is not adequate for routine surveillance of marketed cheese, especially for mixed cheeses, when the amount of milk from different species used for cheese making is unknown.

The detection and quantification of cow milk in the sheep milk and cheese using the commercial ELISAs was performed by **Costa et al., (2008)**, too. The detected value in cheese samples was by 10% lower than the experimental value for QBT ELISA test and by 20 % lower for QGT ELISA test, when more than 40% cow or goat milk was added.

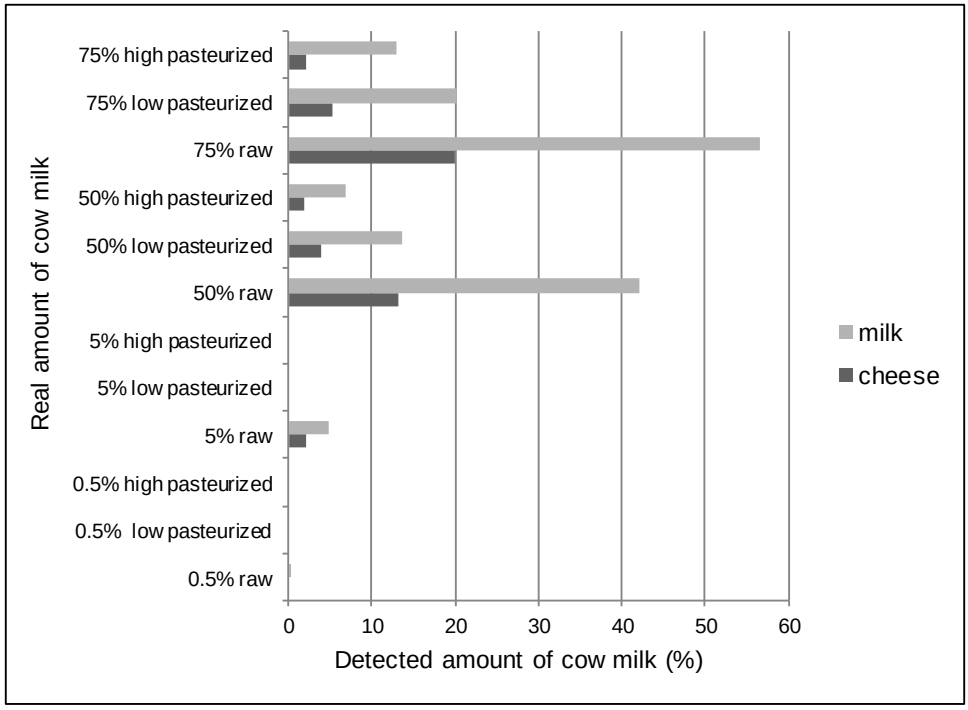


Figure 2 Impact of cow milk heat treatment on its detection in sheep milk and cheese.

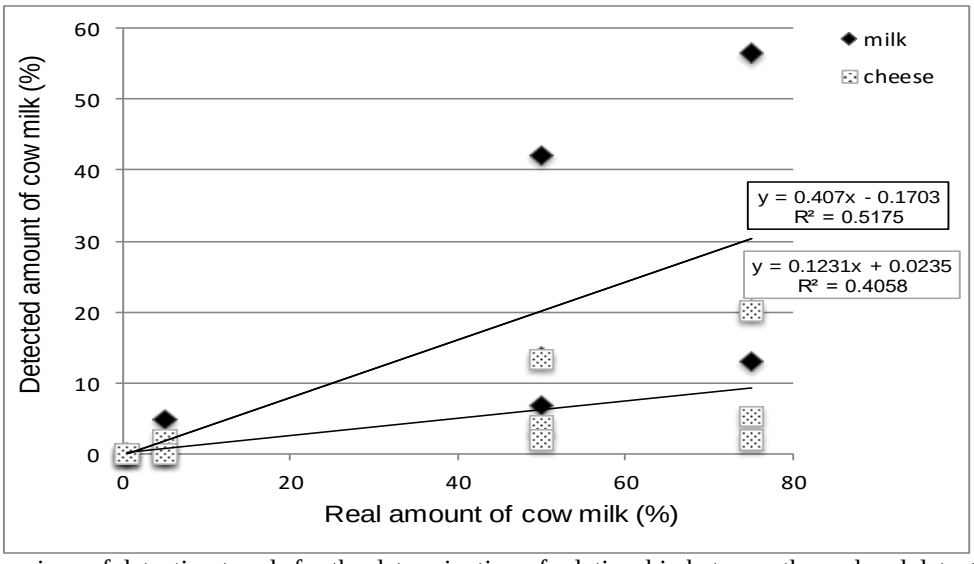


Figure 3 Comparison of detection trends for the determination of relationship between the real and detected percentage of cow milk in sheep milk and cheese (%).

The ELISA tests RC-bovino were subsequently used for quantification of cow milk in 9 samples of commercial "Bryndza". Individual percentage of cow milk in the samples were calculated by interpolating the absorbance values obtained into the calibration curve and using regression equations ($y = 7.3075x - 1.9301$; $R^2 = 0.9995$). The presence of cow milk was confirmed in all analysed samples of bryndza (Table 3). The samples 1 – 8 were evaluated together and the sample 9 was evaluated separately according to the composition differences as given by manufacturers. By ELISA test there were detected from 11.56% (sample 1) to 14.3% (sample 4) cow milk. The coefficient of variation was 9.26% for these 8 samples. The sample 9 „Tatranská bryndza" was specific because of high portion of cow milk. The manufacturer indicates this fact on the labeling (25% of sheep cheese).

In this sample 31.44% cow milk was detected by ELISA. But it can be assumed, that the real addition of cow milk in commercial samples of bryndza was higher than those detected by ELISA. This is based on the previously performed analyses and over mentioned results. Reliability of the ELISA tests and their applicability in the routine analysis was studied by many authors such as **Popelka et al., (2002); Zeleňáková et al., (2008, 2009, 2011); Zarranz and Izco (2007); Costa et al., (2008); Štumr et al., (2008); Brinkhof et al., (2009); Luis et al., (2009); Taylor et al., (2009); Kardar (2010); Sleziaková and Baleková (2010); Xue et al., (2010); Song et al., (2011)** and many others.

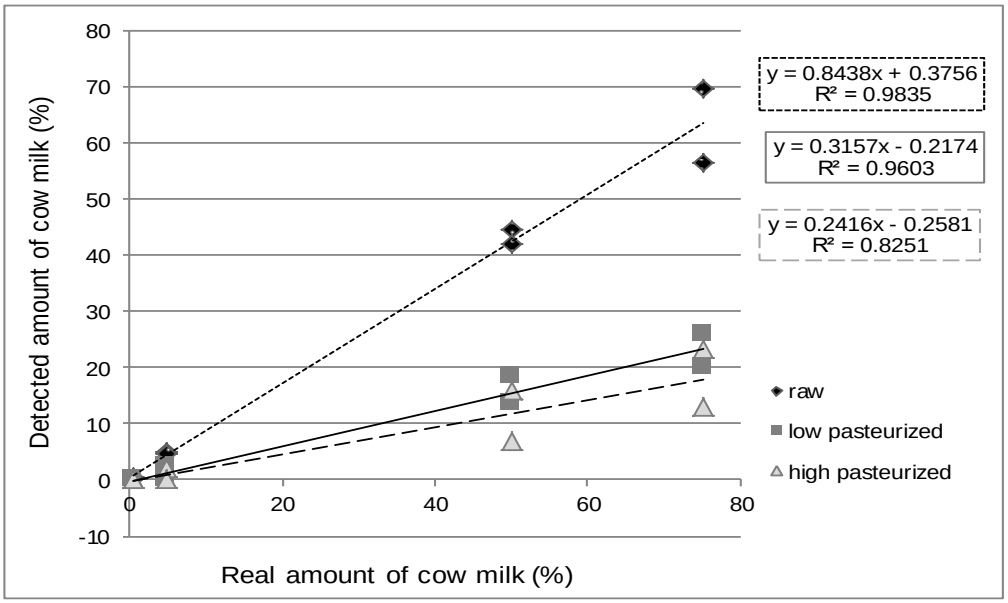


Figure 4 Linear functions with the regression equations for raw and heat-treated cow milk determination in sheep milk (%) amount of cow milk in sheep milk and cheese (%).

Table 3 Samples of the bryndza analysed by the ELISA tests.

Sample number/ manufacturer	Label and composition of bryndza	Quantification of cow milk by ELISA tests
1	Sheep cheese processed from raw milk (min 51%), water, edible salt (max 2.5%), dry matter (min 44%), fat in dry matter (min 48%)	11.56%
2	Stored sheep cheese, cow cheese, edible salt (max 3 %), water, dry matter (min 44%), fat in dry mater (min 48%)	13.91%
3	Stored sheep cheese (min 51%), cow cheese, edible salt (max 3%), water, dry matter (min 44%), fat in dry matter (min 48%)	14.24%
4	Stored sheep cheese (min 51%), cow cheese, edible salt (max 3%), water, dry matter (min 44%), fat in dry matter (min 48%)	14.3%
5	Stored sheep cheese (min 51%), cow cheese, edible salt (max 2%), water, dry matter (min 44%), fat in dry matter (min 48%)	11.95%
6	Sheep cheese processed from raw milk (min 51%), water, edible salt (max 3%), dry matter (min 44%), fat in dry matter (min 4 %)	12.57%
7	Sheep cheese processed from raw milk (min 5 %), cow cheese processed from pasteurized milk, water, edible salt (max 2.5 %), dry matter (min 44%), fat in dry matter (min 48%)	11.63%
8	Mixture of cow and sheep cheese processed from pasteurized milk, water, edible salt (max 2.5%), dry matter (min 44%), fat in dry matter (min 48%)	12.08%
9	Cow cheese, sheep cheese (25%), fat (21%)	31.44%

% – weight percentage, min – minimum, max – maximum.

CONCLUSION

The analyses carried out in laboratory conditions recently, focused on the current situation monitoring of milk and cheese adulteration, have proved the necessity to deal with this issue more thoroughly. Most of the ELISA tests come from abroad (outside Slovakia). Their quality is important for milk producers and processing companies as well as public inspection authorities. The tests should be highly specific, sensitive, reliable, an easy to use, easy to laboratory equipment and of course affordable. As the tests are certified, nobody doubts their quality. Our survey, which we have been performing for a few years, has shown that few milk producers know possibilities of milk and cheese adulteration detection. This situation results in the fact that the producers either don´t do any detection or they use the tests provided by distributors.

The aim of the study was to test the reliability of commercial ELISA tests for raw and heat-treated cow milk detection in the sheep milk and cheese and subsequently to quantify cow milk in commercial "Bryndza". The used ELISA kits are designed for the quantitative determination of cow milk in sheep milk, sheep cheese, goat milk and goat cheese. By ELISA tests was possible to determine these amounts of raw cow milk in sheep milk: 0.5% (0.2%), 5% (4.81%), 50% (42.08%) and 75% (56.52%). The pasteurized samples in different combinations gave lower optical density responses than those prepared from raw milk. The decrease of cow milk amount by 53.53%

and 59.34% (at 5% low and high pasteurized cow milk) and by 62.64% and 66.56% (at 75% low and high pasteurized cow milk) was detected. In next phase of the research, the relationship between the real and detected amount of cow milk (%) in different production stages (milk, cheese) using a regression analysis was examined. However, a lower reliability of the cow milk detection was found and indicated by R^2 values, which ranged from 0.4058 (cheese) to 0.5175 (milk). In practice this means that although individual percentage of cow milk in the sample can be detected (%), but in the unknown sample can´t be clearly confirmed whether the cow milk was raw or heat-treated. In this context, the results can be inaccurate and may not correspond to the real situation. As was noted above, one of the solutions is to set a specific regression curves for each of the heat treatment of analysed milk. The values of determination coefficients were higher than 0.82, which assumes the conditions for the reliable determination of raw or heat-treated cow milk in sheep milk. The only limitation here is the knowledge of cow milk heat treatment.

In total, 9 samples of bryndza were analysed in the monitoring phase of the research with the results of detected cow milk ranged from 11.56% to 14.3%. It can be assumed, that the real addition of cow milk in commercial samples of bryndza was higher than those detected by ELISA.

In conclusion, the analysis has shown that the ELISA tests identified the presence of cow milk, but quantification was not exact because of irreversible changes caused by the manufacturing process. Despite this fact, producer recommended ELISA tests for the detection of sheep milk and cheese adulteration by cow milk. Despite some negatives identified in this study, ELISA tests may find practical application, if they are used only for the qualitative detection of cow milk in other species milks or cheeses. Such detection is important for health, nutritional, technological as well as for economic reasons.

REFERENCES

Asensio, L., González, I., García, T., Martín, R. 2008. Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA). *Food Control*, vol. 19, no. 1, p. 1-8. http://dx.doi.org/10.1016/j.foodcont.2007.02.010

Bobková, A., Židek, R., Flimelová, E., Bobko, M., Fiková, M. 2009. Application of PCR method for milk adulteration and milk products identification. *Potravinarstvo*, vol. 3, no. 1, p. 1-3. ISSN 1337-0960.

Borková, M, Snášelová, J. 2005. Possibilities of Different Animal Milk Detection in Milk and Dairy Products – a Review, *Czech Journal of Food Science*, vol. 23, no. 2, p. 41-50.

Brinkhof, J. M., Houwers, D. J., Moll, L., Dercksen, D., Van Maanen, C. 2009. Diagnostic performance of ELISA and PCR in identifying SRLV-infected sheep and goats using serum, plasma and milk samples and in early detection of infection in dairy flocks through bulk milk testing. *Veterinary Microbiology*, vol. 41 no. 3-4, p. 193-198. http://dx.doi.org/10.1016/j.vetmic.2009.09.060

Commission Regulation (EC) No 273/2008 of 5 March 2008 laying down detailed rules for the application of Council Regulation (EC) No 1255/1999 as regards methods for the analysis and quality evaluation of milk and milk products.

Commission regulation (EC) No 676/2008 of 16 July 2008 registering certain names in the Register of protected designations of origin and protected geographical indications.

Costa, N., Ravasco, F., Miranda, R., Duthoit, M., Roseiro, L. B. 2008. Evaluation of a commercial ELISA method for the quantitative detection of goat and cow milk in ewe milk and cheese. *Small Ruminant Research*, vol. 79, no. 1, p. 73-79. http://dx.doi.org/10.1016/j.smallrumres.2008.07.012

Czerwenka, Ch., Muller, L., Lindner, W. 2010. Detection oft he adulteration of water buffalo milk and mozzarella with cow´s milk by liquid chromatography-mass spectrometry analysis of β-lactoglobulin variants. *Food Chemistry*, vol. 122, no. 3, p. 901-908. http://dx.doi.org/10.1016/j.foodchem.2010.03.034

Edwin K. Lowe, E. K., Anema, S. G., Annie Bienvenue, A., Boland, M. J., Creamer, L. K., Jiménez-Flores, R. 2004. Heat-induced redistribution of disulfide bonds in milk proteins. 2. Disulfide bonding patterns between bovine β-lactoglobulin and κ-casein. *Journal of Agricultural and Food Chemistry*, vol. *52*, no. 25, p. 7669-7680. http://dx.doi.org/10.1021/jf0491254 PMid:15675819

García, T., Martín, R., Rodríguez, E., Morales, P., González, I., Sanz, B., Hernandez, P. E. 1994. Detection of goat´s milk in ewe´s milk by an indirect ELISA. *Food Agricultural Immunology*, vol. 6, no. 1, p. 113-118. http://dx.doi.org/10.1080/09540109409354819

Habánová, M., Lorková, M., Kopčeková, J. 2010. The consumption of Acidophylus drinks and yogurts in selected population of pupils in years 2004 and 2008. *Potravinarstvo*, vol. 3, no. 1, p. 19-23. http://dx.doi.org/10.5219/26

Hurley, I. P., Coleman, R. C., Ireland, H. E., Wiliams, J. H. H. 2006. Use of sandwich IgG ELISA for the detection and quantification of adulteration of milk and soft cheese. *International Dairy Journal*, vol. 16, no. 7, p. 805-812. http://dx.doi.org/10.1016/j.idairyj.2005.07.009

Hurley, I. P., Ireland, H. E., Robert, C. 2004 a. Application of immunological methods for the detection of species adulteration in dairy products. *International Journal of Food Science and Technology*, vol. 39, no. 8, p. 873-878. http://dx.doi.org/10.1111/j.1365-2621.2004.00861.x

Hurley, I. P., Coleman, R. C., Ireland, H. E., Williams, J. H. H. 2004 b. Measurement of bovine IgG by indirect competitive ELISA as a means of detecting milk adulteration. *Journal of Dairy Science*, vol. 87, no. 3, p. 215-221. http://dx.doi.org/10.3168/jds.S0022-0302(04)73195-1

Kardar, A. 2010. The design of a specific-igE ELISA assay kit for major cow´s milk allergens.*Tehran University Medical J.*, vol. 68, no. 3, p. 147-152.

Luis, D. R., Lavilla, M., Sánchez, L., Calvo, M., Pérez, D. M. 2009. Development and evaluation of two ELISA formats for the dteection of β-lactoglobulin in model porcessed and commercial foods. *Food Control*, vol. 20, no. 7, p. 643-647. http://dx.doi.org/10.1016/j.foodcont.2008.09.017

Mayer, H. K., Bürger, J., Kaar, N. 2012. Quantification of cow milk percentage in dairy products – a myth? *Analytical and Bioanalytical Chemistry*, vol. 403, no. 10, p. 303-304. http://dx.doi.org/10.1007/s00216-012-5805-1

Pizzano, R., Nicolai, M. A., Manzo, C., Addeo F. 2011. Authentication of dairy products by immunochemical methods: a review. *Dairy Science and Technology*, vol. 91, no. 2, p. 77-95. http://dx.doi.org/10.1007/s13594-011-0008-7

Popelka P., Popelka P., Horská D., Golian J., Marcinčák S. 2002. Detection of sheep milk and cheeses adulteration using

enzyme immunoanalysis (ELISA). *Slovak Veterinary Journanl*, vol. 27, no. 3, p. 36-37.

Puchades, R., Maquieira, Á. 2013. ELISA tools for food PDO authentication, p. 145-194. In De La Guardia, M., Gonzálves, A. 2013: *Food Protected Designation of Origin: Methodologies and Applications*. Oxford (UK) - Amsterdam (The Netherlands): Elsevier, 800 p. ISBN: 978-0-444-59562-1.

Sleziaková, J., Baleková, S. 2010. Detection of cheese milk and cheese adulteration by imunoenzymatic and molecular methods. Milk safety and quality - current problems: International scientific conference *Hygiena alimentorum XXXI.* Štrbké pleso (Slovak Republic), p. 315-317.

Song, H., Xue, H., Han, Y. 2011. Detection of cow milk in Shaanxi goat´s milk with an ELISA assay. *Food Control*, vol. 22, no 6, p. 883-887. http://dx.doi.org/10.1016/j.foodcont.2010.11.019

Stanciuc, N., Rapeanu, G. 2010. Identification of adulterated sheep and goat cheeses marketed in Romania by immunocromatographic assay. *Food and Agricultural Immunology*, vol. 21, no. 2, p. 157-164. http://dx.doi.org/10.1080/09540100903508683

Suhaj, M., Stankovská, M., Kolek, E. 2010. Detection of cheese and cow cheese in Slovak sheep by isoelectric focusing. *Chemické Listy,* vol. 104, p. 627-630.

Štumr, F., Tomková, K., Šafářová, P. et al. 2008. ELISA tests for determination of allergens in food. *Proteíny:* International scientific conference. Zlín (Czech Republic), p. 180-182.

Taylor, L. S., Nordlee, A. J., Niemann, M. 2009. Allergen immunoassays–considerations for use of naturally incured standards. *Analytical and Bioanalytical Chemistry*, vol. 395, no. 1, p. 83-92. http://dx.doi.org/10.1007/s00216-009-2944-0 PMid:19641908

Xue, H., Hu, W., Son, H., Han, Y., Yang, Z. 2010. Indirect ELISA for detection and quantification of bovine milk in goat milk. *Journal of Food Science and Technology*, vol. 31, no. 24, p. 370-373.

Yeung, J. 2006. Enzyme-linked immunosorbent assay (ELISAs) for detecting allergens in foods. In *Detecting Allergens in Food.* : Woodhead Publishing. 2006, p. 109-124. ISBN: 978-1-85573-728-0.

Zachar, P., Šoltés, M., Kasarda, R., Novotný, J., Novikmecová, M., Marcinčáková, D. 2011. Identification of milk and milk products. *Mljekarstvo*, vol. 61, no. 3, p. 199-207.

Zarranz, M. J., Izco, J. M. 2007. Validation parameters of an ELISA to detect presence of milk from other species. *Milchwissenschaft*, vol. 62, no. 2, p. 159-163.

Zeleňáková, L., Golian, J., Zajác, P. 2008. Application of ELISA tests for the detection of goat milk in sheep milk. *Milchwissenschaft*, vol. 63, no. 2, p. 137-141.

Zeleňáková, L., Šesták, M., Židek, R. 2009. Monitoring of sheep milk and milk products adulteration on common European food market. *Potravinarstvo*, vol. 3, no. 2, p. 69-73.

Zeleňáková, L., Židek, R., Čanigová, M. 2011. Optimization of ELISA method for detection of bovine ß-lactoglobulin in sheep milk and sheep milk products. *Milchwissenschaft*, vol. 66, no. 3, p. 278-281.

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COMPOSITION AND MICROSTRUCTURE ALTERATION OF TRITICALE GRAIN SURFACE AFTER PROCESSING BY ENZYMES OF CELLULASE COMPLEX

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ABSTRACT

It is found that the pericarp tissue of grain have considerable strength and stiffness, that has an adverse effect on quality of whole-grain bread. Thereby, there exists the need for preliminary chemical and biochemical processing of durable cell walls before industrial use. Increasingly used in the production of bread finds an artificial hybrid of the traditional grain crops of wheat and rye – triticale, grain which has high nutritional value. The purpose of this research was to evaluate the influence of cellulose complex (*Penicillium canescens*) enzymes on composition and microstructure alteration of triticale grain surface, for grain used in baking. Triticale grain was processed by cellulolytic enzyme preparations with different composition (producer is *Penicillium canescens*). During experiment it is found that triticale grain processing by enzymes of cellulase complex leads to an increase in the content of water-soluble pentosans by 36.3 – 39.2%. The total amount of low molecular sugars increased by 3.8 – 10.5 %. Studies show that under the influence of enzymes the microstructure of the triticale grain surface is changing. Microphotographs characterizing grain surface structure alteration in dynamic (every 2 hours) during 10 hours of substrate hydrolysis are shown. It is found that the depth and direction of destruction process for non-starch polysaccharides of grain integument are determined by the composition of the enzyme complex preparation and duration of exposure. It is found, that xylanase involved in the modification of hemicelluloses fiber having both longitudinal and radial orientation. Hydrolysis of non-starch polysaccharides from grain shells led to increase of antioxidant activity. Ferulic acid was identified in alcoholic extract of triticale grain after enzymatic hydrolysis under the influence of complex preparation containing cellulase, xylanase and β-glucanase. Grain processing by independent enzymes containing in complex preparation (xylanase and β-glucanase) shows that more significant role in polysaccharide complex composition and grain surface microstructure alteration belongs to xylanase. Grain processing by independent of cellulolytic enzymes may decrease the strength of pericarp tissue of grain and improved sensory characteristics of the bread.

Keywords: triticale; grain; xylanase; microstructure; antioxidant activity

INTRODUCTION

Dietary fiber in cereals is presented by non-starch polysaccharides found in the cell walls and consisting mainly of arabinoxylan and β-glucan **(Jacobs et al., 1998; Gebruers et al., 2008)**. Wheat and rye, as a universal raw material used in baking, became the main objects in studies of the grain pentosans properties. Currently, however, an artificial hybrid of these grains named triticale get wider range of application **(Cauvain et al., 2007)**. Total dietary fiber content of triticale grain is 13 – 16 % depending on the sort. Triticale comprises 6.8% of arabinoxylan, 0.7% of β-glucan and 2.1% of cellulose on average **(Izydorczyk et al., 1995; Barron et al., 2007)**.

Studies show that the molecular structure and the structural organization of arabinoxylans and β-glucans of grain (pentosans) are important determinants of their physical properties, such as solubility in water, viscosity, digestibility. This determines the functionality of said polysaccharides and their physiological functions in the gastrointestinal tract of humans **(Vaikousi et al., 2004; Lazaridou et al., 2007)**. Inclusion of cereals products containing dietary fiber in diet helps reduce cholesterol concentration, that decrease the risk of coronary heart disease **(McIntosh et.al., 1991; Brown et al., 1999)**, improve the glycemic level control for people with type II diabetes **(Lu et al., 2004)**, increases intestinal peristalsis **(Cummings et al., 1992, 2009)**.

Arabinoxylans and β-glucans, along with providing benefits to human health, have the potential to improve the quality of bakery products **(Said et al., 2011)**. There are two types of arabinoxylans: water extractable (about 35% of the total) and non-extractable **(Leggio et al.,, 1999)**. These two fractions differ in physicochemical and functional properties, including water-binding and gel-forming ability **(Courtin et al., 2002)**. Most of the dietary fiber of rye and wheat bran is insoluble **(Grigelmo-Miguel et al., 1999; Van Craeyveld et al., 2009)**. However, water-extractable arabinoxylan is more effective compared to non-extractable in terms of quality improvement and shelf life extension of bread by reducing the effect of staling and starch retrogradation **(Courtin et al., 1999; Said et al., 2011)**.

The pericarp tissue of grain have considerable strength and stiffness, that has an adverse effect on quality of

whole-grain bread **(Antoine et al., 2003)**. There is a growing demand for the usage of sustainable processes of soft biotech processing of plant cell walls, which will replace the chemical treatment **(Ulvskov et al., 2011)**. Usage of a xylanase for the hydrolysis of water-insoluble non-starch polysaccharides of the cell walls leads to improvement in swelling, sensory performance and to deceleration of starch retrogradation process **(Gruppen et al., 1998; Andlaver et al., 2002; Charalampopoulos et al., 2002; Jiang et al., 2005)**.

The purpose of this research was to evaluate the influence of cellulose complex (*Penicillium canescens*) enzymes on composition and microstructure alteration of triticale grain surface, for grain used in baking.

MATERIAL AND METHODOLOGY

Two sorts of triticale grain from different genetic sources were studied. They are «Antaeus» and «Talva 100» (Russian Federation). Dry complex enzyme preparation comprising cellulase, β-glucanase and xylanase, as well as preparations containing individual enzymes (producer is *Penicillium canescens*, The Russian Academy of Sciences' Skryabin Institute of Biochemistry and Physiology of Microorganisms) were used during research. Enzymes had the following activity: cellulase 58711 nkat/g, xylanase 12135 nkat/g, β-glucanase 51317 nkat/g and were given by chemical faculty of Moscow State University **(Sinitsyna et al., 2003)**.

Enzyme preparation in powder was mixed by a magnetic stirrer with a citrate buffer (pH 4.5) for 0.5 hours at a concentration of 0.6 $g.L^{-1}$ before the analysis. This concentration corresponds with the optimum enzyme concentration for bread production from whole triticale grain **(Kuznetsova et al., 2010)**. Whole triticale grain was incubated in enzyme preparation solution with grain-solution ratio of 1:1.5 for 8 hours at 50 °C in thermostat. Duration of cereal substrate hydrolysis determined by the time during which the grain moisture was 40% or more that is required to get the cereal mass with ability to dispersion and allow to use grain raw material for bakery. To save material intact enzyme inactivation wasn't performed after incubation.

Determination of cellulose content, ratio of amorphous and crystalline cellulose and total amount of hemicellulose were carried out according to procedures described by **Ermakov (1972)**. To detect the soluble pentosans, grain sample was analyzed by orcinol-chloride method **(Hashimoto et al., 1987)**.

Concentration determination of low molecular carbohydrates in the grain samples was performed by a chromatographic method with electrochemical detection using liquid chromatograph Agilent 1100 with electrochemical detector ESA Coulochem III. Sugars mixture separation carried out using an anion exchange column with grafted amine phase followed by electrochemical detection.

Microstructural studies were conducted using an electron scanning microscope ZEISS EVO LS. Survey was carried out at an acceleration voltage of 15 kV.

Complex of phenolic compounds was determined by HPLC using MiLiChrome-5 device. Triticale grain ethanolic extract was used, eluent of composition is acetonitrile – water solution of trifluoroacetic acid (pH 2.5, in a ratio of 15:85); elution mode is isocratic, the analysis time is 12 – 25 min, the sample volume – 6.2 ml. Antioxidant activity was determined by spectrophotometric method in alcoholic extract described by **Silva et al., 2005**.

RESULTS AND DISCUSSION

Table 1 shows the research results of dietary fiber content in two sorts of triticale grain.

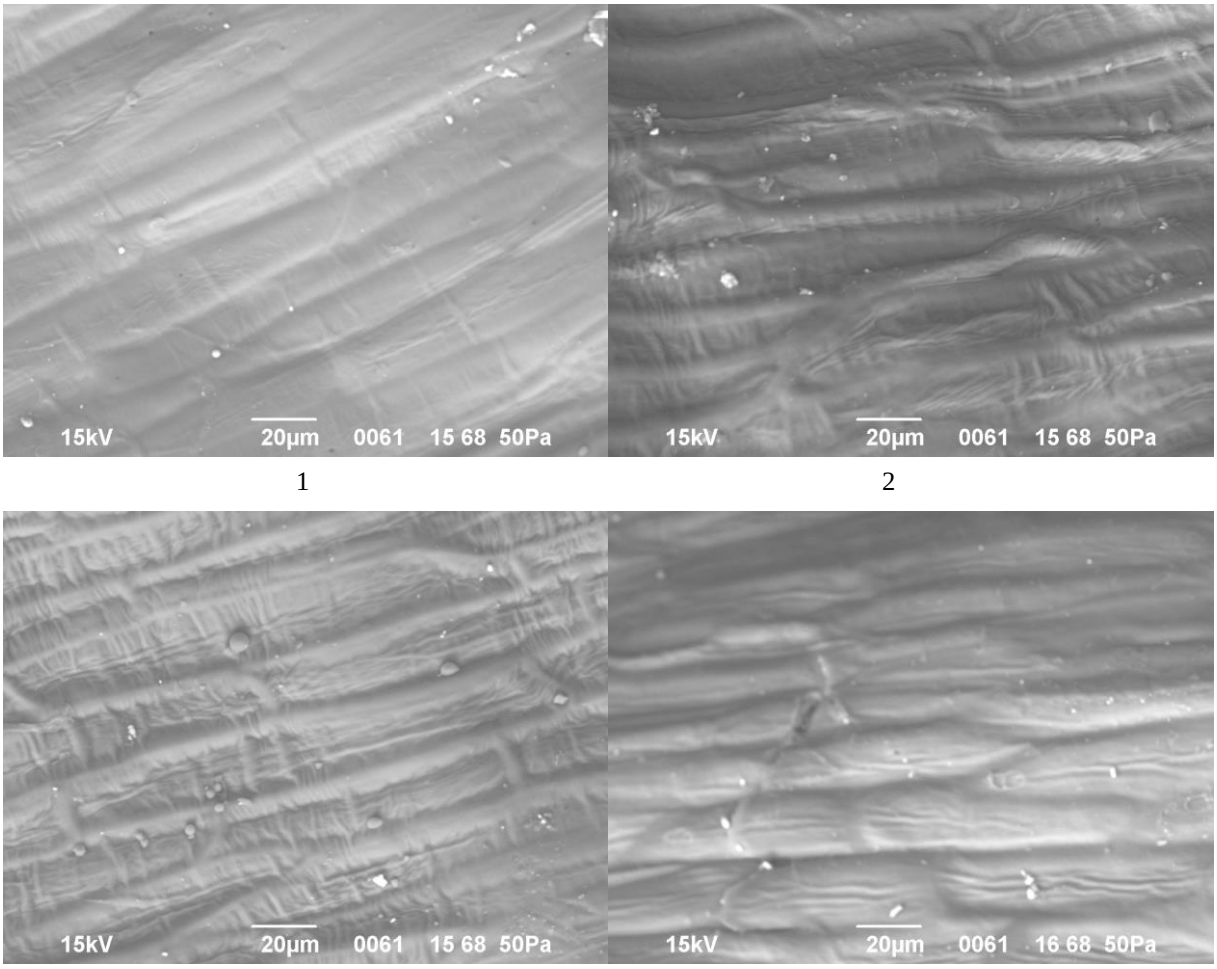
Table 1 Composition of non-starch polysaccharides of dry triticale grain soaked in a citrate buffer and treated with enzyme preparations, in %.

Experiment variation	Cellulose	Ratio of amorphous and crystalline cellulose	Hemicellulose	Soluble pentosans
		Antaeus		
Dry grain	2.20 ±0.04	2.33 ±0.04	7.93 ±0.04	5.46 ± 0.04
Control grain	2.08 ±0.05	2.24 ±0.04	7.24 ±0.05	5.88 ±0.06
Complex preparation	1.96 ±0.03	2.00 ±0.04	6.70 ±0.05	7.60 ±0.03
Xylanase	1.98 ±0.04	2.08 ±0.03	6.96 ±0.04	7.34 ±0.03
β- glucanase	2.04 ±0.03	2.18 ±0.03	7.18 ±0.03	6.66 ±0.05
		Talva 100		
Dry grain	2.14 ±0.04	2.60 ±0.04	7.94 ±0.02	5.84 ±0.03
Control grain	2.02 ±0.03	2.48 ±0.04	7.38 ±0.02	6.15 ±0.02
Complex preparation	1.85 ±0.02	2.21 ±0.04	6.95 ±0.04	7.96 ±0.03
Xylanase	1.90 ±0.05	2.26 ±0.03	7.12 ±0.06	7.68 ±0.03
β- glucanase	2.00 ±0.03	2.34 ±0.03	7.24 ±0.02	6.74 ±0.04

As a result of grain processing by enzyme complex concentration of water-soluble pentosans increased by 36.3 – 39.2%, depending on the sort of grain. Processing of grain by individual enzymes like hemicellulase comprised in complex preparation (xylanase and β-glucanase), showed that more significant role in polysaccharide complex composition of grain surface structures alteration belongs to xylanase. These results are consistent with data

Table 2 Carbohydrate composition of dry triticale grain, soaked in citrate buffer and treated with enzyme preparations, $g.L^{-1}$.

Sugar	Dry grain	Control grain	Complex preparation	Xylanase	β-glucanase
Arabinose	0.00	0.01	0.03	0.02	0.02
Galactose	0.00	0.00	0.00	0.00	0.00
Glucose	0.31	0.35	0.43	0.40	0.37
Xylose	0.00	0.00	0.04	0.02	0.01
Fructose	0.26	0.24	0.22	0.23	0.24
Raffinose	0.00	0.01	0.03	0.03	0.02
Unidentifiedsugar	0.07	0.08	0.12	0.10	0.09
Cellobiose	0.00	0.00	0.00	0.00	0.00
Maltose	1.68	1.98	2.08	2.05	2.02
Total	2.32	2.67	2.95	2.85	2.77



1 2

3 4

Figure 1 Microstructure of triticale grain surface (1 – grain soaked in buffer – control; 2 - treated by complex enzyme preparation; 3 – by xylanase preparation; 4 – by β-glucanase preparation), an increase of x 700. Photo: S. Motyleva, 2014.

from **(Havrlentova et al., 2011)**, where it was found that the level of soluble dietary fiber in wheat bran increases under the influence of enzyme preparations – hemicellulases, especially those containing endoxylanase.

Since varietal differences in non-starch polysaccharides of triticale grain shells content alteration after enzymes processing (cellulase complex *Penicillium canescens*) is not significant, the determination of triticale grain carbohydrate composition by chromatographic method was carried out for the average triticale grain sample, composed of two represented sorts. Hydrolysis of glycosidic linkages in polysaccharides molecules is occurred and partially collapsed matrix carcass nodes,

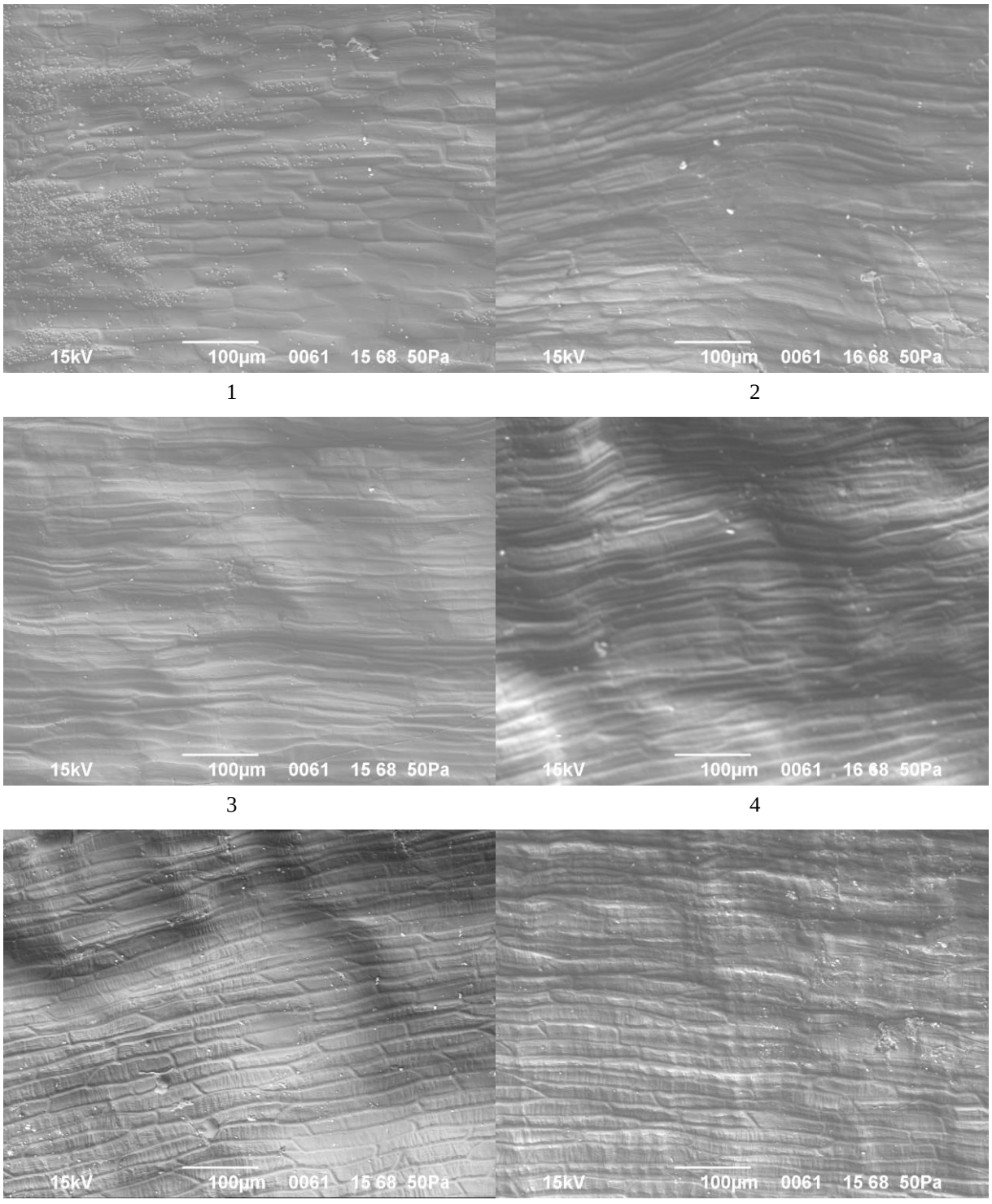


Figure 2 The surface microstructure of triticale grain, soaked in a solution of a complex preparation (cellulase, β-glucanase, xylanase) during different periods (1 – immediately after being placed in a solution; 2 – 2 hours; 3 – 4 hours; 4 – 6 hours; 5 – 8 hours; 6 – 10 hours) x 200 magnification. Photo: S. Motyleva 2014.

wherein substances with low molecular weight and high solubility was formed (see Table 2).

The content of arabinose (0.02 – 0.03 $g.L^{-1}$) and xylose (0.01 – 0.04 $g.L^{-1}$) in grain extracts indicate occurred biochemical processes in arabinoxylan chains. Such processes can be caused by the presence of hydrolyzing glycosidic linkages in the enzyme complex of hemicellulases preparations. Results of chemical composition alteration of the cell walls in wheat grain shells under the influence of xylanase reconciled with scanning electron microscopy **(Tervilä-Wilo et al., 1996; Parkkonen et al., 1997)**. Figure 1 shows microphotographs of dry triticale grain surface structure, soaked in citrate buffer and treated with enzyme preparations.

Xylanase have an influence on both type of hemicellulose fibers with longitudinal and radial orientation. Channels on the surface of the grain shells, having various directions are found. This fact shows that endoxylanases *Penicillium canescens* have much stronger destructive forces for non-starch polysaccharides in outer integument of triticale grain compared to β-glucanase.

Figure 2 shows photographs of the surface microstructure of triticale grain soaked in a solution of a complex preparation for adifferent time.

Microphotographs shows triticale grain surface alteration during hydrolysis by complex enzyme preparation in the dynamics. First of all microfibrils having a longitudinal orientation became bare because hemicellulose shielding layer exposed to degradation influence. Hollows having a radial orientation appear on the surface of the grain shells after 6-8 hours of hydrolysis. It means that deeper processes affecting both arabinoxylan molecules and cellulose matrix microfibrils.

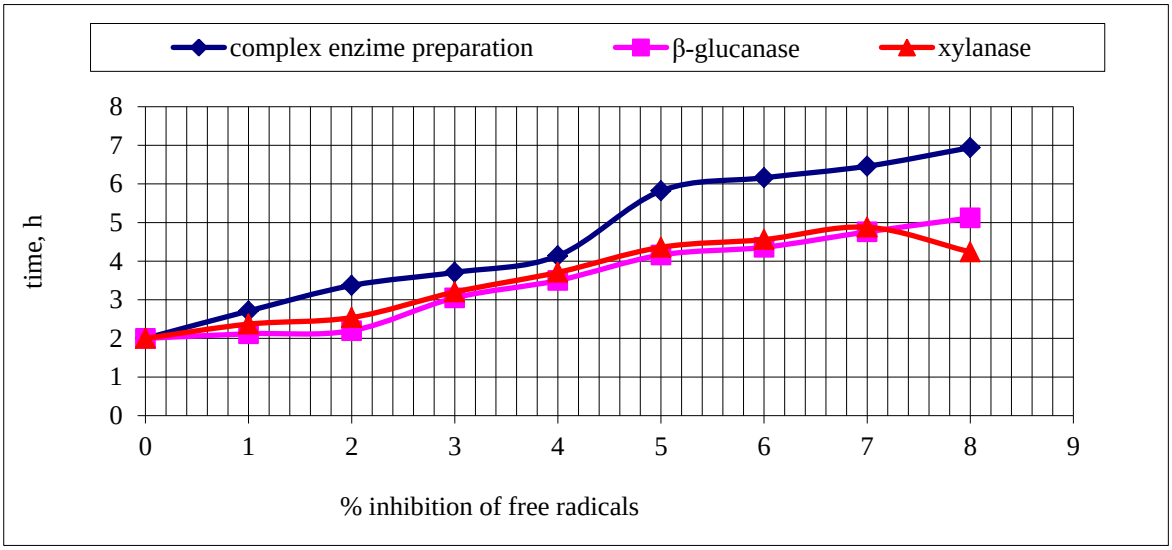


Figure 3 Triticale grain antioxidant activity alteration in the process of enzymatic hydrolysis by cellulase preparations.

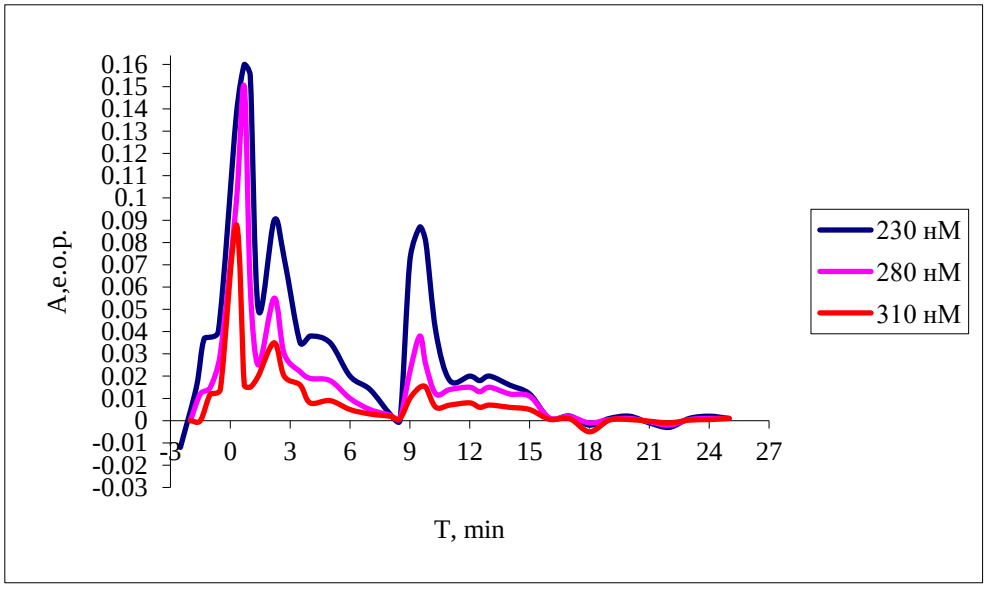


Figure 4 The chromatogram of triticale grain extract after enzymatic hydrolysis with a complex enzyme preparation (cellulase, β-glucanase, xylanase) made of *Penicillium canescens*.

The degradation of xylan from cell walls matrix under the influence of endo-xylanase and β-glucanase leads to destruction of the natural triticale grain shells structure and to increase of the water-soluble pentosans concentration.

Determination of antioxidant activity (Figure 3) for alcoholic extract of triticale grain treated for 8 hours by a complex enzyme preparation, β-glucanase and xylanase, show that the percentage of DPPG free radicals inhibition increasing with extension of hydrolysis duration.

Composition of phenolic compounds in triticale grain extract after enzymatic hydrolysis with a complex enzyme preparation was determined by HPLC method. Chromatogram is shown in Figure 4.

Chromatogram of alcoholic extract allowed to identify organic and hydroxycinnamic acids. Ferulic acid was identified (VR = 9,6; RS = 0,533). These findings are consistent with the results of **(de Vries et al., 2000)**, where stated that after the degradation of xylan chain by endo-xylanase, the antioxidant activity of cereal substrates increases by the release of ferulic acid.

CONCLUSION

During experiment it is found that triticale grain processing by enzymes of cellulase complex leads to an increase in the content of water-soluble pentosans by 36.3 – 39.2% and carbohydrates with a low molecular weight and high solubility. Xylan degradation of the cell walls matrix under the influence of endo-xylanase and β-glucanase leads to the destruction of the natural structure of triticale grain shells, that is consistent with data on the content increase of water-soluble pentosans. Application of cellulase complex enzymes (producer is *Penicillium canescens*) for the treatment of triticale grain increases the content of water-soluble pentosans, low molecular carbohydrates, the antioxidant activity of raw material that has positive implications for the future grain usage in bread baking. Grain surface microstructure alteration leads to modifications of non-starch polysaccharides, that may decrease their strength and improved sensory characteristics of the product.

REFERENCES

Andlaver, W., Furst P. 2002. Nutraceuticals a piece of history, present status and outlook. *Food Research International*. vol. 35, no. 2-3, p. 171-176. http://dx.doi.org/10.1016/S0963-9969(01)00179-X

Antoine, C., Peyron, S., Mobille, F., Lapierre, C., Bouchet, B., Abecassis, J., Rouau, X. 2003. Individual contribution of grain outer layers and their cell wall structure to the mechanical properties of wheat bran. *Journal of Agricultural and Food Chemistry*. vol. 51, no. 7, p. 2026-2033. http://dx.doi.org/10.1021/jf0261598

Barron, C., Surget, A., Rouau, X. 2007. Relative amoutns of tissues in mature what (*Triticum aestivum* L.) grain and their carbohydrate and phenolic acid composition. *Journal of Cereal Science*. vol. 45, no. 1, p. 88-96. http://dx.doi.org/10.1016/j.jcs.2006.07.004

Broun, L., Rosner, B., Willett, W. W., Sacks, F. M. 1999. Cholesterol-lowering effects of dietary fibre: a meta-analysis. *The American Journal of Clinical Nutrition*. vol. 69, p. 30-42. Available at: http://ajcn.nutrition.org/content/69/1/30.full

Cauvain, S. P., Young, L. S. 2007. *Technology of Breadmaking*: Springer US. 397 p. ISBN 978-3-319-14687-4; http://dx.doi.org/10.1007/978-3-319-14687-4

Charalampopoulos, D., Wang, R., Pandella, S. S., Webb, C. 2002. Application of cereals and cereal components in functional food: a review. *International Journal of Food microbiology*, vol. 79, no. 1-2, p. 131-141. http://dx.doi.org/10.1016/S0168-1605(02)00187-3

Courtin, C. M., Delcour, J. A. 2002. Arabinoxylans and endoxylanases in wheat flour bread-making. *Journal of Cereal Science*, vol. 35, no. 3, p. 225-243. http://dx.doi.org/10.1006/jcrs.2001.0433

Courtin, C. M., Roelants, A., Delcour, J. A. 1999. Fractionation–reconstitution experiments provide insight into the role of endoxylanases in bread-making. *Journal of Agricultural and Food Chemistry*, vol. 47, no. 5, p. 1870-1877. http://dx.doi.org/10.1021/jf981178w PMid:10552464

Cummings, J. H., Bingham, S. A., Heaton, K. W., Eastwood M. 1992. Fecal weight, colon cancer risk and the dietary intake of nonstarch polysaccharides (dietary fibre). *Gastroenterology*. vol. 103, no. 6, p. 1783-1789. PMid:1333426

Cummings, J. H., Mann, J. I., Nishida, C., Vorster, H. H. 2009. Dietary fibre: an agreed definition. *Lancet*. vol. 373, no. 9661, p. 366-66. http://dx.doi.org/10.1016/S0140-6736(09)60117-3

De Vries, R. P., Kester, H. C. M., Poulsen, CH. H., Benen, J. A. E., Visser, J. 2000. Synergy between enzymes from *Aspergillus* involved in the degradation of plant cell wall polysaccharides. *Carbohydrate Research*. vol. 327, no. 4, p. 401-410. http://dx.doi.org/10.1016/S0008-6215(00)00066-5

Gebruers, K., Dornez, E., Boros, D., Fras, A., Dynkowska, W., Bedo, Z., Rakszegi, M., Delcour, J. A., Courtin Ch. M. 2008. Variation in the content of dietary fibre and components ther of in wheats in the HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*. vol. 56, no. 21, p. 9740-9749. http://dx.doi.org/10.1021/jf800975w PMid:18921978

Grigelmo-Miguel, N., Martin-Belloso, O. 1999. Comparison of dietary fibre from by-products of processing fruits and greens and from cereals. *Lebensm-Wiss Technology*. vol. 32, no. 8, p. 503-508. http://dx.doi.org/10.1006/fstl.1999.0587

Jacobs, J., Marquart, L., Slavin, J., Kushi, L. 1998. Whole-grain intake and cancer: an explanded review and meta-analysis. *Nutrition and Cancer*. vol. 30, no. 2, p. 85-96. http://dx.doi.org/10.1080/01635589809514647 PMid:9589426

Jiang, Z., Li, X., Yang, S., Li, L., Tan, S. 2005. Improvement of the breadmaking quality of the flour by the hiperthermophilic xylanase B from *Thermotoga Maritime*. *Food Research International*. vol. 38, no. 1, p. 37-43. http://dx.doi.org/10.1016/j.foodres.2004.07.007

Hashimoto, S., Shogren, N. D., Pomeranz, Y. 1987. Cereal pentosans: estimation and significance. I. Pentosans in wheat and milled wheat products. *Cereal Chemistry*. vol. 64, no. 1, p. 30-34. Available at: http://www.aaccnet.org/publications/cc/backissues/1987/documents/64_30.pdf

Havrlentova, M., Petrulakova, Z., Burgarova, A., Gago, F., Hlinkova, A., Sturdik, E. 2011. Cereal β-glucans and their significance for the preparation of functional food: a review. *Czech Journal of Food Sciences*. vol. 29,

no 1, p. 1-14. Available at: http://www.agriculturejournals.cz/publicFiles/34997.pdf

Izydorczyk, M. S., Biliarderis, C. G. 1995. Cereal arabinoxylans: advances in structure and physico-chemical properties. *Carbohydrate Polymers*. vol. 28, no. 1, p. 33-48. http://dx.doi.org/10.1016/0144-8617(95)00077-1

Lazaridou, A., Biliaderis, C. G. 2007. Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. *Journal of Cereal Science*. vol. 46, no. 2, p. 101-118. http://dx.doi.org/10.1016/j.jcs.2007.05.003

Leggio, L. L., Pickersgill, R. W. 1999. Xylanase-oligosaccharide interactions studied by a competitive enzyme assay. *Enzyme and microbial technology*. vol. 25, no. 8-9, p. 701-709. http://dx.doi.org/10.1016/S0141-0229(99)00103-9

Lu, Z. X., Walker, K. Z., Muir, J. G., O'Dea, K. 2004. Arabinoxylan fibre improves metaoblic control in peoplewith Type 2 diabetes.*European Journal of Clinical Nutrition*. vol. 53, p. 621-628. http://dx.doi.org/10.1038/sj.ejcn.1601857

Mcintosh, G. H., Whyte, J., Mcarthur, R., Nestel, P. 1991. Barley and wheat foods: influence on plasma cholesterol concerntations in hypercholesterolemeic men. *The American Journal of Clinical Nutrition*. vol. 53, no. 5, p. 1205-1209. PMid:1850576

Parkkonen, T., Tervila-Wilo, A., Hopeakoski-Nurminen, N., Morgan, A., Poutanen, K., Autio, K. 1997. Changes in Wheat Microstructure Following *in Vitro* Digestion. *Acta Agriculturae Scandinavica - Section B Soil and Plant Science*. vol. 47, no. 1, p. 43-47. http://dx.doi.org/10.1080/09064719709362437

Peterson, K., Nordlang, E., Tornberg, E., Eliasson, A. CH., Buchert, J. 2013. Impact of the cell wall destroying enzymes on the water-holding capacity and solubility of dietary fiber in the rye and wheat bran. *Journal of the science of food and agriculture*. vol. 93, no. 4, p. 882-889. http://dx.doi.org/10.1002/jsfa.5816 PMid:22865289

Said, F., Pasha, I., Anjum, F. M., Sultan, M. 2011. Arabinoxylans and arabinogalactans: a Comprehensive treatise (Review). *Critical Reviews in food science and nutrition*. vol. 51, no. 5, p. 467-476. http://dx.doi.org/10.1080/10408391003681418 PMid:21491271

Silva, B. A., Ferreres, F., Malva, J. O., Dias, A. C. P. 2005. Phytochemical and antioxidant characterization of Hypericum perforatum alcoholic extracts. *Food Chemistry*. vol. 90, no. 1-2, p. 157-167. http://dx.doi.org/10.1016/j.foodchem.2004.03.049

Sinitsyna, O. A., Bukhtoyarov, F. E., Gusakov, A. V., Sinitsyn, A. P., Okunev, O. N., Bekkarevitch, A. O., Vinetsky, Yu. P. 2003. Isolation and properties of major components of *Penicillium canescens* extracellular enzyme complex. *Biochemistry (Moscow)*. vol. 68, no 11, p. 1200-1209. http://dx.doi.org/10.1023/B:BIRY.0000009134.48246.7e

Tervila-Wilo, A., Parkkonen, T., Morgan, A., Hopeakoski-Nurminen, N., Poutanen, K., Heikkinen, P., Autio, K. 1996. *In vitro* digestion of wheat microstructure with xylanase and cellulase from *Trichoderma reesei*. *Journal of Cereal Science*. vol. 24, no. 3, p. 215-225. http://dx.doi.org/10.1006/jcrs.1996.0054

Ulvsov, P., Øbro, J., Hayash, T., Mikkelsen, J. D. 2011. Enzymatic Modification of Plant Cell Wall Polysaccharides. Annual Plant Reviews: Plant Polysaccharides, Biosynthesis and Bioengineering, *Blackwell Publishing Ltd.*, vol. 41, p. 367-387. http://dx.doi.org/10.1002/9781444391015.ch15

Vaikousi, H., Biliaderis, C. G., Izydorczyk, M. S. 2004. Solution flow behavior and gelling properties of water-soluble barley (1→3, 1→4)-β-glucans varying in molecular size. *Journal of Cereal Science*. vol. 39, no. 1, p. 119-137. http://dx.doi.org/10.1016/j.jcs.2003.09.001

Van Craeyveld, V., Holopainen, U., Selinheimo, E., Poutanen, K., Delcour, J. A., Courtin, C. M. 2009. Extensive dry ball milling of wheat and rye bran leads to in situ production of arabinoxylan oligosaccharides through nanoscale fragmentation. *Journal of Agricultural and Food Chemistry*. vol. 57, no. 18, p. 8467-8473. http://dx.doi.org/10.1021/jf901870r PMid:19754173

Ermakov, A. I. 1972. *Methods for biochemical study of plants.* Leningrad: Kolos. 456 p.

Kuznetsova, E. A., Cherepnina, L. V. 2010. Mathematical explanation of the enzyme preparations selection in the technology of triticale grain bread. Storage and processing of agricultural raw materials. M. *"Food Industry"(Moscow)*. no. 2, p. 42-44.

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THE CONTENT OF MERCURY IN VARIOUS TYPES OF CEREALS GROWN IN THE MODEL CONDITIONS

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ABSTRACT

The consumption of cereals in Slovakia but also worldwide is increasing by every year. From 30000 to 50000 tons of mercury circulates throught the biosphere that gets into the atmosphere degassing of the earth's crust and world oceans. Mercury affects CNS and causes its disorders. The high doses of mercury causes a lot of different changes of personality as well as increased agitation, memory lossorinsomnia. It can also affect other organ systems such as the kidney. The exposure level is reflected in the concentration of mercury in blood and urine. The aim of our work was the evaluation of transfer of mercury from sludge to edible part of chosen cereals. The objectives were achieved in simulated conditions of growing pot experiment. We used agricultural soil from the location of Výčapy – Opatovce for the realization of the experiment. The sludge, which was added atvarious doses, was taken from Central Spiš area from locality of Rudňany near the village where minedironore that contains mainly copperand mercury during last few decades was. We used three types of cereals: barley (*Hordeum sativum L.*) variety PRESTIGE, spring wheat (*Triticum aestivum L.*) variety ISJARISSA and oat (*Avena sativa L.*) variety TATRAN. The length of growing season was 90 days. From the obtained results of two years can be concluded that the accumulation of mercury by seed follows wheat < barley < oat. Even though that the oat is characterized by the highest accumulation of mercuryin the seeds, the content did not exceed the maximum level sspecified by The Codex Alimentarius of Slovak Republic. The results shows that the suitable cultivation of the cereals in localities, which are contaminated with heavy metals, especially by mercury, that the high content of mercury in soil do not pose a risk of accumulation of the metal into the cereal grain.

Keywords: cereals; plants; mercury; heavy metals

INTRODUCTION

The cereals are probably the most important source of food for humans and feed foranimals. Consequently, the low level of contamination can affect the health of consumers. Chemical contamination can occur from growing of cereals to their processing and storage **(Alldrick, 2012)**.

The cereals are the most common crops that are grown on arable land of EU. The fifty percent of cereal production in Southern Europe consists of wheat and than barley and maize. Other cereals as oat and rye are grown to a limited amounth **(Finch et al., 2014)**. The consumption of cereals in Slovakia but also worldwide is increasing by every year. The cereals are particularly very important for its nutritional value. The opinion of many experts is that cereals should constitute from 40 to 60 percent of well - balanced diet. Cereals provide most of the calories and proteins consumed worldwide. The current annual world production is more than 2.5 billion tons. This out put is either directly channeled to the food industry or used as animal feed to provide meats, dairy, and poultry products. Among cereals, rice, wheat, and maize yield approximately 89% ofthe total production and constitute the main stay of practically all cultures. The otherless important cereals are barley, oats, sorghum, rye, triticale, and millets. All cereals are strachy foods and contain protein that does not meet the essential aminoacid balance required by growing infants. They are considered a good source of energy, most B vitamins, and dietary fiber when consumed as whole grains **(Serna Saldivar, 2016)**.

The cereals and cereal products are the main sources of carbohydrates in food for humans and feed for animals. Cereal grains are an important source of energy and nutrients in the form of protein, fat, fiber, minerals and vitamins **(Beverly, 2014)**.

The cereals are as well as the most important source of fructans in our daily diet. Nowadays are hotly discussed and compared a lot of different cereals in the terms of fructans structure. Their degradation during processing of food is considered as a potential health benefit. Recent published data suggest that they may also have a prebiotic effect **(Verspreeta et al., 2015)**.

The cereals and cereal bran obtained a significant position as a functional food. They are a source of carbohydrates (arabinoxylan, beta – glucan), phenolic acids (ferulic acid), flavonoids (anthocyanins), oil (γ - oryzanol), vitamins, carotenoids, folates and sterols. Their physico – chemical properties makes them a necesssary ingredient for food fortification. The bran of rice, wheat, oat, barley, millet, rye and corn contain a huge

amount of health-promoting ingredients. The anti-atherogenic, anti-hypertensive and hypoglycemic properties were verified. Further, it was found the effect against oxidative stress. They reduce insulin resistance, prevent the risk of obesity by inducing the feel of fullness **(Patel, 2015)**.

The importance of fiber as a part of a well - balanced diet, has been known for a decades. Soluble fiber such as β-glucan has a significant glycemic effect. The cereals, especially barley and oat are a perfect source of these functional components. Current research suggests that the efficacy of the beta-glucans is also appreciable in the immune system. They have a positive effect as prebiotics **(Koutinas et al., 2014)**. Recent large-scale epidemiological studies have shown that regular consumption of whole grain cereals can reduce the risk of heart disease and certain cancers by 30 percent. One of the factors that increase the functionality of foods is theso-called in digestible resistant starch **(Duchoňová and Šturdík, 2010)**.

The most common toxic heavy metals include Hg, Cd and Pb. The current state of the environment significantly influences gene pool of plants and animals and through food chain and population health and animals **(Cimboláková and Nováková, 2009)**.

Food consumption has been identified as a major source of contaminants income. There are a lot of elements which have a pathological effect on the organism, and are characterized by high toxicity. These contaminants are mercury and arsenic too **(Melo et al., 2008)**. Lead, mercury and cadmium are the elements which have a harmfule ffects on the central nervous system in the development of the child **(Kippler et al., 2012)**. Mercury, as well as other trace elements, moves between different media (i.e. atmospheric aerosol, dust, soil, plants, sediment, in the gas phase, in aqueous solution and solids **(Charlesworth et al., 2011)**.

Mercury mining and its use in products continue to the present. Consumer products containing mercury are batteries, fluorescent lamps, and some cosmetics **(McKelvey et al., 2011; Streetsetal, 2011)**. Mercury and its compounds are toxic to humans and the environment. Mercury is found in various chemical forms and is able to cause a wide variety of clinical effects **(Bernhoft, 2012)**.

The toxicity of mercury and its compounds to humans, such as a taxy, narrow vision, hearing loss and death were firstly described in 1865 **(Grandjean et al., 2010)**. Man receives the highest concentrations of mercury through the food chain and the largest sourceof food consists of animal origin **(Tóth et al., 2012)**.

The high doses of mercury can be fatal to humans, but even relatively low doses can have a serious effect on the nervous system and the development. Nowadays it has been disscused a lot about the harmful effectson the cardiovascular, immune and reproductive systems. Mercury also slow down microbiological activity in soil and under the regulation of classification of ground and surface water is one of the most hazardous substances to health. Mercury is persistent and can change the environment into methylmercury, the most toxic form. The phytotoxicity of mercury depends on its form and sorption. Elemental mercury is a potential source of highly toxic gases. Plants possess different degrees of tolerance of mercury **(Samešová, 2012)**. The plants may be exposed to either direct effect of mercury as antifungal agents, particularly through the crop seed treatment or foliar spray or by an accident. The exposure to mercury may occur through soil, water and air pollution.The concentration of mercury in above – ground parts of plants depends largely on foliar uptake Hg^0 volatilisation from the soil. Wilde dible fungiare characterized by high bioaccumulative ability – they are able to take from substrate and then aggregated up to several tens of its concentrationin soil **(Árvay et al., 2014; Árvay et al., 2015)**.

The factors that are affecting the accumulation of mercury by plants are organic matter contentin the soil or sediments, organic carbon content, redox potential and total metal content. Generally, mercury up takein plants could be related to the degree of soil pollution **(Patra and Sharma, 2000, Tomáš et al., 2012, Árvay et al., 2013; Tomáš et al., 2014)**.

MATERIAL AND METHODOLOGY

The aim of our work was the evaluation of transfer of mercury from sludge to edible part of chosen cereals. The objectives were achieved in simulated conditions of growing pot experiment. We used agricultural soil from the location of Výčapy – Opatovce for the realization of the experiment. The sludge, which was added at various doses, was taken from Central Spiš area from locality of Rudňany near the village where was mined iron ore that contains mainly copperand mercury during last few decades. We used three types ofcereals: barley (*Hordeum sativum L.*) variety PRESTIGE, spring wheat (*Triticum aestivum L.*), variety ISJARISSA and oat (*Avena sativa L.*) variety TATRAN. The length of growing season was 90 days.

Before the establishment growing pot experiment we performed all necessary analyses in soil and sludge. We determined soil reaction, content of nitrogen by Kjeldahl method, phosphorus content, potassium and magnesium contentby Mehlich II solution. Subsequently, we determined the content of heavy metals in the acid mixture HNO_3 and HCl (decomposition by aqua regia) by AA Swith Varian AA240FS (Australia). For analysis of each elelment, we used the multi-element standard SigmaAldrich (Germany).

To every one of all tested pots was weighed 5 kg of soil with 1 kg of silica sand, while the bottom of the container was filled with a small drainage layer of gravel. In each pot was applied the calculated dose of sludge.

Crop shave been harvested at full maturity time and after drying were assessed by mercury by AAS for AMA254 (Czech Republic). Seed samples are analyzed directly without modification.

For statistical evaluation of obtained results was used a statistical program STATISTICA 6.0 Cz. Wetested the resultson the level of descriptive statistical evaluation, and overall visual indication of the level factor, variability and the deviation was expressed in text. For statistical evaluation we used T-test at the level $p \leq 0.05$.

RESULTS AND DISCUSSION

Soil from the locality of Výčapy – Opatovce has analcalic soil reaction with medium level of humidity. It is characterized by good content of phosphorus and potassium and a high content of magnesium.The contents of heavy metals do not exceed the limit values **(Act No. 220/2004 Coll.)**.

Sludge from the locality of Rudňany has a strongly alkaline soil reaction. It is characterized by a very low content of phosphorus and potassium. Mercury content (57.81 $mg.kg^{-1}$) exceeds the maximum permissible amount by 5.78 times **(Act No. 188/2003 Coll.)**.

Mercury content in barley seeds in D variant has increased by 7.9 times in 2013 and 14.2 times in 2014 compared to variant A (soil without addition of sludge). Escalating amount of sludge added to the soilis proportionally reflected in mercury content in the seeds of barley. In 2013 the mercury content in the seeds of barley was almost a half lower than in 2014.

Mercury contentin barley seeds in all variants exceeded the maximum permissible amount specified by The Codex Alimentarius of Slovak republic (CA SR).

Statically significant difference between 2013 and 2014 in mercury content in the seeds of barley was obtained in the variant D. In other variants was not statistically significant difference.

The highest mercury content of seeds of wheat was obtained in the variant D in 2014 where the Hg content in the seeds was higher by 5.7 times than in variant A. The differences between the mercury content of variants C and D was not as significant as in the case of barley. The difference between the highest mercury content of seeds of wheat in variant D in the year 2013 and 2014 had not a

Table 1 Variants of the experiments.

variants	
A	soil 100%
B	soil 90%, sludge 10%
C	soil 80%, sludge 20%
D	soil 70%, sludge 30%

Table 2 The content of microelements in the soil.

MEHLICH II ($mg.kg^{-1}$)			
K	**Ca**	**Mg**	**P**
287.5	6948.0	392.0	587.5

Table 3 The contents of heavy metals in the soil (decomposition by aqua regia) and comparison of **Act No. 220/2004 Coll. of Slovak Republic** ($mg.kg^{-1}$).

	Cd	**Pb**	**Cu**	**Zn**	**Cr**	**Ni**
soil	0.70	18.2	18.4	55.6	17.2	30.6
Act No. 220/2004	0.70	70	60	150	70	50

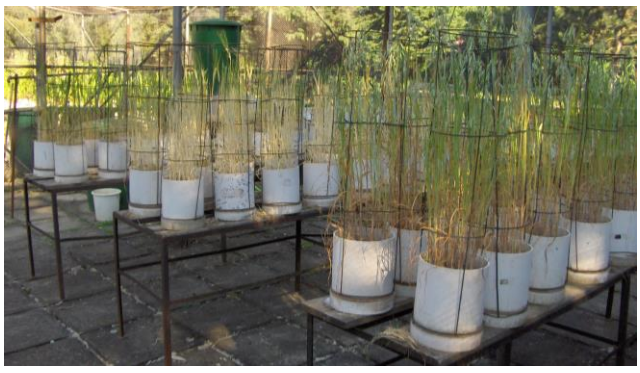

Figure 1 Simulated conditions of growing pot experiment.

Table 5 Mercury content in the seeds of wheat variety Jarissa in 2013 and 2014 ($mg.kg^{-1}$) and the comparison of mercury content with Codex Alimentarius of Slovak republic (CA SR).

	WHEAT	
variants	**2013**	**2014**
A	0.001821	0.001395
B	0.003241	0.001866
C	0.006582	0.004729
D	0.006809	0.007966
CA SR	0.05	0.05

Table 6 Mercury contentin the seeds of oat variety Tatran in 2013 and 2014 ($mg.kg^{-1}$) and the comparison of mercury content with Codex Alimentarius of SR (CA SR) .

	OAT	
variants	**2013**	**2014**
A	0.002971	0.002288
B	0.007252	0.003753
C	0.009468	0.010078
D	0.019722	0.040756
CA SR	0.05	0.05

high level of significance.

Mercury content in wheat seeds in all variants was not exceeded the maximum permissible amount specified by Codex Alimentarius of SR (CA SR) .

Regarding the differences in mercury content in wheat seeds was not obtained statistically significant difference beetween different years.

The largest in take of mercury was obtained in oat seeds. Mercury contentin variant D (maximum addition of sludge) was higher by 17.8 times than in variant A in 2014. In 2013 the mercury content in the seeds of oat was almost a half lower than in 2014. The most significant increase in mercury conten tin the seeds of the oat option C and D was recorded in 2014.

Although oat is characterized by the highest accumulation of mercury in the seeds, the content was not exceeded the maximum permissible amounts specified by Codex Alimentarius of SR (CA SR).

Statically significant difference between 2013 and 2014 in mercury contentin the seeds of oat was obtained in the variants B and D.

Bajčan et al., (2010) measured mercury concentrations in the samples taken agricultural crops grown on all uvial soils in the region of Hont. Hg content in the grains wer in a range of less than 0.0001 mg.kg-1 to 0.0198 1 $mg.kg^{-1}$, what is significantly less mercury as maximum allowable limit for Food (0.05 $mg.kg^{-1}$).

Hg content corresponds to the content in the soil, the greater Hg content in thes oil, the higher the Hg content in the grain cereals. The highest Hg content in the grain weset in the grain of the barley from area Markušovce and 0.2006 $mg.kg^{-1}$, what It represents four times the limit value **(Šabo, 2013).**

In general, the acceptability of the soil for the plants Hg low, tending accumulation in the roots, but the aerial parts of the plants absorbed from the atmosphere directly Hg. To of total Hg in plants has, according to some authors direct deposition up to 90% share. The natural average concentration of Hg in the plants are moved between 0.005 - 0.17 mg of Hg. kg^{-1}, with values of 1 - 3 mg $Hg.kg^{-1}$ are considered phytotoxic **(Toman et al., 2000).**

The following figures show the comparison of the mercury contentin the seeds of commodities in different variants.

Figure 2 shows that the mercury content of the seeds of each cereal in variants B, C and D increases with increasing addition of sludge intot he soil. The smallest storage capacity of mercury was recorded in spring wheat variety ISJARISSA. In a variant D was 0.006809 $mg.kg^{-1}$ of mercury content, which represents almost a half of the amount that has been accumulated by seeds of barley and by 3 times smaller than accumulated amount in the case of seeds of oat in the same variant.

Figure 3 shows a similar situation as Figure 2, thus increasing doses of sludge into the soil affected also by increased mercury content in the seeds of varieties of all crops.The results obtained from the two years can therefore say that in terms of accumulation of mercury seeds equence is as follows wheat < barley < oat.

Increased mercury content in soil in each variant due to the addition of sludge had a statistically significant effect on mercury content in the seeds of all variants ata significance level of $p<0.05$.

For all crops the additions of sludge into the soil have a statistically significant effect on mercury content in different variants ata significance level of $p <0.05$.

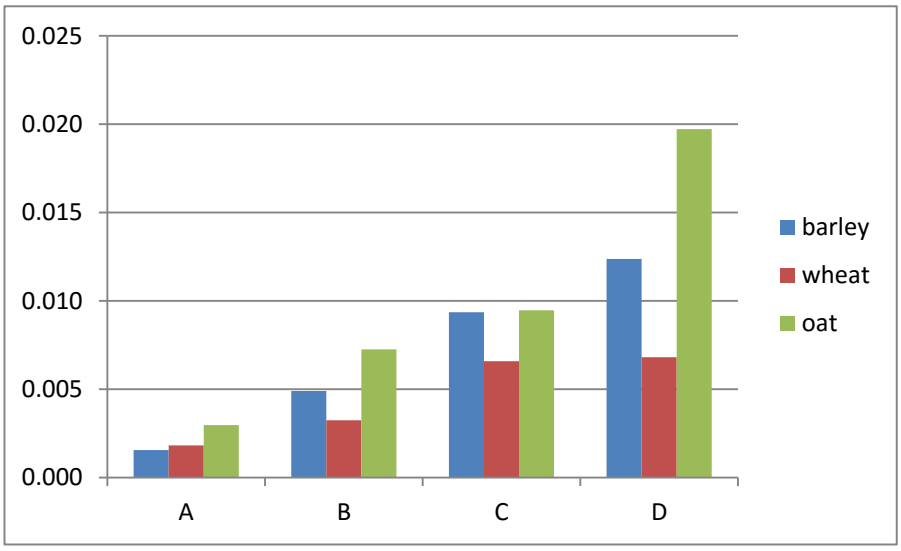

Figure 2 Mercury content in the seeds of various cereals in all variants (mg.kg^{-1}) that were grown in the year 2013.

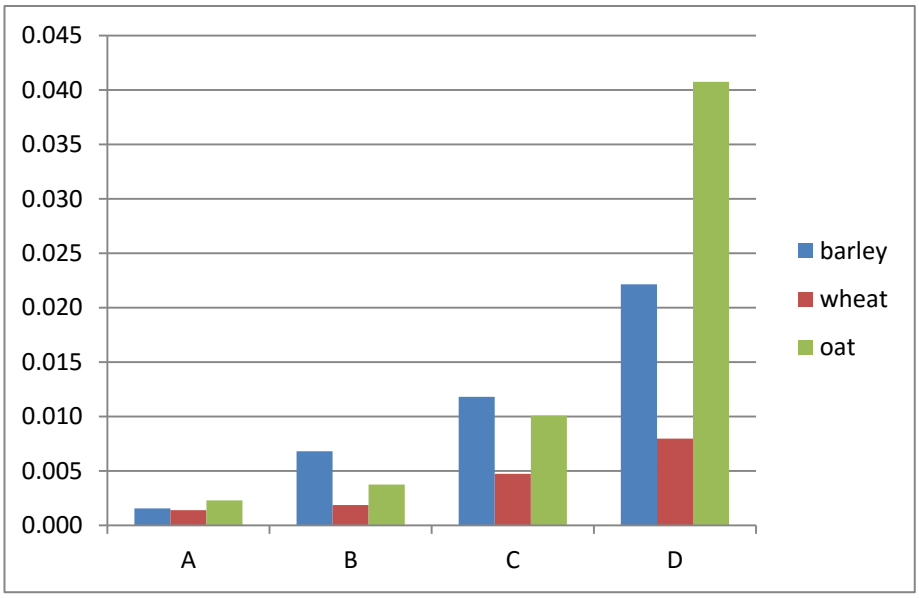

Figure 3 Mercury content in the seeds of various cereals in all variants (mg.kg^{-1}) that were grown in the year 2014.

CONCLUSION

The results showed that the amount of sludge added in specified amounts into the soil increases mercury content in seeds of crops.

Although oat was characterized by the highest accumulation of mercury in the seeds, the content was not exceeded the maximum permissible amount specified by Codex Alimentarius of SR.

From the obtained results of two years can be concluded that the accumulation of mercury by seed follows wheat < barley < oat.

The results showed that the suitable cultivation of the cereals in localities, which are contaminated with heavy metals, especially by mercury, that the high content of mercury in soil do not pose a risk of accumulation of the metal into the cereal grain.

Increasing number of toxic metals in soil leads to an increased content of the emetals in crops and subsequently in animal products. This may have adverse effects on people who consume these products.

There are two main reasons why the contamination of the environment with heavy metals causes concern. First, it can reduce the productivity of plants used as human food and animal feed. Second, it affects the quality of agricultural products.

REFERENCES

Act No. 220/2004 Coll. Of Laws of Slovak Republic. On the conservation and use of agricul- tural land, amending the Act No. 245/2003 Coll. on integrated pollution prevention and control, amending and supplementing of certain acts, as amended.

Act No. 188/2003 Coll. Of Laws of Slovak Republic. about application of sewage sludge and bottom sediments into soil and completing of Law No. 223/2001 Collection of Laws

about wastes and changes and completing of some laws as amended of later Acts.

Alldrick, A. J. 2012. Chemical contamination of cereals. *Chemical Contaminants and Residues in Food*. Campden BRI, UK. p. 421-446. ISBN: 978-0-85709-058-4 http://dx.doi.org/10.1533/9780857095794.3.421

Árvay, J., Tomáš, J., Hauptvogl, M., Kopernická, M., Kováčik, A., Bajčan, D., Massányi, P. 2014. Contamination of wild-grown edible mushrooms by heavy metals in a former mercury mining area. *Journal Environ. Sci*. Health pt. B., vol. 49, no. 11, p. 815-827. http://dx.doi.org/10.1080/03601234.2014.938550

Árvay, J., Tomáš, J., Hauptvogl, M., Massányi, P., Harangozo, Ľ., Tóth, T., Stanovič, R., Bryndzová, Š., Bumbalová, M. 2015. Human exposure to heavy metals and possible public health risks via consumption of wild edible mushrooms from Slovak Paradise National Park, Slovakia. *Journal Environ. Sci.* Health pt. B., vol. 50, p. 838-848. PMid:26357894

Árvay, J., Stanovič, R., Bajčan, D., Slávik, M., Miššík, J. 2013. Content of heavy metals in soil and cropfrom middle Spiš area. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2, p. 1988-1996.

Bajčan, D., Lahučký, L., Tóth, T., Tomáš, J., Árvay, J. 2010. Evaluation of the mercury content in agricultural products from the Hont region. *Potravinarstvo*, vol. 4, special issue, p. 258-263. Available at: http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/3/Bajcan.pdf

Bernhoft, R. A. 2012. Mercury Toxicity and Treatment: A Review of the Literature. *Journal of Environmental and Public Health*, vol. 2012, article ID 460508, 10 p., http://dx.doi.org/10.1155/2012/460508

Beverly, R. L. 2014. Safetyof Food and Beverages: Cereals and Derived Products. *Encyclopedia of Food Safety*, vol. 3 Foods, Materials, Technologies and Risks, p. 309-314. http://dx.doi.org/10.1016/B978-0-12-378612-8.00287-0

Cimboláková, I., Nováková, J. 2009. Heavy metals – the important element of the food chain. *Potravinarstvo*, vol. 3, no. 3, p. 14-16.

Codex Alimentarius of Slovak Republic Potravinový kódex SR 2006 Výnos č.18558/2006-SL Available at: http://www.svps.sk/legislativa/legislativa_kodex.asp

Duchoňová, L., Šturdík, E. 2010. Cereals as basis of preventing nutrition agains tobesity. *Potravinarstvo*, vol. 4, no. 4, p. 6-15.http://dx.doi.org/10.5219/76

Grandjean, P., Satoh H., Murata K., Eto, K. 2010. Adverse effects of methylmercury: environmental health research implications. *Environmental Health Perspectives*, vol. 118, no. 8, p. 1137-1145. http://dx.doi.org/10.1289/ehp.0901757

Charlesworth, S., DeMiguel, E., Ordonez, A. 2011. A review of the distribution of particulate trace elements in urban terrestrial environments and its application to considerations of risk. *Environ Geo chem Health*, vol. 33, no. 2, p. 103-123. http://dx.doi.org/10.1007/s10653-010-9325-7

Kippler, M., Tofail, F., Hamadani, J. D., Gardner, R. M., Grantham-McGregor, S. M., Bottai, M., Vahter, M. 2012. Early-life cadmium exposure and child development in 5-year-old girls and boys: a cohort study in rural Bangladesh. *Environ. Health Perspect.*, vol. 120, no. 10, p. 1462-1468R. http://dx.doi.org/10.1289/ehp.1104431

Koutinas, A. A., Du. C, Lin. C. S. K., Webb C. 2014. Developments in cereal-basedbiorefineries. *Biomass and Waste Supply Chain Exploitation*, vol. 2014, p. 303-334. http://dx.doi.org/10.1533/9780857097385.1.303

McKelvey, W., Jeffery, N., Clark, N., Kass, D., Parsons, P. 2011. Population-based in organic mercury biomonitoring and the identification of skin care products as a source ofexposure in New York City. *Environmental Health Perspectives*, vol. 119, no. 2, p. 203-209. http://dx.doi.org/10.1289/ehp.1002396

Melo, K. Gellein, L. Evje, T. Syversen. 2008. Minerals and trace elements in commercial infant food. *Food and Chemical Toxicology*, vol. 46, no. 10, p. 3339-3342. http://dx.doi.org/10.1016/j.fct.2008.08.007

Patel, S. 2015. Cereal bran fortified-functional foods for obesity and diabetes management: Triumphs, hurdles and possibilities. *Journal of Functional Foods*, vol. 14, no. 24, p. 255-269. http://dx.doi.org/10.1016/j.jff.2015.02.010

Patra, M., Sharma, A. 2000. Mercury toxicity in plants. *Botanical Review*, vol. 66, no. 3, p. 379-422. http://dx.doi.org/10.1007/BF02868923

Samešová, D. 2012. Ťažké kovy v čistiarenských kaloch (Heavy metals in sewage sludge). *Životné prostredie*, vol. 46, no. 5, p. 232-236.

Serna Saldivar, S. O. 2016. Encyclopedia of Food and Health. p. 718-723, ISBN: 978-0-12-384953-3. http://dx.doi.org/10.1016/B978-0-12-384947-2.00128-8

Finch, H. J. S., Samuel, A. M., Lane, G. P. F. 2014. *Lockhart & Wiseman's Crop Husbandry Including Grassland, 13 Cereal*, 9th ed., p. 287-336. http://dx.doi.org/10.1533/9781782423928.3.287

Streets, D. G., Devane, M. K., Lu, Z., Bond, T. C., Sunderland, E. M., Jacob, D. J. 2011. All-time releases of mercury to the atmosphere from human activities. *Environmental Science & Technology*, vol. 45, no. 24, p. 10485-10491. http://dx.doi.org/10.1021/es202765m

Šabo, T. 2013. The content of cadmium, lead and mercury in the grain cereals growing in region of central Spiš. Thesis, Nitra, SPU. 75 p.

Toman, R., Massányi, P., Ducsay, L. 2000. *Kadmium – kontaminant potravového reťazca človeka. Cudzorodé látky v životnom prostredí (Cadmium – contaminant of human food chain.)* Xenobiotics in environmental, Nitra. SPU, p. 208-212, ISBN 80-7137-745-7.

Tomáš, J., Árvay, J., Tóth, T. 2012. Heavymetals in productive parts of agricultural plants. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 1, p. 819-827.

Tomáš, J., Árvay, J., Tóth, T., Vollmannová, A., Kopernická, M., Slávik, M. 2014. The level of crop plants contamination by heavy metals from the historical minesarea. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 3, p. 294-297.

Tóth, T., Andreji, J., Tóth, J., Slávik, M., Árvay, J., Stanovič, R. 2012. Cadmium, lead and mercurycontents in fishes – case study. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 1, p. 837-847.

Verspreeta J., Dorneza E., Endeb W. V. D., Delcoura J. A., Courtina Ch. M. 2015. Cereal grainf ructans: Structure, variability and potential health effects. *Trends in Food Science & Technology*, vol. *43*, p. 32-42. http://dx.doi.org/10.1016/j.tifs.2015.01.006

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HEAVY METALS DETERMINATION IN EDIBLE WILD MUSHROOMS GROWING IN FORMER MINING AREA – SLOVAKIA: HEALTH RISK ASSESSMENT

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ABSTRACT

The aim of the paper is to assess a contamination level of forest substrates and aboveground parts of edible wild mushroom (*M. procera* (Scop.) Singer, *B. recitulatus* Schaeff., *C. cibarius* Fr., *S. grevillei* (Klotzsch) Singer, *A. campestris* L., *R. xerampelina* (Schaeff.) Fr., *L. salmonicolor* R. Heim & Leclair, *C. gibba* (Pers. Ex Fr.) Kumm., *X. chrysenteron* (Bull.) Quél., *M. oreades* (Bolton) Fr.; n = 70) by heavy metals (Cd, Cu, Pb and Zn). The studied location was a broader surroundigs of the historical mining and metal processing area of Banská Bystrica. The collected mushroom samples and underlying substrate samples were analysed using Flame Atomic Absorption Spectrofotometry and Flame Absorption Spectrophotometry with graphite furnace. Bioaccumulation factors (BAF) for individual species and their anatomical parts were calculated from the results obtained. In order to assess a health risk resulting from regular consumption of the mushrooms, provisional tolerable weekly intake (PTWI) was calculated from the results of the monitored heavy metal concentration. Limit values for the studied contaminants (Cd: 0.49 $mg.kg^{-1}$ and Pb: 1.75 $mg.kg^{-1}$ for an individual with an average weight of 70 kg) are defined by FAO and WHO. Our results indicate that *S. grevillei* has a high bioaccumulation ability of Cd. It was confirmed by bioaccumulation factors (BAF_H = 3.47 and BAF_{RFB} = 2.30). The $PTWI_{Cd}$ value was exceeded by 4.11 times. A similar situation occurred in the case of Pb where the highest bioaccumulation factor (BAF_H = 0.24 and BAF_{RFB} = 0.19) was also recorded in the samples of *S. grevillei* and the $PTWI_{Pb}$ value was exceeded by 1.35 times. In general, it can be stated that a consumption of edible wild mushrooms represent a relatively small risk of negative impact on the health of consumers.

Keywords: edible wild mushroom; heavy metal; contamination; bioaccumulation; health risk assessment; Slovakia

INTRODUCTION

Heavy metals are ubiquitous environmental components, the origin of which is natural or anthropogenic **(Jiang et al., 2006; Feng et al., 2003)**. Environmental contamination with heavy metals is increasingly coming to the fore and it is one of the most serious problems of modern society nowadays. Their riskiness arise from the substantial persistence, toxicity and ability to bioaccumulate into environmental components and consequently into the food chain **(Burges et al., 2015; Douay et al., 2013; Roman and Popiela, 2011)**. Long-time industrialization of society and subsequent rapid urbanization lead to an increased amount of xenobiotics and thus also heavy metals in the urban environment **(Szolnoki et al., 2013; Luo et al., 2012)** but also in non-urban areas **(Luo et al., 2014)**, which represents a significant risk to the global ecosystem and the health of human populations **(Siciliano et al., 2009)**.

Some heavy metals (Hg, Cd, Cr, Ni, Pb), arsenic and esential trace elements (Cu, Zn) pose a significant risk to the quality of the environment, which influences on the health of the human population **(Alloway, 2013; Jomová and Valko, 2011)**. They enter the environment via natural activities (volcanic activity, weathering, etc.) and anthropogenic activities (e. g. extraction and processing of minerals, combustion of fossil fuels and waste, etc.). **(Hooda, 2010)**. Cadmium and lead belong to non-essential trace elements and are classified as toxic metals that are harmful to plants, animals and human body even at very low concentrations. They are introduced to the body mostly by inhalation and/or resorption and consequently damage individual systems of the human body **(Timorácká et al., 2011; Silva et al., 2003)**. However, high amounts of the heavy metals can get into the body also by food. Zinc and copper are classified as essential trace elements **(Wuan and Okieimen, 2011; John et al., 2010)**, however they can be toxic to humans in higher concentrations **(Licata et al., 2012)**. They participate in the regulation of various physiological functions, including inflammatory and oxidative processes **(Mocchegiani et al., 2012; Malavolta et al., 2010)**. For example, increased concentration of copper has adverse effects on the activity of the central nervous system and certain physiological processes **(Grandner et al., 2013; Cappuccio et al., 2011)**.

Edible wild mushrooms represent a natural part of forest ecosystems and play an important role in the cyclic pathways of elements and organic matter **(Petkovšek and Pokorny, 2013)**. They are able, together with micro-organisms, to biodegrade substrate and thus utilize waste from agricultural production and/or human activities

(Ouzouni et al., 2009). Some mushroom species are considered as a delicacy in many countries, including countries of Central and Eastern Europe. Fruiting bodies of the mushrooms are popular not only for their texture and flavor, but also for their nutritional properties **(Cheung, 2013; Kalač, 2013)**. They are characterized by low energy value and high concentration of essential biologically valuable elements, specific β-glucans and antioxidant substances **(Kalač, 2013; Kalač, 2009)**. Moreover, they provide a valuable source of fiber, vitamins and minerals such as thiamin, riboflavin, vitamin D, potassium, phosphorus, iron and calcium **(Wang et al., 2014; Falandysz and Borovicka, 2013)**. It has been known for long time that mushrooms are able to accumulate large amounts of heavy metals **(Zhang et al., 2008)**, what makes them ideal for biomonitoring of environmental pollution – particularly contamination of forest ecosystems **(Radulescu et al., 2010)**. There are many factors that influence the presence of metals in mushrooms, for example climate, environmental conditions and concentration of macromolecules in the cell wall of each specific species **(Ostos et al., 2015)**. Studies of the interaction of heavy metals in the system soil/substrate – mycelium showed that mushrooms have several fold higher bioaccumulation capacity to uptake xenobiotics – heavy metals from the substrate compared to higher plants (intake from the atmosphere is negligible) **(Falandysz, 2015; Saba et al., 2015; Zhu et al., 2011; Gursoy et al., 2009)**.

Under natural conditions, the concentration of heavy metals in certain species of edible mushrooms can be higher, even if the soil contamination level is low **(Falandysz et al., 2003)**. The highest concentrations of trace elements are mostly found in the hymenophore, lower values are in the spores and the lowest values are in the stem **(Árvay et al., 2015a; Krasińska and Falandysz, 2015; Falandysz et al., 2007; Alonso et al., 2003)**.

The aim of the paper is to determine the level of transition of the studied heavy metals (Cd, Cu, Pb and Zn) from the substrate into the aboveground parts of edible macroscopic mushrooms collected in the broader area of Banská Bystrica, which is characterized by historic mining and metalworking activity (mining and processing of ore rich in precious metals, copper, lead and associated components: mercury, cadmium, etc.). Bioaccumulation factors (BAF) for individual anatomical parts of mushrooms (hymenophore - H and rest of fruit bodies - RFB) were calculated. Due to the popularity of collecting wild edible mushrooms in Central Europe **(Árvay et al., 2014; Kalač, 2009)**, a health risk arising from their regular consumption was investigated.

MATERIAL AND METHODOLOGY

Study area, sampling and pre-analytical procedure

For the needs of our work, 10 species of the most commonly collected wild mushrooms, which generally represent the most frequently collected mushrooms in Slovakia were chosen. The samples of edible wild mushrooms and substrate (N = 70) were collected in 2014 in the broader area of Banská Bystrica, in the cadastral areas of villages Ľubietová, Radvaň, Malachov, Selce, Nemce, Hrochoť and Podkonice that are characteristic by historical mining and metalworking activity. Identification of the sampling points was made using GPS coordinates (Figure 1). The concentration of heavy metals (Cd, Cu, Pb and Zn) was studied in individual parts of edible wild growing mushrooms. Studied species and their respective sampling frequencies are inculede in Table 1.

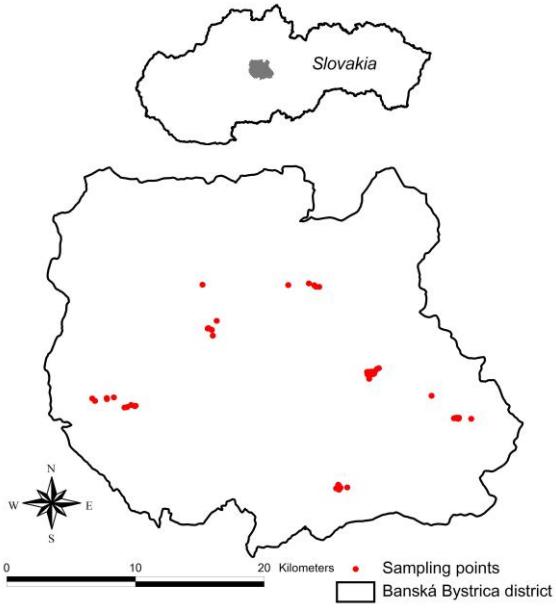

Figure 1 Map of the studied area with sampling points.

Table 1 The heavy metals cencentration (mg.kg^{-1} DM) in substrate.

Species*	N	Cd	Pb	Cu	Zn
		Median ±SD (range)			
Macrolepiota procera (Scop.) Singer	11	2.43 ±0.80 (0.58 – 3.66)	45.3 ±15.7 (28.5 – 88.8)	23.3 ±16.0 (10.0 – 66.9)	97.8 ±31.0 (58.6 – 155)
Boletus reticulatus Schaeff.	9	2.89 ±1.60 (1.76 – 6.94)	49.4 ±12.1 (36.6 – 76.6)	19.4 ±9.0 (11.6 – 38.5)	122 ±66.9 (59.4 – 278)
Cantharellus cibarius Fr.	3	2.68 ±0.60 (2.24 – 3.37)	47.2 ±3.15 (43.7 – 49.8)	20.3 ±8.02 (13.0 – 28.9)	112 ±24.1 (95.2 – 139)
Suillus grevillei (Klotzsch) Singer	11	2.49 ±0.68 (1.66 – 3.51)	41.9 ±16.2 (25.3 – 75.1)	18.1 ±9.91 (3.10 – 38.8)	41.9 ±16.2 (25.3 – 75.1)
Agaricus campestris L.	3	2.74 ±0.19 (2.53 – 2.90)	49.8 ±25.6 (34.4 – 79.3)	31.1 ±17.0 (14.8 – 48.7)	155 ±65.5 (97.0 – 226)
Russula xerampelina (Schaeff.) Fr.	8	2.44 ±0.54 (1.72 – 3.40)	58.2 ±14.5 (41.4 – 76.6)	27.0 ±11.9 (11.3 – 48.1)	58.2 ±14.5 (41.4 – 76.6)
Lactarius salmonicolor R. Heim & Leclair	10	2.82 ±0.41 (2.25 – 3.50)	61.1 ±23.9 (42.7 – 113)	23.2 ±8.84 (13.0 – 43.1)	101 ±24.4 (70.7 – 141)
Clitocybe gibba (Pers. Ex Fr.) Kumm.	3	2.52 ±0.13 (2.34 – 2.66)	43.5 ±4.65 (37.8 – 49.2)	19.8 ±11.7 (11.2 – 36.4)	108 ±28.7 (67.9 – 131)
Xerocomus chrysenteron (Bull.) Quél.	7	2.03 ±0.38 (1.37 – 2.38)	39.3 ±5.23 (31.2 – 47.1)	18.7 ±6.49 (8.40 – 25.8)	39.3 ±5.23 (31.2 – 47.1)
Marasmius oreades (Bolton) Fr.	5	2.22 ±0.43 (1.81 – 2.94)	59.1 ±11.5 (44.1 – 73.6)	25.5 ±10.1 (16.2 – 38.6)	144 ±58.9 (84.9 – 241)

N, number of samples; **SD**, standard deviation; ***Index fungorum (2015)**

Organic and inorganic debris was removed mechanically by ceramic knife and the cap (hymenophore) was separated from the rest of fruit body immediately after collecting of the mushroom samples. Later, the samples were sliced and dried at 45 °C to constant weight. The dried samples were homogenized in a porcelain mortar and then stored in polyethylene bags. After the collection of the mushroom samples, substrate samples were taken from the same spot to a depth of 10 cm. In the laboratory, the substrate samples were dried to a constant weight, and afterwards they were sieved through a sieve with mesh width of 2 mm.

One gram (1 g.) of dried mushroom samples (accuracy to 4 decimal places) were mineralized by 5 cm^3 of concentrated HNO_3 (Merck, Germany) and the same volume of deionized water using microwave mineralization system in MARS X-press 5 (CEM, USA). Afterwards, the sample was filtered through filter paper 390 Filtrak (Munktell, Germany) and filled with deionized water to 50 cm^3. The substrate samples were mineralized the same way as the mushroom samples in the mixtures of HNO_3 and HCl (Merck, Germany) in the ratio 1:1. After the mineralization, the digest was filtered through filter paper 390 Filtrak (Munktell, Germany) and diluted with deionized water to a total volume of 100 cm^3 **(Árvay et al., 2015b; Árvay et al., 2014)**.

Analytical procedure

Quantitative determination of the concentration of the studied trace elements (Cd, Cu, Pb, Zn) was carried out in the mineralized samples by flame atomic absorption spectrometry (F-AAS) in Varian AA 240 FS apparatus (Varian, Australia), by method published in **Árvay et al. (2014)**.

Statistical analysis and risk assessment

All data on the concentration of the studied contaminants in the samples were processed by descriptive statistical analysis at the level of the minimum and maximum values, median values and standard deviation in Statistica 12 software (StatSoft, USA).

Due to the popularity of the collection and subsequent consumption of edible wild mushrooms in Slovakia **(Árvay et al., 2015a; Árvay et al., 2015b; Árvay et al., 2014; Kalač, 2009)**, tolerable weekly intake (PTWI) was calculated, based on the data obtained on the concentration of the studied heavy metals, for a standardized person weighing 70 kg with a consumption of 300 g of fresh edible wild mushrooms per day. The parameter is defined by FAO/WHO (1993) for cadmium and lead separately. The value for cadmium is 0.007 mgkg^{-1} of body weight of a consumer. The value for lead is 0.025 mg.kg^{-1} **(JECFA, 2010; WHO, 1993)**. The legislation does not state PTWI values for the zinc and copper. Due to the high water concentration (which is dependent on weather conditions), generally accepted value of 90% was used for conversion of the water concentration in the mushroom samples **(Kalač, 2009)**.

RESULTS AND DISCUSSION

Table 2 The heavy metals concentration in hymenophore (mg.kg^{-1} DM) and hymenophore and rest of fruit bodies bioaccumulation factors.

Species		Cd	Pb	Cu	Zn
		Median ±SD (range)			
Macrolepiota procera (Scop.) Singer	H	3.98 ±6.48 (0.48-22.9)	6.46 ±3.38 (2.45-13.2)	81.1 ±56.4 (23.7-207)	106 ±63.6 (42.3-247)
	BAF_H	1.64	0.14	3.48	1.08
	BAF_{RFB}	1.34	0.11	2.44	0.83
Boletus reticulatus Schaeff.	H	5.08 ±7.14 (0.66-21.9)	6.58 ±3.63 (2.32-15.0)	57.5 ±34.3 (23.6-122)	226 ±157 (86.9-585)
	BAF_H	1.76	0.13	2.97	1.86
	BAF_{RFB}	1.44	0.11	2.21	1.51
Cantharellus cibarius Fr.	H	0.56 ±0.20 (0.39-0.78)	4.07 ±1.32 (2.55-4.90)	52.1 ±6.57 (47.5-59.6)	75.3 ±3.79 (71.6-79.2)
	BAF_H	0.21	0.09	2.56	0.67
	BAF_{RFB}	0.07	0.06	1.80	0.52
Suillus grevillei (Klotzsch) Singer	H	8.64 ±9.87 (1.72-29.7)	10.1 ±10.0 (1.33-30.0)	42.2 ±36.1 (12.8-138)	118 ±35.2 (71.7-174)
	BAF_H	3.47	0.24	2.33	1.35
	BAF_{RFB}	2.30	0.19	1.69	1.09
Agaricus campestris L.	H	1.44 ±1.12 (0.69-2.72)	6.20 ±5.08 (2.73-12.0)	43.1 ±20.6 (21.3-62.1)	113 ±51.8 (69.7-170)
	BAF_H	0.52	0.12	1.39	0.73
	BAF_{RFB}	0.41	0.09	1.25	0.51
Russula xerampelina (Schaeff.) Fr.	H	2.97 ±3.09 (0.86-10.2)	6.02 ±5.11 (0.97-13.1)	43.8 ±25.3 (15.1-96.5)	93.9 ±40.0 (51.9-182)
	BAF_H	1.22	0.10	1.62	0.84
	BAF_{RFB}	0.83	0.07	0.97	0.57
Lactarius salmonicolor R. Heim & Leclair	H	1.11 ±1.01 (0.50-3.47)	3.17 ±1.08 (1.50-5.67)	15.7 ±7.22 (8.30-31.8)	184 ±132 (45.6-419)
	BAF_H	0.39	0.05	0.67	1.83
	BAF_{RFB}	0.34	0.04	0.46	1.16
Clitocybe gibba (Pers. Ex Fr.) Kumm.	H	5.29 ±5.64 (1.91-11.8)	2.93 ±0.88 (1.93-3.62)	49.6 ±12.6 (37.9-62.9)	131 ±44.7 (83.1-171)
	BAF_H	2.10	0.07	2.50	1.21
	BAF_{RFB}	1.73	0.05	1.99	1.04
Xerocomus chrysenteron (Bull.) Quél.	H	1.84 ±0.78 (0.67-3.22)	6.77 ±3.76 (2.85-12.0)	35.8 ±15.7 (17.5-63.6)	177 ±132 (98.5-448)
	BAF_H	0.90	0.17	1.91	2.10
	BAF_{RFB}	0.71	0.14	1.09	1.61
Marasmius oreades (Bolton) Fr.	H	2.45 ±1.14 (1.08-3.86)	6.93 ±5.86 (1.81-16.9)	34.3 ±36.8 (4.59-96.0)	99.2 ±29.9 (72.6-145)
	BAF_H	1.10	0.12	1.35	0.69
	BAF_{RFB}	0.95	0.08	0.92	0.61

SD, standard deviation; **H**, hymenophore; **BAF_H**, bioaccumulation factor in hymenophore; **BAF_{RFB}**, bioaccumulation factor in rest of fruit bodies.

Heavy metals in the substrate samples

All concentrations of the studied contaminants in the samples of substrates and edible wild mushrooms are given per dry matter (DM). The concentrations of the studied contaminants in the substrate represent an important factor that influences the bioaccumulation ability of individual species of edible wild mushrooms. Therefore, a variable level of translocation of heavy metals into macroscopic mushrooms can be assumed **(Chudzyński *et al.*, 2011)**. The total concentration of the contaminants in the substrate varied within wide ranges (Table 1). The total cadmium concentration in the substrate samples (N = 70) ranged from 0.58 to 6.94 mg.kg^{-1} DM, with the highest concentrations (6.94 mg.kg^{-1} DM) recorded in the substrate samples of *B. recitulatus* (N = 9). The total concentration of lead in the substrate samples (N= 70) ranged between 25.3 – 113 mg.kg^{-1} DM and the highest concentration (113 mg.kg^{-1} DM) was recorded in the substrate samples of *L. salmonicolor* (N = 10). The copper concentration in the samples (N = 70) ranged between 3.10 – 66.9 mg.kg^{-1} DM. The highest concentration (66.9 mg.kg^{-1} DM) was recorded in the substrate samples of *M. procera* (N = 11). The last studied element was zinc, the concentration of which ranged from 25.3 – 278 mg.kg^{-1} DM in all samples, with the highest concentrations (278 mg.kg^{-1}) recorded in the substrate

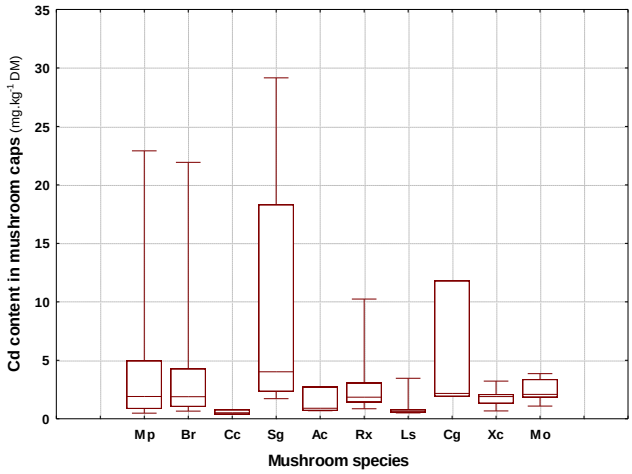

Figure 2 Range (min. – max.) and median, upper and lower quantile values of the cadmium concentration in the caps (mg.kg^{-1} DM) of individual species of edible wild mushrooms. ***MP,*** *M. procera*; ***BR,*** *B. recitulatus*; ***CC,*** *C. cibarius*; ***SG,*** *S. grevillei*; ***AC,*** *A. campestris*; ***RX,*** *R. xerampelina*; ***LS,*** *almonicolor*; ***CG,*** *C. gibba*; ***XC,*** *X. chrysenteron*; ***MO,*** *M. oreades*.

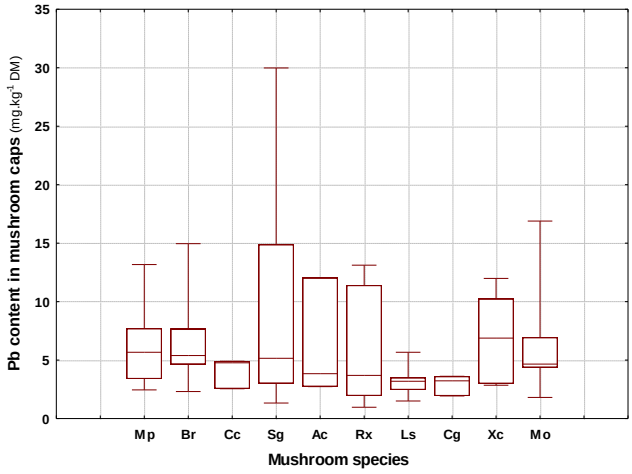

Figure 3 Range (min. – max.) and median, upper and lower quantile values of the lead concentration in the caps (mg.kg^{-1} DM) of individual species of edible wild mushrooms. ***MP,*** *M. procera*; ***BR,*** *B. recitulatus*; ***CC,*** *C. cibarius*; ***SG,*** *S. grevillei*; ***AC,*** *A. campestris*; ***RX,*** *R. xerampelina*; ***LS,*** *L. salmonicolor*; ***CG,*** *C. gibba*; ***XC,*** *X. chrysenteron*; ***MO,*** *M. oreades*.

samples of *B. recitulatus* (N = 9). High variability of the zinc concentration in the substrate indicates significant heterogenity of the zinc concentration in the studied sites.

Heavy metals in mushroom samples

Macroscopic mushrooms are considered to be an important bioaccumulator of xenobiotics (especially heavy metals) **(Árvay *et al.*, 2015a; Islam *et al.*, 2014)**, which was reflected in the concentration of the contaminants in individual anatomical parts of the studied mushroom species. The highest concentration of cadmium was recorded in the samples of *S. grevillei*, where the values in the hymenophore ranged from 8.64 ±9.87 mg.kg^{-1} DM (Figure 2). The ability of the species to bioaccumulate cadmium is the highest among all species (BAF_H = 3.47 and BAF_{RFB} = 2.30). It was confirmed by the findings of **Árvay *et al.*, (2014)**. The cadmium concentration in the hymenophore of individual species was in the following order: *S. grevillei* > *C. gibba* > *B. recitulatus* > *M. procera* > *R. xerampelina* > *M. oreades* > *X. chrysenteron* > *A. campestris* > *L. salmonicolor* > *C. cibarius*.

Similarly, in the case of the lead concentration the maximum values were recorded in the samples of *S. grevillei* (10.1 ±10.0 mg.kg^{-1} DM, 1.33 – 30.0 mg.kg^{-1} DM) (Figure 3). This species had also the highest bioaccumulation factor BAF_H = 0.24 and BAF_{RFB} = 0.19. The lead concentration in the hymenophore of individual species was in the following order: *S. grevillei* > *M. oreades* > *X. chrysenteron* > *B. recitulatus* > *M. procera* > *A. campestris* > *R. xerampelina* > *C. cibarius* > *L. salmonicolor* > *C. gibba*.

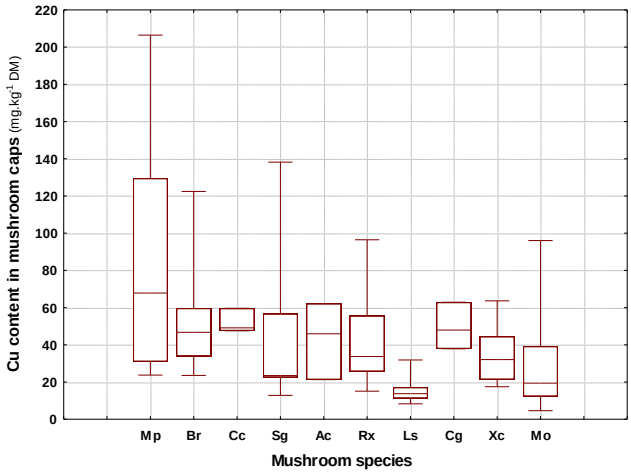

Figure 4 Range (min. – max.) and median, upper and lower quantile values of the copper concentration in the caps (mg.kg^{-1} DM) of individual species of edible wild mushr ooms. ***MP,*** *M. procera*; ***BR,*** *B. recitulatus*; ***CC,*** *C. cibarius*; ***SG,*** *S. grevillei*; ***AC,*** *A. campestris*; ***RX,*** *R. xerampelina*; ***LS,*** *L. salmonicolor*; ***CG,*** *C. gibba*; ***XC,*** *X. chrysenteron*; ***MO,*** *M. oreades*.

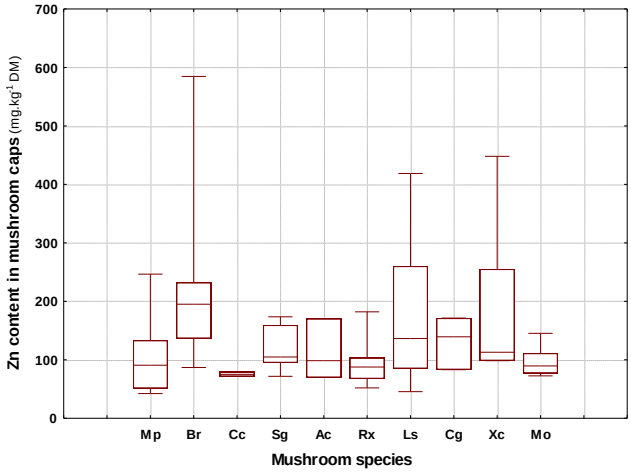

Figure 5 Range (min. – max.) and median, upper and lower quantile values of the zinc concentration in the caps (mg.kg^{-1} DM) of individual species of edible wild mushrooms. ***MP,*** *M. procera*; ***BR,*** *B. recitulatus*; ***CC,*** *C. cibarius*; ***SG,*** *S. grevillei*; ***AC,*** *A. campestris*; ***RX,*** *R. xerampelina*; ***LS,*** *L. salmonicolor*; ***CG,*** *C. gibba*; ***XC,*** *X. chrysenteron*; ***MO,*** *M. oreades*.

Although copper is considered an essential trace element for almost all organisms, its high levels may have a negative impact on physiological processes in the body **(Árvay *et al.*, 2014; Wuana and Okieimen, 2011)**. The highest copper concentration in the samples was recorded in the hymenophore samples of *A. procera* (Scop.) Singer, where the copper concentration was 81.1 ±56.4 mg.kg^{-1} DM (23.7 – 207 mg.kg^{-1} DM) (Figure 4). This species had the highest ability to bioacumulate copper among all species tested (BAF_H = 3.48 and BAF_{RFB} = 2.44). The concentration of copper in individual species was in the following order: *M. procera* > *B. recitulatus* > *C. cibarius* > *C. gibba* > *R. xerampelina* > *A. campestris* > *S. grevillei* > *X. chrysenteron* > *M. oreades* > *L. salmonicolor*.

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Zinc, like copper, is considered an essential trace element. Individual mushroom species showed significant ability to bioaccumulate zinc, with higher accumulation values on locations with lowest zinc cencentration in substrate. The highest zinc concentration was recorded in the samples of *B. recitulatus* Schaeff., with the concentration around 226 ±157 mg.kg^{-1} DM (86.9 – 585 mg.kg^{-1} DM) (Figure 5). The highest ability to bioaccumulate zinc was recorded in the samples of *X. chrysenteron* (BAF_H = 2.10 and BAF_{RFB} = 1.61). The zinc concentration in the hymenophore of individual mushroom species was in the following order: *B. recitulatus* > *L. salmonicolor* > *X. chrysenteron* > *C. gibba* > *S. grevillei* >

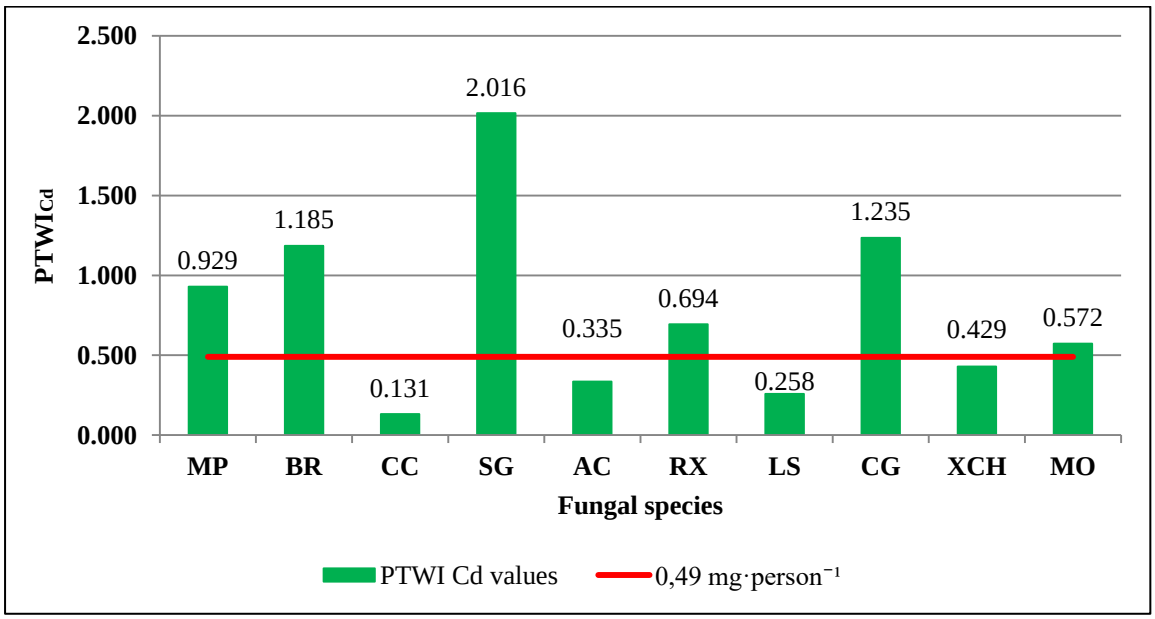

Figure 6 The comparison of weekly intake of cadmium with 300 g of various mushroom species per day to $PTWI_{Cd}$ limit for adult person (0.490 $mg.kg^{-1}$). ***MP,*** *M. procera*; ***BR,*** *B. recitulatus*; ***CC,*** *C. cibarius*; ***SG,*** *S. grevillei*; ***AC,*** *A. campestris*; ***RX,*** *R. xerampelina*; ***LS,*** *L. salmonicolor*; ***CG,*** *C. gibba*; ***XC,*** *X. chrysenteron*; ***MO,*** *M. oreades*.

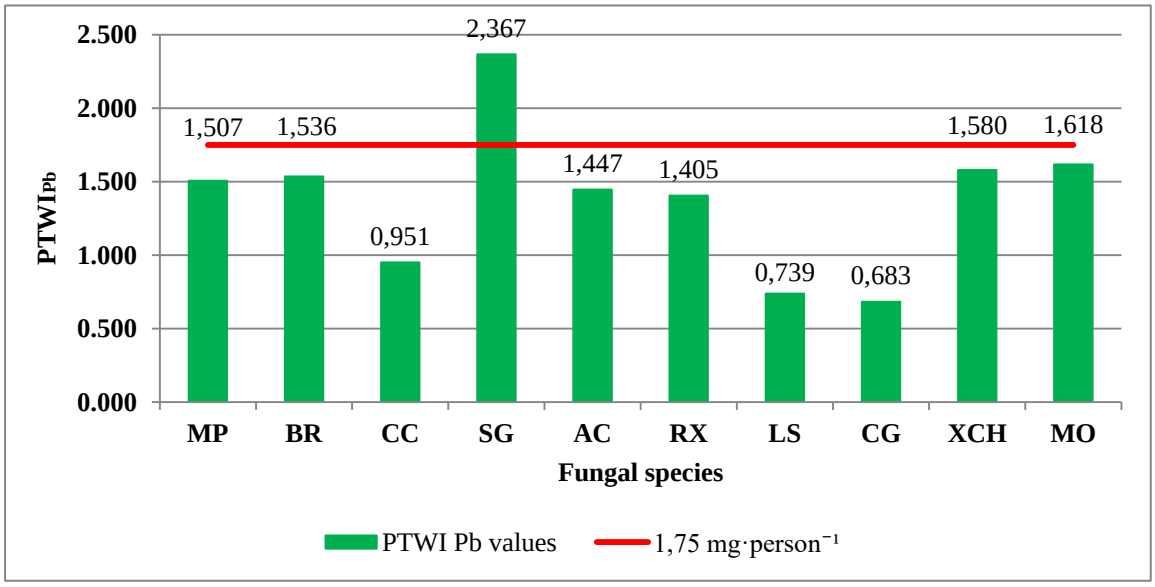

Figure 7 The comparison of weekly intake of lead with 300 g of various mushroom species per day to $PTWI_{Pb}$ limit for adult person (1.750 $mg.kg^{-1}$). ***MP,*** *M. procera*; ***BR,*** *B. recitulatus*; ***CC,*** *C. cibarius*; ***SG,*** *S. grevillei*; ***AC,*** *A. campestris*; ***RX,*** *R. xerampelina*; ***LS,*** *L. salmonicolor*; ***CG,*** *C. gibba*; ***XC,*** *X. chrysenteron*; ***MO,*** *M. oreades*.

A. campestris L. > *M. procera* > *M. oreades* > *R. xerampelina* > *C. cibarius*.

All data on the concentration of the studied contaminants in the substrate and individual anatomical parts of mushrooms are shown in Tables 1 and 2.

Health risk assessment

Provisional tolerable weekly intake (PTWI) is a value set by the FAO and WHO **(JECFA, 2010)** and defined as the maximum quantity of contaminants that may a consumer weighing 70 kg intake per one week. We assumed that the person consumes 300 g fresh mushrooms or 30 g of dried mushrooms per day. The legislation states the following PTWI indices for individual heavy metals: Cd: 0.007 $mg.kg^{-1}$ of bodyweight (0.490 mg $Cd.person^{-1}$) and Pb: 0.025 $mg.kg^{-1}$ of bodyweight (1.750 mg $Pb.person^{-1}$). For the evaluation of the PTWI values of the studied contaminants, their median concentration in the hymenophore were used. The median values were multiplied by the weight of 70 kg. The result was the maximum amount of the contaminants that a consumer can intake per week (Cd: 0.49 mg and Pb: 1.75 mg). The $PTWI_{Cd}$ values were exceeded in several samples. The highest exceedance was recorded in the samples of *S. grevillei* (4.11 fold). In the case of lead, the $PTWI_{Pb}$ values

were exceeded only in the samples of *S. grevillei* (1.35 fold). It indicates a potential risk of intoxication, since it is often collected and consumed species, characterized by significant bioaccumulation ability. The comparison of the calculated $PTWI_{Cd}$ and $PTWI_{Pb}$ values with the defined limit values are shown in Figures 6 and 7.

CONCLUSION

The aim of this study was to assess the contamination level of the substrate and the aboveground part of the edible wild mushroom species collected in the surrounding area of Banská Bystrica characterized by significant mining activity in the past. Macroscopic mushrooms represent a part of the environment that is sensitive to the increased amount of contaminants, which is reflected by their increased concentration in the aboveground parts of wild mushrooms. The results showed that the health risk resulting from the consumption of the studied mushroom species decreases as follows: *M. procera* (Cd) > *R. xerampelina* (Cd) > *S. grevillei* (Cd, Pb) > *B. recitulatus* (Cd, Pb) > *C. gibba* (Cd) > *M. oreades* (Cd, Pb) > *X. chrysenteron* (Cd, Pb) > *A. campestris* (Pb) > *L. salmonicolor* > *C. cibarius*.

REFERENCES

Alloway, B. J. 2013. Introduction. *Alloway, B. J. (Ed.), Environmental Pollution. Heavy Metals in Soils*. Springer, Netherlands, p. 3-9. http://dx.doi.org/10.1007/978-94-007-4470-7_1

Alonso, J., Garcia, M. A., Perez-Lopez, M., Melgar, M. J. 2003. The concentrations and bioconcentration factors of copper and zinc in edible mushrooms. *Archives of Environmental Contamination and Toxicology*, vol. 44, no. 2, p. 180-188. http://dx.doi.org/10.1007/s00244-002-2051-0

Árvay, J., Tomáš, J., Hauptvogl, M., Kopernická, M., Kováčik, A., Bajčan, D., Massanyi, P. 2014. Contamination of wild-grown edible mushrooms by heavy metals in a former mercury-mining area. *Journal of environmental science and health part B: pesticides, food contaminants and agricultural wastes*, vol. 49, no. 11, p. 815-827. http://dx.doi.org/10.1080/03601234.2014.938550

Árvay, J., Tomáš, J., Hauptvogl, M., Massányi, P., Harangozo, Ľ., Tóth, T., Stanovič, R., Bryndzová, Š., Bumbalová, M. 2015a. Human exposure to heavy metals and possible public health risks via consumption of wild edible mushrooms from Slovak Paradise National Park, Slovakia. *J. Environ. Sci. Health pt. B.*, vol. 50, p. 838-848. http://dx.doi.org/10.1080/03601234.2015.1058107

Árvay, J., Záhorcová, Z., Tomáš, J., Hauptvogl, M., Stanovič, R., Harangozo, Ľ. 2015b. Mercury in edible wild-grown mushrooms from historical mining area-Slovakia: bioaccumulation and risk assessment. *J. Microbiol. Biotech. Food Sci.*, vol. 4, special issue 3, p. 1-4. http://dx.doi.org/10.15414/jmbfs.2015.4.special3.1-4

Burges, A., Epelde, L., Garbisu, C. 2015. Impact of repeated single-metal and multimetal pollution events on soil quality. *Chemosphere*, vol. 120, p. 8-15. http://dx.doi.org/10.1016/j.chemosphere.2014.05.037

Cappuccio, F. P., Cooper, D., D'Elia, L., Strazzullo, P., Miller, M. A. 2011. Sleep durationpredicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *Eur. Heart J.*, vol. 32, p. 1484-1492. http://dx.doi.org/10.1093/eurheartj/ehr007

Douay, F., Pelfrêne, A., Planque, J., Fourrier, H., Richard, A., Roussel, H., Girondelot, B. 2013. Assessment of potential health risk for inhabitants living near a former lead smelter. Part 1: metal concentrations in soils, agricultural crops, and home grown vegetables. *Environ. Monit. Assess*, vol. 185, no. 5, p. 3665-3680. http://dx.doi.org/10.1007/s10661-012-2818-3

Falandysz, J., Borovička, J. 2013. Macro and trace mineral constituents and radionuclides in mushrooms: Health benefits and risks. *Applied Microbiology and Biotechnology*, vol. 97, no. 2, p. 477-501. http://dx.doi.org/10.1007/s00253-012-4552-8

Falandysz, J., Kawano, M., Swieczkowski, A., Brzostowski, A., Dadej, M. 2003. Total mercury in wild - grown higher mushrooms and underlying soil from Wdzydze Landscape Park, Northern Poland. *Food Chemistry*, vol. 81, no. 1, p. 21-26. http://dx.doi.org/10.1016/s0308-8146(02)00344-8

Falandysz, J., Kunito, T., Kubota, R., Bielawski, L., Mazur, A., Falandysz, J. J. K., Tanabe, S. 2007. Selected elements in Brown Birch Scaber Stalk Leccinum scabrum. *Journal of Environmental Science and Health, Part A*, vol. 42, no. 14, p. 2081-2088. http://dx.doi.org/10.1080/10934520701626993

Falandysz, J., Zhang, J., Wang, Y. Z., Saba, M., Krasinska, G., Wiejak, A., Li, T. 2015. Evaluation of Mercury Contamination in Fungi *Boletus* species from Latosols, Lateritic red earths, and red and yellow earths in the Circum-Pacific mercurifeous belt of Southwestern China. *PLoS One*, vol. 10, no. 11, p. 1-19. http://dx.doi.org/10.1371/journal.pone.0143608

Feng, X., Tang, S., Shang, L., Yan, H., Sommar, J., Lindqvist, O. 2003. Total gaseous mercury in the atmosphere of Guiyang, PR China. *The Science of the Total Environment*, vol. 304, no. 1-3, p. 61-72. http://dx.doi.org/10.1016/S0048-9697(02)00557-0

Grandner, M. A., Sands-Lincoln, M. R., Pak, V. M., Garland, S. N. 2013. Sleep duration, cardiovascular disease, and proinflammatory biomarkers. *Nat. Sci. Sleep*, vol. 5, p. 93-107. http://dx.doi.org/10.2147/NSS.S31063

Gursoy, N., Sarikurkcu, C., Cengiz, M., Solak, M. H. 2009. Antioxidant activities, metal contents, total phenolic and flavonoid of seven Morchella species. *Food chemistry and toxicology*, vol. 47, no. 9, p. 2381-2388. http://dx.doi.org/10.1016/j.fct.2009.06.032

Hooda, P. S. 2010. *Trace elements in soils.* 1st ed. Chichester, Hooda PS, editor. United Kingdom: John Wiley & Sons Ltd. ISBN 978-1-405-16037-7. http://dx.doi.org/10.1002/9781444319477.ch1

Cheung, P. C. K. 2013. Mini-review on edible mushrooms as source of dietary fiber: preparation and health benefits. *Food Science and Human Wellness*, vol. 2, no. 2-3, p. 162-166. http://dx.doi.org/10.1016/j.fshw.2013.08.001

Chudzyński, K., Jarzyńska, G., Stefańska, A., Falandysz, J. 2011. Mercury content and bio-concentration potential of Slippery Jack, *Suillus luteus*, mushroom. *Food Chemistry*, vol. 125, no. 3, p. 986-990. http://dx.doi.org/10.1016/j.foodchem.2010.09.102

Index Fungorum, 2015. Available at: http://www.indexfungorum.org/Names/Names.asp (accessed Jan. 2016).

Islam, M. S., Han, S., Masunaga, S. 2014. Assessment of trace metal contamination in water and sediment of some rivers in Bangladesh. *Journal of Water and Enviroment Technology*, vol. 12, no. 2, p. 109-121.

JECFA, 2010. Joint FAO/WHO Expert Committee of Food Additives. Summary and conclusions. *Proceedings of the Seventy-second meeting, Rome, 16-25 February 2010.* JECFA/72/SC, Food and Agriculture in Nitra Organization of the United Nations World Health Organization. Issued 16th March 2010.

Jiang, G. B., Shi, J. B., Feng, X. B. 2006. Mercury pollution in China. *Environmental Science and Technology*, vol. 40, p. 3672-3678. http://dx.doi.org/10.1021/es062707c

John, E., Laskow, T. C., Buchser, W. J., Bruce, R. P., Basse, P. H., Butterfield, L. H., Kalinski, P., Lotze, M. T. 2010. Zinc in innate and adaptive tumor immunity. *J. Transl. Med.*, vol. 8, p. 118. http://dx.doi.org/10.1186/1479-5876-8-118

Jomova, K., Valko, M., 2011. Advances in metal-induced oxidative stress and human disease. *Toxicology*, vol. 283, no. 2-3, p. 65-87. http://dx.doi.org/10.1016/j.tox.2011.03.001

Kalač, P. 2009. Chemical composition and nutritional value of European species of wild growing mushrooms: a review. *Food Chemistry*, vol. 113, p. 9-16. http://dx.doi.org/10.1016/j.foodchem.2008.07.077

Kalač, P. 2013. A review of chemical composition and nutritional value of wild growing and cultivated mushrooms. *Journal of science food and Agriculture in Nitra*, vol. 93, no. 2, p. 209-218. http://dx.doi.org/10.1002/jsfa.5960

Kalač, P., Svoboda, L., Havličkova, B. 2004. Contents of detrimental metals mercury, cadmium and lead in wild growing edible mushrooms: A review. *Energy Education Science and Technology*, vol. 13, p. 31-38.

Krasińska, G., Falandysz, J. 2015. Mercury in Orange Birch Bolete *Leccinum versipelle* and soil substratum: bioconcentration by mushroom and probable dietary intake by cosumers. *Environmental Science and Pollution Research (in press).* http://dx.doi.org/10.1007/s11356-015-5331-8

Licata, P., Di Bella, G., Potortì, A. G., Lo Turco, V., Salvo, A., Dugo, G., et al. 2012. Determination of trace elements in goat and ovine milk from Calabria (Italy) by ICP-AES. *Food Additives and Contaminants: Part B: Surveillance*, vol. 5, no. 4, p. 268-271. http://dx.doi.org/10.1080/19393210.2012.705335

Luo, X. S., Yu, S., Zhu, Y. G., Li, X. D. 2012. Trace metal contamination in urban soils of China. *Science of the Total Environment*, vol. 421-422, p. 17-30. http://dx.doi.org/10.1016/j.scitotenv.2011.04.020

Luo, X. S., Ip, C. C. M., Li, W., Tao, S., Li, X. D., 2014. Spatial-temporal variations, sources, and transport of airborne inhalable metals (PM10) in urban and rural areas of northern China. *Atmos. Chem. Phys. Discuss.*, vol. 14, p. 13133-13165. http://dx.doi.org/10.5194/acpd-14-13133-2014

Malavolta, M., Giacconi, R., Piacenza, F., Santarelli, L., Cipriano, C., Costarelli, L. et al. 2010. Plasma copper/zinc ratio: an inflammatory/nutritional biomarker aspredictor of all-cause mortality in elderly population. *Biogerontology*, vol. 11, p. 309-319. http://dx.doi.org/10.1007/s10522-009-9251-1

Mocchegiani, E., Costarelli, L., Giacconi, R., Piacenza, F., Basso, A., Malavolta, M. 2012. Micronutrient (Zn, Cu, Fe)-gene interactions in ageing and inflammatoryage-related diseases: implications for treatments. *Ageing Res. Rev.*, vol. 11, no. 2, p. 297-319. http://dx.doi.org/10.1016/j.arr.2012.01.004

Ostos, C., Pérez-Rodríguez, F., Arroyo, B. M., Moreno-Rojas, R. 2015. Study of mercury content in wild edible mushrooms and its contribution to the Provisional Tolerable Weekly Intake in Spain. *Journal of Food Composition and Analysis*, vol. 37, p. 136-142. http://dx.doi.org/10.1016/j.jfca.2014.04.014

Ouzouni, P. K., Petridis, D., Koller, W. D., Kyriakos, A., Riganakos, K. A. 2009. Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. *Food Chemistry*, vol. 115, no. 4, p. 1575-1580. http://dx.doi.org/10.1016/j.foodchem.2009.02.014

Petkovšek, S. A. S., Pokorny, B. 2013. Lead and cadmium in mushrooms from the vicinity of two large emission sources in Slovenia. *Science of the Total Environment*, vol. 443, p. 944-954. http://dx.doi.org/10.1016/j.scitotenv.2012.11.007

Radulescu, C., Stihi, C., Busuioc, G., Gheboianu, A. I., Popescu, I. V. 2010. Studies concerning heavy metals bioaccumulation of wild edible mushrooms from industrial area by using spectrometric techniques. *Bulletin of Environmental Contamination and Toxicology*, vol. 84, p. 641-646. http://dx.doi.org/10.1007/s00128-010-9976-1

Roman, A., Popiela, E. 2011. Studies of chosen toxic elements concentration in multiflower bee honey. *Potravinarstvo*, vol. 5, no. 2, p. 67-69. http://dx.doi.org/10.5219/134

Saba, M., Falandysz, J., Nnorom, I. C. 2015. Accumulation and distribution of mercury in fruiting bodies by fungus *Suillus luteus* foraged in Poland, Belarus and Sweden. *Environmental Science and Pollution Research*. vol. 2015, p. 1-9. http://dx.doi.org/10.1007/s11356-015-5513-4

Siciliano, S. D., James, K., Zhang, G., Schafer, A. N., Peak, J. D. 2009. Adhesion and enrichment of metals on human hands from contaminated soil at an Arctic urban brownfield. *Environ. Sci. Technol.*, vol. 43, no. 16, p. 6385-6390. http://dx.doi.org/10.1021/es901090w

Da Silva, J. B. B., Borges, D. L. G., Da Veiga, M. A. M. S., Curtius, A. J., Welz, B. 2003. Determination of cadmium in biological samples solubilized with tetramethylammonium hydroxide by electrothermal atomic absorption spectrometry, using ruthenium as permanent modifier, *Talanta*, vol. 60, no. 5, p. 977-982. http://dx.doi.org/10.1016/S0039-9140(03)00182-6

Szolnoki, Z., Farsang, A., Puskás, I. 2013. Cumulative impacts of human activities on urban garden soils: origin and accumulation of metals. *Environmental Pollution*, vol. 177, p. 106-115. http://dx.doi.org/10.1016/j.envpol.2013.02.007

Timoracká, M., Vollmannová, A., Ismael, S. D. 2011. Minerals, trace elements and flavonoids content in white and coloured kidney bean. *Potravinarstvo*, vol. 5, no. 1, p. 56-60. http://dx.doi.org/10.5219/116

Wang, X. M., Zhang, J., Wu, L. H., Zhao, Y. L., Li, T., Li, J. Q., et al. 2014. A mini-review of chemical composition and nutritional value of edible wild-grown mushroom from China. *Food Chemistry*, vol. 151, p. 279-285. http://dx.doi.org/10.1016/j.foodchem.2013.11.062

WHO. 1993. Evaluation of certain food additives and contaminants, 41st Report of the Joint FAO/WHO Expert Committee on Food Additives. Technical Reports Series No 837. World Health Organization, Geneva (Switzerland).

Wuana, R. A., Okieimen, F. E. 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remedisation. *ISRN Ecology, Article ID 402647*, p. 20. http://dx.doi.org/10.5402/2011/402647

Zhang, D., Gao, T., Ma, P., Luo, Y., Su, P. 2008. Bioaccumulation of heavy metal in wild growing mushrooms from Liagshan Yi nationality autonomous prefecture, China.

Journal of Natural Science, vol. 13, no. 3, p. 267-272. http://dx.doi.org/10.1007/s11859-008-0302-2

Zhu, F., Qu, L., Fan, W., Qiao, M., Hao, H., Wang, X. 2011. Assessment of heavy metals in some wild edible mushrooms collected from Yunnan Province, China. *Environmental Monitoring and Assessment*, vol. 179, no. 1, p. 191-199. http://dx.doi.org/10.1007/s10661-010-1728-5

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REDISTRIBUTION OF MINERAL ELEMENTS IN WHEAT GRAIN WHEN APPLYING THE COMPLEX ENZYME PREPARATIONS BASED ON PHYTASE

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ABSTRACT

Biogenic minerals play an important role in the whole human nutrition, but they are included in the grain of the phytates that reduces their bioavailability. Whole wheat bread is generally considered a healthy food, but the presence of mineral elements in it is insignificant, because of weak phytate degradation. From all sources of exogenous phytase the most productive are microscopic fungi. To accelerate the process of transition hard mineral elements are mobilized to implement integrated cellulolytic enzyme preparation based on the actions of phytase (producer is *Penicillium canescens*). Phytase activity was assessed indirectly by the rate of release of phosphate from the substrate. It has been established that the release rate of the phosphoric acid substrate is dependent on the composition of the drug and the enzyme complex is determined by the presence of xylanase. The presented experimental data shows that a cellulase treatment of the grain in conjunction with the β-glucanase or xylanase leading to an increase in phytase activity could be 1.4 – 2.3 times as compared with the individual enzymes. As a result of concerted action of enzymes complex preparation varies topography grain, increase the pore sizes in seed and fruit shells that facilitate the penetration of the enzyme phytase in the aleurone layer to the site of phytin hydrolysis and leads to an increase in phytase activity. In terms of rational parameters of enzymatic hydrolysis, the distribution of mineral elements in the anatomical parts of the grain after processing complex enzyme preparation with the help of X-ray detector EMF miniCup system in a scanning electron microscope JEOL JSM 6390 were investigated. When processing enzyme preparation wheat trend in the distribution of mineral elements, characteristic of grain - the proportion of these elements in the aleurone layer decreases, and in the endosperm increases. Because dietary fiber and phytate found together in the peripheral layers of fiber-rich grains, it is difficult to separate the effects of degradation processes nonstarch polysaccharides and fiberphytate redistribution of polyvalent metal ions. However, studies have shown that phytase - an effective mechanism for regulating mineral nutrient diet. Application of phytase in grain bakery technology will increase the biological value of the product.

Keywords: phytin; phytase; complex enzyme preparation; microstructure; mineral element; grain of wheat

INTRODUCTION

Phytic acid is found in many plant systems. In beans and cereal grain, it is approximately 5.1% by weight. This compound is of vital importance for the successful development of the seed and growth of plants. However, phytic acid is a strong chelator and in the interaction with the polyvalent cations and the formation of complexes with proteins - phytate - reduces the bioavailability of many vital mineral compounds **(Cheryan, 1980; Bergman et al., 1997)**. Studies in animal and human as subjects showed that a diet high in phytic acid leads to a deficiency of zinc, calcium, magnesium, phosphorus. This can cause immunodeficiency and lead to cognitive and growth disorders **(Erdman, 1979)**. At the same time, as anti-nutrients in the human diet, phytates can carry a positive role in the nutrition as antioxidants and anti-cancer drugs **(Graf et al., 1987; Lott et al., 2000; Urbano et al., 2000; Hyun-Joo et al., 2004)**.

Reduction of phytate content in the diet is one way to improve nutrient absorption of mineral elements. This may be achieved through use of cooking methods that lead to activation of the endogenous phytase, through the action of microorganisms, such as yeast in baking, with the proviso that the pH and other environmental conditions are favorable, or by application of exogenous phytase technology **(Lonnerdal, 2002; Lestienne et al., 2005; Eklund-Jonsson et. al., 2006)**. Wholewheat bread, usually considered more healthy food than of high-grade flour because different high content of dietary fiber, vitamins (especially B and E) and biogenic minerals. However, whole-grain bread also contains large amounts of phytate. A reduction in the level of phytate in whole meal flour, rye, oats and wheat after fermentation, a figure only slightly dependent on the temperature of the process **(García-Estepa et al., 1999; Buddrick et al., 2014)**. When making bread from whole grain wheat in establishing pH 5.0 during fermentation phytate level was reduced by 64% **(Türk et al., 1996)**. Phytate degradation to the free ends of phytic acid in the production of almost rye bread with a long fermentation time, but if the bread is made from whole grains, minor degradation of phytate **(McKenzie-Parnell and Davies, 1986; Nielsen et al., 2007)**.

From all sources of exogenous phytase, which have been studied (plants, animals, microorganisms), the most

productive are microscopic fungi **(Wodzinski and Ullah, 1996)**. All commercial phytase preparations containing enzymes of microbial origin, produced by fermentation **(Haefner et al., 2005)**. The main application of phytase are found in feeding monogastric animals **(Madrid et al., 2013)**, but it is also used for the treatment of raw materials destined for human nutrition. This enzyme has already found use in breadmaking, the production of vegetable protein isolates, the wet milling of corn, bran fractionation **(Greiner and Konietzny, 2006)**.

The use of phytase in the art from the bread wheat results in a significant increase in its specific volume and improves the texture and shape. Phytase in baking as improver has two benefits: it improves the nutritional status by reducing the phytate content and promotes activation of endogenous a- amylase, which improves the quality of the product **(Haros et al., 2001)**.

The purpose of the presented work was to study the redistribution of trace elements within the grain by the enzymes of the cellulase complex and phytase (producer *Penicillium canescens*).

MATERIAL AND METHODOLOGY

For the study, we took winter wheat varieties obtained in Moscow 139 Moscow Research Institute of Agriculture "Nemchinovka". Dry using a complex enzyme preparation comprising cellulase, β- glucanase, xylanase, phytase, as well as formulations containing the individual enzymes of the complex or combination thereof (P-215, producing *Penicillium canescens*, IBPM RAS). Enzymes had the following activity: cellulase 58711nkat/g, xylanase 12135 nkat/g, β- glucanase 51317 nkat/g, phytase 205268 nkat/g and were given laboratory physical and chemical transformation of polymers chemical faculty of Moscow State University. MV Lomonosov **(Sinitsyna et al., 2003)**.

Enzyme preparations in powdered form were mixed using a magnetic stirrer with citrate buffer (pH 4.5) for 0.5 hours at a concentration of 0.6 $g.L^{-1}$ in the solution before placing grain. This concentration corresponds to the optimum enzyme in the production of bread from whole grain **(Kuznetsova et al., 2007; 2013).** Whole grain incubated enzyme preparation in solution at the ratio of grains: 1 : 1.5 solution for 8 hours at 50 ±2 °C in an incubator. Modes hydrolysis (t = 50 °C, pH 4.5) are optimum for the operation of the enzymes studied. Duration of cereal substrate hydrolysis determined by the time during which the grain moisture is 40% or more, which is necessary to obtain the grain mass, the ability to undergo dispersion and allow the use of grain raw material for the production of grain bakery. After incubation, the inactivation of enzymes not performed.

Microstructural studies were conducted using an electron scanning microscope ZEISS EVO LS. Survey was carried out at an accelerated voltage of 15 kV.

Phytase activity was assessed indirectly by the rate of release of phosphate from the substrate spectrophotometrically. To a 1 cm^3 of fluid was poured keyhole 1 cm^3 of 10% trichloroacetic acid solution and 2 cm^3 reagent "C" (3.66 g iron sulfate (II) was dissolved in a solution of ammonium molybdate (2.5 g of ammonium molybdate was dissolved in pre- 8 cm^3 of sulfuric acid and adjusted to 250 cm^3 with distilled water). Absorbance of the test solution after 30 minutes of soaking at room temperature for CK-3 for a wavelength of 750 nm in a cuvette with a distance of 1 cm between the faces against distilled water. Calibration curve found the mass concentration of phosphorus using standard aqueous solutions of known concentration of KH_2PO_4. Phytase activity was calculated using the formula:

$$FA = ([PO_4] * 106 * Rrs * Rs) / (M * 103 * t_p), \quad (1)$$

where Rrs - dilution of the enzyme preparation in the reaction mixture;

Rs - pre-dilution of the enzyme preparation (before adding to the reaction mixture);

M - molecular weight phosphate;

t_p - the reaction time.

Determination of trace performed after dry digestion in a muffle furnace at 450 °C and dissolving the ash in the mixture of 10% hydrochloric acid and nitric acid by atomic absorption spectrophotometry, the air-acetylene flame device firm HITACHI 180-80 with deuterium background corrector. For calibration using standard solutions of elements of the company (Merck).

Analysis of the distribution of mineral elements in the anatomical parts of grains and the relative content of mineral elements in the washings were performed using X-ray detector EMF miniCup system in a scanning electron microscope JEOL JSM 6390.

RESULTS AND DISCUSSION

Cereal products provide delivery 20 – 30% minerals (Cu, Zn, Mg, Mn, etc.) in the human diet **(Gyori et al., 1996)**. Table 1 shows the results of the determination of certain mineral element nutrients in wheat.

A number of studies on the processes of distribution of manganese and iron in plant tissues. These elements exhibit a strong affinity for moving organic chelates and complexes. However, when the supply of manganese in

Table 1 Mineral content in wheat grain.

Mineral element	Content [$mg.kg^{-1}$ DM]
Zinc	22.43 ±1.23
Copper	2.13 ±0.13
Manganese	37.50 ±2.10
Iron	64.30 ±4.50
Cobalt	0.04 ±0.01

small plants, its mobility is very limited in tissues. Transfer of iron in plant tissues is difficult. Manganese is a specific component of two enzymes - arginase and phosphotransferase, moreover it increases the activity of certain oxidases. Iron - an essential metal involved in the transformation of the energy required for synthetic processes in the cells. Zinc is part of multiple enzymes - dehydrogenase, peptidases, proteinases and fosfohydrolase. Basic functions related to zinc metabolism of carbohydrates, protein and phosphate. Copper is part of

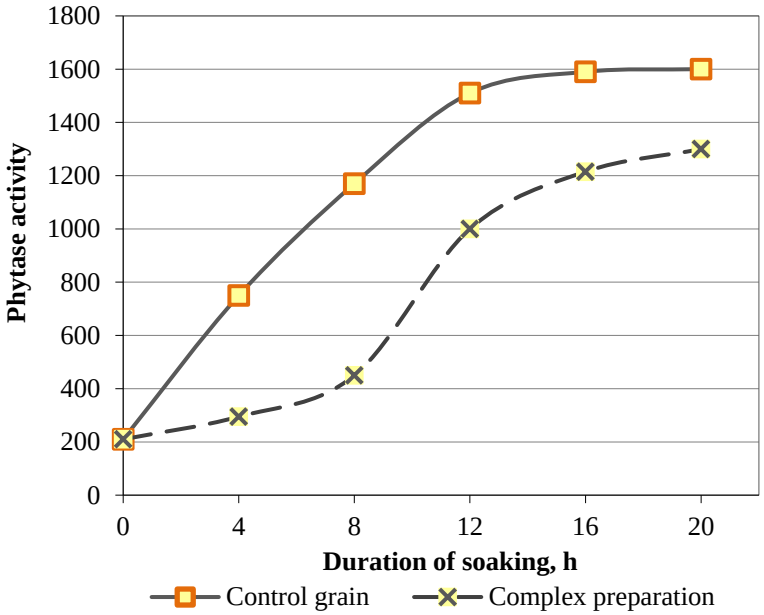

Figure 1 The change in phytase activity of the substrate in the processing of wheat complex enzyme preparation.

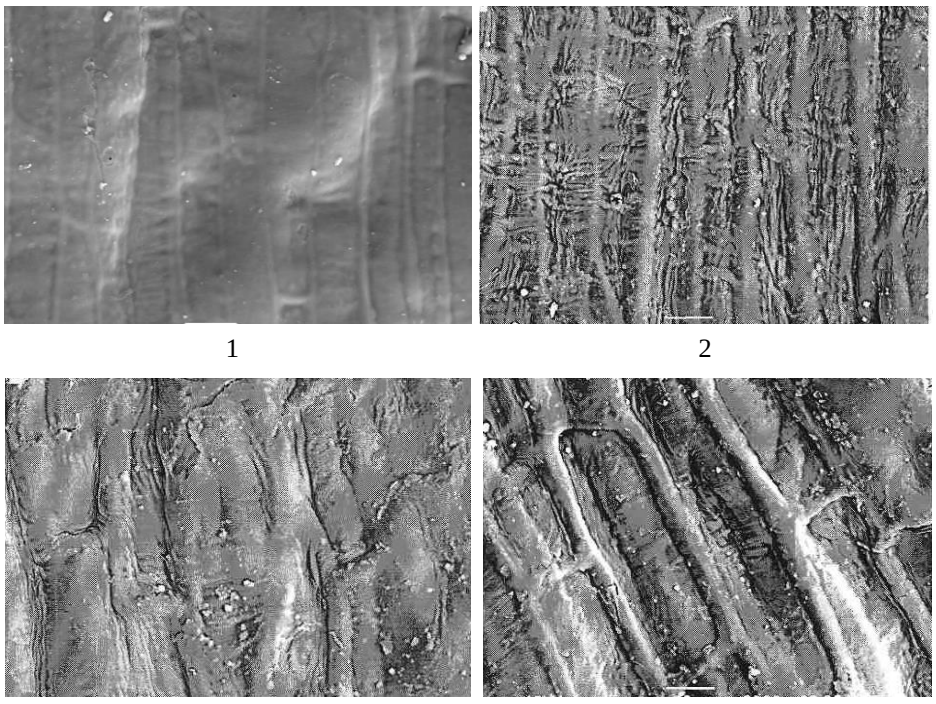

Figure 2 Photomicrographs of the surface of wheat treated with enzymes of the cellulase complex (1 – control without enzyme, 2 – β-glucanase-phytase, 3 – xylanase+phytase, 4 – complex enzyme preparation. An increase of x700. Photo: S. Motyleva, 2013).

enzymes that regulate the processes of respiration, redistribution of carbohydrates, protein metabolism **(Kabata-Pendias and Pendias, 1989)**. Therefore, these trace nutrients plays a key role in the processes of waking up and swelling of the grain as a result of the activation of metabolic processes need to move the movable element in the form increases.

It is known **(Betchel et al., 1981; Jacobsen et al., 1981)**, that it is located in special phytates aleurone grains of the aleurone layer and the embryo and associated biogenic minerals in remote systems. To accelerate the process of transition hard mineral elements are mobilized to implement integrated cellulolytic enzyme preparation based on the actions of phytase.

Treatment of wheat grains complex enzyme preparation during 20 hours was observed changes in the activity of phytase substrate. The experimental data is presented in Figure 1.

Studies have shown that in the first 8 hours of soaking the grain in buffer solution pH 4.5, the activity of phytase substrate is slowly increased as the grains swell and increased by 1.7 times compared to the control. In the period from 8 to 12 hours of hydrolysis observed maximum phytase activity values increase. In the next 8 hours of exposure to the substrate preparation phytase activity in the grain did not undergo significant changes. Phytase activity, the values presented is total value of the substrate activity of endogenous and exogenous phytase.

Table 2 shows the values of the phytase activity of the substrate after 8 hour treatment wheat individual enzymes that are part of a complex enzyme preparation, and their combination in the obligatory presence of exogenous phytase.

From the experimental data presented shows that

Table 2 Effect of enzyme complexes to change the phytase activity of the substrate.

The composition of the enzyme complex	Phytase activity [unit activity]
Cellulase + phytase	685 ±12
β- glucanase + phytase	880 ±21
Xylanase + phytase	940 ±22
Cellulase + β-glucanase + phytase	1050 ±33
Xylanase + cellulase + phytase	1180 ±27

Table 3 Distribution of mineral elements in the anatomical parts of the grain after processing complex enzyme preparation in mass%.

Chemical element	Morphological parts of the grain													
	Germ		The surface of the fruit shell		Fruit shell		Seed coat		Aleurone layer		Endosperm		Barb	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
C+N+O	93.39	98.37	96.77	97.48	99.18	98.95	98.98	99.42	90.08	98.11	99.00	95.86	93.02	94.95
Na	0.03	–	0.01	–	0.03	0.06	0.01	–	–	–	0.02	0.05	0.03	–
Mg	0.10	0.19	0.12	0.22	0.06	0.11	0.08	0.04	2.03	–	0.05	0.08	0.20	0.13
Al	0.06	0.04	0.06	–	0.03	0.05	–	0.01	–	–	0.01	0.01	0.33	-
P	0.31	0.54	0.15	0.13	0.06	0.06	0.04	0.09	3.95	0.25	0.02	0.78	0.30	0.91
S	0.16	0.24	0.18	0.21	0.07	0.10	0.13	0.12	0.13	0.14	0.03	0.93	0.06	0.51
K	0.55	0.21	0.10	0.13	0.08	0.13	0.21	0.02	2.71	0.31	0.09	0.12	0.03	0.32
Ca	0.15	0.06	0.33	0.34	0.10	0.20	0.18	0.10	0.05	0.19	0.01	0.47	0.49	0.61
Cr	0.01	–	0.12	0.14	0,01	0.03	0.01	0.06	–	–	0.05	0.07	0.84	–
Mn	0.92	0.02	–	0.13	–	–	0.01	0.02	0.02	0.23	–	0.24	–	0.32
Fe	0.89	0.03	0.04	0.03	0.02	0.06	0.01	0.02	0.11	0.11	–	0.38	–	0.01
Co	0.02	–	0.08	–	–	0.03	0.01	–	0.03	0.04	0.03	0.13	1.19	0.26
Ni	0.02	0.05	0.03	0.24	0.01	0.01	0.06	–	0.05	–	0.07	0.09	0.14	–
Cu	1.71	–	0.12	0.12	0.10	0.06	0.15	0.03	0.07	0.16	0.10	0.19	2.36	0.45
Zn	1.41	0.06	0.05	0.20	0.03	0.01	0.03	–	0.01	0.24	–	0.46	–	0.28
Se	0.17	0.19	0.20	0.46	0.05	0.05	0.05	–	0.11	0.17	0.06	0.09	0.88	0.46

1 - The distribution of chemical elements in the morphological parts of wheat (control variant), mass%;
2 - Distribution of chemical elements in morphological parts of wheat treated complex enzyme preparation, mass%

a cellulase treatment of the grain in conjunction with the β-glucanase or xylanase leading to an increase in phytase activity could be 1.4 – 2.3 times as compared with the individual enzymes.

This indicates a synergistic effect on the action of enzyme complex which is caused by the action of enzymes on successive substrates entering into the matrix of the cell walls, which is a complex composition of the substrate.

Figure 2 shows photomicrographs of the surface of the wheat treated with the enzyme solution of the test drug under the optimum conditions of hydrolysis.

Pictures were made with a scanning electron microscope at 700x magnification.

Under the action of biocatalysts based cellulases has changed the surface topography of grain. In control variant the relief of the grain surface is parallel strands of cellulose fibrils, hemicellulose polysaccharides layer overlain nature (1). Under the action of the enzyme β-glucanase grain surface topography changes. Denudation observed parallel strands of cellulose microfibrils of varying thickness and tortuosity. Probably destroy exposed top layer of hemicellulose (2).

Xylanase enzyme action causes destruction layer hemicelluloses tissue depth direction. Modification of the surface structures occurs both in longitudinal and radial direction (3). Under the action of the complex enzymes cellulase, β-glucanase and xylanase (4) masonry surface relief grains are formed deep enough, they are represented by parallel strands almost devoid of cuticular crosslinks. As a result of concerted action of enzymes complex preparation varies topography grain, increase the pore sizes in seed and fruit shells that facilitates the penetration of the enzyme phytase in the aleurone layer to the site of phytin hydrolysis and leads to an increase in phytase activity. The article **Haraldsson et al. (2004)** also points to the possibility of combining the degradation of phytate degradation β-glucan under the joint action of phytase and β-glucanase during malting, which is of interest for the production of cereal products with high nutritional value. However, according to our studies listed, phytate degradation to a greater extent due to the presence in the complex enzyme preparation comprising xylanase and phytin hydrolysis, intensity is a maximum when the cereal substrate operates complex enzyme preparation comprising cellulase, β-glucanase, xylanase.

To analyze the distribution of trace elements P, K, Mg, Ca, Fe, Mn in the outer layers of the wheat grain has been used and the X-ray structure analysis. It has been found that the studied elements are concentrated in the aleurone layer. In particular, P, Mg, K were concentrated in the aleurone layer of subcellular particles and outer layers of the wheat grain; Ca was found in abundance in the tissues of the pericarp **(Tanaka et al., 1974)**.

In terms of rational parameters of enzymatic hydrolysis, investigated the distribution of mineral elements in the anatomical parts of the grain after processing complex enzyme preparation with the help of X-ray detector EMF miniCup system in a scanning electron microscope JEOL JSM 6390.

Gained data relative content of mineral nutrient elements are presented in Table 3.

The studies showed that after soaking in solution of the enzyme preparation on the basis of phytase migrates within the mineral grains.

Reduces the number of elements studied in the aleurone layer and significantly increased in the endosperm.

Under the influence of enzyme preparations polysaccharides constituting the matrix of the cell walls are modified, the system is broken native intermolecular bonds between the main structural components of the polysaccharide complex, the process of maceration and partial structures shells fragmentation polymers themselves. This ensures destruction of intercellular substance, leading to the separation of cells, solubilization hydrolysis products. Electrostatic forces arising due to the functional group having affinity for the metal ions at the micelle surface terminate. The process is accompanied by desorption of ions, molecules associated with non-starch polysaccharides. Experimental studies of morphological parts of wheat showed that under the action of biocatalysts based cellulases distribution of chemical elements in the caryopsis changed. There is a tendency in the distribution of mineral elements, characteristic of grain - the proportion of these elements in the aleurone layer (5) is reduced, and in the endosperm (6) - increases. The relative content of nutrients that are part of metalloenzymes and biologically active compounds increased in the endosperm, where during swelling grain intensified oxidative decomposition processes of high- replacement compounds. The chemical elements which have a high mobility, potassium and sodium are moved from the central portion to the peripheral weevil. Because dietary fiber and phytate found together in the peripheral layers of fiber-rich grains, it is difficult to separate the effects of degradation processes nonstarch polysaccharides and fiber phytate redistribution of polyvalent metal ions **(Torre M. et al., 1991)**.

Distribution of chemical elements in the morphological parts of the grain shows that activation occurred own enzyme systems. This is evidenced by the increase in the relative content of sulfur, which is part of the proteins, enzymes and free amino acids, as well as phosphorus, participating in all the processes of metabolism. Increase in the relative amount of phosphorus, sulfur, potassium, magnesium, selenium in the bud indicates the activation of the synthesis of organic compounds necessary for the construction of the developing plant tissues. Reduction in the relative content of trace elements in the aleurone layer speaks about embryonic germ awakening, intensifying the process of synthesis and migration of enzymes in the endosperm. The preferential increase in the endosperm fraction trace indicates that after 8 hours of soaking wheat germination basic feature consists in the biochemical processes in direction towards hydrolysis. The appearance of selenium, known for their antioxidant properties, morphological parts of grains indicates the incorporation of plant protection from the negative effects of oxidation products - free radicals, peroxides and hydroperoxides.

These data confirm that in the hydrolysis of phytin complexes decompose exogenous phytase, phytin formed with mineral elements: calcium, magnesium, iron, copper and zinc. These chemicals migrate into the endosperm where the basic seed and nutrients included in the modification process and replacement of biological

polymers. These experimental data are in agreement with the findings of previous studies that during imbibition of wheat mineral elements (magnesium, calcium and potassium) redistributed from the aleurone layer and mobilized for the development of seedlings **(Eastwood and Laidman, 1971)**.

CONCLUSION

As a result of a complex enzyme preparation based on phytase (producer *Penicillium canescens*) for the treatment of wheat has been a change of the surface microstructure of grain. Microstructural changes and phytase activity indicator substrate, characterizing the rate of release of phosphate caused enzyme complex composition of the drug and determined the presence of xylanase enzyme. Availability phytin phytase is associated with the degree of degradation of the hemicellulose. It has been established that the release rate of the phosphoric acid substrate is dependent on the composition of the drug and the enzyme complex is determined by the presence of xylanase. When processing enzyme preparation wheat trend in the distribution of mineral elements, characteristic of grain - the proportion of these elements in the aleurone layer decreases, and in the endosperm - increases. These data confirm the results of microstructural studies of chemical analysis.

Thus, studies have shown that phytase - an effective mechanism for regulating mineral nutrient diet. Application of phytase in grain bakery technology will increase the biological value of the product.

REFERENCES

Bergman, C. J., Gualberto, D. G., Weber, C. W. 1997. Mineral binding capacity of dephytinized insoluble fiber from extruded wheat, oat and rice brans. *Plant Foods for Human Nutrition*. vol. 51, no. 4, p 295-310. http://dx.doi.org/10.1023/A:1007972205452 PMid:9650723

Buddrick, O., Jones, O. A. H., Cornell, H. J., Small, D. M. 2014. The influence of fermentation processes and cereal grains in wholegrain bread on reducing phytate content. *Journal of Cereal Science*. vol. 59, no. 1, p. 3-8. http://dx.doi.org/10.1016/j.jcs.2013.11.006

Eastwood, D., Laidman, D. L. 1971. The mobilization of macronutrient elements in the germinating wheat grain. *Phytochemistry*. vol. 10, no. 6, p. 1275-1284. http://dx.doi.org/10.1016/S0031-9422(00)84328-9

Eklund-Jonsson, Ch., Sandberg, A.-S., Alminger, M. L. 2006. Reduction of phytate content while preserving minerals during whole grain cereal tempe fermentation. *Journal of Cereal Science*. vol. 44, no. 2, p. 154-160. http://dx.doi.org/10.1016/j.jcs.2006.05.005

Erdman, J. W. 1979. Oilseed phytates: Nutritional implications. *Journal of the American Oil Chemists Society*. vol. 56, no. 8, p. 736-741. http://dx.doi.org/10.1007/BF02663052

García-Estepa, R. M., Guerra-Hernández, E., García-Villanova, B. 1999. Phytic acid content in milled cereal products and breads. *Food Research International*. vol. 32, no. 3, p. 217-221. http://dx.doi.org/10.1016/S0963-9969(99)00092-7

Graf, E., Empson, K. L., Eaton, J. W. 1987. Phytic acid. A natural antioxidant. *Journal of Biological Chemistry*. vol. 262, no. 24, p. 11647-11650. PMid:3040709

Greiner, R., Konietzny, U. 2006. Phytase for food application (Review). *Food Technology and Biotechnology*. vol. 44, no. 2, p. 125-140.

Haefner, S., Knietch, A., Scholten, E., Braun, J., Lohscheidt, M., Zelder, O. 2005. Biotechnological production and applications of phytases. *Short Survey Applied Microbiology and Biotechnology*. vol. 68, no. 5, p. 588-597. http://dx.doi.org/10.1007/s00253-005-0005-y PMid:16041577

Haraldsson, A.-K., Rimsten, L., Alminger, M. L., Andersson, R., Andlid, T., Åman, P., Sandberg, A.-S. 2004. Phytate content is reduced and β-glucanase activity suppressed in malted barley steeped with lactic acid at high temperature. *Journal of the Science of Food and Agriculture*. vol. 84, no. 7, p. 653-662. http://dx.doi.org/10.1002/jsfa.1724

Haros, M., Rosell, C. M., Benedito, C. 2001. Fungal phytase as a potential breadmaking additive. *European Food Research and Technology*. vol. 213, no. 4-5, p. 317-322. http://dx.doi.org/10.1007/s002170100396

Huan-Joo, H.-J., Kim, J.-H., Kim, M.-J. 2004. Comprasion of irradiated phytic acid and other antioxidations for antioxidant activity. *Food Chemistry*. vol. 88, no. 2, p. 173-178. http://dx.doi.org/10.1016/j.foodchem.2004.02.001

Cheryan, M. 1980. Phytic acid interactions in food systems. (Review). *Critical reviews in food science and nutrition*. vol. 13, no. 4, p. 297-335. http://dx.doi.org/10.1080/10408398009527293 PMid:7002470

Kabata-Pendias A., Pendias X. 1989. *Agricultural trace elements in soils and plants*. Moskow: Mir, 439 p.

Kuznetsova, E. A., Karachkina, S. Ya., Prigarina, O. M, Sinitsyn, A. P. 2006. The influence of the enzyme preparation on the basis of phytase in the complex with amber acid on the quality of the grain bread. *Bread Products*. (Moskow), no. 9, p. 62-64.

Kuznetsova, E. A., Cherepnina, L. V., Klepov, R. E., Sinitsyn, A. P., Sinitsyna, O. A. 2012. Preparation influence on a basis phytase on change carbohydrate amylase and lipid complexes of grain raw materials. *Bread Products* (Moskow), no. 10, p. 50-52.

Lestienne, I., Isard-Verniere, Ch., Mounquet, C., Picq, C., Treche, S. 2005. Effects of soaking whole cereal and lequme seeds in iron, zinc and phytate contents. *Food Chemistry*. vol. 89, no. 3. p. 421-425. http://dx.doi.org/10.1016/j.foodchem.2004.03.040

Lonnerdal, B. 2002. Phytic acid-trace element (Zn, Cu, Mn) interactions. *Journal of Food Science and Technology*. vol. 37, no. 7, p. 749-758. http://dx.doi.org/10.1046/j.1365-2621.2002.00640.x

Lott, J. N. A., Ockenden, I., Raboy, V., Batten, G. D. 2000. Phytic acid and phosphorus in crop seeds and fruits: A global estimate (Review). *Seed Science Research*. vol. 10, no. 1, p. 11-33.

Madrid, J., Martínez, S., López, C., Hernández, F. 2013. Effect of phytase on nutrient digestibility, mineral utilization and performance in growing pigs. *Livestock Science*. vol. 154, no. 1-3, p. 144-151. http://dx.doi.org/10.1016/j.livsci.2013.03.003

McKenzie-Parnell, J. M., Davies N. T. 1986. Destruction of phytic acid during home breadmaking. *Food Chemistry*. vol. 22, no. 3, p. 181-192. http://dx.doi.org/10.1016/0308-8146(86)90077-4

Nielsen, M. M., Damstrup, M. L., Dal Thomsen, A., Rasmussen, S. K., Hansen, Å. 2007. Phytase activity and degradation of phytic acid during rye bread making.

European Food Research and Technology. vol. 225, no. 2, p. 173-181. http://dx.doi.org/10.1007/s00217-006-0397-7

Sinitsyna, O. A., Bukhtoyarov, F. E., Gusakov, A. V., Sinitcyn, A. P., Okunev, O. N., Bekkarevitch, A. O., Vinetsky, Yu. P. 2003. Isolation and properties of major components of Penicillium canescens extracellular enzyme complex. *Biochemistry* (Moscow), vol. 68, no. 11, p. 1200-1209. http://dx.doi.org/10.1023/B:BIRY.0000009134.48246.7e

Tanaka, K., Yoshida, T., Kasai, Z. 1974. Distribution of mineral elements in the outer layer of rice and wheat grains, using electron microprobe X-Ray analysis. *Soil Science and Plant Nutrition*. vol. 20, no. 1, p. 87-91. http://dx.doi.org/10.1080/00380768.1974.10433231

Torre, M., Rodriguez, A. R., Saura-Calixto, F. 1991. Effects of dietary fiber and phytic acid on mineral availability. (Review). *Critical reviews in food science and nutrition*. vol. 30, no 1, p. 1-22. http://dx.doi.org/10.1080/10408399109527539 PMid:1657026

Türk, M., Carlsson, N.-G., Sandberg, A.-S. 1996. Reduction in the levels of phytate during wholemeal bread making; effect of yeast and wheat phytases. *Journal of Cereal Science*. vol. 23, no. 3, p. 257-264. http://dx.doi.org/10.1006/jcrs.1996.0026

Urbano, G., Lopez-Jurado, M., Aranda, P., Vidal-Valerde, C., Tenorio, E., Porres, J. 2000. The role of phytic acid in legumes: Antinutrient or beneficial function? *Journal of Physiology and Biochemistry*. vol. 56, no 3, p. 283-294. http://dx.doi.org/10.1007/BF03179796 PMid:11198165

Wodzinski, R. J., Ullah, A. H. J. 1996. Phytase (Review). *Advances in Applied Microbiology*. vol. 42, p. 263-302. http://dx.doi.org/10.1016/S0065-2164(08)70375-7

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MOLECULAR CHARACTERIZATION OF RYE CULTIVARS

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ABSTRACT

The results of molecular analysis of 45 rye taxa (*Secale cereale* L.) represented by agricultural varieties originated from Central Europe and the Union of Soviet Socialist Republics (SUN) are presented. The genetic diversity of rye cultivars by 6 SSR markers was evaluated. Six specific microsatellite primer pairs produced 58 polymorphic alleles with an average of 9.7 alleles per locus. The number of alleles ranged from 6 (*SCM2*) to 14 (*SCM86*). Genetic polymorphism was characterized based on diversity index (DI), probability of identity (PI) and polymorphic information content (PIC). The diversity index (DI) of SSR markers ranged from 0.5478 (*SCM2*) to 0.887 (*SCM86*) with an average of 0.778. The lowest value of polymorphic information content was recorded for *SCM2* (0.484) and the highest value for *SCM86* (0.885) of PIC was detected in *SCM86* with an average of 0.760.The dendrogram of genetic similarity was constructed, based on UPGMA algorithm. The hierarchical cluster analysis divided rye genotypes into 4 main clusters. The first cluster of 14 genotypes was subdivided in two subclusters (1a and 1b) where 50% of genotypes were Czechoslovak origin. The second cluster contained four genotypes were three (75%) of them had Czech or Czechoslovak origin. In the third subcluster separated three rye genotypes of different origin. The rest (24) of rye genotypes in the fourth cluster were divided into two subclusters (4a and 4b) where clearly separated group of Polish (4aa) and Czech and Czechoslovak (4ab) genotypes. Two genotypes of 4aa subcluster (Wojcieszyckie and Dankowskie Nowe) from Poland were genetically the closest. In the dendrogram alle genotypes were differentiated and clustering partially reflects geographic origin of studied rye genotypes. In this experiment, SSRs markers proved to be a high informative and usefull tool in genetic diversity research for the distinguishing and characterization of close related varieties.

Keywords: *Secale cereale* L.; polymorphism; microsatellite; PCR; dendrogram

INTRODUCTION

Common rye (*Secale cereale* L.) is one of the most important cereal crops cultivated in Eastern and Northern Europe **(Targońska et al., 2015)**. Rye (*Secale cereale* L.) is a diploid (2n = 2x = 14) annual, cross-pollinated cereal with an effective gametophytic self-incompatibility system. On a global scale rye (*Secale cereale* L.) is a minor crop, its production being about 5% that of wheat or rice. However, in northern European countries with extreme climatic and poor soil conditions, rye may occupy up to 30% of the acreage **(Altpeter and Konzun, 2007)**. The main advantages of rye over other winter cereals are its excellent tolerance to low temperatures and the ability to realize relatively high grain yields under environmental conditions in which other crops perform poorly. Rye is also known to have the lowest requirements for chemical treatments like fertilizers or pesticides, which makes it an ecologically and economically sound crop for specific regions **(Korzun et al., 2001)**. Moreover, rye offers high contents of many nutritionally favorable compounds such as a whole suite of minerals (Zn, Fe, P), beta-glucans, resistant starch, and bioactive compounds. Rye products are characterized by a high level of dietary fiber **(Andersson et al., 2009)** that may contribute to positive health effects **(Rosén et al., 2011)**.

Molecular markers can provide an effective tool for efficcient selection of desired agronomic traits because they are based on the plant genotypes and thus, are independent of environmental variation. Nowadays, several molecular markers are developed, of which simple sequence repeats (SSRs) or microsatellites are the most widely used types **(Jenabi et al., 2011; Maršálková et al., 2014)**.

Simple sequence repeat (SSR) markers show a relatively good transferability between closely related species **(Botes and Bitalo, 2013)** and they are one of the most promising molecular marker types to identify or differentiate genotypes within a species **(Salem et al., 2008)**. They were successfully used in many plant species, e.g. triticale **(Kuleung et al., 2004; Odrouškova and Vyhnanek, 2013)**, **wheat (Röder et al., 1995; Huang et al., 2002)**, rye **(Khlestkina et al., 2004)**, rice **(Jiang et al, 2010)**, maize **(Ignjatovic-Micic et al., 2015)**, and amaranth **(Žiarovská et al., 2013)**.

Rye SSR markers were first developed over 10 years ago **(Saal and Wricke, 1999; Hackauf and Wehling, 2002,)** and have also been used in studies on genetic diversity **(Shang et al. 2006; Bolibok et al., 2005)**.

The aim of our study was to detect genetic variability among the set of 45 rye genotypes using 6 microsatellite markers.

MATERIAL AND METHODS

Forty five rye (*Secale cereale* L.) genotypes were used in the present study. Seeds of rye were obtained from the Gene Bank of the Slovak Republic of the Plant Production

Research Center in Piešťany and Gene Bank of the Czech Republic of the Crop Research Institute in Prague. Fifteen genotypes of rye came from Czechoslovakia (CSK), another set of fifteen genotypes from Poland (PL), five from Czech Republik (CZ), another five from Hungary (HU) and last five genotypes from Union of Soviet Socialist Republics (SUN). All genotypes are of winter form.

Genomic DNA of rye cultivars was isolated from 100 mg freshly-collected leaf tissue according to GeneJET™ protocol (Fermentas, USA). The concentration and quality of DNA was checked up on 1.0% agarose gel coloured by ethidium bromide and detecting by comparing to λ-DNA with known concentration.

For analysis, six microsatellite primer pairs were chosen according to the literature **(Saal - Wricke, 1999)**. Used primers were localised on 6R, and 7R chromosomes (Table 1). PCR amplification was performed in 20 μL volume containing PCR water, 5 x Green GoTaq® Flexi Buffer, 100 μM dNTP Mix, 0.3 μM primers (Forward and Reverse primer), 1.5 mM $MgCl_2$, 0.4 U *GoTaq® polymerase* (Promega, USA). PCR reactions were performed in a thermocykler (Bio-Rad, USA). The PCR program consisted of these steps: an initial denaturation (1 cycle): 2 min. at 93 °C, (29 cycles) denaturation: 1 min. 93 °C, annealing 2 min. with different temperature for each primer pair and extension 2 min. at 72 °C.

The PCR amplicons (5μL) were resolved by electrophoresis on 6.0% denaturating polyacrylamide gel stained with silver according to **Bassam et al., (1991)**. Final PCR amplicons were scanned in UVP PhotoDoc-t® camera system. The size of alleles was determined by comparing with 10 bp standard lenght marker (Invitrogen: 100 – 330 bp). Each band was treated as a single allele.

Each reproducible band was visually scored for the presence (1) or absence (0) for all genotypes. For determination of the genetic relationships between rye genotypes a dendrogram was used. The dendrogram was constructed based on principle of hierarchical cluster analysis using UPGMA (Unweighted Pair Group Method using arithmetic Averages) algorithm on the basis of Jaccard's coefficient in statistical program SPSS version 17.

Frequencies of incidence of all polymorphic alleles were calculated and used for determination of statistical parameters: diversity index (DI) **(Weir, 1990)**, probability of identity (PI) **(Paetkau et al., 1995)** and polymorphic information content (PIC) **(Weber, 1990)**.

Diversity index (DI):

$$DI = 1 - \sum p_i^2$$

Probability of identity (PI):

$$PI = \sum p_i^4 + \sum_{i=1}^{i=n-1} \sum_{j=i+1}^{n} \left(2 p_i p_j\right)^2$$

Polymorphic information content (PIC):

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 \cdot p_j^2$$

RESULTS AND DISCUSSION

The development of molecular markers has opened up numerous possibilities for their application in plant breeding. Suitable markers for detecting polymorphisms at individual and population levels are SSRs **(Shang et al., 2006; Bolibok et al., 2005, Akhavan et al., 2009)**.

Six rye specific microsatellite primer pairs produced 58 polymorphic alleles with an average of 9.7 alleles per locus. The most polymorphic locus was *SCM86* where 14 polymorphic amplification products were detected. On the other hand the lowest polymorphic locus was *SCM2* with 6 polymorphic alleles.

Jenabi et al., (2011) used fifteen wheat and rye derived microsatellite markers to evaluate genetic variation of the mountain rye *Secale strictum* in Iran and to examine the patterns of diversity related to the varieties and geography. They detected high levels of diversity, with an average number of 6.1 alleles per locus (ranging up to 11) and high level polymorphism with polymorphism rate averaging 0.624 (between populations) and 0.357 (within populations) were observed among 125 individuals from 19 populations collected from various regions of Iran. **Gailîte et al., (2013)** analyzed genetic polymorphism of a set of 9 genotypes originated from Latvia using 12 SSR markers. The number of alleles ranged from 1 to 6 with an average number of alleles per locus 3.4. The results from their study indicate that while the Latvian rye collection is small, the genetic and phenotypic diversity contained within and between the accessions is quite high. **Targońska et al., (2015)** studied genetic diversity among 367 Polish rye accessions using 22 previously published simple sequence repeat (SSR) markers.

Table 1 List and characterization of locus specific microsatellite primers used for SSR analysis.

SSR marker	Forward primer (5′ – 3′)	Reverse primer (5′ – 3′)	Chromosomal location	Anealing temperature
SCM 2	GATGACTATGACTACCAGGATGAA	GGAGTGAGAAGGCCGAGAAG	6R	55 °C
SCM 28	CTGGTCCTGGTCTGGTGGGTC	CGCATCGGGTGTGTCGCATAC	6R	60 °C
SCM 40	CGCATCGGGTGTGTCGCATAC	CACATCTTGGGCCTGACACC	7R	60 °C
SCM 86	CAGATAGATGGGTGTTGTGCG	CTCTTCTCGACATCCACACTCC	7R	60 °C
SCM 101	GCCAGCCGCCACCTTAATTG	AGCCCAACTCTTTCGTGCATG	6R	60 °C
SCM 180	GTTTCGTCCCCGTTGCCATC	ACGTGTCGCTTTCCATTGCCC	6R	60 °C

Table 2 Characteristics of used SSR markers in this study.

SSR marker	Number of alleles	DI	PIC	PI
SCM 2	6	0.547	0.484	0.144
SCM 28	11	0.771	0.764	0.027
SCM 40	13	0.867	0.865	0.004
SCM 86	14	0.887	0.885	0.002
SCM 101	9	0.857	0.851	0.004
SCM 180	5	0.740	0.708	0.022
average	**9.7**	**0.778**	**0.760**	**0.034**

Note: DI - diversity index; PI - probability of identity; PIC - polymorphic information content.

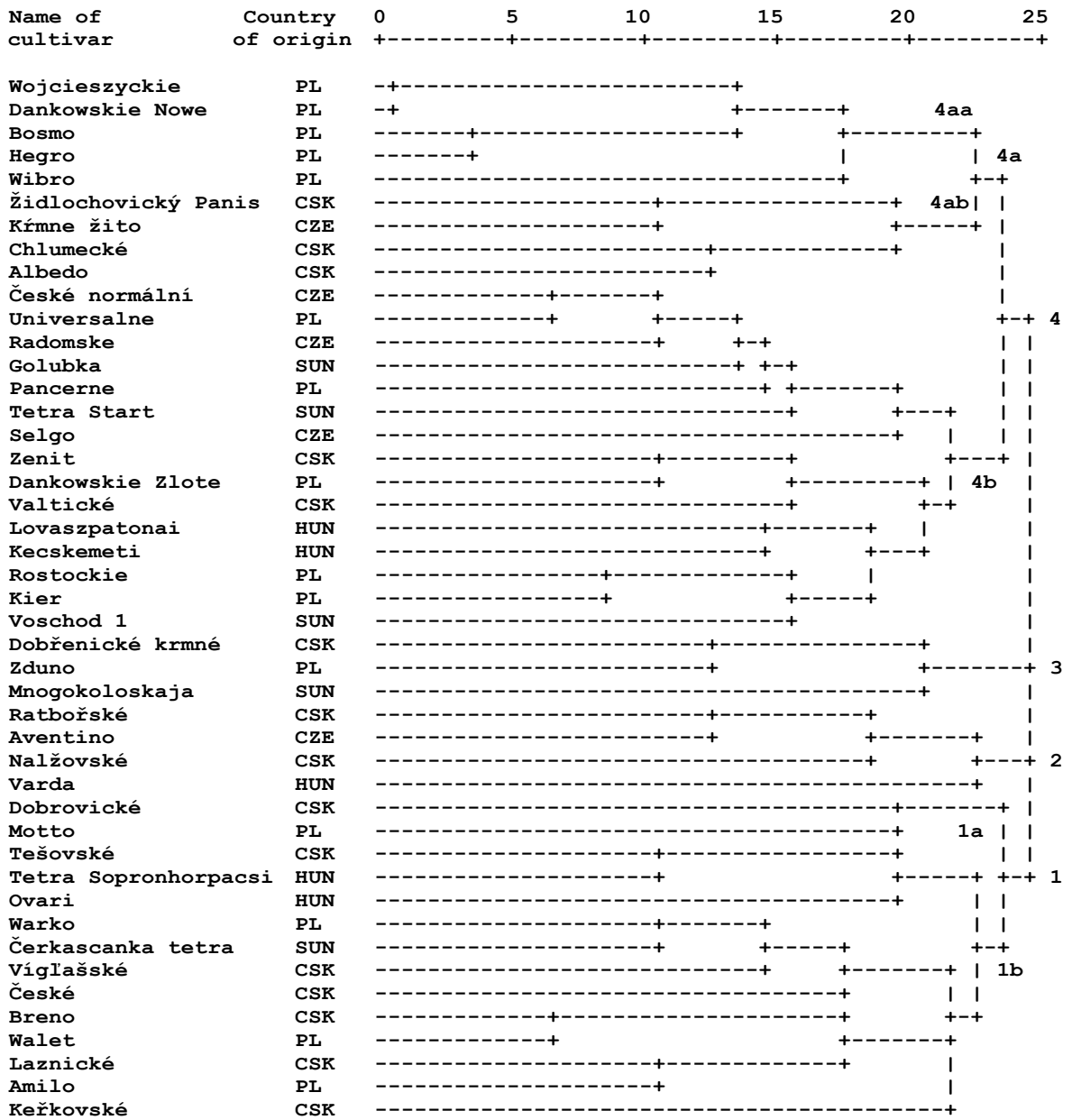

Figure 1 Dendrogram of 45 rye genotypes prepared based on 6 SSR markers.
CSK - Czechoslovakia, CZ - Czech Republic, HU - Hungary, PL - Poland, SUN - Union of Soviet Socialist Republics.

Resulting from the number and frequency of alleles, diversity index (DI), polymorphic information content (PIC) and probabilities of identity (PI) were calculated (Tab. 2). The diversity index (DI) of SSR markers ranged from 0.547 (*SCM2*) to 0.887 (*SCM86*) with an average of 0.778. The lowest value of polymorphic information content was recorded for *SCM2* (0.484) and the highest value for *SCM86* (0.885) of PIC was detected in *SCM86* with an average of 0.760. Only one marker (*SCM2*) reached considerably unfavourable results of DI, PIC, PI and number of alleles compared to average values of tested set. Probability of identity was low ranged from 0.002 (*SCM86*) to 0.144 *(SCM2*) with an average of 0.034 that indicates the possibility to differentiate genetically close genotypes.

Jenabi et al., (2011) found out lower polymorphism in their study. They calculated the within populations PIC value for all microsatellites which ranged from 0.246 to 0.451 with an average of 0.357. **Targońska et al., (2015)** detected the average PIC value for all markers used 0.57. The highest PIC value (0.93) was obtained for SCM152, and the lowest PIC (0.18) was determined for SCM050.

The dendrogram of genetic relationships among 45 rye cultivars based on SSR markers is presented in Figure 1. The hierarchical cluster analysis showed that the rye genotypes were divided into 4 main clusters. The first cluster was divided in two subclusters (1a and 1b). Subcluster 1a contains two genotypes of Czechoslovak and Polish origin. In the subgroup 1b were grouped 12 genotypes which were bred in Czechoslovakia (50%), Poland (25%), Hungary (16.7%) and one coming from Union of Soviet Socialist Republics. The second cluster contained four genotypes were three (75%) of them had Czech or Czechoslovak origin. In the third subcluster separated three rye genotypes of different origin. The rest of rye genotypes in the fourth cluster were divided into two subclusters (4a and 4b). Subcluster 4a was further subdivided into two subclusters, subcluster 4aa with 5 genotypes all coming from Poland and subcluster 4ab with four genotypes of Czech or Czechoslovak origin. Subcluster 4b of 15 genotypes included genotypes of Polish origin (33.3), SUN origin (20%), Czech origin (20%), Czechoslovak origin (13.3) and Hungarian origin (13.3). Two genotypes of 4aa subcluster (Wojcieszyckie and Dankowskie Nowe) from Poland were genetically the closest. We can assume that they have close genetic background (Figure 1).

Targońska et al., (2015) showed that the clustering of rye accessions studied was more weakly correlated with geographic origin than with the source of seeds. **Akhavan et al., (2010)** in the prepared SSR based dendrogram using UPGMA algorithm showed evident broad groupings related to the subspecies. The populations of subsp. *cereale* were mainly grouped but populations belonging to the subsp. *ancestrale* were divided in to two subgroups (groups I and III), indicating higher diversity of the latter subspecies.

CONCLUSION

The objective of this study was to determine the genetic variation among 45 rye varieties using SSR markers. Values of diversity index and polymorphic information content were higher than 0.7 in 83% of SSR markers that means high lever of polymorphism of used markers. WE can recommend them for further analyses. The dendrogram was prepared based on UPGMA algorithm using the Jaccard´s coefficient and divided in to four main clusters. All studied genotypes separated into four clusters. Clustering partially reflected geographic origin of studied rye genotypes. SSR are commonly and extensively used tools for assessment of variability in crops. These marker systems are efficient due to their locus specificity, reproducibility and reliability, for analysis of molecular differentiation and for resolving taxonomic problems in plants. Our result showed appreciably high genetic diversity among the rye genotypes studied. This survey showed the high genetic diversity within the European rye genepool as an important source for crop breeders, and indicated that there is value in sampling for useful genes for crops improvement.

REFERENCES

Akhavan, A., Saeidi, H., Rahiminejad, M. R. 2009. Genetic diversity of Secale cereale L. in Iran as measured using microsatellites. *Genetic Resources and Crop Evolution*, vol. 57, no. 3, p. 415-422. http://dx.doi.org/10.1007/s10722-009-9480-9

Altpeter, F., Konzun, V. 2007. Rye. *Biotechnology in Agriculture and Forestry*, vol. 59, p. 107-117. http://dx.doi.org/10.1007/978-3-540-36752-9_5

Andersson, R., Fransson, G., Tietjen, M., Åman, P. 2009. Content and molecular-weight distribution of dietary fiber components in wholegrain rye flour and bread. *Journal of Agricultural and Food Chemistry*, vol. 57, no. 5, p.2004-2008. http://dx.doi.org/10.1021/jf801280f

Bassam, B. J., Caetano-Anolles, G., Gresshoff. P. M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry*, vol. 196, no. 1, p. 80-83. http://dx.doi.org/10.1016/0003-2697(91)90120-i

Bolibok, H., Rakoczy-Trojanowska, M., Hromada, A., Pietrzykowski, R. 2005. Efficiency of different PCR-based marker systems in assessing genetic diversity among winter rye (*Secale cereale* L.) inbred lines. *Euphytica*, vol. 146, no. 1, p. 109-116. http://dx.doi.org/10.1007/s10681-005-0548-0

Botes, W. C., Bitalo D. 2013. Identification, evaluation and optimalization of a minimum simple sequence repeat marker set for triticale breeding. *Journal of Apllied Biology and Biotechnology*, vol. 1, no. 4, p.16-23.

Gailîte, A., Gaile, A., Gaile, I., Voronova, A., Veinberga, I., Kokare, A., Ruòìis, D. E. 2013. Genotypic assessment of the Latvian rye (*Secale cereale* L.) collection. *Proceedings of the Latvian Academy of Sciences*, vol. 67, p. 264-267. http://dx.doi.org/10.2478/prolas-2013-0046

Hackauf, B., Wehling, P. 2002. Identification of microsatellite polymorphisms in an expressed portion of the rye genome. *Plant Breeding*, vol. 121, no. 1, p. 17-25. http://dx.doi.org/10.1046/j.1439-0523.2002.00649.x

Huang, X. Q., Börner, A., Röder, M. S., Ganal, M. W. 2002. Assesing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theoretical Applied Genetics*, vol. 105, no. 4, p. 699-707. http://dx.doi.org/10.1007/s00122-002-0959-4

Ignjatovic-Micic, D., Ristic, D., Babic, V., Andjelkovic, V., Vancetovic, J. 2015. A simple SSR analysis for genetic diversity estimation of maize landraces. *Genetika*, vol. 47, no. 1, p. 53-62. http://dx.doi.org/10.2298/gensr1501053i

Jenabi, T., Saeidi, H., Rahiminejad, M. R. 2011. Biodiversity of *Secale strictum* in Iran measured using microsatellites. *Genetic Resources and Crop Evolution*, vol. 58, no. 4, p. 497-505. http://dx.doi.org/10.1007/s10722-010-9593-1

Jiang, S. K., Huang, C., Zhang, X. J., Wang, J. Y., Chen, W. F., Xu, Z. J. 2010. Development of Highly Informative Microsatellite (SSR) Marker Framework for Rice (*Oryza sativa* L.) Genotyping. *Agricultural Sciences in China.*, vol. 9, no. 12, p. 1697-1704. http://dx.doi.org/10.1016/s1671-2927(09)60268-6

Khlestkina, E. K., Than, M. H. M., Pestsova, E. G., Röder, M. S., Malyshev, S. V., Korzun, V., Börner, A. 2004. Mapping of 99 new microsatellite-derived loci in rye (*Secale cereale* L.) including 39 expressed sequence tags. *Theoretical Apllied Genetics.*, vol. 109, no. 4, p. 725-732. http://dx.doi.org/10.1007/s00122-004-1659-z

Korzun, V., Malyshev, S., Voylokov, A. V., Börner, A. 2001. A genetic map of rye (*Secale cereale* L.) combining RFLP, isozyme, protein, microsatellite and gene loci. *Theoretical and Applied Genetics*, vol. 102, no. 5, p. 709-717. http://dx.doi.org/10.1007/s001220051701

Kuleung, C., Baenziger, P. S., Dweikat, I. 2004. Transferability of SSR markers among wheat, rye and triticale. *Theoretical and Applied Genetics*, vol. 108, no. 6, p. 1147-1150. http://dx.doi.org/10.1007/s00122-003-1532-5

Maršálková, L., Židek, R., Pokoradi, J., Golian, J., Belej, Ľ. 2014. Genetic diversity and relatedness among seven red deer (*Cervus Elaphus*) populations. *Potravinarstvo*, vol. 8, no. 1, p. 15-19. http://dx.doi.org/10.5219/320

Ondroušková, J., Vyhnánek, T. 2013. Study of genetic variability of triticale varieties by SSR markers. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2, p. 2366-2368.

Paetkau, D., Calvert, W., Stirling, I., Strobeck, C. 1995. Microsatellite analysis of population structure in Canadian polar bears., *Molecular Ecology*, vol. 4, no. 3, p. 347-354. http://dx.doi.org/10.1111/j.1365-294X.1995.tb00227.x PMid:7663752

Persson, K., von Bothmer, B. 2002. Genetic diversity in landraces of rye (*Secale cereale* L.) from Northern part of Europe by using allozymes. *Hereditas*, vol. 136, no. 1, p. 29-38. http://dx.doi.org/10.1034/j.1601-5223.2002.1360105.x

Röder, M. S., Plaschke, J., König, S. U., Börner, A., Sorrels, M. E., Tanksley, S. D., Ganal, M. 1995. Abundance, variability and chromosomal location of mocrosatellites in wheat. *Molecular Genetics and Genomics*, vol. 246, no. 3, p. 327-333. http://dx.doi.org/10.1007/bf00288605

Rosén, L. A. H., Östman, E. M., Shewry, P. R., Ward, J. L., Andersson, A. A. M., Piironen, V., Lampi, A. M., Rakszegi, M., Bedö, Z., Björck, I. M. E. 2011. Postprandial glycemia, insulinemia, and satiety responses in healthy subjects after whole grain rye bread made from different rye varieties. *Journal of Agricultural and Food Chemistry*, vol. 59, no. 22, p. 12139-12148. http://dx.doi.org/10.1021/jf2019825

Saal, B., Wricke, G. 1999. Development of simple sequence repeat markers in rye (*Secale cereale* L.). *Genome*, vol. 42, no. 5, p. 964-972. http://dx.doi.org/10.1139/gen-42-5-964

Salem, K. F. M., El-Zanaty, A. M., Esmail, R. M. 2008. Assessing wheat (*Triticum aestivum L.*) genetic diversity using morphological characters and microsatellite markers. *World Journal of Agricultural Sciences*., vol. 4, no. 5, p. 538-544.

Shang, H. Y., Wei, M. Y., Wang, X. R., Zheng, Y. L. 2006. Genetic diversity and phylogenetic relationships in the rye genus *Secale* L. (rye) based on *Secale cereale* microsatellite markers. *Genetics and Molecular Biology*, vol. 29, no. 4, p. 685-691. http://dx.doi.org/10.1590/s1415-47572006000400018

Targońska, M., Bolibok-Brągoszewska, H., Rakoczy-Trojanowska, M. 2015. Assessment of Genetic Diversity in Secale cereale Based on SSR Markers. *Plant Journal Moecular Biology Report*, p. 1-15. http://dx.doi.org/10.1007/s11105-015-0896-4

Weber, J. L. 1990. Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics*, vol. 7, no. 4, p. 524-530. http://dx.doi.org/10.1016/0888-7543(90)90195-z

Weir, B. S. 1990. Genetic data analysis. Sinauer Associated, Sunderland: Massachusetts, p. 445. PMCid:PMC249124

Žiarovská, J., Ražná, K., Labajová, M. 2013. Using of Inter Microsatellite Polymorphism to evaluate gamma-irradiated Amaranth mutants. *Emirates Journal of Food and Agriculture*, vol. *25*, p. 673-681. http://dx.doi.org/10.9755/ejfa.v25i9.15879

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EVALUATION OF CAROTENOIDS, POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY IN THE SEA BUCKTHORN FRUIT JUICE

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ABSTRACT

Due to the content of biologically active substances, sea buckthorn (*Hippophae rhamnoides* L.) has become the object of great interest of both, experts and the general public. It is appreciated particularly for the high content of vitamins and other biologically active substances, not only in berries but also in leaves and bark. The aim of the study was to evaluate the nutritional quality of sea buckthorn juice prepared from different varieties of sea buckthorn based on the content of total carotenoids, polyphenols and antioxidant activity. In this study we used varieties Hergo, Tytti, Vitaminaja, Raisa, Askola, Dorana, Slovan, Leikora, Bojan, Terhi and Masličnaja. Content of different components was quantified using spectrophotometry. The total carotenoids content expressed as β-carotene content in juice ranged from 50.63 mg.100 g^{-1} DM to 93.63 mg.100 g^{-1} DM, the highest content was in variety Askola and the lowest one in Terhi. Total polyphenols content determined by Folin-Ciocalteu method ranged from 13.03 mg GAE. dm^{-3} DM to 25.35 mg GAE. dm^{-3} DM. The highest content was identified in juice of variety Dorana and the lowest one in Raisa. The antioxidant activity quantified by the FOMO method ranges from 45.11 g AA. dm^{-3} DM to 108.77 g AA. dm^{-3} DM. The highest antioxidant activity was determined in juice of Dorana and the lowest in variety Bojan.

Keywords: sea buckthorn; sea buckthorn juice; carotenoids; polyphenols; antioxidant activity

INTRODUCTION

The first remarks about sea buckthorn are several centuries old. In traditional Chinese medicine it was used in the years 618-907 AD against cough, to improve blood circulation, to help with digestive problems, to relieve pain. Leaf extract was used in Mongolia to treat colitis **(Guliyev et al., 2004)**. Sea buckthorn caught considerable attention in Russia, where it has been seen as a very important plant for its healing and regenerative effects **(Bajer, 2014)**. Nowadays the public interest in sea buckthorn as a special dietary supplement is growing, mainly for its nutritional and health-related effects **(Yang and Kallio, 2002)**.

Sea buckthorn is native to Asia and very large Eurasian area at different altitudes. It is a unique plant that is currently domesticated in different countries, in particular China, Russia, Germany, Finland, Romania, France, Nepal, Pakistan and India **(Selvamuthukumaran et al., 2007)**. Sea buckthorn belongs to the less demanding timber species in terms of location requirements. The most suitable for its growth is light sandy clay soil but it thrives also in arid, semi-arid and fragile mountain areas. From the temperature point of view, it withstands high daytime temperatures in summer and severe winter frosts **(Letchamo et al., 2007)**.

Although almost all parts of the sea buckthorn plant are used, the fruit is the most valuable product, together with seeds. Sea buckthorn berries contain almost all water soluble vitamins and fat soluble vitamins and many other substances necessary for the human body. They contain pectin, essential oils, tannins, organic acids, oils, minerals and other substances. Significant sugars in sea buckthorn berries are glucose, fructose and xylose. From the organic acids there are mainly malic and quinic acids. Research has shown that Russian berries have relatively lower concentration of organic acids (2.1 to 3.2 g 100 mL^{-1} juice) than Finnish and Chinese genotypes holding the highest concentrations of organic acids (3.5 – 9.1 g 100 mL^{-1} juice) **(Bal et al., 2011)**. **Raffo et al., (2004)** state that the sour varieties contain predominantly malic acid (46.6 $mg.g^{-1}$) and quinic acid (28.2 $mg.g^{-1}$). **Bal et al., (2011)** reported that the fruits have a high proportion of aspartic acid and glutamic acid, but it is necessary to mention also the high content of essential amino acids, especially lysine, valine, threonine, methionine, leucine, izoleucine, tryptophan, phenylalanine.

Sea buckthorn is an excellent source of natural antioxidants **(Bal et al., 2011)**. The most important antioxidant in sea buckthorn juice is vitamin C **(Rosch et al., 2003)**. The authors report that one berry covers the recommended daily dose of vitamin C. The highest concentration of vitamin C is in Chinese subspecies *H. sinensis,* 2500 mg.100 g^{-1}. European subspecies of *H. rhamnoides* berries contain more than 360 mg.100 g^{-1} of vitamin C **(Bal et al., 2011)**. **Ercisli et al., (2007)** reported that there is lower content of ascorbic acid in varieties which are not cultivated. Vitamin E in sea buckthorn berries is found in the form α, β, γ,

δ - tocopherols **(Yang and Kallio, 2002)**. The content of vitamin E varies from 8.0 mg to 16 mg 100 g^{-1} of fruit. Is is mainly in pulp oil (100 to 160 mg.100 g^{-1} of oil) and seed oil (105 to 120 mg.100 g^{-1}) **(Bajer, 2014)**. According to **Rosch et al., (2003)**, the content of vitamin E in the seed oil corresponds to 61-113 mg.100 g^{-1} and the content in the juice oil varies from 162 to 255 mg.100 g^{-1}.

Carotenoids give sea buckthorn typical yellow to orange colour. Therefore, the oil from the pulp contains more carotenoids than seed oil. The most active representative of carotenoids is β-carotene. In addition to β-carotene in sea buckthorn, there are also lycopene, zeaxanthin, β-kryptoxanthin **(Yang and Kallio, 2002b)**.

In sea buckthorn berries there were identified 12 types of flavonoids: quercetin, kaempferol, hesperidin, rutin, and others. Myricetin, citrine, catechin and others are also present. Those are secondary plant metabolites that are beneficial for the body due to its antioxidant character **(Chen et al., 2013; Bal et al., 2011)**. **Arimboor et al., (2006)** reported that sea buckthorn berries and leaves contain 9 phenolic acids: gallic acid, protocatechuic, salicylic, p-hydroxybenzoic, vanillic, caffeic, cinnamic, p-coumaric and ferulic.

The aim of this study was to determine the content of total carotenoids, polyphenols and antioxidant activity of sea buckthorn juice from selected varieties.

MATERIAL AND METHODOLOGY

The study reviewed 11 varieties of sea buckthorn – Hergo, Tytti, Vitaminaja, Raisa, Askola, Dorana, Slovan, Leikora, Bojan, Terhi, Masličnaja, which were obtained from the Central Control and Testing Institute for Agriculture - State department of fruit variety testing in Veľké Ripňany. German varietis were represented by Hergo, Askola, Dorana, Leikora. Russian originating were varieties Vitaminaja and Masličnaja, Finnish varieties were represented by varieties Tytti, Terhi and Raisa and Slovak by Bojan and Slovan.

Harvesting of berries for the analysis was carried out in the second half of September, when the fruits had characteristic deep orange colour. Due to fine surface structure of berries and their vulnerability, the full branches with berries were collected. The shoots were then frozen at -18 °C and were stored in the freezer until the analysis was run. Analyses were performed within 48 hours after harvesting. Before the analysis, we separated berries from the shoots in frozen state by gently shaking them off. Frozen sea buckthorn berries of different varieties were partially thawed and then pressed to obtain sea buckthorn juice, which was then subject to analysis.

Content of total carotenoids expressed in β- carotene was analysed at a wavelength of 455 nm by spectrophotometer UV VIS Jenway model 6405 UV / VIS in accordance with the methodology STN 12136 Determination of total carotenoids content and individual carotenoid fractions. Samples were extracted in acetone followed by capturing carotenoids in the petroleum ether solution.

Polyphenolic substance content was determined by spectrophotometry at a wavelength of 700 nm by Folin – Ciocalteu method **(Singleton and Rossi, 1965)** and was measured as equivalent content of gallic acid. The method is based on reaction of the Folin - Ciocalteu reagent with polyphenols, with leads to formation of blue colour product. The intensity of the blue colour is proportional to the content of the polyphenols.

The antioxidant activity was determined by the FOMO method **(Prieto et al., 1999)**. The principle of the method is the reduction of Molybdenum (VI^{+}) to Molybdenum (V^{+}) by activity of the reducing component in the phosphorus presence. There is a green Phosphomolybdic complex which colour intensity is measured at a wavelength of 695 nm by spectrophotometer. The reductive ability of compounds can be expressed as ascorbic acid (AA) content, which is needed to achieve the same reduction effect.

The results were processed by the statistical package Statistica 8.0 (Statsoft, Inc., Tulsa, USA). The differences between the samples were followed by Tukey's HSD test and correlation dependence between evaluation indicators by using Pearson‘s correlation coefficient.

RESULTS AND DISCUSSION

Sea buckthorn fruits are rich in carotenoids, the most active representative is β-carotene, whose average content was mentioned by **Bajer (2014)** 1.8-3.9 g mg.100 g^{-1} fruit. In our samples the content of carotenoids in sea buckthorn juice ranged from 5.87 mg.100 g^{-1} in variety Terhi to 12.07 mg.100 g^{-1} in Askola (Table 1).

After the conversion of carotenoids to 100 % dry mater, the content of carotenoids ranged from 50.63 mg.100 g^{-1} dry matter in variety Terhi to 93.63 mg.100 g^{-1} dry matter in Askola. Carotenoid content in samples decreased in the following order Askola > Vitaminaja > Hergo > Leikora > Doran > Tytti > Slovan > Bojan > Raisa > Masličnaja > Terhi. Tukey's HSD test determined the lowest content of carotenoids in the juice of varieties Terhi and Masličnaja, among which there was no statistically significant difference. The highest content of carotenoids was in juice of German variety Askola, which was statistically significantly different from any other samples. High levels of carotenoids content over 70 mg.100 g^{-1} dry matter we also found in samples of juices from German varieties Dorana, Leikora, Hergo. From Russian varieties a high content of carotenoids in juice was evaluated variety Vitaminaja. Slovak varieties Slovan and Bojan were lower in content of carotenoids.

Yang and Kallio (2002) state the total content of carotenoids in sea buckthorn berries from 1.0 to 200.0 mg.100 g^{-1} and β-carotene content from 0.2 to 17.0 mg.100 g^{-1}. **Eccleston et al., (2002)** determined the total content of carotenoids in sea buckthorn juice at the amount of 7.3 ml mg.100 mL^{-1} and β-carotene formed 3.3 mg. 100 mL^{-1}. **Kuruczek et al., (2012)** analyzed nine Russian varieties of sea buckthorn and the maximum levels of carotenoids were found in varieties Aromatnaya (28.97 mg.100 g^{-1} fresh weight), Arumnyj (21.51 mg.100 g^{-1}) and Botanicheskaya (14.2 mg.100 g^{-1}). In the study of **Raffo et al., (2004)** in German varieties of sea buckthorn Askola, Hergo and Leikora a presence of major carotenoid zeaxanthin (3-15 mg.100 g^{-1}), β-carotene (0.3-5 mg.100 g^{-1}) and ß-cryptoxanthin (0.5-1.9 g mg.100 g^{-1}) were found.

Table 1 Rating of carotenoids content in sea buckthorn juice.

Varieties	Total carotenoids (mg.100 g^{-1})	Total carotenoids (mg.100 g^{-1} DM)
Terhi	5.87	50.63[a]
Masličnaja	6.23	53.23[b]
Raisa	9.89	64,20[b]
Bojan	8.29	64.27[b]
Slovan	8.56	67,92[c]
Tytti	8.98	69.64[cd]
Dorana	8.21	71.40[de]
Leikora	9.66	73.76[e]
Hergo	10.56	77.07[f]
Vitaminaja	11.99	87.55[g]
Askola	12.08	93.63[h]

NOTE: [a-h] means indicated by the same letter are insignificantly different at P >0.05; DM – dry matter.

In these three varieties increased concentrations of carotenoids were observed while berry ripening. **Andersson (2009)** in his work examines the content of carotenoids in sea buckthorn berries. In his experiments he used varieties Ljubitelskaja, originating in Russia and BHi 72587, BHi 72588, BHi 727102 from the Swedish University of Agricultural Sciences Balsgard. The main carotenoids occurring in sea buckthorn berries include lutein, zeaxanthin, β cryptoxanthin, lycopene, γ-carotene, β-carotene. The author quantified that the total carotenoid content ranged from 1.5 to 18.5 g mg.100 g^{-1}, in variety Ljubitelskaja it was 5.9 mg.100 g^{-1}, in BHi 72587 15.1 mg.100 g^{-1}, in BHi 72588 13.8 mg.100 g^{-1}, in variety BHI 727102 9.4 mg.100 g^{-1}. **Mőrsel et al., (2014)** provided the total content of polyphenols and carotenoids in sea buckthorn and orange juices. The highest content of β-carotene (18.65 mg.100 mL^{-1}) and polyphenolic substances (156.65 mg.100 mL^{-1}) were found in sea buckthorn juice. In orange juice the content of β-carotene was 11.68 mg.100 mL^{-1} and polyphenols 140.73 mg.100 mL^{-1}.

While evaluating the polyphenols content we identified the lowest one expressed in mg GAE.dm^{-3} (gallic acid equivalent) in the juice of varieties Raisa (2.00 g GAE.dm^{-3}) and the highest in the juice of Dorana (2.92 g GAE.dm^{-3}) (Table 2). After conversion of polyphenols content into dry weight, monitored substances were found within the values from 13.3 g GAE.dm^{-3} dry matter in a sample of the varieties Raisa to 25.35 g GAE.dm^{-3} dry matter in the juice of Dorana. Polyphenols content in the studied samples of sea buckthorn juice declined in the following order of Dorana > Hergo > Leikora > Vitaminaja > Masličnaja > Tytti > Terhi > Slovan > Bojan > Askola > Raisa (Table 2).

Tukey's test proved statistically significant differences in polyphenols content in the evaluated juice samples. The difference was not statistically significant between the juices of Slovan and Terhi. Similarly like in the carotenoids evaluation, the high levels of polyphenols were found in juices of German varieties Dorana, Hergo,

Table 2 Rating of polyphenol content in sea buckthorn juice.

Varieties	Total polyphenols (g GAE.dm^{-3})	Total polyphenols (g GAE.dm^{-3} DM)
Raisa	2.00	13.03[a]
Askola	2.05	15.88[b]
Bojan	2.11	16.34[c]
Slovan	2.24	17.81[d]
Terhi	2.07	17.88[d]
Tytti	2.36	18.31[e]
Masličnaja	2.30	19.68[f]
Vitaminaja	2.72	19.89[g]
Leikora	2.64	20.13[h]
Hergo	2.84	20.76[i]
Dorana	2.92	25.35[j]

NOTE: [a-h] means indicated by the same letter are insignificantly different at P >0.05, DM – dry matter.

Leikora. Variety Askola, reaching the highest levels of carotenoids did not belong to varieties with a high content of polyphenols. Correlation analysis proved that there is no correlation dependence between the polyphenol and carotenoiod contents in sea buckthorn juice.

Bončiková et al., (2012) followed the content of total polyphenols in apple varieties Topaz, Pinova, Jonagold and Idared. Analyzing the detected value of 496.7 mg. kg^{-1} in a variety Idared after 842.2 mg. kg^{-1} in a variety Topaz. While determining the antioxidants in sea buckthorn juice **Eccleston et al., (2002)** declare flavonoids content in the amount of 1.182 mg.100 mL^{-1}, isorhamnetin-rutinoside formed 355 mg. 100 mL^{-1}, isorhamnetin-glucoside 142 mg. 100 mL^{-1}, guercetin-glycoside and guercetin-rutinoside 35 mg.100 mL^{-1}. **Arimboor et al., (2006)** dealt with processing of the fresh sea buckthorn berries and their chemical evaluation and indicated that the pure sea buckthorn juice containing no oil is characteristic by a high content of vitamin C (168.3-184.0 mg.100 g^{-1}) and polyphenols (2392-2821 mg.100 g^{-1}). **Raffo et al., (2004)** demonstrated the presence of flavonoids isorhamnetine (350-660 g mg.100 g^{-1}, quercetin (30-100 mg.100 g^{-1}) and kaempferol (2-5 mg.100 g^{-1}) in the German varieties Askola, Hergo and Leikora. **Gutzeit et al., (2007)** in their study isolated certain flavonoids from the juice of sea buckthorn using highspeed countercurrent liquid chromatography. Isorhamnetin-3-O-β-D-glucoside (95 mg), isorhamnetin -3-O-β-rutinoside (10 mg), guercetin-3-O-β-D-glucoside (5 mg) were separated from 4.1 g of the crude ethyl acetate extract. **Rop et al., (2014)** investigated and determined in the fruit of sea buckthorn the total content of polyphenols, flavonoids and antioxidant activity. The following samples were analyzed – varieties of Czech origin – Botanicky, Buchlovicky, of German origin – Hergo, Leikora and Russian origin – Ljubitelna, Trofimovskij. Polyphenolic substances content were measured spectrophotometrically by Folin – Ciocalteu method. Total polyphenol content was detected in the range from 8.62 g GAE. kg^{-1} dry matter in variety Buchlovicky to 14.17 g GAE. kg^{-1} dry matter in variety Trofimovskij. In variety Hergo it was 9.65 g GAE. kg^{-1} dry matter and in variety Leikora 9.74 g GAE. kg^{-1} dry matter, which are the values lower to what we have found in our work.

In the samples of sea buckthorn juice antioxidant activity was assessed by using the FOMO method. The antioxidant activity of the samples was expressed in $mg.dm^{-3}$ equivalent of ascorbic acid (AA). The values of antioxidant activity of sea buckthorn juice samples ranged from 12.51 g $AA.dm^{-3}$ in varieties Dorana to 5.82 g $AA.dm^{-3}$ in Bojan (Table 3).

After conversion to dry weight we identified statistically the highest antioxidant activity in the juice of variety Dorana (107.88 g $AA.dm^{-3}$ dry matter). Evaluated varieties according to antioxidant activity formed six homogeneous groups. Relatively high levels of antioxidant activity in addition to variety Dorana were also found in juices of Slovan Masličnaja. Also varieties Leikora, Terhi and Vitaminaja achieved higher levels of antioxidant activity. Average values we found in the juice of varieties. Hergo and Askola, with no statistically significant difference, and variety Tytti. The lowest antioxidant activity we found in the juice of varieties Raisa and Bojan.

Kuruczek et al., (2012) devoted their study to analysis of antioxidant activity of crude extracts from sea buckthorn berries. Analyses were performed using spectrophotometric methods FRAP and DPPH and nine Russian sea buckthorn varieties grown in Poland were examined. The highest values of antioxidant activity by DPPH method the authors found in varieties Avgustinka (45.78%), Aromatnaya (45.37%) Arumnyj (44.08%), Prozachnaya (39.06%) and by the FRAP method it was in the varieties Botanicheskaya (1892 μmol.L^{-1}) Avgustinka (819 μmol.L^{-1}), Luchistaya (676 μmol.L^{-1}), Aromatnaya (648 μmol.L^{-1}). **Rop et al., (2014)** investigated the antioxidant activity of botanical varieties Botanicky, Buchlovicky, Hergo, Leikora, Ljubitelna, Trofimovskij. The highest antioxidant activity using DPPH method was found in the Russian variety Ljubitelna (18.11 g TEAC kg^{-1}), the lowest in the Czech variety Botanicky (11.26 g TEAC kg^{-1}). In the German variety Hergo authors found antioxidant activity 11.58 g TEAC kg^{-1} and in Leikora 11,50 g TEAC kg^{-1}.

Table 3 Evaluation of antioxidant activity of sea buckthorn juice.

Varieties	Total antioxidant activity (g $AA.dm^{-3}$)	Total antioxidant activity (g $AA.dm^{-3}$ DM)
Bojan	5.82	45.12^{a}
Raisa	7.01	45.53^{a}
Tytti	7.91	61.32^{b}
Hergo	9.04	65.98^{c}
Askola	7.80	68.21^{c}
Leikora	9.91	75.67^{d}
Terhi	8.84	76.22^{d}
Vitaminaja	10.51	77.95^{d}
Masličnaja	10.22	88.05^{e}
Slovan	11.16	89.06^{e}
Dorana	12.23	108.77^{f}

NOTE: $^{a-f}$ means indicated by the same letter are insignificantly different at $P > 0.05$, DM – dry matter.

Table 4 Correlation matrix between the monitored parameters, depending on the results of the Pearson's correlation coefficient.

	Polyphenols	Antioxidant activity
carotenoids (mg.100 g^{-1} DM)	0.08	-0.02
polyphenols (g.dm^{-3} DM)		**0.79****

NOTE: ** Correlations are significant at the level P <0.01; n= 33, DM – dry matter.

Yildiz et al. (2012) analyzed samples of seven genotypes of sea buckthorn from Turkey. They assessed their polyphenols content and antioxidant activity. The total polyphenols content was between 213 mg GAE.100 g^{-1} and 262 mg GAE.100 g^{-1}. Analyses of antioxidant activity by DPPH method showed the high value in the sea buckthorn genotypes, on average 94.2% inhibition of DPPH radical. **Ivanišová et al., (2015)** analyzed antioxidant activity in sea buckthorn and its products (fruit, sea buckthorn tea, oil and juice. The highest activity by DPPH method was observed in oil 8.75 mg TEAC. g^{-1}. Antioxidant activity by phosphomolybdenum method was in the range of 111.59 mg TEAC. g^{-1} to 196.4 mg TEAC. g^{-1}, with higher rates in sea buckhorn tea.

Using Pearson correlation coefficients, we watched a relationship between assessed parameters of sea buckthorn juice quality. We found statistically significant positive correlation between polyphenols content and antioxidant activity. Between the polyphenols and carotenoids contents and carotenoids content and antioxidant activity of sea buckthorn juice we did not find correlation dependence (Table 4).

CONCLUSION

Sea buckthorn (Hippophae rhamnoides L.) has become a product of interest thanks to its content of biologically active substances. It is appreciated especially for the high content of vitamins not only in fruit but also in its leaves or bark which are characterized by healing effects. Sea buckthorn fruits are unique due to the large amount of vitamins, vitamin C, tocopherol, minerals, ß-carotene, flavonoids and organic acids. Sea buckthorn juice is rich in vitamin C, indicating its antioxidant effects. The goal of this research was to assess the content of total carotenoids, polyphenols and antioxidant activity in juice of selected sea buckthorn varieties In this work juice samples of 11 sea buckthorn varieties were evaluated – Hergo, Tytti, Vitaminaja, Raisa and Asko Doran Slovan, Leikora, Bojan, Terhi, Masličnaja. The total carotenoids expressed in β-carotene content in the evaluated sample juices ranged from 50.63 mg.100 g^{-1} dry matter in variety Terhi to 93.63 mg.100 g^{-1} dry matter in Askola. The lowest content of polyphenols we found in the juice of the variety Raisa (13.03 g GAE.dm^{-3} dry matter) and the highest in the sample of Dorana (25.35 g GAE.dm^{-3} dry matter). The values of antioxidant activity of sea buckthorn juice samples ranged from 45.12 g AA.dm^{-3} dry matter of the Bojan sample to 108.77 g AA.dm^{-3} dry matter in the sample of Dorana. Among evaluated varieties Dorana juice was characterized by a high quality of content of nutritionally important substances. It clearly reached the highest content of polyphenolic compounds and high antioxidant activity.

REFERENCES

Andersson, S. C. 2009. Carotenoids, Tocochromanols and Chlorophylls in Sea Buckthorn Berries (*Hippophae rhamnoides*) and Rose Hips (*Rosa* sp.). Swedish University of Agricultural Sciences (Alnarp, Swedish), Available at: http://pub.epsilon.slu.se/2091/1/ThesisAndersson.pdf

Arimboor, R., Venugopalan, W., Sarinkumar, K., Arumughan, C., Sawhney, R. C. 2006. Integrated processing of fresh Indian sea buckthorn (*Hippophae rhamnoides*) berries and chemical evaluation of products. *Journal of the Science of Food and Agriculture*, vol. 86, no. 14, p. 2345-2353. http://dx.doi.org/10.1002/jsfa.2620

Bajer, J. 2014. Sea buckthorn. 1st ed. Prague: Mladá fronta, 156 p. ISBN 978-80-204-3385-5.

Bal, M. L., Meda, V., Naik, N. S., Satya, S. 2011. Sea buckthorn berries: A potential source of valulable nutrients for nutraceuticals and cosmocenticals. *Food Research International*, vol. 44, p. 1718-1727. http://dx.doi.org/10.1016/j.foodres.2011.03.002

Bončíková, D., Tóth, T., Tomáš, J., Suleiman, D., Tóth, J., Slávik, M. 2012. Effective antioxidant phenolic compounds in selected varieties of apples. *Potravinarstvo*, vol. 6, no. 4, p. 11-15. http://dx.doi.org/10.5219/222

Eccleston, C., Baoru, Y., Tahvonen, R., Kallio, H., Rimbach, G. H., Minihane, A. M. 2002. Effects of an antioxidant-rich juice (*Sea buckthorn*) on risk factors for coronary heart disease in humans. *Journal of Nutritional Biochemistry*, vol. 13, no. 6, p. 346-354. http://dx.doi.org/10.1016/S0955-2863(02)00179-1

Ercisli, S., Orhan, E., Ozdemir, O., Sengul, M. 2007. The genotypic effects on the chemici composition and antioxidant aktivity of sea buckthorn (*Hippophae rhamnoides* L.) berries grown in Turkey. *Scientia Horticulturae*, vol. 115, no. 1, p. 27-33. http://dx.doi.org/10.1016/j.scienta.2007.07.004

Guliyev, V. B., Gul, M., Yildirim, A. 2004. *Hippophae rhamnoides* L.: chromatographic methods to determine chemical composition, used in traditional medicine and pharmacological effect. *Journal of chromatography*, vol. 812, no. 1/2, p. 291-307. http://dx.doi.org/10.1016/j.jchromb.2004.08.047

Gutzeit, D., Wray, V., Winterhalter, P., Jerz, G. 2007. Preparative isolation and purification of flavonoids and protocatechuic acid from Sea Buckthorn juice concentrate (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) by high-speed couter-current chromatography. *Chromatographia*, vol. 65, no. 1/2, p. 1-7. http://dx.doi.org/10.1365/s10337-006-0105-60009-5893/07/01

Chen, CH., Xu, M. X., Chen, Yang-Yu, Y. M., Wem, Y. F., Zhang, H. 2013. Identification, quantification and antioxidant activity of acylated flavonol glycosides from sea buckthorn (*Hippophae rhamnoides ssp. sinensis*). *Food Chemistry*,

vol. 141, no. 3, p. 1573-1579. http://dx.doi.org/10.1016/j.foodchem.2013.03.092

Ivanišová, E., Kačániová, M., Frančáková, H., Blašková, M., Brindza, J. 2015. Sea Buckthorn – importantsource of biologically active compounds. *Agrobiodiversity for improving nutrition, health and life quality (Proceedings of scientif works),* p. 284-288. ISBN 978-80-552-1379-8

Kuruczek, M., Swiderski, A., Mech-Nowak, A., Król, K. 2012. Antioxidant capacity of crude extracts containing carotenoids from the berries of various cultivars of Sea buckthorn (*Hippophae rhamnoides* L.). *Acta Biochemica Polonica*, vol. 59, no. 1, p. 135-137. PMid:4022914

Letchamo, W., Molnar, T., Funk, C. R. 2007. Eco-genetic variations in biological activities of sea berry. *Acta Horticulturae* (ISHS), vol. 756, p. 229-244. ISSN 0567-7572.

Mőrsel, J.-T., Zubarev, Y., Eagle, D. 2014. *Sea buckthorn. Research for a promising crop: A look at recent developments in cultivations, breeding, technology, health and enviroment.* Bood – Books and Demand, p. 76-78. ISBN 3732299864.

Prieto, P., Pineda, M., Aguilar., M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, vol. 269, p. 337-341.

Raffo, A., Paoletti, F., Antonelli, M. 2004. Changes in sugar, organic acid, flavonol and carotenoid composition during ripening of berries of three seabuckthorn (*Hippophae rhamnoides* L.) cultivars. *European Food Research and Technology*, vol. 219, no. 4, p. 360-368. http://dx.doi.org/10.1007/s00217-004-0984-4

Rop, O., Ercisli, S., Mlcek, J., Jurikova, T., Hoza, I. 2014. Antioxidant and radical scavenging activities in fruits of 6 sea buckthorn (*Hippophae rhamnoides* L.) cultivars. *Turkish Journal of Agriculture and Forestry*, vol. 38, no. 2, p. 224-232. http://dx.doi.org/10.3906/tar-1304-86

Rosch, D., Bergmann, H., Knorr, D., Kroh, L. W. 2003. Structure-Antioxidant Efficiency Relationships of Phenolic Compounds and Their Contribution to the Antioxidant Activity of Sea Buckthorn Juice. *Journal of Agriculture and Food Chemistry*, vol. 51, no. 15, p. 4233-4239. http://dx.doi.org/10.1021/jf0300339

Selvamuthukumaran, M., Khanum, F., Bawa, S. A. 2007. Development of sea buckthorn mixed fruit jelly. *International Journal of Food Science and Technology*, vol. 42, no. 4, p. 403-410. http://dx.doi.org/10.1111/j.1365-2621.2006.01233.x

Singleton, V. L., Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic – phosphotun gstic acid reagents. *American. Journal of Enology and Agriculture*, vol. 14, no. 3. p.144-158.

Yang, B., Kallio, H. 2002. Composition and physiological effects of sea buckthorn (*Hippophae*) lipids. *Trends in Food Sciene Technology*, vol. 13, no. 5, p. 160-167. http://dx.doi.org/10.1016/S0924-2244(02)00136-X

Yildiz, H., Sengul, M., Celík, F., Ercisli, S., Duralija, B. 2012. Bioactive Content of Sea Buckthorn (*Hippophae rhamnoides* L.) Berries Turkey. *Agriculture Conspectus Scientificus*, vol. 77, no. 1, p. 53-55.

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INCIDENCE OF BACTERIA AND ANTIBACTERIAL ACTIVITY OF SELECTED TYPES OF TEA

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ABSTRACT

The purpose of this study was to determine *in vitro* antibacterial activity of selected teas (Assam: Indian black tea from *Camellia sinensis*, Pu-erh: darkpu-erh (shu) from *Camellia sinensis*, Sencha: Japanese green tea from *Camellia sinensis*) against five species of pathogenic microorganisms. In our study, we determined the total viable count (TVC), yeasts (Y) and *Enterobacteriaceae* counts (E). MALDI-TOF MS Biotyper was used for identification of colonies after cultivation. Evaluation of the antimicrobial activity was performed by disc diffusion method, well diffusion method and detection of minimum inhibitory concentration (MIC). For antibacterial activity against *Escherichia coli* CCM 2024, *Yersinia enterocolitica* CCM 5671, *Klebsiella pneumonie* CCM 2318, *Staphylococus aureus* CCM 2461 and *Bacillus thurigiensis* CCM19 were detected. The inhibition zones were measured in mm in disc diffusion method and well diffusion method. The MIC of the individual extracts was measured spectrophotometrically. The high number of total viable count was found in Pu-erh tea (2.1 log CFU.g^{-1}) and lowest number was found in Assam tea (0.7 log CFU.g^{-1}). The high number of *Enterobacteriacea* was found in Pu-erh tea (2.03 log CFU.g^{-1}) and lowest in Assam tea (0 log CFU.g^{-1}). The higher number of yeasts was found in Pu-erh tea (1.83 log CFU.g^{-1}) and lowest in Assam tea (0.3 log CFU.g^{-1}). Mass spectrometry revealed the presence of seven Gram positive bacteria *Bacillus cereus, B. mycoides, B. pumilus, Enterococcus durans, Staphylococcus epidermis, S. hominis, S. warneri*, four Gram negative bacteria *Acinetobacter junii, Hafnia alvei, Klebsiella pneumoniae, Sphingomonas* spp. and two yeast - *Candida glabrata, Cryptococcus albidus*. The results show that certain tea extracts are particularly active against various pathogenic bacteria. Tea extracts (Sencha, Rooibos, Mate, Assam) were found to have the strongest antibacterial activity against *Staphylococcus aureus* CCM 2461.

Keywords: bacteria; antibacterial activity; MALDI TOF MS Biotyper; tea

INTRODUCTION

Tea is a popular beverage due to its inherent liquor and flavour characteristics. Two major types of tea available in the market are green tea and black tea. Linnaeus first classified the tea plant as Thea sinensis and later named as (*Camellia sinensis* (L) O. Kuntze). The native place of tea plants is claimed as the area touching Nagaland, Manipur along Assam and Burma frontier in the west, even though China in the east touching southwardly through the hills of Burma, Thailand and Vietnam. India is the second largest producer of black CTC tea with a production of 1135.07 million kilograms in the year 2012. It supplies approximately 26% of global back tea demand. Moreover, Assam tea estates manufacture more than 50% of all Indian black tea production. Taste of the tea liquor and appearance of the made tea are two major characteristics quality parameters of black tea **(Dutta and Baruah, 2014)**.

Pu-erh black tea, which is subjected to a long-time of secondary oxidization and fermentation (post-fermented), is defined as a new type of tea in recent years **(Liang et al., 2005)**. Pu-erh black tea, originally produced in the Yunnan province of China, is used as a health beverage to prevent a variety of diseases. First parching crude green tea leaves (*Camellia sinensis* var. *assamica* (L.) obtain Pu-erh black tea O. Kuntze; *Theaceae*) and then undergoes secondary fermentation with microorganisms such as *Aspergillus niger* (postfermented). During the fermentation process, catechins are oxidized into quinone by polyphenol oxidase and then condensed to form bisflavanol, theaflavin, thearubigen, and other high molecular components **(Wang et al., 2010)**. These are regarded as the biologically important active components of Pu-erh black tea which may be responsible for its acclaimed health benefits. Examples of such health benefits include hypocholesterolemia, anti-obesity **(Fujita and Yamagami, 2008a, 2008b)**, anti-atherosclerosis **(Hou et al., 2009)** and anti-mutagenicity **(Wang et al., 2011a,b)**. It is generally believed that the popularity of Pu-erh black tea is linked to its long history of use, especially in Asia, and its health benefits.

Tea quality is important for its market value and is defined by colour, freshness, strength, and aroma. To date, approximately 600 volatiles have been described in black

tea, with fewer numbers in oolong and green tea, due to the lesser degree of fermentation when producing these teas, and thereby tea quality influences a certain market percentage. Fresh tea leaves of *Camellia sinensis* are steamed immediately after plucking to produce Japanese green tea (Sencha). Endogenous enzymes involved in aroma formation are inactivated by the steam treatment, producing low aroma contents. The commercial value of Sencha is mainly evaluated by umami (taste) and fresh green odor, whereas flowery and fruityodor is essential for black tea or semi-fermented tea (oolong tea). The volatile compounds in green tea, black tea and semifermented tea have been intensively analysed **(Katsuno et al., 2014)**. The potent odorants of Japanese and Chinese green teas and black tea have been investigated based on aroma extract dilution analysis (AEDA) **(Kumazawa and Masuda, 2002; Kumazawa et al., 2006)**. To enrich green tea with more aroma attributes, a selection of raw materials (tea cultivars) can be employed. Amongst several types of Sencha aroma-rich green teas, made from *C. sinensis* cultivars, Kohshun and Shizu 7132 have a sweet odor **(Yang et al., 2009)**, and are becoming popular in Japan.

Despite the significant role of herbal teas in improving nutrition and health, there have been reports of microbial contamination and adverse effects resulting from their consumption. These include neurological, cardiovascular and hematological hazards **(Palmer et al., 2003)**. Toxin-producing microbial contaminants are often the cause of these adverse effects. Therefore, it is important to identify the microbial contaminants of herbal tea products as indicators of safety and quality **(Schweiggert et al., 2005)**. A few reports demonstrating microbial contamination of medicinal herbs from various parts of the world exist in the literature. **Rizzo et al., (2004)** indicated that medicinal plants in Argentina harbored toxigenic fungi such as *A. flavus*, *A. parasiticus* and several members of the Genus *Fusarium*.

In recent years, much attention has been focused on the role of tea flavonoids in the promotion of health, especially of catechins **(Ivanišová et al. 2013; Ivanišová et al., 2015 a,b)**. In plants, these metabolites are involved in their protection against several pathogens including insects, bacteria, fungi, and viruses. In the human organism, these polyphenols may exert health promoting properties, mainly antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial activities **(Dias et al., 2013; Sharangi, 2009; Silva, 2012; Wheeler, 2004)**.

The purpose of this study was to determine *in vitro* the antibacterial activity of selected teas (Assam: Indian black tea from*Camellia sinensis*, Pu-erh: dark pu-erh (shu) from*Camellia sinensis*, Sencha: Japanese green tea from *Camellia sinensis*) against five species of pathogenic microorganisms. In our study, we determined the total viable count, number of yeasts and number of*Enterobacteriaceae* genera.

MATERIAL AND METHODOLOGY

For microbial analysis and antimicrobial activity threeselected teas (Assam: Indian black tea from*Camellia sinensis*, Pu-erh: dark pu-erh (shu) from*Camellia sinensis*, Sencha: Japanese green tea from *Camellia sinensis*) were used.

Microbiological analysis

Five grams of the tea was sampled using sterile scalpels and forceps, immediately transferred into a sterile stomacher bag, containing 45 mL of 0.1% peptone water (pH 7.0), and homogenized for 60 s in a Stomacher at room temperature. Microbiological analyses were conducted by using standard microbiological methods. Total viable count (TVC) were determined using Plate Count Agar (PCA, Oxoid, UK) after incubation for 2 days at 35°C. For *Enterobacteriaceae*, Violet red bile glucose agar (VRBL, Oxoid, UK) were inoculated with sample suspension and incubated at 37°C for 24 h. Number of yeasts (Y) were determined using Tryptic Glucose Yeast agar (TGYA, Oxoid, UK). Inoculated plates were incubated for 5 days at 25°C. All plates were examined for typical colony types and morphology characteristics associated after the incubation.

We used MALDI-TOF Mass Spectrometer (Bruker Daltonics, Germany) for identification of bacteria and yeasts isolated from tea samples. After incubation of yeasts at 25°C for 5 days, isolated colonies were picked and suspended in 300 µL of sterile distilled water and mixed thoroughly. 900 µL of absolute ethanol was added. The mixture was centrifuged at 13 000 × g for 2 min. After the supernatant was discarded, the pellet was centrifuged again. Residual ethanol was completely removed by pipetting and the pellet was allowed to dry at room temperature. Subsequently 10 µL of formic acid (70%) was added and mixed with the pellet with a sterile toothpick. Next, 10 µL of acetonitrile (100%) was added and mixed thoroughly. The solution was centrifuged at maximum speed for 2 minutes again, and 1 µL of the supernatant was spotted on a polished MALDI target plate (Bruker Daltonics, Germany). Immediately after drying 1 µL of the matrix solution was added to each spot and allowed to air dry. The matrix used was a saturated solution of α-cyano-4-hydroxycinnamic acid (HCCA) (Bruker Daltonics, Germany) dissolved in 50% acetonitrile with 0.025% trifluoroacetic acid (TFA). The matrix solution preparation (2.5 mg of HCCA) contains 500 µL of acetonitrile, 475 µL of ultra-pure water and 25 µL of trifluoroacetic acid. Next added 250 µL of this solution to the 2.5 mg of HCCA. Samples were then processed in the MALDI-TOF MS (Microflex LT/SH, Bruker Daltonics, Germany) with flex Control software and results obtained with Real-time Classification software (RTC) by used database "Taxonomy" (Bruker Daltonics, Germany).

Antimicrobial activity

The dry materials were crushed, weighed out to 10 g and soaked separately in 100 mL of ethanol p.a. (96%, Sigma, Germany) during two weeks at room temperature in the dark. Exposure to sunlight was avoided to prevent the degradation of active components. Then, ethanolic tea extracts were subjected to evaporation under reduced pressure at 40 °C in order to remove the ethanol (Stuart RE300DB rotary evaporator, Bibby scientific limited, UK, and vacuum pump KNF N838.1.2KT.45.18, KNF, Germany).

For antibacterial activity, bacteria *Escherichia coli* CCM 2024, *Yersinia enterocolitica* CCM 5671, *Klebsiella pneumonie* CCM 2318, *Staphylococus aureus* CCM 2461

and *Bacillus thurigiensis* CCM19 were used. Bacteria were collected from Czech Collection of Microorganisms. The bacterial cultures were cultivated in Muller Hinton broth (Imuna, Slovakia) at 37 °C.

Antimicrobial activity of tea extract was determined using a disc diffusion method and well diffusion method. The MIC of the individual extracts was measured spectrophotometrically. Briefly, a 100 μL of the test bacteria were grown in 10 ml of fresh media until they reached a count of approximately of 10^5 cells.mL^{-1}. An amount of 100 μL of the microbial suspension was spread onto Mueller Hinton agars. Each antimicrobial assay was performed in at least triplicate.

RESULTS AND DISCUSSION

Green tea is produced from tea leaves that have not undergone the process of fermentation. Until recently, the world trade in tea focused almost exclusively on black tea **(Ošťádalová et al., 2014)**.

The number of microorganisms identified in tea is shown in Figure 1. The highest number of total viable count was found in Pu-erh tea (2.1 log CFU.g^{-1}) and the lowest in Assam tea (0.7 log CFU.g^{-1}). The high number of *Enterobacteriacea* was found in Pu-erh tea (2.03 log CFU.g^{-1}) and the lowest in Assam tea (0 log CFU.g^{-1}). The higher number of yeasts was found in Pu-erh tea (1.83 log CFU.g^{-1}), while the in Assam tea (0.3 log CFU.g^{-1}). Mass spectrometry revealed the presence of seven Gram positive bacteria *Bacillus cereus, B. mycoides, B. pumilus, Enterococcus durans, Staphylococcus epidermis, S. hominis, S. warneri*, four Gram negative bacteria *Acinetobacter junii, Hafnia alvei, Klebsiella pneumoniae, Sphingomonas* spp. and two yeast *Candida glabrata, Cryptococcus albidus*.

Microflora of tea was studied before. The microorganisms identified in tea wer *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas flourescens, Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcencens, Salmonella typhimurium* and *Escherichia coli*, respectively. Fungal isolates were identified as *Aspergillus niger, Aspergillus flavus, Penicillium expansum, Rhizopus stolonifer*, and *Fusarium solanii*, respectively **(Omogbal and Ikenebomeh, 2013)**. This finding indicates that tea could be contaminated with large numbers of different microorganisms.

Mbata et al. 2005 reported that daily consumption of green tea can kill gram positive *Staphylococcus aureus* and other harmful bacteria. Also it has been reported that the green tea contain catechin and polyphenols. These compounds have been found to possess antibacterial and antiviral action as well as anticarcinogenic and antimutagenic properties. This suggests that these compounds could be responsible for the inhibitory of *L. monocytogenes*.

The crude ethanolic extract of white tea exhibited moderate antimicrobial activity against *Sigella sonnei* (11.0 mm), *Pseudomonas aeroginosa* (10.0 mm), *Escherichia coli* (9.0 mm) and *Bacillus cereus* (8.0 mm) at 500 μg.disc^{-1} **(Ur Rashid et al., 2013)**.

The antibacterial activity detected with disc diffusion method is shown in Figure 2. The best antibacterial activity against the tested bacteria with disc diffusion method was found against *Stapylococcus aureus* for Sencha tea (5 mm). The highest antibacterial activity against *Escherichia coli* with disc diffusion method was found for Assam tea (1 mm). The highest antibacterial activity against *Yersinia enterocolitica* was found for Assam and Pu-erh tea (2 mm). The higher antibacterial activity against *Klebsiela pneumonie* was found for Assam and Sencha tea (1 mm). The higher antibacterial activity against *Bacillus thurigiensis* was found for Pu-erh tea (2 mm).

The results of the study showed that the tea extract of *Camellia sinensis* indicates the presence of potent antibacterial activity, which confirms its use against the pathogenic microorganisms. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. Disk diffusion method did not produce recordable results for all the three type of tea leaves against the pathogens. Among these the methanolic extract of fresh green tea exhibited greater antimicrobial activity. The methanol extracts of the test plant produced larger zones of inhibition against the bacteria. These observations may be attributed to green tea catechin compounds and polyphenols. These compounds have been found to possess antibacterial action **(Saikia et al., 2006)**.

The microorganisms which were found to be sensitive to fresh green tea extracts were *E. coli, Enterococcus faecalis, Staphylococcus aureus, Candida albicans* and *Pseudomonas aeruginosa* **(Archana and Abraham, 2011)**.

It has been documented that green tea contains catechin and polyphenols which are highly sensitive to the oxidation process. The catechin and polyphenols have been found to possess antibacterial and antiviral action as well as anticarcinogenic and antimutagenic properties. These compounds could be responsible for the inhibition of pathogens. The antibacterial effects of tea polyphenols (TPP) extracted from Korean green tea (*Camellia sinensis*) against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) were evaluated in the previous study. The earlier works by **Mabe et al. (1999)** showed that tea catechins have an antibacterial effect against *H. pylori* and may have a therapeutic effect against gastric mucosal injury induced by this organism.

The antibacterial activity with well diffusion method is shown in Figure 3. The best antibacterial activity against tested bacteria with well diffusion method was found against *Bacillus thurigiensis* for Sencha tea.

The agar-well diffusion method was used for the concentrations of 100, 200, 300, and 400 mg.mL^{-1}, respectively. Results showed that the minimum inhibitory concentration of tea alcohol extract was 400 mg.mL^{-1} with inhibition zone of 20 mm. The extract decreased the bacterial viable count since it showed a visible decrease to $<5\times10^6$ CFU.mL^{-1} after 24 hours of incubation. Black tea extract also had the ability to completely inhibit *Pseudomonas* growth on blood agar and inhibited protease activity and adhesion **(Flayyih et al. 2013)**.

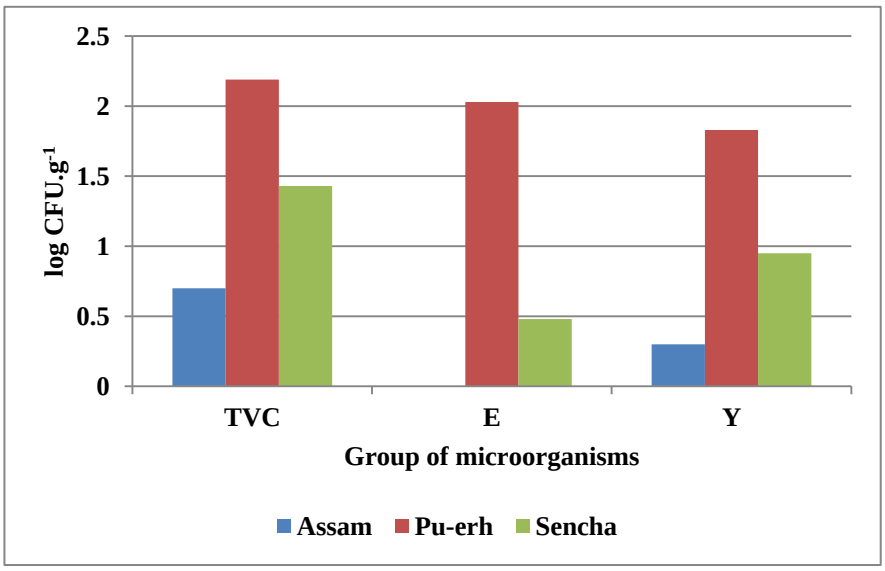

Figure 1 The number of microorganisms in log CFU.g^{-1} in tea samples.

NOTE: TVC - total viable count, E - *Enterocacteriaceae*,Y - yeasts.

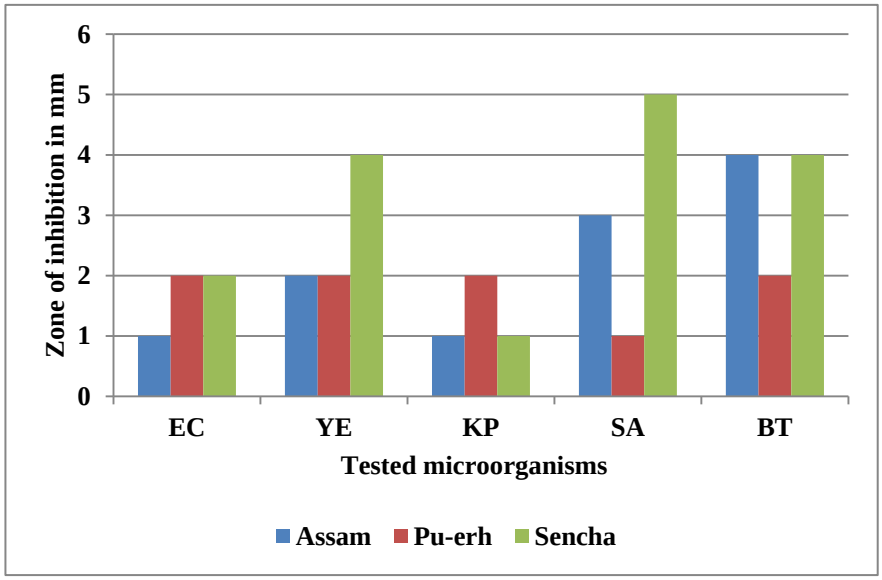

Figure 2 Antimicrobial activity of selected tea against bacteria by disc diffusion method.

NOTE: EC - *Escherichia coli*, YE - *Yersinia enterocolitica*, KP – *Klebsiella pneumonie*, SA – *Staphylococus aureus*, BT - *Bacillus thurigiensis.*

In the study of **Radji et al. 2013** the MIC of green tea extract against MRSA was 400 µg.mL^{-1}, while the MIC for MDR– *P. Aeruginosa* was 800 µg.mL^{-1}. The anti-bacterial activity of green tea extract is comparable to standard antibiotic. The activity of 16 µg of green tea extract against the laboratory strain of *S. Aureus* ATCC 25923 was comparable to that of commercially available oxacillin (1 µg), whereas the activity of 16 µg green tea extract was comparable to that of commercially available gentamicin (10 µg) against the laboratory strain *P. Aeruginosa* ATCC 27853, even though green tea extract was slightly less effective. Green tea extract showed good antimicrobial activity against MRSA and MDR – *P. aeruginosa*, although both of these bacteria have been resistant to multiple classes of antibiotics.

The polyphenol contents of green tea have been reported to inhibit the varieties of pathogenic bacterial growth such as *Helicobacter pylori*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Salmonella typhi*, *Shigella dysenteriae*, *Shigella flexneri* and *Vibrio cholera*. Polyphenols in green tea were also found to be effective against human immunodeficiency virus, hepatitis, and influenza viruses. Dental caries and periodontal diseases are two the most prevalent plaques associated with oral infectious diseases produced by endogenous oral flora. *S. mutans* and *S. sobrinus* are known as the main etiological

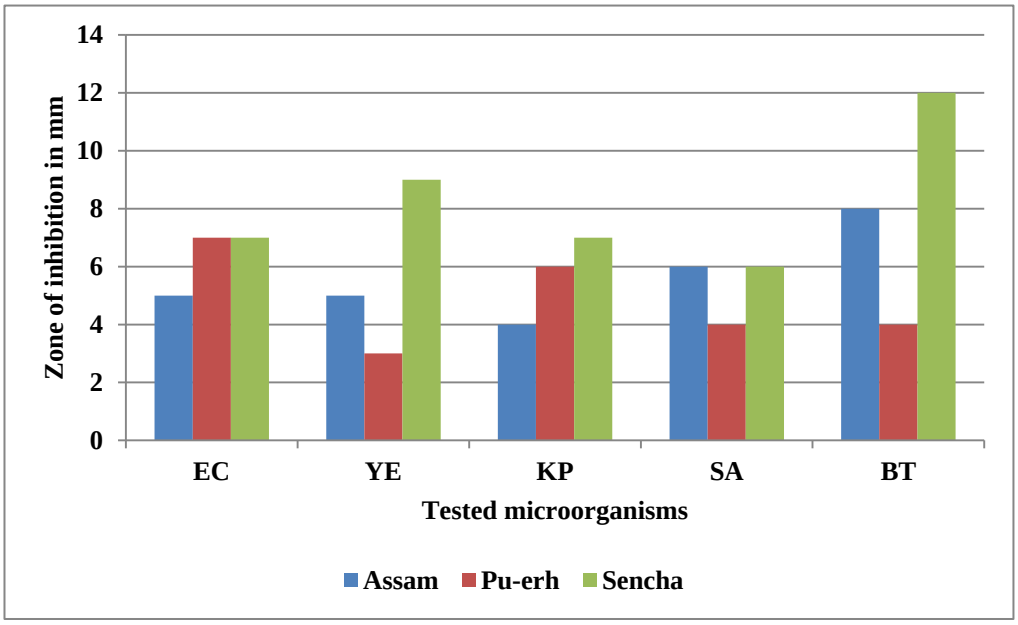

Figure 3 Antimicrobial activity of selected tea against bacteria in mm.

NOTE: *EC - Escherichia coli, YE - Yersinia enterocolitica, KP - Klebsiella pneumonie, SA - Staphylococcus aureus, BT - Bacillus thurigiensis.*

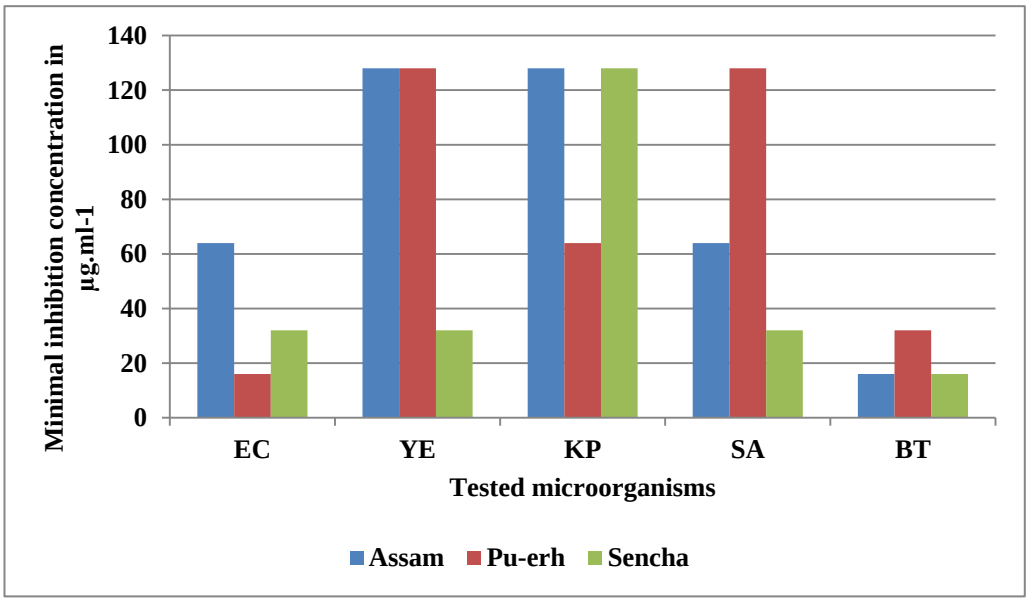

Figure 4 Minimal inhibition concentration of selected tea against bacteria in µg.mL^{-1}

NOTE: *EC - Escherichia coli, YE - Yersinia enterocolitica, KP - Klebsiella pneumonie, SA - Staphylococcus aureus, BT - Bacillus thurigiensis.*

agents of dental caries. These cariogenic bacteria adhere to the tooth surface and produce a sticky glycocalyx film composed of glucan resulting from the action of glucosyltransferase on dietary sucrose. Accumulation of bacteria causes dental plaque formation within which there is continuing acid production by the bacterial plaque **(Araghizadehet al., 2013)**. Tea extract exhibited the inhibitory effect also to those microorganisms.

The minimum inhibitory concentration (MIC) of the ethanol extract is shown in Figure 4. The lowest antibacterial activity was typical for Pu-erh against *Escherichia coli*.

CONCLUSION

The mass spectrometry for identification of tea microflora was used and altogether the presence of seven Gram-positive and four Gram-negative bacteria species were revealed. Tea extracts exhibited the antimicrobial activity, thus have a potential antimicrobial activity against microorganisms even agains the pathogens.

REFERENCES

Araghizadeh, A., Kohanteb, J., Fani, M. M. 2013. Inhibitory activity of green tea (*Camellia sinensis*) extract on some clinically isolated cariogenic and periodontopathic bacteria. *Medical principes and practice*, vol. 22, p. 368-372. http://dx.doi.org/10.1159/000348299P PMid:23485656

Archana, S., Abraham, J. 2011. Comparative analysis of antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens. *Journal of Applied Pharmaceutical Science*, vol. 1, no. 8, p. 149-152. Available at: http://www. japsonline.com/admin /php/uploads/230_pdf.pdf

Dias, T., Tomás, R. G., Teixeira, N. F., Alves, M. G., Oliveira, P. F., Silva, B.M. 2013. White Tea (*Camellia sinensis* (L.)): Antioxidant Properties and Beneficial Health Effects. *International Journal of Food Science. Nutr. Dietetics*, vol. 2, p. 1-15.

Fujita, H., Yamagami, T. 2008a. Efficacy and safety of Chinese black tea (Pu-Ehr) extract in healthy and hypercholesterolemic subjects. *Annals of Nutrition and Metabolism*, vol. 53, p. 33-42. http://dx.doi.org/ 10.1159/000153006PMid:18769024

Fujita, H., Yamagami, T. 2008b. Extract of black tea (pu-ehr) inhibits postprandial rise in serum cholesterol in mice, and with long term use reduces serum cholesterol and low density lipoprotein levels and renal fat weight in rats. *Phytotherapy Research*, vol. 22, p. 1275-1281. http://dx.doi.org/10.1002/ptr.2477 PMid:18570239

Ivanišová, E., Frančáková, H., Dráb, Š., Benčová, S. 2015b. Elderberry as important source of antioxidant and biologically active compounds. *Proceedings International Scientific and Professional Conference 15th Ružiška Days, Today Science, Tomorrow Industry*, Vukovar, Croatia. p. 212-221. ISBN 978-953-7005-36-8.

Ivanišová, E., Frančáková, H., Ritschlová, P., Dráb, Š., Solgajová, M., Tokár, M. 2015a. Biological activity of apple juice enriched by herbal extracts. *JMBFS*, vol. 4, no. special issue 3, p. 69-73. http://dx.doi.org/ 10.15414/jmbfs.2015.4.special3.69-73

Ivanišová, E., Tokár, M., Mocko, K., Mendelová, A., Bojňanská, T., Mareček, J. 2013. Antioxidant activity of selected plant products. *JMBFS*, vol. 2, no. Special issue 1, p. 1692-1703. Available at: http://www.jmbfs.org/wp-content/uploads/2013/06/68_jmbs_ivanisova_fbp_f.pdf

Katsuno, T., Kasuga, H., Kusano, Y., Yaguchi, Y., Tomomura, M., Cui, J., Yang, Z., Baldermann, S., Nakamura, Y., Ohnishi, T., Mase, T., Watanabe, N. 2014. Characterisation of odorant compounds and their biochemical formation in green tea with a low temperature storage process. *Food Chemistry*, vol. 148, p. 388-395. http://dx.doi.org/10.1016/j.foodchem.2013.10.069 PMid:24262573

Kumazawa, K., Masuda, H. 2002. Identification of potent odorants in different green tea varieties using flavor dilution technique. *Journal of Agricultural and Food Chemistry*, vol. 50, p. 5660-5663. http://dx.doi.org/ 10.1021/jf020498jPMid:12236694

Kumazawa, K., Wada, Y., and Masuda, H. 2006. Characterization of epoxydecenal isomers as potent odorants in black tea (Dimbula) infusion. *Journal of Agricultural and Food Chemistry*, vol. 54, p. 4795-4801. http://dx.doi.org/10.1021/jf0603127PMid:16787030

Liang, Y., Zhang, L., Lu, J. 2005. A study on chemical estimation of pu-erh tea quality. *Journal of the Science of Food and Agriculture*, vol. 85, p. 381-390. http://dx.doi.org/10.1002/jsfa.1857

Mabe, K., Yamada, M., Oguni, I., Takahashi, T. 1999. *In vitro* and *in vivo* activities of tea catechins against Helicobacter pylori. *American Journal of Microbiology*, vol. 43, no. 7, p. 1788-1791. PMid:10390246

Mbata, T., Debiao, L., Saikia, A. 2005. Antibacterial activity of the crude extract of Chinese green tea (*Camellia Sinensis*) on *Listeria monocytogenes*. *The Internet Journal of Microbiology*, vol. 2, no. 2, p. 1-4. Available at: http://www.ajol.info/ index.php/ajb/article/viewFile/58725/47051

Omogbai, B. A., Ikenebomeh, M. 2013. Microbiological charasteristics and phytochemical screening of some herbal teas in Nigeria. *European Scientific Journal*, vol. 9, no. 18, p. 1857-7881.

Ošťádalová, M., Tremlová, B., Straka, I., Pokorná, J., Čáslavková, P. 2014. Evaluation of significant pigments in green teas of different origin. *Potravinarstvo*, vol. 8, no. 1, p. 221-227. http://dx.doi.org/10.5219/344

Palmer, M. E., Haller, C., Mckinney, P. E., Klein-Schwartz. W., Tschirgi, A., Smolinske, S. C., Woolf, A., Sprague, B. M., Bartlett, W. D., Landzberg, B. R. 2003. Adverse events associated with dietary supplements: an observational study. *Lancet*, vol. 361, p. 101-106. http://dx.doi.org/10.1016/s0140-6736(03)12227-1 PMid:12531576

Dutta, P. P. Baruah, D. C. 2014. Drying modelling and experimentation of Assam black tea (*Camellia simensis*) with producer gas as a fuel. *Applied Thermal Engineering*, vol. 63, no. 2, p. 495-502. http://dx.doi.org/10.1016/ j.applthermaleng.2013.11.035

Radji, M., Agustama, R. A., Elya, B., Tjampakasari, C. R. 2013. Antimicrobial activity of green tea extract against isolates of methicillin–resistant*Staphylococcus aureus*and multi–drug resistant*Pseudomonas aeruginosa*. *Asian Pacific Journal of Tropical Biomedicine*, vol. 3, no. 8, p. 663-667. http://dx.doi.org/10.1016/s2221-1691(13)60133-1 PMid:23905026

Rashid, M. M. U., Sayeed, M. A., Hassan, M. M., Jainul, M. A., Azam, S., Rahman, M. M. Antidepressant and Antibacterial Activities of Camellia sinensis (White Tea). 2013. *e-Journal of Science & Technology (e-JST)*, vol. 4, no. 8, p. 39-46.

Rizzo, I., Vedoya, G. and Maurutto, S. 2004. Assessment of toxigenic fungi on Argentinean medicinal plants. *Microbiology Research*, vol. 159, p. 113-120. http://dx.doi.org/10.1016/j.micres.2004.01.013PMid:15293944

Sharangi, A. 2009. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.) – A review. *Food Research International*, vol. 42, no. 5-6, p. 529-535. http://dx.doi.org/10.1016/j.foodres.2009.01.007

Schweiggert, U., Mix, K., Schieber, A. and Carle, R. 2005. An innovative process for the production of spices through immediate thermal treatment of the plant material. *Innovative Food Science*, vol. 6, p. 143-153. http://dx.doi.org/10.1016/j.ifset.2004.11.006

Silva, B. M. 2012. *Tea Consumption and Health*. Banerjee, G., Ed.; Nova Science Publishers: New York, EUA, p. 23-38. ISBN: 978-1-61942-721-1.

Flayyih, T., Hanaa, S. Y. and Subhi, I. M. 2013. Antimicrobial Effects of Black Tea (*Camellia sinensis*) on *Pseudomonas Aeruginosa* Isolated from Eye Infection May. *Iraqi Journal of Science*, vol. 54, no. 2, p. 255-265.

Wang, D., Xiao, R., Hu, X., Xu, K., Hou, Y., Zhong, Y., Meng, J., Fan, B., Liu, L. 2010. Comparative safety evaluation of Chinese Pu-erh green tea extract and Pu-erh black tea extract in Wistar rats. *Journal of Agricultural and Food Chemistry*, vol. 58, p. 1350-1358. http://dx.doi.org/10.1021/jf902171hPMid:20028013

Wang, D., Xu, K., Zhong, Y., Luo, X., Xiao, R., Hou, Y., Bao, W., Yang, W., Yan, H., Yao, P., Liu, L. 2011a. Acute and subchronic oral toxicities of Pu-erh black tea extract in Sprague-Dawley rats. *Journal of Ethnopharmacology*, vol. 134, no. 1, p. 156-164. http://dx.doi.org/10.1016/j.jep.2010.11.068 PMid:21134434

Wang, D., Zhong, Y., Luo, X., Wu, S., Xiao, R., Bao, W., Wheeler, D.; W, W. 2004. The Medicinal Chemistry of Tea. *Drug Development Research*, vol. 61, p. 45-65. http://dx.doi.org/10.1002/ddr.10341

Yang, W., Yan, H., Yao, P., Liu, L. G. 2011b. Pu-erh black tea supplementation decreases quinocetone-induced ROS generation and oxidative DNA damage in Balb/c mice. *Food and Chemical Toxicology*, vol. 49, p. 477-484. http://dx.doi.org/10.1016/j.fct.2010.11.028 PMid:21112366

Yang, Z., Kinoshita, T., Tanida, A., Sayama, H., Morita, A., Watanabe, N. 2009. Analysis of coumarin and its glycosidically bound predcursor in Japanese green tea having sweet-herbaceous odour. *Food Chemistry*, vol. 114, no. 1, p. 289-294. http://dx.doi.org/10.1016/j.foodchem.2008.09.014

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THE CONTENT OF TOTAL POLYPHENOLS IN DIFFERENT VARIETIES OF *SOLANUM TUBEROSUM* GROW IN SPIŠ AREA

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ABSTRACT
Potatoes can be classified into groups of foods that are consumed regularly and in relatively large quantities, they are an essential source of polyphenol compounds. Phenolic compounds are the predominant antioxidants in nutrition and their study is currently being paid much attention. These antioxidants act synergistically; polyphenol compounds protect vitamin C and β-carotene, which in turn helps to increase the effect of vitamin E. Potatoes are very popular vegetables in Slovakia, not only in terms that they are easy to prepare, but also by the fact that they combine the wholesomeness of cereals and delicacy and characteristic chemical composition of vegetables. It is important that they find their place in our diet. Nutritional value of potatoes is determined by the content of nutrients such as protein, starch, fat, minerals, and absence of toxins, as well as by a significant content of bioactive components from the group of polyphenols. The study was performed in order to analyse 7 Slovak potato varieties from Spiš area, according to biologically active compounds: such as polyphenols. The content of total polyphenols was determined by the method of Lachman et al., (2003). The lowest determined content of total polyphenol (mg.kg^{-1} dry matter) in locality Spišský Štvrtok was measured in a variety Victoria (795.05 mg.kg^{-1} dry matter) and the highest content of total polyphenols in locality Spišský Štvrtok was measured in variety Laura (1238.42 mg.kg^{-1} dry matter). In the locality Odorín was determine the lowest content of total polyphenols in variety Red Anna (974.09 mg.kg^{-1} dry matter) and the highest content of total polyphenols was determined in variety Laura (978.95 mg.kg^{-1} dry matter). Between all varieties in locality Spišský Štvrtok was confirmed the statistically significant difference in the influence of the variety in the contents of total polyphenols (mg.kg^{-1} DM). This varietal dependence was not appear in samples taken in the locality Odorín. The total polyphenols content of the potatoes can be influenced by other factors, for example locality. In this case, there were statistically significant differences in the content of total polyphenols in variety Laura obtained from two different localities.

Keywords: potato; variety; total polyphenol; compound; Spiš area

INTRODUCTION

Eatable potatoes have an important role in the production of agricultural crops and also of produced food raw materials. The potatoes are frequently consumed in Europe but sometimes overlooked nutritional quality of this staple crop. *Solanum tuberosum* follows only rice and wheat in world importance as a food crop for human consumption. Cultivated potatoes have spread from the Andes of South America where they originated to 160 countries around the world. Consumption of fresh potatoes has declined while processed products have increased in popularity.

According to **Frančáková, et al., (2001)**, potato tuber is growing for its rhizone tubers, which is known as potatoes. Potatoes filled in human nutrition mainly for volume function, than eating function and protective function. Potatoes are an important food, industrial raw materials, feed and a major agricultural crops with high yield potentially useful biomass. As the potato becomes a staple in the diets of an increasing number of humans, small differences in potato nutritional composition will have major impacts on population health (**Camire et al., 2009)**.

According to **Lisińska (2006)** the nutritive value of potato is relatively high, because of protein content and composition (high percentage of essential amino acids: lysine, leucine, phenyloalanine, threonine and valine). Potato is also characterised by high amounts of starch, and lower content of sugars, minerals (K, Mg, Fe, Cu, J, P) and vitamins of group B, folic acid, fat-soluble vitamins E, K, and carotenoids, which may be converted into vitamin A **(Wroniak, 2006)**. The content of vitamins in tubers is not high, however 200 g of potatoes covers much of the daily requirement for these compounds, especially vitamin B6 (20 – 26%), vitamin B1 (12 – 20%), niacin (10 – 20%), folic acid (4 – 12%), and pantothenic acid (10%) **(Lisińska and Leszczyński, 1989)**. According to **Astley (2003)** *Solanum tuberosum* is an excellent source of vitamin C and other biologically active substances, such as polyphenols and flavonoids, which are commonly described as antioxidants. These substances have beneficial influence on human organism, as they protect against cardiovascular disease, and cancer, as well as reduce blood cholesterol level. The potato is a carbohydrate-rich, energy-providing food with little fat. Potato protein content is fairly low but has an excellent biological value of 90 – 100. Potatoes are particularly high in vitamin C and are a good source of several B vitamins and potassium. The skins provide

Table 1 Characterictics of soil and nutrient content.

Point of delivery	pH (KCl)	C_{ox}	mold	P	K	Ca	Mg
		%			(mg.kg^{-1})		
Matejovce	5.75	1.56	2.69	36.27	191.03	2780	193.50
Spišský Štvrtok	5.22	2.74	3.21	30.23	178.38	1710	180.0
Odorín	5.19	2.22	2.83	82.71	179.75	1590	161.0

substantial dietary fiber. Many compounds in potatoes contribute to antioxidant activity and interest in cultivars with pigmented flesh is growing. Potato tubers present in human nutrition an important source of antioxidants. According to **Musilová et al., (2010)** in the potatoes are the greatest extent represented polyphenols (1226 – 4405 mg.kg^{-1}) and ascorbic acid (170 to 990 mg.kg^{-1}), carotenoids (4 – 4.5 mg.kg^{-1}), α-tocopherol (0.5 to 2.8 mg.kg^{-1}), in small amounts of selenium (0.1 mg.kg^{-1}) and α-lipoic acid. Polyphenols are important sources in potatoes. They are divided into two main groups: phenolic acids and flavonoids, which create from 1/3 to 2/3 of all antioxidants **(Tapiero et al., 2002**; **Musilová et al., 2013)**.

Polyphenols are a group of plant secondary metabolites that are markedly represents in the diet of humans and animals. The main factor responsible for the delayed research on polyphenols is the variety and the complexity of their chemical structure **(D'Archivio et al., 2007**; **Hegedűsová et al., 2015)**.

Lachman et al., (2013) present that phenolic compounds are the most commonly used group of antioxidants, most of which is represented by the chlorogenic acid isomers, and caffeic acid. Polyphenolic substances contained in foods of plant origin are at present pursued plant components. According to **Suli et al., (2014)** polyphenols are found in normal foods of plant origin in varying amounts. The content of phenolic compounds in natural materials is quite variable, depending on each type of crop, but also of their varieties. According to **Mareček et al., (2013)** in the varietal composition, we have to take more account of varietal differences, especially in the carbohydrates content and in the options of processing to different products. Polyphenol content is conditioned by genetically influenced and agronomic soil and weather or environmental conditions. **André et al., (2009)** classed the polyphenols into a group of natural antioxidants. About the effect of polyphenolic substances on human health is constantly debated in professional and general level, with views on the action of these agents are not completely uniform **(Lachman et al., 2013**; **Volnová et al., 2015)**. Content of total polyphenolic compounds and anthocyanins is dissimilar at different stages of tuber maturity; it is affected by different environmental conditions, e.g. longer days and lower temperatures **(Reyes et al., 2004)** or ecological way of cultivation **(Hamouz et al., 2005)**.

The aim of this research was to evaluate a set of seven potato varieties and watched the content of total polyphenols in different varieties of *Solanum tuberosum* grow in Spiš area.

MATERIAL AND METHODOLOGY

Material: For analyses we used seven potato varieties from Spiš area: Victoria, Laura (Spišský Štvrtok), Belana, Laura (Odorín), Red Anna, Marabel, Malvína (Matejovce), which were analysed for the content of biologically active compounds: total polyphenols and potato varieties were collected from Spiš area. Each variety was removed from four places of our area of interest.

Methods: analysis of potatoes: Total polyphenols were determined by the method of **Lachman et al., (2003)** and expressed in mg eq. gallic acid per kg dry matter. Gallic acid is usually used as a standard unit for phenolics content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteau reagent. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing an aliquot of extract. The content was mixed and a sodium carbonate solution (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min. and the absorbance was measured at 765 nm of wave length against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

Analysis of soil: In each locality we determined exchange soil reaction (pH/KCl) – was determined oxidimetry %, with using translation method of Ťurin, Cox carbon content (%) and mold (%) – were determined oxidimetry %, with using translation method of Ťurin, and content of macroelements (mg.kg^{-1}) – we set by Mehlich II method, analytical method for the determination of output was atomic absorption spectrophotometer (AAS Varian AA Spectr DUO 240FS/240Z/UltrAA). We evaluated the indicators based on the Code of Good Agricultural Practice **(Bielek, 1996, Decree no. 338/2005 Coll.)** Results were statistically evaluated by the Analysis of Variance (ANOVA – Multiple Range Tests, Method: 95.0 percent LSD) using statistical software STATGRAPHICS (Centurion XVI.I, USA) and the regression and correlation analysis (Microsoft Excel) was used.

RESULTS AND DISCUSSION

The results of the analysis of different locations are referred to Table 1. Soil from Matejovce area have been weakly alkaline, with middle content of mold, very low content of phosphorus, middle content of potassium and good content of magnesium (193.50 mg.kg^{-1}). The soil from locality Odorín is alkaline, with middle content of mold, high content of P, middle content of K and good content of Mg and soil from Spišský Štvrtok area is alkaline too, with middle content of mold, with good content of phosphorus, middle content of K and good content of magnesium. Nowadays, the great emphasis is placed on research of polyphenols from plant extracts, as well as their biological activity **(Arnal et al., 2012; Duchnowicz et al., 2012**; **Stojadinovic et al., 2013**; **Zhang et al., 2013)**. The polyphenols are the most abundant antioxidants in the

Table 2 Multiple Range Tests for the locality effect on the total polyphenols content (mg.kg^{-1} DM) in potato tubers (Spišský Štvrtok locality).

Variety	Count	Mean	Homogenous groups
Victoria	16	795.05 ±108.92	X
Belana	16	956.75 ±129.54	X
Laura	16	1238.42 ±23.31	X

Method: 95,0 percent LSD.

Table 3 ANOVA Table for total polyphenol content by variety.

Source	Sum of Squares	Df	Mean Square	F-Ratio	*P*-Value
Between groups	1.61096E6	2	805480.0	82.79	0.0000
Within groups	437810.0	45	9729.1		
Total (Corr.)	2.04877E6	47			

human diet **(Bystrická et al., 2010)**. They are known to exhibit stronger antioxidant activity than monophenols **(Troszynska et al., 2002)**. In our work, we watched the locality effect on the total polyphenols content in different varieties of potatoes. The results of analyzes of individual samples have statistically processed (Table 2 – 7)

As we can see in Table 2, determined values of total polyphenol content in three different varieties were in range from 795.05 mg.kg^{-1} DM (locality Spišský Štvrtok, Victoria variety) to 1238.42 mg.kg^{-1} DM (Spišský Štvrtok area, Laura variety). Minimal measured values in variety Victoria was 614.72 mg.kg^{-1} DM, in variety Belana was 722.72 mg.kg^{-1} DM and in variety Laura was 1202.72 mg.kg^{-1} DM. Maximal measured value in variety Victoria was 993.28 mg.kg^{-1} DM, in variety Belana was 1112.12 mg.kg^{-1} DM and in variety Laura was 1268.88 mg.kg^{-1} DM. Statistically significant differences in total polyphenols content between individual variety is confirmed. The differences were between varieties Victoria – Belana and Laura, between varieties Belana – Victoria and Laura and between variety Laura – Belana and Victoria.

Between the content of total polyphenols in varieties of locality Odorín were only minimal differences, while the lowest and highest TPC we have established in variety Laura (minimum: 883.12 mg.kg^{-1} DM and maximum 1037.76 mg.kg^{-1} DM, which is almost 18% difference). The difference between the lowest and highest average value of TPC is only 0.5%. These contents of total polyphenols in testing varieties were determined only with minimal difference. There were not statistically significant differences between different varieties. The content of total polyphenols was not different in average

Based on the results of the statistical evaluation it can be stated that there are significant differences in potatoes of the same variety Laura from different areas (Odorín and Spišský Štvrtok). The TPC in variety Laura from locality Spišský Štrvtok was nearly about 27% higher as from Odorín area. Minimal measured value was in variety Laura 883.12 mg.kg^{-1} DM from locality Odorín, and maximum was in variety Laura 1268.88 mg.kg^{-1} DM from locality Spišský Štvrtok.

The average TPC of all samples middle early varieties was in the variety Victoria 795.05 mg.kg^{-1} DM and standard deviation was 108.92, in variety Belana was average value of TPC 956.75 mg.kg^{-1} DM and standard deviation was about 129.54 and in the last variety Laura from Spišský Štvrtok was average value TPC 1238.42 mg.kg^{-1} DM and standard deviation was 23.31. Based on these results it can be assumed correlation between the location and the total polyphenol content in potatoes, which is confirmed by the results of many authors who deal with the issue **Reddivari et al., (2007)**; **Hamouz et al., (2007)**. **Burgos et al., (2013)** as one of the key factors indicate variety and the conditions in their processing, too. **Lachman et al., (2008)** also confirm the significant effect of locality, which have a high content of TPC and higher

Table 4 Multiple Range Tests for the effect of variety on the total polyphenols content (mg.kg^{-1} DM) in potato tubers (locality Odorín).

Variety	Count	Mean	Homogenous groups
Red Anna	16	974.09 ±42.44	X
Marabel	12	977.79 ±31.77	X
Laura	16	978.95 ±54.16	X

Method: 95,0 percent LSD

Table 5 ANOVA Table for total polyphenol content by variety.

Source	Sum of Squares	Df	Mean Square	F-Ratio	*P*-Value
Between groups	203.218	2	101.609	0.05	0.9506
Within groups	82124.7	41	2003.04		
Total (Corr.)	82327.9	43			

Table 6 Multiple Range Tests for the effect of variety Laura on the total polyphenols content (mg.kg^{-1} DM) in potato tubers (Odorín and Spišský Štvrtok localities).

C.locality	Count	Mean	Homogenous groups
Odorín	16	978.95	X
Sp. Štvrtok	16	1238.42	X

Method: 95,0 percent LSD

Table 7 ANOVA Table for total polyphenols content by variety Laura.

Source	Sum of Squares	Df	Mean Square	F-Ratio	*P*-Value
Between groups	538597.0	1	538597.0	309.82	0.0000
Within groups	52152.3	30	1738.41		
Total (Corr.)	590750.0	31			

yields of potatoes in the area, which had the lowest average annual temperature and minimum daily temperatures. Further the author notes, that the content of total polyphenols can be influenced by variety. This fact is confirmed by many other authors. According to **Navarre et al., (2011)** a difference in the content of total polyphenols may be caused, for example, genotype or varietal affiliation. The influence of variety as an important factor influence the polyphenol content in potato tubers is confirmed by our results.

Pawelzik et al., (1999) and **Friedman (1997)** determined a significant effect of variety on TP content, which has already been confirmed by our results.

CONCLUSION

Polyphenols are secondary metabolites of plants with antioxidant properties.

The potato is one of the richest sources of antioxidants in the human diet. The main antioxidants are polyphenols (123 – 441 mg 100 g^{-1}), ascorbic acid (8 – 54 mg 100 g^{-1}), carotenoids (up to 0.4 mg 100 g^{-1}) and tocopherols (up to 0.3 mg 100 g^{-1}). L-Tyrosine, caffeic acid, chlorogenic acid and ferulic acid are amongst the main polyphenols, which have about twice the level in the skin compared with the flesh of the potato. In terms of chemical structure, it is a diverse group of chemically related substances, which are divided into several classes and subclasses. Technological processes used in the food production, storage and the meals treatment lead to changes in polyphenol content in foods. These factors together effect the representation of polyphenols in foods and also their utility.

Content of polyphenols is especially affected by variety, year of cultivation, stress factors (mechanical damage of tubers, attack of pathogens or action of light on tubers) and by cooking treatment. In a lesser extent the effect of locality, potassium fertilization, storage temperature, γ-irradiation and other factors could be involved, but there is only a little demonstrable empirical evidence in the literature references.

Polyphenolic exceed biological activity in the human body, among others they can take active part in the removal of free radicals, metal ion chelatation as well as affect enzyme activity and protein availability. Although their health beneficial properties, polyphenolic compounds are prevalent, between others, coronary heart disease, cancer, inflammatory diseases.

Nutritional value of potatoes is influenced by the content of nutrients, absence of toxic substances and presence of biologically active polyphenols, which are responsible for antioxidant activity of this vegetable. Potato is easy to prepare, widespread and versa, as it combines energy value of cereals and chemical composition typical for vegetables. It is therefore very important to include it in our everyday diet.

In our work we deal with the research of changes to the total polyphenols content in different varieties of potatoes. In conclusion we can say that the lowest content of total polyphenols we found in a variety Victoria of locality Spišský Štvrtok and the highest content of total polyphenols we have established in a Laura variety of locality Spišský Štvrtok. Total polyphenols content was statistically significant in the area Spišský Štvrtok and statistically not significant in the area Odorín. So the effect of locality on the content of total polyphenols in potato tubers in variety Laura was statistically significant.

REFERENCES

André, C. M., Oufir, M., Hofmann, L., Hausman, J. F., Rodez, H., Everse, D. 2009. Influence of environment and genotype on polyphenol compounds and in vitro antioxidant capacity of native Andrean potatoes (*Solanum Tuberosum L.*). *Journal of Food Composition and Analysis*, vol. 22, no. 6, p. 517-524. http://dx.doi.org/10.1021/jf062740i

Arnal, N., Alaniz, M. J. T., Marra, C. A. 2012. Natural polyphenols may ameliorate damage induced by copper overload. *Food and Chemical Toxicology*, vol. 50, no. 2, p. 415-422. http://dx.doi.org/10.1016/j.fct.2011.10.037

Astley S. B. 2003. Dietary antioxidants – Past, present and future? *Trends in Food Science and Technology*, vol. 14, no. 3, p. 93-98. http://dx.doi.org/10.1016/s0924-2244(02)00281-9

Bielek, P. 1996. *Ochrana pôdy. Kódex správnej poľnohospodárskej praxe (Soil protection. Codex of good agricultural praxtice).* Bratislava : VÚPÚ. ISBN 80-85361-21-3.

Burgos, G., Amoros, W., Munoa, L., Sosa, P., Cayhualla, E., Sanchez, C., Díaz, C., Bonierbale, M. 2013. Total phenolic, total anthocyanin and phenolic acid concentrations and antioxidant activity of purple-fleshed potatoes as affected by boiling. *Journal of Food Composition and Analysis*, vol. 30, no. 1, p. 6-12. http://dx.doi.org/10.1016/j.jfca.2012.12.001

Camire M. E., Kubow, S., Donnelly, D. J. 2009. Potatoes in human health. *Critical Reviews in Food Science and Nutrition*, vol. 49, no. 10, p. 823-840. http://dx.doi.org/10.1080/10408390903041996

D'archivio, M., Filesi, C., Benedetto, R., Gargiulo, R., Giovannini, C., Masella R. 2007. Polyphenols, dietary sources and bioavailability, *Ann. Ist. Super Sanità*, vol. 43, no. 4., p. 348-361.

Duchnowicz, P., Bors, M., Podsedek, A., Koter-Michalak, M., Broncel, M. 2012. Effect of polyphenols extracts from *Brassica* vegetables on erythrocyte membranes (*in vitro* study). *Enviromental Toxicology and Pharmacology*, vol. 34, no. 3, p. 783-790. http://dx.doi.org/10.1016/j.etap.2012.09.008

Frančáková, H. 2001. Zemiaky – suroviny a potravina. In *Hodnotenie surovín a potravín rastlinného pôvodu* (Potatoes – raw material and food. In *Evaluation of raw materials and food of plant origin*). Nitra : SPU, 156 p. ISBN 80-7137-886-0.

Hamouz, K., Lachman, J., Čepl, J., Dvořák, P., Pivec, V., Prášilová, M. 2007. Site conditions and genotype influence polyphenol content in potatoes. *Horticultural Science*, vol. 52, no. 3, p. 163-188. http://dx.doi.org/10.1016/j.foodchem.2011.07.077

Hegedűsová, A., Mezeyová, I., Timoracká, M., Šlosár, M., Musilová, J., Juríková, T. 2015. Total polyphenol content and antioxidant capacity changes in dependence on chosen garden pea varieties. *Potravinarstvo*, vol. 9, no. 1, p. 1-8. http://dx.doi.org/10.5219/412

Lachman, J., Hamouz, K., Dvořák, P., Pivec, V. 2005. The effect of ecological growing on the potatoes yield and quality. *Plant Soil Environment*, vol. 51, no. 9, p. 397-402. http://dx.doi.org/10.1016/j.scienta.2008.03.030

Lisińska, G. 2006. Technological value and quality of polish potatoes. *Zesz. Probl. Post. Nauk. Roln*. vol. 511, p. 81-94. http://dx.doi.org/10.1016/j.foodchem.2005.03.035

Lisińska G., Leszczyński W. 1989. Potato science and technology. *Elsevier Applied Science London*, vol. 42, no. 8, p. 326. http://dx.doi.org/10.1002/star.19900420821

Mareček, J., Frančáková, H., Bojňanská, T., Fikselová, M., Mendelová, A., Ivanišová, E. 2013. Carbohydrates in varieties of stored potatoes and influence of storage on quality of fried products. *Journal of Microbiology, Biotechnology and Food Sciences*, sp. Issue, no. 2, 1744-1753. http://dx.doi.org/10.15414/jmbfs.2015.4.special3.106-108

Musilová, J., Poláková, Z., Peltznerová, L. 2010. Potato polyphenols. *Potravinarstvo*, vol. 4, no. 2, p. 87-93. http://dx.doi.org/10.5219/305

Musilová, J., Lachman, J., Bystrická, J., Poláková, Z., Kováčik, P., Hrabovská, D. 2013. The changes of the polyphenol content and antioxidant activity in potato tubers (solanum tuberosum l.) Due to nitrogen fertilization. *Potravinarstvo*, vol. 7, no. 1, p. 164-170. http://dx.doi.org/10.5219/305

Navarre, D. A., Pillai, S. S., Sakja, R., Holden, J. 2011. HPLC profiling of phenolics in diverse potato genotypes. *Food Chemistry*, vol. 127, no. 1, p. 34-41. http://dx.doi.org/10.1016/j.foodchem.2010.12.080

Pawelzik E., Delgado E., Poberezny J., Rogoziňska I. 1999: Effect of different climatic conditions on quality of certain German and Polish potato varieties. *Potato research*, vol. 48, no. 3-4, p. 635-636. http://dx.doi.org/10.1007/bf02742374

Reddivari, L., Hale, A. L., Miller, J. C. Jr. 2007. Genotype, Location, and Year Influence Antioxidant Activity, Carotenoid Content, Phenolic Content, and Composition in Specialy Potatoes. *Journal of Agricultural and Food Chemistry*, vol. 55, no. 20, p. 8073-8079. http://dx.doi.org/10.1007/s12230-010-9150-7

Stojadinovic, M., Radosavljevic, J., Ognjenovic, J., Vesic, J., Prodic, I., Stanic-Vucinic, D., Velickovic, T. C. 2013. Binding afinity between dietary polyphenols and β-lactoglobulin negatively correletes with the protein susceptibility to digestion and total antioxidant activity of complexes formed. *Food Chemistry*, vol. 136, no. 3-4, p. 1263-1271. http://dx.doi.org/10.1016/j.foodchem.2012.09.040

Süli, J., Homzová, K., Sobeková, A., Bujdošová, Z., Hrušková, T. 2014. Polyphenolic compounds in foods. *Výživa*, vol. 17, no.3, p. 162-170. http://dx.doi.org/10.1016/b978-0-12-397934-6.00012-7

Troszynska, A., Estrella. I., Lopez-Amores, M. L., Hernandez T. 2002. Antioxidant Activity of Pea (Pisum sativum L.) Seed Coat Acetone Extract. *LWT - Food Science and Technology*, vol. 35, no. 2, p. 158-164. http://dx.doi.org/10.1006/fstl.2001.0831

Volnová, B., Musilová, J., Hrabovská, D., Bystrická, J., Kavalcová, P., Kopernická, M. 2015. The research of total polyphenols content and their changes in different varieties of potatoes. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 4, no. 3, p. 181-184.

Wroniak J., 2006. Nutritional benefits of potato. *Ziemn. Pol.* vol. 2, p. 17-20. http://dx.doi.org/10.15193/zntj/2012/85/079-092

Zhang, G., Hu, M., He, L., Fu, P., Wang, L., Zhou, J. 2013. Optimalization of microwave-assisted enzymatic extraction of polyphenols from waste peanut shells and evaluation of its antioxidant and antibacterial activities *in vitro*. *Food and Bioproducts Processing*, vol. 91, no. 2, p. 158-168. http://dx.doi.org/10.1016/j.fbp.2012.09.003

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CHARACTERIZATION OF PROTEIN FRACTIONS AND ANTIOXIDANT ACTIVITY OF CHIA SEEDS (*Salvia Hispanica* L.)

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ABSTRACT

Chia seed (*Salvia hispanica* L.) is an annual herbaceous plant categorized under *Lamiaceae* family. Chia seeds were investigated as a source of proteins and natural antioxidants. It is a potential alternative source of high quality protein, fats, carbohydrates, high dietary fibre, vitamins and mineral elements. The objective of this study was to evaluate chia seed from protein content and antioxidant acivity and highlight the quality of this pseudocereal. A crude protein, moisture content, content of protein fractions, total antioxidant capacity (TAC) and superoxide dismutase activity of chia seeds and food products containing chia seeds were determined. The protein content of chia seeds ranged from 2.9% to 4.6% dry matter from that albumins and globulins ranged from 54.6% to 62.8%. Chia is poor in a prolamines (<15%). Various chia seeds showed differences in their SOD activity and exhibited the high antiradical activity against 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The highest antioxidant capacity was found in sample chia seeds from Bolivia (1.46 mM TEAC.g^{-1} in the dry matter) and the lowest values of antioxidant activity was estimated in sample chia seeds from Argentina (1.05 mM TEAC.g^{-1} in the dry matter). The highest SOD activity was determined in sample chia from Argentina (2191.8 U.g^{-1} in the dry matter). The lowest SOD activity was found in sample chia-bio from Argentina (754.0 U.g^{-1} in the dry matter.). It makes them potentially suitable for use in the gluten-free diet of coeliac people and it can be used as a potential ingredient in health food because of its high antioxidant activity.

Keywords: chia seed; protein; protein fraction; antioxidant activity

INTRODUCTION

In recent years, demand for food with multiple health benefits has increased. There is an interest to introduce a new food to prevent various disorders **(Mohd Ali et al., 2012)**.

During recent decades, it has been demonstrated worldwide increase in allergies and intolerances to certain foods, which is associated with nutrition, lifestyle, economic growth and urbanization **(Gilissen et al., 2014)**. For example, the most common food-induced enteropathy, caused by intolerance to cereal proteins (gluten), is coeliac disease, also termed coeliac sprue. Large number of coeliac patients is also lactose-intolerant. It leads to mineral, vitamin and protein deficiencies in the diet suitable for them **(Arendt et al., 2011)**.

Chia seeds (*Salvia hispanica* L.) are one of the potential alternative sources of high quality protein, fats, carbohydrates, high dietary fibre, vitamins and mineral elements. They also contain a high amout of antioxidants, and therefore are reintroduced to diets to provide health benefits for patients and healthy persons **(Segura-Campos et al., 2014)**.

Salvia hispanica L. is a plant of the *Lamiaceae* or *Labiatae* family native of central and southern America, and grows in arid climates. It can grow up to 1 meter tall, has opposite arranged leaves and small flowers (Figure 1). It produces a small white and dark seeds **(Mohd Ali et al., 2012)**, which are considered a pseudocereals and an oilseeds (Figure 2) **(Sandoval-Oliveros and Paredes-Lopéz, 2012)**.

Due to the composition of seeds, they present a good alternative source of proteins for humans. Chia seeds contain a higher amount of proteins (19-23%) than other traditionally used grains, such as wheat (14%), barley (9.2%), oats (15.3%), corn (14%) and rice (8.5%) **(Monroy-Torres et al., 2008; Sandoval-Oliveros and Paredes-Lopéz, 2012)**.

Determinant of quality proteins is digestibility. It is the amount of protein absorbed into the body relative to the amount that was consumed. Protein digestibility of c hia flour is 79.8%, according to **Monroy-Torres et al., (2008)**, as well as cereals processed for direct consumption (corn, wheat, oats, etc.).

Chia seeds are also rich in natural antioxidants, especially phenolic compounds such as chlorogenic acids, caffeic acids, kaempferol and quercetin. All of the mentioned characteristics may reduce cardiovascular diseases, regulate an intestinal transit or prevent of some diseases such as type II diabetes and some types of cancer **(Sandoval-Oliveros and Paredes-Lopéz, 2012)**.

The main objective of the present study is to characterize and evaluate a content of proteins, protein fractions and antioxidant activity in Slovakia commercially available chia seeds.

MATERIAL AND METHODOLOGY

Chia seeds (*Salvia hispanica* L.) were obtained from local markets in Nitra, Slovak Republic. Three types of chia seeds from different producers were used for analysis. The first sample of chia seeds is originating in Bolivia, the

Figure 1 *Salvia hispanica* L. plant, **(Mohd Ali et al., 2012)**.

Figure 2 Chia seed, **(Mohd Ali et al., 2012)**.

second sample of chia was originating in Argentina and as produced by ecological farming (bio). The last sample of chia was harvested also in Argentina (conventional farming). For analysis, we used a bio-raw apricot flapjack (containing 4% of chia seeds) and chia spelled biscuits (containing 3% of chia seeds). Flours and food products for analyses were prepared by milling (BOSCH, MKM 6000).

Moisture content was determined according to the ICC Standard Method No. 110/1 for cereals and cereal products. Approximately 8 g of each sample of seeds were weighed into special aluminium dishes and dried until constant weight, using a moisture analyzer KERN DBS 60-3.

For determination of crude protein content was used 500 mg of each sample of milled chia flours and products with chia seeds. Nitrogen content was measured by the Kjeldahl method according to the **ICC Standard Method No. 105/2 (1994)**. The samples were digested in a Kjeldahl Digestion Unit type DK6 (Velp Scietifica), using cupric sulfate and potassium sulfate as catalysts. The digested samples were than destilled using UDK 127 Destilation Unit (Velp Scientifica) and the destilates were titrated with H_2SO_4 (c = 0,1 M). The protein content was calculated as nitrogen x conversion factor f (N x 6.25).

For extraction of protein fractions was used 2500 mg of each sample of chia flours and milled food products. Fractionation of proteins (albumin, globulins, prolamins and glutelins) was carried out according to the Golenkov, using modification of the method reported by **Michalik (2002)**. The protein content of the isolated fractions was assessed by Kjeldahl method.

In this study the QUENCHER procedure was used to measure the total antioxidant capacity (TAC) using $ABTS^+$ assay **(Serpen et al., 2012)**. All three samples of chia seeds needed to be diluted at 1:1 (w/w) with cellulose. ABTS was dissolved in deionized water to a concentration of 7 mM. The radical cation of ABTS was obtained by reaction with 2.45 mM potassium persulfate and allowing the stock solution to stand in the dark at room temperature for at least 12 hours **(Re et al., 1999)**. The working solution of $ABTS^+$ was prepared by diluting 10 mL of $ABTS^+$ stock solution with approximately 800 mL of a water/ethanol (50:50, v/v) mixture. The working solution absorbance was 0.750 – 0.800 at 734 nm **(Sargi et al., 2013)**. Ten (±1.0) mg of powdered sample was weighed into a centrifuge tube having 15 mL capacity. The reaction was started by adding 10 mL of $ABTS^+$ working solution. The tube was shaken rigorously for 1 minute and placed on shaker in the dark. The mixture was shaken at 350 rpm at room temperature on the shaker (ThermoMixer C, Eppendorf) to facilitate the surface reaction between the solid samples and $ABTS^+$ solution. After exactly 30 minutes for ABTS probe from the first introduction of radical/oxidant solution onto solid samples, centrifugation (Avanti J-25, Beckman Coulter) was performed at 9,200 x g for 2 minutes. Optically clear supernatants were transferred into spectrophotometric cuvette and the absorbance values were measured at 734 nm for ABTS assay (6705 UV/VIS spectrophotometer, JENWAY). The TAC of samples determined with ABTS assay were calculated in mmol of Trolox equivalent antioxidant capacity (TEAC) per g of sample using the calibration curves **(Serpen et al., 2012)**.

In this study the diagnostic Ransod set (RANDOX, Great Britain) was used for the determination of superoxide dismutase activity. The principle of the method was based on the xanthine and xanthine oxidase that produce superoxide radicals reacting with tetrazolium salt to red formasan. SOD activity is determined as a degree of inhibition of this reaction which occurs at 37 °C. Following preparation was identical both for the prepared yeast samples and standards from which the calibration curve was constructed **(Březinová Belcredi et al., 2010)**.

The chia seeds were homogenized in chilled 0.1 M sodium phosphate buffer (pH 7.4) to prepare a 10% homogenate. The homogenate was centrifuged at 10,000 x g at 4 °C for 10 minutes and the supernatant was used for assays **(Sangeetha, 2010)**. The sample (0.05 mL) and the substrate (1.7 mL) were added into a cuvette and the mixture was carefully blended. Reaction was started by addition of xanthine oxidase (0.25 mL). The cuvette was placed into the spectrophotometer and an absorbance of 505 nm was measured. The first absorbance was measured after 30 seconds (A_1) and the second after 3 minutes (A_2). The result was converting to SOD units/g of sample.

RESULTS AND DISCUSSION

The moisture content, the total protein content and the proportion of the protein fractions of chia seeds and products with chia are summarized in the Table 1.

The moisture content of chia samples ranged from 5.8 to 6.72%. These results are within the range of 4.5% to 6.8% reported by numerous authors **(Monroy-Torres et al. 2008; Coorey et al. 2012; Sandoval-Oliveros and Paredes-Lopéz 2012; Segura-Campos et al. 2014)**.

Chia seeds are characterized by a high protein content. Studies according to **Monroy-Torres et al., (2008)**, **Sandoval-Oliveros and Paredes-Lopéz (2012)** and **Segura-Campos et al., (2014)**, describe the value of protein content of 15 – 23%. In the present study, protein content of all samples of chia flour was very low and ranged from 2.9 to 4.6%. It could be caused by using unmodified chia flours. **Sandoval-Oliveros and Paredes-Lopéz (2012)** used defatted and dried flours of mucilage-free chia seeds for the same analysis, with the result 23% of proteins in dry solids.

After protein extaction and fractionation by solubility, all fractions were quantified by Kjeldahl method. The proportion obtained from chia 1 (Bolivia) was 55.8% of crude albumins and globulins, 13.8% of prolamins, 9.5% of glutelins, whereas 20.9% of the protein wasn´t recovered. The proportion obtained from chia 2 (bio-chia, Argentina) was 62.8% of crude albumins and globulins, 14.2% of prolamins, 15.1% of glutelins, whereas 7.9% of insoluble residue. In the chia 3 (Argentina), the content of albumins was 54.6%, 12.5% were prolamins, 15.2% were glutelins and the content of insoluble residue was 17.7%. Albumins and globulins were the most abundant (from 55.8% to 62.8%) followed by glutelins (9.5% – 15.2%), and prolamins (12.5 – 14.2%). The values are similar to those reported by **Sandoval-Oliveros and Paredes-López (2012)**, excluding the values of the insoluble residues (7.9 – 20.9%), which were higher.

Palenčárová and Gálová (2009) investigated the proportion of each protein fraction in selected cereals. Compared to their results, chia seeds contained double amount of nutritionally valuable fraction of storage proteins, albumins and globulins, compared to the common used cereals (wheat 25.4%, barley 27.12%, rye 41.34% and oats 20.22%). The content of the celiac active prolamin fraction was twice lower, compared to wheat (36.7%), barley (32.57%) and rye (28.75%). The prolamin content in chia was also lower than that of oats (16.65%). The glutelins content was determined lower, and content of insoluble residues was detected higher in chia seeds to that of above-mentioned cereals. The values of mentioned protein fractions are also consistent with those reported by **Gálová et al., (2011)**.

The dominance of albumin and globulin fractions was proved in pseudocereals such as amaranth **(Hricová et al., 2011)** and buckwheat **(Guo and Yao, 2006)**. The only difference found between amaranth (*Amaranthus cruentus*) and chia was a higher proportion of glutelins and lower proportion of prolamins in chia seeds. On the other hand, the protein fractions of chia and buckwheat (*Fagopyrum tataricum*) were very similar, except insoluble residues that were not mentioned by authors **Guo and Yao (2006)**.

From the results shown in Table 1 it follows that a content of crude protein detected in chia biscuits and apricot chia flapjack was 1.3% and 1.5%, respectively. Both types of chia meals presented a various composition of protein fractions. The main protein fraction corresponded to glutelins (41.9%) and fractions of albumins and globulins (39.3%) in spelt chia biscuits and apricot flapjack, respectively. The food products contain low level of prolamins, 11.9% in spelt biscuits and 17.3% in apricot flapjack. The differences were caused by various compositions of these meals, which represent a complex food composed of several different nutritionally valuable constituents. Spelt and oat, the two main components of the products, were also different in the nutritional value, thus in the composition of protein fractions **(Socha et al., 2010)**.

The values of a crude protein in products were also different than those found for chia seed samples. From the nutrition point of view, chia seeds are better source of valuable proteins, compared to food products containing insignificant amount of chia seeds.

In the present study was used TAC measurement by QUENCHER method. The QUENCHER procedure eliminates time-consuming extraction steps, which assists to build a unique database and ease of comparison for the TAC of different food types. The solvent composition of probe radical solution had a significant influence on TAC measured by direct QUENCHER. In this procedure, the solvent not only acted as a reactant carrier but also a food matrix solubilizer **(Serpen et al., 2012)**.

Based on the results of trolox standard curve (Figure 3) it was possible to calculate the trolox equivalent antioxidant capacity for chia seeds.

Table 1 The total protein content and the proportion of the protein fractions of chia seeds and products containing chia.

Sample (Coutry of Origin)	Content of Chia seeds (%)	Crude Protein (%)	Albumins and Globulins (%)	Glutelins (%)	Insoluble Residue (%)
Chia (Bolivia)	100	2.9	55.8	9.5	20.9
Chia, bio (Argentina)	100	3.6	62.8	15.1	7.9
Chia (Argentina)	100	4.6	54.6	15.2	17.7
Apricot flapjack (United Kingdom)	4	1.5	39.3	26.2	17.2
Chia spelled biscuits (Slovakia)	3	1.3	26.9	41.9	19.3
n*	-	3	3	3	3

Figure 3 Percentage quenching of absorbance at 734 nm as a function of Trolox concentrations built by using $ABTS^{+}$ working solution.

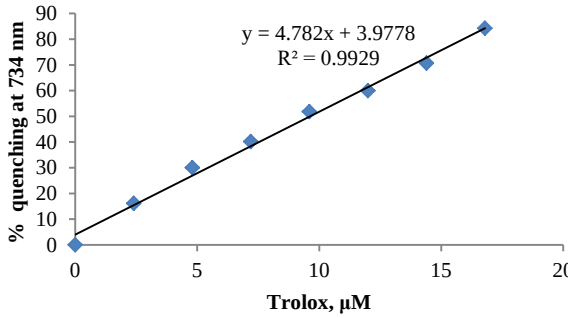

Figure 4 Calibration curve using for calculation of SOD activity.

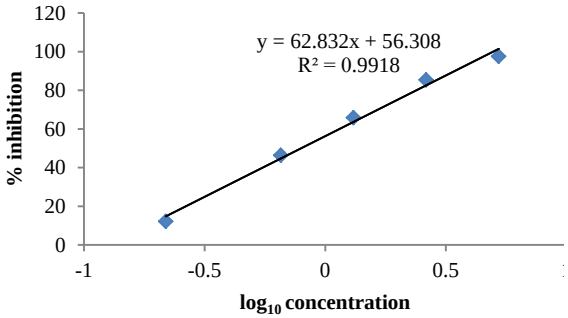

Table 2 Antioxidant capacity ($ABT^{\cdot+}$) and superoxide dismutase activity of chia seeds in the dry matter.

Samples (Country of Origin)	ATBS (mM TEAC.g^{-1} d.w.)	SOD (U.g^{-1} d.w.)	Moisture (%)
Chia (Bolivia)	1.46	1231.4	5.80
Chia, bio (Argentina)	1.13	754.0	6.72
Chia (Argentina)	1.05	2191.8	6.62
n*	3	3	3

* The number of repetitions, TEAC = trolox Equivalent Antioxidant Capacity, d.w. = dry weight.

Based on results (Table 2) it can be concluded that the highest antioxidant capacity was found in sample chia seeds from Bolivia (1.46 mM TEAC.g^{-1} in the dry matter) and the lowest values of antioxidant activity was estimated in sample chia seeds from Argentina (1.05 mM TEAC.g^{-1} in the dry matter). As seen from the Table 2, obtained data showed that there was a difference between TAC of tested samples which can be caused by a different variety or growing conditions.

Serpen et al., (2012) used the QUENCHER procedure for the $ABTS^{+\cdot}$ assay and they determined the antioxidant capacity of some seeds such as wheat (17.0 mM TEAC.kg^{-1} in the dry matter.), rice (14.9 mM TEAC.kg^{-1} in the dry matter) and rye (32.7 mM TEAC.kg^{-1} in the dry matter). These results are lower than those found for all seeds in present study for the $ABTS^{+\cdot}$ assay.

Sargi et al., (2013) determined the antioxidant capacity of chia seeds 2.56 mM TEAC.g^{-1} in the dry matter. These results are higher than those found for seeds in present study. **Vázques-Ovando et al., (2009)** found that antioxidant activity in the fiber-rich fraction of chia was 488 µM TEAC.g^{-1} in the dry mater, **Marineli et al., (2014)** reported for Chilean chia seeds 436 µM TEAC.g^{-1} and for Argentina chia meals **Capitani et al., (2012)** reported 557.2 µM TEAC.g^{-1} in the dry matter.

Chia is considered a seed with high antioxidant capacity, because is loaded with high amount of phenolic compounds **(Martínez-Cruz and Peredes-López, 2014)**. In this study was measured activity of superoxide dismutase, which protects the organism against the oxidatie damage caused by active oxygen forms **(Piterková et al., 2005)**. In this study was used a standard curve for determintion of SOD activity (Figure 4).

The highest SOD activity was determined in sample chia from Argentina (2191.8 U.g^{-1} in the dry matter). The lowest SOD activity was found in sample chia-bio from Argentina (754.0 U.g^{-1} in the dry matter.). There is no exact information about SOD activity in chia seed, so **Kolahi-Ahari (2006)** determined SOD activity in different species of kiwifruit on the level about 40 U.g^{-1} fresh weight. **Březinová-Belcredi et al., (2010)** detected superoxide dismutase activity in grain samples of 12 varieties and lines of spring barley in the interval 62 – 147 U.g^{-1} in the dry matter. In comparisom with these results it can be concluded that SOD activity in chia seeds is very high. According to the results in Table 2 it is evident that SOD activity in chia tested samples was very hight. Detection of hight antioxidant activity and SOD activity in chia seeds indicate the chia seeds to be a potential ingredient in health food products such as nutrition bars or cookies.

CONCLUSION

Based on the current research findings, chia seed is a good source of valuable protein fractions (albumins and globulins) and antioxidant compounds. The content of prolamins is low (<15%) what makes chia seeds potentially useful in the preparation of gluten-free products suitable for celiacs. The isolation and preparation of selected compounds from chia seeds could be used to produce potent natural antioxidants or ingredients with commercial applications in pharmacy, food industry or as a dietary suplements.

REFERENCES

Arendt, E. K., Moroni, A., Zannini, E. 2011. Medical nutrition teraphy: use of sourdough lactic acid bacteria as a cell factory for delivering functional biomolecules and food ingredients in gluten free bread. *Microbial Cell Factories*,

vol. 10, suppl. 1, p. 1-7. http://dx.doi.org/10.1186/1475-2859-10-S1-S15 PMCid:PMC3231922

Březinová Belcredi, N., Ehrenbergerová, J., Vaculová, K. 2010. Antioxidační aktivita enzymu superoxiddismutasy v zrnu ječmene jarního. *Kvasný průmysl*, vol. 56, no. 3, p. 128.

Capitani, M. I., Spotorno, V., Nolasco, S. M., Tomás, M. C. 2012. Physicochemical and functional characterization of by-products from chia (*Salvia hispanica* L.) seeds of Argentina. *LWT - Food Science and Technology*, vol. 45, no. 1, p. 94-102.

Gilissen, L., Van Der Meer, I., Smuldwers, M. 2014. Reducing the incidence of allergy and intolerance to cereals. *Journal of Cereal Science*, vol. 59, no. 3, p. 337-345. http://dx.doi.org/10.1016/j.jcs.2014.01.005

ICC Standard Method No. 110/1. 1976. Determination of moisture content of cereals and cereal products (Practical Method). International Association for Cereal Science and Technology, Vienna.

ICC Standard Method No. 105/2. 1994. Determination of crude protein in cereals and cereal products for food and for feed. International Association for Cereal Science and Technology, Vienna.

Kolahi-Ahari, A. 2006. *A study of superoxide dismutase activity and superoxide production in kiwifruit*. A thesis for degree of M.Sc., Canterbury : University of Canterbury, p. 39.

Marineli, Rd. S., Moraes, É. A., lenquiste, S. A., Godoy, A. T., Eberlin, M. N., Maróstica, M. R. Jr. 2014. Chemical characterization and antioxidant potential of Chilean chia seeds and oil (Salvia hispanica L.). *LWT – Foof Science an Technology*, vol. 59, no. 2, p. 1304-1340.

Martínez-Cruz, O., Peredes-López, O. 2014. Phytochemical profile and nutraceutical potential of chia seeds (*Salvia hispanica* L.) by ultra high performance liquid chromatography. *Journal of Chromatography A*, 1346, p. 43-48. http://dx.doi.org/10.1016/j.chroma.2014.04.007 PMid:24811150

Michalík, I. 2002. Unifikovaná metóda diskontinuálnej frakcionácie bielkovinového komplexu zrna obilnín (Unified method of discontinuous fractionation of protein complex in grain cereals). *Poľnohospodárstvo*, vol. 48, no. 7., p. 333-341.

Mohd Ali, N., Keong Yeap, S., Yong Ho, W., Kee Beh, B., Wei Tan, S., Guan Tan, S. 2012. The promising future of Chia, Salvia hispaica L. *Journal of Biomedicine and Biotechnology*, vol. 2012, no. 1, p. 1-9.

Monroy-Torres, R., Mancilla-Escobar, M. L., Gallaga-Solórzano, J. C., Medina-Godoy, S., Santiago-García, E. J. 2008. Protein digestibility of chia seed Salvia hispanica L. *Revysta Salud Pública y Nutrición*, vol. 9, no. 1, p. 1-6.

Piterkova, J., Tomankova, K., Luhova, L., Petřivalsky, M., Peč, P. 2005. Oxidativni stres: Lokalizace tvorby aktivnich forem kysliku a jejich degradace v rostlinnem organismu. *Chemické Listy*, vol. 99, p. 455-466.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, vol. 26, no. 9/10, p. 1231-1237. http://dx.doi.org/10.1016/S0891-5849(98)00315-3

Sandoval-Oliveros, M. R., Paredes-Lopéz, O. 2012. Isolation and Characterization of Proteins from Chia Seeds (Salvia hispanica L.). *Journal of Agricultural and Food Chemistry*, vol. 61, no. 11, p. 193-201.

Sangeetha, R. 2010. Activity of superoxide dismutase and catalase in fenugreek (Trigonella foenum-graecum) in response to carbendazim. *Indian Journal of Pharmaceutical Sciences*, vol. 72, no. 1, p. 116-118. http://dx.doi.org/10.4103/0250-474X.62248 PMid:20582202

Sargi, S. C., Silva, B. C., Santos, H. M. C., Montanher, P. F., Boeing, J. S., Santos-Júnior, O. O., Souza, N. E., Visentainer, J. V. 2013. Antioxidant capacity and chemical composition in seeds rich in omega -3: chia, flax, and perilla. *Food Science and Technology (Campinas)*, vol. 33, no. 3. http://dx.doi.org/10.1590/s0101-20612013005000057

Segura-Campos, M. R., Ciau-Solís, N., Rosado-Rubio, G., Chel-Guerrero, L., Betancur-Ancona, D. 2014. Physicochemical characterizations of chia (Salvia hispanica) seed oil from Yucatán, Mexico. *Agricultural Sciences*, vol. 5, no. 3, p. 220-226. http://dx.doi.org/10.4236/as.2014.53025

Serpen, A., Gökmen, V., Fogliano, V. 2012. Solvent effects on total antioxidant capacity of foods measured by direct QUENCHER procedure. *Journal of Food Composition and Analysis*, vol. 26, no. 1-2, p. 52-57. http://dx.doi.org/10.1016/j.jfca.2012.02.005

Socha, P., Raždíková, A., Urminská, D. 2010. Optimalizácia stanovenia prítomnosti celiakálne aktívnych bielkovín v cereáliách a pseudocereáliách (Optimization of celiatic disease active proteins estimation in cereals and pseudocereals). *Potravinarstvo*, vol. 4, special no., p. 497-505. Available at: http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/5/Socha.pdf

Vazques-Ovando, A., Rosado-rubio, G., Chel-Guerrero, L., Betancur-Ancona, D. 2009. Physicochemical properties of a fibrous fraction from chia (Salvia hispanica L.). *LWT-Food Science and Technology*, vol. 42, no. 1, p. 168-173.

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ANTIFUNGAL ACTIVITY OF LEMON, EUCALYPTUS, THYME, OREGANO, SAGE AND LAVENDER ESSENTIAL OILS AGAINST *ASPERGILLUS NIGER* AND *ASPERGILLUS TUBINGENSIS* ISOLATED FROM GRAPES

Miroslava Císarová, Dana Tančinová, Juraj Medo

ABSTRACT

Today, it is very important to find out the protection of products of natural origin as an alternative to synthetic fungicides. The promising alternative is the use of the essential oils (EOs). Essential oils from plants have great potential as a new source of fungicide to control the pathogenic fungi.The main objective of this study was evaluation of the antifungal activity of lemon (*Citrus lemon* L.), eucalyptus (*Eucalyptus globulus* LABILL.), thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.) sage (*Salvia officinalis* L.) and lavender (*Lavandula angustifolia* MILLER.) EOs against *Aspergillus niger* and *Aspergillus tubingensis* isolated from grapes and their ability to affect the growth. It was tested by using the vapor contact with them. At first both tested isolates were identified by using PCR method. Sequence data of 18S rRNA supported the assignment of these isolates to the genus *Aspergillus* and species *A. niger* (ITS region: KT824061; RPB2: KT824060) and *A. tubingensis* (ITS region: KT824062; RPB2: KT824059). Second, EO antifungal activity was evaluated. The effect of the EO volatile phase was confirmed to inhibit growth of *A. niger and A tubingensis*. EOs were diluted in DMSO (dimethyl sulfoxide) final volume of 100 µL. Only 50 µL this solution was distributed on a round sterile filter paper (1 x 1 cm) by micropipette, and the paper was placed in the center of the lid of Petri dishes. Dishes were kept in an inverted position. The essential oils with the most significant activity were determined by method of graded concentration of oils - minimum inhibitory doses (MIDs). The most effective tested EOs were oregano and thyme oils, which totally inhibited growth of tested isolates for all days of incubation at 0.625 $\mu L.cm^{-3}$ (in air) with MFDs 0.125 $\mu L.cm^{-3}$ (in air). Lavender EO was less active aginst tested strains (MIDs 0.313 $\mu L.cm^{-3}$). The results showed that the tested EOs had antifungal activity, except lemon and eucalyptus. Sage EO was the only one which decelerated the radial growth of colony of both tested strains after all days of cultivation in comparison with a control sets. Our study provides the support that essential oils can be used to control plant pathogens such as *A. niger* and *A. tubingensis*.

Keywords: *Aspergillus*; essential oils; antifungal activity; vapor

INTRODUCTION

Fruit deterioration is a key postharvest problem because fungal spoilage can cause great economic losses. Grape, as a perishable fruit, is susceptible to fungal infection, especially from *Aspergillus niger* which causes a disease called black mold, one of the major causes of rapid and extensive deterioration of table grapes during the harvest and the major obstacle for storage **(dos Santos et al., 2012; de Sousa et al., 2013)**. *Aspergillus niger*, the most important member of *Aspergillus* subgenus *Circumdati* section *Nigri*, is primarily a plant pathogenic fungi responsible for deterioration of stored food material, as well as *Aspergillus tubingensis*, which includes species that morphologically resemble *Aspergillus niger* **(Samson et al., 2000)**. In addition, the genus *Aspergillus* and its species are producers of several mycotoxins. *A. flavus* and *A. parasiticus* are the main aflatoxins-producing species, while production of ochratoxin A is mainly associated with *Aspergillus carbonarius* and *A. niger* or *Nigri* section species, which has also been reported to produce fumunosin, sterigmatocystin, cyclopiazonic acid and patulin **(Plascencia-Jatomea et al., 2014)**. Spoilage and poisoning of food by fungi are the major problem for food industry and consumers. Decay may increase post harvest losses up to 50% without fungicide treatment. However, the use of synthetic fungicides is becoming more restrictive and thus alternative treatments need to be developed to reduce environmental risk and satisfy the demands of consumer groups **(Phillips et al., 2012)**. This negative consumer perception of chemical preservatives drives attention towards natural alternatives **(Sharma and Tripathi, 2008)**. Due to an increasing risk of chemical contamination upon the application of synthetic fungicides to preserve fresh fruits and vegetables, essential oils are gaining increasing attention **(Farzaneh et al., 2015)**.

Essential oils are aromatic and volatile liquids extracted from plants. The chemicals in essential oils are secondary metabolites, which play an important role in plant defense as they often possess antimicrobial properties **(Hyldgaard et al., 2012)**. Some of EOs have been reported to be active *in vitro* against *A. niger* such as lemongrass **(Tzortzakis and Economakis, 2007)** and *Matricaria chamomilla* flower **(Tolouee et al., 2010)**. A number of EO components have been registered by the European Commission for use as flavourings in food stuffs **(Commission Decision of 23 January, 2002)**. Some EO formulations are currently used

as food preservatives and are kept in the category "GRAS" in view of their favourable safety profile. Being volatile in nature, such EOs may be used as plant-based fumigants for the stored food commodities. Hence, EOs may play a significant role in overcoming storage losses and in enhancing food shelf life **(Prakash et al., 2015)**.

The objective of this study was evaluation of the antifungal activity of 6 EOs by using vapor contact against the fungal species of the genus *Aspergillus* section *Nigri* isolated from grapes in Slovakia.

MATERIAL AND METHODOLOGY

Fungal isolation and identification

Two isolates of black aspergilly, *Aspergillus niger KMi-116-LR* and *Aspergillus tubingensis KMi-144-LR* isolated from grapes, were used. These isolates belong to the collection of microorganisms at the Department of Microbiology of the Slovak Agricultural University in Nitra. They were inoculated on Czapek Yeast Autolysate Agar (CYA) **(Samson et al., 2002)** dishes.

Culturing conditions and DNA extraction

Single spore fungal isolates grown on SDA (Pancreatic Digest of Casein 5 $g.L^{-1}$, Peptic Digest of Meat 5 $g.L^{-1}$, Glucose 40 $g.L^{-1}$, Agar 15 $g.L^{-1}$, BioLife, Italy, Srl) plates (2 weeks, 26 °C, 16/8 light regime) were used for DNA extraction. DNA was extracted using a ZR fungal/bacterial DNA extraction kit (Zymo Research Corp. USA, CA). Identification of isolates was based on 18S rDNA-ITS1-5.8S rDNA-ITS2-28S rDNA region (ITS). We used also partial sequences of second largest subunit of DNA dependent RNA polymerase II (RBP2) because ITS region has low discrimination power among species in *Aspergillus* sect. *nigri*. Amplification reactions were carried out in 25 μL volumes containing: 200 mM dNTPs, 1x dreamTaq buffer, 0.5 unit DreamTaq DNA polymerase (Life technologies, USA), 0.5 mM of corresponding primer, and 0.5 μL DNA. Conditions of PCR reactions were following: initial denaturation at 95 °C for 3 min, 35 cycles were performed consisting of denaturation at 95 °C for 30 s, annealing at corresponding temperature for each primer set for 45 s, and extension at 72 °C for 90 s, final step was 10 min incubation at 72 °C. PCR reactions were carried out in a Biorad MJ mini thermal cycler (BioRad Corp., USA, CA). Primers used for PCR and sequencing of ITS region were ITS1 and ITS4 **(White et al., 1990)**. Primer pair for PCR amplifdication was partial RPB2 where RPB2-5F and RPB2-7cR and sequencing primer was RPB-6F **(Liu et al.,**

Table 1 The major constituents of essential oils analyzed by Calendula company a.s.

Essential oils	Compound	Amount (%)
Lemon	β-pinene	7.0 – 17
	sabinene	1.0 – 3.0
	limonene	56 – 78
	γ-terpinene	6.0 – 12
	β-caryophyllene	max. 0.5
	neral	0.3 – 1.5
	α-terpineol	max. 0.6
	neryl acetate	0.2 – 0.9
	geranial	0.5 – 2.3
	geranyl acetate	0.1 – 0.8
Oregano	carvacrol	min. 50
Lavender	limonene	<1.0
	cineole	<2.5
	3-octanone	0.1 – 2.5
	camphor	<1.2
	linalool	20 – 45
	linalyl acetate	25 – 46
	terpinen-4-ol	0.1 – 6.0
	lavandulyl acetate	>0.2
	lavandulol	>0.1
	α-terpineol	<2.0
Thyme	ρ-cimene	40 ±3
	thymol	32 ±2
Eucalyptus	α-pinene	9.0
	β-pinene	max. 1.5
	sabinene	max 0.3
	α-phellandrene	max. 1.5
	limonene	12
	1,8-cineole	min. 70
	camphor	max. 0.1
Sage	1,8-cineole	min. 5.0
	thujone	min. 15.0
	borneole	min. 5.0

Note: max. (maximum), min. (minimum).

1999). PCR products were cleaned-up by ExoI/FastAP (Life technologies, USA) and sent to Macrogen (Korea) for Sanger sequencing. Acquired sequences were assembled and processed using the Seaview software **(Gouy et al., 2010)**. Isolates were identified by comparison with records in genbank database using genbank BlastN tool. (http://blast.ncbi.nlm.nih.gov/). Sequences of both used isolates was deposited in genbank database under folowing accesion numbers: *A. niger* isolate KMi-116-LR, ITS: KT824061; RPB2: KT824060. *A. tubigensis* isolate KMi-144-LR, ITS: KT824062; RPB2: KT824059.

Essential plant oils

The essential oils used in this study were extracts of lemon (*Citrus lemon* L.), eucalyptus (*Eucalyptus globulus* LABILL.), thyme (*Thymus vulgaris* L.), oregano (*Origanum vulgare* L.) sage (*Salvia officinalis* L.) and lavender (*Lavandula angustifolia* MILLER.), they all were supplied by Calendula company a.s. (Nová Ľubovňa, 238 A, Slovakia). The gas chromatography analysis of the main components of each essential oils were determined by Calendula company a.s. (Table 1). Essential oils were extracted by hydro distillation and its quality and stability were certified by suppliers.

Antifungal activity of essential oils

The antifungal activity of selected EOs was investigated by microatmosphere method. The test was performed in sterile Petri dishes (Ø 90 mm) containing 15 mL of CYA. Evaluation by filter paper was made by the method adapted from **Guynot et al., (2003)**. First, all EOs were tested in highest concentration (0,625 µL.cm^{-3} of air). EOs were diluted in DMSO (dimethyl sulfoxide) final volume of 100 µL. Only 50 µL of this solution was distributed on a round sterile filter paper (1 x 1 cm) by micropipette, and the paper was placed in the center of the lid of Petri dishes. Dishes were kept in inverted position. Filter paper discs impregnated with dimethyl sulfoxide (DMSO) were only used as a control to confirm no solvent effect of bioactivity. Each fungus was inoculated in the center of Petri dishes with needle – inoculated. Dishes were tightly sealed with parafilm and incubated for fourteen days at 25 ±1 °C (three replicates were used for each treatment). Diameters (Ø mm) of the growing colonies were measured at the 3rd, 7th, 11th and 14th day with a ruler. Essential oils able to inhibit each fungus (visible inhibition- non growth of fungus) were used in the following test.

Minimum inhibitory doses (MIDs)

After incubation, the minimum inhibitory doses (MIDs) of EOs with the most significant activity were recorded by the method adapted from **Kloucek et al., (2012)**. The essential oils with the most significant activity were determined by method of graded concentration of oils. EOs dissolved in DMSO were prepared at different concentrations (0.500, 0.313, 0.188, 0.125, 0.063 µL.cm^{-3} of air). Cultivation was carried out at the 25 ±1 °C and measured after 14 days. The MID (expressed as microlitres of EOs per volume unit of atmosphere above the organism growing on the agar surface) was defined as the lowest concentration of the oil which did not permit any visible growth after 14 days in comparison with control sets.

Statistical analysis

All analyses were performed in triplicate and the results were expressed as the mean of the data obtained in each replicate. Statistical analyses were performed with descriptive statistics (mean and standard deviation) and inferential tests (ANOVA followed by 95.0% Tukey HSD test) to determine statistically significant differences ($p < 0.05$) between treatments.

RESULTS AND DISCUSSION

Contamination of grapes and grape products by *Aspergillus* section *Nigri* is known to occur widely. The fungal species *Aspergillus niger*, *Aspergillus tubingensis*, and *Aspergillus carbonarius* are included within this section and during their growth are able to produce mycotoxins **(Somma et al., 2012)**.

The objective of this study was to find the activity of the volatile phase of lemon, oregano, lavender, eucalyptus, thyme and sage essential oils against the fungal growth of *Aspergillus niger* and *Aspergillus tubingensis*. First, all EOs were tested at the higherst concentration (0,625 µL.cm^{-3}). Both tested strains, *Aspergillus niger* (Figure 1) and *A. tubingensis* (Figure 2) were sensitive in treatment with oregano, lavender and thyme EOs, which completely inhibited their growth after all days of cultivation (14 days). Strain *A. niger* was not sensitive in treatment with lemon EO, as same as *A. tubingensis*. Eucalyptus EO had very similar antifungal activity against both tested strains. *A. niger* showed the most significant sensibility to the sage EO at the highest concentration (0,625 µL.cm^{-3}) after 7 days of cultivation. *A. tubingensis* seems to be more resistant in treatment with sage EO. It was inhibited by sage EO only after 3 days of cultivation in a comparison with control sets and *A. niger* strain.

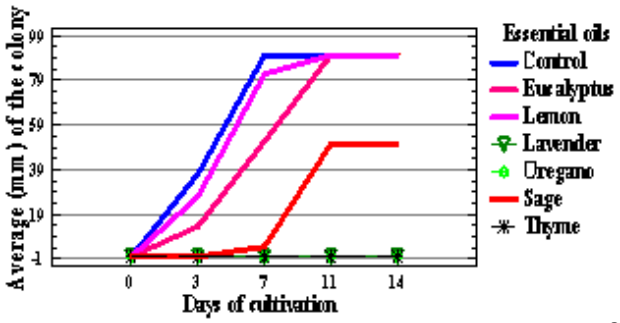

Figure 1 Antifungal activity of tested EOs (0.625 µL.cm^{-3}) to *Aspergillus niger* KMi-116-LR.

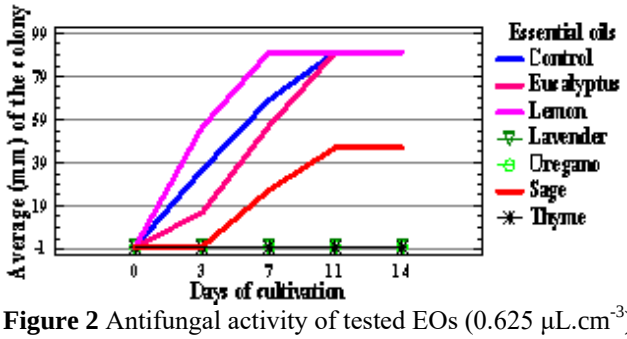

Figure 2 Antifungal activity of tested EOs (0.625 µL.cm^{-3}) to *Aspergillus tubingensis* KMi-144-LR.

Table 2 Effect of different concentrations of lavender, oregano and thyme essential oils on radial growth inhibition (after 14 days) of *A. niger* and *A. tubingensis*.

Conc. µL.cm^{-3}	***Aspergillus niger*** KMi-116-LR			***Aspergillus tubingensis*** KMi-144-LR		
Essential oils	**Lavender**	**Oregano**	**Thyme**	**Lavender**	**Oregano**	**Thyme**
0.500	0^{a} ±0	0^{a} ±0	0^{a} ±0	0^{a} ±0	0^{a} ±0	0^{a} ±0
0.313	0^{a} ±0	0^{a} ±0	0^{a} ±0	0^{a} ±0	0^{a} ±0	0^{a} ±0
0.188	24.50^{b} ±2.29	0^{a} ±0	0^{a} ±0	7.67^{b} ±2.08	0^{a} ±0	0^{a} ±0
0.125	34.67^{c} ±8.39	0^{a} ±0	0^{a} ±0	22.67^{c} ±6.43	0^{a} ±0	0^{a} ±0
0.063	66.67^{e} ±20.82	7.33^{a} ±0.58	45.67^{d} ±16.01	36.00^{d} ±8.54	6.50^{ab} ±1.80	44.67^{e} ±0.76
Control	90^{f} ±0	90^{f} ±0	90^{f} ±0	90^{f} ±0	90^{f} ±0	90^{f} ±0

* Data in the column followed by different letters are significantly different in 95% Tukey HSD test.

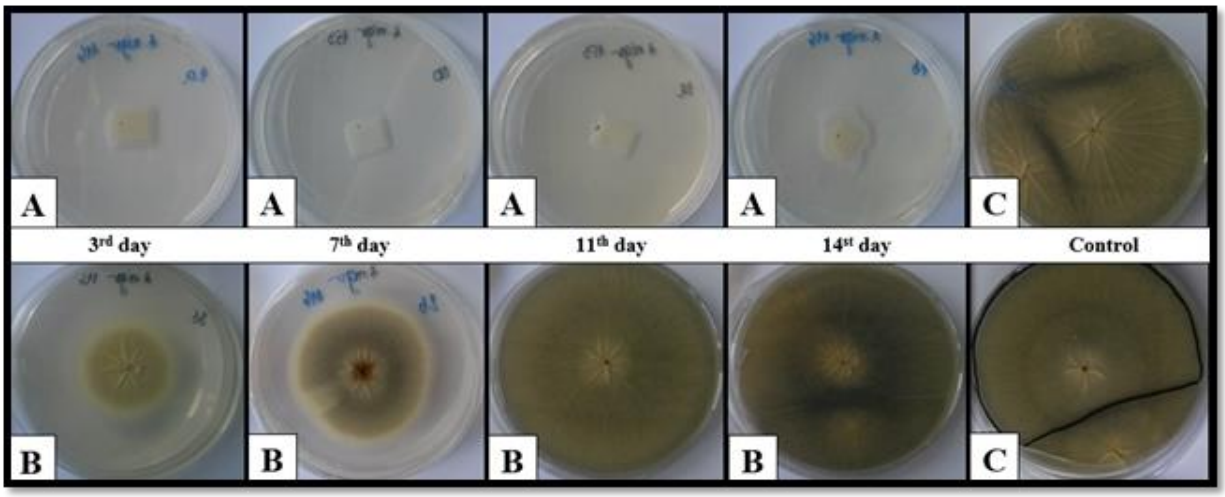

Figure 3 Antifungal activity of oregano (A) and lemon essential oils (B) against *Aspergillus niger*; (C) control.

Pinto et al., (2007) in their study also demonstrated similar results of antifungal activity of sage EO against fungi, but different results were found by **Suhr and Nielsen (2003)** where sage EO showed very poor inhibitor effects. Our results showed that all tested EOs have antifungal activity, except lemon and eucalyptus EOs, and demonstrated significant differences between each other (p <0.001). **Velázquez-Nuñez et al., (2013)** studied antifungal activity of citrus essential oils. They reported the minimum inhibitory concentration for the growth of *A. flavus* by direct addition 16.000 mg.L^{-1}, while for the vapor contact 8000 mg of EO mg.L^{-1} in air. For the both studied methods, growth of *A. flavus* decreased with increasing EO concentration. Further, studies have also documented that eucalyptus and lemon essential oils are effective even against fungal strains in vapor contact, e.g.: *Aspergillus niger*, *A. flavus*, *Penicillium chrysogenum and P. verrucosum* **(Viuda-Martos et al., 2008)**, *A. clavatus*, *A. niger*, etc. **(Su et al., 2006)**. Regarding to previous studies, this study demonstrated that lemon and eucalyptus EOs were not effective against tested strains in comparison with other tested EOs (sage, oregano, lavender and thyme). Also **Vilela et al., (2009)** reported that eucalyptus EOs and its major compound 1,8-cineole demonstrated very poor fungicidal activity against *A. flavus* and *A. parasiticus* in both contact and headspace volatile exposure assays.

In this study the most effective EOs were able to inhibit growth of tested strains all days of cultivation at the highest concentration (0.625 µL.cm^{-3}) and were used for determination of MIDs. Among all oils tested, thyme, oregano and lavender oils proved to be the best inhibitor of the black aspergilly. Results are showed in Table 2. The best results (MIDs 0.125 µL.cm^{-3}) (p <0.05) for both, *A. niger* and *A. tubingensis* showed oregano and thyme EOs.

In study of **Combrinck et al., (2011)** thyme EO proved to be the most effective inhibitor, totally inhibiting all of the pathogens tested at concentrations of 1000 µL.L^{-1} and lower, with the exception of a resistant *Penicillium* strain. Several researchers **(Stević et al., 2014; Kocić-Tanackov et al., 2012; Zabka et al., 2014)** found hight inhibitory effect of oregano EOs against fungi, too.

In our study, *A. niger* showed visible growth after 14 days only in treatment with lavender EO with a higher MIDs value 0.313 µL.cm^{-3}, as same as *A. tubingensis* (MIDs 0.313 µL.cm^{-3}) (p <0.05). **Soylu et al., (2010)** tested

rosemary and lavender EOs against *Botrytis cinerea*, and they also found that rosemary and lavender EOs were inhibitory at relatively higher concentrations (25.6 μg.mL^{-1}). Also **Daferera et al., (2003)** demonstrated that lavender, rosemary, sage, and pennyroyal essential oils have less inhibitory activity against tested fungal species. Although the concentrations of oils tested in this work were not the same. But antifungal activity of tested EOs depends on concentration of EOs, cultivation time and used method. In a previous study conducted by **Goñi et al., (2009)** behavior of clove EO was not the same in direct contact and vapor phase. **Bluma et al., (2009)** demonstrated that the vapor generated by 5000 μL.L^{-1} of poleo oil significantly reduced growth of *Aspergillus* section *Flavi* in the order of 78.0%, whereas the dose of 3000 μL.L^{-1} completely inhibited fungal development in the direct contact assay **(Bluma and Etcheverry, 2008)**. In study of **Velázquez-Nuñez et al., (2013)** direct addition of orange peel EO had a rapid effect on *A. flavus* growth, but exposure to orange peel EO vapors was more effective, requiring lower concentrations of EO to inhibit mold growth. They concluded that vapor contact is an alternative when essential oils (EO's) and microorganisms are placed separately in some sealed environment.

CONCLUSION

As a conclusion, volatile substances from oregano, thyme (MIDs 0.125 μL.cm^{-3}) and lavender (MID 0.313 μL.cm^{-3}) essential oils had a potential antifungal activity against tested strains of black aspergilly. Results showed that the tested EOs had antifungal activity, except lemon and eucalyptus EOs in comparison with control sets. In spite of the fact that sage EO showed only weak antifungal activity, and was able only to delayed growth of *A. niger* (after 7 days of cultivation) and *A. tubingensis* (after 3 days) could be used in food preservation, but further research is needed. Our study gives support that essential oils can be used to control plant pathogens such as *A. niger* and *A. tubingensis*.

REFERENCES

Bluma, R., Landa, M. F., Etcheverry, M. 2009. Impact of volatile compounds generated by essential oils on *Aspergillus* section *Flavi* growth parameters and aflatoxin accumulation. *Journal of the Science of Food and Agriculture*, vol. 89, no. 9, p. 1473-1480. http://dx.doi.org/10.1002/jsfa.3611

Bluma, R. V., Etcheverry, M. G. 2008. Application of essential oils in maize grain: Impact on *Aspergillus* section *Flavi* growth parameters and aflatoxin accumulation. *Food Microbiology*, vol. *25*, no. 2, p. 324-334. http://doi.org/10.1016/j.fm.2007.10.004

Combrinck, S., Regnier, T., Kamatou, G. P. P. 2011. *In vitro* activity of eighteen essential oils and some major components against common postharvest fungal pathogens of fruit. *Industrial Crops and Products*, vol. 33, no. 2, p. 344-349. http://doi.org/10.1016/j.indcrop.2010.11.011

Commission Decision of 23 January (2002) amending Commission Decision 1999/ 217/EC as regards the register of flavouring substances used in or on foodstuffs. 2002/113/EC: Official Journal L49 20/02/2002. p. 1-160.

Daferera, D. J., Ziogas, B. N., Polissiou, M. G. 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. *Crop Protection*, vol. 22, no. 1, p. 39-44. http://dx.doi.org/10.1016/s0261-2194(02)00095-9

De Sousa, L. L., de Andrade, S. C. A., Athayde, A. J. A. A., de Oliveira, C. E. V., de Sales, C. V., Madruga, M. S., de Souza, E. L. 2013. Efficacy of *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils in combination to control postharvest pathogenic Aspergilli and autochthonous mycoflora in *Vitis labrusca* L. (table grapes). *International Journal of Food Microbiology*, vol. 165, no. 3, p. 312-318. http://doi.org/10.1016/j.ijfoodmicro.2013.06.001

Dos Santos, N. S. T., Athayde Aguiar, A. J. A., de Oliveira, C. E. V., Veríssimo de Sales, C., de Melo e Silva, S., Sousa da Silva, R., de Souza, E. L. 2012. Efficacy of the application of a coating composed of chitosan and *Origanum vulgare* L. essential oil to control *Rhizopus stolonifer* and *Aspergillus niger* in grapes (*Vitis labrusca* L.). *Food Microbiology*, vol. 32, no. 2, p. 345-353. http://doi.org/10.1016/j.fm.2012.07.014

Farzaneh, M., Kiani, H., Sharifi, R., Reisi, M., Hadian, J. 2015. Chemical composition and antifungal effects of three species of *Satureja* (S. *hortensis*, *S. spicigera*, and *S. khuzistanica*) essential oils on the main pathogens of strawberry fruit. *Postharvest Biology and Technology*, vol. 109, p. 145-151. http://doi.org/10.1016/j.postharvbio.2015.06.014

Goñi, P., López, P., Sánchez, C., Gómez-Lus, R., Becerril, R., Nerín, C. 2009. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food Chemistry*, vol. 116, no. 4, p. 982-989. http://doi.org/10.1016/j.foodchem.2009.03.058

Gouy, M., Guindon, S., Gascuel, O. 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, vol. 27, no. 2, p. 221-224. http://doi.org/10.1093/molbev/msp259

Guynot, M. E., Ramos, A. J., Setó, L., Purroy, P., Sanchis, V., Marín, S. 2003. Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products. *Journal of Applied Microbiology*, vol. 94, no. 5, p. 893-899. http://doi.org/10.1046/j.1365-2672.2003.01927.x

Hyldgaard, M., Mygind, T., Meyer, R. L. 2012. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology*, vol. 3, no. 12, p. 1-24. http://doi.org/10.3389/fmicb.2012.00012

Kloucek, P., Smid, J., Frankova, A., Kokoska, L., Valterova, I., Pavela, R. 2012. Fast screening method for assessment of antimicrobial activity of essential oils in vapor phase. *Food Research International*, vol. 47, no. 2, p. 161-165. http://doi.org/10.1016/j.foodres.2011.04.044

Kocić-Tanackov, S., Dimić, G., Tanackov, I., Pejin, D., Mojović, L., Pejin, J. 2012. The inhibitory effect of oregano extract on the growth of *Aspergillus* spp. and on sterigmatocystin biosynthesis. *LWT - Food Science and Technology*, vol. 49, no.1, p. 14-20. http://doi.org/10.1016/j.lwt.2012.04.013

Liu, Y. J., Whelen, S., Hall, B. D. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. *Molecular Biology and Evolution*, vol. 16, no. 12, p. 1799-1808. http://doi.org/10.1016/j.foodres.2011.07.035

Phillips, C. A., Laird, K., Allen, S. C. 2012. The use of Citri-V™® - An antimicrobial citrus essential oil vapour for the control of *Penicillium chrysogenum*, *Aspergillus niger* and *Alternaria alternata in vitro* and on food. *Food Research*

International, vol. 47, no. 2, p. 310-314. http://doi.org/10.1016/j.foodres.2011.07.035

Pinto, E., Salgueiro, L. R., Cavaleiro, C., Palmeira, A., Gonçalves, M. J. 2007. *In vitro* susceptibility of some species of yeasts and filamentous fungi to essential oils of *Salvia officinalis*. *Industrial Crops and Products*, vol. 26, no. 2, p. 135-141. http://doi.org/10.1016/j.indcrop.2007.02.004

Plascencia-Jatomea, M., Yépiz Goméy, S. M., Veley-Haro, J. M. 2014. Chapter 8 - *Aspergillus* spp. (Black Mold), In: Bautista- Baños, S. *Posth Harvest Decay- Control Strategies*. 2014. Printed in USA : Academic Press Publication, p. 267-282. ISBN 978-0-12411552-1. http://dx.doi.org/10.1016/b978-0-12-411552-1.00008-9

Prakash, B., Kedia, A., Mishra, P. K., Dubey, N. K. 2015. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities - Potentials and challenges. *Food Control*, vol. 47, p. 381-391. http://doi.org/10.1016/j.foodcont.2014.07.023

Samson, A. R., Hoekstra, E. S., Frisvad, J. C., Filtenborg, O., 2002. *Introduction to Food and Airborne Fungi, Sixth Edition.* CBS-Utrecht : Netherlands. p. 64-97. http://dx.doi.org/10.1007/s11046-005-4201-1

Sharma, N., Tripathi, A. 2008. Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiological Research*, vol. 163, no. 3, p. 337-344. http://doi.org/10.1016/j.micres.2006.06.009

Somma, S., Perrone, G., Logrieco, A. F. 2012. Diversity of black aspergilli and mycotoxin risks in grape, wine and dried vine fruits. *Phytopathologia Mediterranea*, vol. 51, no. 1, p. 131-147. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.09.021

Soylu, E. M., Kurt, Ş., Soylu, S. 2010. *In vitro* and *in vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. *International Journal of Food Microbiology*, vol. 143, no. 3, p.183-189. http://doi.org/10.1016/j.ijfoodmicro.2010.08.015

Stević, T., Berić, T., Šavikin, K., Soković, M., Godevac, D., Dimkić, I., Stanković, S. 2014. Antifungal activity of selected essential oils against fungi isolated from medicinal plant. *Industrial Crops and Products*, vol. 55, p. 116-122. http://doi.org/10.1016/j.indcrop.2014.02.011

Su, Y. C., Ho, C. L., Wang, E. I., Chang, S. T. 2006. Antifungal activities and chemical compositions of essential oils from leaves of four eucalyptus. *Taiwan Journal For. Sciences*. vol. 21, p. 49-61. http://dx.doi.org/10.1002/ffj.1685

Suhr, K. I., Nielsen, P. V. 2003. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. *Journal of Applied Microbiology*, vol. 94, no. 4, p. 665-674. http://doi.org/10.1046/j.1365-2672.2003.01896.x

Tolouee, M., Alinezhad, S., Saberi, R., Eslamifar, A., Zad, S. J., Jaimand, K., Razzaghi-Abyaneh, M. 2010. Effect of *Matricaria chamomilla* L. flower essential oil on the growth and ultrastructure of *Aspergillus niger* van Tieghem. *International Journal of Food Microbiology*, vol. 139, no. 3, p. 127-133. http://doi.org/10.1016/j.ijfoodmicro.2010.03.032

Tzortzakis, N. G., Economakis, C. D. 2007. Antifungal activity of lemongrass (*Cympopogon citratus* L.) essential oil against key postharvest pathogens. *Innovative Food Science and Emerging Technologies*, vol. 8, no. 2, p. 253-258. http://doi.org/10.1016/j.ifset.2007.01.002

Velázquez-Nuñez, M. J., Avila-Sosa, R., Palou, E., López-Malo, A. 2013. Antifungal activity of orange (*Citrus sinensis* var. Valencia) peel essential oil applied by direct addition or vapor contact. *Food Control*, vol. 31, no. 1, p. 1-4. http://doi.org/10.1016/j.foodcont.2012.09.029

Vilela, G. R., de Almeida, G. S., Dapos-Arce, M. A. B. R., Moraes, M. H. D., Brito, J. O., da Silva, M. F. D. G. F., da Gloria, E. M. 2009. Activity of essential oil and its major compound, 1,8-cineole, from *Eucalyptus globulus* Labill., against the storage fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. *Journal of Stored Products Research*, vol. 45, p. 108-111. http://doi.org/10.1016/j.jspr.2008.10.006

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Perez-Álvarez, J. 2008. Antibacterial activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. *Journal of Food Safety*, vol. 28, no. 4, p. 567-576. http://doi.org/10.1111/j.1745-4565.2008.00131.x

White, T. J., Bruns, S., Lee, S., Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications*, vol. 64, no. 1, p. 315-322. http://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zabka, M., Pavela, R., Prokinova, E. 2014. Antifungal activity and chemical composition of twenty essential oils against significant indoor and outdoor toxigenic and aeroallergenic fungi. *Chemosphere*, vol. 112, p. 443-448. http://doi.org/10.1016/j.chemosphere.2014.05.014

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ANTIOXIDANT ACTIVITY, PHENOLIC CONTENT AND COLOUR OF THE SLOVAK CABERNET SAUVIGNON WINES

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ABSTRACT

Antioxidants are specific substances that oxidize themselves and in this way they protect other sensitive bioactive food components against destruction. At the same time, they restrict the activity of free radicals and change them to less active forms. Grapes and wine are a significant source of antioxidants in human nutrition. One of the most important group occuring in grapes and wines are polyphenols. Many of phenolic compounds have been reported to have multiple biological activities, including cardioprotective, anti-inflammatory, anti-carcinogenic, antiviral and antibacterial properties attributed mainly to their antioxidant and antiradical activity. Therefore, it is important to know the content of polyphenols and their antioxidant effects in foods and beverages. Twenty-eight Cabernet Sauvignon wine samples, originated from different Slovak vineyard regions, were analyzed using spectrophotometry for the content of total polyphenols, content of total anthocyanins, antioxidant activity and wine colour density. Determined values of antioxidant activity in observed wines were within the interval 69.0 – 84.2% inhibition of DPPH (average value was 78.8% inhibition of DPPH) and total polyphenol content ranged from 1,218 to 3,444 mg gallic acid per liter (average content was 2,424 mg gallic acid.L^{-1}). Determined total anthocyanin contents were from 68.6 to 430.7 mg.L^{-1} (average content was 220.6 mg.L^{-1}) and values of wine colour density ranged from 0.756 to 2.782 (average value was 1.399). The statistical evaluation of the obtained results did not confirm any linear correlations between total polyphenol content, resp. total anthocyanin content and antioxidant activity. The correlations between total polyphenol content and total anthocyanin content, resp. the content of total anthocyanins and wine colour density were strong. The results confirmed very strong correlations between wine colour density and total polyphenol content, resp. antioxidant activity.

Keywords: polyphenol; antioxidant activity; anthocyanin; red wine; Cabernet Sauvignon

INTRODUCTION

Phenolic compounds are the most abundant secondary metabolites present in the plant kingdom. They possess a common structure comprising an aromatic benzene ring with one or more hydroxyl substituents. They represent a large and diverse group of molecules including two main families: the flavonoids based on common C6-C3-C6 skeleton and the non-flavonoids. In plant, they play a role in growth, fertility and reproduction and in various defence reactions to protect against abiotic stress like UV-light or biotic stresses such as predator and pathogen attacks. They also constitute basic components of pigments, essences and flavors **(Weisshaar and Jenkins 1998; Winkel-Shirley, 2002)**. Recent interest, however, in food phenolics has increased greatly because of the antioxidant and free radical-scavenging abilities associated with some phenolics and their potential effects on human health **(Bravo, 1998)**. Many of phenolic compounds (resveratrol, quercetin, rutin, catechin, proanthocyanidins) have been reported to have multiple biological activities, including cardioprotective, anti-inflammatory, anti-carcinogenic, antiviral and antibacterial properties attributed mainly to their antioxidant and antiradical activity **(Lorrain et al., 2013)**.

Grapes and grape products (mainly wines and juices) are a rich source of phenolic compounds. From the clue of "French paradox", polyphenolics from grapes and red wines attracted the attention of scientists to define their chemical composition and quantity **(Urpi-Sarda et al., 2009)**. Globally, red wines contain more phenolic compounds than white wines. It is caused by the technology of winemaking, when making white wines the grapes' skin is removed before fermentation **(Beer et al., 2006)**. The total polyphenols in wine besides variety of grapes, locality of growing, climatic conditions, are affected also by procedure of winemaking: length of contact of stum with grapes's skin, mixing, temperature, content of SO_2, pH value, content of alcohol etc. **(Villano et al., 2006; Lachman and Šulc 2006)**.

Cabernet Sauvignon (CS) is perhaps best known, most popular and one of most cultivated blue grapevine varieties in the world. This variety gives a lower harvest, wines are full-bodied, higher acids and polyphenols content (tannins and dyes) and excellent aging potential. Variety has traditionally mixing with other blue sort to achieve overall softer feel and a more balanced wine taste. Colder climate of Central Europe often makes the aroma of Slovak Cabernets with flavour of green pepper and grass

denouncing the lack of ripeness of the grapes. Cabernet Sauvignon is grown mainly in southwestern France, where this variety spread around the world (northern Italy, USA, South Africa, Australia, South America). In Slovakia, CS grown at about 13% of the areas planted with blue grapevine varieties and CS is the third most cultivated blue variety after Blaufränkisch and St. Laurent **(Ďurčová, 2011**; **Šajbidorová, 2012)**.

The purpose of this study was to determine and evaluate chosen antioxidant and sensory properties (the content of total polyphenols, content of total anthocyans, antioxidant activity and wine colour density) and their mutual correlations in red wine samples – Cabernet Sauvignon, originated from different Slovak vineyard areas.

MATERIAL AND METHODOLOGY

Chemicals and instruments

All analysed parameters – total polyphenol content, total anthocyanin content, antioxidant activity and wine colour density in wines were analyzed using UV/VIS spectrophotometry (spectrophotometer Shimadzu UV/VIS – 1240, *Shimadzu, Japan*). The chemicals used for all analysis were: Folin-Ciocalteau reagent, monohydrate of gallic acid p.a., anhydrous natrium carbonate p.a., citric acid p.a., dodecahydrate of disodium hydrogen phosphate, 35% hydrochloric acid p.a., ethanol p.a., methanol p.a., 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical p.a.

Samples

Analysed, bottled, red, especially quality and dry wines

Table 1 Characteristics of analysed Cabernet Sauvignon wine samples.

Sample	Producer	Vineyard area	Vintage	Quality
LC-1	Vitis Pezinok / Hubert J.E. Sereď	Little Carpathian	2008	quality
LC-2	Bočko Víno, Šenkvice	Little Carpathian	2008	quality
LC-3	VPS, Pezinok	Little Carpathian	2010	quality
LC-4	Víno Jano, Limbach	Little Carpathian	2009	quality
LC-5	Villa Víno Rača, Bratislava	Little Carpathian	2013	quality
SS-1	Vitis Pezinok / Hubert J.E. Sereď	South Slovak	2007	quality
SS-2	Villa Víno Rača, Bratislava	South Slovak	2008	quality
SS-3	Víno Matyšák, s.r.o., Pezinok	South Slovak	2010	quality
SS-4	VINIDI, s.r.o., Bratislava	South Slovak	2008	late harvest
SS-5	Vinárske závody Topoľčianky	South Slovak	2010	quality
SS-6	Hubert J.E., Sereď	South Slovak	2007	quality
SS-7	Malokarpatská vinohrad. spol., Pezinok	South Slovak	2009	quality
N-1	Víno Nitra, Nitra	Nitra	2009	quality
N-2	Chateau Modra, Modra	Nitra	2009	late harvest
N-3	Vinárske závody Topoľčianky	Nitra	2006	quality
N-4	Vinárske závody Topoľčianky	Nitra	2009	quality
N-5	Víno Nitra, Nitra	Nitra	2009	quality
N-6	Mrva a Stanko, Trnava	Nitra	2011	grapes selection
ES-1	J&J Ostrožovič, Veľká Tŕňa	East Slovak	2009	quality
ES-2	PD Vinohrady, Choňkovce	East Slovak	2008	late harvest
ES-3	PD Vinohrady, Choňkovce	East Slovak	2007	grapes selection
ES-4	Pivnica Tibava, Tibava	East Slovak	2008	quality
ES-5	Pivnica Tibava, Tibava	East Slovak	2009	quality
CS-1	Agro Movino, Veľký Krtíš	Central Slovak	2009	quality
CS-2	Agro Movino, Veľký Krtíš	Central Slovak	2010	quality
CS-3	Agro Movino, Veľký Krtíš	Central Slovak	2011	grapes selection
CS-4	Agro Movino, Veľký Krtíš	Central Slovak	2011	quality
CS-5	L. Korcsog, Korvinum, Rykynčice	Central Slovak	2011	late harvest

Cabernet Sauvignon (CS) and their characteristics are mentioned in Table 1. Wine samples with origin in various Slovak vineyard areas (VA) were purchased in retail network, to provide that analysed samples of wine would have the same properties as wines that are consumed by common consumers (properties of wine affected by various factors, such as period and conditions of storage or distribution of wine).

Antioxidant activity determination

Antioxidant activity (AA) was assessed by method of **Brand-Williams et al., (1995)** using of DPPH (1,1-diphenyl-1-picrylhydrazyl) radical. Absorbance was read at 515.6 nm and antioxidant effectiveness was expressed as % inhibition of DPPH (quantitative ability of tested compound to remove in certain period a part of DPPH radical).

Determination of total polyphenol content

Total polyphenol content (TPC) was determined by modified method of **Singleton and Rossi (1965)**. 0.1 mL of wine sample was pipetted into 50 mL volumetric flask and diluted with 5 mL of distilled water. To diluted mixture 2.5 mL Folin-Ciocalteau reagent was added and after 3 minutes 7.5 mL of 20% solution of Na_2CO_3 was added. Then the sample was filled with distilled water to volume 50 mL and after mixing left at the laboratory temperature for 2 hours. By the same procedure the blank and calibration solutions of gallic acid were prepared. Absorbance of samples solutions was measured against blank at 765 nm. The content of total polyphenols (TP) in wines was calculated as amount of gallic acid equivalent (GAE) in mg per 1 litre of wine.

Determination of total anthocyanin content

Total anthocyanin content (TAC) was assessed by modified pH differential method of **Lapornik et al., (2005)**. The principle of this method is reduction of the pH of wine samples with hydrochloric acid to values 0.5 – 0.8 associated with the transformation of all anthocyans to red colored flavilium cation. The content of total anthocyanins (TA) was calculated from the difference absorbance values of both solutions (origin and acidified) and expressed as the amount of anthocyans in mg per 1 liter of wine.

Determination of wine colour density

Wine colour density (WCD) was assessed by method of **Sudrand (1958)** as the sum of the absorbance at 420 nm and 520 nm. The absorbance of the wine samples was measured in 0.2 cm path lenth glass cells.

All analyses were performed as four parallels.

Statistical analysis

Statistical analysis was performed using the software Statistica 6.0 (StatSoft, Czech Republic) and the results were evaluated by analysis of variance ANOVA.

RESULTS AND DISCUSSION

All studied parameters – the content of total polyphenols, the content of total anthocyanins, antioxidant activity and wine colour density of the Slovak wines Cabernet Sauvignon are described in Table 2.

Antioxidant activity in analysed wine samples was in range 69.0 – 84.2% inhibition of DPPH. Average value of AA was 78.8% inhibition of DPPH. The average value of AA in Cabernet Sauvignon wines is a slightly lower than we found out in the other two major Slovak red wines Blaufränkisch – 83.3% and St. Laurent – 81.2% inhibition of DDPH **(Bajčan et al., 2012)**, but slightly higher compared to Slovak Alibernet wine samples – 74.0% inhibition of DPPH **(Bajčan et al., 2015)**. Similar results of AA reported **Slezák (2007)** and **Špakovská et al., (2012)**, who found out AA in Slovak wines – Cabernet Sauvignon in range from 71.6 to 90.9% inhibition of DPPH. On the basis of value of AA an order could be as following: wines from Little Carpathian VA > wines from East Slovak VA > wines from Central Slovak VA > wines from Nitra VA > wines from South Slovak VA. Gained results did not exert statistically significant differences (at significance level $p = 0.05$) between values of antioxidant activity in wines made in various vineyard areas in Slovakia.

Total polyphenol content in analysed wine samples was in the range from 1,218 to 3,444 mg GAE.L^{-1}. Average content of TP was 2,424 mg GAE.L^{-1}. The average content of total polyphenols in wines - Cabernet Sauvignon is a little higher than we found out in the other two major Slovak varietal red wines Blaufränkisch – 2,003 mg GAE.L^{-1} and St. Laurent – 2,297 mg GAE.L^{-1} **(Bajčan et al., 2012)**. On the other hand, average content of TP in Slovak Cabernet Sauvignon wines was much lower than we determined in Alibernet wines – 3,057 mg GAE.L^{-1} **(Bajčan et al., 2015)**. The results are similar to results reported by **Slezák (2007)** and **Špakovská et al., (2012)**, who found out the content of TP in Slovak wines – Cabernet Sauvignon in range from 2,150 to 3,102 mg GAE.L^{-1}. Other (foreign) scientists **(Kondrashov et al., 2009; Burin et al., 2010**; **Yoo et al., 2011)** analyzing TPC in CS wines reported also very similar results (1,453 – 3,589 mg GAE.L^{-1}). **Cliff et al., (2007)** reported much lower average value of TPC (1,055 mg GAE.L^{-1}) in CS wines originated in British Columbia, Canada what is probably due to cold weather and lack of mature grapes. According to the average value of TPC an order for wines could be as following: wines from Central Slovak VA > wines from South Slovak VA > wines from Nitra VA > wines from Little Carpathian VA > wines from East Slovak VA. Gained results exerted statistically significant differences (at significance level $p = 0.05$) between TPC in wines made in East Slovak VA and TPC in wines made in Central Slovak VA, resp. South Slovak VA.

Total anthocyanin content in analysed wine samples was in the range from 68.6 to 430.7 mg.L^{-1}. Average content of TA was 220.6 mg.L^{-1}. The average TAC in wines Cabernet Sauvignon is significantly lower than we found out in the other three Slovak varietal red wines Blaufränkisch – 266.1 mg.L^{-1}, St. Laurent – 264 mg.L^{-1} and Alibernet – 403 mg.L^{-1} (**Bajčan et al., 2015; Tóth et al., 2011)**. According to the average value of TAC an order for wines could be as following: wines from Central Slovak VA > wines from Nitra VA > wines from Little Carpathian VA > wines from South Slovak VA > wines from East Slovak VA. Gained results exerted statistically significant differences between TAC in wines made in

East Slovak VA and TAC in wines made in Central Slovak VA, resp. Nitra VA.

Wine colour density in analysed wine samples was in range from 0.756 to 2.782. Average value of WCD was 1.399. The average value of WCD in wines Cabernet Sauvignon is a little higher than we found out in the other two major Slovak varietal red wines Blaufränkisch – 1.110 and St. Laurent – 1.224 **(Tóth et al., 2011)**. But on the other hand, average value of WCD in Slovak Cabernet Sauvignon wines was much lower than we determined in Alibernet wines – 2.317 **(Bajčan et al., 2015)**. This is the first study monitoring WCD in Slovak wines Cabernet Sauvignon, so we can´t compare our data with other scientists. The results are little higher to results reported by **Poiana et al., (2007)**, who found out WCD in Romanian wines - Cabernet Sauvignon in range from 0.708 to 1.474 (average value – 1.206).

According to the average value of WCD an order for

Table 2 The content of total polyphenols (TPC), content of total anthocyanins (TAC), antioxidant activity (AA) and wine colour density (WCD) in analysed wines.

Sample	TPC mg GAE.L^{-1}	TAC Mg.L^{-1}	AA %	WCD
LC-1	2,206 ±22	82.5 ±2.7	82.9 ±2.7	1.059 ±0.006
LC-2	1,926 ±23	246.3 ±3.7	79.1 ±3.3	1.182 ±0.004
LC-3	2,667 ±46	246.9 ±4.2	82.1 ±3.8	0.896 ±0.011
LC-4	2,237 ±117	151.1 ±5.3	80.1 ±2.5	1.177 ±0.015
LC-5	2,642 ±30	282.4 ±2.8	79.8 ±0.8	1.449 ±0.012
Average LCVA	***2,336 ±308^{a}***	***201.8* ±85.8^{a}**	***80.8 ±1.6^{a}***	***1.153* ±0.237^{a}**
SS-1	2,215 ±46	68.6 ±3.1	79.5 ±2.6	1.137 ±0.021
SS-2	2,267 ±46	208.2 ±1.6	81.8 ±1.4	1.064 ±0.009
SS-3	2,966 ±46	292.7 ±2.8	77.1 ±2.6	1.385 ±0.012
SS-4	2,634 ±22	206.4 ±7.4	75.9 ±1.7	1.608 ±0.008
SS-5	2,886 ±22	330.8 ±7.7	76.6 ±2.0	1.861 ±0.008
SS-6	3,365 ±22	111.8 ±7.4	73.5 ±3.8	1.927 ±0.019
SS-7	2,118 ±44	152.3 ±2.5	80.5 ±2.5	1.053 ±0.015
Average SSVA	***2,636 ±461^{b}***	***195.8* ±97.0^{b}**	***77.8 ±3.1^{b}***	***1.434* ±0.323^{b}**
N-1	1,632 ±69	103.9 ±0.7	81.1 ±1.7	1.096 ±0.006
N-2	2,747 ±44	330.0 ±2.1	76.2 ±2.0	1.801 ±0.014
N-3	2,513 ±46	272.1 ±5.6	80.0 ±3.9	1.426 ±0.018
N-4	2,885 ±68	293.5 ±3.5	69.0 ±1.8	2.782 ±0.023
N-5	2,628 ±23	162.6 ±6.7	84.2 ±4.0	1.076 ±0.005
N-6	2,798 ±43	363.3 ±8.4	77.4 ±1.0	1.968 ±0.021
Average NVA	***2,534 ±495^{c}***	***254.2* ±102.5^{c}**	***78.0 ±3.2^{c}***	***1.691* ±0.674^{c}**
ES-1	1,270 ±23	147.5 ±3.8	71.2 ±4.8	1.066 ±0.011
ES-2	2,206 ±22	84.9 ±3.2	81.9 ±1.2	1.159 ±0.010
ES-3	2,268 ±44	77.3 ±2.1	79.9 ±1.3	1.105 ±0.017
ES-4	2,230 ±22	120.6 ±5.3	83.8 ±2.0	0.888 ±0.009
ES-5	1,218 ±23	111.8 ±2.5	78.7 ±2.7	0.756 ±0.008
Average ESVA	***1,838 ±451^{bd}***	***108.4* ±30.2^{cd}**	***79.1 ±5.4^{d}***	***0.995* ±0.173^{bd}**
CS-1	2,409 ±23	236.8 ±3.1	79.9 ±1.7	1.175 ±0.004
CS-2	2,359 ±46	421.2 ±23.9	82.4 ±2.9	1.509 ±0.016
CS-3	3,444 ±91	430.7 ±9.5	74.4 ±3.1	2.095 ±0.022
CS-4	2,873 ±46	341.1 ±5.6	78.8 ±3.7	1.805 ±0.023
CS-5	2,275 ±31	299.8 ±4.9	78.6 ±1.1	1.667 ±0.017
Average CSVA	***2,672 ±502^{d}***	***345.9* ±83.3a^{d}**	***78.8 ±3.4^{e}***	***1.650* ±0.397^{d}**
Total average	**2,424 ±537**	**220.6 ±106.4**	**78.8 ±3.7**	**1.399 ±0.483**

NOTE: Values of TPC, TAC, AA and WCD are expressed as arithmetic average ±standard deviation.
[a-e] Values with the same letters denote significant differences (p <0.05) among vineyard areas.
LCVA – Little Carpathian vineyard area, SSVA – South Slovak vineyard area, NVA – Nitra vineyard area, ESVA – East Slovak vineyard area, CSVA – Central Slovak vineyard area.

wines could be as following: wines from Nitra VA > wines from Central Slovak VA > wines from South Slovak VA > wines from Little Carpathian VA > wines from East Slovak VA. Gained results exerted statistically significant differences (at significance level $p = 0.05$) between WCD in wines made in East Slovak VA and WCD in wines made in Central Slovak VA, and South Slovak VA.

In order to investigate the mutual relations between analyzed parameters, the linear regressions were obtained. The statistical evaluation of the obtained results did not confirm any linear correlations between TPC and AA, resp. TAC and AA (r = -0.255, resp. r = -0.279) at significance level $p < 0.1$. This is not in the agreement with the study of **Burin et al., (2010)**, **Kondrashov et al., (2009)** and **Balík et al., (2008)** who found out very strong linear correlations between TPC, resp. TAC and AA in wines and grape juices. Explanation lies in the differences in the methodology of AA determination. The correlations between TPC and TAC (r = 0.542), resp. TAC and WCD (r = 0.600) were highly significant at significance level $p < 0.01$. **Cioroi and Musat (2007)** reported stronger correlation between TPC and TAC (r = 0.739 and 0.771) in red wines. The statistical evaluation of the obtained results confirmed very highly significant correlations at significance level $p < 0.001$ between WCD and TPC, resp. WCD and AA (r = 0.697, resp. r = - 0.714).

CONCLUSION

Slovak red wines – Cabernet Sauvignon have high antioxidant activity (average value 78.8% inhibition of DPPH), high content of healthy useful phenolic compounds (average value of TPC 2,424 mg GAE.L^{-1}), moderate value of TAC (average value 220.6 mg.L^{-1}) and good colour (average value of WCD 1.399). The results showed statistically significant differences for 3 studied parameters (TPC, TAC and WCD) in wines made in some vineyard areas in Slovakia. On the basis of statistical evaluation of our results, statistically significant correlations were demonstrated between wine colour density and other 3 parameters (TPC, TAC and AA), resp. between TPC and TAC.

REFERENCES

Bajčan, D., Čéryová, S., Tomáš, J. 2012. Antioxidant properties of the bestselling Slovak red wines. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 1, no. 4, p. 455-465.

Bajčan, D., Šimanský, V., Tóth, T., Árvay, J. 2015. Colour, Phenolic content and antioxidant activity of the Slovak Alibernet red wine samples. In *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 4, special no. 3, p. 5-8.

Balík, J., Kyseláková, M., Vrchotová, N., Tříska, J., Kumšta, M., Veverka, J., Híc, P., Toušek, J., Lefnerová, D. 2008. Relations between polyphenols content and antioxidant activity in vine grapes and leaves. *Czech Journal of Food Science*, vol. 26, p. S25-S32.

Beer, D., Joubert, E., Marais, J., Manley, M. 2006. Unravelling the total antioxidant capacity of Pinotage wine: contribution of phenolic compounds. *Journal of Agricultural and Food Chemistry*, vol. 54, no. 8, p. 2897-2905. http://dx.doi.org/10.1021/jf052766u

Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie*, vol. 28, no. 1, p. 25-30. http://dx.doi.org/10.1016/s0023-6438(95)80008-5

Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition reviews*, vol. 56, no. 11, p. 317-333. http://dx.doi.org/10.1111/j.1753-4887.1998.tb01670.x

Burin, V. M., Falcao, L. D., Chaves, E. S., Gris, E. F., Preti, L. F., Bordignon-Luiz, T. 2010. Phenolic composition, colour, antioxidant activity and mineral profile of Cabernet Sauvignon wines. *International Journal of Food Science and Technology*, vol. 45, no. 7, p. 1505-1512. http://dx.doi.org/10.1111/j.1365-2621.2010.02296.x

Cioroi, M., Musat, C. L. 2007. Investigations on the correlations between polyphenol content from red wines and their antioxidant activity. *Cercetari Agronomice in Moldova*, vol. 132, no. 4, p. 35-41.

Cliff, M. A., King, M. C., Schlosser, J. 2007. Anthocyanin, phenolic composition, colour measurement and sensory analysis of BC commercial red wines. *Food Research International*, vol. 40, no. 1, p. 92-100. http://dx.doi.org/10.1016/j.foodres.2006.08.002

Ďurčová, E. 2011. Sauvignon blanc a Cabernet sauvignon. *Vinič a vino*, vol. 11, p. 63-64.

Kondrashov, A., Ševčík, R., Benáková, H., Kostířová, H., Štípek, S. 2009. The key role of grape variety for antioxidant capacity of red wines. *The European e-Journal of Clinical Nutrition and Metabolism*, vol. 4, no. 1, p. 41-46. http://dx.doi.org/10.1016/j.eclnm.2008.10.004

Lachman, J., Šulc, M. 2006. Phenolics and antioxidant activity of wines during the winemaking process. *Bornimer Agrartechnische Berichte*, vol. 55, p. 161-168.

Lapornik, B., Prošek, M., Wondra, A. G. 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering*, vol. 71, no. 2, p. 214-222. http://dx.doi.org/10.1016/j.jfoodeng.2004.10.036

Lorrain, B., Ky, I., Pechamat, L., Teissedre, P. L. 2013. Evolution of analysis of polyphenols from grapes, wines, and extracts. *Molecules*, vol. 18, no. 1, p. 1076-1100. http://dx.doi.org/10.3390/molecules18011076 PMid:23325097

Poiana, M. A., Moigradean, D., Gergen, I., Harmanescu, M. 2007. The establishing the quality of red wines on the basis of chromatic characteristics. *Journal of Agroalimentari Proccesses and Technologies*, vol. 13, no. 1, p. 199-208.

Singleton, V. L., Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, vol. 16, no. 3, p. 144-158.

Slezák, F. 2007. Preserving of antioxidant components in wines from Little Carpathian region: research report. Biocentrum Modra and VÚP Bratislava, Modra, Slovakia. 19 p.

Sudrand, P. 1958. Interpretation des d´absorption des vins rouges. *An. Technology and Agriculture*, vol. 7, p. 203-208.

Šajbidorová, V. 2012. Grapes, grape wine: situation and outlook report to 31. 7. 2012. Research Institute of Agricultural and Food Economics, Bratislava, Slovakia, 40 p.

Špakovská, E., Marcinčák, S., Bača, M., Turek, P. 2012. Polyphenolic content and antioxidative activity of wines from Sobrance region. *Potravinarstvo*, vol. 6, no. 3, p. 32-35. http://dx.doi.org/10.5219/204

Tóth, T., Bajčan, D., Vollmannová, A., Trebichalský, P., Lahučký, L., Miššík, J. 2011. The phenolic content and antioxidant activity of varietal Slovak red wines. *Chemiské Listy*, vol. 105, no. S6, p. 1029-1030.

Urpi-Sarda, M., Monagas, M., Khan, N., Lamuela-Raventos, R. M., Santos-Buelga, C., Sacanella, E., Castell, M., Permanyer, J., Andres-Lacueva, C. 2009. Epicatechin, procyanidins, and phenolic microbial metabolites after cocoa intake in humans and rats. *Analytical and Bioanalytical Chem*istry, vol. 394, no. 6, p. 1545–1556. http://dx.doi.org/10.1007/s00216-009-2676-1

Villano, D., Fernández-Pachón, M. S., Troncoso, A. M., García-Parrilla, M. C. 2006. Influence of enological practices on the antioxidant capacity and total polyphenols. *Food Chemistry*, vol. 95, no. 3, p. 394-404. http://dx.doi.org/10.1016/j.foodchem.2005.01.005

Weisshaar, B., Jenkins, G. I. 1998. Phenylpropanoid biosynthesis and its regulation. *Current Opinion in Plant Biology*, vol. 1, no. 3, p. 251-257. http://dx.doi.org/10.1016/S1369-5266(98)80113-1

Winkel-Shirley, B. 2002. Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology*, vol. 5, no. 3, p. 218-223. http://dx.doi.org/10.1016/S1369-5266(02)00256-X

Yoo, Y. J., Prenzler, P. D., Saliba, A. J., Ryan, D. 2011. Assessment of some Australian red wines for price, phenolic content, antioxidant activity and vintage in relation to functional food prospects. *Journal of Food Science*, vol. 76, no. 9, p. 1355-1364. http://dx.doi.org/10.1111/j.1750-3841.2011.02429.x

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DETERMINATION OF HEAVY METALS CONCENTRATION IN RAW SHEEP MILK FROM MERCURY POLLUTED AREA

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ABSTRACT

The paper focuses on determining the content of monitored contaminants (Cd, Cu, Hg, Pb and Zn) in 53 samples of raw sheep milk collected in 2013 and 2014 on the sites Poráč and Matejovce nad Hornádom (middle Spiš). The area is characterized by historical mining and metalworking activity (mining and processing of polymetallic ores rich in Hg, Cd and Pb). Currently, the area is one of the most mercury contaminated areas in Central Europe. All statistical analyses were carried out using the statistical software Statistica 10.0 *(*Statsoft, USA). Descriptive data analysis included minimum value, maximum value, arithmetic mean and standard deviation. The results of the studied contaminant content show that the limit value for cadmium (10 $\mu g.kg^{-1}$) was exceeded in 25 samples. In the case of lead, the limit value of 20 $\mu g.kg^{-1}$ was exceeded in 16 cases. The limit value for copper (0.4 $mg.kg^{-1}$) was exceeded in one case. The limit value for zinc is not defined by a legislative standard. The risk level of the studied contaminants in the samples of raw sheep milk decreases as follows: Cd > Pb > Hg > Cu > Zn. It can be concluded that frequent and long-term consumption of the raw sheep milk originating from the studied sites poses a health risk. The content of the contaminants in the milk and their eventual transition into dairy products should be monitored over a longer term in more detail.

Keywords: former mercury mining area; health hazard; heavy metal; raw sheep milk; Slovakia

INTRODUCTION

Heavy metals and/or trace elements are ubiquitous components of the environment that may be of natural origin: volcanic activity, fires, geogenic origin **(Rutter et al., 2008)**, or anthropogenic origin: metal industry, mining, heavy industry, transportation **(Cui et al., 2005; Navarro et al., 2008; Singh et al., 2005)**. Increasing level of environmental contamination is directly correlated with the level of industrialization **(Tubaro and Hungerford, 2007)**. Metalworking industry and mining of minerals that contain hazardous heavy metals represent a major risk of the environmental contamination, especially of local nature.

Consuming local food poses the greatest risk of intoxication of the consumers by heavy metals that consequently affects their health. **Loutfy et al., (2006)** reported that consumers receive 90% of the total amount of heavy metals by consumption of food from contaminated areas. As a result, human exposure to toxic metals has become a major health risk. Chronic intake of heavy metals above their safe threshold by humans and animals has damaging effects and can cause non-carcinogenic hazards, such as neurologic involvement, headache and liver disease **(John and Andrew, 2011; Lai et al., 2010)**.

Children are particularly sensitive to increased concentrations of heavy metals (especially Hg, Cd and Pb) and arsenic because their tissues and organs accumulate high concentrations of contaminants reflecting in their health during the process of their development and growth. Central nervous system is especially sensitive due to its progressive development and even small amounts of heavy metals can cause irreversible processes resulting in mental retardation and behavioral disorders **(Ataro et al., 2008)**.

Milk and dairy products contain many essential nutrients and their regular consumption is recommended, especialy for young children **(Maas et al., 2011)**. Sheep raw milk has a higher content of essential vitamins and minerals than cow's milk and could be used to cater to consumers' appetite for healthy and safer products **(Bogdanovičová et al., 2015)**. Ovine milk is the most completed natural fluid, one of the most important basic and healthiest raw materials, which plays important role in the dairy nutrition of all population **(Lačanin et al., 2015)**. However, milk and dairy products may contain varying amounts of different toxic contaminants, especially heavy metals **(Ataro et al., 2008)**. In recent years, several reports have indicated the presence of heavy metals in milk and other dairy products **(Kazi et al., 2009; Soylak et al., 2005; Tuzen et al., 2008)**. Due to the fact that milk and milk products are very common food, it is necessary to make great efforts to control the content of the monitored contaminants and at the same time to monitor the quality of individual environmental components that are the main sources of heavy metals in the human food chain **(Caggiano et al., 2005)**.

The paper focuses on the evaluation of the contamination level of raw sheep milk by heavy metals (Cd, Cu, Hg, Pb

and Zn). The studied area was formerly characterized by important mining and metal processing activities **(Angelovičová and Fazekašová, 2014)**. Currently, the area is considered as one of the most contaminated sites by mercury, cadmium and lead in Slovakia, but also in Central Europe, which is significantly reflected in the quality of the grown vegetables **(Slávik et al., 2014)**, edible wild mushrooms **(Árvay et al., 2015; Svoboda et al., 2006)**. Thus it is assumed that it will be reflected in the quality and contamination level of the monitored raw sheep milk, the production of which belongs to the major and characteristic agricultural activities in the area.

MATERIAL AND METHODOLOGY

Samples collection

Samples of fresh sheep milk (N = 53) were obtained during 2013 and 2014 from identical individuals in two locations: Poráč (N = 20) and Matejovce nad Hornádom (N = 33). Immediately after the milking, the samples were temporarily stored in PE centrifuge bottles (50 cm^3) and frozen. Just before the analysis, the milk samples were defrosted at room temperature, filtered, homogenized and subsequently analytically processed.

Pre-analytical and analytical procedure

Frozen samples of the sheep milk were defrosted at room temperature just before the analysis. Subsequently, the samples were homogenized by shaking and 2 g were weighed and poured into mineralization tubes. The homogenized sheep milk samples were mineralized in a closed system of microwave digestion using Mars X-Press 5 (CEM Corp., Matthews, NC, USA) in a mixture of 5 cm^3 of HNO_3 (SupraPUR, Merck, Darmstadt, Germany) and 5 cm^3 of deionized water (0.054 $\mu S.cm^{-1}$) from Simplicity185 (Millipore SAS, Molsheim, France). Digestion conditions for the applied microwave system comprised of the heat, which ran up to 150 °C for 10 minutes and was kept at the constant temperature for 10 minutes. A blank sample was carried out in the same way. The sample was subsequently filtered through a quantitative filter paper Filtrak 390 (Munktell & Filtrak GmbH, Bärenstein, Germany) and filled up with deionized water to a volume of 50 cm^3 **(Árvay et al, 2014)**.

The contents of the studied contaminants were determined by flame atomic absorption spectrometry: F-AAS (Cu and Zn) on the SpectrAA 240 FS (Varian Inc., Mulgrave, VIC, Australia), electrothermal atomic absorption spectrometry: GF-AAS (Cd and Pb) with Zeemann background correction on the SpectrAA 240 Z (Varian Inc., Mulgrave, VIC, Australia). Total mercury content (THg) was determined directly in the liquid milk samples (200 µL) by a selective mercury analyzer AMA-254 (Altec, Praque, Czech Republic) based on CV–AAS. Detection limit for F-AAS was 2.0, 0.6 $\mu g.kg^{-1}$ for Cu and Zn, respectively. Detection limit for GF-AAS was 10.0 $ng.kg^{-1}$ for both Cd and Pb. Detection limit for mercury was 1.5 $ng.kg^{-1}$. Certipur® (Merck, Darmstadt, Germany) calibration solution was used for the calibration of all instruments.

Statistical analysis and risks assessment

All statistical analyses were carried out using the statistical software Statistica 10.0 (Statsoft, USA). Descriptive data analysis included minimum value, maximum value, arithmetic mean and standard deviation. The limit of the statistical significance was set up at $p < 0.05$ for all descriptive statistical analyses. To evaluate a health risk resulting from the consumption of raw sheep milk, the obtained data on the content of the studied contaminants were compared with limit values defined by the Codex Alimentarius of the Slovak Republic **(PK SR, 2006)** and EC Regulation 1881/2006 **(EC, 2006)**.

Table 1 Heavy metals in sheep milk samples with descriptive statistics.

Year of collection	Number of samples	Heavy metals in sheep milk samples Median ±SD (range)				
		Hg $\mu g.kg^{-1}$	**Cd** $\mu g.kg^{-1}$	**Pb** $\mu g.kg^{-1}$	**Cu** $mg.kg^{-1}$	**Zn** $mg.kg^{-1}$
		Matejovce nad Hornádom				
2013	19	0.138 ±0.587 (0.079 – 0.286)	1.66 ±1.86 (0.71 – 9.07)	17.3 ±46.5 (ND – 193)	0.14 ±0.06 (0.08 – 0.26)	3.41 ±1.63 (1.02 – 8.04)
2014	14	0.220 ±0.121 (0.063 – 0.450)	8.35 ±9.31 (2.95 – 30.5)	13.6 ±28.3 (ND – 93.6)	0.16 ±0.08 (0.10 – 0.36)	5.51 ±1.07 (4.43 – 7.93)
		Poráč				
2013	10	0.061 ±0.016 (0.036 – 0.079)	12.9 ±4.33 (9.19 – 22.2)	7.85 ±33.9 (6.05 – 113)	0.07 ±0.44 (0.02 – 1.47)	5.53 ±0.99 (3.53 – 6.01)
2014	10	0.068 ±0.025 (0.025 – 0.103)	22.2 ±13.3 (10.1 – 52.9)	12.5 ±9.83 (5.73 – 39.5)	0.12 ±0.14 (0.02 – 0.51)	5.64 ±1.28 (4.10 – 7.97)
Maximum Allowable Levels		**50**[a]	**10**[a]	**1000**[a] **20**[b]	**0.4**[a]	--

NOTE: ND – not detected, SD – standard deviation.

[a] Maximum allowable levels of monitored heavy metals - Codex allimentarius of Slovakia **(PKSR, 2006)**.

[b] Maximum allowable levels of monitored heavy metals - Commission regulation (EC) 1881/2006 **(EC, 2006)**.

RESULTS AND DISCUSSION

The contents of the studied heavy metals together with the basic statistical indicators are shown in Table 1. Due to the fact that the sites of interest were characterized in the past by intensive extraction and processing of mercury **(Árvay et al., 2014)**, mercury is considered to be the main heavy metal in terms of quality assessment of the raw sheep milk in this paper. Its content varied in relatively wide intervals within the years, as well as the sites. The highest concentration in terms of the site was recorded in Matejovce nad Hornádom where the mean value of Hg was 0.138 $\mu g.kg^{-1}$ of the raw sheep milk in 2013 and 0.220 $\mu g.kg^{-1}$ in 2014. The mean value of the Hg content in the milk from Poráč was about one order of magnitude lower: 0.061 $\mu g.kg^{-1}$ in 2013 and 0.068 $\mu g.kg^{-1}$ in 2014. The data are balanced also within the set, as evidenced by the lower standard deviation (Table 1) in comparison with the variability of the Hg values obtained from Matejovce. Such significant differences in the content of the studied contaminant from the sites that are about 2 km apart are due to a significant difference in the atmospheric distribution of emissions from the sources. This is confirmed by other studies **(Angelovičová and Fazekašová, 2014; Svoboda et al., 2000)**. The mercury content in the milk samples from the both sites did not exceed the maximum level of 50 $\mu g.kg^{-1}$ set by the Codex Alimentarius SR **(PKSR, 2006)**.

The content of cadmium, which is an accompanying element in polymetallic ores mined in the area of interest ranged in a much wider intervals. It is evidenced by the extremely high standard deviations (Table 1). The mean values of Cd content were 9.12 $\mu g.kg^{-1}$ (2013) and 22.2 $\mu g.kg^{-1}$ (2014) in the Poráč area and 1.66 $\mu g.kg^{-1}$ (2013) and 8.35 $\mu g.kg^{-1}$ (2014) in the Matejovce area. The highest concentration of cadmium was recorded in the Poráč area in 2014 (52.9 $\mu g.kg^{-1}$). Large differences in the cadmium content in the sheep milk samples can be caused by several factors such as: seasonality, climatic conditions and variability of feed ration **(Rahimi, 2013)**, since the samples were obtained during outdoor breeding and pasturing. Hygiene standard defined by the Codex Alimentarius sets the contaminant content at 10 $\mu g.kg^{-1}$. The obtained results show that the limit value was exceeded in 6 out of 53 samples taken in Matejovce in 2014. In the Poráč, 9 samples exceeded the limit value in 2013 and 10 in 2014. It can be stated that the cadmium content exceeded the limit value in almost 50% of all samples of the raw sheep milk.

The lead content in the milk samples varied at different intervals, depending on the site. The mean Pb content was 7.85 $\mu g.kg^{-1}$ (2013) and 12.5 $\mu g.kg^{-1}$ (2014) in the Poráč area. Similarly to the cadmium, the lead content varied widely, which was reflected in the standard deviations (Table 1). In comparison with Matejovce, where the Pb content varied in a higher concentration: 3.17 $\mu g.kg^{-1}$ (2013) and 13.6 $\mu g.kg^{-1}$ (2014), the Poráč area seems to be less risky. However, the results show that both sites pose a potential risk resulting from the sheep milk consumption, since the limit value (20 $\mu g.kg^{-1}$) set by the EC Regulation 1881/2006 **(EC, 2006)** was exceeded in 9 (2013) and 3 samples (2014) taken from Matejovce and in 2 samples (2013 and 2014) taken from Poráč. Codex Alimentarius SR does not state a maximum allowed content of lead.

Copper and zinc are considered essential micronutrients and their content in food is desirable in an optimal amount **(Maas et al., 2011)**. The copper content in the milk samples varied in a relatively low concentrations compared with zinc. The mean value of the copper content was 0.07 $mg.kg^{-1}$ (2013) and 0.12 $mg.kg^{-1}$ (2014) in the samples from the Poráč site and 0.14 $mg.kg^{-1}$ (2013) and 0.16 $mg.kg^{-1}$ (2014) in the samples from the Matejovce site. Codex Alimentarius SR set the maximum level of copper to 0.40 $mg.kg^{-1}$. The limit value was not exeeded in any samples on the mean level. However, in the case of individual samples, the limit was exceeded in one sample from the Poráč site taken in 2013 (1.47 $mg.kg^{-1}$). It can be concluded that in terms of the copper content, consumption of the sheep milk does not pose a health risk. The mean values of the zinc content varied at higher levels. In the samples taken from the Poráč site, the Zn content was 5.53 $mg.kg^{-1}$ (2013) and 5.64 $mg.kg^{-1}$ (2014). The Zn content recorded in the samples taken from the Matejovce site was 3.41 $mg.kg^{-1}$ (2013) and 5.51 $mg.kg^{-1}$ (2014). The content of Zn was relatively balanced as evidenced by the relatively small standard deviations (Table 1). Due to the fact that no legislative standard defines limit values for Zn in milk, it is not possible to make conclusion on the hygienic quality of the sheep milk in terms of zinc content.

CONCLUSION

Evaluation of the contamination level of agricultural products and food ingredients is important in terms of maintaining an adequate health safety of human food chain components, especially in areas that are significantly contaminated by risk elements such as heavy metals. The contents of the studied contaminants in the raw sheep milk samples taken from two areas: Poráč and Matejovce nad Hornádom, ranged in various levels posing different degrees of health risk resulting from consumption of the milk. Mercury was assumed to pose the highest health risk, however, the hygiene standards for this element were not exceeded. The most hazardous contaminant was cadmium. The maximum allowed level of Cd (10 $\mu g.kg^{-1}$) was exceeded in 25 out of 53 samples. The limit value of lead (20 $\mu g.kg^{-1}$) was exceeded in 16 cases. The copper content exceeded the limit value (0.4 $mg.kg^{-1}$) in one case. The limit value for zinc is not defined by any legislation. It can be concluded that regular consumption of the sheep milk, in connection with intake of the studied contaminants from other sources, may pose a health risk in the long term. Therefore, it is necessary to monitor the contaminants in the milk, as well as milk products in long-term and more detail.

REFERENCES

Angelovičová, L., Fazekašová, D. 2014. Contamination of the soil and water environmentu by heavy metals in the former mining area of Rudňany, Slovakia. *Soil Water Research*, vol. 9, no. 1, p. 18-24.

Ataro, A., McCrindle, R. I., Botha, B. M., McCrindle, C. M. E., Ndibewu, P. P. 2008. Quantification of trace elements in raw cow´s milk by inductively coupled plasma mass

spectrometry (ICP-MS). *Food Chemistry*, vol. 111, no. 1, p. 243-248. http://dx.doi.org/10.1016/j.foodchem.2008.03.056

Árvay, J., Tomáš, J., Hauptvogl, M., Kopernická, M., Kováčik, A., Bajčan, D., Massányi, P. 2014. Contamination of wild-grown edible mushrooms by heavy metals in a former mercury-mining area. *Journal of Environmental Science and Helath – Part B*, vol. 49, no. 11, p. 815-827. http://dx.doi.org/10.1080/03601234.2014.938550 PMid:25190556

Árvay, J., Tomáš, J., Hauptvogl, M., Massányi, P., Harangozo, Ľ., Tóth, T., Stanovič, R., Bryndzová, Š., Bumbalová, M. 2015. Human exposure to heavy metals and possible public health risks via consumption of wild edible mushrooms from Slovak Paradise National Park, Slovakia. *Journal of Environmental Science and Helath – Part B*, vol. 50, no. 11, p. 838-848. http://dx.doi.org/10.1080/03601234.2015.1058107

Bogdanovičová, K., Skočková, A., Šťástková, Z., Koláčková, I., Karpíšková, R. 2015. The Bacteriological quality of goat and ovine milk. *Potravinarstvo*, vol. 9, no.1, p. 72-76. http://dx.doi.org/10.5219/438

Caggiano, R., Sabia, S., D´Emilio, M., Macchiato, M., Anastasio, A., Ragosta, M. 2005. Letal levels in fodder, milk, dairy products, and tissue sampled in ovine farms of southern Italy. *Environmental Research*, vol. 99, no. 1, p. 48-57. http://dx.doi.org/10.1016/j.envres.2004.11.002 PMid:16053927

Cui, Y., Zhu, Y., Zhai, R., Huang, Y., Qja, Y., Liang, J. 2005. Exposure to metal mixtures and human health impacts in a contaminated area in Nanning, China. *Environment International*, vol. 31, no. 6, p. 784-790. http://dx.doi.org/10.1016/j.envint.2005.05.025 PMid:15979144

European Commission – EC, 2006. Commission regulation (EC) No. 1881/2006 of 19. December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, L 364.

John, G. F., Andrew, B. A. 2011. Lead isotopic study of the human bioaccesibility of lead in urban soil from Glasgow, Scotland. *Sci. Tot. Environ.*, vol. 409, p. 4958-4965. http://dx.doi.org/10.1016/j.scitotenv.2011.08.061 PMid:21930292

Kazi, T. G., Jalbani, N., Baig, J. A., Kandhro, G. A., Afridi, H. H, Arain, M. B. 2009. Assessment of toxic metals in raw and processed milk samples using electrothermal atomic absorption spectrophotometer. *Food and Chemical Toxicology*, vol. 47, no. 9, p. 2163-2169. http://dx.doi.org/10.1016/j.fct.2009.05.035 PMid:19500636

Lačanin, I., Dušková, M., Kladnická, I., Karpíšková, R. 2015. Occurrence of enterococcus spp. isolated from the milk and milk products. *Potravinarstvo*, vol. 9, no. 1, p. 258-262. http://dx.doi.org/10.5219/476

Lai, H. Y., Hseu, Z. Y., Chen, T. C., Chen, B. C., Guo, H. Y., Chen, Z. S. 2010. Health risk-based assessment and management of heavy metals-contaminated soil sites in Taiwan. *International Journal of Environmental Research and Public Health*, vol. 7, no. 10, p. 3595-3614. http://dx.doi.org/10.3390/ijerph7103596 PMid:21139851

Loutfy, N., Fuerhacker, M., Tundo, P, Raccanelli, S., El Dien, A. G., Ahmed, M. T. 2006. Dietary intake of dioxins and dioxins-like PCBs, due to the consumption of dairy products, fish/seafood and meat from Ismalia city, Egypt. *Science of the Total Environment*, vol. 370, no. 1, p. 1-8. http://dx.doi.org/10.1016/j.scitotenv.2006.05.012 PMid:16806402

Maas, S., Lucot, E., Gimber, F., Crini, N., Badot, P. M. 2011. Trace metals in raw cow´s milk and assessment of transfer to Comté cheese. *Food Chemistry*, vol. 129, no. 1, p. 7-12. http://dx.doi.org/10.1016/j.foodchem.2010.09.034

Navarro, M. C., Perey-Sirvent, C., Martinez-Sanchez, M. J., Vidal, J., Tovar, P. J., Bech, J. 2008. Abandoned mine sites as a source of contamination by heavy metals: a case study in a semi-arid zone. *Journal of Geochemical Exploration*, vol. 96, no. 1-2, p. 183-193. http://dx.doi.org/10.1016/j.gexplo.2007.04.011

PKSR, 2006. Potravinový kódex Slovenskej republiky, výnos č. 18558/2006-SL. Nyjvyššie prípustné množstvá kontaminantov v potravinách platné v Slovenskej republike z 11. septembra 2006. (Food Codex of Slovak republic, decree no. 18558/2006-SL. Maximal alloved levels of contaminants in food in Slovak republic, from 11th of Sepbember 2006), Available at: http://www.svssr.sk/dokumenty/legislativa/18558_2006.pdf.

Rahimi, E. 2013. Lead and cadmium concentrations in goat, cow, sheep, and buffalo milks from different regions of Iran. *Food Chemistry*, vol. 136, no. 2, p. 389-391. http://dx.doi.org/10.1016/j.foodchem.2012.09.016 PMid:23122075

Rutter, A. P., Schauer, J. J., Lough, G. C., Snyder, D. C., Kolb, C. J., Von Klooster, S. E., Rudolf, T., Manolopoulos, H., Olson, M. L. 2008. A comparison of speciated atmospheric mercury at an urban center and an upwind rural location. *Journal of Environmental Monitoring*, vol. 10, no. 1, p. 102-108. http://dx.doi.org/10.1039/B710247J PMid:18175023

Singh, A. N., Zeng, D. H., Chen, F. S. 2005. Heavy metal concentrations in redeveloping soil mine spoil under plantationof certain native woody speciesin dry tropical environment, India. *Journal of Environmental Science*, vol. 17, no. 1, p. 169-174.

Slávik, M., Tóth, T., Árvay, J., Kopernická, M., Harangozo, Ľ., Stanovič, R., Trebichalský, P., Kavalcová, P. 2014. The heavy metals content in vegetables from middle Spiš area. *Journal of Microbiology, Biotechnology and Food Science*, vol. 2, special no., p. 277-280.

Soylak, M., Saracoglu, S., Tuzen, M., Mendil, D. 2005. Determination of trace metals in mushroom samples from Kayseri, Turkey. *Food Chemistry*, vol. 92, no. 4, p. 649-652. http://dx.doi.org/10.1016/j.foodchem.2004.08.032

Svoboda, L., Havlíčková, B., Kalač, P. 2006. Contents of cadmium, mercury and lead in ideble mushrooms growing in a historical silver-mining area. *Food Chemistry*, vol. 96, no. 4, p. 580-585. http://dx.doi.org/10.1016/j.foodchem.2005.03.012

Svoboda, L., Zimmermannová, K., Kalač, P. 2000. Concentration of mercury, cadmium, lead and copper in fruiting bodies of edible mushrooms in an emission area of a copper smelter and a mercury smelter. *Science of the Total Environment*, vol. 246, no. 1, p. 61-67. http://dx.doi.org/10.1016/S0048-9697(99)00411-8

Tubaro, A., Hungerford, J 2007. *Environmental Toxicology*. In: Gupta, RC. (Ed.), Veterinary Toxicology: Basic and clinical principles. Academic, New York, NY, USA, p. 663-725.

Tuzen, M., Saracoglu, S., Soylak, M. 2008. Evaluation of trace element contents of powdered beverages from Turkey.

Journal of Food Nutrition Research, vol. 47, no. 3, p. 120-124.

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BIOACCUMULATION OF CADMIUM BY SPRING BARLEY (*HORDEUM VULGARE* L.) AND ITS EFFECT ON SELECTED PHYSIOLOGICAL AND MORPHOLOGICAL PARAMETERS

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ABSTRACT

Heavy metals and other toxic elements in the environment, mainly located in soil and groundwater, have a significant effect on plant and its productivity that has a huge attention in recent years. Accumulation of heavy metals in soil cause toxicity to plants, and contaminate the food chain. The industrial areas, as well as developing countries have been contaminated with high concentration of heavy metals. Main sources of contamination are mining and other industrial processes, as well as military and or lanfills, sludge dumps or waste disposal sites. The heavy metals are very dangerous to environment and pose serious danger to public health by entering throught the food chain or into drinking water. Phytoextraction is one way how to remove the contaminants from soil by plants. Phytoextraction of heavy metals is a technology that has been studied for several years. It is more ecological and cheaper way how to clean our environment.Several plant species are known becauce they hyperaccumulate a high contents of metals from the soil. The accumulators are mainly herbaceous species, crops and nowadays angiosperm trees with a high growth such as poplars or willows. We have focused on the determination of some morphological (lenght and weight of roots and biomass) and physiological (contents of dry mass and number of lief stomata) characteristics and the determination of the bioaccumulation factor and the translocation factor of cadmium by spring barley (*Hordeum vulgare* L.). Imprints of leaves were evaluated using an optical microscope Axiostar Plus, Carl Zeiss, lens CP Achromat 40x/0.65, eyepiece PI 10x / 18, Canon Utilities Software Zoom Browser EX 4.6 and hardware Acer Travel Mate 4600, Canon Power Shot A95. The density of stomata was evaluated on an area of 1 mm^2. Samples of the dried plants (leaves and roots) were mineralized by acid digestion using microwave digestion device MARS X - press 5. The end of determination to obtain the cadmium content was performed by atomic absorption spectrometer Varian 240 Z with GTA120 graphite furnace. The effect of contamination by cadmium to germination, length of leaves and number of stomata on abaxial side of leaf was confirmed. The contaminated soil by cadmium does not pose a risk of heavy metal entry into the feed and food chain by spring barley (*Hordeum vulgare* L.).

Keywords: stomata; barley; phytoextraction; cadmium; heavy metals;

INTRODUCTION

Trace metals in the aquatic environment can be traced to both natural and anthropogenic sources. Trace metals are classified as being light or heavy with densities less or greater than 5 $g.cm^{-3}$. Natural and anthropogenic activities usually result in gaseous emissions and wastewater discharges into the environment. When these substances in the emissions and effluent discharged into the environment are in very minute amounts or in low concentrations and are toxic to plants and animals and have short residence time in the environment, they are described as contaminants **(Tyokumbur and Okorie, 2014)**.

Heavy metals are extremely persistent in the environment because they are not biodegradable and may not be broken down by chemical oxidation or through thermal processes. Some metals are essential for plant growth. Very high or low contents of some heavy metals may be inhibitory to plant growth **(Ochonogor and Atagana, 2014)**.

Heavy metals are inorganic chemical hazards. The most contaminated sites are by lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni). Soils are the major sink for heavy metals released into the environment by. Their total concentration in soils persists for a long time after their introduction. Changes in their chemical forms (speciation) and bioavailability are possible **(Maslin and Maier, 2000)**.

Heavy metal contamination of soil may pose risk human's health and the ecosystem through soil, the food chain, drinking ground water, reduction in food quality (safety and marketability) via phytotoxicity, reduction in land usability for agricultural production causing food insecurity, and land tenure problems. The adequate protection and restoration of soil ecosystems contaminated by heavy metals require their characterization and remediation **(Wuana and Okieimen, 2011)**.

Cadmium (Cd^{2+}) is a highly toxic trace element whose presence in the environment is cxaused by human

activities. It is taken up by roots via essential metal transporters **(Cohen et al., 1998; Lasat et al., 2000; Pence et al., 2000)**.

After longer exposure to heavy metal decreases growth rate by affecting various aspects of plant physiology, as well as decreases carbon assimilation that can lead to wilting **(Perfus - Barbeoch et al., 2002)**.

Plants throughout their life cycle experience various types of environmental stresses (such as drought, salinity, high temperature, cold, heavy metal and other similar stresses) due to their sessile nature. Among these stresses, salinity stress has become the limiting factor for the productivity of agricultural crops by affecting germination, plant vigor and finally crop yield **(Munns and Tester, 2008; Zhang et al., 2011; Arif Shafi Wani, 2013)**.

Cadmium is toxic to many plant species even at very low concentration. It is mainly generated from smelting industries, abrasion of automobile tires, burning of diesel and heating oils and from phosphate fertilizers originated by aerobically digested sewage sludge. Concerning to its effects on plants, Cd is accumulated in them and interacts with several physiological processes such as photosynthetic, respiratoryand nitrogen metabolism, resulting in poor growth and low biomass. Furthermore, Cd is associated with oxidative stress and it can result in the production of free radicals and active oxygen species **(Puertas-Mejı´a et al., 2010)**.

The role of oxidative stress in metal toxicity has been assessed by measuring alterations in the redox metabolic components of stressed plants. Over the past few years major progress has been achieved, particularly by comparing metal tolerant and/or metal hyperaccumulator genotypes with their non-tolerant relatives and by using transgenic plants that overexpress or lack specific redox elements. These approaches provided novel insight into the relationship between metal sensitivity and cellular redox imbalance **(Sharma, 2008)**. Metal ions may directly interfere with the metabolic activities by altering the conformation of proteins, for example enzymes, transporters or regulator proteins, owing to their strong affinities as ligands to sulfhydryl and carboxylic groups. This is taken to be a major cause for metal imposed toxic effects **(Sharma, 2004)**. Stress factors generally applied at higher levels may cause irreversible changes in physiological processes as stomatal closure or slowing down the biochemical processes. Low levels of toxic metals such as cadmium also slow growth and affect biochemical processes. Strength and duration of stress exposure can also cause permanent changes. In addition to toxic metals, changes in the membranes of plant cells are mostly affected by water stress, changes in temperature and by frost. Toxicity of the metals (such as cadmium), can cause an accumulation in tissues, which consequently affects the metabolism of plants, particularly the photosynthetic apparatus **(Lachman et al., 2015)**.

The mechanisms of cadmium (Cd) uptake and tolerance in plants have been studied extensively, but a clear understanding of what controls the translocation of Cd to aboveground tissues is lacking. One approach to better understanding the factors that control Cd accumulation and distribution is to determine where Cd is bound as it travels from the root surface to aboveground parts **(Akhter et al., 2014)**.

Accumulation and translocation of the environmental pollutants as cadmium was evaluated in different parts of plants. Although roots comprise usually only a little part of whole plant biomass, they consistently contain 70 – 100% of the whole plant metal burden **(Lachman et al., 2004)**.

The effect of Cd on transpiration of water from leaves has been studied extensively. At low concentrations, Cd increased the permeability of the leaf cuticle and increased transpiration in sugar beet. At high concentrations, Cd induced stomatal closure and decreased leaf transpiration in mustard (*Brassica juncea* L.), barley (*Hordeum vulgare* L.), and lettuce (*Lactuca sativa* L.). However, the mechanism of Cd-induced stomatal closure is still poorly understood. Some studies reported increased production of abscisic acid (ABA) with increased Cd-exposure and suggested that ABA might regulate stomata closure in Cd-stressed conditions, however, in ABA-insensitive mutants of Arabidopsis thaliana L. Cd^{2+} affected guard cell regulation in an ABA-independent manner by entering the cytosol via Ca^{2+} channels **(Akhter and Macfie, 2012)**.

Environmental contamination by Cd in human food typically comes from crops and contaminated water. The effects of Cd range from shortness of breath, effects on respiratory system, vomiting and diarrhoea, kidney damage and renal failure, bone damage, Itai-Itai disease (osteomalacia), to low birth weight and increase in abortions **(Stanbrough et al., 2013)**.

Crops grown in contaminated soil may accumulate Cd in different plant parts, such as root, leaf, grain etc., and consumers may develop a number of Cd-related chronic diseases. It is recommended to keep Cd concentrations below regulatory guidelines in vegetables, fruits, grains and other agricultural products to avoid metal toxicity. Because the concentration of Cd in edible plant tissues is not always directly proportional to the concentration of Cd in the soil, understanding the mechanisms of Cd accumulation and translocation in plants is important to ensuring food safety **(Akhter and Macfie, 2012)**.

On the other hand cereals are main foods in many countries, as human foods or as animal feeds. Epidemiological studies indicate that the consumption of whole - grain and whole - grain products is related to reduction in total mortality, coronary heart disease mortality, diabetes and cancer incidense. These beneficial effects are attributed to the bioactive factors in cereal grain such as non digestible carbohydrates and phytochemicals **(Ivanišová et al., 2010)**. Cereals and pseudocereals have a significant role in human nutrition. They are source of specific carbohydrates, proteins, lipids, fibre and wide spectrum of vitamins and minerals. Cereals and pseudocereals may also contain some antinutritional factors, such as phytic acid, polyphenols, trypsin inhibitors and inhibitors of α-amylase. These are responsible for reducing of protein and carbohydrate digestibility and decreasing accessibility of minerals due to complex formation **(Kocková and Valík, 2011)**.

The legislation should respect environmental protection and public health, at national and international level **(Kabata - Pendias and Pendias, 2001).**

MATERIAL AND METHODOLOGY

The aim of our work is the evaluation of selected morphological (lenght and weight of roots and biomass) and physiological (contents of dry mass and number of lief stomata) characteristics and the determination of the bioaccumulation factor and the translocation factor of cadmium by spring barley (*Hordeum vulgare* L.).

The seeds were germinated in Petri dishes on a filter paper for 48 hours in the dark with temperature 25 °C and 80% air humidity. After 2 days 100 germinated seeds were transferred into each container filled with 950 g of washed silica sand. The containers were watered firstly by Hoagland's solution **(Hoagland and Arnon, 1950)** and after that on alternate days as needed with destilled water. The water – soluble $CdCl_2$. 2.5 H_2O was added to containers to obtain the application of 1, 5 and 25 mg.kg^{-1}. The control treatments (0 mg.kg^{-1}) had no added heavy metal. The plants were grown with supplementary lihting 16/8 hours photoperiod and controlled temperature 20 – 25 °C. The plants were harvested after four weeks of cultivation. They were cleaned, washed with deionized water and separated into roots and aerial parts.

Imprints of leaves have been transferred to a glass slide and preparations were made for further analysis. Microreliefs we collected in the central part of the leaf on adaxial (upper) and abaxial (lower) side. Preparations were evaluated using an optical microscope Axiostar Plus, Carl Zeiss, lens CP Achromat 40x/0.65, eyepiece PI 10x / 18, Canon Utilities Software Zoom Browser EX 4.6 and hardware Acer Travel Mate 4600, Canon Power Shot A95. The density of stomata was evaluated on an area of 1 mm^2.

Cadmium concentrations were obtained by treating the samples by 10 cm^3 of aqua regia (2.5 cm^3 HNO_3 and 7.5 cm^3 HCl) using microwave digestion unit Mars X-press 5 (CEM Corp., USA). The mineralization was carried out in teflon vessels. The concentrations were measured by atomic absorption spectrometry (AAS) in a Varian AA 240 Z (Varian, Australia) with GTA120 graphite furnace.

The significance of selected parametres was verified by LSD test. We used Pearson correlation coefficients at significance level of p <0.05 (weak statistical significance) and p <0.01 (very strong statistical significance) by STATGRAPHICS Plus 5.1.

RESULTS AND DISCUSSION

In the experiment, the barley plants showed visual symptoms of the external toxic effect of metal, such as leaf discoloration and dehydration.

The changes in dry matter content of roots and leaves, and the length of the leaves indicate that the plant react to changing of environmental conditions **(Piršelová et al., 2010)**.

Strong statistical significance was confirmed between contamination by cadmium with (Table 1):

- germination → low negative correlation,
- length of the leaves→ high negative correlation,

Weak statistical significance was confirmed between contaminanation by cadmium with (Table 2):

- weight of biomass→ low negative correlation.

The negative impacts of heavy metals on plants are decreasing of seed germination, lipid content, enzyme activity and plant growth, the inhibition of photosynthesis or reduction of chlorophyll production **(Gardea-Torresdey et al., 2005; Akpor et al., 2014)**.

The adaxial (Figure 1) and abaxial (Figure 2) side preparations of the barley leaves were evaluated using an optical microscope. Very strong statistical significance was between contaminanation by cadmium with (Table 3) number of leaf stomata on abaxial side → high positive correlation. After microwave digestion of the harvested biomass (roots and leaves) of spring barley (*Hordeum vulgare* L.) was measured the content of Cd and the obtained results are shown in Figure 3 and Figure 4.

The cadmium content in roots in first variant (application 1 mg.kg^{-1} of $CdCl_2$. 2.5 H_2O) was 219.39 ±68.65 mg.kg^{-1}, in second variant (application 5 mg.kg^{-1} of $CdCl_2$. 2.5 H_2O) was 489.38 ±140.41 mg.kg^{-1} and in third variant (application 25 mg.kg^{-1} of $CdCl_2$. 2.5 H_2O) was 2064.36 ±108.32 mg.kg^{-1} of dry mass.

Table 1 Cadmium effect on the germination and length of the leaves of barley.

Contamination by Cd (mg.kg^{-1})	Germination (%)	Length of the leaves (cm)
0	94	29.40
1	91	28.60
5	88	24.90
25	84	23.90

Table 2 Cadmium effect on the dry weight of the roots and leaves of barley.

Contamination by Cd (mg.kg^{-1})	Dry weight of the roots (g)	Dry weight of the leaves (g)	Total biomass (g)
0	0.8902	1.4850	2.3752
1	0.7232	1.4848	2.2080
5	0.4792	1.2613	1.7405
25	0.6400	1.2850	1.9250

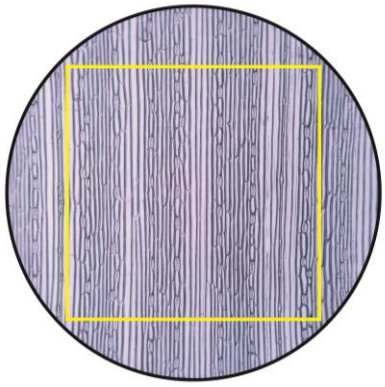

Figure 1 Detail of the adaxial (upper) side of spring barley (*Hordeum vulgare* L.) leaf on an area of 1 mm^2.

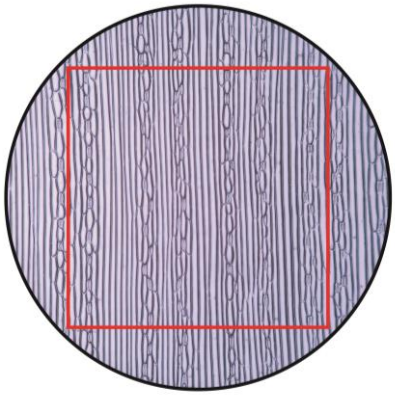

Figure 2 Detail of the abaxial (lower) side of spring barley (*Hordeum vulgare* L.) leaf on an area of 1 mm^2.

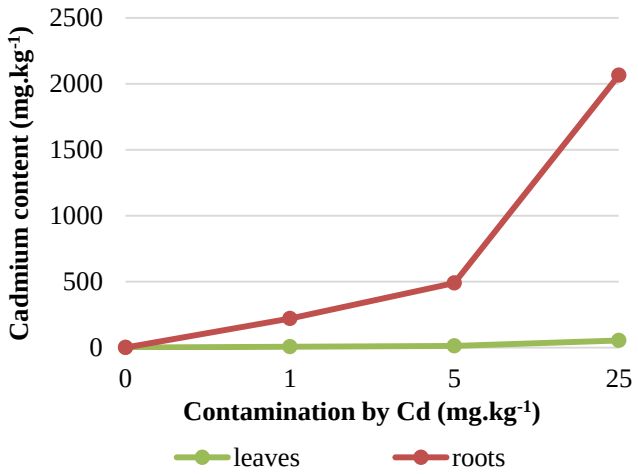

Figure 3 The evaluation of cadmium content in the roots and leaves of barley.

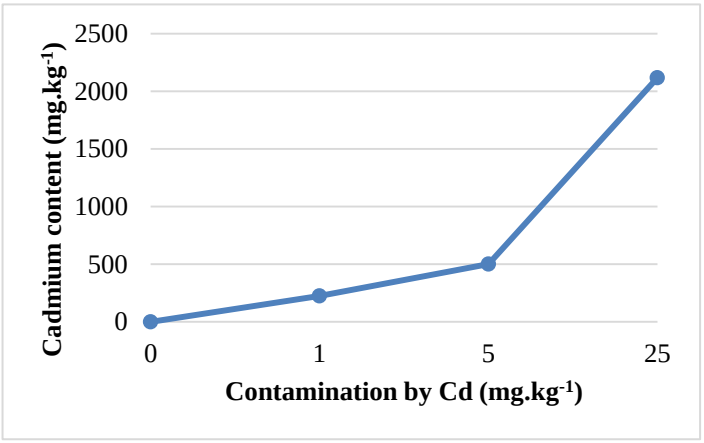

Figure 4 The evaluation of cadmium content in the barley.

The content of cadmium in leaves varied over a value in first variant 5.57 ±0.29 mg.kg^{-1}, in second variant 11.68 ±2.14 mg.kg^{-1} and in third variant 52.93 ±6.73 mg.kg^{-1} of dry mass.

The cadmium content in different parts of the plant increases proportionally with an increasing application of heavy metal. The cadmium content in the root system was up to 40 times higher than the aboveground part of barley.

The bioaccumulation factor has been used as an effective way to show the potencial of the plants for phytoremediation. It is the indicator of the ability of metal accumulation by plants. A good accumulator plant should

Table 3 Cadmium effect on the number of leaf stomata in the central part of the barley leaf on adaxial (upper) and abaxial (lower) side.

Contamination by Cd ($mg.kg^{-1}$)	Number of lief stomata per $1mm^2$ (adaxial side)	Number of lief stomata per $1mm^2$ (abaxial side)
0	59	51
1	53	58
5	59	63
25	60	67

Table 4 Bioaccumulation factor and the translocation factor of barley according to contamination by cadmium.

Contamination by Cd ($mg.kg^{-1}$)	BF of the roots (%)	BF of the leaves (%)	TF of the barley x 100
0	-	-	-
1	219.39	5.57	2.54
5	97.88	2.34	2.39
25	82.57	2.12	2.56

have a bioaccumulation factor lower than 100%. The translocation factor discribes in which part of plant body is the highest accumulation of contaminant **(Kherbani et al., 2015).**

The bioaccumulation factor was calculated as:

BF = Cd content in the plant/ Cd content in the soil.

The translocation factor was described as:

TF = Cd content in the leaves/ Cd content in the roots.

In our case, the maximum bioaccumulation factor of roots was obtained with the value 97.88% to a 5 $mg.kg^{-1}$ for Cd.

The cadmium content in the leaves of barley was much lower than in the rooots.Translocation factor is too small, so spring barley is very interesting plant for phytoextraction. The results are shown in Table 4.

In acid soils, cadmium is more mobile and less able to return the adsorption to sediments and minerals, rocks and sand. Adsorption of cadmium dependents on its concentration, pH of the soil solution, soil type, duration of exposure and the concentration of complexing ligand. Cadmium is an element that is highly mobile in acidic soil. The mobility increases with decreasing pH, fertilizing by acid fertilizers and low content of organic matter in the soil **(Trebichalský et al., 2010)**.

Phytoextraction of heavy metals is a technology that has been studied for several years. It is more ecological and cheaper way how to clean our environment.

Several plant species are known becauce they hyperaccumulate a high contents of metals from the soil, they are able to store particularly high amounts of heavy metals in aboveground organs. The accumulators are mainly herbaceous species, crops and nowadays angiosperm trees with a high growth such as poplars or willows. Woody species now represent attractive models since they have a higher biomass and a more important root system to decontaminate soils deeper than herbaceous plants **(Saladin, 2015)**.

CONCLUSION

Periodical monitoring of plants should be encouraged especially crops from areas that are grown and harvested next to the mining or industrial areas and the geochemical anomalies. The heavy metals may enter the leaves via the stomata.

The effect of contamination by cadmium to germination, length of leaves and number of stomata on abaxial side of leaf was confirmed.The contaminated soil by cadmium do not pose a risk of heavy metal entry into the feed and food chain by spring barley (*Hordeum vulgare* L.).

REFERENCES

Akpor, O. B., Ohiobor, G. O., Olaolu, T. D. 2014. Heavy metal pollutants in wastewater effluents: Sources, effects and remediation. *Advances in Bioscience and Bioengineering*. vol. 2, no. 4, p. 37-43. http://dx.doi.org/10.11648/j.abb.20140204.11

Akher, M. F., Macfie S. M. 2012. Species-Specific relationship between Transpiration and Cadmium Translocation in Lettuce, Barley and Radish. *Journal of Plant Studies*. vol. 1, no. 1, p. 1-13. http://dx.doi.org/10.5539/jps.v1n1p2

Akher, M. F., Omelon, Ch. R., Gordon, R. A., Moser, D., Macfie S. M. 2014. Localization and chemical speciation of cadmium in the roots of barley and lettuce. *Environmental and Experimental Botany*. vol. 100, no. 1, p. 10-19. http://dx.doi.org/10.1016/j.envexpbot.2013.12.005

Wani, A. S., Ahmad, A., Hayat, S., Fariduddin, Q. 2013. Salt-induced modulation in growth, photosynthesis and antioxidant system in two varieties of Brassica juncea. *Saudi Journal of Biological Sciences*, vol. 20, no. 2, p. 183-193. http://dx.doi.org/10.1016/j.sjbs.2013.01.006 PMid:23961235

Cohen, C. K., Fox, T. C., Garvin, D. F., Kochian, L. V. 1998. The role of iron-deficiency stress responses in stimulating heavy-metal transport in plants. *Plant Physiology*. vol. 116, p. 1063-1072. http://dx.doi.org/10.1104/pp.116.3.1063

Gardea-Torresdey, J. I., Peralta-Videa, J. R., Rosa, G. D., Parsons, J. G. 2005. Phytoremediation of heavy metals and

study of the metal coordination by X-ray absorption spectroscopy. *Coordination Chemistry Reviews.* vol. 249, no. 17-18, p. 1797-1810. http://dx.doi.org/10.1016/j.ccr.2005.01.001

Hoagland, D. R., Arnon, D. I. 1950. The water – culture method for growing plants without soil. *California Agricultural Experiment Station Circular*, vol. 347, p. 1-32.

Ivanišová, E., Ondrejovič, M., Dráb, Š., Tokár, M. 2011. The evaluation of antioxidant activity of milling fractions of selected cereals grown in the year 2010. *Potravinarstvo*, vol. 5, no. 4, p. 28-33. http://dx.doi.org/10.5219/163

Kabata-Pendias, A., Pendias, H. 2001. *Trace Metals in Soils and Plants*, 2nd ed., CRC Press, Boca Raton, Florida, USA.

Kherbani, N., Abdi, N., Lounici, H. 2015. Effect of cadmiumand zinc on growing barley. *Journal of Environmental Protection*, vol. 2, no. 2, p. 160-172. http://dx.doi.org/10.4236/jep.2015.62018

Kocková, M., Valík, Ľ. 2011. Potencial of cereals and pseudocereals for lactic acid fermentations. *Potravinarstvo*, vol. 5, no. 2, s. 27-40. http://dx.doi.org/10.5219/127

Lachman, J., Dudjak, J., Miholová, D., Kolihová, D., Pivec. V. 2004. Effect of cadmium stress on the uptake and distribution of microelements copper and zinc in plant parts of barley (*Hordeum sativum* L.). *Scientia Agriculturae Bohemica*, vol. 35, no. 3, p. 81-86.

Lachman, J., Kotíková, Z., Zámečníková, B., Miholová, D., Száková, J., Vodičková, H. 2015. Effect of cadmium stress on barley tissue damage and essential metal transport into plant. *Open Life Sciences*, vol. 10, no. 1, p. 30-39. http://dx.doi.org/10.1515/biol-2015-0004

Lasat, M. M., Pence, N. S., Garvin, D. F., Ebbs, S. D., Kochian, L. V. 2000. Molecular physiology of zinc transport in the Zn hyperaccumulator Thlaspi caerulescens. *Journal of Experimental Botany.* vol. 51, no. 342, p. 71-79. http://dx.doi.org/10.1093/jexbot/51.342.71

Maslin, P., Maier, R. M. 2000. Rhamnolipid-enhanced mineralization of phenanthrene in organic-metal co-contaminated soils, *Bioremediation Journal*, vol. 4, no. 4, p. 295-308. http://dx.doi.org/10.1080/10889860091114266

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology.* vol. 59, no. 1, p. 651-681. http://dx.doi.org/10.1146/annurev.arplant.59.032607.092911

Ochonogor, R. O., Atagana, H. I. 2014. Phytoremediation of Heavy Metal Contaminated Soil by Psoralea Pinnata. *International Journal of Environmental Science and Developement*, vol. 5, no. 5, p. 440-443. http://dx.doi.org/10.7763/ijesd.2014.v5.524

Pence, N. S., Larsen, P. B., Ebbs, S. D., Letham, D. L., Lasat, M. M., Garvin, D. F., Eide, D., Kochian, L. V. 2000. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator Thlaspi caerulescens. *Proceedings of the National Academy of Sciences*, vol. 97, no. 9, p. 4956-4960. http://dx.doi.org/10.1073/pnas.97.9.4956

Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A., Forestier, C. 2002. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *The Plant Journal.* vol. 32, no. 4, p. 539-548. http://dx.doi.org/10.1046/j.1365-313x.2002.01442.x

Piršelová, B., Lukáč, P., Dobroviczká, T., Mészáros, P., Libantová, J., Moravčíková, J., Matušíková, I. 2010. Vplyv iónov kadmia na vybrané morfologicko-fyziologické charakteristiky bôbu obyčajného cv. Aštar. (Effects of cadmium ions on selected morphological and physiological characteristics of faba bean cv. Aštar). *Potravinarstvo*, vol. 4, special issue, p. 337-341. Available at: http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/3/Pirselova.pdf

Puertas-Mejía, M. A., Ruiz-Díez, B., Fernández-Pascual, M. 2010. Effect of cadmium ion excess over cell structure and functioning of Zea mays and Hordeum vulgare. *Biochemical Systematics and Ecology*, vol. 38, no. 3, p. 285-291. http://dx.doi.org/10.1016/j.bse.2010.02.001

Saladin, G. 2015. Phytoextraction of heavy metals: The potential efficiency of conifers. *Soil Biology.* vol. 44. p. 333-353. http://dx.doi.org/10.1007/978-3-319-14526-6_18

Sharma, S. S., Kaul, S., Metwally, A., Goyal, K. C., Finkemeier, I., Dietz, K. J. 2004. Cadmium toxicity to barley (*Hordeum vulgare*) as affected by varying Fe nutritional status. *Plant Science*, vol. 166, no. 5, p. 1287-1295. http://dx.doi.org/10.1016/j.plantsci.2004.01.006

Sharma, S. S., Dietz K. J. 2008. The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Science*, vol. 14, no. 1, p. 43-50. http://dx.doi.org/10.1016/j.tplants.2008.10.007

Stanbrough, R., Chuaboonmee, S., Palombo E. A., Malherbe, F., Bhave, M. 2013. Heavy Metal Phytoremediation Potential of a Heavy Metal Resistant Soil Bacterial Isolate, Achromobacter sp. Strain AO22. *APCBEE Procedia*, vol. 5, p. 502-507. http://dx.doi.org/10.1016/j.apcbee.2013.05.085

Trebichalský, P., Molnárová, J., Musilová, J., Bystrická, J., Bajčan, D. 2010. Obsah kadmia a olova v zrne jačmeňa siateho s ohľadom na dodržanie ekologickej rovnováhy pestovateľského prostredia. (Cadmium and lead contents in barley grains from the view point of ecologicla balance cultivation environment). *Potravinarstvo*, vol. 4, special issue, p. 358-363. Available at: http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/3/Trebichalsky.pdf

Tyokumbur, E. and Okorie, T. 2014. Toxic trace metal contamination (Arsenic, Cadmium and Lead) of Sarotherodon melanotheron (Rupell, 1852) from Alaro stream in Ibadan. *Journal of Food and Nutrition Sciences*, vol. 2, no. 6, p. 258-261. http://dx.doi.org/10.11648/j.jfns.20140206.13

Wuana, R. A., Okieimen, F. E. 2011. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecology.* vol. 2011, p. 1-20. http://dx.doi.org/10.5402/2011/402647

Zhang, H. J., Dong, H. Z., Li, W. J., Zhang, D. M. 2011. Effects of soil salinity and plant density on yield and leaf senescence of field - grown cotton. *Journal of Agronomy and Crop Science.* vol. 198, no. 1, p. 27-37. http://dx.doi.org/10.1111/j.1439-037x.2011.00481.x

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MICROBIOLOGICAL QUALITY OF CHICKEN THIGHS MEAT AFTER APPLICATION OF ESSENTIAL OILS COMBINATION, EDTA AND VACCUM PACKING

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ABSTRACT

The aim of the present work to monitoring chicken the microbiological quality of vaccum packaged thighs after treatment by ethylenediaminetetraacetate (EDTA), anise (*Pimpinella anisum*), spearmint (*Mentha spicata* var. *crispa*), thyme (*Thymus vulgaris* L.) oregano (*Origanum vulgare* L.) essential oils and stored in at 4 ±0.5 °C for a period of 16 days. The following treatments of chicken thighs were used: air-packaged control samples, control vacuum-packaged samples, vacuum-packaging with EDTA solution 1.5% w/w, control samples, vacuum-packaging after treatment with *Pimpinella anisum, Mentha spicata* var. *crispa* essential oil at concentrations 0.2% v/w, vacuum-packaging after treatment with *Thymus vulgaris* L., *Origanum vulgare* L. essential oil at concentration 0.2% v/w. The quality assessment of all samples was done microbiologically and following microbiological parameters were detected: the anaerobic plate count, *Enterobacteraceae* counts, lactic acid bacteria and *Pseudomonas* spp. counts. The number of anaerobic plate count ranged from 3.69 log CFU.g^{-1} in all tested group on 0 day to 5.68 log CFU.g^{-1} on 16 day in control group stored in air condition. The number of lactic acid bacteria ranged from 2.00 log CFU.g^{-1} in all tested group on 0 day to 4.82 log CFU.g^{-1} on 16 day in group with oregano, thyme essential oils combination. *Enterobacteriacea* counts in chicken thighs was 0.68 log CFU.g^{-1} on 0 day to 7.58 CFU.g^{-1} on 16 day in air-packed meat samples. The *Pseudomonas* spp. was not found in all tested samples. Among the antimicrobial combination treatments examined in this work, the as application of vacuum packaging, EDTA and essential oils treatment was the most effective against the growth of *Enterobactericeae*, inhibitory effect on anaerobic plate count also was observed. The results of this present study suggest the possibility of application the *Pimpinella anisum*, *Mentha spicata* var. *crispa*, *Thymus vulgaris* L., *Origanum vulgare* L. essential oil of as natural food preservatives and potential sources of antimicrobial ingredients for food industry for chicken thighs meat treatment.

Keywords: meat; microorganisms; essential oils; vaccum; EDTA

INTRODUCTION

Poultry meat is a very popular food commodity around the world due to its low cost of production, low fat content, high nutritional value, distinct flavor **(Barbut, 2001; Patsias et al., 2008)**. The diverse nutrient composition of meat makes it an ideal environment for the growth and prolifiration of meat spoilage micro-organisms, as well as food-borne pathogens **(Zhou et al., 2010)**. Therefore is essential to apply adequate preservation technologies to extend the shelf life of perishable meat products which is a major concern for the meat industries **(Wang et al., 2004)**.

Special attention in poultry meat production is paid to the fact that live animals are hosts to a large number of different microorganisms residing on their skin, feathers and in the alimentary tract. During the slaughter a majority of these microorganisms are eliminated, but subsequent contamination is possible at any stage of the production process including contamination from feather plucking and evisceration equipment, washing before storage, cooling or freezing. Microorganisms from the environment, equipment and operators' hands also can contribute to contamination of meat. During the processing the changes in the microflora of meat are reported from, in general, Gram-positive rods (micrococci) to Gram-negative bacteria including Enterobacteriaceae, *Pseudomonas* spp., which were isolated the most frequently. Industrial poultry slaughterhouses have a particular technological process, the individual stages of which are not in conformity with modern principles of hygienic meat production and processing. Factors, which alter the mcrobiological quality of poultry meat can occur during the all processing steps **(Kozačinski et al., 2006)**.

Naturally occurring antimicrobial compounds have good potential to be applied as food preservatives. Essential oils, other extracts from plants, herbs, spices, some of their constituents have shown antimicrobial activity against different food pathogens and spoilage microorganisms **(Bakkali et al., 2008; Burt, 2004; Holley and Patel, 2005)**. Plants, plants products have been claimed to have

health-promoting effects, which may be related to the antioxidant activity *in vivo* **(Ivanišová et al., 2013; Ivanišová et al., 2015a, b)**.

Anise (*Pimpinella anisum* L.), which belongs to the family *Apiaceae*, is an important spice, medicinal plant used for pharmaceutics, perfumery and food industry. The fruits as well as the essential oils are characterized by antispasmodic, antioxidant, antimicrobial, insecticidal and antifungal effects **(Gülcin et al., 2003; Özcan, Chalchat, 2006; Tepe et al., 2006; Tirapelli et al., 2007)**. Its fruits which are called aniseed contain around 1.5-5.0% of essential oil mainly composed of volatile phenylpropanoids like trans-anethole with around 90% **(Tabanca et al., 2005)**. In addition, the essential oil of the anise fruit also contains a small proportion of estragol, anisaldehyde, himachalene and cis-anethole **(Omidbaigi et al., 2003; Tabanca et al., 2006)**.

The genus *Mentha* of the family *Lamiaceae* comprises about 19 species, 13 natural hybrids, is widely distributed across the Europe, Africa, Asia, Australia and North America **(Kumar et al., 2011)**. *Mentha spicata* L., commonly known as spearmint, is a native of Africa, temperate Asia and Europe. It is an herbaceous, rhizomatous, perennial plant growing up to 40x130 cm in height. A literature review shows the antifungal effect of *M. spicata* EO (essential oil) against some food-poisoning fungi **(Sokovic et al., 2009)**, other storage insects **(Lee et al., 2002)**, but reports are lacking about this EO's ability to counter aflatoxin production.

Antimicrobial activity of thyme or oregano essential oil incorporated edible films have been evaluated by a number of researchers, however, limited data exist on the application of antimicrobial edible films incorporated with essential oils in real food systems **(Seydim and Sarikus, 2006; Chi et al., 2006; Oussalah et al., 2006; Du et al., 2008)**. Among *Lamiaceae* species, oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.), wild thyme (*Thymus serpyllum* L.) have been studied widely for their antioxidant activity due to the high content of phenolic compounds **(Vichi et al., 2001; Zandi and Ahmadi, 2000)**.

The aim of this study was to investigate the effects of anise, spearmint, thyme, oregano essential oils and ethylenediaminetetraacetate in combination with vacuum packaging on the microbiological properties of chicken thighs.

MATERIAL, METHODOLOGY

Preparation of samples

To evaluate the antimicrobial activity of essential oils the chicken thigh with skin for each experimental group was taken. The chicken thigh fresh samples with were prepared as follow: for air-packaging (AC, control samples) chicken thigh fresh meat was packaged to polyethylene bags and stored aerobically at 4 ±0.5°C; for vacuum-packaged (VPC, control samples) chicken thigh fresh meat was packaged to polyethylene bags, stored anaerobically in vacuum at 4 ±0.5°C; for vacuum-packed samples with EDTA solution 1.5% w/w (VPEC, control samples) chicken thigh was treated with EDTA for 1 min, then packaged to polyethylene bags, stored anaerobically in vacuum at 4 ±0.5°C; for vacuum-packed samples treated with *Pimpinella anisum* + *Mentha spicata* var. *crispa* 0.20% v/w (VP+PAO+MSO) chicken thigh was treated with anise in combination with mint oil for 1 min, packaged to polyethylene bags, stored anaerobically in vacuum at 4 ±0.5°C; for vacuum-packed samples treated with *Thymus vulgaris* L. In combination with *Origanum vulgare* L. 0.20 % v/w (VP+TVO+OVO) chicken thigh was treated with essential oil for 1 min, packaged to polyethylene bags, stored anaerobically in vacuum at 4 ±0.5°C. For sample packaging, a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic) was used. Each sample was packaged immediately after treatment. EDTA solution (pH 8.0, 99.5% purity, analytical grade, Invitrogen, USA) was prepared at final concentration of 50 mM and used in treatment of chicken thigs samples. Anise, spearmint, thyme and oregano essential oils (Hanus, Nitra, Slovakia) was added to coat the surface of chicken thigh on both sides of each sample using a micropipette. Final concentration of 0.2% v/w of EO was used for treatment.

Microbiological analysis

An amount of 10 g (10 cm^2) of the chicken thigh was sampled using sterile scalpels, forceps and immediately transferred into a sterile stomacher bag containing 90 mL of 0.1% peptone water (pH 7.0) and homogenized for 60 s in a Stomacher at room temperature. Sampling and microbiological testing was carried out after certain time intervals: 0, 4, 8, 12, 16 days of experiment. Chicken thighs were stored in vacuum packaging at 4 ±0.5 °C. Microbiological analyses were conducted with accordance to standard microbiological methods. Anaerobic plate count (APC) was determined on Plate Count Agar (PCA, Oxoid, UK) after incubation for 48 h at 35 °C in anaerobic conditions. For *Pseudomonas* spp., 0.1 mL from prepared chicken meat suspension was spread onto the Pseudomonas Isolation agar (PIA, Oxoid, UK). After inoculation PIA was incubated for 48 h at 25 °C. For lactic acid bacteria enumeration, a 1.0 mL of sample was inoculated onto Rogosa, Sharpe agar (MRS, Oxoid, UK), Inoculated agar was incubated for 48-78 h at 37 °C in an aerobic atmosphere supplemented with carbon dioxide (5% CO_2). For *Enterobacteriaceae* counts, a 1.0 mL of sample was transferred into 10 mL of molten (45 °C) Violet Red Bile Glucose agar (VRBL, Oxoid, UK). After setting, a 10 mL molten medium was added to cover the suspension. Inoculated VRBL agars were incubated at 37 °C for 24 h. All plates were examined for typical colony appearance and morphology characteristics associated with each medium applied for cultivation of microorganisms.

RESULTS, DISCUSSION

Essential oils have not only antibacterial properties, but their application in meat can affect some meat characteristics as well. Based on antibacterial properties of EOs, type of affected pathogen, some essential oils are better than others for application in meat industry. Concentration of essential oils, which should be added to meat in order to prevent the oxidation, proliferation of foodborne pathogens, or to extend shelf-life by inhibition of background microflora, is usually higher than one used

in *in vitro* conditions because of interaction with meat components **(Bošković et al., 2013)**.

Anaerobic plate count (AC) values for the tested groups of chicken thigh are showed in Figure 1. The initial anaerobic plate count value of chicken thigh was 3.69 log CFU.g^{-1} on 0 day and the number of microorganisms increases to 5.68 log CFU.g^{-1} on 16 day in control group stored in air condition. In control group stored in vacuum packaging the AC counts were from 3.69 log CFU.g^{-1} on 0 day to 5.12 log CFU.g^{-1} on 16 day of experiment. In control group stored in vacuum packaging and EDTA treated the AC ranged from 3.69 log CFU.g^{-1} on 0 day to 4.78 log CFU.g^{-1} on 16 day. In the group after treatment with anise and spearmint essential oils combination, AC ranged from 3.69 log CFU.g^{-1} on 0 day to 4.56 log CFU.g^{-1} on 16 day. In group after treatment with thyme and oregano essential oils combination, the AC ranged from 3.69 log CFU.g^{-1} on 0 day to 4.45 log CFU.g^{-1} on 16 day. The lowest number on APC on 16 days was found in the group treated with oregano and thyme essential oil combination (4.45 log CFU.g^{-1}).

In study of **Radha Krishnan et al., (2014)**, *Enterobacteriaceae*, a psychrotrophic facultative anaerobic bacterial group, formed a substantial part of the chicken meat microbial flora and reached the final counts of 4.68, 3.76 for samples from the initial count of 3.32 log10 CFU.g^{-1}. For other samples, final counts were obtained as 4.59, 4.41, 3.91, 4.26, 4.51, 4.01, 4.11, 3.84 log10 CFU.g^{-1} for, samples respectively. **Radha Krishnan et al., (2014)** confirmed that the bacterial counts obtained from spice treated samples were lower than those from the control samples. It is important to point out, that the samples treated with combination of different spice extracts showed lower counts in comparison with the samples treated with extracts of individual spices.

The results of **Kačániová et al., (2015)** study suggest the possibility of using the essential oil of *Pimpinella anisum* L. And *Mentha piperita* as natural food preservatives and potential source of antimicrobial ingredients for meat. Among the treatments of antimicrobial combination examined in this work, the application of vacuum packaging, EDTA and essential oils treatment were the most effective against the growth of lactic acid bacteria, *Enterobactericeae.* Inhbitory effect on total viable count also was observed. Based on microbiological analyses, treatments with *Pimpinella anisum* L. and *Mentha piperita* essential oils resulted in shelf-life extension in comparison with the control samples. The similar results were found in our study in group with combination of anise, spearmint essential oils were used.

The primary objective of chilling poultry is to reduce microbial growth to a level that will maximize both food safety and shelf life **(Popelka et al., 2014)**. Hovewer, psychrotrophic nature of lactic acid bacteria enhancing their survival and multiplying on meat and supporting the spoilage of products. Lactic acid bacteria (LAB) values for the tested groups of chicken thigh are showed in Figure 2. The initial TVC value of chicken thigh was 2.00 log CFU.g^{-1} on 0 day. The number of lactic acid bacteria ranged from 2.00 log CFU.g^{-1} in all tested group on 0 day to 4.82 log CFU.g^{-1} on 16 day in group treated with oregano and thyme essential oils combination.

In control group stored in air condition, the number of LAB ranged from 2.00 log CFU.g^{-1} on 0 day to 3.98 log CFU.g^{-1} on 16 day. In control group stored in vacuum packaging LAB counts ranged from 2.00 log CFU.g^{-1} on 0 day to 4.12 log CFU.g^{-1} on 16 day. In control group stored in vacuum packaging after EDTA treatment, LAB ranged from 2.00 log CFU.g^{-1} on 0 day to 4.23 log CFU.g^{-1} on 16 day.

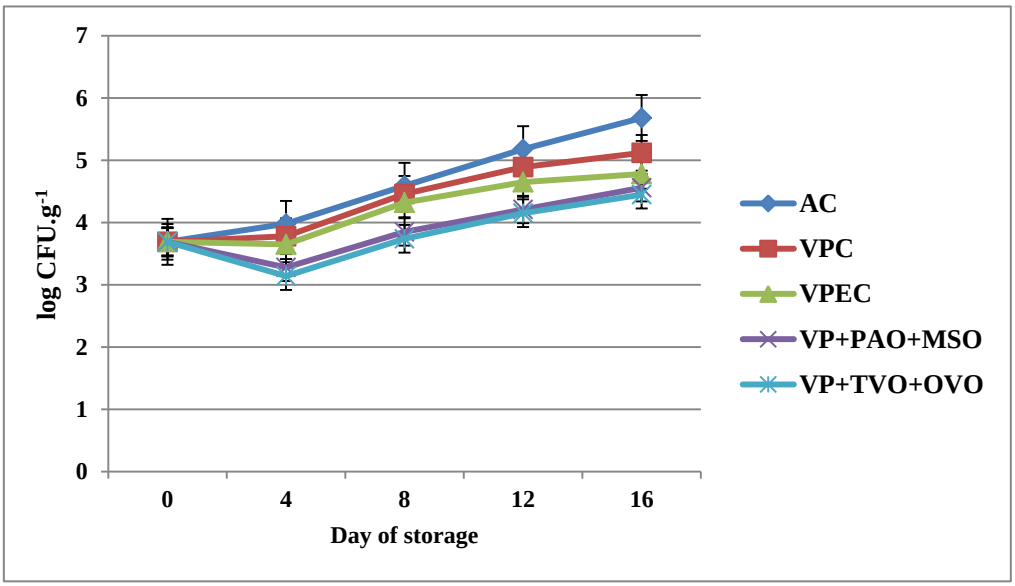

Figure 1 Changes (log CFU.g^{-1}) in population of anaerobic plate count in chicken thigh stored in air (AC); stored in vacuum (VPC); stored in vacuum packaging with EDTA (VPEC); stored in vacuum packaging after treatment with *Pimpinella anisum* + *Mentha spicata* var. *crispa* 0.20% v/w combination (VP+PAO+MSO); stored in vacuum packaging after treatment with *Thymus vulgaris* L. + *Origanum vulgare* L. 0.20 % v/w, combination (VP+TVO+OVO).

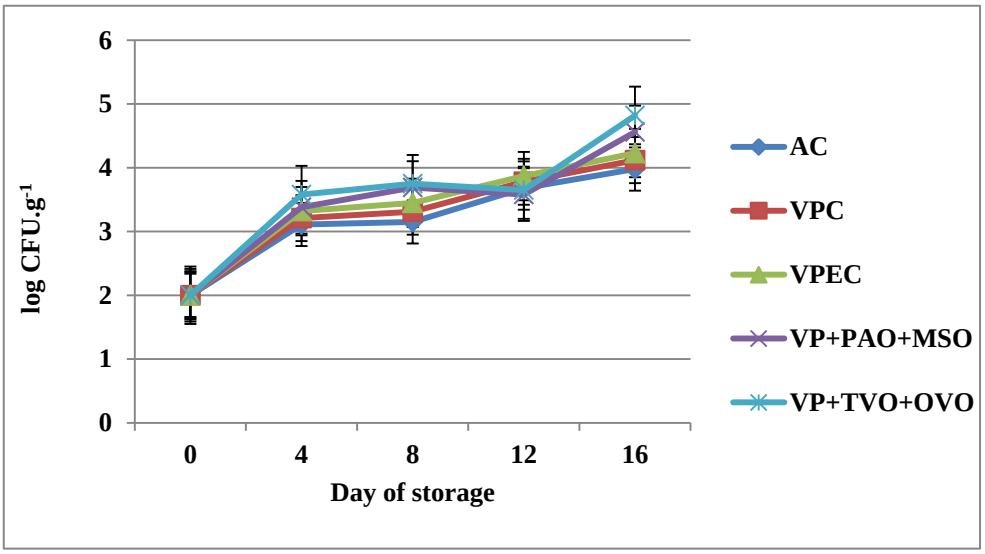

Figure 2 Changes (log CFU.g^{-1}) of lactic acid bacteria counts in chicken thigh stored in air (AC); stored in vacuum (VPC); stored in vacuum packaging with EDTA (VPEC); stored in vacuum packaging after treatment with *Pimpinella anisum* + *Mentha spicata* var. *crispa* 0.20% v/w combination (VP+PAO+MSO); stored in vacuum packaging after treatment with *Thymus vulgaris* L. + *Origanum vulgare* L. 0.20 % v/w, combination (VP+TVO+OVO).

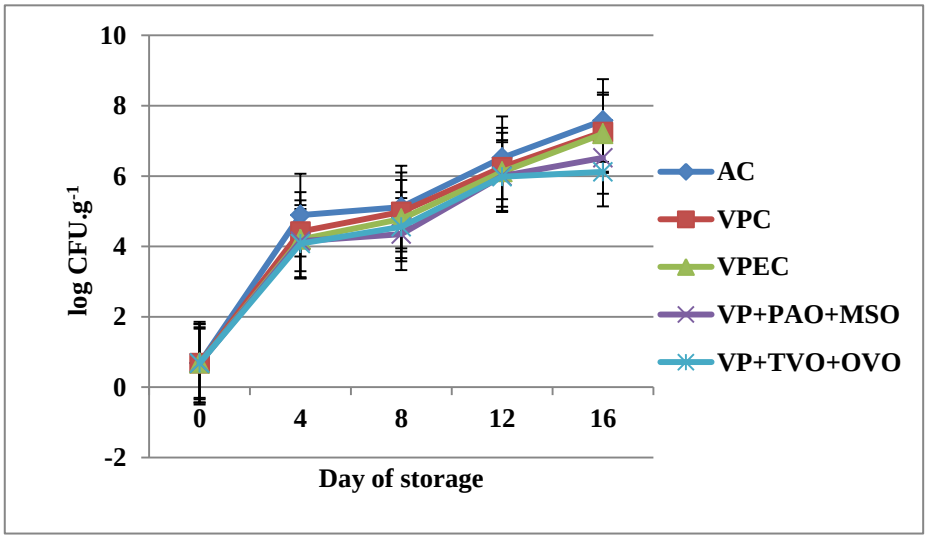

Figure 3 Changes (log CFU.g^{-1}) in population of *Enterobacteriaceae* in chicken thigh stored in air (AC); stored in vacuum (VPC); stored in vacuum packaging with EDTA (VPEC); stored in vacuum packaging after treatment with *Pimpinella anisum* + *Mentha spicata* var. *crispa* 0.20% v/w combination (VP+PAO+MSO); stored in vacuum packaging after treatment with *Thymus vulgaris* L. + *Origanum vulgare* L. 0.20 % v/w combination (VP+TVO+OVO).

In the group after treatment with anise and spearmint essential oils combination, number of LAB ranged from 2.00 log CFU.g^{-1} on 0 day to 4.56 log CFU.g^{-1} on 16 day. In the group after treatment with oregano and thyme essential oils combination ranged from 2.00 log CFU.g^{-1} on 0 day to 4.82 log CFU.g^{-1} on 16 day.

LAB behaves as facultative anaerobes and able to grow under high concentrations of CO_2. Thus they constitute a substantial part of the natural microflora of VP meats. LAB are recognized as the important competitors to other spoilage related microbial groups under VP/MAP conditions **(Castellano et al., 2004; Doulgeraki et al., 2011; Zhang et al., 2009)**. Particularly, *Lactobacillus* spp., *Carnobacterium* spp., *Leuconostoc* spp. are associated to the spoilage of refrigerated raw meat **(Nychas, Skandamis, 2005)**. More species of lactobacilli can be found during the storage under the vacuum at 4°C including *Lb. algidus* beyond *Lb. sakei*. The results of **Ntzimani et al. (2010)** indicate that LAB was an important part of the precooked chicken microflora, irrespective of the packaging conditions, the antimicrobial treatment combination. The latter observations could probably help to explain their rapid growth between days 0, 2 of storage. This is also in agreement with LAB growth in beef stored under MAP at 5°C **(Skandamis and Nychas, 2001)**.

Enterobacteriaceae counts of the tested groups of chicken thigh are showed in Figure 3. The initial *Enterobacteriacea* genera value of chicken thigh was 0.68 log CFU.g^{-1} on 0 day. Presences of these bacteria were found on all groups at 16 day. The number of *Enterobacteriaceae* genera ranged from 0.68 log CFU.g^{-1} in all tested groups of samples on 0 day to 7.58 log CFU.g^{-1} on 16 day in control group stored in air condition. In control group stored in air condition the number of *Enterobacteriaceae* genera ranged from 0.68 log CFU.g^{-1} on 0 day to 7.58 log CFU.g^{-1} on 16 day. In control group stored in vacuum packaging, *Enterobacteriaceae* counts ranged from 0.68 log CFU.g^{-1} on 0 day to 7.25 log CFU.g^{-1} on 16 day. In control group stored in vacuum packaging after EDTA treatment, *Enterobacteriaceae* counts ranged from 0.68 CFU.g^{-1} on 0 day to 7.20 log CFU.g^{-1} on 16 day. In the group of chicken thigh treated with anise and spearmint essential oils combination *Enterobacteriaceae* counts ranged from 0.68 log CFU.g^{-1} on 0 day to 6.52 log CFU.g^{-1} on 16 day. In the group of chicken thigh treated with oregano and thyme essential oils combination, *Enterobacteriaceae* counts ranged from 0.68 log CFU.g^{-1} on 0 day to 6.12 log CFU.g^{-1} on 16 day.

Enterobacteriaceae grew under vacuum packaging conditions at a slower rate than under aerobic packaging. This is in agreement with the results of **Chouliara et al., (2007)**, who reported that both MAP, oregano oil had a strong effect in the reduction of *Enterobacteriaceae* counts. On day 9 of storage, the use of oregano oil at its lower concentration (0.1%), had practically no effect on *Enterobacteriaceae* counts while the higher concentration (1%) gave a reduction of more than 6 log CFU.g^{-1}. On the same day, the *Enterobacteriaceae* counts were reduced by 1.5 log CFU.g^{-1} (MAP 1), 1.8 log CFU.g^{-1} (MAP 1, oregano oil 0.1%), more than 6 log CFU.g^{-1} (MAP 1, oregano oil 1%), 3.4 log CFU.g^{-1} (MAP2), 4.3 log CFU.g^{-1}g (MAP 2, oregano oil 0.1%), more than 6 log CFU.g^{-1} (MAP 2, oregano oil 1%).

Growth of the *Enterobacteriaceae* was completely inhibited after thyme essential oil treatment was applied and final counts (ca. 4.0 log CFU.g^{-1}) were reduced (ca. 3 log cycle) significantly (p <0.05) at the end of the storage period (day 12) in Giatrakou et al. (2010) study. The explanation of this was the antibacterial effects of the essential oils applied the study and this is in agreement with the results of the present study. Thymol essential oil treatment also produced the lower bacterial counts as compared to the control samples during the storage that is in agreement with our results.

Pseudomonas spp. were not isolated in the present study from all samples grou were tested. It is now well established that *Pseudomomas* spp. may form a significant part of the spoilage microflora of chicken meat stored under refrigeration **(Jay et al., 2005)**.

Among the treatments used for improving the shelf-life of products examined in the study of **Pavelkova et al., 2014**, the application of EDTA, oregano oil and thymus oil were the most effective against the growth of Gram-negative bacteria. Inhibitory effect on total viable count and LAB also was identified. Based on microbiological analyses, treatments with oregano and thymus oil combination produced a shelf-life extension of 8-9 days in comparison to the control samples. The ability of vacuum packaging to inhibit a growth of spoilage organisms is well documented, but many pathogenic organisms are less affected in this process. Therefore, the combined effect of essential oils as oregano and thymus including vacuum packaging on the safety of the meat could be investigated.

CONCLUSION

The results of the present study suggest the possibility of using the essential oil of anise, spearmint, thymol, oregano as natural food preservatives and potential source of antimicrobial ingredients for meat. Among the combinations of treatments, which may pose antimicrobial activity and examined in the present work, the use of modified storage condition as vacuum packaging, treatment with EDTA and essential oils were the most effective against the growth of lactic acid bacteria, *Enterobactericeae* family. Also the growth of anaerobic microorganisms were inhibited. Based on microbiological analyses, the treatment with anise, spearmint, thyme, oregano essential oils resulted in shelf-life extension as compared to the control samples. The combined effect of four essential oils, EDTA, vacuum packaging can significantly contribute the shelf-life and safety of the chicken thigh.

REFERENCES

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. 2008. Biological effects of essential oils – A review. *Food, Chemical Toxicol*ogy, vol. 46, p. 446-475. http://dx.doi.org/10.1016/j.fct.2007.09.106

Barbut, S. 2001. Poultry Products Processing. *An Industry Guide*. London : CRC Press, 560 p. ISBN 9781587160608 http://dx.doi.org/10.1201 /9781420031744

Bošković, M., Baltić, Ž. M., Ivanović, J., Durić, J., Lončina, J., Dokmanović, M., Marković, R. 2013. Use of essential oils in order to prevent foodborne illnesses caused by pathogens in meat. *Tehnologija mesa*, vol. 54, no. 1, p. 14-20. http://dx.doi.org/10.5937/tehmesa1301014B

Burt, S. 2004. Essential oils: their antibacterial properties, potential applications in foods – a review. *International Journal of Food Microbiology*, vol. 94, p. 223-253. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022

Castellano, P. H., Holzapfel, W. H., Vingolo, G. M. 2004. The control of *Listeria innocua*, *Lactobacillus sakei* in broth, meat slurry with the bacteriocinogenic strain *Lactobacillus casei* CRL705. *Food Microbiology*, vol. 21, no. 3, p. 291-298. http://dx.doi.org/10.1016/j.fm.2003.08.007

Doulgeraki, A. I., Paramithiotis, S., Nychas, G. J. E. 2011. Characterization of the *Enterobacteriaceae* community that developed during storage of minced beef under aerobic or modified atmosphere packaging conditions. *International Journal of Food Microbiology*, vol. 145, no. 1, p. 77-83. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.11.030

Du, W. X., Olsen, C. W., Avena-Bustillos, R. J., Mchugh, T. H., Levin, C. E., Friedman, M. 2008. Antibacterial activity against *E. coli* O157:H7, physical properties, storage stability of novel carvacrol-containing edible tomato films. *Journal of Food Science*, vol. 73, p. M378-M383. http://dx.doi.org/10.1111/j.1750-3841.2008.00892.x

Giatrakou, V., Ntzimani, A., Savvaidis, I. N. 2007. Effect of chitosan, thyme oil on a ready to cook chicken product. *Food Microbiology*, vol. 27, p. 132-136. http://dx.doi.org/10.1016/j.fm.2009.09.005

Gülcin, I., Oktay, M., Kirecci, E., Küfrevioglu, Ö. I. 2003. Screening of antioxidant, antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chemistry*, vol. 83, p. 371-382. http://dx.doi.org/10.1016/s0308-8146(03)00098-0

Holley, R. A., Patel, D. 2005. Improvement in shelf-life, safety of perishable foods by plant essential oils, smoke antimicrobials. *Food Microbiology*, vol. 4, p. 273-292. http://dx.doi.org/10.1016/j.fm.2004.08.006

Chi, S., Zivanovic, S., Penfield, M. P. 2006. Application of chitosan films enriched with oregano essential oil on bologna-active compounds, sensory attributes. *Food Science, Technology International*, vol. 12, p. 111-117. http://dx.doi.org/10.1177/1082013206063845

Chouliara, E., Karatapanis, A., Savvaidis, I. N., Kontominas, M. G. 2007. Combined effect of oregano essential oil, modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 °C. *Food Microbiology*, vol. 24, p. 607-617. http://dx.doi.org/10.1016/j.fm.2006.12.005

Ivanišová, E., Frančáková, H., Dráb, Š., Benčová, S. 2015[a]. Elderberry as important source of antioxidant, biologically active compounds. In *Proceedings International Scientific, Professional Conference 15th Ružiška Days, Today Science Tomorrow Industry*, Vukovar, Croatia. p. 212, 221. ISBN978-953-7005-36-8.

Ivanišová, E., Frančáková, H., Ritschlová, P., Dráb, Š., Solgajová, M., Tokár, M. 2015[b]. Biological activity of apple juice enriched by herbal extracts. *Jounal of Microbiology, Biotechnology and Food Sciences*, vol. 4, no. special issue 3, p. 69-73. http://dx.doi.org/10.15414/jmbfs.2015.4.special3.69-73

Ivanišová, E., Tokár, M., Mocko, K., Mendelová, A., Bojňanská, T., Mareček, J. 2013. Antioxidant activity of selected plant products. *Jounal of Microbiology, Biotechnology and Food Sciences*, vol. 2, no. special issue 1, p. 1692-1703.

Jay, J. M., Loessner, M. J., David, A. 2005. *Modern Food Microbiology*, 7th ed. New York, USA: Springer Science Inc. http://dx.doi.org/10.1007/bf03174975

Kačániová, M., Petrová, Mellen, M., Čuboň, J., Haščík, Hleba, L., Terentjeva, M., Kunová, S., Blaškovičová, H. 2015. Impact of anise (*Pimpinella anisum*), mint (*Mentha piperita*) essential oils to microbial activity in chicken meat. *Jounal of Microbiology, Biotechnology and Food Sciences*, vol. 4, no. special issue 1, p. 28-31. http://dx.doi.org/10.15414/jmbfs.2015.4.special1.28-31

Kozačinski, L., Hadžiosmanovič, M., Zdolec, N. 2006. Microbiological quality of poultry meat on the Croatian market. *Veterinarski Arhiv*, vol. 76, p. 305-313.

Kumar, P., Mishra, S., Malik, A., Satya, S. 2011. Insecticidal properties of *Mentha* species: A review. *Industrial Crops, Products*, vol. 34, p. 802-817. http://dx.doi.org/10.1016/j.indcrop.2011.02.019

Lee, B., Lee, S., Annis, P. C., Pratt, S. J., Park, B., Tumaalii, F. 2002. Fumigation toxicity of essential oils, monoterpenes against the red flour beetle, *Tribolium castaneum* Herbst. *Journal Asia-Pacific Entomology*. vol. 5, p. 237-240. http://dx.doi.org/10.1016/s1226-8615(08)60158-2

Ntzimani, A. G., Giatrakou, V. I., Savvaidis, I. N. 2010. Combined natural antimicrobial treatments (EDTA, lysozyme, rosemary, oregano oil) on semi cooked coated chicken meat stored in vacuum packages at 4 °C: Microbiological, sensory evaluation. *Innovative Food Science, Emerging Technologies*, vol. 11, no. 1, p. 187-196. http://dx.doi.org/10.1016/j.ifset.2009.09.004

Nychas, G. J. E., Skandamis, P. 2005. Fresh meat spoilage, modified atmosphere packaging (MAP). In: Sofos, J. N. (Ed.), *Improving the Safety of Fresh Meat*, Cambridge: CRC/ Woodhead Publishing Limited, p. 461-502. ISBN 978-1-85573-955-0 http://dx.doi.org/10.1533/9781845691028.2.461

Omidbaigi, R., Hadjiakhoondi, A., Saharkhiz, M. 2003. Changes in content, chemical composition of *Pimpinella anisum* L. oil at various harvest time. *Journal of Essential Oil Bearing Plants*, vol. 6, p. 46-50. http://dx.doi.org/10.1080/0972-060x.2003.10643328

Oussalah, M., Caillet, S., Salmieri, S., Saucier, L., Lacroix, M. 2006. Antimicrobial effects of alginate-based film containing essential oils for the preservation of whole beef muscle. *Journal of Food Protection*, vol. 69, p. 2364-2369. http://dx.doi.org/10.1021/jf049389q

Özcan, M. M., Chalchat, J. C. 2006. Chemical composition and antifungal effect of anise (*Pimpinella anisum* L.) fruit oil at ripening stage. *Annals of Microbiology*, vol. 56, p. 353-358. http://dx.doi.org/10.1007/bf03175031

Patsias, A., Badeka, A. V., Savvaidis, I. N., Kontominas, M. G. 2008. Combined effect of freeze chilling, MAP on quality parameters of raw chicken fillets. *Food Microbiology*, vol. 25, no. 4, p. 575-581. http://dx.doi.org/10.1016/j.fm.2008.02.008

Pavelková, A., Kačániová, M., Horská, E., Rovná, K., Hleba, L., Petrová, J. 2014. The effect of vacuum packaging, EDTA, oregano, thyme oils on the microbiological quality of chicken's breast. *Anaerobe*, vol. 29, p. 128-133. http://dx.doi.org/10.1016/j.anaerobe.2013.09.002

Popelka, P., Pipová, M., Nagy, J., Nagyová, A., Fečkaninová, A., Figeľ, J. 2014. The impact of chilling methods on microbiological quality of broiler carcasses. *Potravinarstvo*, vol. 8, 2014, no. 1, p. 67-71. http://dx.doi.org/10.5219/327

Radha Krishnan, K., Babuskin, S., Azhagu Saravana Babu, P., Sasikala, M., Sabina, K., Archana, G., Sivarajan, M., Sukumar, M. 2014. Antimicrobial, antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of Food Microbiology*, vol. 171, p. 32-40. http://dx.doi.org/10.1016/j.ijfoodmicro.2013.11.011

Seydim, A. C., Sarikus, G. 2006. Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary, garlic essential oils. *Food Research International*, vol. 39, p. 639-644. http://dx.doi.org/10.1016/j.foodres.2006.01.013

Skandamis, P., Nychas, G. J. 2001. Effect of oregano essential on microbiological, physicochemical attributes of minced meat stored in air, modified atmospheres. *Journal of Applied Microbiology*, vol. 91, vol. 6, p. 1011-1022. http://dx.doi.org/10.1046/j.1365-2672.2001.01467.x

Sokovic, M. D., Vukojevic, J., Marin, P. D., Brkic, D. D., Vajs, V., Griensven, L. J. 2009. Chemical composition of essential oils of *Thymus*, *Mentha* species, their antifungal activities. *Molecules* vol. 14, p. 238-249. http://dx.doi.org/10.3390/molecules14010238

Tabanca, N., Demirci, B., Kirimer, N., Baser, K. H. C., Bedir, E., Khan, I. A., Wedge, D. E. 2005. Gas chromatographic–mass spectrometric analysis of essential oil from *Pimpinella aurea*, *Pimpinella corymbosa*, *Pimpinella peregrina*, *Pimpinella puberula* gathered from Eastern, Southern Turkey. *Journal of Chromatography A*, vol. 1097, p. 192-198. http://dx.doi.org/10.1016/j.chroma.2005.10.047

Tabanca, N., Demirci, B., Kirimer, N., Baser, K. H. C., Bedir, E., Khan, I. A., Wedge, D. E. 2006. Gas chromatographic–mass spectrometric analysis of essential oil from *Pimpinella* species gathered from Central, Northern

Turkey. *Journal of Chromatography A*, vol. 1117, p. 194-205. http://dx.doi.org/10.1016/j.chroma.2006.03.075

Tepe, B., Akpulat, A. H., Sokmen, M., Daferera, D., Yumrutas, O., Aydin, E., Polissiou, M., Sokmen, M. 2006. Screening of the antioxidative, antimicrobial properties of the essential oil of *Pimpinella anisetum*, *Pimpinella flabellifolia* from Turkey. *Food Chemistry*, vol. 97, p. 719-724. http://dx.doi.org/10.1016/j.foodchem.2005.05.045

Tirapelli, C. R., deAndrade, C. R., Cassano, A. O., De Souza, F. A., Ambrosio, S. R., Costa, F. B., Oliveria, A. M. 2007. Antispasmodic, relaxant effects of the hydroalcoholic extract of *Pimpinella anisum* (*Apiaceae*) on rat anococcygeous smooth muscle. *Journal of Enthopharmacology*, vol. 110, p. 23-29. http://dx.doi.org/10.1016/j.jep.2006.08.031 PMid:17027208

Vichi, S., Zitterl-Eglseer, K., Jugl, M., Fraz, C. 2001. Determination of the presence of antioxidants deriving from sage, oregano extracts added to animal fat by means of assessment of the radical scavenging capacity by photo chemiluminescence analysis. *Nahrung/Food*, vol. 45, p. 101-104. PMid:11379280

Wang, S. H., Chang, M. H., Chen, T. C. 2004. Shelf-life, microbiological profile of chicken wing products following sous vide treatment. *International Journal. Poultry Science*, vol. 3, no. 5, p. 326-332. http://dx.doi.org/10.3923/ijps.2004.326.332

Zandi, P., Ahmadi, L. 2000. Antioxidant effect of plant extracts of *Labiatae* family. *Journal of Food Science Technology*, vol. 37, p. 436-439.

Zhang, H., Kong, B., Xiong, Y. L., Sun, X. 2009. Antimicrobial activities of spice extracts against pathogenic, spoilage bacteria in modified atmosphere packaged fresh pork, vacuum packaged ham slices stored at 4 °C. *Meat Science*, vol. 81, no. 4, p. 686-692. http://dx.doi.org/10.1016/j.meatsci.2008.11.011

Zhou, G. H., Xu, X. L., Liu, Y. 2010. Preservation technologies for fresh meat – a review. *Meat Science*, vol. 86, no. 1, p. 119-128. http://dx.doi.org/10.1016/j.meatsci.2010.04.033

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HERBICIDAL EFFECT IN RELATION TO THE ACCUMULATION OF MACROELEMENTS AND ITS REGULATION BY REGULATORS OF POLYAMINE SYNTHESIS

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ABSTRACT

Stress effects of triazine herbicide on cumulating of important macroelements (phosphorus, potassium, calcium and magnesium) into the grain of barley variety Kompakt, as well as the elimination of its negative effect through the addition of regulators of polyamine synthesis (γ-aminobutyric acid and propylenediamine) were investigated in pot trial. These morphoregulators are degrading products of polyamines and hypothetically after foliar application they should support their biosynthesis which increased level act against stress in plants. Application of the herbicide alone in comparison to control variant reduced the contents of all mentioned macroelements in grain of barley and also in variants, where the mixtures of herbicide with regulators of polyamine biosynthesis were applied, also the values of contents of all macroelements (except of magnesium) in barley grain were reduced (in comparison to this variant). It could be summarized that the presence of regulators in mixtures with triazine herbicide in comparison to control variant had not positive effects on contents of these biogenic elements in grain. By the comparison of variant with the applied herbicide with variants, where also regulators of polyamine synthesis were applied, there was the most positive influence of these mixtures of morphoregulators on statistically non-significant accumulation of phosphorus into generative organs of spring barley and in the case of positive accumulation of magnesium into these plant tissues there was statistically significant relation only after application of mixtures of herbicide with propylenediamine. Positive influence on accumulation of calcium was evaluated only after using of mixtures of herbicide with propylenediamine (statistically significant relation was recorded at the dose 29.6 g.ha^{-1}).

Keywords: barley; polyamines; triazine herbicide

INTRODUCTION

Triazine herbicides are widely used against broad leaf weeds and crops and in tree seedling nurseries. Triazines are primarily soil applied herbicides. Further research showed that triazine herbicides when taken up by the root move rapidly to the top by apoplastic movement. It concentrates first in the internal veinal areas and finally in the margin of the leaf **(Parveen et al., 2002)**. They are photosynthetic inhibitors and cause chlorosis and desiccation of green tissues. However all these effects are observed in light and not in the dark. Atrazine is a chloroamino triazine herbicide. It is a selective, pre-emergence herbicide for control of many grasses and broad leaf weeds in maize, sorghum, sugar cane and many table crops and increases the yield of crop **(Shah et al., 2000)**.

The great majority of herbicides act by inhibiting a specific plant enzyme essential for metabolism, whereas the remainder, including auxinic herbicides, act as general inhibitors **(Powles and Yu, 2010; Cabrito et al., 2011)**.

Polyamines are small positively charged aliphatic molecules ubiquitous in almost all life forms. These compounds have been implicated in a wide range of life processes in plants including seed germination, growth, floral initiation, floral development, pathogen defenses, and environmental stress responses **(Martin-Tanguy and Aribaud, 1994; Walters, 2003; Palavan-Unsal, 1995)**. Despite extensive studies on polyamine metabolism, the exact role that these compounds play in plant physiology remains unclear **(Tiburcio et al., 1997)**. In plants, polyamines are involved in various physiological events such as development, senescence and stress responses. **(Gill and Tuteja, 2010; Ramakrishna and Ravishankar, 2011)**. Endogenous polyamines could contribute to plant stress tolerance as part of defense mechanisms or adaptation programs that help plant organism to cope with the negative stress consequences **(Todorova et al., 2015)**.

High cellular levels of polyamines correlate with plant tolerance to a wide array of environmental stresses. Moreover, as compared with susceptible plants, stress-tolerant ones generally have a large capacity to enhance polyamine biosynthesis in response to abiotic stress **(Gill and Tuteja, 2010)**.

Conversely, treatments with polyamine biosynthesis inhibitors reduce stress tolerance, but this effect is reversed by concomitant application of exogenous polyamines. The influence of polyamines on in vitro morphogenetic response and caffeine biosynthesis were reported in Coffea canephora. Apart from primary metabolic functions, external feeding of certain polyamines are known to act as elicitors **(Kumar et al., 2008)**.

In addition, uncommon polyamines, like homospermidine, 1,3-diaminopropane, cadaverine and canavalmine have been detected in a large number of biological systems, including plants, animals, algae and

bacteria. At the physiological pH, polyamines are found as cations. This polycationic nature of polyamines is one of their important properties effectuating their biological activities. Large body of evidence suggested that plant transformation with genes of polyamines biosynthetic enzymes or the exogenous application of polyamines such as putrescine, spermidine and spermine results in abiotic stress tolerance in various plants **(Valero et al., 2002)**.

Ali (2000) reported that exogenous application of putrescine reduced the net accumulation of Na^+ in different organs of *Atropa belladonna* subjected to salinity stress. Putrescine alleviated the adverse effect of NaCl during germination and early seedling growth and increased the alkaloids as well as endogenous putrescine of *A. belladonna*. **Lutts et al., (1996)** reported that putrescine increased the growth and the leaf tissue viability of salttreated plants in all cvs. of *Oryza sativa*. They suggested that this positive effect was associated with an increase in ethylene biosynthesis through an increase in ACC content and a suppression of NaCl-induced inhibition of ACC conversion to ethylene and suggested the involvement of putrescine in salinity tolerance in rice. **Ndayiragije and Lutts (2006)** studied the possible role of exogenous application of polyamines on *Oryza sativa* and noted that addition of polyamines in nutritive solution reduced plant growth in the absence of NaCl and did not afford protection in the presence of NaCl. Polyamine-treated plants exhibited a higher K^+/Na^+ ratio in the shoots, suggesting an improved discrimination among monovalent cations at the root level, especially at the sites of xylem loading. Putrescine induced a decrease in the shoot water content in the presence of NaCl, while spermidine and spermine had no effects on the plant water status. In contrast to spermidine, spermine was efficiently translocated to the shoots.

GABA is a non-protein amino acid with some functional properties for human health such as lowing blood pressure and regulating heart rate **(Mody et al., 1994)**. GABA is widely present in prokaryotic and eukaryotic organisms **(Yang et al, 2015)**. In recent years, GABA-enriched foods have become popular, such as GABA-tea **(Syu et al., 2008)**, GABA-brown rice **(Komatsuzaki et al., 2007)**, GABA-soy bean sprouts **(Guo et al., 2012)**. In plant cells, GABA is synthesized via the α-decarboxy1ation of glutamate (Glu) in an irreversible reaction which is cata1yzed by glutamate decarboxylase (GAD) **(Bown et al., 1997)**. This metabolic pathway is called GABA shunt. In addition, GABA can also be formed via γ-aminobutyraldehyde intermediate from polyamine degradation reaction where diamine oxidase (DAO) is the key enzyme **(Wakte et al., 2011)**. Researches on GABA accumulation in germinating seeds focus on GABA shunt **(Bai et al., 2009; Mae et al., 2012)**, but little information is available on polyamine degradation pathway **(Xing et al., 2007)**. In the majority of germinating seeds, stressful conditions such as hypoxia **(Guo et al., 2011)**, salt stress **(Widodo et al., 2009)** and drought **(Kramer et al., 2010)** can strongly increase GABA content. During fava bean germination under non-stress condition, GABA content increased slightly **(Yang et al., 2011)**, but it increased significantly when germinating under hypoxia stress **(Yang et al., 2013)**. Under these stressful conditions, the relationship between GABA shunt and polyamine degradation pathway is still not clear.

MATERIAL AND METHODS

In pot experiment 6 kg of substrate (soil:sand – 4:2) was weighed. Analyses done in soil used in experiment are shown in Table 1. It was sown 30 plants which were thinned into 20 pieces after post-emergence. At the phase of early tillering plants were foliar treated (after 25 days) in the control treatment with the water (Table 2), in other

Table 1 Agrochemical characteristics of soil (horizons 0 – 0.2m).

Soil reaction	Humus content	Content of nutrients			
		N_{an}	P	K	Mg
(pH/KCl)	(%)	(mg.kg^{-1})	(mg.kg^{-1})	(mg.kg^{-1})	(mg.kg^{-1})
7.03	2.34	8.7	54.3	178.35	407.8

Table 2 Variants of the pot experiment.

VARIANT NUMBER	FOLIAR TREATMENT
1	Control: 9.0 mL water
2	Triazine herbicide 0.5 l.ha^{-1}: 1.0 mL water solution of triazine herbicide +8.0 mL water
3	Triazine herbicide 0.5 l.ha^{-1} +GABA 500 g.ha^{-1}: 1.0 mL water solution of triazine herbicide +4.7 mL 20 mM solution GABA +3.3 mL water
4	Triazine herbicide 0.5 l.ha^{-1} +PDA 59.2 g.ha^{-1}: 1.0 mL water solution of triazine herbicide +3.8 mL 2 mM solution PDA +4.2 mL water
5	Triazine herbicide 0.5 l.ha^{-1} +PDA 29.6 g.ha^{-1}: 1.0 mL water solution of triazine herbicide +1.9 mL 2 mM solution PDA +6.1 mL water

NOTE: PDA – 1,3-propylenediamine, GABA – γ-aminobutyric acid.

variants with triazine herbicide alone (the active ingredient is cyanazine with chemical formula 2-(4-chloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropiono-nitrile), or its mixture with γ-aminobutyric acid (GABA) with dose 500 $g.ha^{-1}$, in another variant with 1,3-propylenediamine (PDA) with dose of 59.2 $g.ha^{-1}$, and in last variant with the PDA in the amount of 29,6 $g.ha^{-1}$. The plants were watered with constant volume in all pots.

Crops were harvested in full ripeness, 2 g of barley grain after homogenization were mineralized in 20 mL of nitric acid with 5 mL of perchloric acid and after filtration the filtrate was afterwards filled to volume 50 mL. Then the contents of potassium, calcium and magnesium were determined by method of flame AAS with VARIAN (AAS Varian AA Spectr DUO 240FS/240Z/UltrAA, manufacturer Varian Australia Pty Ltd, A.C.N. 004 559 540, Mulgrave, Australia). Phosphorus was determined by method of Gonzáles **(John, 1970)** – 0.5 mL of above mentioned solution in 50 mL volumetric flask was filled with water till mark, 1 mL of ascorbic acid was added and 4 mL of solution with extraction agent containing sulphuric acid, ammonium molybdate and potassium antimonyl tartrate hemihydrate. Solution was mixed and after two hours the absorbance at 670 nm on UVmini-1240, UV-VIS Spectrophotometer, SHIMADZU, Japan (UV-1800), was measured against distilled water. Final values of phosphorus content in barley grain were defined from calibration curve of standards absorbance.

Results were evaluated by statistical program Statgraphics 4.0 (Statpoint Technologies, Inc., Czech republic), the data were analyzed by means of one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Application of herbicide alone in comparison to control variant (Table 3) reduced the contents of all macroelements in barley grain (values of our tested macroelements in barley grain percentually declined in interval 13 – 29%) – statistically significant in the case of phosphorus and potassium and also in variants with applied mixtures of herbicide with regulators of polyamine biosyntheis; the values of all macroelements contents (except of magnesium) in grain of barley were also reduced (in comparison to first variant.

It could be summarized that the presence of regulators in mixtures with triazine herbicide in comparison to control variant had not positive effects on contents of these biogenic elements (P, K, Ca) in grain (Table 3). Evaluating of magnesium cumulating had following summarise: the most positive statistically significant influence was in variants with applied propylenediamine (PDA). By the comparison of variant with the applied herbicide with variants, where also regulators of polyamine synthesis were applied, there was the most positive influence of these mixtures of morphoregulators on statistically non-significant accumulation of phosphorus into generative organs of spring barley and in the case of positive accumulation of magnesium into these plant tissues there was statistically significant relation only after application of mixtures of herbicide with PDA. Positive influence on accumulation of calcium was evaluated only after using of mixtures of herbicide with PDA (statistically significant relation was recorded at the dose 29.6 $g.ha^{-1}$). Only the uptake of potassium into barley grain was not affected positively by regulators of polyamine synthesis when compared to variant, where triazine herbicide was used alone (in mentioned cases this influence was statistically non-significant).

Cereals in Slovak republic, as well as in European Union have important representation in structure of plant production **(Kračmár et al., 2014; Tomka et al., 2010)**. Cereal agricultural production is limited by a wide array of abiotic and biotic stress factors including weeds, drought, cold, heat, salinity, imbalances in mineral nutrition, viral, and others, often acting in combinations under field conditions.

Since pesticide stress has not yet been extensively examined at the grain macroelements level, no data on this topic are currently available in the literature. Therefore, this study provides the first data and a framework for further investigation. Several researches have suggested that crop selectivity to triazine herbicides or their residues might be improved by exploiting the natural variability that is clearly preset in plants, either by searching for varietal differences in triazine tolerance or by altering the genetic structure of the crop by repeated artificial selection for genuine resistance.

Treatment of spring barley variety Kompakt with triazine herbicide and its mixtures with regulators of polyamine biosynthesis affected the accumulation of macroelements. From the theoretical point of view it could be presumed that applied amounts of regulators of polyamine synthesis will not directly affect the content of nutrients in plant, because they do not contain mentioned inorganic macroelements (P, K, Ca, Mg), because they are organic compounds. Changes in accumulation of macroelements in spring barley grain are probably caused by influencing of translocation of nutrients in plant, or by influencing of

Table 3 Contens of macroelements in grain of barley.

Variant Number	Content of nutrients ($mg.kg^{-1}$)				Dry matter (%)
	P	K	Ca	Mg	
1	4672.9B	8627.7B	122.6A	1452.1A	94.10B
2	3652.6A	6316.9A	87.0A	1275.4A	94.00B
3	3926.5A	5990.5AB	80.2A	1406.7AB	93.85B
4	3674.7AB	5378.3AB	96.7AB	1481.1B	93.42A
5	3825.7B	4490.3A	106.1B	1622.3B	93.52B

NOTE: Letters in table stand for statistical significance in columns (p <0.01). Their conformity means that the values are statistically non-significant and different letters characterize statistically significance.

metabolism of compounds groups, or physiology of plant and subsequent change in ability of plant to uptake nutrients from soil solution.

Triazine herbicides affect biochemical processes in plant, also energetic processes in cells which with their presence in tissues are obviously inhibited. Macroelement phosphorus is the part of structures $H_2PO_4^-$ and HPO_4^{2-} that are the part of important compounds NADP and $NADPH^+$, as well as phosphate fragments are in macroergic bonds. Our experiment confirmed this fact and also there was minimal influence of PDA and GABA on reduction of stress induced by herbicide presence.

Function of potassium in metabolism of spring barley plants is versatile: affects managing with water and improves health state and grain quality. Triazine herbicide which acts in plants destructive has great impact also on its uptake into barley grain.

Similarly, Pakistani authors **(Perveen et al., 2002)** found out that the contents of potassium, phosphorus and sodium were in roots and in shoots of bean plants (*Vigna radiate* (L.)) decreased after the application of triazine herbicide. The authors have explained it by injuries of tissues in plants after application of these pesticides. Potassium is involved in the protein synthesis, cell membrane and ionic balance, opening of stomata and other plant movements **(Hale and Orcutt, 1987)**.

Not only macroelements contents have decreasing tendency after the application of triazine herbicide, but this decrease was evaluated also by other important organic compounds in plants. In experiment carried out by Indian scientists **(Khan et al, 2006)** it was obvious that the application of herbicide isoproturon significantly decreased the values of protein in grain of wheat *Triticum aestivum*. Also significant decline of chlorophyl content (with bound magnesium) was also recorded **(Yin et al, 2008**; **Nemat et al., 2008)**, even by low concentration of isoproturon in plants.

As well as in the case of phosphorus, also the cumulating of potassium into barley grain the morphoregulators have not reducing effect on stress induced by triazine herbicide presence.

The role of Ca^{2+} as one of the nutrients and as a key ion in maintaining the structural rigidity of the cell walls as well as in membrane structure and function has been known for a long time **(Reddy et al., 2011; Hepler, 2005)**. During the last three decades, numerous studies have shown that Ca^{2+} is an important messenger in eliciting responses to diverse signals, including many biotic and abiotic signals **(McAinsh and Pittman, 2009; DeFalco et al., 2010)**. It appears that plants use Ca^{2+} as a messenger more than any other known messengers in plants. This is evident from the fact that nearly all signals (developmental, hormonal, and stresses) cause changes in cellular Ca^{2+}, primarily in the cytosol and, in some cases, in the nucleus and other organelles.

These herbicides belong to groups of photosynthesis inhibitors – their effectiveness lies in inhibition of photosynthetic electron transfer by disabling of photochemic reaction II. level known as Hill reaction. The most probable place of chlorophyll inhibition by photosynthesis is 5-membered ring of chlorophyll. By the bond of herbicide on keto-, resp. enol- form of five 5-membered ring, the chlorophyll inhibits the transfer of electrons. Changes as consequence of destruction of photosynthetic apparatus (inhibition of photosynthetic electron process) in plant are induced. Main photoreceptors of green plants are chlorophylls a + b which contain magnesium complex of reduced porfirine. This fact could explain our decline in magnesium that is the part of these important organic structures. The experiment revealed more positive influence of regulators of polyamine biosynthesis on increase of magnesium content in generative organs of tested cereal, because they have more positive influence on protection of photosynthetic structure by inhibiting of chemical bonds of triazine herbicide formation with elements from chlorophyll.

Recently, GABA acts an important function in plant stress responses **(Saito et al., 2008)**. This compound inhibits not only the influence of herbicide on plants, but also reduces harm pathogens. In experiment of **Okada and Matsubara (2012)** where added GABA and arginine (0.1, 1% w/v) into the Fusarium root rot (Fusarium oxysporum f. sp. asparagi, MAFF305556, SUF1226) in vitro suppressed further rot extension. GABA was also patented in USA as important antistressor **(Plant Health Care Inc., 2009)**.

CONCLUSION

Negative influence of triazine herbicide on accumulation of tested macroelements (P, K, Ca, Mg) into barley grain variety Kompakt was recorded. The application of herbicide mixtures with regulators of polyamine biosynthesis in comparison to control variant did not improve this accumulation (except of macroelement magnesium content). In our experiment there was more positive influence of used PDA and GABA in combination with triazine herbicide only in comparison to variant, where the herbicide alone was applied and in the case of statistically non-significant accumulation of phosphorus, statistically significant relation of macroelements uptake into barley grain was evaluated, in the case of magnesium accumulation into barley grain with mixtures of herbicide with PDA and calcium only at the dose of this morphoregulator 29.6 $g.ha^{-1}$. The uptake of potassium into barley grain was not positively affected by regulators of polyamine synthesis in comparison to variant where triazine herbicide was used alone.

REFERENCES

Ali, R. M. 2002. Role of putrescine in salt tolerance of Atropa belladonna plant. *Plant Science*, vol. 152, no. 2, p.173-179. http://dx.doi.org/10.1016/S0168-9452(99)00227-7

Bai, Q. Y., Chai, M. Q., Gu, Z. X., Cao, X. H., Li, Y. Liu, K. L. 2009. Effects of Components in Culture Medium on Glutamate Decarboxylase Activity and Gamma-aminobutyric Acid Accumulation in Foxtail Millet (Setaria italica L.) during Germination. *Food Chemistry*, vol. 116, no. 1, p. 152-157. http://dx.doi.org/10.1016/j.foodchem.2009.02.022

Bown, A. W., Shelp, B. J. 1997. The Metabolism and Functions of γ-aminobutyric Acid. *Plant Physiol*ogy, vol. 115, no. 1, p. 1-5. PMid:12223787

Cabrito, T., Remy, E, Teixeira, M., Duque, P., Sá-Correia, I. 2011. Resistance to Herbicides in the Model Organisms Saccharomyces cerevisiae and Arabidopsis thaliana: The

Involvement of Multidrug Resistance Transporters. In Kortekamp, A. *Herbicides and Environment*. InTech : Rijeka, Croatia, 746 p. ISBN 978-953-307-476-4. http://dx.doi.org/10.5772/13057

DeFalco, T. A., Bender, K. W., Snedden, W. A. 2010. Breaking the code: Ca^{2+} sensors in plant signalling. *Biochemical Journal*, vol. 425, no. 1, p. 27-40. http://dx.doi.org/10.1042/BJ20091147 PMid:20001960

Gill, S. S., Tuteja, N. 2010. Polyamines and abiotic stress tolerance in plants. *Plant Signaling and Behavior*, vol. 5, no. 1, p. 26-33. http://dx.doi.org/10.4161/psb.5.1.10291 PMid:20592804

Guo, Y., Chen, H., Song, Y., Gu, Z. 2011. Effects of Soaking and Aeration Treatment on γ-Aminobutyric Acid Accumulation in Germinated Soybean (Glycine max L.). *European Food Research and Technology*, vol. 232, no. 5, p. 787-795. http://dx.doi.org/10.1007/s00217-011-1444-6

Guo, Y., Yang, R., Chen, H., Song, Y. Gu, Z. 2012. Accumulation of γ-Aminobutyric Acid in Germinated Soybean (Glycine max L.) in Relation to Glutamate Decarboxylase and Diamine Oxidase Activity Induced by Additives under Hypoxia. *European Food Research and Technology*, vol. 234, no. 4, p. 679-687. http://dx.doi.org/10.1007/s00217-012-1678-y

Hale, M. G., Orcutt, D. M. 1987. *The Physiology of Plant Under Stress*. 1st Ed., Jhon Wiley and Sons : New York, USA, p. 428.

Hepler, P. K. 2005. Calcium: A central regulator of plant growth and development. *Plant Cell*, vol. 17, no. 8, p. 2142-2155. http://dx.doi.org/10.1105/tpc.105.032508

John, M. K. 1970**.** Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Science*, vol. 109, no. 4, p. 214-220. http://dx.doi.org/10.1097/00010694-197004000-00002

Khan, M. S., Chaudhry, P., Wani, P. A., Zaidi, A. 2006. Biotoxic effects of the herbicides on growth, seed yield, and grain protein of greengram. *Journal of Applied Sciences and Environmental Management*, vol. 10, no. 3, p. 141-146. http://dx.doi.org/10.4314/jasem.v10i3.17333

Komatsuzaki, N., Tsukahara, K., Toyoshima, H., Suzuki, T., Shimizu, N., Kimura, T. 2007. Effect of Soaking and Gaseous Treatment on GABA Content in Germinated Brown Rice. *Journal of Food Engineering*, vol. 78, no. 2, p. 556-560. http://dx.doi.org/10.1016/j.jfoodeng.2005.10.036

Kráčmar, S., Vojtíšková, P., Švec, P., Kubáň, V., Krejzová, E., Bittová, M., Svobodová, B. 2014. Chemical composition of buckwheat plant parts and selected buckwheat products. *Potravinarstvo*, vol. 8, no. 1, p. 247-253. http://dx.doi.org/10.5219/385

Kramer, D., Breitenstein, B., Kleinwachter, M. Selmar, D. 2010. Stress Metabolism in Green Coffee Beans (Coffea arabica L.): Expression of Dehydrins and Accumulation of GABA during Drying. *Plant Cell Physiology*, vol. 51, no. 4, p. 546-553. http://dx.doi.org/10.1093/pcp/pcq019 PMid:20208063

Kumar, V., Giridhar, P., Chandrashekar, A., Ravishankar, G. A. 2008. Polyamines influence morphogenesis and caffeine biosynthesis in in vitro cultures of Coffea canephora P ex Fr. *Acta Physiologiae Plantarum*, vol. 30, no. 30, p. 217-23. http://dx.doi.org/10.1007/s11738-007-0110-x

Lutts, S., Kinet, J. M., Bouharmont, J. 1996. Ethylene production in relation to salinity by leaves of rice (*Oryza sativa* L.) tolerance and exogenous putrescine application. *Plant Science*, vol. 116, no. 1, p. 15-25. http://dx.doi.org/10.1016/0168-9452(96)04379-8

Mae, N., Makino, Y., Oshita, S., Kawagoe, Y., Tanaka, A., Aoki, K., Kurabayashi, A., Akihiro, T., Akama, K. and Koike, S. 2012. Accumulation Mechanism of γ-aminobutyric Acid in Tomatoes (Solanum lycopersicum L.) under Low O_2 with and without CO_2. *Journal of Agricultural and Food Chemistry*, vol. 60, no. 4, p. 1013-1019. http://dx.doi.org/10.1021/jf2046812

Martin-Tanguy, J., Aribaud, M. 1994. Polyamine metabolism, floral initiation and floral development in chrysanthemum (Chrysanthemum morifolium Ramat.), *Plant Growth Regulation*, vol. 15, no. 1, p. 23-31. http://dx.doi.org/10.1007/BF00024673

McAinsh, M. R., Pittman, J. K. 2009. Shaping the calcium signature. *New Phytologist*, vol. 181, no. 2, p. 275-294.

Mody, I., De Koninck, Y., Otis, T. Soltesz, I. 1994. Bridging the Cleft at GABA Synapses in the Brain. *Trends in Neurosciences*, vol. 17, no. 12, p. 517-525. http://dx.doi.org/10.1111/j.1469-8137.2008.02682.x PMid:19121028

Nemat Alla, M. M., Hassan, N. M., El-Bastawisy, Z. M. 2008. Changes in antioxidants and kinetics of glutathione-S-transferase of maize in response to isoproturon treatment. *Plant Biosystems*, vol. 142, no. 1, p. 16-20. http://dx.doi.org/10.1080/11263500701872135

Ndayiragije, A., Lutts, S. 2006. Exogenous putrescine reduces sodium and chloride accumulation in NaCl-treated calli of the salt-sensitive rice cultivar I Kong Pao. *Plant Growth Regulation*, vol. 48, no. 1, p. 51-63. http://dx.doi.org/10.1007/s10725-005-4825-7

Okada, T., Matsubara, Y. 2012. Tolerance to Fusarium Root Rot and the Changes in Free Amino Acid Contents in Mycorrhizal Asparagus Plants. *HortScience*, vol. 47, no. 6, p. 751-754. http://dx.doi.org/10.2503/jjshs1.81.257

Palavan-Unsal, N. 1995. Stress and polyamine metabolism, *Bulgarian Journal of Plant Physiology*, vol. 21, no. 1-3, p. 3-14.

Plant Health Care, Inc: *Gaba/pesticidal combinations.* Patent owner: Gregory Keith Lewis. PCT/US2009/054489. United States Patent. Patent no WO2010022251 A2. 2009-08-20.

Perveen**,** R. S., Shaukat, S. S.**,** Naqvi, I. I. 2002. Effect of Atrazine on Carbohydrates, Potassium, Sodium, Phosphate and Amino Acids Contents in Bean *Vigna radiate* (L.) Wilczek. *Asian Journal of Plant Sciences*, vol. 1, no. 5, p. 552-553. http://dx.doi.org/10.3923/ajps.2002.552.553

Powles, S. B., Yu, Q. 2010. Evolution in action: plants resistant to herbicides. *Annual Review of Plant Biology*, vol. 61, p. 317-47. http://dx.doi.org/10.1146/annurev-arplant-042809-112119

Ramakrishna, A., Ravishankar, G. A. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling and Behavior*, vol. 6, no. 11, p. 1720-1731. http://dx.doi.org/10.4161/psb.6.11.17613 PMid:22041989

Reddy, A. S. N., Ali, G. S., Celesnik, H., Day, I. S. 2011. Coping with Stresses: Roles of Calcium- and Calcium/Calmodulin-Regulated Gene Expression. *American Society of Plant Biologists*, vol. 23, no. 6, p. 2010-2032.

Saito, T., Matsukura, C., Sugiyama, M., Watahiki, A., Ohshima, I., Iijima, Y., Konishi, C., Fujii, T., Inai, S., Fukuda, N., Nishimura, S., Ezura, H. 2008. Screening for γ-aminobutyric acid (GABA)-rich tomato varieties. *Japanese Society for Horticultural Science*, vol. 77, no. 3, p. 242-250. http://dx.doi.org/10.2503/jjshs1.77.242

Shah, S. M., Khan, M. A., Mirza, M. Y. 2000. Effect of pre-emergence herbicide on soybean yield. *Sarhad Journa of Agriculture*, vol. 16, p. 57-59.

Syu, K. Y., Lin, C. L., Huang, H. C. Lin, J. K. 2008. Determination of Theanine, GABA, and Other Amino Acids in Green, Oolong, Black, and Pu-erh Teas with Dabsylation and High-performance Liquid Chromatography. *Journal of Agriculture and Food Chemistry*, vol. 56, no. 17, p. 7637-7643. http://dx.doi.org/10.1021/jf801795m

Tiburcio, A. F., Altabella, T., Borrel, T., Masgrau, C. 1997. Polyamines metabolism and its regulation, *Physiologia Plantarum*, vol. 100, no. 3, p. 664-674. http://dx.doi.org/10.1034/j.1399-3054.1997.1000330.x

Tomka, M., Bradová, J., Gálová, Z. 2010. Differentiation of barley genotypes using esterases as protein markers. *Potravinarstvo*, vol. 4, special no., p. 516-522. Available at: http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/5/Tomka.pdf

Todorova, D., Z. Katerova, V. Alexieva, I. Sergiev. 2015. Polyamines – possibilities for application to increase plant tolerance and adaptation capacity to stress. *Genetics and Plant Physiology*, vol. 5, no. 2, p. 123-144. http://dx.doi.org/10.1079/9781780642734.0194

Valero, D., Perez-Vicente, A., Martinez-Romero D., Castillo S., Guillen F., Serrano M. 2002. Plum storability improved after calcium and heat postharvest treatments: role of polyamines. *Journal of Food Sciences*, vol. 67, no. 7, p. 2571-2575. http://dx.doi.org/10.1111/j.1365-2621.2002.tb08778.x

Wakte, K. V., Kad, T. D., Zanan, R. L., Nadaf, A. B. 2011. Mechanism of 2-acetyl-1-pyrroline Biosynthesis in Bassia latifolia Roxb. Flowers. *Physiology and Molecular Biology of Plants*, vol. 17, no. 3, p. 231-237. http://dx.doi.org/10.1007/s12298-011-0075-5

Walters, D. 2003. Resistance to plant pathogens: possible roles for free polyamines and polyamine catabolism, *New Phytologist*, vol. 159, no. 1, p. 109-115. http://dx.doi.org/10.1046/j.1469-8137.2003.00802.x

Widodo, Patterson, J. H., Newbigin, E., Tester, M., Bacic, A., Roessner, U. 2009. Metabolic Responses to Salt Stress of Barley (Hordeum vulgare L.) Cultivars, Sahara and Clipper, which Differ in Salinity Tolerance. *Journal of Experimental Botany*, vol. 60, no. 14, p. 4089-4103. http://dx.doi.org/10.1093/jxb/erp243

Xing, S. G., Jun, Y. B., Hau, Z. W., Liang, L. Y. 2007. Higher Accumulation of Gamma-aminobutyric Acid Induced by Salt Stress through Stimulating the Activity of Diarnine Oxidases in Glycine max (L.) Merr. Roots. *Plant Physiology Biochemistry*, vol. 45, no. 8, p. 560-566. http://dx.doi.org/10.1016/j.plaphy.2007.05.007

Yang, R., Chen, H., Gu, Z. 2011. Factors Influencing Diamine Oxidase Activity and γ-aminobutyric Acid Content of Fava Bean (Vicia faba L.) during Germination. *Journal of Agricultural and Food Chemistry*, vol. 59, no. 21, p. 11616-11620. http://dx.doi.org/10.1021/jf202645p

Yang, R., Guo, Q., Gu, Z. 2013. GABA Shunt and Polyamine Degradation Pathway on γ-aminobutyric Acid Accumulation in Germinating Fava Bean (Vicia faba L.) under Hypoxia. *Food Chemistry*, vol. 136, no. 1, p. 152-159. http://dx.doi.org/10.1016/j.foodchem.2012.08.008

Yang, R., Yin, Y., Gu, Z. 2015. Polyamine Degradation Pathway Regulating Growth and GABA Accumulation in Germinating Fava Bean under Hypoxia-NaCl Stress. *Journal of Agricultural Science and Technology*, vol. 17, no. 2, p. 311-320.

Yin, X. L., Jiang, L., Song, N. H., Yang, H. 2008. Toxic reactivity of wheat (*Triticum aestivum)* plants to herbicide isoproturon. *Journal of Agricultural and Food Chemistry*, vol. 56, no. 12, p. 4825-4831. http://dx.doi.org/10.1021/jf800795v PMid:18522406

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YEAST DIVERSITY IN NEW, STILL FERMENTING WINE "FEDERWEISSER"

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ABSTRACT

The aim of this study was to isolate and identify yeasts in different new wine "federweisser" samples. We collected the samples at the end of the August 2015 and in the middle of the September 2015. Used 15 new wine samples in this study (5 white and 10 red) were from the local Slovak winemakers. Irsai Oliver (3), Moravian Muscat (2), Agria/Turan (1), Dornfelder (3), Blue Frankish (3), Pinot Noir (1) and Saint Laurent (2). Three cultivation media were used for detection of yeasts in "federweisser" samples. Malt extract agar base (MEA), Wort agar (WA) and Wild yeast medium (WYM) were used for the cultivation of yeasts. Cultivation was performed by spread plate method. Ethanol/formic acid extraction procedure was used for preparation of samples. MALDI-TOF Mass Spectrometer (Microflex LT/SH) (Bruker Daltonics, Germany) was used for the identification of yeasts. We identified seven different strains of *Saccharomyces cerevisiae* (23; 70%), two strains of *Kloeckera apiculata* [teleomorph *Hanseniaspora uvarum*] (7; 21%), and one strain of *Pichia kluyveri* (1; 3%), *Pichia occidentalis* [anamorph *Candida sorbosa*] (1; 3%) and *Metschnikowia pulcherrima* (1; 3%) in 15 new wine "federweisser" samples. *Saccharomyces cerevisiae* was dominant species in each new wine sample, and formed creamy convex colonies with circular edge. *Metschnikowia pulcherrima* formed convex to pulvinate, circular white-pink colored colonies, *Kloeckera apiculata* formed flat, circular smooth colonies with turquoise center with gray edge, *Pichia occidentalis* formed irregular pulvinate light-cream colored colonies, and *Pichia kluyveri* formed turquoise, convex, undulate and smooth colonies on Malt extract agar base with bromocresol green.

Keywords: new wine; yeasts; *Saccharomyces cerevisiae*; MALDI-TOF MS

INTRODUCTION

Federweisser wine is grape must which is just undergoing the process of fermentation. Grape must is the juice of the wine grapes which is gained after the pressing of grapes. After corresponding treatment and storing, the must would become wine after finishing the process of fermentation. Because of this, Federweisser is not specially produced as some kind of drink but as an early product of wine production. The fermentation causes the splitting of the fructose of the grapes in alcohol and carbon dioxide. Because of the yeasts and bacteria in the must, the fermentation goes on very quickly. That is why Federweisser is drinkable only a couple of days. But the cool storage can lengthen the process of fermentation. In the refrigerator, Federweisser can be kept about 10 days. The grape must is considered "Federweisser" wine as soon as the alcohol concentration is about 4 to 6%. At the beginning, it tastes quite sweet. During the process of fermentation, the sweetness subsides. Due to the concentration of carbon dioxide, Federweisser tastes very prickly and tangy. Because of the high carbon dioxide concentration, a corking or air tight closure of the Federweisser is not possible. Especially in the past, this caused a transportation problem. Federweisser could only be offered regionally and was limited. Grape must is inoculated with a pure culture of yeasts (*S. cerevisiae*), usually 10-20 g.100 L^{-1} of must. Federweisser is very good for cold or warm drinking **(Malík et al., 2012)**.

Yeasts are found throughout nature. However, they do not occur randomly, but are found in specific habitats where different species form communities **(Lachance and Starmer, 1998)**. Within the winemaking environment (habitat), the vineyard (grape surfaces) and cellar (equipment surfaces and must) can be considered specialized niches where the wine related yeasts can form communities **(Polsinelli et al., 1996)**. The yeast species found in different niches associated with grape growth (vineyards) and wine production (wineries, grape must, fermentation and wine) can be arbitrarily divided into two groups, i.e. the *Saccharomyces* group and the non-*Saccharomyces* group. The *Saccharomyces* group with its primary representative, *Saccharomyces cerevisiae*, is present on grape skins in low numbers **(Rankine, 1972; Török et al., 1996, König et al., 2009)**, and on winery equipment and in fermenting must in greater numbers **(Fugelsang et al., 2007)**. Non-*Saccharomyces* yeast are part of the natural microbiota present on grapes, and harvesting and winemaking equipment, and are present at least during the early stages of fermentation **(Fleet and Heard, 1993; Renouf et al., 2005, 2007)**. While generally incapable of completing alcoholic fermentation, their application in co-inoculation or sequential inoculation with *S. cerevisiae* is increasingly popular **(Ciani et al., 2006; Ciani and Maccarelli, 1998; Comitini et al., 2011; Jolly et al., 2006; Soden et al., 2000)**, particularly for their effects on wine composition, flavour and aroma **(Benito et al., 2011; Ciani et al., 2006; Comitini et al., 2011; Cordero Otero et al., 2003; Di Maio et al., 2012;**

Domizio et al., 2011; Garcia et al., 2002; Jolly et al., 2006, 2014; Magyar and Toth, 2011; Morata et al., 2012; Soden et al., 2000; Toro and Vazquez, 2002).

The fermentation of grape must is a complex microbiological process that involves interactions between yeasts, bacteria, and filamentous fungi **(Fleet, 2007; Fugelsang and Edwards, 2007)**. Yeasts, which play a central role in the winemaking process, are unicellular fungi that reproduce by budding **(Ribéreau-Gayon et al., 2006)**. More than 100 yeast species have been isolated from grapes, must and wine **(König et al., 2009)**. The predominant species on the grape is *Kloeckera apiculata*, which may represent more than 50% of the flora obtained from the fruit **(Fugelsang and Edwards, 2007)**. Other species of obligate aerobic or weakly fermentative yeasts with very limited alcohol tolerance may also be found in lesser proportions. These belong to the genera *Candida*, *Cryptococcus*, *Debaryomyces*, *Issatchenkia*, *Kluyveromyces*, *Metschnikowia*, *Pichia*, and *Rhodotorula* **(Fleet and Heard, 1993; Ribéreau-Gayon et al., 2006)**. The growth of these species, known collectively as non-*Saccharomyces* yeasts (or wild yeasts), is limited to the first 2 or 3 days of fermentation, after which they die as a result of ethanol toxicity. As these yeasts disappear, highly fermentative strains of the species *Saccharomyces cerevisiae* and *Saccharomyces bayanus* begin to multiply until they become solely responsible for alcoholic fermentation. The yeasts present in the must during the first few hours after filling the tanks belong to the same genera as those found on the grapes, predominantly *Kloeckera* (*Hanseniaspora*). In these spontaneous vinification conditions, *Saccharomyces* yeasts begin to develop after around 20h and are present alongside the grape-derived yeast flora. After 3rd or 4th day of fermentation, *Saccharomyces* yeasts predominate and are ultimately responsible for alcoholic fermentation **(Ribéreau-Gayon et al., 2006)**. This change in the yeast population is linked to the increasing presence of ethanol, the anaerobic conditions, and the use of sulfites during harvesting and in the must, the concentration of sugar, and the greater tolerance of high temperatures shown by *S. cerevisiae* compared with other yeasts **(Fleet and Heard, 1993; Fleet, 2007)**. *S. cerevisiae* comprises numerous strains with varying biotechnological properties **(Ribéreau-Gayon et al., 2006)**.

The aim of this study was to isolate and identify yeasts in different Slovak new wine "federweisser" samples.

MATERIAL AND METHODOLOGY

Federweisser samples, Spread plate method and Cultivation media

Samples of new wine "federweisser" were collected at the end of the August 2015 and in the middle of the September 2015 from local Slovak winemakers. Samples (apx.100 mL) were collected into 200 mL sterile plastic bottles with screw caps, and immediately stored at 8 ±1 °C in refrigerator. Bottle caps have been released, because the carbon dioxide (CO_2) was still produced by yeasts. Collected and stored samples (No. 15) were diluted with sterile physiological saline (0.85%), and dilution 10^{-4} and 10^{-5} were used for next analysis. 100 µL each dilution (10^{-4}, 10^{-5}) was placed on the surface of solidified agar media. The spread plate method was used for isolation of yeasts in federweisser samples. Samples were obtained from white (5) and red new wines (10). Irsai Oliver (3), Moravian Muscat (2), Agria/Turan (1), Dornfelder (3), Blue Frankish (3), Pinot Noir (1) and Saint Laurent (2). Three cultivation media were used for detection of yeasts in federweisser samples. Malt extract agar base (MEA) (BioMark™, India); Wort agar (HiMedia®, India) and Wild Yeast medium (HiMedia®, India). MEA has been enriched with glucose (CentralChem®, Slovakia) (50 g.L^{-1}), yeast extract (Conda, Spain) (3 g.L^{-1}) and acid base indicator bromocresol green (Sigma-Aldrich®, USA) (0.020 g.L^{-1}) (pH 3.8-5.4 yellow to blue). Yeasts were cultivated on Petri dishes at 25 °C for 5 days in aerobic conditions.

Identification of yeasts

We used MALDI-TOF Mass Spectrometer (Bruker Daltonics, Germany) for identification of yeasts isolated from federweisser samples. After incubation of yeasts at 25 °C for 5 days, isolated colonies were picked and suspended in 300 µL of sterile distilled water and mixed thoroughly. 900 µL of absolute ethanol was added. The mixture was centrifuged at 13 000 × g for 2 min. After the supernatant was discarded, the pellet was centrifuged again. Residual ethanol was completely removed by pipetting and the pellet was allowed to dry at room temperature. Subsequently 10 µL of formic acid (70%) was added and mixed with the pellet with a sterile toothpick. Next, 10 µL of acetonitrile (100%) was added and mixed thoroughly. The solution was centrifuged at maximum speed for 2 minutes again, and 1 µL of the supernatant was spotted on a polished MALDI target plate (Bruker Daltonics, Germany). Immediately after drying 1 µL of the matrix solution was added to each spot and allowed to air dry. The matrix used was a saturated solution of α-cyano-4-hydroxycinnamic acid (HCCA) (Bruker Daltonics, Germany) dissolved in 50% acetonitrile with 0.025% trifluoroacetic acid (TFA). The matrix solution preparation (2.5 mg of HCCA) contains 500 µL of acetonitrile, 475 µL of ultra-pure water and 25 µL of trifluoroacetic acid. Next added 250 µL of this solution to the 2.5 mg of HCCA. Samples were then processed in the MALDI-TOF MS (Microflex LT/SH, Bruker Daltonics, Germany) with flex Control software and results obtained with Real-time Classification software (RTC) by used database "Taxonomy" (Bruker Daltonics, Germany).

RESULTS AND DISCUSSION

After cultivation time, we obtained results from number of CFU (colony forming unit) in 100 µL of new wine sample of each used decimal dilutions. For better interpretation of results logarithmic conversion was applied on numerical results. Natural logarithm (Log_e) was used in Microsoft® Office Excel program by function LN. The highest number of yeasts cultivated on malt extract agar (MEA) was found in sample number thirteen Pinot Noir 6.43 log CFU.100 µL^{-1} and the lowest number of yeasts cultivated on MEA was present in the third sample Moravian Muscat 4.62 log CFU.100 µL^{-1}. The highest number of yeasts cultivated on Wort agar (WA) was found also in sample number 13 Pinot Noir 6.39 log CFU.100 µL^{-1}, but the lowest number of yeasts

Table 1 Number of yeasts in federweisser samples in log CFU.100 μL^{-1}.

	Cultivation media	MEA		WA		WYM	
No.	**Variety**	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}
1.	*Agria/Turan*	6.12	5.29	6.34	5.74	6.04	5.39
2.	*Irsai Oliver*	6.07	5.35	6.27	5.69	6.00	4.75
3.	*Moravian Muscat*	5.91	4.62	6.07	5.57	6.03	5.08
4.	*Irsai Oliver*	6.32	5.89	6.18	5.38	5.74	5.51
5.	*Blue Frankish*	6.30	ND	6.20	ND	5.63	ND
6.	*Irsai Oliver*	6.27	5.81	6.28	5.54	6.29	6.05
7.	*Blue Frankish*	6.31	ND	6.25	ND	6.33	ND
8.	*Moravian Muscat*	6.38	5.98	6.30	5.78	5.70	5.24
9.	*Blue Frankish*	6.37	6.06	6.03	5.75	5.82	5.61
10.	*Dornfelder*	6.39	6.09	6.28	5.76	5.66	5.12
11.	*Saint Laurent*	5.98	5.07	6.18	5.77	4.25	2.89
12.	*Dornfelder*	6.23	5.86	6.24	5.75	5.56	4.57
13.	*Pinot Noir*	6.43	6.14	6.39	6.18	ND	ND
14.	*Saint Laurent*	6.51	6.19	6.29	5.77	5.11	4.72
15.	*Dornfelder*	6.01	5.84	5.92	5.65	5.25	4.20

NOTE: **MEA**: Malt extract agar, **WA**: Wort agar, **WYM**: Wild yeast medium, **ND**: not detected.

cultivated on WA was present in the fourth sample Irsai Oliver 5.38 log CFU.100 μL^{-1}. The highest number of yeasts cultivated on Wild yeast medium (WYM) was found in sample number seven Blue Frankish 6.33 log CFU.100 μL^{-1} and the lowest number of yeasts cultivated on WYM was present in the fifteenth sample Dornfelder 4.20 log CFU.100 μL^{-1}. Table 1 contains results from microbiology of new wines obtained by spread plate method with used specific decimal dilutions 10^{-4} and 10^{-5}. Yeasts were countable at these two used dilutions. In this study we identified seven different strains of *Saccharomyces cerevisiae* (23), two strains of

Table 2 Yeast species in new wine "federweisser" samples.

No.	Variety	Species identified by MALDI-TOF MS
1.	**Turan/Agria**	*Saccharomyces cerevisiae* WS LLH
		Saccharomyces cerevisiae 991400574
		Kloeckera apiculata DSM 70788
2.	**Irsai Oliver**	*Saccharomyces cerevisiae* WS LLH
		Saccharomyces cerevisiae DSM 1334
3.	**Moravian Muscat**	*Saccharomyces cerevisiae* DSM 3798
		Saccharomyces cerevisiae WS LLH
		Kloeckera apiculata DSM 2768
4.	**Irsai Oliver**	*Saccharomyces cerevisiae* DSM 70868
5.	**Blue Frankish**	*Saccharomyces cerevisiae* DSM 1334
		Metschnikowia pulcherrima CBS 610NT
		Pichia kluyveri MY890_09
		Kloeckera apiculata DSM 2768
		Saccharomyces cerevisiae WS LLH
6.	**Irsai Oliver**	*Kloeckera apiculata* DSM 2768
		Pichia occidentalis CBS 1910
		Saccharomyces cerevisiae WS LLH
		Saccharomyces cerevisiae DSM 1334
7.	**Blue Frankish**	*Kloeckera apiculata* DSM 70788
		Saccharomyces cerevisiae DSM 1334
		Saccharomyces cerevisiae WS LLH
8.	**Moravian Muscat**	*Saccharomyces cerevisiae* WS LLH
		Kloeckera apiculata DSM 2768
		Saccharomyces cerevisiae CBS 1171
9.	**Blue Frankish**	*Kloeckera apiculata* DSM 2768
		Saccharomyces cerevisiae CBS 1171
10.	**Dornfelder**	*Saccharomyces cerevisiae* DSM 1334
11.	**Saint Laurent**	*Saccharomyces cerevisiae* DSM 1334
12.	**Dornfelder**	*Saccharomyces cerevisiae* DTY3
		Saccharomyces cerevisiae DSM 1334
13.	**Pinot Noir**	*Saccharomyces cerevisiae* DSM 70868
14.	**Saint Laurent**	*Saccharomyces cerevisiae* CBS 1171
15.	**Dornfelder**	*Saccharomyces cerevisiae* DSM 70868

Kloeckera apiculata (7), and one strain of *Pichia kluyveri* (1), *Pichia occidentalis* (1) and *Metschnikowia pulcherrima* (1) in fifteen federweisser samples. *Pichia kluyveri* was identified in Blue Frankish sample number five and *Pichia occidentalis* (anamorph *Candida sorbosa*) in sample number six (Irsai Oliver). We also identified one strain of *Metschnikowia pulcherrima* in sample number five (Blue Frankish).

The most common species in new wine samples was *Saccharomyces cerevisiae* and we identified seven different strains namely: DSM 1334, DSM 3798, DSM 70868, DTY3, CBS 1171, WS LLH and strain 991400574. Second most common species in new wine samples was *Kloeckera apiculata* (*Hanseniaspora uvarum*). *K. apiculata* was found in 7 new wine samples, two different strains (DSM 2768 and DSM 70788). Seven different strains of *Saccharomyces cerevisiae* was found in 15 new wine samples, what can be seen in Table 2.

S. cerevisiae is the most important yeast for wine production and is responsible for the metabolism of grape sugar to alcohol and CO_2. For these reasons *S. cerevisiae* is often simply referred to as "the wine yeast" **(Fleet, 1993; Pretorius et al., 1999; Swiegers and Pretorius, 2005)**. From all of identified yeasts, *Saccharomyces cerevisiae* was the dominant species, and we identified this species in all 15 new wine samples (70%). Grapes contain different species of yeast belongs to non-*Saccharomyces* yeasts such as *Kloeckera* (dominant genera), *Metschnikowia, Candida, Pichia, Rhodorotula, Aureobasidium* etc. *Saccharomyces* yeasts are not present in grape surface, or present in very low levels (less than 50 $CFU.mL^{-1}$) **(Prakitchaiwattana et al., 2004; Combina et al., 2005; Raspor et al., 2006; König et al., 2009)**.

When alcoholic fermentation starts non-*Saccharomyces* yeast population decrease. After the start of alcoholic fermentation when the ethanol concentration reaches 5 to 6% these yeast will be die **(Fugelsang et al., 2007)**. As fermentation progresses, the levels of these yeasts decrease, while that of *Saccharomyces* increases **(Fleet and Heard 1993)**. By the end of fermentation, *Saccharomyces* is the majority of the yeasts found, and often the only yeast isolated. New, still fermenting wine contains 4 to 6% ethanol and mostly contains only *Saccharomyces cerevisiae*, which is always predominant in new wines. But yeasts as *Kloeckera, Metschnikowia, Candida, Pichia* etc. can be identified in new wine samples in low populations. Some winemakers use commercial pure cultures and the others prefer to encourage the growth of some non-*Saccharomyces* yeasts early in alcoholic fermentation but eventually inoculate with *Saccharomyces* **(Fugelsang et al., 2007)**.

We identified except *S. cerevisiae* also *Kloeckera apiculata* in 7 new wine samples (21%) in lower population. Very interesting was that we isolated and identified only 3 another species of yeasts: *Metschnikowia pulcherrima* (3%), *Pichia kluyveri* (3%) and *Pichia occidentalis* (3%). In study **Kántor et al., (2015)** bromocresol green was used as a supplement also in Malt extract agar base (MEA) from BioMark™ (India). But in that study, cultivation media was not supplemented with yeast extract and glucose, only bromocresol green was added. After sterilization by autoclaving, that medium had a dark blue color. However in this study we supplemented Malt extract agar base (BioMark™, India) with yeast extract and glucose, and after sterilization by autoclaving had medium olive-green color. Malt extract agar base (BioMark™, India) contains only malt extract (30 $g.L^{-1}$), mycological peptone (5 $g.L^{-1}$) and agar (15 $g.L^{-1}$). Supplementation was desired in this case with glucose, yeast extract and bromocresol green. Yeasts grow very well in this modified medium, and bromocresol green is very helpful in differentiating of yeasts. Figure 1 shows the different colony morphology of 4 yeast species grown on MEA in sample number 5 (Blue Frankish). As you can see, the number of *Saccharomyces cerevisiae* was the highest, then *Kloeckera apiculata* and after that *Pichia kluyveri* and

Figure 1 Yeast species isolated from new wine "Federweisser" (Sample no. 5, dilution 10^{-4}).

only one colony on this petri dish belonged to *Metschnikowia pulcherrima*. *M. pulcherrima* produced maroon colored pigment called pulcherrimin, which was visible from the bottom of the petri dish. *Metschnikowia pulcherrima* formed convex to pulvinate, circular white-pink colonies. *Kloeckera apiculata* formed flat, circular smooth colonies with turquoise center with gray edge. *Pichia kluyveri* formed turquoise, convex, undulate and smooth colonies and *Pichia occidentalis* formed irregular pulvinate light-cream colonies.

CONCLUSION

In this study we isolated and identified yeast species in 15 Slovak new wine "Federweisser" samples. We identified the yeast isolates by MALDI-TOF mass spectrometry biotyper (Bruker Daltonics, Germany). The most dominant species was *Saccharomyces cerevisiae* which was isolated from all 15 new wine samples, which was a very good result. By mass spectrometry we identified 7 different strains of *S. cerevisiae*. The second most common species was *Kloeckera apiculata* (*Hanseniaspora uvarum*) found in 7 new wine samples (2 strains). We also identified other non-*Saccharomyces* yeasts such as *Metschnikowia pulcherrima* (1 strain), *Pichia occidentalis* (1 strain) and *Pichia kluyveri* (1 strain).

REFERENCES

Benito, S., Morata, A., Palomero, F., Gonzales, M. C., Suarez-Lepe, J. A. 2011. Formation of vinyl phenolic pyranoanthocyanins by *Saccharomyces cerevisiae* and *Pichia guillermondii* in red wines produced following different fermentation strategies. *Food Chemistry*, vol. 124, no. 1, p. 15-23. http://dx.doi.org/10.1016/j.foodchem.2010.05.096

Ciani, M., Beco, L., Comitini, F. 2006. Fermentation behaviour and metabolic interactions of multistarter wine yeast fermentations. *International Journal of Food Microbiology*, vol. 108, no. 2, p. 239-245. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.11.012

Ciani, M., Maccarelli, F. 1998. Oenological properties of non-*Saccharomyces* yeasts associated with winemaking. *World Journal of Microbiology and Biotechnology*, vol. 14, no. 2, p. 199-203. http://dx.doi.org/10.1023/a:1008825928354

Combina, M., Mercado, L., Borgo, P., Elia, A., Joofre, V., Ganga A., Martinez, C., Catania, C. 2005. Yeasts associated to Malbec grape berries from Mendoza, Argentina. *Journal of Applied Microbiology*, vol. 98, no. 5, p. 1055-1061. http://dx.doi.org/10.1111/j.1365-2672.2005.02540.x

Comitini, F., Gobbi, M., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., Ciani, M. 2011. Selected non-Saccharomyces wine yeasts in controlled multistarter fermentations with *Saccharomyces cerevisiae*. *Food Microbiology*, vol. 28, no. 5, p. 873-882. http://dx.doi.org/10.1016/j.fm.2010.12.001

Cordero Otero, R., Ubeda, J. F., Brionez-Perez, A. I., Potgieter, N., Villena, M. A., Pretorius, I. S., Van Rensburg, P. 2003. Characterization of the b-glucosidase activity produced by enological strains of non-*Saccharomyces* yeast. *Journal of Food Science*, vol. 68, no. 8, p. 2564-2569. http://dx.doi.org/10.1111/j.1365-2621.2003.tb07062.x

Di Maio, S., Genna, G., Gandolfo, V., Amore, G., Ciaccio, M., Oliva, D. 2012. Presence of *Candida zemplinina* in Sicilian musts and selection of a strain for wine mixed fermentations. *South African Journal of Enology and Viticulture*, vol. 33, p. 80-87.

Domizio, P., Romani, C., Lencioni, L., Comitini, F., Gobbi, M., Mannazzu, I., Ciani, M. 2011. Outlining a future for non-*Saccharomyces* yeasts: Selection of putative spoilage wine strains to be used in association with *Saccharomyces cerevisiae* for grape juice fermentation. *Internationl Journal of Food Microbiology*, vol. 147, no. 3, p. 170-180. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.03.020

Fleet, G. H. 1993. The microorganisms of winemaking – isolation, enumeration and identification. In: Fleet, G.H. (ed). *Wine microbiology and biotechnology*. Harwood Academic Publishers : Switzerland, p. 1-25. ISBN 9780415278508

Fleet, G. H. 2007. Wine. In M. P. Doyle, & L. R. Beuchat (Eds.), *Food microbiology: Fundamentals and frontiers* (3rd ed.), ASM Press : Washington, DC, USA, p. 863-890. http://dx.doi.org/10.1128/9781555815912.ch41

Fleet, G. H., Heard, G. 1993. Yeasts - Growth during fermentation. In: Fleet, G. (Ed.), *Wine microbiology and biotechnology*. Taylor & Francis Inc. : New York, USA, p. 27-54. ISBN 9781555816261 http://dx.doi.org/10.1128/9781555818463

Fugelsang, K. C., Edwards, C. G. 2007. *Wine microbiology: Practical applications and procedures*. Springer : New York, NY, USA, 393 p. ISBN: 978-0-387-33341-0 http://dx.doi.org/10.1007/978-0-387-33349-6

Garcia, A., Carcel, C., Dulau, L., Samson, A., Aguera, E., Agosin, E., Gunata, Z. 2002. Influence of a mixed culture with *Debaryomyces vanriji* and *Saccharomyces cerevisiae* on the volatiles of a Muscat wine. *Journal of Food Science*, vol. 67, no. 3, p. 1138-1143. http://dx.doi.org/10.1111/j.1365-2621.2002.tb09466.x

Jolly, N. P., Augustyn, O. P. H., Pretorius, I. S. 2006. The role and use of non-Saccharomyces yeasts in wine production. *South African Journal of Enology and Viticulture*, vol. 27, no. 1, p. 15-39.

Jolly, N. P., Varela, C., Pretorius, I. S. 2014. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Research*, vol. 14, no. 2, p. 215-237. http://dx.doi.org/10.1111/1567-1364.12111

Kántor, A., Kačániová, M., Kluz, M. 2015. Natural microflora of wine grape berries. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 4, no. 1, p. 32-36. http://dx.doi.org/10.15414/jmbfs.2015.4.special1.32-36

König, H., Fröhlich, J., Unden, G. 2009. *Biology of Microorganisms on Grapes, in Must and in Wine*, Springer-Verlag : Berlin Heidelberg, Germany, 378 p. ISBN 978-3-540-85462-3. http://dx.doi.org/10.1007/978-3-540-85463-0

Lachance, M. A., Stramer, W. T. 1998. *Ecology of yeasts*. In: Kurtzman, C.P. & Fell, J.W. (eds). The yeasts, a taxonomic study. Elsevier Science Publishers : Amsterdam, Netherland, p. 21-30. ISBN: 978-0-444-81312-1 http://dx.doi.org/10.1016/b978-044481312-1/50007-1

Magyar, I., Toth, T. 2011. Comparative evaluation of some oenological properties in wine strains of *Candida stellata*, *Candida zemplinina*, *Saccharomyces uvarum* and *Saccharomyces cerevisiae*. *Food Microbiology*, vol. 28, no. 1, 94-100. http://dx.doi.org/10.1016/j.fm.2010.08.011

Malík, F., et al. 2012. Vinársky lexikón. (1st ed.): Fedor Malík a syn : Modra, Slovakia, 144 p. ISBN: 978-80-97086-30-5.

Morata, A., Benito, S., Loira, I., Palomero, F., Gonzales, M. C., Suarez-Lepe, J. A. 2012. Formation of pyranoanthocyanins by *Schizosaccharomyces pombe* during the fermentation of red must. *International Journal of Food*

Microbiology, vol. 159, no. 1, p. 47-53. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.08.007

Polsinelli, M., Romano, P., Suzzi, G., Mortimer, R. 1996. Multiple strains of *Saccharomyces cerevisiae* on a single grape vine. *Letters of Applied Microbiology*, vol. 23, no. 2, p. 110-114. http://dx.doi.org/10.1111/j.1472-765x.1996.tb00043.x

Prakitchaiwattana, C. J., Fleet, G. H., Heard, G. M. 2004. Application and evaluation of denaturing gradient gel electrophoresis to analyse the yeast ecology of wine grapes. *FEMS Yeast Research*, vol. 4, no. 8, p. 865-877. http://dx.doi.org/10.1016/j.femsyr.2004.05.004

Pretorius, I. S., Van der Westhuizen, T. J., Augustyn, O. P. H. 1999. Yeast biodiversity in vineyards and wineries and its importance to the South African wine industry. *South African Journal of Enology and Viticulture*, vol. 20, no. 2, p. 61-74.

Rankine, B. C. 1972. Influence of yeast strain and malolactic fermentation on composition and quality of table wines. *American Journal of Enology and Viticulture*, vol. 23, no. 4, p. 152-158.

Raspor, P., Milek, D. M., Polanc, J., Smole Mozina, S., Cadez, N. 2006. Yeasts isolated from three varieties of grapes cultivated in different locations of the Dolenjska vine-growing region, Slovenia. *International Journal of Food Microbiology*, vol. 109, no. 1-2, p. 97-102. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.01.017

Renouf, V., Claisse, O., Lonvaud-Funel, A. 2005. Understanding the microbial ecosystem on the grape berry surface through numeration and identification of yeast and bacteria. Aust. *Journal of Grape and Wine Research*, vol. 11, no. 3, p. 316-327. http://dx.doi.org/10.1111/j.1755-0238.2005.tb00031.x

Renouf, V., Strehaiano, P., Lonvaud-Funel, A. 2007. Yeast and bacteria analysis of grape, wine and cellar equipments by PCR-DGGE. *Journal International des Sciences de la Vigne et du Vin*, vol. 41, no. 1, p. 51-61.

Ribéreau-Gayon, P., Dubourdieu, D., Donéche, B., Lonvaud, A. 2006. *Handbook of enology*, Vol. 1. The microbiology of wine and vinifications (2nd ed.), John Wiley & Sons : Chichester, UK. ISBN: 0-470-01034-7.

Soden, A., Francis, I. L., Oakey, H., Henschke, P. A. 2000. Effects of co-fermentation with *Candida stellata* and *Saccharomyces cerevisiae* on the aroma and composition of Chardonnay wine. *Australian Journal of Grape and Wine Research*, vol. 6, no. 1, p. 21-30. http://dx.doi.org/10.1111/j.1755-0238.2000.tb00158.x

Swiegers, J. H., Pretorius, I. S. 2005. Yeast modulation of wine flavour. *Advances in Applied Microbiology*, vol. 57, p. 131-175. http://dx.doi.org/10.1016/s0065-2164(05)57005-9

Toro, M. E., Vazquez, F. 2002. Fermentation behaviour of controlled mixed and sequential cultures of *Candida cantarelli* and *Saccharomyces cerevisiae* wine yeasts. *World Journal of Microbiology and Biotechnology*, vol. 18, p. 347-354. http://dx.doi.org/10.1023/A:1015242818473

Török, T., Mortimer, R. K., Romano, P., Suzzi, G., Polsinelli, M. 1996. Quest for wine yeasts – an old story revisited. *Journal of Industrial Microbiology & Biotechnology*, vol. 17, no. 3, p. 303-313. http://dx.doi.org/10.1007/bf01574705

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RISK OF CONTAMINATION OF WILD BERRIES FROM UPPER ORAVA REGION BY CADMIUM

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ABSTRACT

The upper Orava region is located at the North Slovakia, near of potential sources of environmental contamination due by mining of coal, zinc and lead ores. The aim of the study was to evaluate the risk of consumption of wild forest fruit from Upper Orava region from the aspect of cadmium content. Ten sampling points were found by random search. From these points samples of soil, leaves and fruits of wild berries (9 samples of blueberries *Vaccinium Myrtillus* and 1 sample of strawberries *Fragaria Vesca*) were collected. In soil samples the active soil reaction (pH/H_2O) ranged from 3.53 (strong acidity) to 4.56 (extremly strong acidity), and the determined percentage of humus ranged from 1.66 (low humic soil) to 4.90 (high humic soil). In two soil samples the total content of cadmium determinated in soil extracts by *aqua regia* exceeded limit 0.70 mg.kg^{-1} given by the legislation in tne Slovak Republic. In three soil samples the determined content of cadmium mobile forms determined in soil extracts by NH_4NO_3 exceeded the limit 0.10 mg.kg^{-1}. The content of Cd determined in leaves as well as in fruits was evaluated according to Food Codex of the Slovak Republic. Only in one sample of leaf samples the limit 1.00 mg.kg^{-1} was exceeded. The other leaf samples are safely when used as an ingredient in tea mixtures. On the other hand even in 7 fruit samples the limit 0.05 mg.kg^{-1} was exceeded. This fruit can pose a risk for the human organism when is directly consumed as well as may negatively affect the human health when is used as raw materials in the food industry.

Keywords: Upper Orava; heavy metals; cadmium; soil; wild berries; leaves

INTRODUCTION

The upper Orava region is located on the North Slovakia, surrounded by Orava reservoir, near South Poland border (under 20 km).

In South Poland, close to the Silesia-Cracow region, seven of twenty-seven ecological hazardous areas of Poland are located. Mining of coal, zinc and lead ores cause a great additional threat to the natural environment and the human population. A considerable set of data is available on the air pollution in the city of Cracow and the whole area of Katowice voivodship **(Godzik, 1993)**.

These two towns are from the investigated area 120 km respectively 140 km far. So, the air pollution caused by long-distance transfer from these industrial centres can be a potencial source of contamination by heavy metals in the environment of the Upper Orava region.

Heavy metal pollution is released into the environment by various anthropogenic activities, such as industrial manufacturing processes, domestic refuse and waste materials **(Guala et al., 2010)**. Soils contaminated with heavy metals cause many environmental and human health problems calling for an effective technological solution. Many sites around the world remain contaminated because it is expensive to clean them up by available technologies.

Anthropogenic pollution caused by heavy metals entering into the plant is subsequently passed into the food chain with the consequence in hazards to human health **(Krížová, 2009)**.

Cadmium, a by-product of zinc production, is one of the most toxic elements to which man can be exposed at work or in the environment. Once absorbed, Cd is efficiently retained in the human body, in which it accumulates throughout life. Cd is primarily toxic to the kidney, but it can also cause prostate and renal cancer as well as the bone demineralization **(Bernard, 2008)**.

In Slovakia there are many areas with natural resources of some forest fruit, such as raspberries, blueberries, blackberries, lingonberries, etc. These fruits contain vitamins, minerals and polyphenolics compounds which are resistant against unsuitable climatic conditions and they can adapt to more severe soil-climatic conditions. Upper Orava region belongs to the Slovakian areas with an occurrence of wild forest berries. Forest fruit such as blueberries, forest strawberries, raspberries, cranberries etc. are often collected by people for their flavor, color and bioactive components which have a positive effect to the human health **(Nile and Park 2014)**. According to **Häkkinen et al., (1999**) small forest fruits, both wild or bred, are traditional part of Finnish consumers, with significant content of biological active non-nutrients, but also of essential nutritive components. The essential elements (K, Ca, P, Mg, Al, B, Cu, Fe, Na, Mn and Zn) are important components of highbush blueberries, while suitable fact for human organism is low content of Na **(Bushway et al., 2006)**. **Prior et al., (1998)** consider blueberries as one of the richest sources of antioxidant

phytonutrients, while composition and content of phenolic compounds in blueberries have changed in relation to variety, period, as well as to locality of growing **(Giovanelli and Burati, 2009)**.

The aim of the study was to evaluate the risk of consumption of wild forest fruit from Upper Orava region from the aspect of Cd content.

MATERIAL AND METHODOLOGY

The experiment was realized in region Upper Orava, in cadasters of villages: Malé Borovce, Habovka, Zábiedovo, Brezová, Vitanová and area near Orava reservoir (Figure 1). Samples were collected in June 2014. The exact coordinates of sampling sites are presented in Table 1. The average annual temperature is 6 °C (12.5 °C during vegetation) and the average annual rainfall is 800 – 900 mm (550 mm during vegetation).

Samples of soil, fruits (9 samples of blueberries (*Vaccinium Myrtillus*) and 1 sample of wild strawberries (*Fragaria vesca*)) and leaves (can be used as a tea mixture) were taken from individual sampling points. The soil samples were taken from the upper horizon.

The active soil reaction pH/H_2O was determined electrometrically (691 pH Meter, Metrohm, Swiss), and content of oxidizable carbon (C_{OX}, %) was determined using volumetric method according to Tjurin (H_2SO_4: Merck, Germany, $K_2Cr_2O_7$: Merck, Germany; $(NH_4)_2Fe(SO_4)_2$•$6H_2O$: Merck, Germany) while a content of humus (Hum., %) was calculated from C_{OX} content.

Pseudototal content of cadmium including all the form besides residual metal fraction was assessed in soil extract by *aqua regia* (HCl: CentralChem, Slovakia, HNO_3: Merck, Germany) and content of mobile forms in soil extract by NH_4NO_3 (c = 1 mol.dm^{-3}, Merck, Germany)). Used analytical method was flame AAS (AAS Varian AA Spectr DUO 240 FS/240Z/UltrAA, Varian, Australia).

The determined values were compared with limits given by **European Commission Regulation no. 1881/2006** as well as **Slovak decree no. 220/2004 of coll.**

Homogenized berry samples (4 g) were mineralized in a closed system of microwave digestion using Mars X-Press 5 (CEM Corp., USA) in a mixture of 5 mL HNO_3 (Suprapur, Merc, Germany) and 5 mL deionized water (0.054 μS.cm^{-1}) from Simplicity 185 (Millipore, UK). Metal determinations were performed in a Varian AA240Z (Varian, Australia) atomic absorption spectrometer with Zeeman background correction. The graphite furnace technique was used for the Cd determination. The obtained results were expressed as mg.kg^{-1} FM. Gained results were evaluated according to hygienic limit for Cd content in fruit given by the **Food Codex of the Slovak Republic**. Each analysis was done in 4 repetitions.

Statistical processing of the results was carried out using software Statgraphics Centurion XVI.I. One-way analysis of variance (α = 0.05) was used. Mean comparisons between investigated parameters were done by the LSD test.

RESULTS AND DISCUSSION

Soil samples:

In soil samples the active soil reaction (pH/H_2O) ranged from 3.53 (strong acidity) to 4.56 (extremly strong acidity), data are available in Table 1. **Römkens et al. (1998) and Barančíková (1998)** reported that Cd and Zn solution concentrations were higher in forest soils and were strongly increased below pH 5.5 even despite the low total metal content. **Kawabata et al., (2011)** presented similar pH values to our results in soil used for blueberry production. On the other hand, **Maliníková et al., (2013)** presented higher pH values of forest soil (5.28-7.67). The

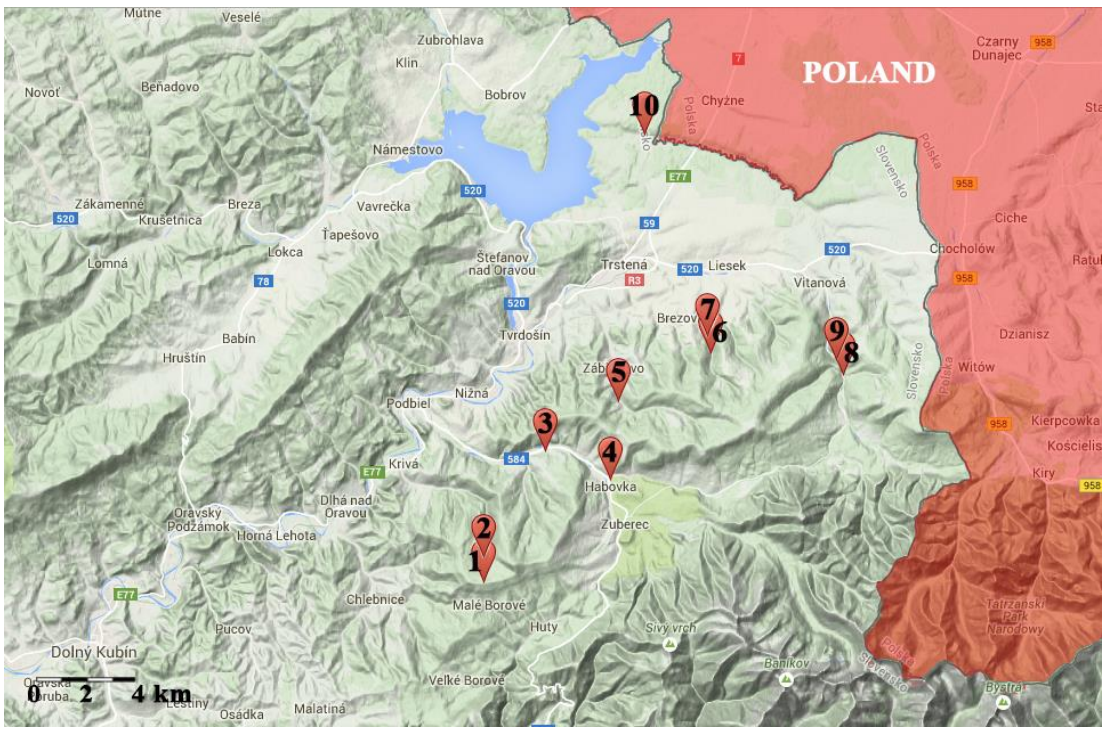

Figure 1 Investigated area and sampling points.

% of humus in soil samples was in range 1.66 (low humic soil) to 4.90 (high humic soil), data are available in Table 1. **Fanrong (2011)** reported a positive correlation between organic mater content and bioavailability of heavy metals in soil. The total cadmium content in soil samples (determinated in soil extracts by *aqua regia*) was in the range 0.12 – 1.45 mg.kg^{-1} (Table 1). In two soil samples (Samples no. 1 and 3) the total content of cadmium exceeded the limit value 0.70 mg.kg^{-1} given by **European Commission Regulation no. 1881/2006** as well as **Slovak decree no. 220/2004 of coll.** (1.45 and 0.73 mg.kg^{-1} respectively). Several studies focused on the monitoring of the environmental contamination of the soils in Slovakia were published. **Musilová et al., (2015)**, **Vilček et al., (2012), Tomáš et al., (2009)** determined the total Cd content in soil samples in intervals 0.65 – 6.73 mg.kg^{-1}, 0.36 – 12.26 mg.kg^{-1}, 0.34 – 0.40 mg.kg^{-1} respectively. **Taraškevičius et al., (2013)** presented values of the Cd contents in soil extracts of *aqua regia* in some European soils in range 0.07 – 12.8 mg.kg^{-1}. In Table 1 also values of content of mobile cadmium forms determined in soil extracts by NH_4NO_3 are presented. The values were compared to the limit value 0.10 mg.kg^{-1} given by **European Commission Regulation no. 1881/2006** as well as **Slovak decree no. 220/2004 of coll**. In Sample no. 1 (0.15 mg.kg^{-1}), Sample no. 2 (0.10 mg.kg^{-1}) and Sample no. 8 (0.13 mg.kg^{-1}) the determined content of cadmium mobile forms exceeded the limit. **Musilová et al. (2015), Vilček et al. (2012), Tomáš et al. (2009)** also determined contents of cadmium mobile forms in soils of Slovakia. They determined values in range 0.029 – 0.236 mg.kg^{-1}, 0.02 – 0.78 mg.kg^{-1}, 0.5 – 0.7 mg.kg^{-1} respectively, similar to our results.

Leaf samples:

The determined Cd content in leaves of investigated forest fruit (Table 2) was compared to hygienic limit 1.00 mg.kg^{-1} for Cd content in tea mixtures given by the **Food Codex of the Slovak Republic**. Only in Sample no. 6 (2.02 mg.kg^{-1} DM) the limit was exceeded, all other leaf samples have the determined Cd content under the hygienic limit. Despite that finding our results indicate a significantly higher degree of Cd accumulation in leaves than in fruits.

Fruit samples:

On the other hand even in 8 fruit samples the limit 0.05 mg.kg^{-1} given for small berries by the **Food Codex of the Slovak Republic** was exceeded (Table 2), whereas in 1 Sample (no. 2) the Cd content was lower than the detection limit. **Von Hoffen et al., (2014)** determined Cd content of blackberries in range 0.004 – 0.18 mg.kg^{-1} FM. On the other hand, **Reimann et al., (2001)** presented significantly lower values (0.009 mg.kg^{-1}) of Cd content in blueberries compared to our results. According to **Wieczorek et al., (2010)** the concentration of Cd in wild berries, ranged from 6 to 49 μg.kg^{-1} fresh weight.

Cadmium (Cd) is a toxic heavy metal that can accumulate in the human body and the environment for

Table 1. Analysis of soil samples from Upper Orava region, July 2014.

No.	Sample	GPS coordinates	Active soil reaction [pH H2O]	Cox [%]	% of humus in soil samples [%]	Total Cd content in soil samples [mg.kg^{-1} DM]		Content of mobile forms of Cd in soil samples [mg.kg^{-1} DM]	
						Average	SD	Average	SD
1	Blueberries	N 49° 14.186´´ E 19° 31.819´´	3.53	2.84	4.90	1.45	±0.01	0.15	±0.02
2	Blueberries	N 49° 14.791´´ E 19° 31.826´´	4.11	1.79	3.09	0.37	±0.01	0.10	±0.01
3	Blueberries	N 49° 17.265´´ E 19° 33.997´´	4.48	1.54	2.66	0.73	±0.01	0.08	±0.00
4	Blueberries	N 49° 16.613´´ E 19° 36.374´´	4.56	2.53	4.36	0.34	±0.01	0.03	±0.01
5	Blueberries	N 49°18.442´´ E 19° 36.637´´	4.23	1.56	2.69	0.27	±0.02	0.05	±0.01
6	Blueberries	N 49° 19.607´´ E 19° 40.058´´	4.31	1.39	2.39	0.25	±0.01	0.05	±0.01
7	Blueberries	N 49° 19.957´´ E 19° 39.881´´	4.35	1.47	2.54	0.14	±0.01	0.06	±0.02
8	Blueberries	N 49° 19.053´´ E 19° 44.552´´	3.89	2.26	3.90	0.57	±0.02	0.13	±0.02
9	Blueberries	N 49° 19.412´´ E 19° 44.520´´	4.44	0.97	1.66	0.12	±0.01	0.07	±0.01
10	Strawberries	N 49° 24.775´´ E 19° 37.613´´	4.00	1.61	2.78	0.17	±0.01	0.09	±0.01
	Limit value *					**0.70**		**0.10**	

NOTE: * limit given by European Commission Regulation no. 1881/2006 as well as Slovak decree no. 220/2004 of coll.

Table 2. Cd contents in samples collected in Upper Orava region (fruits and leaves) and transfer factors (soil-fruit and soil-leaves), July 2014.

No.	Sample	Cd content in fruit samples [mg.kg^{-1} FM]		Cd content in leaves samples [mg.kg^{-1} DM]		Transfer factors soil - fruit	Transfer factors soil - leaves
		Average	SD	Average	SD		
1	Blueberries	0.09 e	±0.01	0.60 cd	±0.01	1.70	0.25
2	Blueberries	UDL**	-	0.89e	±0.02	-	0.11
3	Blueberries	0.05 c	±0.01	0.57 bc	±0.01	1.73	0.14
4	Blueberries	0.08 e	±0.00	0.92 e	±0.01	0.30	0.03
5	Blueberries	0.05 c	±0.01	0.52 a	±0.01	0.92	0.09
6	Blueberries	0.07 d	±0.01	2.02 f	±0.06	0.78	0.03
7	Blueberries	0.04 b	±0.01	0.61 d	±0.03	1.54	0.09
8	Blueberries	0.05 bc	±0.01	0.56 b	±0.01	2.82	0.23
9	Blueberries	0.05 c	±0.01	0.60 d	±0.02	1.53	0.12
10	Strawberries	0.07	±0.00	0.97	±0.01	1.35	0.09
	P-value	0.0000		0.0000			
	F-ratio	42.19		1413.93			
Limit value *		**0.05**		**1.00**			

NOTE: Average values marked with the same letter are not significantly different ($p < 0.05$)
* limit value given by Food Codex of Slovakia.
** Cd content under detection limit.

lengthy periods **(Zhang et al., 2014)** and due its exposure the toxic effects in a variety of structures such as kidneys, liver and central nervous system including proteinuria, glucosuria, and aminoaciduria with final renal dysfunction are confirmed **(Xu et al., 2013)**.

The high concentration of heavy metals in soils is usually reflected by higher concentrations of metals in plants, and consequently in animal and human bodies **(Buszewski et al., 2000)**.

The transfer of soil pollutants into the plants causes many physiological disorders. The degree of heavy metal mobility, activity and bioavailability and consequently plant uptake is influenced by many factors such soil reaction, temperature, redox potential, cation exchange capacity of solid phase, competition with other metal ions, ligation by anions, composition and quantity of the soil solution **(Wopereis et al., 1988)**.

To characterize quantitatively the transfer of an element from soil to plant, the soil–plant Partition Coefficient or Transfer Factor (TF) or Concentration Ratio or Biological Accumulation Coefficient (BAC) that expresses the ratio of contaminant concentration in plant parts to concentration in dry soil is used **(Chojnacka et al., 2005)**. In Table 2 the calculated values of TF are presented. Generally, the transfer factors calculated for soil-leaves transfer were low. This may be because only the accumulation of metals in the leaves were studied more metals could have accumulated in the root **(Olayinka et al. 2011)**. The transfer factors calculated for soil-fruit transfer were higher. The higher the value of the TF, the more mobile/available the metal is **(Olayinka et al., 2011)**.

CONCLUSION

In two soil samples from the Upper Orava region the total content of cadmium exceeded limit 0.70 mg.kg^{-1} and in three soil samples the determined content of cadmium mobile forms exceeded the limit 0.10 mg.kg^{-1}, which can be put into context with extremely strong soil reaction. This factor increases the release of mobile forms in the soil environment. Wild berries such as blueberries (*Vaccinium Myrtillus*) and wild strawberries (*Fragaria Vesca*) have a positive effect to the human health because of their content of bioactive and chemoprotective components as well as an antioxidant activity. On the other hand it is necessary to monitor content of cadmium or other heavy metals in this fruit which is affected by soil-ecological conditions. Heavy metals become toxic for the human organism, when they entering into the food chain. Eating wild berries from the region of Upper Orava may present a potential risk for the human health. Our results indicate a significantly higher degree of Cd accumulation in leaves than in fruits, even though the limit for tea mixture was exceeded only in one sample.

It is necessary to monitor the soil content of hazardous elements in territory of Upper Orava as well as their transfer into plants and the food chain because of food safety.

REFERENCES

Barančíková, G. 1998. Návrh účelovej kategorizácie pozemkov v SR z hľadiska citlivosti na znečistenie ťažkými kovmi (Proposal of appropriate categorization of land in the SR in terms of susceptibility to contamination by heavy metals). *Rostlinná výroba*, vol. 44, no. 3, p. 117-122.

Bernard, A. 2008. Cadmium & its adverse effects on human health. *Indian Journal of Medical Research*, vol. 128, p. 557-564. PMid:19106447

Bushway, R. J., Mc Gann, D. F., Cook, W. P., Bushway, A. A. 2006. Mineral and vitamin content of lowbush blueberries (*Vaccinium angustifolium Ait.*). *Journal of Food Science*, vol. 48, no. 6, p. 1878. http://dx.doi.org/10.1111/j.1365-2621.1983.tb05109.x

Buszewski, B., Jastrzębska, A., Kowalkowski, T., Górna-Binkul, A. 2000. Monitoring of Selected Heavy Metals Uptake by Plants and Soils in the Area of Toruń, Poland. *Polish Journal of Environmental Studies*, vol. 9, p. 511-515.

Chojnacka, K., Chojnacki, A., Gorecka, H. 2005. Bioavailability of potentially toxic metals from polluted soils to plants. *Science of the Total Environment*, vol. 337, no. 1-3, p. 175-182. http://dx.doi.org/10.1016/j.scitotenv.2004.06.009

Commission Regulation (EC) No. 1881/2006, 19. December 2006, that define maximum of levels for certain contaminants in foodstuffs. OJ L 314M , 1.12.2007, p. 558–577.

Fanrong, Z., Shafagat, A., Haitao, Z., Younan, O., Boyin, Q., Feibo, W., Gouping, Z. 2011 The influence of pH and organic mater content in paddy soil on heavy metal availability and their uptake by rice plants. *Environmental Pollution*, vol. 159, no. 1, p. 84-91. http://dx.doi.org/10.1016/j.envpol.2010.09.019

Foodstuffs Code of the Slovak Republic, Annex. 1 of the X. Head, from Second part. Contaminants in foodstuffs, Part A Chemical substances. Decree of the Ministry of Agriculture of the Slovak Republic No.: 18558/2006-SL. Available at: http://www.svps.sk/dokumenty/legislativa/18558_2006.pdf

Giovanelli, G., Buratti, S. 2009. Comparison of polyphenolic composition and antioxidant activity of wild Italian bluberries and some cultivated varieties. *Food Chemistry*, vol. 112, p. 903-908. http://dx.doi.org/10.1016/j.foodchem.2008.06.066

Godzik, S., Szudzuj, J., Poborski, P. 1993. Environmental pollution in south Poland and effects. *Folia Med Cracov*. vol. 34, no. 1-4, p. 9-18. PMid:8175068

Guala, D. S., Vega, F. A., Covelo, E. F. 2010. The dynamics of heavy metals in plant–soil interactions. *Ecological Modelling*, vol. 221, p. 1148-1152. http://dx.doi.org/10.1016/j.ecolmodel.2010.01.003

Häkkinen, S., Heinonen, M., Kärenlampi, S., Mykkänen, H., Ruuskanen, J., Törrö, R. 1999. Screening of seleceted flavonoids and phenolic acids in 19 berries. *Food Research International*, vol. 32, p. 345-353. http://dx.doi.org/10.1016/S0963-9969(99)00095-2

Kawabata, A., Deenik, J., Hamasaki, R., Lichty, J., Nakamoto, S. 2011. *Acidification of Volcanic Ash Soils From Maui and Hawai'i Island for Blueberry and Tea Production*. Honolulu (HI): University of Hawaii. 7 p. (Soil and Crop Management; AS-5). Available at: http://hdl.handle.net/10125/33267

Krížová, L., Vollmannová, A., Margitánová, E., Árvaj, J., Szabóová, G. 2009. Rizikové kovy v drobnom lesnom ovocí (Risk metals in the small forest fruit). *Potravinarstvo*, vol. 3, no. 4, p. 43-47.

Maliníková, E., Kukla, J., Kuklová, M., Balážová, M. 2013. Altitudinal variation of plant traits: morphological characteristics in Fragaria vesca L. (Rosaceae). *Annals of Forest Research*, vol. 56, no. 1, p. 79-89.

Musilova, J., Arvay, J., Vollmannova, A., Toth, T., Tomas, J. 2015. Environmental contamination by heavy metals in region with previous mining activity. In: *Proceeding of the 14th International Conference on Environmental Sciences and Technology*. Rhodes, Greece, 3-5 September 2015. Available at: http://cest.gnest.org/cest15proceedings/public_html/papers/cest2015_00067_poster_paper.pdf

Nile, S. H., Park, S. W. 2014. Edible berries: bioactive components and their effect on human health. *Nutrition*, vol. 30, no. 2, p. 134-144. http://dx.doi.org/10.1016/j.nut.2013.04.007 PMid:24012283

Olayinka K. O., Oyeyiola A. O., Odujebe F. O., Oboh B. 2011. Uptake of potentially toxic metals by vegetable plants grown on contaminated soil and their potential bioavailability using sequential extraction. *Journal of Soil Science and Environmental Management*, vol. 2, no. 8, p. 220-227.

Prior, R. L., Wu, X. L., Gu, L. W., Hager, T. J., Hager, A., Howard, L. R. 2008. Whole berries versus berry anthocyanins: Interactions with dietary fat levels in the C57BL/6J mouse model of obesity. *Journal of Agricultural and Food Chemistry*, vol. 56, issue 3, p. 647-653. http://dx.doi.org/10.1021/jf071993o PMid:18211017

Reimann, C., Koller, F., Frengstad, B., Kashulina, G., Niskavaara, H. Englmaier, P., 2001. Comparison of the element composition in several plant species and their substrate from a 1 500 000 km^2 area in Northen Europe. *Science of The Total Environment*, vol 278, no. 1-3, p. 87-112. http://dx.doi.org/10.1016/s0048-9697(00)00890-1.

Römkens, P., Salomons, W. 1998. Cd, Cu AND Zn solubility in arable and forest soils: consequnces of land use changes for metal mobility and risk assessment. *Soil science*, vol. 163, no. 11, p. 859-871.

Slovak decree No. 220/2004 of the coll. as amended. Law on the protection and use of agricultural land. Part: Limit values of the hazardous substances in agricultural soil. Available at: http://www.zakonypreludi.sk/zz/2004-220

Taraškevičius, R., Zinkutė, R., Stakėnienė, R., Radavičius, M. 2013. Case Study of the Relationship between Aqua Regia and Real Total Contents of Harmful Trace Elements in Some European Soils. *Journal of Chemistry*, vol. 2013, p. 5. http://dx.doi.org/10.1155/2013/678140

Tomáš, J., Čéry, J., Melicháčová, S., Árvay, J., Lazor, P., 2009. Monitoring of Risky Elements in Zone of Pollution Strážske Area. *Czech Journal of Food Sciences*, vol. 27, special issue. p. 397-400.

Vilček J., Hronec O., Tomáš J. 2012. Risk elements in soils of burdened areas of eastern Slovakia. *Polish Journal of Environmental Studies*, vol. 21, no. 5, p. 1429-1436.

Von Hoffen, L., Säumel, I. 2014. Orchards for edible cities: Cadmium and lead content in nuts, berries, pome and stone fruits harvested within the inner city neighbourhoods in Berlin, Germany. *Ecotoxicology and environmental safety*, vol. 101, p. 233-239. http://dx.doi.org/10.1016/j.ecoenv.2013.11.023 PMid:24507151

Wieczorek, J., Petrzak, M., Osowski, A., Wieczorek, Z. 2010. Determination of lead, cadmium, and persistent organic pollutants in wild and orchard-farm-grown fruit in northeastern Poland. *Journal of Toxicology, Environment and Health*, vol. 73, p. 1236-1243. http://dx.doi.org/10.1080/15287394.2010.492009 PMid:20706949

Wopereis, M. C., Gascuel-Odoux, C., Bourrie, G., Soignet, G. 1988. Spatial variability of heavy metals in soil on a one-hectare scale. *Soil Science*, vol. 146, p. 113-118. http://dx.doi.org/10.1097/00010694-198808000-00009

Xu, D., Chen, Z., Sun, K., Yan, D., Kang, M., Zhao, Y. 2013. Effect of cadmium on the physiological parameters and the subcellular cadmium localization in the potato (*Solanum tuberosum* L.). *Ecotoxicology and Environmental Safety*, vol. 97, p. 147-153. http://dx.doi.org/10.1016/j.ecoenv.2013.07.021 PMid:23938042

Zaujec, A. et al. 2009. *Pedológia a základy geológie*, Nitra : Slovak University of Agriculture, Slovakia, ISBN 978-80-552-0207-5.

Zhang, W. L., Du, Y., Zhai, M. M., Shang, Q. 2014. Cadmium exposure and its health effects: A 19-year follow-up study of a polluted area in China. *Science of the Total Environment*, vol. 470-471, p. 224-228. http://dx.doi.org/10.1016/j.scitotenv.2013.09.070 PMid:24140693

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THE EXTENSION OF SHELF-LIFE OF CHICKEN MEAT AFTER APPLICATION OF CARAWAY AND ANISE ESSENTIAL OILS AND VACUUM PACKAGING

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ABSTRACT

The effect of caraway (CEO) and anise (AEO) essential oils as well as vacuum packaging (VP) in extending of the shelf life of fresh chicken breast meat stored at 4 °C was investigated. CEO and AEO were used at concentrations 0.2% v/w with and without VP. Microbiological properties of chicken breast meat were monitored over a 16 day period. The microbiological parameters as the anaerobic plate count (AC), *Enterobacteraceae*, lactic acid bacteria and *Pseudomonas* spp. counts were detected. The anaerobic plate counts ranged from 2.77 log CFU.g^{-1} in all tested group on 0 day to 5.45 log CFU.g^{-1} on 16 day in control group stored in air condition. The number of lactic acid bacteria ranged from 3.20 log CFU.g^{-1} in all tested group on 0 day to 4.75 log CFU.g^{-1} on 16 day in control group stored in air condition. *Enterobacteriaceae* counts ranged from 0.00 to 4.25 log CFU.g^{-1} on 16 day in control group stored in air condition. The number of *Pseudomonas* spp. ranged from 0.00 log CFU.g^{-1} in all tested group on 0 day to 2.65 log CFU.g^{-1} on 16 day in control group stored in air condition. Statistically significant differences ($p \leq 0.001$) were found among tested group in all tested microorganisms. Among the antimicrobial combination treatments were examined in the study, the as application of vacuum packaging, EDTA, and essential oils were the most effective against the growth of lactic acid bacteria and *Enterobactericeae* and to a less extent on anaerobic plate count. The results of this present study suggest the possibility of using the essential oil of caraway and anise as natural food preservatives and potential source of antimicrobial ingredients for chicken breast meat.

Keywords: bacteria; caraway and anise essential oils; vacuum; EDTA; chicken breast

INTRODUCTION

Special attention in poultry meat production is paid to the fact that live animals are hosts of a large number of different microorganisms residing on their skin, feathers or in the alimentary tract. Majority of these microorganisms are eliminated during the slaughter, but subsequent contamination is possible at any stage of the production process. Contamination may occur from feather plucking and evisceration equipment, washing prior to storage as cooling, or during the freezing. Microorganisms from the environment, equipment and operators' hands can contaminate meat. During the slaughter, the changes in composition of microflora occur from, in general, Gram-positive rods and micrococci to, most frequently, Gram-negative bacteria, including *Enterobacteriaceae*, *Pseudomonas* spp. Industrial poultry slaughterhouses have a particular technological process, the individual stages of which are not in conformity with modern principles of hygienic meat production and processing so there are various possibilities for contamination of chicken meat **(Kozačinski et al., 2006)**. Poultry meat is a highly perishable food commodity providing an almost perfect medium for microbial growth including both spoilage and pathogenic microorganisms **(Jay et al., 2005**) therefore the microbial contamination during the poultry meat processing is very crucial.

Meat production is one of the major activities in Europe. The main type of meat produced is pork (48.7%) followed by poultry (23.6%) and bovine (23.3%). Meat and meat products present an ideal substrate supporting the growth of several spoilage and pathogenic bacteria. Moreover, meat and poultry products have frequently been found to be contaminated with pathogens **(Mor-Mur and Yuste, 2010)**. The pathogens ability to grow at refrigerator temperatures helps the organism to evolve from a low initial to an infective dose level during the storage of refrigerated foods, including those originally harbouring the pathogen and those, post-heat treatment, contaminated **(Ray, 2001)**.

It is well known that packaging makes food more convenient and gives the food greater safety assurance from microorganisms, biological and chemical changes so that the packaged foods may have a longer shelf life. As a result, packaging has become an indispensable element in the food manufacturing process. In order to meet the huge demand of the food industry, there has been a remarkable growth in the development of food packaging in the past decades **(Tsigarida and Nychas, 2001)**.

Aromatic plants and herbal products have been used worldwide as natural additives for medicinal purposes because they have been accepted by consumers. Various biological activitive compounds sharing antioxidative, anticoccidial, immunostimulating or antimicrobial properties have been identified in these plants **(Ivanišová et al., 2013; Ivanišová et al., 2015 a,b)**.

Carum carvi, which is also known as caraway, is one of the oldest spices cultivated in Europe. Nowadays, it is cultivated from northern temperate to tropical climates, including countries such as Jamaica, India, Canada, the United States of America and Australia. In India, this spice is known as *Kashmiri jeera*. The dried ripe fruits (schizocarp) of *C. carvi* L. family *Apiaceae* (*Umbelliferae*) are extensively being used in folk medicine as a carminative, found to be effective against spasmodic gastrointestinal complaints, irritable stomach, indigestion, lack of appetite and dyspepsia in adults, and in relieving flatulent colic of infants. The volatile oils from *C. carvi* have also been used as an anti ulcerogenic, antitumor, antiproliferative and antihyperglycemic agent. The seeds of *C. carvi* have been used in alternative medicine as a laxative, in colic treatment, and as a mouth freshener **(Thippeswamy et al., 2013)**.

Anise (*Pimpinella anisum* L.) a member of the *Apiaceae* family, is an annual aromatic plant, native to Iran, India, Turkey and many other warm region in the world. Anise seed possesses eugenol trans-anethole, methylchavicol, anisaldehyde, estragole, coumarins, scopoletin, umbelliferone, estrols, terpene hydrocarbons, polyenes, and polyacetylenes. Most of the plant parts such as fruits, seeds, and essential oil contain compounds with proven antiparasitic and digestion stimulating, antifungal and antipyretic, antioxidant, antimicrobial, anthelmintic and hypocholesterolemic properties **(Yazdi et al., 2014)**.

The present study was undertaken to determine the effect of vacuum packaging combined with caraway or anise essential oil tratment on microbiological properties of chicken breast meat stored at 4 °C.

MATERIAL AND METHODOLOGY

Preparation of samples

Chicken breast samples (totally 30) for microbiological analysis were used in this study.

To evaluate the antimicrobial activity of essential oils the chicken breast with skin of each experimental group was taken. The chicken breast fresh samples were prepared as follow: for air-packaging (AC, control samples) chicken breast fresh meat was packaged to polyethylene bags and stored aerobically at 4 °C; for vacuum-packaged (VC, control samples) chicken breast fresh meat was packaged to polyethylene bags and stored anaerobically in vacuum at 4 °C; for vacuum-packed samples with EDTA solution 1.5% w/w (VPEC, control samples) chicken breast fresh meat was treated with EDTA for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum at 4 °C; for vacuum-packed samples with *Carvum carvi* 0.20% v/w (VP+CEO) chicken breast fresh meat was treated with caraway oil for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum at 4 °C; for vacuum-packed samples with *Pimpinella anisum* L. 0.20% v/w, (VP+AEO) chicken breast fresh meat was treated with anise oil for 1 min and packaged to polyethylene bags and stored anaerobically in vacuum at 4 °C. For sample packaging a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic) was used and each sample were packed immediately after treatment.
EDTA solution (pH 8.0, 99.5% purity, analytical grade, Invitrogen, USA) was prepared at final concentration of 50 mM Caraway and anise essential oils (Calendula, Nová Ľubovňa, Slovakia) was added to coat chicken breast surface (both sides) of each sample using a micropipette.

Microbiological analysis

An amount of 10 g (10 cm^2) of the chicken breast was sampled using sterile scalpels and forceps, immediately transferred into a sterile stomacher bag, containing 90 mL of 0.1% peptone water (pH 7.0), and homogenized for 60 s in a stomacher at room temperature. Sampling was carried out on 0, 4, 8, 12 and 16 days of experiment. Microbiological analyses were conducted by using standard microbiological methods. Anaerobic plate count (AC) was determined using Plate Count Agar (PCA, Oxoid, UK) after incubation for 48 h at 35 °C under anaerobically condition. For *Pseudomonas* spp., 0.1 mL from serial dilutions of chicken homogenates was spread onto the surface of Pseudomonas Isolation agar (PIA, Oxoid, UK). *Pseudomonas* spp. enumerated after incubation for 48 h at 25 °C. For lactic acid bacteria, Rogosa and Sharpe agar (MRS, Oxoid, UK) was inoculated with a 1.0 mL of sample suspension. Inoculated plates were incubated for 48-78 h at 37 °C in an aerobic atmosphere supplemented with carbon dioxide (5% CO_2). For *Enterobacteriaceae*, a 1.0 mL of sample was transferred into 10 mL of molten (45 °C) Violet Red Bile Glucose agar (VRBL, Oxoid, UK). Inoculated plates were incubated at 37 °C for 24 h. All plates were examined for typical colony types and morphology characteristics associated with each medium applied for incubation. Enumeration of all tested groups of bacteria was performed in triplicate.

Figures were created Microsoft® EXCEL 2013. Data for the mean from each replication was calculated and all data were log transformed. Statistical analysis were done with STATGRAPHICS 5 software (UMEX GmbH Dresden, Germany). Confectionary Student's Tukey HSD test was calculated for differences in numbers of bacteria and samples were accepted as significantly different at $p \leq 0.001$.

RESULTS AND DISCUSSION

Food contamination by microorganisms and their development and, hence, the food decontamination possibilities represent a serious problem. Chemical agents to prevent microbial growth and various additives that are used in food industries are considered to be potentially harmful to human health. In seeking of possible alternatives, the antimicrobial compounds of natural origin sharing antibacterial activities originated from plants currently are studied intensively worldwide.

Spices are aromatic plants that are widely used in the food industry and culinary food preparation for flavouring. However, their essential oils and extracts can contribute to control of the growth of harmful microorganisms. It is

necessary the spice to be effective enough to ensure that the product is safe and also have acceptable sensory characteristics **(Dimič et al., 2012)**. The primary objective of chilling poultry safety enssurance is to reduce microbial growth to a level that will improve both food safety and shelf life **(Popelka et al., 2014)**. The anaerobic plate count ranged from 2.77 log CFU.g^{-1} in all tested group on 0 day to 5.45 log CFU.g^{-1} on 16 day in control group stored in air condition. In control group stored vacuum packaged AC ranged from 2.77 log CFU.g^{-1} on 0 day to 5.25 log CFU.g^{-1} on 16 day. In control group stored vacuum packaged after EDTA treatment, AC ranged from 2.77 log CFU.g^{-1} on 0 day to 5.21 log CFU.g^{-1} on 16 day. After treatment with caraway essential oil, AC ranged from 2.77 log CFU.g^{-1} on 0 day to 4.20 log CFU.g^{-1} on 16 day and after treatment with anise essential oil ranged from 2.77 log CFU.g^{-1} on 0 day to 4.15 log CFU.g^{-1} on 16 day. Statistically significant differences ($p \leq 0.001$) of anaerobic plate count were found among all tested group at all tested days except AC and VP+CEO, VC and VP+CEO, VC and VP+AEO, VP+CEO and VP+AEO on 4th day; VC and VPEC on 8th and 16th day; VP+CEO and VP+AEO on 16th day. Anaerobic plate count (AC) values for the tested groups of chicken breast are showed in Figure 1.

Many herbs and spices have been recognized for their preservative or medicinal properties for millennia. Essential oils present in plant matter have been attributed as principal sources of compounds exhibiting antimicrobial activity, which has been illustrated against bacteria and fungi. The understanding of antimicrobial mechanisms of action of EO has led to increased interest to the specific compounds responsible for this activity, specifically those phenolic in nature **(Davidson et al., 2013)**.

The initial LAB value of chicken breast was 3.20 log CFU.g^{-1} on 0 day. In AC samples the LAB counts ranged from 3.20 log CFU.g^{-1} to 4.75 log CFU.g^{-1} on 16 day. In control group VC samples LAB count ranged from 3.20 log CFU.g^{-1} on 0 day to 4.52 log CFU.g^{-1} on 16 day. In VPEC samples LAB ranged from 3.20 log CFU.g^{-1} on 0 day to 4.38 log CFU.g^{-1} on 16 day. In VP+CEO samples the counts of LAC ranged from 3.20 log CFU.g^{-1} on 0 day to 3.05 log CFU.g^{-1} on 16 day and in VP+AEO ranged from 3.20 log CFU.g^{-1} on 0 day to 3.00 log CFU.g^{-1} on 16 day. Lactic acid bacteria (LAB) values for the tested groups of chicken breast are showed in Figure 2. Statistically significant differences ($p \leq 0.001$) of lactic acid bacteria numbers were found among all tested group at all tested days except VP+CEO and VP+AEO on 16th day.

Lactic acid bacteria are found to be more resistant to the cytotoxic effects of essential oils. **Rodríguez et al., (2009)** suggest that a fact that LAB are present and grow on phenol containing plants, and therefore have adapted in order to successfully colonize such antagonistic substrates like one of the reason of LAB resistance to phenolics is because. Degradation capabilities of phenolic compounds by LAB have also been described, although the number of studies is still limited.

The use of spices and spice blends in many food products that already contain high levels of similar seasonings has also been examined. Ideally, reengineering of food formulations that contain high levels of spices such as oregano or thyme seasonings could take advantage of the already present sources of essential oils. Unfortunately, some work has shown that spices stimulate the growth and acid production of LAB **(Shelef, 1983)**.

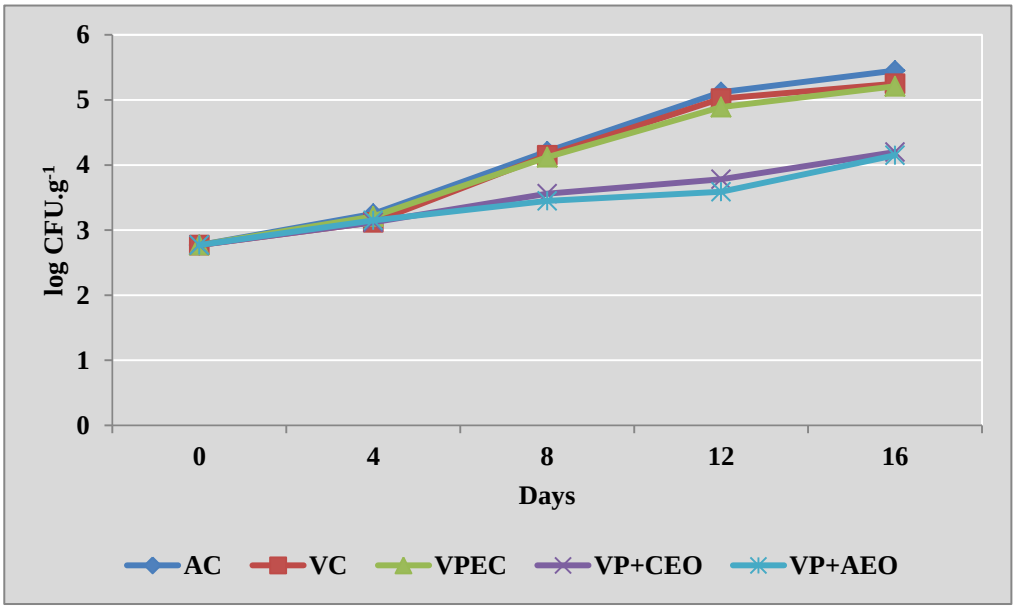

Figure 1 Changes (log CFU.g^{-1}) in population of anaerobic plate count in chicken breast stored in air (AC); stored in vacuum (VC); stored vacuum packaged with EDTA (VPEC); stored under vacuum packaged with *Carvum carvi* 0.20% v/w (VP+CEO); stored vacuum packaged with *Pimpinella anisum* L. 0.20% v/w (VP+AEO).

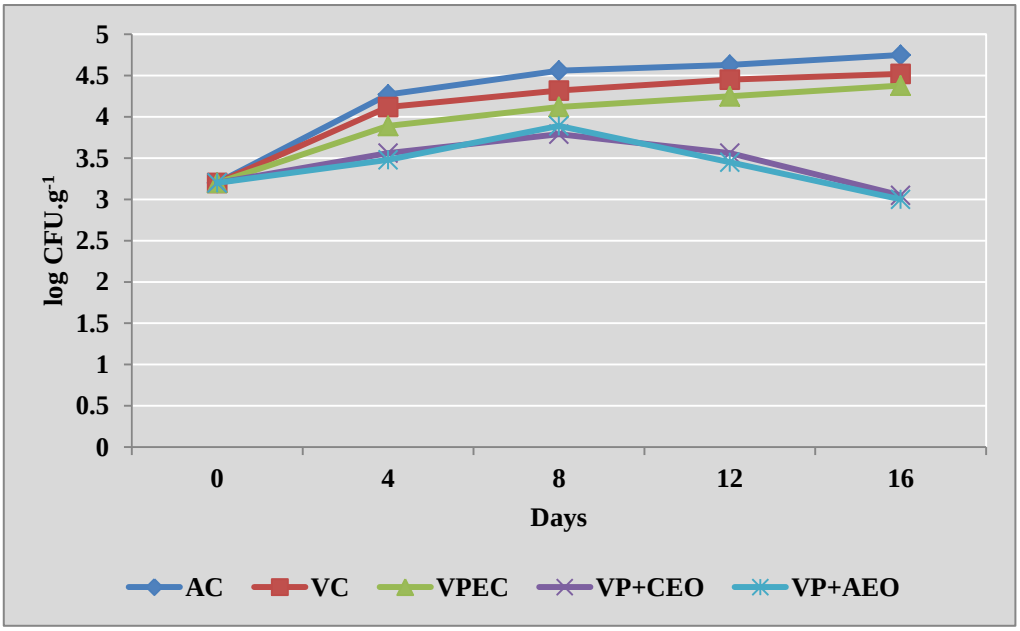

Figure 2 Changes (log CFU.g^{-1}) of lactic acid bacteria in chicken breast stored in air (AC); stored in vacuum (VC); stored vacuum packaged with EDTA (VPEC); stored vacuum packaged with *Carvum carvi* 0.20% v/w (VP+CEO); stored vacuum packaged with *Pimpinella anisum* L. 0.20% v/w (VP+AEO).

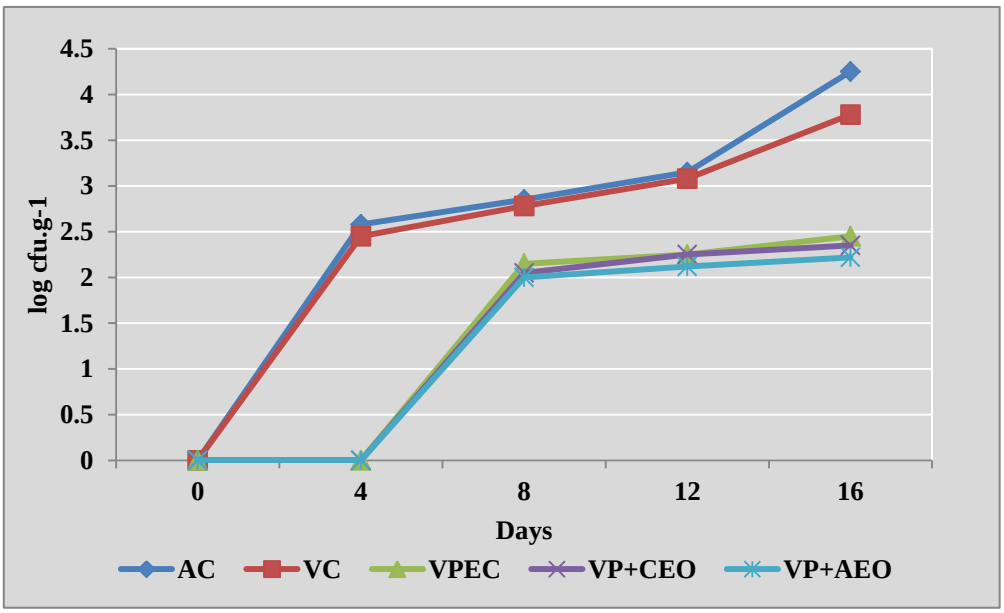

Figure 3 Changes (log CFU.g^{-1}) in *Enterobacteriaceae* counts in chicken breast stored in air (AC); stored in vacuum (VC); stored vacuum packaged with EDTA (VPEC); stored vacuum packaged with *Carvum carvi* 0.20% v/w (VP+CEO); stored vacuum packaged with *Pimpinella anisum* L. 0.20% v/w (VP+AEO).

The chemical composition and physical characteristics of meat makes it a suitable environment for bacterial growth, which includes bacteria such as LAB, *Pseudomonas*, and foodborne pathogens. LAB spoilage in meats is a relevant problem as they are facultative anaerobes that can grow and continue to spoil foods under chilled conditions **(Fratianni et al., 2010; Pyrgotou et al., 2010)**.

Fratianni et al. (2010) treated fresh strips of chicken breast meat with an agar slurry solution containing 0.5% thyme and balm essential oils for 15 min. Samples were stored for 21 days at 4 °C. Thyme was incredibly effective to control the LAB growth for the period of 16 days; 21-day counts were only 0.8×10^3 CFU.mL^{-1}, which was consistent throughout the entire 3 weeks of experiment. The antibacterial effect of balm oil was much less evident until the day 21, with balm oil closely matching the untreated control up until that point. *Salmonella* on the treated chicken was very sensitive to balm oil, while thyme oil very effectively reduced the growth of *E. coli*.

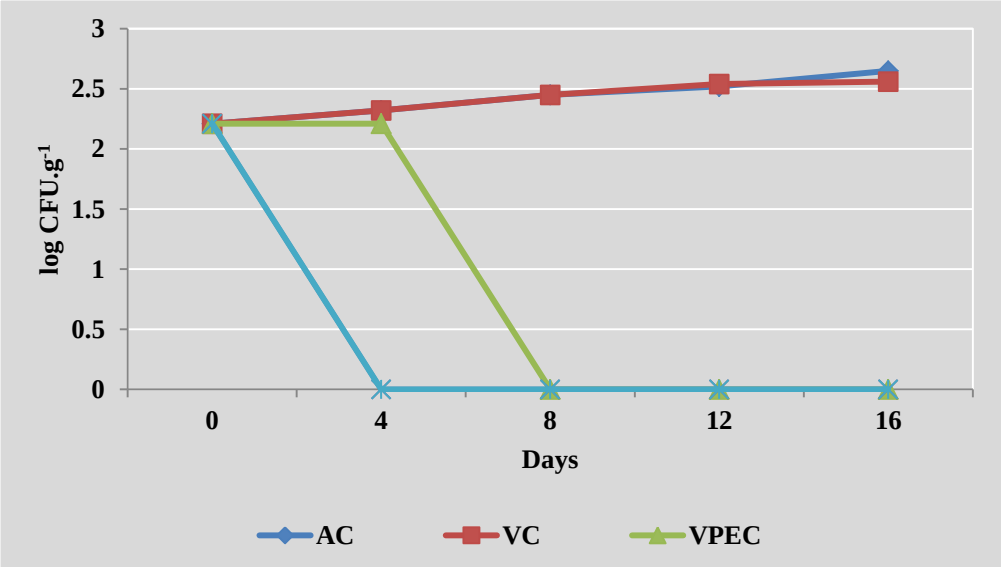

Figure 4 *Pseudomonas* count (log CFU.g^{-1}) in chicken breast stored in air (AC); stored in vacuum (VC); stored in vacuum packaging with EDTA (VPEC); stored vacuum packaged with *Carvum carvi* 0.20% v/w (VP+CEO); stored vacuum packaged with *Pimpinella anisum* L. 0.20% v/w (VP+AEO).

Enterobacteriaceae counts ranged from 0.0 log CFU.g^{-1} in all tested group on 0 day to 4.25 log CFU.g^{-1} on 16 day in AC group. In VP group *Enterobacteriaceae* counts ranged from 0.00 log CFU.g^{-1} on 0 day to 3.78 log CFU.g^{-1} on 16 day. In VPEC group *Enterobacteriaceae* counts ranged from 0.00 log CFU.g^{-1} on 0 day to 2.45 log CFU.g^{-1} on 16 day. In the group with caraway essential oil treatment *Enterobacteriaceae* counts ranged from 0.00 log CFU.g^{-1} on 0 day to 2.35 log CFU.g^{-1} on 16 day and in group treated with anise essential oil from 0.00 log CFU.g^{-1} on 0 day to 2.22 log CFU.g^{-1} on 16 day. Statistically significant differences ($p \leq 0.001$) of *Enterobacteriaceae* genera number were found among all tested group at all tested days except VPEC and VP+CEO, VPEC and VP+CEO, VP+CEO and VP+AEO on 4th day; AC and VC, VPEC and VP+CEO, VPEC and VP+CEO, VP+CEO and VP+AEO on 8th; VPEC and VP+CEO on 12th day. *Enterobacteriaceae* genera values for the tested groups of chicken breast are showed in Figure 3.

Generally, the Gram-positive bacteria were more sensitive to essential oils or antibacterial compounds than Gram-negative bacteria, which is in agreement with previous reports **(Dorman and Deans, 2000; Burt, 2004; Shan et al., 2007)**. This resistance could be ascribed to the structure of the cellular walls of Gram-negative bacteria, mainly with regard to the presence of lipoproteins and lipopolysaccharides that form a barrier to restrict entry of hydrophobic compounds **(Cox and Markham, 2007)**.

Pseudomonas spp. counts ranged from 0.00 log CFU.g^{-1} in all tested group on 0 day to 2.65 log CFU.g^{-1} on 16 day in AC group. In VC group *Pseudomonas* spp. ranged from 0.00 log CFU.g^{-1} on 0 day to 2.56 log CFU.g^{-1} on 16 day. In another tested groups on 16 day *Pseudomonas* spp. were not found. Statistically significant differences ($p \leq 0.001$) of anaerobic plate count were found among all tested group at all tested days except AC and VC on 4th, 8th 12th day; VPEC and VP+CEO, VPEC and VP+CEO on 8th, 12th, 16th day; VP+CEO and VP+AEO on 4th, 8th 12th, 16th day. *Pseudomonas* spp. values for the tested groups of chicken breast are showed in Figure 4.

Numerous studies documented the inhibitory effects of some essential oils and extracts of spices, plants, or their major active constituents on the bacteria - *Escherichia coli*, *Aeromonas* spp., *Enterococcus faecalis*, *Salmonella enterica Typhimurium*, *Staphylococcus aureus*, *Shigella* spp., *Bacillus* spp., *Listeria monocytogenes*, *Micrococcus spp.*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptococcus* spp., *Lactobacillus* spp., *Enterobacter* spp. with Gram-positive bacteria as generally more sensitive than Gram-negative bacteria **(Amensour et al., 2010; Bagamboula et al., 2003; Baidar et al., 2004; Celiktas et al., 2007,Faleiro et al., 2003; Moreira et al., 2005; Skočibušić et al., 2006; Sokmen et al., 2004; Veldhuizen et al., 2007; Viuda-Martos et al., 2008)**.

CONCLUSION

Caraway and anise essential oils exhibited good antimicrobial properties against anaerobic bacteria, lactic acid bacteria and *Enterobacteriacea* at 0.2% concentration. Essential oils and their components may provide a solution for the growing demand of natural preservation methods that require minimal processing of meat. Even more exciting is the fact that these essential oils are already approved for use in foods, meaning that once the issues of application and concentration are resolved and food producers can almost immediately begin using essential oils in their food formulations. Future work must comprise studies that determine which essential oils are most appropriate for preservation, what concentrations and delivery methods are most appropriate and effective, and what foods or packaging methods are most ideal for reformulation or reengineering to take advantage of the antimicrobial activity of essential oils.

REFERENCES

Amensour, M., Bouhdid, S., Fernandez-Lopez, J., Idaomar, M., Senhaji, N. S., Abrini, M. 2010. Antibacterial activity of extracts of *Myrtus communis* against food-borne pathogenic and spoilage bacteria. *International Journal of Food Properties*, vol. 13, p. 1215-1224. http://dx.doi.org/10.1080/10942910903013399

Bagamboula, C. F., Uyttendaele, M., Debevere, J. 2003. Antimicrobial effect of spices on *Shigella sonnei* and *Shigella flexneri*. *Journal of Food Protection*, vol. 66, no. 4, p. 668-673. PMid:12696694

Baidar, H., Sagdic, O., Tahsin, Oskan, G., Karadogan, T. 2004. Antibacterial activity and composition of essential oils from *Origanum*, *Thimbra* and *Satureja* species with commercial importance in Turkey. *Food Control*, vol. 15, p. 169-172. http://dx.doi.org/10.1016/s0956-7135(03)00028-8

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology*, vol. 94, no. 3, p. 223-253. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022

Celiktas, Y. O., Kocabas, E. E. H., Bedir, E., Sukan, F. V., Ozek, T., Baser, K. H. C. 2007. Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chemistry*, vol. 100, no. 2, p. 553-559. http://dx.doi.org/10.1016/j.foodchem.2005.10.011

Cox, S. D., Markham, J. L. 2007. Susceptibility and intrinsic tolerance of *Pseudomonas aeruginosa* to selected plant volatile compounds. *Journal of Applied Microbiology*, vol. 103, p. 930-936. http://dx.doi.org/10.1111/j.1365-2672.2007.03353.x

Davidson, P. M., Critzer, F. J., Taylor, T. M. 2013. Naturally occurring antimicrobials for minimally processed foods. *Annual Review of Food Science and Technology*, vol. 4, p. 163-190. http://dx.doi.org/10.1146/annurev-food-030212-182535

Dimić, G. R., Kocić-Tanackova, S. D., Jovanovb, O. O., Cvetković, D. D., Markova, S. L., Velićanskia, A. S. 2012. Antibacterial activity of lemon, caraway and basil extracts on *Listeria* spp. *Acta Periodica Technologica*, vol. 43, p. 239-246. http://dx.doi.org/10.2298/apt1243239d

Dorman, H. J. D., Deans, S. G. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, vol. 88, p. 308-316. http://dx.doi.org/10.1046/j.1365-2672.2000.00969.x

Faleiro, M. L., Miguel, M. G., Ladeiro, F., Venacio, F., Tavares, R., Bruto, J. C., Figueiredo, A. C., Barroso, J. G., Pedro, L. G. 2003. Antimicrobial activity of essential oils from Potuguese endemic species of *Thymus*. *Letters in Applied Microbiology*, vol. 36, no. 1, p. 35-40. http://dx.doi.org/10.1046/j.1472-765x.2003.01259.x

Fratianni, F., De Martino, L., Melone, A., De Feo, V., Coppola, R., Nazzaro, F. 2010. Preservation of chicken breast meat treated with thyme and balm essential oils. *Journal of Food Science*, vol. 75, no. 8, p. M528-M535. htttp://dx.doi.org/10.1111/j.1750-3841.2010.01791.x

Ivanišová, E., Tokár, M., Mocko, K., Mendelová, A., Bojňanská, T., Mareček, J. 2013. Antioxidant activity of selected plant products. *Journal of Microbiology Biotechnology and Food Sciences*, vol. 2, Special issue 1, p. 1692 -1703.

Ivanišová, E., Frančáková, H., Ritschlová, P., Dráb, Š., Solgajová, M., Tokár, M. 2015a. Biological activity of apple juice enriched by herbal extracts. *Journal of Microbiology Biotechnology and Food Sciences*, vol. 4, special issue 3, p. 69-73. htttp://dx.doi.org/10.1111/j.1750-3841.2010.01791.x

Ivanišová, E., Franćáková, H., Dráb, Š., Benčová, S. 2015b. Elderberry as important source of antioxidant and biologically active compounds. *Proceedings International Scientific and Professional Conference 15th Ružiška Days, Today Science Tomorrow Industry*, Vukovar, Croatia. p. 212. ISBN 978-953-7005-36-8.

Jay, J. M., Loessner, M. J., Golden, David, A. 2005. *Modern food microbiology* 7th ed. Springer Science Inc. : New York, NY, USA. ISBN 978-0-387-23413-7

Kozačinski, L., Hadžiosmanovič, M., Zdolec, N. 2006. Microbiological quality of poultry meat on the Croatian market. *Veterinarski Arhiv*, vol. 76, p. 305-313.

Moreira, M. R., Ponce, A. G., del Walle, C. E., Rouza, S. I. 2005. Inhibitory parameters of essential oils to reduce a food borne pathogen. *LWT – Fod Science and Technology*, vol. 38, no. 5, p. 565-570. htttp://dx.doi.org/10.1016/j.lwt.2004.07.012

Mor-Mur, M., Yuste, J. 2010. Emerging bacterial pathogens. Meat and Poultry: An Overview. *Food Bioprocess and Technology*, vol. 3, p. 24-35. http://dx.doi.org/10.1007/s11947-009-0189-8

Popelka, P., Pipová, M., Nagy, J., Nagyová, A., Fečkaninová, A., Figeľ, J. 2014. The impact of chilling methods on microbiological quality of broiler carcasses. *Potravinarstvo*, vol. 8, 2014, no. 1, p. 67-71. htttp://dx.doi.org/10.5219/327

Pyrgotou, N., Giatrakou, V., Ntzimani, A., Savvaidis, I. N. 2010. Quality assessment of salted, modified atmosphere packaged rainbow trout under treatment with oregano essential oil. *Journal of Food Science*, vol. 75, no, 7, p. M406-M411. http://dx.doi.org/10.1111/j.1750-3841.2010.01724.x

Ray, B. 2001. *Fundamental food microbiology* (2nd ed.). CRC Press : Boca Raton, FL, USA.

Rodríguez, H., Curiel, J. A., Landete, J. M., de las Rivas, B., de Felipe, F. L., Gómez-Cordovés, C., Mancheño, J. M., Muñoz, R. 2009. Food phenolics and lactic acid bacteria. *International Journal of Food Microbiology*, vol. 132, no. 2-3, p. 79-90. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.03.025

Shan, B., Cai, Y. Z., Brooks, J. D., Corke, H. 2007. Antibacterial properties and major bioactive components of cinnamon stick (*Cinnamomum burmannii*): activity against foodborne pathogenic bacteria. *Journal of Agriculture and Food Chemistry*, vol. 55, p. 5484-5490. http://dx.doi.org/10.1021/jf070424d

Shelef, L. A. 1983. Antimicrobial effects of spices. *Journal of Food Safety*, vol. 6, p. 29-44. http://dx.doi.org/10.1111/j.1745-4565.1984.tb00477.x

Skočibušić, M., Bezić, N., Dunkić, V. 2006. Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis. growing in Croatia. *Food Chemistry*, vol. 96, p. 20-28. http://dx.doi.org/10.1016/j.foodchem.2005.01.051

Sokmen, A., Gulluce, M., Akpulat, H. A., Deferera, D., Tepe, B., Polission, M., Sokmen, M., Sahin, F. 2004. The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. *Food Control*, vol. 15, p. 627-634. http://dx.doi.org/10.1016/j.foodcont.2003.10.005

Thippeswamy, N. B. Akhilender Naidu, K., Achur, R. N. 2013. Antioxidant and antibacterial properties of phenolic extract from *Carum carvi* L. *Journal of Pharmacy*

Research, vol. 7, no. 4, p. 352-357. http://dx.doi.org/10.1016/j.jopr.2013.03.028

Tsigarida, E., Nychas, G. J. E. 2001. Ecophysiological attributes of *Lactobacillus* sp. and *Pseudomonas* sp. on sterile beef fillets in relation to storage temperature and film permeability. *Journal of Applied Microbiology*, vol. 90, p. 696-705. http://dx.doi.org/10.1046/j.1365-2672.2001.01292.x

Veldhuizen, E. J. A., Creutzberg, T. O., Burt, S. A., Haagsman, H. P. 2007. Low temperature and binding to food components inhibit the antibacterial activity of carvacrol against *Listeria monocytogenes* in steak tartare. *Journal of Food Protection*, vol. 70, no. 9, p. 2127- 2132. PMiD:17900092

Viuda-Martos, M., Ruiz-Navajas, Y., Fernandez-Lopez, J., Perez-Alvarez, J. 2008. Antibacterial activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinsnsis* L.) essential oils. *Journal of Food Safety*, vol., 28, no. 4, p. 567-576. http://dx.doi.org/10.1111/j.1745-4565.2008.00131.x

Yazdi, F. F., Ghalamkari, G., Toghiani, M., Modaresi, M., Landy, N. 2014. Anise seed (*Pimpinella anisum* L.) as an alternative to antibiotic growth promoters on performance, carcass traits and immune responses in broiler chicks. *Asian Pacific Journal of Tropical Disease*, vol. 4, no. 6, p. 447-451. http://dx.doi.org/10.1016/s2222-1808(14)60604-6

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COMPARABLE EFFICIENCY OF DIFFERENT EXTRACTION PROTOCOLS FOR WHEAT AND RYE PROLAMINS

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ABSTRACT

The identification and quantification of cereal storage proteins is of interest of many researchers. Their structural or functional properties are usually affected by the way how they are extracted. The efficiency of extraction process depends on the cereal source and working conditions. Here, we described various commonly used extraction protocols differing in the extraction conditions (pre-extraction of albumins/globulins, sequential extraction of individual protein fractions or co-extraction of gluten proteins, heating or non-heating, reducing or non-reducing conditions). The total protein content of all fractions extracted from commercially available wheat and rye flours was measured by the Bradford method. Tris-Tricine SDS-PAGE was used to determine the molecular weights of wheat gliadins, rye secalins and high-molecular weight glutelins which are the main triggering factors causing celiac disease. Moreover, we were able to distinguish individual subunits (α/β-, γ-, ω-gliadins and 40k-γ-, 75k-γ-, ω-secalins) of wheat/rye prolamins. Generally, modified extraction protocols against classical Osborne procedure were more effective and yields higher protein content in all protein fractions. Bradford measurement led into underestimation of results in three extraction procedures, while all protein fractions were clearly identified on SDS-PAGE gels. Co-extraction of gluten proteins resulted in appearance of both, low-molecular weight fractions (wheat gliadins and rye secalins) as well as high-molecular weight glutelins which means that is not necessary to extract gluten proteins separately. The two of three extraction protocols showed high technical reproducibility with coefficient of variation less than 20%. Carefully optimized extraction protocol can be advantageous for further analyses of cereal prolamins.

Keywords: extraction; prolamins; wheat; rye

INTRODUCTION

Cereal baked products are predominantly manufactured from wheat or rye flours. Storage non-enzymatically active proteins (prolamins), namely gliadins in wheat and secalins in rye, together with glutelin polymers represent the main triggering factor of celiac disease **(van den Broeck et al., 2011)**. Celiac disease is an inflammatory disorder that mainly affects the small intestine with typical gastrointestinal or extraintestinal symptoms **(Kaukinen et al., 2014)**. So far, the only therapy for celiac disease is lifelong gluten-free diet avoiding any products from wheat, rye, barley, their crossbred varieties and possibly oats **(Zingone et al., 2010)**. Majority of patients following strict gluten-free diet continue to suffer from symptoms, therefore to avoid contamination of gluten-free products by gluten and tighten labeling of such products is a priority.

According to the mobility in polyacrylamide gels, wheat gliadins are subdivided into α/β-, γ- and ω-subunits **(Wieser, 2007)** while rye secalins comprise from γ- and ω-subunits **(Shewry, 2004)**. Wheat α/β- and γ-gliadins as well as rye 40k-γ-secalins belong to the group of monomeric polypeptides with low molecular weight of approx. 28-45 kDa. Wheat ω-gliadins, rye 75k-γ-secalins and rye ω-secalins have molecular weight of approx. 50-80 kDa **(van Eckert et al., 2010)**. Glutelin polymers of wheat are generally subdivided into low-molecular weight glutenin subunits (LMW-GS) and high-molecular weight glutenin subunits (HMW-GS) **(van den Broeck et al., 2009)**, and glutelins of rye are represented by the HMW secalins **(Wieser, Koehler, 2008)**. These proteins have elevated content of two amino acids, glutamine (35% in wheat) and proline (15% in wheat), which makes them highly resistant to degradation by gastrointestinal proteolytic enzymes **(Gregorini et al., 2009)**. Therefore, analysis of structural or functional properties of wheat/rye prolamins requires an appropriately optimized extraction protocol.

Generally, based on different solubility, cereal proteins can be classified into water/salt-soluble albumins and globulins, alcohol-soluble prolamins, and high-molecular weight glutelins soluble in diluted acid/base solutions **(Osborne, 1924; Mamone et al., 2011)**. Various extraction protocols usually consisted initially of removing albumins and globulins **(Kruger et al., 1988)** or exploited co-extraction of gluten proteins (wheat gliadins and glutenins) without pre-extraction of salt soluble proteins **(van den**

Broeck et al., 2009). The differences among prolamin extraction methods involved various temperature conditions as well. Extractability of prolamins is almost unaffected by heating up to 75-80 °C **(Wieser, 1998)** which is the major problem of heat-processed foods. One way how to increase the extractability of prolamins is using a reducing agent to the extractant. However, reducing conditions are more suitable for low-molecular weight α/β- and γ-gliadins in wheat bearing 3-4 intramolecular disulphide bonds comparing to cysteine-free ω-gliadins **(Wieser, 1998)**.

Here, we investigated various extraction protocols of wheat/rye flour proteins involving different sequential extraction steps (pre-extraction of albumins and globulins, co-extraction of gluten proteins, sequential extraction of gliadins/secalins and glutelins) as well as different conditions (heating and non-heating, reducing and non-reducing).

MATERIAL AND METHODOLOGY

Biological Material

Commercially available wheat and rye flours were obtained from mill house Vitaflora (Kolarovo, Slovakia). Gliadin standard was purchased from Sigma-Aldrich (St. Louis, USA).

All extraction protocols were optimized for milligram quantities in Eppendorf tubes and extractions were performed in technical duplicates.

Extraction of cereal proteins according to Osborne (1924)

Cereal proteins were extracted using 1.5 ml of solvent per 50 mg flour by continuous mixing (Roller Mixer SRT9D, Stuart, Staffordshire, UK) at 60 rpm for 1 hour at room temperature. Albumin and globulin fractions were extracted with 0.5 M NaCl, the salt was then removed by distilled water, and finally, prolamins were extracted with 70% (v/v) aqueous ethanol. After each step, supernatant was centrifuged at 9000 x *g* for 15 min at room temperature.

Extraction of cereal proteins according to Osborne (1924) and further modified by Weiss et al. (1993)

To obtain salt soluble protein extract, flours (375 mg) were firstly extracted with 1.5 ml 50 mM Tris-HCl (pH 8.8) containing 1.5% (w/v) polyvinylpolypyrrolidone for 1 hour at 4 °C with vortexing at 15-min intervals. Centrifugation was carried out at 20,000 x *g* for 20 min at 4 °C. The extraction step for salt soluble proteins was repeated at the same conditions. Supernatants were pooled and referred to as "albumin/globulin" fraction. To remove buffers, pellet was re-suspended and washed in distilled water. Alcohol soluble proteins were extracted twice with 1.5 mL of 75% (v/v) aqueous ethanol by continuous mixing (Roller Mixer SRT9D, Stuart, Staffordshire, UK) at 60 rpm for 2 hours at room temperature. After centrifugation at 20,000 x *g* for 20 min at 4 °C, both supernatants referred to as "prolamin" fraction were pooled. The rest of ethanol was removed by re-suspending the pellet in distilled water. Finally, the "glutelin" extract was obtained by addition of 1.5 mL of SDS-DTT buffer (50 mM Tris-HCl, pH 8.8, 1% SDS 0.5% DTT) and extracted for 1 hour at room temperature with vortexing at 15-min intervals, followed by centrifugation at 20000 x *g* for 20 min at 4 °C.

Extraction of cereal proteins according to van den Broeck et al. (2009)

The two-step gluten extraction procedure was carried out at protein sample/extraction buffer ratio 1:10 (w/v). Pre-extraction of wheat gliadins and rye secalins was performed with 50% aqueous iso-propanol (v/v) by continuous mixing (Roller Mixer SRT9D, Stuart, Staffordshire, UK) at 60 rpm for 30 min at room temperature, followed by centrifugation at 10,000 x *g* for 10 min at room temperature. The residual pellet was extracted twice with 50% aqueous iso-propanol, 50 mM Tris-HCl, pH 7.5 containing 1% (w/v) DTT (ratio 1:10) for 30 min at 60 °C with vortexing every 5-10 min, followed by centrifugation at 10,000 x *g* for 10 min at room temperature. After each step, samples were properly re-suspended by mixing and sonicated for 10 min in an ultrasonic bath (Sonorex Digitec, Bandelin, Berlin, DE). Supernatants were pooled and referred to as "two-step gluten extract".

Measurement of total protein content

All protein fractions from each extraction protocol were divided into few aliquots (300 µL) and precipitated with 5 volumes of ice-cold 1 M ammonium acetate in methanol incubated at -30 °C overnight. The next day precipitate was centrifuged at 5,000 x *g* for 10 min at 4 °C, washed 2-times with ice-cold 1 M ammonium acetate in methanol, and pellet was dried using vacuum concentrator (Concentrator Plus, Eppendorf, Hamburg, DE). One aliquot was reconstituted in 100 µL of solubilisation buffer (8 M Urea, 50 mM DTT) and used to determine the protein concentration using Bradford Solution for Protein Determination (Applichem, Darmstadt, DE) according to manufacturer´s instructions with BSA as a standard. Protein quantification was performed in technical duplicate (n = 2) using BioDrop DUO spectrophotometer (Biochrom Ltd, Cambridge, UK).

SDS-PAGE analysis

The second aliquot after ammonium acetate precipitation was reconstituted in 100 µL of buffer for electrophoresis (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol) and analyzed by Tris-Tricine SDS-PAGE under reducing conditions according to the Schägger-von Jagow method **(Schägger and von Jagow, 1987)**. Proteins (10 µg/lane) were separated using BioRad MiniProtean Tetra Cell system (Bio-Rad Laboratories, Hercules, USA), followed by silver staining **(Blum et al., 1987)**. Gels were scanned using a Bio-Rad GS-800 Densitometer (Bio-Rad Laboratories, Hercules, USA) and saved as TIFF format.

RESULTS AND DISCUSSION

The aim of our work was to assess the efficiency of various extraction protocols focusing on wheat and rye flour prolamins. Both flours are routinely used in Slovak bakery industry. Many extraction protocols have recently been developed **(Singh et al., 1991; Weiss et al., 1993; DuPont et al., 2005; van den Broeck et al., 2009)** for cereal proteins, mainly wheat gliadins.

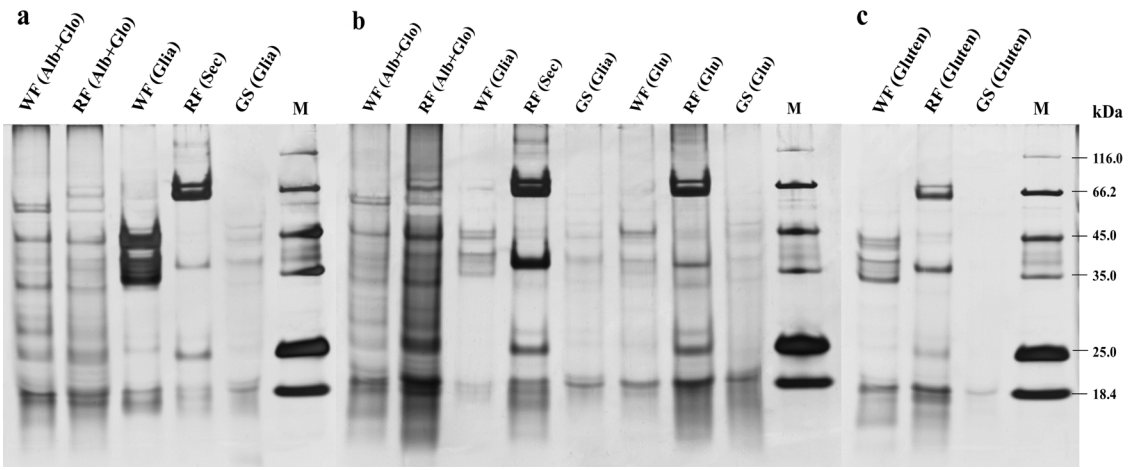

Figure 1 SDS-PAGE analysis of wheat (WF) and rye (RF) flour protein fractions followed by silver staining using different extraction protocols (a) Osborne, 1924; (b) Osborne, 1924 further modified by Weiss et al., 1993 and (c) van den Broeck et al., 2009. Gliadin standard (GS) was used as a control. Abbreviations in parenthesis refer to the protein fractions of wheat and rye flour as well as gliadin standard after each step of extraction: Alb+Glo – albumins and globulins; Glia – gliadins; Sec – secalins; Glu – glutelins; Gluten – gluten extract; M – marker.

However, pilot study of **Osborne (1924)** based on different solubility of proteins is most widely used extraction protocol. Generally, albumin and globulin fractions are soluble in water/salt solutions, prolamins are soluble in alcohols and high-molecular weight glutelins are soluble in diluted acid/base solutions. According to **Osborne (1924)** procedure the average protein content (n = 2) of wheat gliadins was only 0.03 mg.mL^{-1} (±0.01) and rye secalins 0.03 mg.mL^{-1} (±0.00) after removal of salt soluble albumins/globulins (Figure 2). To evaluate the efficiency of gliadin/secalin extraction we assigned gliadin standard as a control sample for measurement. Ethanol extraction of gliadin standard resulted in average protein content (n = 2) of 2.89 mg.mL^{-1} (±0.01) suggesting incomplete extraction of wheat/rye prolamins from flours.

SDS-PAGE analysis revealed that Osborne procedure was successful in extraction of prolamins (Figure 1a) indicating few strong bands of approx. 30-45 kDa (α/β- and γ-gliadins) and 66.2 kDa (75k-γ-secalins) in wheat and rye flours, respectively. Electrophoretic profile of gliadin standard is poorly visible (Figure 1a), most likely due to the incomplete solubility in SDS-PAGE buffer. The average protein content (n=2) of albumins/globulins was 0.06 mg.mL^{-1} (±0.02) and 0.18 mg.mL^{-1} (±0.00) in wheat and rye flours, respectively. The obtained results suggested that Bradford determination underestimates real amount of protein content comparing to SDS-PAGE analysis. Contrasted differences between Bradford measurement and electrophoretic profiling could also be assigned to very low technical reproducibility (e.g. 23% error between wheat gliadins duplicates in Bradford measurement).

The Osborne procedure was further modified **(Weiss et al., 1993)** by separated extraction of low-molecular weight subunits (gliadins in wheat, secalins in rye) and high-molecular weight glutelins, as well as by the addition of reducing agent at non-heated conditions. The average protein content (n = 2) after two sequential extraction steps was 1.76 mg.mL^{-1} (±0.01) and 0.13 mg.mL^{-1} (±0.01) of wheat gliadins and rye secalins, respectively. Albumins/globulins were also sequentially extracted; after pooling the average protein content (n = 2) was 1.08 mg.mL^{-1} (±0.06) in wheat and 0.76 mg.mL^{-1} (±0.02) in rye flours. These results indicated that salt soluble albumins/globulins in rye flour are more abundant comparing to secalins (Figure 2). Glutelins were most represented in both, wheat and rye flours (Figure 2) with average protein content (n=2) of 2.32 mg.mL^{-1} (±0.07) and 0.87 mg.mL^{-1} (±0.04), respectively.

However, SDS-PAGE analysis revealed that glutelins extracted at reducing conditions have similar molecular weights (Figure 1b) but with less intensity of α/β- and γ-gliadins (35-40 kDa) as well as 40k-γ-secalins (one band of approx. 37 kDa). These results are in agreement with general statement that glutelins are high-molecular weight subunits **(van den Broeck et al., 2009)** and are co-extracted together with monomeric gliadins and secalins. After glutelin extraction using reducing agent, rye 75k-γ-secalins (two bands of approx. 66.2 kDa) represented dominant fraction (Figure 1b) with highest protein content, 0.87 mg.mL^{-1} (±0.04). The same conclusions were also achieved in a study of **Gellrich et al., (2003)**. While the average protein content (n = 2) of gliadin standard was very high, 8.03 mg.mL^{-1} (±0.37) in gliadin extract and 2.44 mg.mL^{-1} (±0.01) in glutelin extract, electrophoretic profile indicated its impaired solubility in SDS-PAGE buffer (Figure 1b).

The last protocol used in our study **(van den Broeck et al., 2009)** differs from previous in simultaneous two-step co-extraction of gluten proteins (gliadins/secalins and glutelins) under reducing conditions at higher temperature (60 °C) and without removal of albumins/globulins. The average protein content (n = 2) in wheat gluten extract was markedly lower, 0.77 mg.mL^{-1} (±0.01), comparing to **Weiss et al., (1993)** modification protocol (Figure 2), probably due to the underestimation of results using Bradford measurement. In case of rye gluten extract, the average protein content (n = 2) was 0.50 mg.mL^{-1} (±0.08).

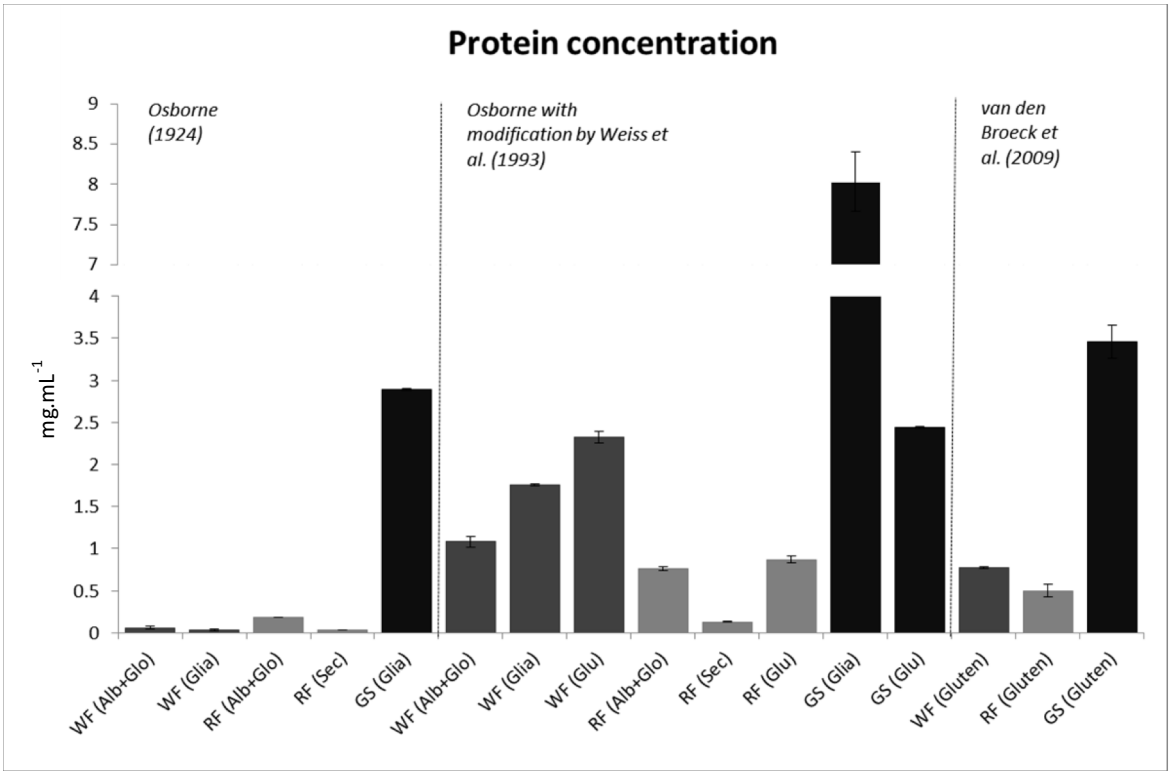

Figure 2 Protein concentration (mg.mL^{-1}) of wheat (WF) and rye (RF) flour protein fractions using Bradford solution for protein determination in three different extraction protocols. Gliadin standard (GS) was used as a control. Abbreviations in parenthesis refer to the protein fractions of wheat and rye flour as well as gliadin standard after each step of extraction: Alb+Glo – albumins and globulins; Glia – gliadins; Sec – secalins; Glu – glutelins; Gluten – gluten extract. Data presented is averages ± standard deviation (n = 2). All error bars are included.

SDS-PAGE analysis revealed typical bands of approx. 30-45 kDa (α/β- and γ-gliadins) and 37 kDa (40k-γ-secalins) in wheat and rye flours, respectively (Figure 1c). Moreover, 75k-γ-secalins (two bands of approx. 66.2 kDa) were also detected (Figure 1c) in rye gluten extract. The average protein content (n = 2) of gliadin standard in gluten extract was 3.46 mg.mL^{-1} (±0.20). Similarly, to previous protocols, separation of gliadin standard on polyacrylamide gel was insufficient due to the incomplete solubility in SDS-PAGE buffer (Figure 1c).

Precisely optimized extraction protocol is a critical step to analyze cereal proteins many of which causing allergies or food intolerances. In our study we aimed to compare different extraction protocols for wheat/rye prolamins as a main triggering factor in celiac disease. Generally, wheat contain higher amount of prolamins than rye which was proved by e.g. fractionation of protein complex **(Mickowska et al., 2012)**. Both, wheat and rye prolamins has highest content of two amino acids, glutamine and proline **(Mickowska et al., 2012)** suggesting their poor digestibility by gastric enzymes. As a result, Glu- and Pro-rich peptides containing T-cell stimulating epitopes are occurred that can cause celiac disease. Ancient varieties of wheat and rye could also be harmful **(Ciclitira et al., 2005; Hybenova et al., 2013)** as they are genetically similar with amino acid composition comparable to modern varieties. However recent studies **(van den Broeck et al., 2010)** revealed that e.g. presence of the Glia-α9 epitope was lower in the wheat landraces.

In our study we aimed to analyze prolamin extract from commercially available wheat and rye flours. According to Bradford method, the total protein content was slightly lower comparing to other studies **(van den Broeck et al., 2009)**. The differences could be attributed by using the different wheat varieties. Moreover, wheat/rye flours used here were milled during different conditions (procedure not described) which probably resulted in a loss of proteins, for instance, wheat ω-gliadin fractions with molecular weight of 50-80 kDa were almost unable to detect in SDS-PAGE gels. Contrary, **van den Broeck et al., (2009)** described that ω-gliadins/D-type LMW-GS fractions were abundantly presented in all wheat varieties. In summary, the efficiency of extraction protocol depends not only on the cereal protein source, but also on working conditions and analytical method of their identification/quantification.

CONCLUSION

Various extraction protocols with different working conditions examined here were generally efficient in extraction of wheat/rye flour prolamins. Although, the pilot Osborne procedure yields in lower protein content using Bradford measurement comparing to SDS-PAGE analysis, it is still considered as an effective method due to its rapid and simple nature. Up to date, several modifications of extraction conditions are under investigation using multiple extraction steps or reducing agents. These protocols are usually time consuming, however, carefully optimized conditions can reduce not only time but also can increase the protein content in extracts. In some cases, the huge amount of starting material is required for analysis. Therefore, in our study we optimized all extraction

protocols for milligram quantities using Eppendorf tubes. Except the Osborne procedure, the two protocols used here showed high technical reproducibility according to Bradford with coefficient of variation less than 20%. Assuming above mention facts, van den Broeck protocol is a good choice for simultaneous co-extraction of gluten proteins from wheat/rye flours.

REFERENCES

Blum, H., Beier, H., Gross, H. J. 1987. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels, *Electrophoresis*, vol. 8, p. 93-99. http://dx.doi.org/10.1002/elps.1150080203

van den Broeck, H. C., America, A. H. P., Smulders, M. J. M., Bosch, D., Hamer, R. J., Gilissen, L. J. W. J., van der Meer, I. M. 2009. A modified extraction protocol enables detection and quantification of celiac-disease related gluten proteins from wheat. *Journal of Chromatography B*, vol. 877, p. 975-982. http://dx.doi.org/10.1016/j.jchromb.2009.02.035

van den Broeck, H. C., de Jong, H. C., Salentijn, E. M. J., Dekking, L., Bosch, D., Hamer, R. J., Gilissen, L. J. W. J., van der Meer, I. M., Smulders, M. J. M. 2010. Presence of celiac disease epitopes in modern and old hexaploid wheat varieties: wheat breeding may have contributed to increased prevalence of celiac disease, *Theoretical and Applied Genetics*, vol. 121, no. 8, p. 1527-1539. http://dx.doi.org/10.1007/s00122-010-1408-4

van den Broeck, H. C., Gilissen, L. J. W. J., Smulders, M. J. M., van der Meer, I. M., Hamer, R. J. 2011. Dough quality of bread wheat lacking α-gliadins with celiac disease epitopes and addition of celiac-safe avenins to improve dough quality. *Journal of Cereal Sciences*, vol. 53, p. 206-216. http://dx.doi.org/10.1016/j.jcs.2010.12.004

Ciclitira, P. J., Ellis, H. J., Lundin, K. E. A. 2005. Gluten-free diet – what is toxic? *Best Practice & Research Clinical Gastroenterology*, vol. 19, no. 3, p. 359-371. http://dx.doi.org/10.1016/j.bpg.2005.01.003

DuPont, F. M., Chan, R., Lopez, R., Vensel, W. H. 2005. Sequential extraction and quantitative recovery of gliadins, glutenins, and other proteins from small samples of wheat flour. *Journal of Agricultural and Food Chemistry*, vol. 53, p. 1575-1584. http://dx.doi.org/10.1021/jf048697l

van Eckert, R., Bond, J., Rawson, P., Klein, Ch. L., Stern, M., Jordan, T. W. 2010. Reactivity of gluten detecting monoclonal antibodies to a gliadin reference material. *Journal of Cereal Sciences*, vol. 51, no. 2, p. 198-204. http://dx.doi.org/10.1016/j.jcs.2009.11.012

Gellrich, C., Schieberle, P., Wieser, H. 2003. Biochemical characterization and quantification of the storage protein (secalin) types in rye flour. *Cereal Chemistry*, vol. 80, no. 1, p. 102-109. http://dx.doi.org/10.1094/CCHEM.2003.80.1.102

Gregorini, A., Colomba, M., Ellis, H. J., Ciclitira, P. J. 2009. Immunogenicity characterization of two ancient wheat α-gliadin peptides related to coeliac disease. *Nutrients*, vol. 1, no. 2, p. 276-290. http://dx.doi.org/10.3390/nu1020276

Hybenova, E., Štofirová, J., Mikulajová, A. 2013. Celiac disease and gluten-free diet. *Potravinarstvo*, vol. 7, no. 1, p. 95-100. http://dx.doi.org/10.5219/276

Kaukinen, K., Lindfors, K., Mäki, M. 2014. Advances in the treatment of coeliac disease: an immunopathogenic perspective. *Nature Reviews Gastroenterology & Hepatology*, vol. 11, p. 36-44. PMid:23917697

Kruger, J. E., Marchylo, B. A., Hatcher, D. 1988. Preliminary assessment of a sequential extraction scheme for evaluating quality by reversed-phase high-performance liquid chromatography and electrophoretic analysis of gliadins and glutenins. *Cereal Chemistry*, vol. 65, no. 3, p. 208-214.

Mamone, G., Picariello, G., Addeo, F., Ferranti, P. 2011. Proteomic analysis in allergy and intolerance to wheat products. *Expert Review of Proteomics*, vol. 8, no. 1, p. 95-115. http://dx.doi.org/10.1586/epr.10.98

Mickowska, B., Socha, P., Urminská, D., Cieślik, E. 2012. The comparison of prolamins extracted from different varieties of wheat, barley, rye and triticale species: amino acid composition, electrophoresis and immunodetection, *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 1, no. 4, p. 742-752. ISSN 1338-5178.

Osborne, T. B. 1924. *The Vegetable Proteins*, 2nd ed., Longmans Green and Co., London, UK. 154 p.

Schägger, H., von Jagow, G. 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Analytical Biochemistry*, vol. 166, no. 2, p. 368-379. http://dx.doi.org/10.1016/0003-2697(87)90587-2

Shewry, P. R. 2004. Improving the protein content and quality of temperate cereals: wheat, barley and rye. In Çakmak, I. and Welch, R. M. I*mpacts of Agriculture on Human Health and Nutrition.* EOLSS Publishers Co Ltd. : Oxford, UK. ISBN 978-1848265448.

Singh, N. K., Shepherd, K. W., Cornish, G. B. 1991. A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. *Journal of Cereal Science*, vol. 14, no. 3, p. 203-208. http://dx.doi.org/10.1016/S0733-5210(09)80039-8

Weiss, W., Vogelmeier, C., Görg, A. 1993. Electrophoretic characterization of wheat grain allergens from different cultivars involved in bakers' asthma. *Electrophoresis*, vol. 14, no. 8, p. 805-816. http://dx.doi.org/10.1002/elps.11501401126

Wieser, H. 1998. Investigations on the extractability of gluten proteins from wheat bread in comparison with flour. *Zeitschrift für Lebensmitteluntersuchung und - Forschung A*, vol. 207, no. 2, p. 128-132. http://dx.doi.org/10.1007/s002170050306

Wieser, H. 2007. Chemistry of gluten proteins. *Food Microbiology*, vol. 24, no. 2, p. 115-119. http://dx.doi.org/10.1016/j.fm.2006.07.004

Wieser, H., Koehler, P. 2008. The biochemical basis of celiac disease. *Cereal Chemistry*, vol. 85, no. 1, p. 1-13. http://dx.doi.org/10.1094/CCHEM-85-1-0001

Zingone, F., Capone, P., Ciacci, C. 2010. Celiac disease: Alternatives to a gluten free diet. *World Journal of Gastrointestinal Pharmacology and Therapeutics*, vol. 1, no. 1, p. 36-39. PMid:21577293

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EVALUATION OF PRIMARY AND SECONDARY METABOLITES IN SELECTED VARIETIES OF POTATOES

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ABSTRACT

The aim of study was to determine primary and secondary metabolites in selected varieties of potatoes. Potatoes (*Solanum tuberosum* L.) are good source of bioactive compounds, mainly phenols as one of the most important components. The chemical composition with reducing sugar, starch, ascorbic acid, total polyphenol and flavonoid content were analysed in five potato varieties (Agria, Marabel, Red Anna, Picasso, Princess). Values of dry matter content ranged from 20.34 to 23.64%. In terms of tubers storage, its content above 20% is required. The highest level of starch was detected in variety Princess (16.82%). The lowest reducing sugar content was recorded by variety Marabel (0.08%). Similarly, low values reached varieties Princess (0.12%), Agria (0.14) and Red Anna (0.16%). These would be appropriate to use for food processing and for production of fried potato chips or fries. Variety Red Anna reached the highest amount of vitamin C (73.72 mg.kg^{-1}). The lower levels of this vitamin showed tubers of varieties Picasso (35.02 mg.kg^{-1}) and Princess (36.89 mg.kg^{-1}). The antioxidant activity was measured with radical scavenging assays using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical as well as phosphomolybdenic assay. Potato varieties contained high levels of total polyphenols (0.474 – 1.550 mg GAE per dry weight) and flavonoids (1.407 – 15.933 µg QE per dry weight). The consumption of potatoes can provide nutritional value along with antioxidant potential that can be helpful for proper functioning of the body physiological systems. Statistical evaluation by the single factor analysis of variance detected high significant impact of variety on the content of all the analytical parameters in evaluated varieties of potato tubers.

Keywords: starch; reducing sugars; antioxidant activity; polyphenols; flavonoids

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fifth most important crop worldwide after sugar cane, maize, wheat and rice with production of >364 million tons in 2012 **(FAO, 2014)**. Potato spread to Europe from the America in the late 1500s **(Camire et al., 2009)** and immediately became very important for human nutrition in the "Old Word" as well. Nowadays, potatoes are cultivated in more than 160 countries with more than 4000 cultivars **(Hils and Pieterse, 2007)**. Potatoes are rich in carbohydrate and provide significant quantities of proteins, minerals (iron) and vitamins (B complex and vitamin C), dietary fiber, and antioxidants which vary with variety, storage conditions, growing season, soil type, and pre-harvest nutrition **(Singh and Kaur, 2009)**. Nowadays, potatoes have received substantial interest as a valuable source of antioxidants because they contain a variety of secondary metabolites including phenols and are consumed in relatively high amounts **(Wegener and Jansen, 2013)**. Phenols have been associated with certain health benefits such as inhibition of cholesterol accumulation in blood, reduction of the risk of coronary heart disease, prevention of some types of cancer, and retardation of macular degeneration among others **(Kita et al., 2013)**. In potatoes, most of the phenols are present between their cortex and peel, while their content reduces towards the center of the tuber **(Friedman, 1997)**. Chlorogenic acid and caffeic acid are two of the most prominent phenolic acids reported in potato followed by protocatechuic acid, *t*-cinnamic acid, *p*-coumaric acid, ferulic acid, vanillic acid, gallic acid, syringic acid, and salicylic acid **(Reddivari et al., 2007)**.

Antioxidant activity and total phenolics are different between potato cultivars. Bioactive composition of potato compared to other vegetables is low but since potato form a substantial part of our daily diet, it is therefore important to screen and identify those genotypes which are high in antioxidants **(Kaur and Aggarwal, 2014)**.

The aim of this study was to evaluate primary (reducing sugar content, starch content), secondary metabolites (polyphenols, flavonoids, ascorbic acid content) and antioxidant activity in selected varieties of potatoes.

MATERIAL AND METHODOLOGY

Plant material

Potatoes were grown on a field nursery at the Department of Environmental Protection and Organic Farming (DEPOF) in Spišská Belá (Slovakia). The used genotypes of potatoes were: Agria, Marabel, Red Anna, Picasso and Princess. This list includes one of the most cultivated varieties in Slovak Republic. Samples with peel were before the measurement crushed to mash, lyophilized

(IISHIN Freeze Dryer, IISHIN lab. Co. Ltd.) and then stored at 4°C in refrigerator. These varieties from the crop year of 2014 were assessed 4 weeks after the harvest. Storage of tubers was carried out in cooling box at 6 °C and under relative humidity 85%. Material samples were weighed on Mettler Toledo Analytical Balances.

Chemicals

All chemicals used were of analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Sample preparation

0.5 g of milling fractions was extracted with 20 mL of 80% ethanol for 20 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 20 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids).

Dry matter content

Dry matter content of potato varieties was measured in samples of around 10 g by pre-drying at 65 °C for 3 hours and by drying at 105 °C for 3 hours to the constant weight (WTB Binder drying oven). Weight of the dried sample was converted to the initial fresh mass.

Starch content

10 g of sample was hydrolysed in a boiling water bath at 100 °C using 100 mL of 1.422% hydrochloric acid. Each solution was then treated and cleaned with 2 mL of 15% potassium ferrocyanide and 2 mL of 30% zinc sulphate solution. Optical activity of filtrate sample was measured on P3001RS Automatic Digital Polarimeter (°Sx1.775), (A. KRÜSS Optronic GmbH, Germany).

Reducing sugar content

Determination of reducing sugars was performed by Schoorl method using Fehling´s solutions I and II. There was used around 5 g of weighed sample. Titration of sample was done with sodium thiosulfate solution. Reducing sugar content was determined by the consumption difference between the blank titer and average sample titer (Schoorl table).

Ascorbic acid content

Vitamin C was extracted from homogenized samples by the metaphosphoric acid solution. Dehydro-L-ascorbic acid was reduced to L-ascorbic acid. The total vitamin C content was determined by HPLC method with UV detection at 265 nm (Agilent 1220, Agilent Technologies, USA). Dosing of samples was realized on Agilent Autosampler (Agilent Technologies, USA) by injection.

Radical scavenging activity

Radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(Yen and Chen, 1995)**. The extracts (0.5 mL) were mixed with 2 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). Absorbance of the sample extract was determined using the spectrophotometer Jenway (6405 UV/Vis, Jenway, Staffordshire, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10-50 $mg.L^{-1}$; R^2 = 0.983) was used as the standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Reducing power

Reducing power of samples was determined by the phosphomolybdenum method of **Prieto et al., (1999)** with slight modifications. The mixture of sample extract (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M) and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, Jenway, Staffordshire, England). Trolox (10-1000 $mg.L^{-1}$; R^2=0.998) was used as the standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Total polyphenol content

Total polyphenol content of potato extracts was measured by the method of **Singleton and Rossi, (1965)** using Folin-Ciocalteu reagent. 0.2 mL of each sample extract was mixed with 0.2 mL of the Folin-Ciocalteu reagent, 2 mL of 20% (w/v) sodium carbonate and centrifugated at 10000 g (Neofuge VS – 100 BN, China) for 10 min. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, Jenway, Staffordshire, England). Gallic acid (5-250 $mg.L^{-1}$; R^2 = 0.999) was used as the standard and the results were expressed in $mg.g^{-1}$ gallic acid equivalents.

Total flavonoid content

Total flavonoids were determined using the modified method of **(Quettier – Deleu et al., 2000)**. 2 mL of sample extract was mixed with 0.4 mL of 5% (w/v) ethanolic solution of aluminium chloride. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, Jenway, Staffordshire, England). Quercetin (0.5-20 $mg.L^{-1}$; R^2 = 0.999) was used as the standard and the results were expressed in $\mu g.g^{-1}$ quercetin equivalents.

Statistical analysis

Statistical software SAS 9.2 and Enterprise Guide 3.0 was used for the statistical evaluation. On the other hand, single factor analysis of variance was used to assess the impact of variety on dry matter, starch, reducing sugar, vitamin C, polyphenol and flavonoid content. Statistical significance was measured with Tukey´s test (p <0.5).

RESULTS AND DISCUSSION

Dry matter content

Dry matter content of potato tubers ranges in Slovak climate conditions from 20 to 25%. Its higher content is in terms of storage a stabilizing factor. However, it may have negative effect on potato taste properties. It usually also correlates with the starch content. Our evaluated cultivars reached values of dry matter content from 20.34% to 23.64 (Table 2) while the highest amount showed variety Princess that is therefore suitable for long-term storage. Dry matter content is affected by the climate conditions, fertilization and variety **(Poberezny and Wszelaczynska, 2011)**.

Starch content

Starch content is highest in tubers after harvest. During the storage, its content gradually decreases because of degradation to simple sugars and it is also consumed

during breathing of tubers. Its values in assessed cultivars were in the range from 14.13 to 16.82% (Table 2). These values can be rated in growing conditions of Central European region as appropriate or greater. Higher starch content is suitable for the production of potato chips. During the heat treatment, starch starts to gelatinize and tubers may rupture **(Šimková et al., 2013)**.

Reducing sugar content

Reducing sugars are an important factor in food processing of potato tubers for fried chips. To help prevent the Maillard reaction accompanied by undesirable sensoric symptoms, low reducing sugar content is appropriate. Ideal are values up to 0.20%. This requirement was, except for Picasso (0.25%), fulfilled by all of the varieties (Table 2). These can be used for the production of potato chips. Lower storage temperatures support an increase in the content of reducing sugars **(de Quadros et al., 2010)** which must be therefore monitored during the storage.

Ascorbic acid content

Vitamin C is nutritional ingredient that characterizes potatoes as an important crop with antioxidant activity **(Külen et al., 2013)**. The highest content is in the fresh tubers, whilst heat treatment reduces its amount. Among the assessed cultivars the highest content showed tubers of variety Red Anna (Table 2) with purple skin (73.72 $mg.kg^{-1}$). Conversely, variety Picasso reached its lowest value (35.02 $mg.kg^{-1}$). The amount of vitamin C decreases due to storage conditions.

Antioxidant activity

The antioxidant potential of potato cultivars was determined on the basis of scavenging activity of the stable free radicals DPPH and reducing ability by phosphomolybdenum assay (Figure 1 and Figure 2). Agria, Red Anna and Princess cultivars contained highest antioxidant activity – 1.556; 1.316 and 1.028 mg TEAC per 100 g^{-1} dry matter for DPPH and phospomolybdenum method (19.071; 23.450 and 23.428 mg TEAC per 100 g^{-1} dry matter) respectively. Among the tested potatoes Marabel and Picasso cultivars showed lower antioxidant potential. Red Anna belongs to the cultivars with red peel; Agria, Marabel, Picasso and Princess are potatoes with brown peel. Extract prepared from red peel potatoes have stronger antioxidant activity than brown peel, probably due to the strong effect of anthocyanins. Previously, it was expounded that the antioxidant activity of ethanolic and aqueous potato extract has activity 62.3% and 62.5%, respectively **(Kaur and Kapoor, 2002)**. **Karadeniz et al., (2005)** reported similar activity (70%) of the potato extracts 70% for same sample weight. **Ezekiel et al., (2013)** reported that potatoes showed 94% scavenging activity towards hydroxyl radicals, and almost complete inhibition of superoxide radicals. Antioxidant activity of potatoes is mainly caused by their chlorogenic, protocatechuic and caffeic acid content. Chlorogenic acid from potatoes has been found to be an effective inhibitor of lipid oxidation **(Al-Shaikan et al., 1995)**. Numerous investigations reported that potato has applicable amount of antioxidant that possess significant inhibition ability **(Karadeniz et al., 2005)**. Genotype and growth conditions, such as water availability, light quality and temperature, affect the synthesis and accumulation of antioxidants in potatoes. Peeling the potato considerably reduced its antioxidant activity. According to **Ezekiel et al., (2013**) potatoes contain relatively low amount of total phenolic acids, but they have high antioxidant activity compared to other fruits and vegetables.

Total polyphenol content

The results of the Folin–Ciocalteu assay are shown in Table 1. Among the evaluated cultivars Agria and Red Anna had the highest gallic acid equivalent (1.550 $mg.g^{-1}$; 0.977 $mg.g^{-1}$), followed by Princess and Marabel cultivars (0.675 $mg.g^{-1}$ and 0.524 $mg.g^{-1}$). In Picasso variety was observed the lowest value of total polyphenol content – 0.474 $mg.g^{-1}$. The variations of polyphenol content between varieties may result from genotypes and harvest locations that influence the accumulation of phenolic compounds by synthesizing different quantities and/or types of phenolics **(Lachman et al., 2009)**. Earlier, **Karadeniz et al., (2005)** reported that the polyphenol content of potato is 32.44 ±6.07 mg GAE.100 g^{-1}. Previously, **Al-Saikhan et al. (1995)** also published that potato contains 11.41 –27.47 mg GAE.100 g^{-1} total polyphenol content. Generally, it is considered that edible part of potato accounts 40% of the total polyphenol content **(Chu et al., 2002)**, while amount of conjugated polyphenols in potato is 57.9±13.4% **(Vinson et al., 1998)**.

Polyphenols are distributed mostly between the cortex and peel tissues of the potato. Potato peel contains about ten times as much polyphenols as potato flesh. About 50% of the phenolic compounds may be found in peel and adjoining tissues while the rest decreases in concentration from the outside towards the centre of the potato tuber. Chlorogenic acid constitutes up to 90% of the total polyphenolic content of potatoes **(Friedman 1997)**. Other phenolic acids include protocatechuic, sinapic, coumaric and vanillic acids. Other polyphenolic compounds present in potatoes include anthocyanins, flavanones (naringenin and eriodictyol), flavan-3-ols (catechin and epicatechin) and flavonols (kaempferol and sometimes quercetin glycosides) **(Lewis, 1999)**. Many of these compounds are present in fairly low concentrations. The phenols can be recovered from the skin portion, which is discarded as waste during potato processing and can be used for value addition in different food products **(Navarre et al., 2009; Bončíková et al., 2012; Musilová et al., 2015)**.

Total flavonoid content

Flavonoid content of evaluated potatoes (Table 1) ranged from 1.417 to 15.933 µg/g QE. Red Anna variety contains the highest flavonoid content, due to the presence of anthocyanins in the peel. Anthocyanins are a sub-group within the flavonoids and present in substantial amounts in pigmented flesh potatoes. Anthocyanin levels between 5.5 and 35 mg/100 g fresh weight in potatoes have been reported **(Brown, 2008)**. Red peel and purple or red-fleshed cultivars has twice of the flavonoid concentration of white-fleshed cultivars and their concentrations are considerably higher in skin. In potatoes was reported presence of these flavonoids: catechin, epicatechin, erodictyol, kaempeferol, naringenin and rutin. The potato flavonols content is not significantly high, but these can be considered as a valuable source of these compounds because of their high consumption **(Tudela et al., 2002)**.

Table 1 Total polyphenol and flavonoid content in selected varieties of potatoes.

Sample	Total polyphenols content (mg GAE.g^{-1})	Total flavonoids content (µg QE.g^{-1})
Agria	1.550 ±0.08	14.709 ±1.91
Marabel	0.524 ±0.05	6.301 ±0.71
Red Anna	0.977 ±0.01	15.933 ±1.47
Picasso	0.474 ±0.04	1.417 ±0.27
Princess	0.675 ±0.03	3.547 ±0.95

NOTE: GAE (gallic acid equivalent); QE (quercetin equivalent); ± (standard deviation of the mean).

Table 2 Components contained in potato tubers.

Sample	Dry matter content (%)	Starch content (%)	Reducing sugar content (%)	Vitamin C (mg.kg^{-1})
Agria	22.49 ±0.20	16.12 ±0.14	0.14 ±0.02	56.32 ±1.15
Marabel	21.92 ±0.11	15.72 ±0.25	0.08 ±0.01	49.60 ±0.84
Red Anna	20.34 ±0.06	14.31 ±0.03	0.16 ±0.01	73.72 ±2.59
Picasso	20.98 ±0.26	14.79 ±0.28	0.25 ±0.01	35.02 ±1.33
Princess	23.64 ±0.19	16.82 ±0.16	0.12 ±0.01	36.89 ±0.89

NOTE: ± (standard deviation of the mean).

Table 3 Single factor analysis of variance for selected parameters of potato tubers (Tukey test), Major effect: variety.

Sample	Degrees of Freedom	Sum of Squares	Mean Squares	F-test (F-ratio)	Significance (p-value)
Dry matter	4	19.96473333	4.99118333	52.95	0.0001^{+++}
Starch	4	13.29029333	3.07257333	27.79	0.0001^{+++}
Reducing sugar	4	0.04710667	0.01177667	28.49	0.0001^{+++}
Vitamin C	4	3995.890067	748.972517	110.87	0.0001^{+++}
Total polyphenols	4	1469.900440	367.475110	279.13	0.0001^{+++}
Total flavonoids	4	0.32515160	0.08128790	89.15	0.0001^{+++}

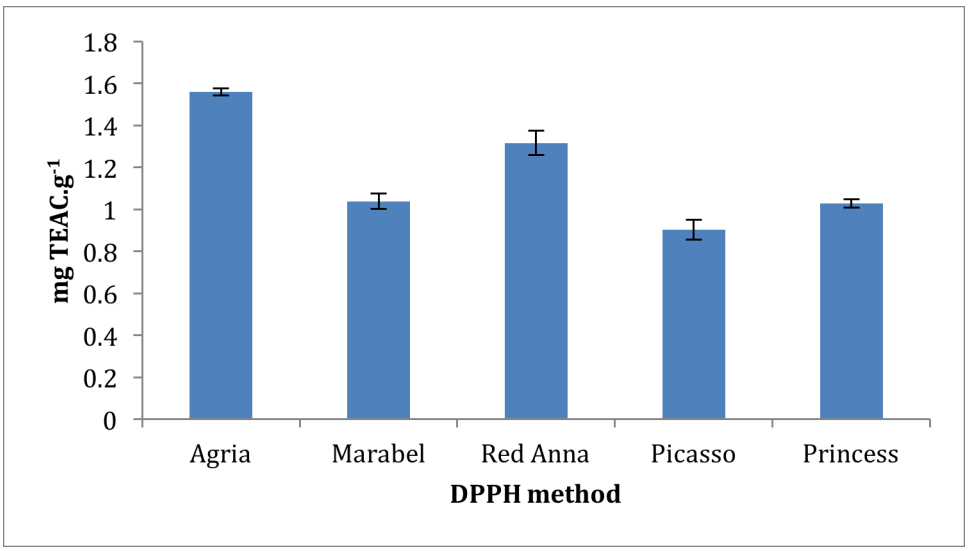

Figure 1 Antioxidant activity of potatoes determined by DPPH method (TEAC – Trolox equivalent antioxidant capacity).

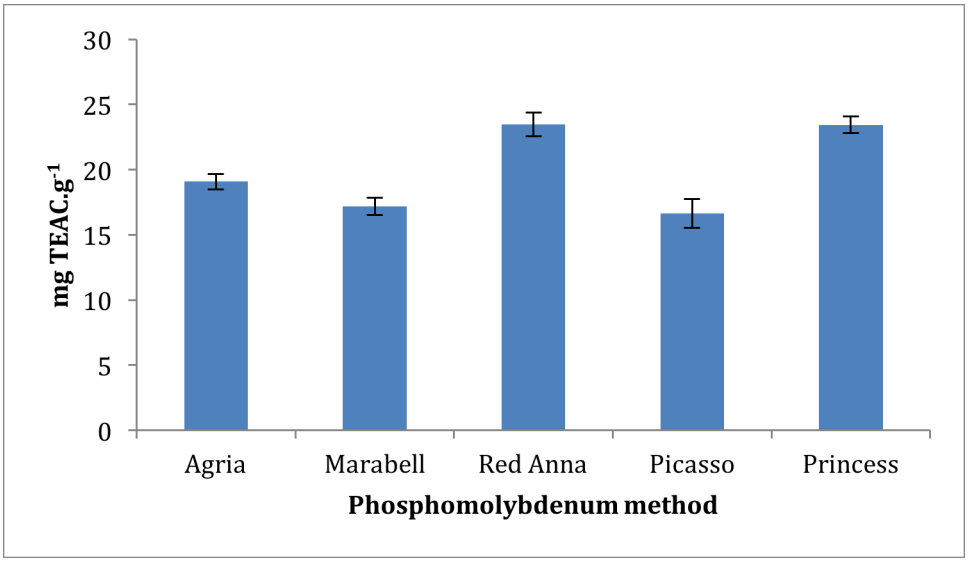

Figure 2 Antioxidant activity of potatoes determined phosphomolybdenum method (TEAC – Trolox equivalent antioxidant capacity).

It can be stated, that the amounts of flavonoids are not proportional to total polyphenol content in evaluated samples. Each potato cultivar contained different levels of these bioactive compounds, which is in general agreement with results of other authors, because the content of phenolic compounds depends on potato cultivar, weather, soil and agrotechnical conditions **(Hamouz et al., 1999; Gumul et al., 2011)**. The results of statistical evaluation are listed in Table 3. Statistical evaluation using the single factor analysis (ANOVA, SAS 9.2) confirmed highly statistically significant influence on the content of all the analyzed compounds.

CONCLUSION

From the data in this study, it can be concluded that potatoes are rich sources of primary and secondary metabolites. The variety Red Anna and Agria showed higher biological activity (antioxidant activity, polyphenols and flavonoids) in comparison with other varieties. The content of dry matter and starch measured in evaluated tubers predetermines these cultivars for long-term storage. Low values of reducing sugar content in varieties Agria, Marabel, Princess and Red Anna is desired parameter for the production of potato chips. Potato tubers are in addition a valuable source of vitamin C (variety Red Anna). Phytochemicals in potatoes can be used for development of functional foods or nutraceuticals. Considering the large quantities in which potatoes are consumed throughout the world, they could be a very good vehicle for addressing some health related problems.

There was statistically high significant impact of variety on dry matter, starch, reducing sugar, vitamin C, polyphenol and flavonoid content. The results confirmed a significant influence of varietal characteristics on examined components of potato tuber cultivars. Above results are an original compact research focused on technological parameters, biologically active compounds and antioxidant activity of selected potato varieties grown in Slovakia. The results provide innovative findings in the area of potato quality research.

REFERENCES

Al-Saikhan, M. S., Howard, L. R., Miller, J. C. 1995 Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum* L.). *Journal of Food Science* vol. 60, p. 341-343. http://dx.doi.org/10.1111/j.1365-2621.1995.tb05668.x

Bončíková, D., Tóth, T., Tomáš, J., Suleiman, D., Tóth, J., Slávik, M. 2012. Effective antioxidant phenolic compounds in selected varieties of apples. *Potravinarstvo*, vol. 6, p. 11-15. http://dx.doi.org/10.5219/222

Brown, C. R., Durst, R. W., Wrolstad, R., De Jong, W. 2008. Variability of phytonutrient content of potato in relation to growing location and cooking method. *Potato Research*, vol. 51, p. 259-270. http://dx.doi.org/10.1007/s11540-008-9115-0

Camire, M. E., Kubow, S., Donnelly, A. J., 2009. Potatoes and human health. *Critical Reviews in Food Science and Nutrition,* vol. 49, no. 10, p. 823-840. http://dx.doi.org/10.1080/10408390903041996

Chu, Y. F., Sun, J., Wu, X., Liu, R. H. 2002. Antioxidant and antiproliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry*, vol. 50, p. 6910-6916. PMid:12405796

Ezekiel, R., Singh, N., Sharma, S., Kaur, A. 2013. Beneficial phytochemicals in potato – a review. *Food Research International*, vol. 50, p. 487-496. http://dx.doi.org/doi:10.1016/j.foodres.2011.04.025

FAO, 2014. FAOSTAT Crop production. [cit. 2015-10-12] Available at: http://faostat.fao.org

Friedman, M. 1997. Chemistry, biochemistry, and dietary role of potato polyphenols. A review. *Journal of Agricultural and Food Chemistry*, vol. 45, p. 1523-1540. http://dx.doi.org/10.1021/jf960900s

Gumul, D., Ziobro, R., Noga, M., Sabat, R. 2011. Characterisation of five potato cultivars according to their nutritional and pro-health componets. *Acta Scientiarum Polonorum Technologia Alimentaria*, vol. 10, p. 77-81. PMid:22232530

Hamouz K., Lachman J., Vokal B., Pivec V. 1999. Influence of environmental conditions and way of cultivation on the polyphenol and ascorbic acid content in potato tubers. *Rostlinní výroba*, vol. 45, p. 293.

Hils, U., Pieterse, L. 2007. World Catalogue of Potato Varieties. Agrimedia Press, Agrimedia, GmBH, Clenze, Germany.

Karadeniz, F., Burdurlu, H. S., Koca, N., Soyer, Y. 2005. Antioxidant activity of selected fruits and vegetables grown in Turkey. *Turkish Journal of Agriculture and Forestry*, vol. 89, p. 297-303.

Kaur, C., H. C. Kapoor. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology*, vol. 37, p.153-161. http://dx.doi.org/10.1046/j.1365-2621.2002.00552.x

Kaur, S., Aggarwal, S. 2014. Evaluation of antioxidant phytochemicals in different genotypes of potato. *International Journal of Engineering Research and Applications*, vol. 4, p. 167-172.

Kita, A., Bakowska-Barczak, A., Hamouz, K., Kułakowska, K., Lisińska, G. 2013. The effect of frying on anthocyanin stability and antioxidant activity of crisps from red- and purple-fleshed potatoes (*Solanum tuberosum* L.). *Journal of Food Composition and Analysis*, vol. 32, p. 169-175. http://dx.doi.org/10.1016/j.jfca.2013.09.006

Külen, O., Stushnoff, C., Holm, D. G. 2013. Effect of cold storage on total phenolics content, antioxidant activity and vitamin C level of selected potato clones. *Journal of the Science of Food and Agriculture*, vol. 93, no. 10, p. 2437-2444. http://dx.doi.org/10.1002/jsfa.6053 PMid:23400895

Lachman J., Hamouz, K., Šulc, M., Orsak, M., Pivec, V., Hejtmankova, A., Dvořak, P., Čepl, J. 2009. Cultivar differences of total anthocyanins and anthocyanidins in red and purple-fleshed potatoes and their relation to antioxidant activity. *Food Chemistry*, vol. 114, p. 836-843. http://dx.doi.org/10.1016/j.foodchem.2008.10.029

Lewis, C. E., Walker, J. R. L., Lancaster, J. E. 1999. Changes in anthocyanin, flavonoid and phenolic acid concentrations during development and storage of coloured potato (*Solanum tuberosum* L.) tubers. *Journal of the Science of Food and Agriculture,* vol. 79, p. 311-316. http://dx.doi.org/10.1002/(SICI)1097-0010(199902)79:2<311::AID-JSFA199>3.0.CO;2-Q

Musilová, J., Bystrická, J., Volnová, B., Lednický, P. 2015. Dependence among total polyphenols content, total antioxidant capacity and heavy metals content in potatoes. *Potravinarstvo,* vol. 9, no. 1, p. 515-522. http://dx.doi.org/10.5219/532

Navarre, D. A., Goyer, A., Shakya, R. 2009. Nutritional value of potatoes. Vitamin, phytonutrient and mineral content. In Singh, J. and Kaur, L., *Advances in potato chemistry and technology*, Academic Press, Elsevier : USA, p. 395-424. ISBN 978-0-12-374349-7. http://dx.doi.org/10.1016/b978-0-12-374349-7.00014-3

Poberezny, J., Wszelaczynska, E. 2011. Effect of bioelements (N, K, Mg) and long-term storage of potato tubers on quantitative and qualitative losses part II. Content of dry matter and starsch. *Journal of Elementology,* vol. 16, no. 2, p. 237-246. http://dx.doi.org/10.5601/jelem.2011.16.2.07

Prieto, P., Pineda, M., Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, vol. 269, p. 337-341. http://dx.doi.org/10.1006/abio.1999.4019 PMid:10222007

Quadros, D. A., Lung, M. C., Ferreira, S. M. R., Freitas, R. J. S. 2010. Frying quality of potato, with regard to reducing sugar and non-reducing sugar, during storage at room temperature. *Acta Scientarum-Technology*, vol. 32, no. 4, p. 439-443.

Quettier-Deleu, CH., Gressier, B., Vesseur, J., Dine, E., Brunet, C., Luyckx, M., Cazin, M., Cazin, J. C., Bailleul, F 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology*, vol. 1-2, p. 35-42. http://dx.doi.org/10.1016/S0378-8741(00)00196-3

Reddivari, L., Hale, A., Miller, J. 2007. Genotype, location, and year influence antioxidant activity, carotenoid content, phenolic content, and composition in specialty potatoes. *Journal of Agricultural and Food Chemistry*, vol. 55, p. 8073-8079. http://dx.doi.org/10.1021/jf071543w PMid:17760413

Singh, J., Kaur, L. 2009. *Advances in potato chemistry and technology*. Academic Press, Elsevier : USA, 508 p. ISBN 978-12-374349-7.

Singleton, V. L., Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Agricultural*, vol. 6, p. 144-158.

Šimková, D., Lachman, J., Hamouz, K. 2013. Effect of cultivar, location and year on tota starch, amylose, phosphorus content and starch grain size of high starch potato cuktivars for food and industrial processing. *Food Chemistry.* vol. 141, no. 4, p. 3872-3880. http://dx.doi.org/10.1016/j.foodchem.2013.06.080

Tudela, J. A., Cantos, E., Espin, J. C., Tomas-Barberan, F. A., Gil, M. I. 2002. Induction of antioxidant flavonol biosynthesis in fresh-cut potatoes. Effect of domestic cooking. *Journal of Agricultural and Food Chemistry*, vol. 50, p. 5925-5931. http://dx.doi.org/10.1021/jf020330y PMid:12358461

Vinson, J. A., Hao, Y., Xuehui, S., Zubik, L. 1998. Phenol antioxidant quantity and quality in foods: Vegetables. *Journal of Agricultural and Food Chemistry*, vol. 46, p. 3630-3634. http://dx.doi.org/10.1021/jf980295o

Wegener, Ch. B., Jansen, G. 2013. Antioxidants in different potato genotypes: effect of drought and wounding stress. *Agriculture*, vol. 3, no. 1, p. 131-146. http://dx.doi.org/10.3390/agriculture3010131

Yen, G. C., Chen, H. Y. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural Food Chemistry*, vol. 43, p. 27-32. http://dx.doi.org/10.1021/jf00049a007

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LUNASIN DETECTION IN COLOURED WHEAT GENOTYPE

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ABSTRACT

Lunasin is a biologically active protein, composed of 43 amino acid residues. There has been proven many health-promoting effects of lunasin peptide. The most important health benefits include: anti-hypertension, antioxidant activity, cancer prevention or therapy. It was also demonstrated anti-inflammation, hypocholesterolemic activity, anti-obesity and immunomodulation. The focus of our research is to summarize the discovery, characterization and biological activities of lunasin, which will provide a reference for the future development and utilization of lunasin, and a basis for exploring the underlying mechanisms of these health-beneficial functions. Lunasin was first isolated in 1987 at Niigata University School of Medicine in Japan, during the screening of protease inhibitors from soybean seeds. It was subsequently found in other beans, grains and herbal plants, including wheat, barley, rye, triticale. Concentration of lunasin is ranging from 0.013 to 70.5 mg protein lunasin/g of protein. Big step forward in the understanding of the lunasine operating mechanism in the fight against cancer has arisen after study on cloning of the soybean lunasin gene and subsequent transfection into mammalian cells which led to the discovery that the lunasin gene can disrupt mitosis and induce chromosome breakage, ultimately leading to cell apoptosis. The main goal of our work was to evaluate collection of wheat with unusual grain colour for presence of lunasin gene. DNA was extracted by commercial kit and lunasin gene was detected by PCR reaction. Our results showed presence of lunasin gene detected by 3 combinations of 2 sets of primer pair and indicated lunasin peptide presence in cereal grains. These findings are necessary to confirmed by proteome analysis.

Keywords: cancer; coloured wheat; gene detection; lunasin; PCR

INTRODUCTION

Civilization diseases are one of the most worldwide problems of mankind. Cancer is the largest and the most widespread illness. Surgical treatment was the most effective, in past, but there are a lot of less invasive methods of cancer healing, in presence. New substances originated from plants or animals, which show chemopreventive effects, are shown by ongoing studies **(Hernández-Ledesma et al., 2009)**.

Carcinogenesis is a process which consists of combination of multiple heritable and environmental factors. Epidemiological studies shown, that cancer appearance and mortality significantly varied across the world. Cancer remains the main cause of mortality in western world. These parts of world where is diet centered on plant foods tending to have a lower rate of cancer, but prevalence of cancer is rising rapidly in one generation after their emigration to the western countries. This indicates that genetic factors are not the primary factors that cause cancer and modification of nutritional habits and lifestyle, as well as, consumption of foods containing bioactive components can offer a significant protection against carcinogenesis **(Hernándes-Ledesma et al. 2009)**.

Lunasin is one of these substances, which produce not only a lot of positive effects on human organism, but also anticancer activity. Lunasin is biological active peptid, which consist of 43 amino acids. There has been confirmed, that lunasin protected cells against chemical transformation induced by chemical carcinogens and virus and ras oncogenes. Mechanism of lunasin action is based on balance influence between acetylation and deacetylation of histones. This mechanism may cause cell death, because in this case lunasin acts as a tumour suppressor which is tightly bounded on deacetylated histones in cell nucleus and have ability to influence cancer cells apoptotically and cytotoxic **(Chang et al., 2014)**.

In vitro studies, animal treatment and epidemiologic researches showed that soy consumption is in connection with decreasing of some cancer types **(Hernández-Ledesma et al., 2009)**.

Hsieh et al., (2010) reported, that the first animal model confirmed preventive properties against chemical carcinogen-induced skin cancer in mice. Lunasin also play role as an active cancer preventive agent in treatment of human breast cancer. Lunasin in combination with aspirin arrest the cell cycle in the S- and G-phases, respectively, acting synergistically to induce apoptosis which was achieved by modulating the expression of genes encoding G1 and S-phase regulatory proteins.

Lunasin is a soybean derived peptide with a MW of 5.5 kDa and contains 9 aspartic acid residues on C domains, cell adhesion motifs consit of 3 amino acids residues (arginin – glycine – aspartic acid) and predicted helix with structural homology to a conserved region of chromatin binding

proteins. Lunasin is not fully digested in gastrointestinal system, but is absorbed intact, reaching target tissues. The biological activity of lunasin depends on cultivar, environmental factors and processing conditions, which in turn affected its concentration **(Wang et al., 2008)**.

Lunasin has been discovered in most of soybean varieties and its concentration ranged from 4.4 to 70.5 mg lunasin in one gram of protein **(Hernándes-Ledesma et al., 2009)**.

Jeong et al., (2007) detected lunasin in wheat using mass spectrometry. They determined 14 amino acids fragment (KQLQGVNLTPCEKH) with m/z 656, 8640 Da. This fragment corresponded to 12-25 amino acids fragment of soy albumin subunits, which was identified as a lunasin peptide.

The main goal of our research was to detected lunasin gene in collection of coloured wheat grain.

MATERIAL AND METHODOLOGY

There was analysed collection of 8 genotypes of wheat grain (Table 1) with unusual grain colour.

DNA isolation was performed from wheat grain by commercial kit GeneJET TM (Fermentas). Isolation protocol was modified for isolation DNA not only from fresh tissue, but also from grain. Modification contains supplementation of Lysis buffer A with 2% (w/v) polyvinylpyrrolidone. Wheat grains of each cultivar (up to 100 mg) were grinded in liquid nitrogen using a mortar and pestle. Then were grounded plant tissue powder transferred into the tubes with the prealiquoted Lysis buffer A (with PVP). The rest of extraction steps were held according standard procedure with usage of Lysis buffer B, RNase A. Purificatrion of extracted DNA were realized in spin column tubes with utilization of Plant g-DNA binding solution which anchored extracted DNA on spin column membrane. Wash buffer I and Wash buffer II purified anchored DNA. Elution of DNA from membrane to solution was realized by Elution buffer. The quantity and quality of puried DNA were measured by nanophotometer and visualisated by 1.5% horizontal agarose electorphoresis.

GoTaq® Green Master Mix from Promega Company was used for Polymerase chain reaction (PCR) DNA amplification. GoTaq® Green Master Mix is a premixed ready-to-use solution containing bacterially derived Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. PCR amplification protocol of DNA fragments were realized using primer pairs (Table 2) and their combination (Table 3) according to **Dinelli et al., (2014)**. 25 µL of reaction mix was prepared on ice and contained 12.5 µL of GoTaq® Green Master Mix, 2.5 µL of 10 µM upstream primer, 2.5 µL of 10 µM downstream primer, 2.5 µL of nuclease-free water and 5 µL of 0.025 $ng.\mu L^{-1}$ DNA template. Standard PCR procedure consists of 2 min initial denaturation step at 95 °C for activation of reaction mix. Product amplification contained 3 subsequent steps. 20 s denaturation of DNA at 95 °C, 30 s anelation of primers at 54 °C and 30 s polymerization of DNA fragmets at 72 °C. These 3 steps repeat 50 times. The last step of PCR procedure is 5 min final extension at 72 °C. Quality of amplyfied product were confirmed by 1.5% horizontal agarose electrophoresis.

DNA fragments were separated in 8% vertical polyacrylamide gel electrophoresis for 360 min at 500V in Hoeffer SE 660 electrophoretic system and visualised by silver staining.

Table 1 List of analysed genotypes.

	Name	Species	Originated	Colour
1	Barevná 25 – modrá	*Triticum aestivum* L.	CZ	**Blue**
2	Trojzrnka	*Triticum aestivum* L.	CZ	**Red**
3	Mnohokvietková	*Triticum aestivum* L.	CHINA	**Red**
4	Tr.Etiopicum Jakubz	*Triticum aestivum* L.	Ethiopia	**Purple**
5	Tr. Etiopicum araratica-červená	*Triticum aestivum* L.	Ethiopia	**Purple**
6	UC 66094	*Triticum aestivum* L.	USA	**Blue**
7	Koniny-červená	*Triticum aestivum* L.	CZ	**Red**
8	Modré zrno	*Triticum aestivum* L.	CZ	Blue

Table 2 Nukleotid sequences of used primers.

Name	Primer type	Sequence
Lun1	forward	**AAATGGCANCACCAGNA**
	revers	**CGTCATCATCATNATCGTNA**
Lun2	forward	**GATANCTGCCNCAAGCA**
	revers	**TCTTNTCCATNATGTGCTTCTC**

Table 3 Primer pairs combinations.

Number	Primer pair combination
1	Lun1 F x Lun1 R
2	Lun1 F x Lun2 R
3	Lun2 F x Lun1 R
4	Lun2 F x Lun2 R

Electrophoretic separation and visualisation of DNA fragments were performed according **Bassam et al., (1991)**.

Visualised DNA fragments were captured by UVP digital imagine system and detected by Doc-IT LS software from UVP Jena, Germany.

RESULTS AND DISCUSSION

Lunasin is a novel, cancer-preventive peptide whose efficacy against chemical carcinogens and oncogenes has been demonstrated in mammalian cells and in a skin cancer mouse model. Isolated and characterized in soy, lunsin peptide is also documented in barley, wheat, tritikale, rye and oat **(Lumen et al., 2005)**.

The characterisation of cDNAs encoding lunasin shows that it corresponds to the small subunit of the soybean 2S albumin. The biological activity of lunasin has led to searches for related peptides in other plant species, including reported isolation from *Solanum*, amaranthus seeds, Brazil nut, sunflower and cereal seeds.

The identity of the peptides in wheat was confirmed by partial sequences which match exactly to the soybean sequence over stretches of 14 amino acids **(Mitchell et al., 2013)**.

We therefore decided to search for lunasin gene sequence aroud colour wheat genotype by utilization of 2 sets of primers developed by **Dinelli et al., (2014)**.

Our results show that utilization of primer pair Lun1 forward and Lun1 revers showed presence of 121 bp length DNA fragment. This primers pair combination seems to be suitable for lunasin gene detection, because we were able to detect lunasin gene in all of genotypes (Figure 1 and Figure 2).

Primer pair combination Lun1 forward and Lun2 revers not provide any fragment with desirable length 85 bp Using of this primer pair combination is contradictory and has to be tested in future.

Primer pair combination Lun2 forward and Lun1 revers was used for detection of DNA fragment with length 103 bp. There were obtained presence of desired DNA fragment in each genotype of evaluated wheat collection (Figure 3 and 4).

Application of primer pair combination Lun2 forward and

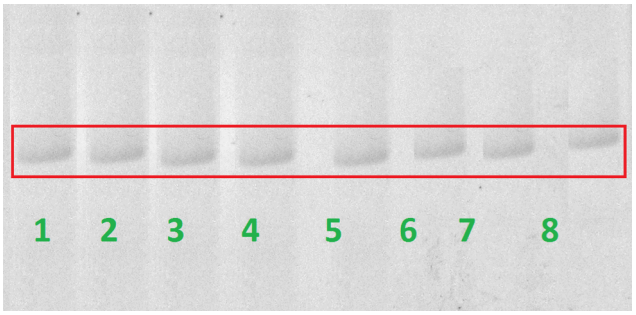

Figure 3 Lunasin gene detection with primer pair combination F2R1 – 103 bp.

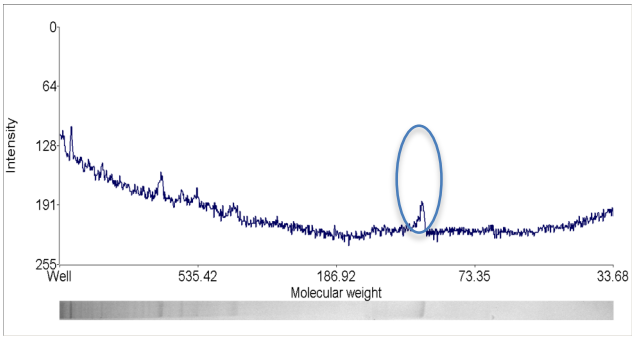

Figure 4 Barevná 25 – F2R1.

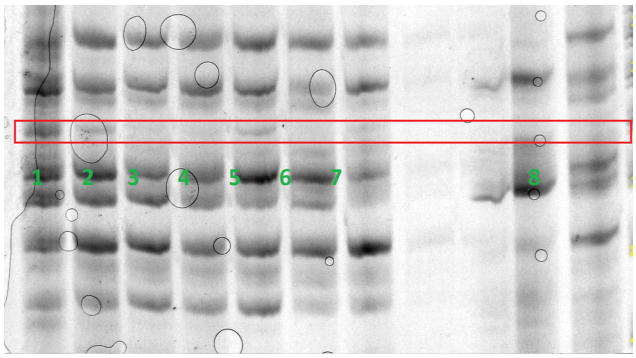

Figure 1 Lunasin gene detection with primer pair combination F1R1 – 121 bp.

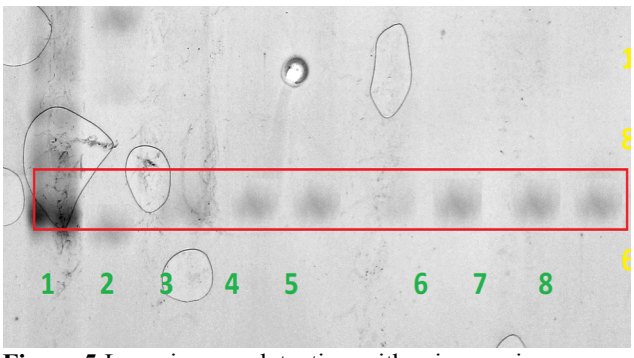

Figure 5 Lunasin gene detection with primer pair combination F2R2 – 67 bp.

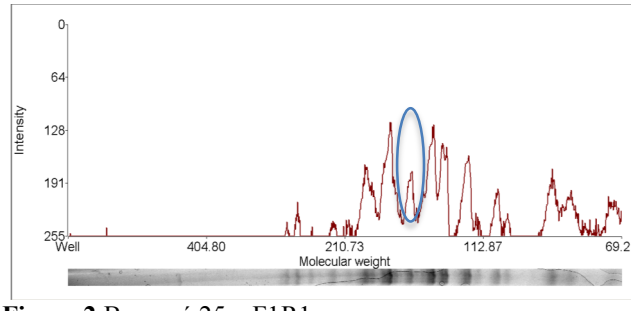

Figure 2 Barevná 25 – F1R1.

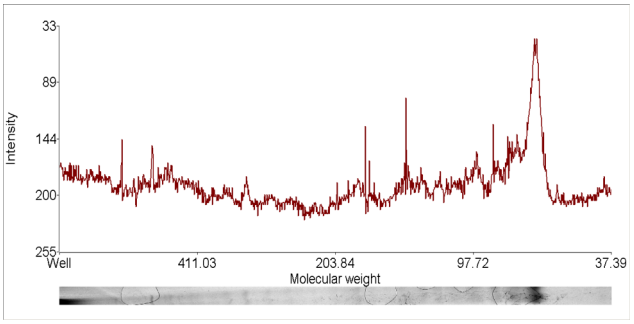

Figure 6 Barevná 25 – F2R2.

Lun2 revers provide detection of lunasin gene fragment with length 67 bp. This primer pair combination was suitable for detection of lunasin gene fragment in all genotype (Figure 5 and Figure 6).

We focused on confirmation of **Dinelli et al., (2014)** results in our research. **Dinelli et al., (2014)** monitored, that although there was positive presence of lunasin peptide in wheat proteome analysis, no gene coding this peptide was detected. However, **Jeong et al., (2009)** and **Maldonado-Cervantes et al., (2010)** observed lunasin peptide in proteome analysis and postulated also presence of gene coding this peptide.

Our results showed presence of gene coding lunasin peptide, but are in controversy with **Dinelli et al., (2014)** results. These lunasin gene detection findings require analysis of wheat proteome for detection of lunasin peptide to confirm expression of monitored DNA fragment.

Nakurte et al., (2012) and **Mitchell et al., (2013)** observed presence of lunasin peptide in cereals and their results indicated importance of mass spectrometry in cereal proteome analysis to confirm lunasin gene detection.

Presence of lunasin in triticale (X *Triticosecale* Wittmack) confirmed by **Nakurte et al., (2012)** indicated, that triticale is the most lunasin-rich cereal. The greatest lunasin content was 6.46 $mg.g^{-1}$ in the grain of triticale genotype 0002-26. In comparison, the highest lunasin content in rye variety Dankovske Diament was 1.5 $mg.g^{-1}$ of grain and the highest lunasin content in the winter wheat variety Fredis was 0.23 $mg.g^{-1}$ of grain. They conclude that triticale can play significant role as functional food, with great potential for the use of triticale products in human and animal diets.

Results of **Nakurte et al., (2012)**, which detected lunasin peptide in wheat and triticale corresponds to our observation about presence of lunasin gene in colour wheat genotype.

Jeong et al., (2009) focused their research on identification of lunasin peptide in rye (*Secale cereale* L.) cultivars. Lunasin was present in 15 out of 21 cultivars of analyzed rye cultivars. Lunasin present in rye crude protein preparation was stable to pepsin and pancreatin in *in vitro* digestion. They concluded that lunasin in rye is bioavailable and that consumption of rye may play an important role of cancer prevention in rye consuming population. Wheat is close relative to rye and therefore is possibility of wheat utilization in cancer prevention. Our results indicate presence of lunasin gene in wheat. Although lunasin peptide presence in wheat is contradictory, observation obtained by **Nakurte et al., (2012)** and **Jeong et al., (2007)** are in agreement with our observations.

Lunasin peptide detection in oat genotypes (*Avena sativa* L.) was performed by **Nakurte et al., (2013)**. Lunasin was detected using LC-MS/MS analysis. They observed genotype-related fluctuations in the lunasin content. The highest lunasin level was 0.197 $mg.g^{-1}$ of grain. There was also no correlation between lunasin and protein content, but genotype-dependent variations of the lunasin content was demonstrated during different years. Therefore, is very important to study influence of farming system, crop management and climate conditions on lunasin content in cereals as well as clarifying if consumption of lunasin-containing foods plays as important role in cancer and cardiovascular disease prevention.

Jeong et al., (2010) also elucidated role of cereals in cancer prevention. They reported the prevalence; bioavailability and bioactivity of lunasin from barley.

The liver and kidney of rats were fed with lunasin-enriched barley and inhibits the activities of histone acetyl transferases.

These findins and our results indicated that lunasin is prevalent in cereals and is bioavailable and bioactive. Consuption of cereals could play an important role of cancer prevention in cereal-consuming populations.

Recombinant production of the therapeutic peptide lunasin was widely studied by **Kyle at al., (2012)**. They used a pET28 vector to express cellulose binding domain (CBD)-lunasin fusion with a hexahistidine tag and Tobacco Etch Virus protease site, to allow protease-mediated release of native lunasin. The use of CBD as a fusion partner gave high protein yields by autoinduction, with lunasin release by TEV protease cleavage. This approach could provide a potentially valuable route for production of this therapeutic peptide.

CONCLUSION

Utilization of 2 sets of primer pair in 4 combinations showed suitability of F1R1, F2R1 and F2R2 primer pair combination for detection of lunasin gene. Identification of lunasin gene may be used for chromosome site identification and genetic manipulation with promotor to enhance gene activity and production of desirable level of peptide.

REFERENCES

Bassam, B. J, Caetano-Anollés, G., Gresshoff, P. M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry*, vol. 196, no. 1, p. 80-83. http://dx.doi.org/10.1016/0003-2697(91)90120-I

Chang, H. C., Lewis, D., Tung, C. Y., Han, L., Henriquez, S. M. P., Voiles, L., Lupov, I. P., Pelloso, D., Sinn, A. L., Pollok, K. E., de Lumen, B. O., Li, F., Blum, J. S., Srivastava, S., Robertson, M. J. 2013. Soypeptide lunasin in cytokine immunotherapy for lymphoma. *Cancer Immunol Immunother*, vol. 63, no. 3, p. 283-295. http://dx.doi.org/10.1007/s00262-013-1513-8 PMid:24363024

Dinelli, G., Bregola, V., Bosi, S., Fiori, J., Gotti, R., Simonetti, E., Trozzi, C., Leoncini, E., Prata, C., Massaccesi, L., Malaguti, M., Quinn, R., Hrelia, S. 2014. Lunasin in wheat: A chemical and molecular study on its presence or absence. *Food Chemistry*, vol. 151, p. 520-255. http://dx.doi.org/10.1016/j.foodchem.2013.11.119 PMid:24423565

Hernández-Ledesma, B., Hsieh, C. C., de Lumen, B. O. 2009. Lunasin, a novel seed peptide for cancer prevention. *Peptides*, vol. 30, no. 2, p. 426-430. http://dx.doi.org/10.1016/j.peptides.2008.11.002 PMid:19056440

Hsieh, C. C., Hernández-Ledesma, B., de Lumen, B. O. 2010. Lunasin, a novel seed peptide, sensitizes human breast cancer MDA-MB-231 cells to aspirin-arrested cell cycle and induced apoptosis. *Chemico-Biological Interactions*, vol. 186, no. 2, p. 127–134. http://dx.doi.org/10.1016/j.cbi.2010.04.027 PMid:20457246

Jeong, H. J., Jeong, J. B., Hsieh, C. C., Hernández-Ledesma, B., de Lumen, B. O. 2010. Lunasin is prevalent in barley and is bioavailable and bioactive in In Vivo and In Vitro studies.

Nutrition and Cancer, vol. 62, no. 8, p. 1113-1119. http://dx.doi.org/10.1080/01635581.2010.515529 PMid:21058199

Jeong, H. J., Jeong, J. B., Kim, D. S., Park, J. H., Lee, J. B., Kweon, D. H., Chung, G. Y., Seo, E. W., de Lumen, B. O. 2007. The cancer preventive peptide lunasin from wheat inhibits core histone acetylation. *Cancer Letters*, vol. 255, no. 1, p. 42-48. http://dx.doi.org/10.1016/j.canlet.2007.03.022 PMid:17481808

Jeong, H. J., Lee, J. R., Jeong, J. B., Park, J. H., Cheong, Y., de Lumen, B. O. 2009. The cancer preventive seed peptide lunasin from rye is bioavailable and bioactive. *Nutrition and Cancer*, vol. 61, no. 5, p. 680-686. http://dx.doi.org/10.1080/01635580902850082 PMid:19838942

Kyle, S., James, K. A. R., McPherson, M. J. 2012. Recombinant production of the therapeutic peptide lunasin. *Microbial Cell Factories*, vol. 11, no. 28. http://dx.doi.org/10.1186/1475-2859-11-28

Lumen, B. O. 2005. Lunasin: A cancer-preventive soy peptide. *Nutrition Reviews*, vol. 63, no. 1, p. 16-21. http://dx.doi.org/10.1111/j.1753-4887.2005.tb00106.x PMid:15730231

Maldonado-Cervantes, E., Jeong, H. J., León-Galván, F., Barrera-Pacheco, A., De León-Rodríguez, A., González de Mejia, E., de Lumen, B. O., Barba de la Rosa, A. P. 2010. Amaranth lunasin-like peptide internalizes into the cell nucleus and inhibits chemical carcinogen-induced transformation of NIH-3T3 cells. *Peptides*, vol. 31, no 9, p. 1635-1642. http://dx.doi.org/10.1016/j.peptides.2010.06.014 PMid:20599579

Mitchell, R. A. C., Lovegrove, A., Shewry, P. R. 2013. Lunasin in cereal seeds: What is the origin? *Journal of Cereal Science*, vol. 57, no. 3, p. 267-269. http://dx.doi.org/10.1016/j.jcs.2013.01.013 PMid:24817784

Nakurte, I., Kirhnere, I, Namniece, J., Saleniece, K., Krigere, L., Mekss, P., Vicupe, Z., Bleidere, M., Legzdina, L., Muceniece, R. 2013. Detection of the lunasin peptide in oats (Avena sativa. L). *Journal of Cereal Science*, vol. 57, no. 3, p. 319-324. http://dx.doi.org/10.1016/j.jcs.2012.12.008

Nakurte, I., Klavins, K., Kirhnere, I., Namniece, J., Adlere, L., Matvejevs, J., Kronberga, A., Kokare, A., Strazdina, V., Legzdina, L., Muceniece, R. 2012. Discovery of lunasin peptide in triticale (X Triticosecale Wittmack). *Journal of Cereal Science*, vol. 56, no. 2, p. 510-514. http://dx.doi.org/10.1016/j.jcs.2012.04.004

Wang, W., Dia, V. P., Vasconez, M., de Meija E. G., Nelson, R. L. 2008. Analysis of soybean protein-derived peptides and the effect of cultivar, environmental conditions, and processing on lunasin concentration in soybean and soy products. *Journal of AOAC International*, vol. 91, no. 4, p. 936-946. PMid:18727556

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EFFECT OF THE ADDITION OF HYDROCOLLOIDS ON THE RHEOLOGICAL AND BAKING PROPERTIES OF THE PRODUCTS WITH ADDED SPELT FLOUR (*TRITICUM SPELTA* L.)

Tatiana Bojňanská, Jana Šmitalová, Alena Vollmannová

ABSTRACT

The paper presents the results of the evaluation of the effect of additives on the rheological properties of composite flour made of wheat flour in the amount of 70% and spelt flour at 30%. As additives guar gum (0.5% by weight of flour) and xanthan gum (0.16% by weight of flour) were used. Properties of produced control dough and doughs with hydrocolloids were evaluated by means of rheological appliances by Farinograph, Extenzograph, Amylograph and Rheofermentometer. Based on the observed results it can be concluded that the addition of xanthan gum has a positive effect on increasing of farinographic water absorption capacity, extension of dough development time and dough stability and generally positively affected farinographic properties. The addition of guar gum has improved especially extensographic properties as extensographic energy and extensographic resistance. Based on amylographic evaluation of control doughs and doughs with additives it can be stated that in the dough with guar gum the amylographic maximum has slightly increased. Hydrocolloid guar gum contributed to an increased retention capacity of dough observed. Based on our measurements we can indicate that addition of guar and xanthan gum contributed to an increased rheological quality of doughs prepared with addition of flour from spelt wheat. With reference to the baking experiment it was found that the use of hydrocolloids has a positive effect on the improvement of the baking properties, in particular larger volume, specific volume, and the volume yield of the dough with the addition of guar and xanthan gum compared to the control. Our results showed that aditives significantly influenced rheological qualities of dough and a baking quality of products. These findings thus allow optimizing the recipe in order to increase the technological quality of leavened bakery products.

Keywords: hydrocolloids; guar gum; xanthan gum; rheological properties; frozen dough

INTRODUCTION

Spelt wheat (*Triticum spelta* L.), family (Poaceae) is like common wheat (*Triticum aestivum* L.) classified as hexaploid wheat with 42 chromosomes and a six-rowed ear. Contrary to the common wheat spelt wheat has the husk that protects the grain against pests, insects and microbial contamination **(Krkošková et al., 2011; Filipčev et al., 2014)**. Spelt wheat can be described as old European cultural wheat that is currently grown in Western Europe, in Austria, Germany, Belgium, Switzerland and northern Spain. Its popularity is increasing due to the lower cultivation demands, and it is also suitable to be grown in foothill areas with poor soil and excess rainfall. It is used in various forms - such as grains, also for production of pasta, crackers, bread and beer **(Bojňanská et al., 2002)**.

The dry matter of common wheat grain is composed of saccharides in an amount of about 70%, with the most important part of the saccharides being starch at 60% – 65%, which consists of amylose (26% – 28%) and amylopectin (72% – 74%). The proportion of oligosaccharides of the total saccharides content is about 2% to 3%. The amount of total dietary fibre in grains varies from 9% to 12%, of which about 2% are made up of soluble fibre and the rest being insoluble fibre **(Feillet, 2000; Pruska-Kedzior et al., 2008; Escarnost et al., 2012)**.

The seed of spelt wheat contains about 15% of protein and the albumin portion is 13% of the total protein content, 3% is of globulins, 40% of prolamins, and 45% glutelins **(Krkošková et al., 2005; Pruska-Kedzior et al., 2008)**. It was found that the digestibility of spelt proteins is better in comparison to wheat protein, but the difference is minimal **(Ranhotra et al., 1995)**. The amount of essential amino acids of the total amino acids in common wheat and spelt wheat differs only marginally, and this difference is not statistically significant suggesting a very similar protein quality **(Grela, 1996; Abdel-Aal et Hucl, 2002). Bojňanská et al. (2002)** indicated that the amount of gluten varies in the range from 30% to 52%.

The grain of spelt wheat contains approximately 3% of fat, predominantly located in the embryo, aleurone, and at the lesser degree in endosperm (about 1.5% of the total lipids) **(Delcour et Hoseney, 2010)**. Fatty acids are represented mainly by linoleic acid (60%) and palmitic acid (17%), and these are lower amounts compared to their content in common wheat. Another major fatty acid is

oleic acid (18% in spelt wheat which is 6% more than in common wheat) **(Escarnot et al., 2012)**.

Spelt wheat is similarly to the common wheat a source of B group vitamins with approximately 1.5% concentrated mainly in germ. **Ranhort et al., (1995)** found out comparable amounts of thiamine and riboflavin in the grains of spelt wheat and common wheat. According to findings by **Bojňanská et al., (2002)** the amount of minerals varies between 1.8% and 2.3% depending on the variety and the year. The similar results reported also **Ranhort et al., (1995)** and **Ruibal-Mendiet et al., (2005)**.

Based on these findings it can be concluded that spelt wheat is therefore technologically and nutritionally suitable raw material for the use in bakery products affecting also their sensory characteristics.

Hydrocolloids are high molecular and hydrophilic biopolymers performing several functions in the food industry. The most significant features are their ability to control the rheological properties and texture of food. In the baking industry they are added in particular to stabilize emulsions, suspensions and foams and to improve the processing properties. Among other properties they have the ability to inhibit starch retrogradation, retain moisture, improve the overall structure, and slow down aging of products. They can be used as a substitute for fat and eggs **(Arozarena et al., 2001; Collar et al., 1999; García - Ochoa et al., 2000; Příhoda et al., 2003; Kohajdová et al., 2008 a, b; Magala et al., 2011; Rodge et al., 2012; Šedivý et al., 2013; Eduardo et al. 2014; Qiu et al., 2015)**. An important positive feature is that the use of hydrocolloids even in small quantities (less than 1% by weight of flour), has a significant impact on enhanced ability of dough to bind water, increase the volume of products, slow retrogradation of starch and thus extend the shelf life of bakery products **(Collar et al., 1999; Khan et al., 2007; Škara et al., 2013)**.

Guar gum is defined as the ground endosperm of the seeds of natural strains of the guar plant, *Cyamopsis tetragonolobus* (L.) Taub. (family *Leguminosae*) and it consists of hydrocolloidal polysaccharides of high molecular weight composed of galactopyranose and mannopyranose combined through glycosidic linkages, which may be described chemically as galactomannan **(FC SR, Decree 1)**. The plant grows mostly in India and Pakistan, but since 1950 it has also been grown in Texas and Arkansas as a commercial commodity **(Achayuthakan et Suphantharika, 2008). Kohajdová et al., (2008a)** reported that bread with addition of guar gum had after baking better baking properties, such as a higher volume and improved sensory quality, particularly more attractive appearance and aroma, softer crumb and firmer crust.

Xanthan gum is an exocellular polysaccharide of microbial origin that is produced by aerobic fermentation of sugar by the bacterium *Xanthomonas campestris*. China is its largest producer in the world **(Hojerová et al., 2005; Achayuthakan et Suphantharika, 2008; FC SR Decree 2; Tao et al., 2012)**. The main chain of xanthan gum is formed by β-D- (1,4) glucose units and the side chains are composed by residues of D-glucuronic acid and two mannose moieties D **(Velíšek, 2002)**. Xanthan gum has an impact on strengthening links between flour proteins and thereby firming the dough structure. Baked products with a xanthan gum have larger volume, and optimum shape in comparison to products without this additive. During dough preparation xanthan gum binds to the starch and by that slows down its retrogradation, which is to be said by experts one of the main reasons of products staling **(Collar et al., 1999; Arozarena et al., 2001; Rosell et al., 2001; Gimeno et al., 2004; Ashwini et al., 2009)**.

MATERIAL AND METHODOLOGY

In the study, the effect of the addition of hydrocolloids of xanthan and guar gum to composite flour from spelt wheat on the rheological properties of dough was addressed. The results were compared with objective baking properties of baked products.

To prepare control loaves wheat flour T 650 was used. The second group of loaves was made from composite flour, based on wheat flour T 650 in an amount of 70% (Mlyn Pohronský Ruskov a.s., Hlavná 76, 935 62 Pohronský Ruskov, Slovakia) with an addition of spelt wholemeal flour at 30% (company J. Vince s.r.o., 925 91 Kráľová nad Váhom 320, Slovak Republic). According to recipe fresh compressed yeast was used (Trenčianske droždie, Old Herold Hefe, s.r.o., Bratislavská 36, 911 05 Trenčín, SR). In wheat and composite flour, the moisture was determined (%) **(ICC Standards No. 110/1 (1976))**, as well as content of crude protein (%) **(ICC Standard No. 105/2, (1994))** and content of ash (%) **(ICC Standard No. 104/1 (1990))**. Rheological measurements of prepared composite flour and wheat flour were made by means of *Farinograph-E*, Brabender OhG, Duisburg, Germany **(ICC Standrd 115/1 (1992), AACC Method 54-21 (1995))**. Based on these measurements following characteristics were determined: farinographic flour water absorption capacity (%), dough development time (min), dough stability (min), farinographic quality number. By means of *Extensograph-E*, Brabender OhG, Duisburg, Germany **(ICC - Standard 114/1 (1992), AACC Method 54-10 (1995))** extensographic energy (cm^2), extensographic tensibility (mm) and extensographic maximum (EU) were determined. By means of *Amylographe-E,* Brabender OhG, Duisburg, Germany **(ICC-Standard 126/1, AACC Method 22-10 (1995))** the initial gelatinization temperature (°C), the maximum gelatinization temperature (°C) and the amylographic maximum (AU) were determined.

The recipe of dough, which was tested in rheofermentometer consisted of 250 g of composite flour, fresh yeast in an amount of 2.8%, which was dispersed in water and added to flour during dough preparation in farinograph. After the first minute salt was added in an amount of 2%, and mixing continued for six minutes. The volume of added water depended on flour water absorption capacity to produce dough of optimal consistency. 315 g of dough was then inserted into Rheofermentometer Rheo F4 (Tripette & Renaud Chopine, Villeneuve-la-Garenne, France) **(AACC Method 89-01.01)** in order to determine during a three-hour test *total volume* (cm^3), *volume of* CO_2 *lost* (cm^3), *retention volume* (cm^3), *retention coefficient R* (%) (ratio of the volume that was detained in the dough to the total volume of produced CO_2).

Experimental loaves were prepared from a mixture of flour (350 g of wheat and 150 g of soy flour), sucrose (5 g), salt (9 g), yeast (20 g) and water addition based on

farinographic water absorption capacity. Bread experiment was carried out without the use of enzyme-active substances and other improvement agents. The development of dough took place in a laboratory mixer Diosna SP 12. After that the dough was elaborated and formed into loaves that stayed yeasted in a yeasting room for 20 minutes at temperature of 30 °C and were baked in an oven Miwe Condo at 240 °C with steaming (baking time 20 min). The baked loaves were evaluated by objective methods and the volume of products (cm^3), a specific volume of products ($cm^3.100g^{-1}$), volume yield ($cm^3.100g^{-1}$ flour), cambering (the ratio between height and width) were determined.

RESULTS AND DISCUSSION

The function of hydrocolloids lies in their ability to modify dough and improve its rheological properties, thus contribute to the maintenance of its quality **(Yaseen et al., 2010)**. Another important function of the gums is their ability to enhance the absorption of water in the baked products **(Mandala et al., 2008)** resulting in effecting the rheological properties of the dough and the extended shelf life of the products as they prevent migration of water during the staling of bread **(Fanta et al., 1996; Collar et al., 1999, Kohajdová et al., 2009)**.

Table 1 shows the effect of the addition of guar and xanthan gum on farinograph characteristics of composite flour. The addition of 0.5% of guar gum reduced farinograph water absorption capacity and development of dough compared to samples without this additive. **Rodge et al., (2012)** found that the addition of guar gum worked on shortening the time of dough development and extending dough stability. The application of 0.16% of xanthan gum we have implemented within our experiments did not significantly affect the water absorption capacity of the mixture, but contributed to the prolongation of the dough development. We have found that the addition of guar and xanthan gum had a positive effect on the stability of the dough extension and on the farinographic quality number, which was significantly higher (by 30) than in the control sample. The fact that the xanthan gum in the recipe contributes to increase of farinographic water absorption capacity, extension of dough development and its stability was also confirmed by **Rosell et al., (2001)** and **Davari-Ketilateh et al., (2013)**.

Time of dough development depends on the amount and quality of gluten, granularity of flour and the level of grinding and is determined primarily by the process of gluten hydration **(Dodok et Szemes, 1998)**. Strong flour is according **Muchová (2007)** defined as one that will during processing bind large amount of water, will reach optimum rheological properties slowly and retains them for a long time.

The results presented in Figure 1, which expresses amylographic evaluation of samples, show that the addition of guar gum and xanthan gum in all observed samples caused a slight decrease in the initial gelatinization temperature in comparison to a control sample without the gums. Hydrocolloids according to **Mandala (2012)** impact the acceleration of gelatinization and slow down the retrogradation and our results are consistent with these findings. We also noted that the addition of xanthan gum did not affect the maximum temperature of gelatinization.

The optimum value of amylographic maximum should vary in wheat flours between 300 AU and 650 AU, which is a sign of optimum amylase activity and products from these flours are identified as technologically very good **(Dodok et Szemes, 1998; Šedivý et al., 2013)**. Very high curve values of amylographic maximum above 800 AU predict flours with low amylase activity, and thus we can assume that the products from them would have very dry crumb with potential cracks and bland taste.

The results presented in Figure 2 describing extensographic parameters of composite flour with the addition of spelt flour and hydrocolloids show that with increasing time of maturing the value of extensographic energy has increased. Optimal values according to **Šedivý et al., (2013)** are between 90 cm^2 and 300 cm^2. After thirty minutes of maturing the highest value of extensographic energy was in the sample with guar gum at 94 cm^2, then the sample with xanthan gum at 84 cm^2 and lowest in the control sample without hydrocolloids at 80 cm^2. After thirty minutes of maturing the highest value of extensographic energy was in the sample with guar gum at 94 cm^2, then the sample with xanthan gum at 84 cm^2 and lowest in the control sample without hydrocolloids at 80 cm^2. Based on our findings it can be concluded that the best value of extensographic energy was in samples with guar gum.

Table 1 Farinographic parameters of composite flour (70% T 650 and 30% spelt wheat flour with addition of hydrocolloids).

Flour sample	farinographic flour water absorption capacity %	dough development time min	dough stability min	farinographic quality number
70%T650 + 30% T. spelt flour control	62.0	5.3	7.7	101
70%T650 + 30% T. spelt flour +0,5% guar gum	51.0	3.8	9.6	131
70%T650 + 30% T. spelt flour +0,16% xanthan gum	62.1	7.2	10.6	131

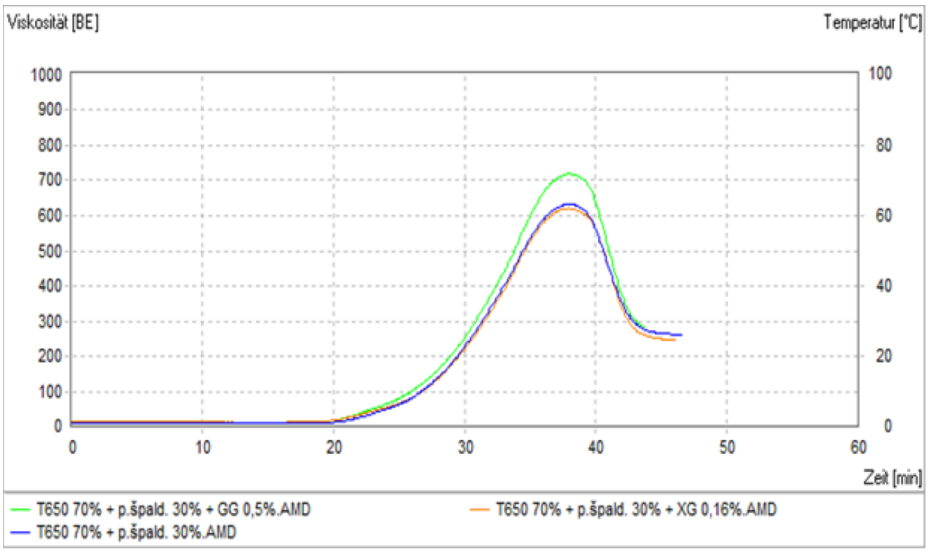


Figure 1 Amylographic parameters of composite flour with addition of spelt wheat and hydrocolloids.

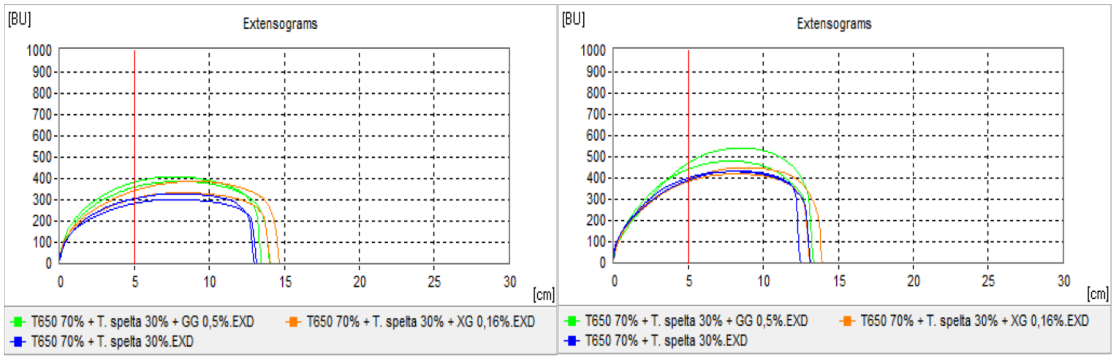
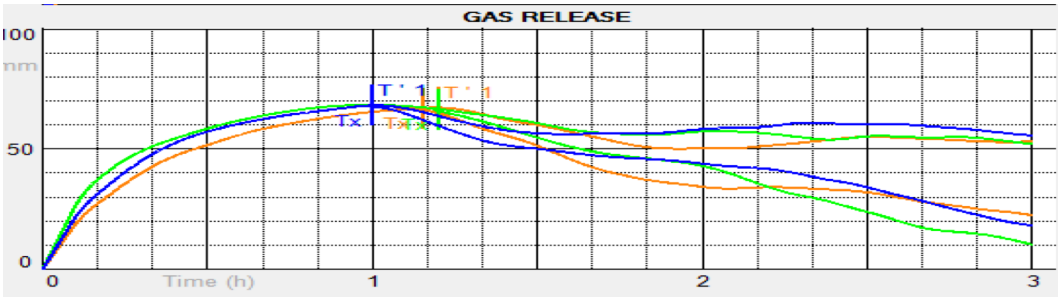


Figure 2 Extensographic parameters of composite flour with addition of spelt wheat and hydrocolloids after 15 and 30 minutes of maturing.



Picture 3 Reofermentometric parameters of composite flour with addition:

Spelt wheat ——, Guar gum ——, Xantan gum ——.

Higher value of extensographic resistance is a sign of strong gluten of good quality, which is mechanically resistant during manipulation with dough **(Příhoda et al., 2003)**. We found that with increasing time of maturing the dough resistance and extensographic maximum have increased which can be considered a positive result.

A comparison of the effect of hydrocolloids on extensographic maximum and dough resistance shows that guar gum had a significant impact on increasing of these values and it predicts products with a good volume.

For leavened bakery products the high elongation of dough is not desirable **(Dodok et Szemes, 1998)**. Optimal values for wheat flour are according to **Šedivý et al., (2013)** from 120 mm to 200 mm (±8 mm). With increasing of dough maturing time there was a slight decrease of elongation spotted in a control sample as well as in dough sample with the addition of hydrocolloids. In dough from composite flour with the addition of guar gum after fifteen and thirty minutes of maturing the elongation has increased in comparison to control.

Table 2 Results of bakery experiment with composite flour of 70% T 650 and 30% flour from spelt wheat with addition of hydrocolloids.

	70% T650+30% T. spelt flour control	*70% T650+30% T. spelt flour + 0,5% guar gum*	*70% T650+30% T. spelt flour + 0,16% xanthan gum*
Loaf volume cm^3	200.0	212.5	212.5
Specific loaf volume (cm^3.100g)	226.7	240.0	242.5
Volume recovery (cm^3.100g flour)	320.0	340.0	340.0
Recovery of product (%)	141.1	141.5	140.4
Baking loss %	14.6	13.8	14.6

Similar results describing that the addition of guar gum influenced the increasing of elongation were published by **Ribotta et al., (2004)**. Addition of hydrocolloids increased dough resistance during manipulation, which is from technological view point of significant importance.

Reofermentometric evaluation, the results of which are presented in Figure 3 showed that the addition of guar and xanthan gum does not significantly affect dough retention capacity. It can be regarded as positive that in dough with the addition of xanthan gum the breaking point occurred about nine minutes later, and in dough with guar gum even 12 minutes later than in the control dough without additives. Similar results in which the guar gum demonstrated itself as an additive delaying the time at which a break point occurs, and which increases retention were also found in the previous research with doughs made from flour of common wheat.

Results of experimental baking presented in Table 2 show that guar and xanthan gum positively influenced the total volume of products, the volume yield and specific volume compared with products without hydrocolloids. Similar results regarding the positive impact of hydrocolloids on baking properties were published by **Kohajdová et al., (2008a)**. Table 2 also shows that the addition of hydrocolloids did not influence the baking yield and baking loss during baking, since they were in all variants at a comparable level.

CONCLUSION

Summarizing and comparing the results of rheological evaluation of composite flour with the addition of spelt flour showed that the addition of guar gum extended dough stability time and increased dough farinograph number of quality compared to the control.

Significant improvement of the farinograph characteristics was observed in dough with the addition of xanthan gum, and this finding is very positive. Addition of guar gum in an amount of 0.5% positively influenced and improved extensographic properties of composite flour. Guar gum contributed to the increase of amylographic maximum, but in this case it can be considered as negative because an excessive increase of this value predicts reduced sensory quality of baked products.

The use of xanthan gum, in contrast to guar gum optimised amylographic properties, and thus an excellent quality of the crumb can be predicted. Evaluation of composite flours in reofermentometer showed that using of guar gum may slightly increase dough retention capacity, which is technologically positive finding, because maintaining of fermentation gas is a key factor to ensure sufficient volume of bakery products. Based on the evaluation of baking experiment it can be concluded that the loaves containing added guar and xanthan gum achieved compared to a sample without additives better indicators of bakery quality, which was reflected mainly by higher volume, specific volume and volume yield.

REFERENCES

AACC Method 89-01.01, Standard for measurement of yeast activity and gas production. Method for using Tripette & Renaud Chopine the Rheofermentometer Rheo F4.

Abdel-Aal, E. S. M., Hucl, P. 2002. Amino acid composition and in vitro protein digestibility of selected ancient wheats and their end products. *Journal of Food Composition and Analysis*, vol. 15, no. 6, p. 737-747. http://dx.doi.org/10.1006/jfca.2002.1094

Achayuthakan, P., Suphantharika, M. 2008. Pasting and rheological properties of waxy corn starch ad affected by guar gum and xanthan gum. *Carbohydrate Polymers*, vol. 71, no. 1, p. 9-17. http://dx.doi.org/10.1016/j.carbpol.2007.05.006

Arozarena, I., Bertholo, H., Empis, J., Bunger, A., De Sousa, I. 2001. Study of the total replacement of eff by white lupine protein, emulsifiers and xanthan gum in yellov cakes. *European Food Research and Technology*, vol. 213, no. 4-5, p. 312-316. http://dx.doi.org/10.1007/S002170100391

Ashwini, A., Jyotsna, R., Indrani, D. 2009. Effect of hydrocolloids and emulsifiers on the rheological, microstructural and quality characteristics of eggless cake. *Food Hydrocolloids*, vol. 23, no. 3, p. 700-707. http://dx.doi.org/10.1016/j.foodhyd.2008.06.002

Bojňanská, T., Frančáková, H. 2002. The use of spelt wheat (*Triticum spelta* L.) for baking application. *Rostlinná výroba*, vol. 48, no. 4, p. 141-147.

Collar, C., Andreu, P., Martínez, J. C., Armero, E. 1999. Optimization of hydrocolloid addition to improve wheat bread dough functionality: a response surface methodology study. *Food Hydrocolloids*, vol. 13, no. 6. p. 467-475. http://dx.doi.org/10.1016/S0268-005X(99)00030-2

Davari Ketilateh, M., Aziz, M., Fazeli, F. 2013. Effect of hydrocolloids (Tragacanth and Xanthan) on frozen dough characteristics and volumetric (hamburger) bread. *Iranian*

Journal of Nutrition Sciences and Food Technology, vol. 7, no. 5, p. 301-309.

Delcour, J. A., Hoseney, R. C. 2010. *Principles of cereal science and technology*. 3rd ed., American Association of Cereal Chemists : St Paul, MN, USA. p. 1-257. ISBN 978-1-891127-63-2. http://dx.doi.org/10.1094/9781891127632

Dodok, L., Szemes, V. 1998. *Laboratórne kontrolné metódy pre pekársku a cukrársku prax* (Laboratory test methods for bakery and confectionery practice). Gomini : Pezinok, Slovakia, 77 p.

Eduardo, M., Svanberg, U., Ahrné, L. 2014. Effect of Hydrocolloids and Emulsifiers on Baking Quality of Composite Cassava-Maize-Wheat Breads. *International Journal of Food Science*, vol. 2014, p. 9. http://dx.doi.org//10.1155/2014/479630

Escarnot, E., Jacquemin, J. M., Agneessens, R., Paquot, M. 2012. Comparative study of the content and profiles of macronutrients in spelt and wheat, a review. *Biotechnology, Agronomy, Society and Environment*, vol. 16, no. 2, p. 243-256.

Fanta, G. F., Christianson, D. D. 1996. Starch-hydrocolloid composites prepared by steam jet cooking. *Food Hydrocolloids*, vol. 10, no. 2, p. 173-178. http://dx.doi.org/10.1016/S0268-005X(96)80032-4

FC SR Decree (1) *of Ministry of agriculture and rural development of the Slovak Republic and Ministry of health of the Slovak Republic No. 08936/2010-OL of 15 March 2010 that changes the decree of these ministries of 11 February 2008 No. 04650/2008 - OL, which publishes the second part, twelfth chapter of the Food codex of the Slovak Republic governing additives in foods.* Available at: http://www.svssr.sk/dokumenty/legislativa/8936_2010.pdf

FC SR Decree (2) *of Ministry of agriculture and rural development of the Slovak Republic and Ministry of health of the Slovak Republic No. 04650/2008 OL of 11 February which publishes the second part, twelfth chapter of the Food codex of the Slovak Republic governing additives in foods.* Available at: http://www.svssr.sk/dokumenty/legislativa/4650_2008.pdf

Feillet, P. 2000. *Le grain de blé, composition et utilisation*. Paris : Institut National de la Recherche Agronomique. 2000. p. 308.

Filipčev, B., Šimurina, O., Boroža-Solarov, M. 2014. Combined effect of xylanase, ascorbic and citric acid in regulating the quality of bread made from organically grown spelt cultivars. *Journal of Food Quality*, vol. 37, no. 3, p. 185-195.http://dx.doi.org/10.1111/jfq.12081

Garciá-Ochoa, F., Santosa, V. E., Casasb J. A., Gómez, E. 2000. Xanthan gum: production, recovery, and properties. *Biotechnology Advances*, vol. 18, no. 7, p. 549-579. http://dx.doi.org/10.1016/S0734-9750(00)00050-1

Grela, E. R. 1996. Nutrient composition and content of antinutritional factors in spelt (*Triticum spelta* L.) cultivars. *Jounal of the Science of Food and Agriculture*, vol. 71, no. 33, p. 399-404. http://dx.doi.org/10.1002/(SICI)1097-0010(199607)71:3<399::AID-JSFA609>3.0.CO;2-Q

Hojerová, J., Štern, P., Zysmlye, R. 2005. The rheology of food hydrocolloids. *Bulletin of Food Research*, vol. 44, no. 1-2, p. 83-99.

ICC - Standard 114/1, 1992, AACC Method 54-10:1995, Method for using the Brabender Extensograph.

ICC - Standard 126/1, AACC Method 22-10:1995, Method for using the Brabender Amylograph.

ICC - Standrd 115/1, 1992, AACC Method 54-21:1995, Method for using the Brabender Farinograph.

ICC Standard No. 104/1: 1990 Determination of ash in cereals and cereal products.

ICC Standard No. 105/2: 1994 Determination of crude protein.

ICC Standard No. 110/1:1976 Determination of the moisture content of cereals and cereal products (Practical method).

Khan, T., Parh, J. K., Kwon, J. H. 2007. Functional biopolymers produced by biochemical technology considering applications in food engineering. *Korean Journal of Chemical Engineering*, vol. 24, no. 5, p. 816-826.

Kohajdová, Z., Karovičová, J. 2008a. Influence of hydrocolloids on quality of baked goods. *Acta Scientiarum Polonorum Technologia Alimentaria*, vol. 7, no. 2, p. 43-49.

Kohajdová, Z., Karovičová, J., Gajdošová, Ž. 2008b. Význam hydrokoloidov v pekárstve (Importance of hydrocolloids in bakery industry). *Potravinarstvo*, vol. 2, no. 3, p. 9-18. Available at: http://www.potravinarstvo.com/dokumenty/potravinarstvo_no3_2008.pdf

Kohajdová, Z., Karovičová, J., Schmidt, Š. 2009. Significance of emulsifiers and hydrocolloids in bakery industry. *Acta Chimica Slovaca*, vol. 2, no. 1, p. 46-61.

Krkošková, B. 2011. Naše pôvodné zabudnuté obilniny - špalda, pšenica, jačmeň (Our original forgotten cereals - spelt, wheat, barley). *Liečivé rastliny*, vol. 48., no. 3. Available at: http://www.liecive.herba.sk/index.php/archiv/rok-2011/3-2011/nae-povodne-zabudnute-obilniny-spalda-psenica-jacmen

Krkošková, B., Buráková, E., Macová, E. 2005. Charakteristika nutričných a profylaktických zložiek pšenice špaldy (Characteristics of nutrient and prophylactic ingredients of Triticum spelta). *Bulletin potravinárskeho výskumu (Bulletin of Food Research)*, vol. 44, no. 3-4, p. 185-193.

Magala, M., Kohajdová, Z., Karovičová, J. 2011. Staling of bakery products. *Potravinarstvo*, vol. 5, no. 2, p. 49-54. http://dx.doi.org/10.5219/124

Mandala, I. G. 2012. Viscoelastic properties of starch and non-starch thickeners in simple mixtures or model food. In de Vicente, J. *Viscoelasticity – From Theory to Biological Applications*, InTech : Rijeka, Croatia, p. 217-236, ISBN 978-953-51-0841-2. http://dx.doi.org/10.5772/50221

Mandala, I. G., Kapetanakou, A., Kostaropoulos, A. 2008. Physical properties of breads containing hydrocolloids stored at low temperature: II-Effect of freezing. *Food Hydrocolloids*, vol. 22, no. 8, p. 1443-1451. http://dx.doi.org/10.1016/j.foodhyd.2007.09.003

Muchová, Z. 2007. *Technológia spracovania cereálií* (Cereal Technologies). 2nd ed., SPU : Nitra, Slovakia. 192 p. ISBN 978-80-8069-980-2

Příhoda, J., Humpolíková, P., Novotná, D. 2003. *Základy pekárenské technologie* (The bacis of bakery technogy). Pekař a cukrář s.r.o. : Prague, Czech Republic, 363 p. ISBN 80-902922-1-6.

Pruska-Kedzior, A., Kedzior, Z., Klockiewicz-Kaminska, E. 2008. Comparison of viscoelastic properties of gluten from spelt and common wheat. *European Food Research of Technol*ogy, vol. 227, no. 1, p. 199-207.

Qiu, CH., Zhao, M., McClements, D. J. 2015. Improving the stability of wheat protein-stabilized emulsions: Effect of pectin and xanthan gum addition *Food Hydrocolloids*, vol. 43, p. 377-387. http://dx.doi.org/10.1016/j.foodhyd.2014.06.013

Ranhotra, G. S., Gelroth, J. A., Glaser, B. K., Lorenz, K. J. 1995. Baking and nutritional qualities of spelt wheat sample. *LWT - Food Science and Technology,* vol. 28, no. 1, p. 118-122. http://dx.doi.org/10.1016/S0023-6438(95)80022-0

Ribotta, P. D., Pérez, G. T., León, A. E., Añón, M. C. 2004. Effect of emulsifier and guar gum on microstructural, rheological and baking performance of frozen bread dough. *Food Hydrocolloids*, vol. 18, no. 2, p. 305-313. http://dx.doi.org/10.1016/S0268-005X(03)00086-9

Rodge, A. B., Sonkamble, S. M., Salve, R. V., Syed Imran Hashmi. 2012. Effect of hydrocolloid (guar gum) incorporation on the quality characteristics of bread. *Journal of Food Processing & Technology* vol. 3, no. 2, p. 1-7. http://dx.doi.org/10.4172/2157-7110.1000136

Rosell, C. M., Rojas, J. A., Benedito de Barber, C. 2001. Influence of hydrocolloids on dough rheology and bread. *Food Hydrocolloids*, vol. 15, no. 1, p. 75-81. http://dx.doi.org/10.1016/S0268-005X(00)00054-0

Ruibal-Mendieta, N. L., Delacroix, D. L., Mignolet, J. M. P., Marques, C., Rozenberg, R. Petitjean, G., Habib-Jiwan, J. L., Meurens, M., Qeentin-Leclerco, J., Delzenne, N. M., Larondelle, Y. 2005. Spelt (*Triticum aestivum* ssp. *spelta*) as a source of breadmaking flours and bran naturally enriched in oleic acid and minerals but not phytic acid. *Journal of Agricultural and Food Chemistry*, vol. 53, no. 7, p. 2751-2759. http://dx.doi.org/10.1021/jf048506e

Šedivý, P., Dostál, J., Kovaříková, D., Martinek, V. 2013. *Pekařská technologie I. Suroviny* (The bakery technology I. Ingredients). Pekař a cukrář s.r.o. : Prague, Czech Republic, 238 p. ISBN 978-80-903913-7-6

Škara, N., Novotni, D., Čukelj, N., Smerdel, B., Ćurić, D. 2013. Combined effects of inulin, pectin and guar gum on the quality and stability of partially baked frozen bread. *Food Hydrocolloids*, vol. 30, no. 1, p. 428-436. http://dx.doi.org/10.1016/j.foodhyd.2012.06.005

Tao, F., Wang, X., Ma, C., Yang, CH., Tang, H., Gai, Z., Xu, P. 2012. Genome Sequence of Xanthomonas campestris JX, an Industrially Productive Strain for Xanthan Gum *Journal of Bacteriology*, vol. 194, no. 17, p. 4755-4756. http://dx.doi.org/10.1128/JB.00965-12

Velíšek, J. 2002. *Chemie potravin 1* (*Food chemistry I*), 2nd ed., Ossis : Tábor, Czech Republic, 344 p. ISBN 80-86659-00-3

Yaseen, A. A., Shouk, A. E. H. A., Ramadan, M. T. 2010. Corn-Wheat Pan Bread Quality as Affected by Hydrocolloids. *Journal of American Science,* vol. 6, no. 10, p. 684-690.

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EFFECT OF DIFFERENT PHYTOGENIC ADDITIVES ON OXIDATION STABILITY OF CHICKEN MEAT

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ABSTRACT

The aim of the study was to evaluate the oxidative stability (TBARS method) of breast and thigh muscle after application of feed mixtures enriched by phytogenic additives. The experiment started with 150 pieces one-day-old chicks of Cobb 500 hybrid combination. They were divided into one control (C) and two experimental groups (1st EG and 2nd EG). Each group included 50 chicks. In experimental groups, feed additives were applied as followed: 100 mg.kg^{-1} Agolin Poultry (in the 1st EG) and 500 mg.kg^{-1} Agolin Tannin Plus (in the 2nd EG). Experimental broiler chickens were fed during 42 days by *ad libitum*. Chicken meat samples of breast and thigh muscle were analysed in the 1st day, 1st, 2nd, 3rd, 4th, 5th and 6th month of storage in frozen storage at -18 °C. We recorded positive influence on chicken meat oxidative stability in all experimental groups with application of phytogenic feed additives. Obtained results showed that applied phytogenic additives had positive influence on oxidative stability of breast and thigh muscles. At the end of frozen storage (in 6th month), we found higher malondialdehyde (MDA) values and lower oxidative stability (p <0.05) of breast muscle in control group (0.167 mg.kg^{-1}) compared to experimental groups (from 0.150 mg.kg^{-1} in 1. EG to 0.155 mg.kg^{-1} in 2. EG). In the thigh muscle, we found similar tendency of oxidative changes as in the breast muscle. At the end of frozen storage (in the 6th month), MDA average values of thigh muscle were higher (p <0.05) in control group (0.181 mg.kg^{-1}) compared to experimental groups (1. EG 0.164 mg.kg^{-1} and 2. EG 0.169 mg.kg^{-1}). Significant differences (p <0.05) between the control and experimental groups were found from the 5th month of storage in thigh and breast muscle. Obtained results indicate positive influence of phytogenic additives applied in chicken nutrition, namely on stabilization of fatty substance to degradation processes.

Keywords: phytogenic additives; chicken meat; oxidative stability

INTRODUCTION

Phytogenic feed additives (PFA) are commonly defined as plant-derived compounds incorporated into diets to improve the productivity of livestock through amelioration of feed properties, promotion of the animal's production performance, and improving quality of food derived from those animals. Although this definition is driven by purpose of use, other terms are commonly used to classify the vast variety of phytogenic compounds, mainly with respect to origin and processing, such asherbs (flowering, nonwoody, and nonpersistent plants), spices (herbs with intensive smell or taste commonly added to human food), essential oils (volatile lipophilic compounds derived by cold expression or by steam or alcohol distillation), or oleoresins (extract derived by nonaqueous solvents). Within phytogenic feed additives, the content of active substance in products may vary widely, depending on the plant part used (e.g. seeds, leaf, root, or bark), harvesting season, and geographical origin. The technique for processing (e.g. cold expression, steam distillation, extraction with nonaqueous solvents, etc.) modifies the active substances and associated compounds within the final product **(Windisch et al., 2008; Jacela et al., 2010)**. This is class of feed additives is at present used to a great extent as alternatives to the antibiotic growth promoters in poultry and swine nutrition **(Wati et al., 2015)**.

Aromatic plants, also known as herbs and spices, have been used in the Middle East since approximately 5000 BC for their preservative and medical properties, in addition to enhancing the aroma and flavour of foods **(Chang, 2000)**. Their use continues undiminished today and according to the World Health Organization (WHO) nearly 80% of the planet population, especially in developing countries still depends on plant produced medicines for their healthcare **(Grubik-Fakim, 2006)**. Currently, there is an increasing interest in using herbs and spices in animal nutrition, in order to replace the use of antibiotics and ionophore anticoccidials, especially after the ban of antibiotics feed additives within the European Union countries in 2006 and discussions to restrict their use outside Europe **(Greathead, 2003; Windisch et al., 2008; Hashemi and Davoodi, 2010; Yitbarek, 2015)**.

The nutritional properties of poultry meat are highly valued; it is a meat with low fat content and less saturated fatty acid than the most ruminant tissues **(Starčevič et al., 2015)**. At average broilers have from 3.5 to 5.0% of fatty tissuses. Poultry fat contain higher amount of polyunsatured fatty acids than fatty tissues other slaughtered animals. Exactly, polyunsatured fatty acids are the most sensible fractions to oxidation processes. Lipid oxidation oxidation in meat is one of the reasons for quality degradation during storage. This process is associated with the presence of free radicals that lead to

the production of aldehydes responsible for the development on rancid flavours and changes in the colour of meat **(Fasseas et al., 2007)**. The rate of oxidation increases in result of the following: (1) high intake of oxidized lipids and prooxidants; (2) deterioration of sensitive polyunsaturated fatty acids (polyunsatured fatty acids); and (3) low intake of antioxidative nutrients. In muscle foods, oxidative reactions continue postmortem and are a leading cause of quality deterioration during processing and storage. With a relatively high proportion of PUFA, poultry meat is more susceptible to oxidative processes, specifically lipid oxidation, than beef or pork **(Smet et al., 2008)**. Lipid oxidation is a major cause of meat quality deterioration which lowers the functional, sensory and nutritive values of meat and neat products; and therefore, consumer's acceptability **(Bou et al., 2004)**. Oxidative stability of poultry meat is influenced not only by bird genotype but also feeding, rearing practices and the degree of muscle tissue damages during preslaughter, e.g. physical damage, early post-mortem conditions, pH and carcass temperature **(Morissey et al., 1998; Zamora and Hildago, 2001)**. These factors could by manipulated by supplementing the animal diet with phytogenic compounds such as different essential oils and polyphenols to improve animal productivity and the quality of food derived from those animals **(Lee et al., 2003; Jang et al., 2004; Okuda, 2005)**.

Phytogenic feed additives are often applied into the feed mixtures, because they improve the taste and odour of feed and subsequently, body weight gain and feed intake are increased and feed conversion is improved, too **(Angelovičová et al., 2010)**. Phytogenic feed additives enhance productivity through the improvement of digestibility, nutrient absorption and elimination of pathogens residents in the animal gut **(Athanasiadou et al., 2007)**. Digestive stimulation by phytogenic additives is achieved through stimulation of salvia secretion, liver, pancreas and intestine enzymes activities, intestine function and morphohistology and metabolism **(Perič et al., 2010)**. Antioxidant effects of plant extracts may be used to slow or prevent the fat oxidation in food products **(Rababah et al., 2004)**. Application of oils and plant extracts in poultry nutrition is important for health state of animals and animal performance as well as for oxidative stability of produced meat **(Frankič et al., 2009)**. Antioxidant activity of plants and their extracts is directly correlated with phenols content **(Chrpová et al., 2010)**. Several studies about phytogenic additives in poultry nutrition were published, mainly about application of aromatic herbs like a cloves **(Isabel and Santos, 2009)**, a rosemary **(Šperňáková et al., 2007)**, a cinnamon **(Ciftci et al., 2010)**, an anise **(Al-Kassie, 2008)**, an oregano **(Fiková et al., 2009)** and a salvia **(Hernandez et al., 2004)**.

The aim of the experiment was to determine the oxidative stability in the most valuable parts of chicken carcasses (Cobb 500 hybrid combination) during the frozen storage (6 months) after application of phytogenic feed additives Agolin Poultry, Agolin Tannin Plus, in their diet.

MATERIAL AND METHODOLOGY

Animals and diets

The experiment was undertaken in poultry test station Zamostie Company. The experiment started with 150 pieces of one-day-old hybrid chicks Cobb 500, which were divided into 3 groups (n = 50): control (C) and 2 experimental groups (1^{st} EG and 2^{nd} EG).

Experimental broiler chickens were fed during 42 days by *ad libitum* system with feed mixtures: BR1 starter feed mixture (until the 10^{th} day of age), BR2 growth feed mixture (from 11^{th} to 20^{th} day of age), BR3 growth feed mixture (from 21^{st} to 35^{th} day of age) and BR4 final feed mixture (from 36^{th} to 42^{nd} day of age). Feed mixtures were produced with coccidiostats in powder form.

Nutritional value (Table 1) of feed mixture was the same in each group during the whole experiment. However, the diet of broiler chickens in experimental groups were supplemented by feed additives on base of acids and plant essential oils: Agolin Poultry at a dose of 100 $mg.kg^{-1}$ (1^{st} EG); Agolin Tannin Plus at a dose of 500 $mg.kg^{-1}$ (2^{nd} EG).

Sample analysis

At the end of feeding (day 42^{th}) from each group were selected 10 pieces of chicken for slaughter analysis. Slaughtering and cutting of chickens were undertaken in the Department of animal products evaluation and processing. To determine changes in lipid degradation (determination of thiobarbiturates numbers, TBA) the samples of chickens were boned and thigh and breast muscle packed into polyethylene bags and stored for 6 months at -18 °C.

TBARS analysis

TBA value expressed in number of malondialdehyde (MDA) was measured in the process of first storage day of 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} months. TBA number was determined according to **Marcinčák et al., (2006)**. Absorbance of samples was measured at a wavelength of 532 nm on UV-VIS spectrophotometer T80 (PG Limeted Instruments, UK). Results were calculated as the amount of MDA in 1 kg of sample.

RESULTS AND DISCUSSION

The lipids in poultry exhibit a higher degree of unsaturation compared with red meat, because of a relatively high content of phospholipids. The degree of unsaturation of phospholipids in subcellular membranes is an important factor in the determination of oxidative stability of meats. The oxidative potential increases as the degree of unsaturation of lipids in meat increases **(Coetzee and Hoffman, 2001)**. The oxidation of lipids is influenced by the addition of antioxidant substances. The practical application of antioxidants can be difficult from the point of view of hygiene and technology. It is much better when natural antioxidants are incorporated in feed mixes **(Kušev et al., 1996)**.

Table 1 Composition of the diets.

Ingredients (%)	Starter (1st to 10th day of age)	Grower I (11th to 20th day of age)	Grower II (21st to 35th day of age)	Finisher (36th to 42nd day of age)
Maize	46.33	48.50	50.05	50.91
Wheat	14.00	15.00	15.00	15.00
Soybean meal (45% CP[1])	30.00	26.60	28.00	26.70
Fish meal (72% CP[1])	2.50	2.00	-	-
Dried blood	2.00	2.00	-	-
Soybean oil	1.00	1.80	2.80	3.00
Monocalcium phosphate	1.60	1.25	1.30	1.48
Calcium carbonate	1.37	1.55	1.50	1.56
Fodder salt	0.20	0.30	0.35	0.35
Lysine	0.27	0.15	0.15	0.16
Methionine	0.27	0.18	0.17	0.20
Threonine	0.09	0.10	0.08	0.07
Vitamin premix	0.05	0.04	0.04	0.03
Micromineral premix	0.04	0.04	0.04	0.04
Enzyme phytase	0.015	0.015	0.015	0.015
Wheat meal	0.215	0.12	0.10	0.135
Maxiban (Narasin+Nicarbasin)	0.05	-	-	-
Sacox (salinomycin sodium)	-	0.055	0.055	-
Analysed composition (g.kg^{-1})				
Crude protein	220.00	207.00	197.00	188.00
Fibre	20.00	24.00	28.00	29.00
Lysine	14.00	12.50	12.50	11.50
Methionine	6.00	5.20	5.20	5.00
Ca	9.00	8.50	8.50	8.50
P (non-phytate)	4.20	4.00	4.00	4.00
Na	1.60	1.60	1.60	1.60
$^{2}ME_N$ (MJ kg^{-1})	12.30	12.75	13.15	13.15

Legend: ^{1}CP – Crude protein, $^{2}ME_N$ – Metabolizable energy.

Table 2 Effect of frozen storage (-18 ºC) on the concentration of MDA (mg.kg^{-1}) in breast muscle (mean ±*SD*).

Time of storage	Group		
	Control	1. EG	2. EG
Day – 1	0.108 ±0.009^{a}	0.101 ±0.010^{a}	0.098 ±0.008^{a}
Month – 1	0.119 ±0.009^{a}	0.117 ±0.0009^{a}	0.117 ±0.009^{a}
Month – 2	0.127 ±0.009^{a}	0.124 ±0.010^{a}	0.126 ±0.009^{a}
Month – 3	0.137 ±0.015^{a}	0.131 ±0.006^{a}	0.131 ±0.008^{a}
Month – 4	0.143 ±0.006^{a}	0.139 ±0.012^{ab}	0.137 ±0.010^{b}
Month – 5	0.155 ±0.006^{a}	0.144 ±0.006^{ab}	0.147 ±0.013^{b}
Month – 6	0.167 ±0.010^{a}	0.150 ±0.018^{b}	0.155 ±0.011^{b}

The results of the oxidation stability determined in breast muscle of chickens COBB 500 during 6 months storage at -18 °C are shown in Table 2. Immediately after slaughtering and processing of poultry samples we recorded low values of MDA. Obtained results indicate that addition of antioxidants had effect on reducing of oxidation processes in meat. Process of production of meat products (cutting, grinding, and mixing) causes degradation of muscle membrane system and has a strong influence on oxidation of intracellular fat, primarly phospolipids **(Bystrický and Dičáková, 1998)**. During freeze storage of the breast muscles (6 months) were detected increased content of MDA in comparison to the first day of storage. During whole period of freeze storage were higher values of MDA determined in control group compare to experimental groups. The higher average MDA value determined in breast muscles of broiler chicken hybrid combination COBB 500 was in samples of control group (0.167 mg.kg^{-1}) compared to experimental groups E2 (0.155 mg.kg^{-1}) and E1 (0.150 mg.kg^{-1}) after 6-month of freezing storage. Significantly higher values of MDA were determined in control group compare to experimental group from fifth month to the end of storage. Reached results oxidation stability breast muscle during freeze storage are in accordance with **Ahadi et al., (2010)**; **Marcinčák et al., (2010)**.

Table 3 Effect of frozen storage (-18 ºC) on the concentration of MDA (mg.kg^{-1}) in thigh muscle (mean ±*SD*).

Time of storage	Group		
	Control	1.EG	2.EG
Day – 1	0.129 ±0.013^{a}	0.125 ±0.011^{a}	0.120 ±0.008^{a}
Month – 1	0.132 ±0.009^{a}	0.129 ±0.005^{a}	0.128 ±0.009^{a}
Month – 2	0.139 ±0.004^{a}	0.135 ±0.005^{a}	0.136 ±0.010^{a}
Month – 3	0.148 ±0.011^{a}	0.143 ±0.011^{a}	0.146 ±0.015^{a}
Month – 4	0.160 ±0.012^{a}	0.151 ±0.012^{ab}	0.156 ±0.015^{b}
Month – 5	0.171 ±0.011^{a}	0.159 ±0.014^{ab}	0.163 ±0.008^{b}
Month – 6	0.181 ±0.021^{a}	0.164 ±0.013^{b}	0.169 ±0.009^{b}

Trend of thigh muscle oxidation stability of chicken hybrid combination COBB 500 was during 6 months of freeze storage similar than in breast muscle. The results of the oxidation stability determined in thigh muscle of chickens COBB 500 during 6 months storage at -18 °C are shown in Table 3. The higher average MDA value determined in thigh muscles was in samples of control group (0.181 mg.kg^{-1}) compared to experimental groups E1 (0.164 mg.kg^{-1}) and E2 (0.169 mg.kg^{-1}) after 6-month of frozen storage. Significantly higher values of MDA were determined in control group compare to experimental groups from fifth month to the end of storage. Higher amount of MDA in thigh muscle compare to breast muscle is due to by higher amount of fat occurred in thigh muscle **Botsoglou et al., (2002)**.

Reached results of oxidation stability determined in chicken meat of hybrid combination COBB 500 after phytogenic additives addition in their diet are in accordance with **Imik et al., (2010)** and **Rahimi et al., (2011)**. The possibilities of using alternative feed supplements containing various antioxidant active substances for poultry which increase the oxidation stability of the meat during its period of freeze storage are showen in works of **Skřivan et al., (2010)**; **Karaalp and Genc (2013)**.

Botsoglou et al., (2007) reported that a higher concentration of antioxidants in poultry meat has the effect of reducing lipid oxidation, i.e. there is a reduction in MDA values during chilling and refrigeration storage, which was confirmed by our findings. Also **Samouru et al., (2007)** and **Ramos Avila et al., (2013)** state that the degradation pathways of fatty substances play one of the main causes of foods deterioration and unpleasant odours. This factor is also responsible for the loss of flavour, texture, appearance, nutritional value of food, increases the drop losses, pigment, polyunsaturated fatty acids, fat-soluble vitamins, reduces the quality of meat intended for human consumption and ultimately reduces its stability, shelf life and safety.

CONCLUSION

Results achieved in the experiment show that the addition of different phytogenic feed additives (Agolin Poultry and Agolin Tannin Plus) in feed mixture for broiler chickens had a significantly ($p \leq 0.05$) positive impact on the reduction of oxidative processes in the breast and thigh muscles during 6 months freeze storage at -18°C.

REFERENCES

Ahadi, F., Chekani-Azar, S., Shahryar, H. A., Lotfi, A., Mansoub, N. H., Bahrami, Y. 2010. Effect of Dietary Supplementation with Fish Oil with Selenium or Vitamin E on Oxidative Stability and Consumer Acceptability of Broilers Meat. *Global Veterinaria*, vol. 4, p. 216-221.

Al-Kassie, G. A. M. 2008. The Effect of Anise and Rosemary on Broiler Performance. *International Journal of Poultry Science*, vol. 7, no. 3, p. 243-245. http://dx.doi.org/10.3923/ijps.2008.243.245

Angelovičová, M., Kačaniová, M., Angelovič, M., Lopašovský, Ľ. 2010. Použitie tymianovej silice per os na produkciu výkrmových kurčiat. *Potravinarstvo*, vol. 4, special no., p. 127-132. Available at: http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/2/Angelovicova.pdf

Athanasiadou, S., Githiori, J., Kyriazakis, I. 2007. Medicinal plants for helminth parasite control: facts and fiction. *Animal*, vol. 1, no. 9, p. 1392-1400. http://dx.doi.org/10.1017/s1751731107000730

Avila-Ramos, F., Pro-Martínez, A., Sosa-Montes. E., Cuca-García,. J. M., Becerril-Pérez, C., Figueroa-Velasco, J. L., Ruiz-Feria, C. A., Hernández-Cázares, A. S., Narciso-Gaytán, C. 2013. Dietary supplemented and meat-added antioxidants effect on the lipid oxidative stability of refrigerated and frozen cooked chicken meat. *Poultry Science*, vol. 92, no. 1, p. 243-249. http://dx.doi.org/10.3382/ps.2012-02409

Botsoglou, N. A., Christaki, E., Fletouris, D. J., Florou-Paneri, P., Spais, A. B. 2002. The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. *Meat Science*, vol. 62, no. 2, p. 259-265. http://dx.doi.org/10.1016/s0309-1740(01)00256-x

Botsoglou, N. A., Govaris, A. Giannenas, I. Botsoglou, E., Papapageorgiou, G. 2007. The incorporation of dehydrated rosemary leave in therations of turkeys and their impact on the oxidative stability of the produced raw and cooked meat. *International Journal of Food Science and Technology*, vol. 58, no. 4, p. 312-320. http://dx.doi.org/10.1080/09637480701228583

Bou, R., Guardiola, F., Tres, A., Barroeta, A. C., Codony, R. 2004. Effect of dietary fish oil, -tocopheryl acetate, and zinc supplementation on the composition and consumer acceptability of chicken meat. *Poultry Science*, vol. 83, no. 2, p. 282-292. http://dx.doi.org/10.1093/ps/83.2.282

Bystrický P., Dičáková Z. 1998. Animal fats in foods. *Slovak Veterinary Journal*, vol. 23, no. 1, p. 1-46.

Ciftici, M., Simsek, U. G., Yuce, A., Yilmaz, O., Dalkilic, B. 2010. Effects of Dietary Antibiotic and Cinnamon Oil Supplementation on Antioxidant Enzyme Activities, Cholesterol Levels and Fatty Acid Compositions of Serum

and Meat in Broiler Chickens. *Acta Veterinaria Brno*, vol. 79, no. 1, p. 33-40. http://dx.doi.org/10.2754/avb201079010033

Coetzee G. J. M., Hoffman L. C. 2001. Effect of dietary vitamin E on the performance of broilers and quality of broiler meat during refigerated and frozen storage. *South African Journal of Animal Science*, vol. 31, no. 3, p. 161-175. http://dx.doi.org/10.4314/sajas.v31i3.3799

Fasseas, M. K., Mountzourism, K. C., Tarantilis, P. A., Polissiou, M., Zervas, G. 2007. Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chemistry*, vol. 106, no. 3, p. 1188-1194. http://dx.doi.org/10.1016/j.foodchem.2007.07.060

Fikselová, M., Židek, R., Bobková, A., Angelovičová, M., Golian, J., Bobko, M., Lopašovský, Ľ., Zeleňáková, L. 2009. Detection of materials with antioxidant activity by modern analytic system. *Acta Fytotechnica et Zootechnica*, vol. 12, p. 157-162.

Frankič, R., Voljč, M., Salobir, J., Rezar, V. 2009. Use of herbs and spices and their extracts in animal nutrition. *Acta agriculturae Slovenica*, vol. 94, no. 2, p. 95-102.

Greathead, H. 2003. Plants and plant extracts for improving animal productivity. *Proceedings of the Nutrition Society*, vol. 62. no. 2, p. 279-290. http://dx.doi.org/10.1079/pns2002197

Gurib-Fakim, A. 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, vol. 27, no. 1, p. 1-93. http://dx.doi.org/10.1016/j.mam.2005.07.008

Hashemi, S. R., Davoodi, H. 2010. Phytogenics as New Class of Feed Additive in Poultry Industry. *Journal of Animal and Veterinary Advances*, vol. 9, no. 17, p. 2295-2304. http://dx.doi.org/10.3923/javaa.2010.2295.2304

Hernandez, F., Madrid, J., Garcia, V., Orengo, J., Megias, M. D. 2004. Influence of two plant extracts on broiler performance, digestability and digestive organ size. *Poultry Science*, vol. 83, no. 2, p. 169-174. http://dx.doi.org/10.1093/ps/83.2.169

Chang, J. 2000. Medicinal herbs: Drugs or dietary supplements? *Biochemical Pharmacology*, vol. 59, no. 3, p. 211-219. http://dx.doi.org/10.1016/s0006-2952(99)00243-9

Chrpová, D., Kourimská, L., Gordon, M. H., Heřmanová, V., Roubičková, I., Panek, J. 2010. Antioxidant activity of selected phenols and herbs used in diets for medical conditions. *Czech Journal of Food Science*, vol. 28, no. 4, p. 317-325.

Imik, H., Atasever, M. A., Koc, M., Atasever, M. A., Ozturan, K. 2010. Effect of dietary supplementation of some antioxidants on growth performance, carcass composition and breast meat characteristics in quails reared under heat stress. *Czech Journal Animal Science*, vol. 55, no. 5, p. 209-220.

Isabel, B., Santos, Y. 2009. Effects of dietary organic acids and essential oils on growth performance and carcass characteristics of broiler chickens. *Journal of Applied Poultry resersch*, vol. 18, no. 3, p. 472-476. http://dx.doi.org/10.3382/japr.2008-00096

Jacela, J. Y., DeRouchey, J. M., Tokach, M. D., Goodband, R. D., Robert, D., Nelssen, J. L., Renter, D. G., Dritz, S. S. 2010. Feed additives for swine: Fact sheets – flavors and mold inhibitors, mycotoxin binders, and antioxidants. *Journal of Swine Health and Production*, vol. 18, no. 1, p. 27-32.

Jang, I. S., Ko, Y. H., Yang, H. Y., Ha, J. S., Kim, J. Y., Kang, J. Y., Yoo, D. H., Nam, D. S., Kim, D. H., Lee, C. Y. 2004. Influence of Essential Oil Components on Growth Performance and the Functional Activity of the Pancreas and Small Intestine in Broiler Chickens. *Asian-Australasian Journal of Animal Sciences*, vol. 17, no. 3, p. 394-400. http://dx.doi.org/10.5713/ajas.2004.394

Karaalp, M., Genc, N. 2013. Bay laurel (*Laurus nobilis* L.) in Japanese quails feeding. 2. Fatty acid content and oxidative stability of breast meat. *Bulgarian Journal of Agricultural Science*, vol. 19, p. 606-610.

Kušev J., Jantošovič J., Šály, J., Kozák M. 1996. The effect of vitamin E on the quality of fat component of broiler chicken meat. Veterinarni Medicína. – Czech, vol. 41, no. 5, p. 139-142.

Marcinčák, S., Popelka, P., Bystrický, P., Hussein, K., Hudecová, K. 2005. Oxidative stability of meat and meat products after feeding of broiler chickens with additional amounts of vitamine E and rosemary. *Meso*, vol. 7, no. 1, p. 34-39.

Marcinčák, S., Popelka, P., Šimková, J., Marcinčáková, D., Martonová, M. 2010.Oxidative stability of chilled chicken meat after feeding of selected plants. *Potravinarstvo*, vol. 4, no. 3, p. 46-49. http://dx.doi.org/10.5219/38

Lee, K.-W., Everts, H., Kappert, H. J., Frehner, M., Losa, R., Beynen, A. C. 2003. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. *British Poultry Science*, vol. 44, no. 3, p. 450-457. http://dx.doi.org/10.1080/0007166031000085508

Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. 1998. Lipid stability in meat and meat products. *Meat Science*, vol. 49, supplement 1, p. S73-S86. http://dx.doi.org/10.1016/s0309-1740(98)90039-0

Okuda, T. 2005. Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochemistry*, vol. 66, no. 17, p. 2012-2031. http://dx.doi.org/10.1016/j.phytochem.2005.04.023

Perić, L., Milošević, N., Žikić, D., Bjedov, S., Cvetković, D., Markov, S., Monhl, M., Steiner, T. 2010. Effects of probiotic and phytogenic products on performance, gut morphology and cecal microflora of broiler chickens. *Archiv Tierzucht*, vol. 53, no. 3, p. 350-359.

Rababah, T. M., Hettiarachchy, N. S., Horax, R. 2004. Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, 60 ginger, rosemary, gotukola, and ginkgo extracts, vitamin E, and tertbutylhydroquinone. *Journal of Agricultural and Food Chemistry*, vol. 52, no. 16, p. 5183-5186. http://dx.doi.org/10.1021/jf049645z

Rahimi, S., Karmad Azad, S., Karimi Torshizi, M. A. 2011. Omega-3 Enrichment of Broiler Meat by Using Two Oil Seeds. *Journal of Agricultural Science and Technology*, vol. 13, no. 3, p. 353-365.

Samouris, G. I., Bampidis, V. A., Sossidou, E., Zantopoulos, N. 2007. Lipid oxidation of raw and cooked turkey breast meat during refrigerated storage. *Archiv für Geflügelkunde*, vol. 71, no. 1, p. 41-44.

Skřivan, M., Dlouhá, G., Englmaierová, M., Červinková, K. 2010. Effects of different levels of dietary supplemental caprylic acid and vitamin E on performance, breast muscle vitamin E and A, and oxidative stability in broilers. *Czech Journal of Animal Science*, vol. 55, no. 4, p. 167-173.

Smet, K., Raes, K., Huyghebaert, G., Haak, L., Arnouts, S., De Smet, S. 2008. Lipid and protein oxidation of broiler meat as influenced by dietary natural antioxidant supplementation. *Poultry Science*, vol. 87, no. 8, p.1682-1688. http://dx.doi.org/10.3382/ps.2007-00384

Šperňáková, D., Máté, D., Rózańska, H., Kováč, G. 2007. Effects of dietary use of rosemary powder and a-tocopherol on performance of chicken, inhibition of lipid oxidation

during storage at chilling conditions and increasing of meat quality. *Bulletin of the Veterinary Institute in Pulawy*, vol. 51, p. 585-589.

Starčević, K., Krstulović, L., Brozić, D., Maurić, M., Stojević, Z., Mikulec, Ž.,Bajić, M., Mašek, T. 2014. Production performance, meat composition and oxidative susceptibility in broiler chicken fed with different phenolic compounds. *Journal of the Science of Food and Agriculture*, vol. 95, no. 6, p. 1172-1178. http://dx.doi.org/10.1002/jsfa.6805

Wati, T., Ghosh, T. K., Syed, B., Haldar, S. 2015. Comparative efficacy of a phytogenic feed additive and an antibiotic growth promoter on production performance, caecal microbial population and humoral immune response of broiler chickens inoculated with enteric pathogens. *Animal Nutrition*, vol. 1, no. 3, p. 213-219. http://dx.doi.org/10.1016/j.aninu.2015.08.003

Windisch, W., Schedle, K., Plitzner, C, Kroismayr, A. 2008. Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science*, vol. 86, no. 14, 140-148. http://dx.doi.org/10.2527/jas.2007-0459

Yitbarek, M. S. 2015. Phytogenics As Feed Additives In Poultry Production: A Review. *International Journal of Extensive Research*, vol.3, p. 49-60.

Zamora, R., Hidalgo, F. J. 2001. Inhibition of Proteolysis in Oxidized Lipid-Damaged Proteins. *Journal of Agricultural and Food Chemistry*, vol. 49, no. 12, p. 6006-6011. http://dx.doi.org/10.1021/jf0102719

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IDENTIFICATION OF DIFFERENCES IN CHEMICAL COMPOSITION AMONG WHOLE STICK AND SLICED NITRAN SALAMIS TROUGH PRINCIPAL COMPONENT ANALYSIS

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ABSTRACT

The subject of this work was to examine differences in chemical composition of sliced and whole stick Nitran salamis, purchased from various manufacturers. Nitran salamis are traditional dry fermented meat products of Slovak origin. Taking into account variations in raw materials, production process and potential adulteration, differences in chemical composition within one brand of salami from different manufacturers might be expected. Ten salamis were determined for basic chemical composition attributes and Principal Component Analysis was applied on data matrix to identify anomalous ones. It has been shown that six attributes, namely: protein without collagen of total protein, total protein, total meat, total fat, collagen of total protein and NaCl, were the most important for salamis as first two Principal Components together explained 70.16% of variance among them. Nitran D was found to be the most anomalous salami, as had the lowest value of protein without collagen of total protein (14.14% ±0.26%), total protein (17.42% ±0.44%), total meat (120.29% ±0.98%) and the highest one of total fat (50.85% ±0.95%), collagen of total protein (18.83% ±0.50%) and NaCl (9.55% ±1.93%), when compared to its whole stick variant Nitran C and other samples. In addition to collagen of total protein content, Nitran D together with Nitran A, F and H did not satisfied the legislatively determined criterion, which is ≤16%. This suggested that extra connective tissues were added to intermediate products, which resulted in high variability and inferior quality of final products. It is a common practice in the meat industry to increase the protein content or water binding properties of meat products.

Keywords: PCA; Nitran salami; quality; protein; collagen

INTRODUCTION

Salamis are dry fermented meat products that are popular across most of European countries **(Fabbri and Cevoli, 2015)**. Such countries or their geographic regions produce characteristic salamis trough traditional manufacturing processes. In brief, meat (pork and beef) and fat are minced and mixed with salt, curing agents (nitrate and nitrite), spices, herbs, sugar, starter cultures and other additives such as non-meat proteins **(Fongaro et al., 2015; Cevoli et al., 2014)**. The mixture is stuffed into natural or artificial casing and then subjected to fermentation and drying (ripening) stage **(Fongaro et al., 2015)**. During these phases, physical, chemical and microbiological transformations take place in salami **(Jerković et al., 2010; Martín-Sanchéz et al., 2011)**, gradually giving a product with characteristic colour, flavour, taste and texture **(Papavergou et al., 2012)**. The degree of changes and the final quality of salami depend on product formulation, the variations in raw meat used, the starter culture and processing conditions **(Marino et al., 2015; Van Schalkwyk et al., 2011, Zajác et al., 2015)**.

After ripening, when the desired characteristics are reached, the product can leave the ripening room and is ready to be placed in market **(Fongaro et al., 2015)**. However, the physical and biochemical activities inside the salamis are not stopped at this phase and proceed at a rate depending on several factors, mainly temperature. In particular, further water lost can be avoided by using of modified packaging atmosphere **(Tabanelli et al., 2013)**. Taking into account variations in raw materials, production process and potential adulteration, differences in chemical composition within one brand of salami from different manufacturers might be expected.

The subject of study was to identify the differences in chemical composition of traditional Slovak Nitran salamis in relation to the manufacturer and variant (i.e. either whole sticks or slices packaged in modified atmosphere) using Principal Component Analysis (PCA).

The PCA is multivariate statistical method used for the identification of the most important directions of variability in a multivariate data matrix and presenting the results graphically. This technique has already been used by **Bianchi et al., (2007)** who discriminated between the two kinds of Italian salamis according to profile of volatile compounds. **Herranz et al., (2008)** applied the PCA on the fatty acid profile in order to separate Milano-type salamis into different groups. **Van Schalkwyk et al., (2011)** performed PCA on chemical composition of salamis from game meat, in order to examine differences and consumer acceptability. **Corral et al., (2013)** used this technique to examine the relationship among reduction of salt content and textural parameters, chemical composition and physical properties of Italian salamis.

MATERIAL AND METHODOLOGY

Samples

Ten Nitran salamis from five different manufacturers were purchased from local supermarkets in Nitra, Slovakia. From each manufacturer, Nitran salami was purchased as whole stick and slices packaged in modified atmosphere. Salamis were labelled and assigned by codes according to variant (W = whole stick, S = slices) and manufacturer (number 1 – 5) (Table 1).

Table 1 Codes for salamis.

Salamilabel	Variant	Manufacturer
Nitran A	W	1
Nitran B	S	1
Nitran C	W	2
Nitran D	S	2
Nitran E	W	3
Nitran F	S	3
Nitran G	W	4
Nitran H	S	4
Nitran I	W	5
Nitran J	S	5

Chemical analysis

Analysis of chemical composition was accomplished at Department of Food Hygiene and Safety, SUA, Nitra, Slovakia. Determined attributes were as follows: the content of water (W), ash (A) and NaCl according to ISO 1442:1997, ISO 937:1998 and ISO 1841-1:1996, respectively; content total fat (TF) by acid hydrolysis and ether extraction according to AOAC 991.36; content of total protein (TP) by Kjeldahl method according to AOAC 2011.11 (content of nitrogen multiplied by factor 6.25) and content of hydroxyproline (H) according to ISO 3496:1994. The content of collagen (C) was calculated by multiplying of H with factor 8. The TP was used to calculate the protein without collagen as percentage of total protein (P-CTP), the collagen as percentage of total protein (CTP). Apparent total meat content (TM) was calculated according to **McLean (1999)**. Each determination was performed in triplicate and results represent mean values with standard deviations (SDs).

Statistical analysis

The means and SDs of numeric data were computed using Microsoft Office Excel 2010. The PCA analysis was then performed on mean values of numeric data for 9 attributes (without H) using the TANAGRA 1.4.50 software. In order to enchance the interpretation of principal components (PCs), both the CTR coefficients (contributions of points to dimensions) and the correlation coefficients among attributes were calculated within the PCA.

RESULTS AND DISCUSSION

Table 2 summarises the means and SDs of the measurements.

The correlation coefficients among attributes are shown in Table 3. There existed several strong correlations among some attributes. Besides the positive and moderate correlation with P-CTP, TM and A, TP correlated with 3 attributes (NaCl, CTP and TF) negatively and 2 attributes (C and W) slightly. The weak correlation among TP-related attributes and W was expected, as salamis are basically dried and should have low content of water after drying and ripening stage **(Corral et al., (2013)**.

Table 2 Means and standard deviations of measurements for salamis attributes.

Sample	NaCl (%)	A (%)	W (%)	TF (%)	H (%)	C (%)	TP (%)	P-CTP (%)	CTP (%)	TM (%)
Nitran A	3.99 ±1.19	5.32 ±0.04	35.06 ±0.55	36.62 ±1.33	0.52 ±0.08	4.16 ±0.97	23.19 ±0.18	19.03 ±0.21	17.94 ±0.44	148.12 ±2.49
Nitran B	3.44 ±0.75	4.39 ±0.18	31.33 ±0.91	43.12 ±1.13	0.37 ±0.05	2.96 ±0.17	20.84 ±0.10	17.88 ±0.97	14.20 ±0.97	142.31 ±1.78
Nitran C	1.19 ±0.58	5.02 ±0.27	31.82 ±1.07	39.29 ±0.82	0.31 ±0.02	2.48 ±0.22	23.10 ±0.58	20.62 ±0.58	10.74 ±0.82	149.05 ±1.85
Nitran D	9.55 ±1.93	4.68 ±0.05	28.09 ±0.51	50.85 ±0.95	0.41 ±0.12	3.28 ±0.41	17.42 ±0.44	14.14 ±0.26	18.83 ±0.50	120.29 ±0.98
Nitran E	3.30 ±1.33	4.88 ±0.17	34.87 ±0.16	38.19 ±0.42	0.44 ±0.10	3.52 ±0.24	24.94 ±0.15	21.40 ±0.74	14.13 ±1.21	159.51 ±1.59
Nitran F	3.61 ±1.80	4.40 ±0.11	35.44 ±1.27	36.46 ±0.74	0.48 ±0.06	3.84 ±0.45	22.12 ±0.62	18.28 ±0.23	17.36 ±1.32	141.13 ±0.71
Nitran G	1.25 ±0.36	4.89 ±0.20	33.82 ±0.46	39.52 ±0.60	0.41 ±0.22	3.28 ±0.22	21.75 ±0.34	18.47 ±0.34	15.08 ±0.71	131.26 ±0.57
Nitran H	3.85 ±1.17	4.70 ±0.30	22.80 ±1.50	49.79 ±0.31	0.51 ±0.03	4.08 ±0.70	24.62 ±0.83	20.54 ±0.63	16.57 ±0.94	162.01 ±1.14
Nitran I	3.65 ±1.05	4.58 ±0.09	33.37 ±0.46	39.44 ±1.04	0.38 ±0.02	3.04 ±0.56	20.81 ±0.70	17.77 ±0.50	14.61 ±1.24	133.00 ±1.82
Nitran J	4.42 ±0.86	5.45 ±0.13	23.29 ±0.38	47.95 ±0.42	0.44 ±0.05	3.48 ±0.28	22.25 ±0.51	18.77 ±0.22	15.64 ±0.35	144.50 ±0.66

Table 3 Correlation coefficients among attributes of chemical composition of Nitran salamis.

	P-CTP	TP	NaCl	TM	CTP	TF	C	W	A
P-CTP	1.00								
TP	0.97	1.00							
NaCl	-0.75	-0.65	1.00						
TM	0.90	0.93	-0.46	1.00					
CTP	-0.57	-0.35	0.71	-0.31	1.00				
TF	-0.39	-0.35	0.62	-0.14	0.31	1.00			
C	0.12	0.36	0.22	0.34	0.75	0.04	1.00		
W	0.07	0.04	-0.35	-0.14	-0.14	-0.91	-0.11	1.00	
A	0.29	0.32	-0.11	0.23	-0.05	0.02	0.16	-0.22	1.00

Table 4 Results from the PCA analysis for the first five PCs.

Principal component	Eigen value	Proportion of variance explained (%)	Cumulative variance explained (%)
1	4.02	44.91	44.91
2	2.27	25.25	70.16
3	1.55	17.24	87.40
4	0.84	9.36	96.76
5	0.24	2.75	99.51

The results of the PCA analysis are presented in Table 4. Four PCs were extracted that accounted for 96.76% of the total variation. The first 3 of these PCs explain together 87.40% of total variation. In other words, these PCs are the most important, because 87.40% of total variance for Nitran salamis, in the 9 considered attributes, can be condensed into three new attributes (PCs). The eigen value of PC correspond with its importance.

For example, when **Bianchi et al., (2007)** performed the PCA on the class of aldehydes, the first two PCs accounting for the 68.00% of the variance, allowed to group the salamis according to their kind. **Herranz et al., (2008)** analysed nutritional indices in Milano salamis using 4 attributes and found that first two PCs for salamis explained 76.50% of the total variation. **Van Schalkwyk et al., (2011)** found the first two PCs analysing variables of sensory, microbiological, textural and physicochemical, from matured game salamis explained 86.74% of the total variability of those measurements. In Italian salamis, **Corral et al., (2013)** reported that 57.87% of total variation is explained by the first two PCs with measurements using number of parameters including fat, protein and water content.

Table 5 shows that all attributes of salamis had similar proportion (correlation value) in the 1st PC except for C, W and A. After P-CTP, the most important attributes for the 1st PC were TP, TM, TF, CTP and NaCl. So, the 1st PC is mainly defined by these attributes, while the 2nd one is mainly described by C, TF, TM and W. The 3rd PC the best describes differences in TF, CTP, W and C among the samples. The 4th PC is predominantly defined by A, as that had little importance in the previous PCs. Ultimately, the last 5th PC explains the smallest proportional variance among attributes.

Figure 1, Figure 2 and Figure 3 display the correlation scatterplot of attributes on first four PCs. The attributes are interpreted according to the correlations among each other (Table 3) and each PC (Table 5). Thus, attributes close to each other are positively correlated, attributes separated 180° are negatively correlated, whereas if they are separated by 90° they are independent.

The Figure 1 displays that P-CTP is the most positively correlated with TP and TM. On the other hand, this attribute group is negatively correlated with TF, CTP and NaCl, which are, by contrary, positively correlated to each other. The 2nd PC is the best characterized by C and W because they are placed farthest from its origin. The 3rd PC shows that TF is in the highest negative correlation with C (Figure 2). The Figure 3 indicates the independence of A from other attributes.

The most valuable asset of the CTR coefficients (Table 6) to the PCA consists in their utility, when finding the samples that contributed to the particular PC markedly is

Table 5 Correlation coefficients in the eigen vectors (loadings) for the five first PCs, with percent and total percent contributions to explained variance.

Attribute	PC 1		PC 2		PC 3		PC 4		PC 5	
	ρ	% (tot.%)	ρ	% (tot.%)	ρ	% (tot.%)	ρ	% (tot.%)	ρ	% (tot.%)
P-CTP	0.96	93 (93)	0.22	5 (98)	0.07	1 (99)	-0.07	1 (99)	0.04	0 (99)
TP	0.91	83 (83)	0.38	15 (98)	-0.09	1 (99)	-0.08	1 (100)	0.01	0 (100)
NaCl	-0.86	74 (74)	0.30	9 (84)	-0.08	1 (84)	-0.01	0 (84)	0.39	16 (100)
TM	0.79	63 (63)	0.50	26 (89)	0.02	0 (89)	-0.24	6 (95)	0.19	4 (99)
CTP	-0.64	41 (41)	0.46	22 (63)	-0.60	37 (99)	0.01	0 (99)	-0.07	1 (100)
TF	-0.58	34 (34)	0.56	32 (66)	0.54	30 (96)	-0.15	3 (99)	-0.02	0 (99)
C	0.01	0 (0)	0.71	52 (52)	-0.68	46 (98)	-0.05	0 (98)	-0.12	2 (100)
W	0.28	8 (8)	-0.70	50 (58)	-0.61	38 (96)	0.08	1 (97)	0.15	3 (99)
A	0.26	7 (7)	0.41	18 (24)	0.13	2 (26)	0.85	73 (100)	0.04	0 (100)
Variation explained	4.04	45 (45)	2.27	25 (70)	1.55	17 (87)	0.84	9 (97)	0.24	3 (100)

Table 6 CTR coefficients of samples to each PC.

Salami	CTR to PC1	CTR to PC2	CTR to PC3	CTR to PC4	CTR to PC5
Nitran A	1.37	4.02	29.53	22.63	1.27
Nitran B	0.54	6.57	3.34	14.07	0.00
Nitran C	13.68	9.97	23.87	2.54	3.60
Nitran D	66.15	0.05	0.37	0.24	10.97
Nitran E	15.51	0.23	1.84	0.89	33.41
Nitran F	0.02	1.93	25.84	9.15	2.34
Nitran G	0.23	6.90	0.28	4.65	41.04
Nitran H	1.40	41.73	1.85	25.59	4.84
Nitran I	0.78	12.16	0.01	0.45	0.23
Nitran J	0.33	16.45	13.06	19.78	2.31

needed, i.e. to uncover the anomalous parameters of the samples in which they differ in each other (Table 5).

According to the CTR coefficients for 1st PC it can be noted that variance in P-CTP, TP, TM, TF, CTP and NaCl is mainly given by opposition between Nitran D (CTR = 66.15) and remaining samples of Nitran salamis (CTR <16.00) (Table 6). Numeric data confirm this, as Nitran D had the lowest content of P-CTP, TP and TM (14.14%, 17.42% and 120.29%, respectively) and the highest one of TF, CTP and NaCl (50.85%, 18.83% and 9.55%, respectively).

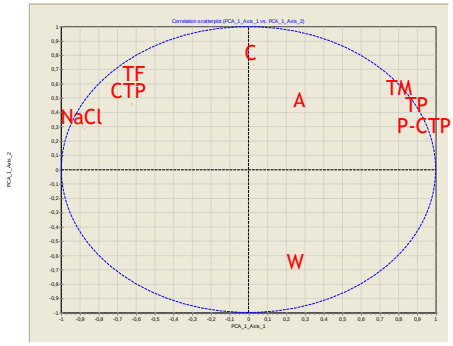

Figure 1 Correlation scatterplot – PC1 vs. PC2.

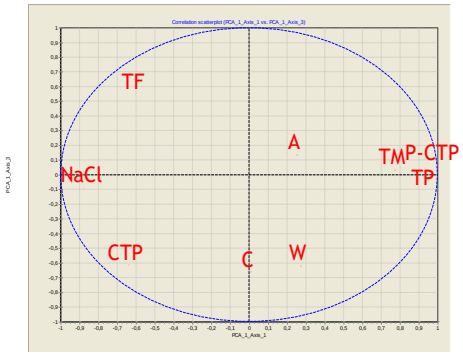

Figure 2 Correlation scatterplot – PC1 vs. PC3.

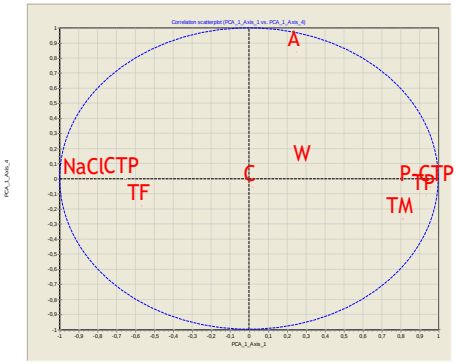

Figure 3 Correlation scatterplot – PC1 vs. PC4.

Collagen content is used as an index of the quality for fermented and dried meat products **(da Silva et al., 2015)**. However, the total content is limited by regulatory agencies **(Sentandreu and Sentandreu, 2014)**. According to **Decree of the Ministry of Agriculture of the Slovak Republic and the Ministry of Health of the Slovak Republic no. 1895/2004-100 establishing a chapter of the Food Codex of the Slovak Republic regulating meat products (2005)**, fermented and dried meat products have to contain maximally 16% CTP. Thus, the values above this limit indicate extra addition of collagen or its hydrolysates, which is a common practice in the meat industry to increase the protein content or water binding properties of meat products **(Sentandreu and Sentandreu, 2014)**. Nitran D was not the most in accordance with this criterion among the salamis (CTP = 18.83%). On the contrary, Nitran E, C and H belonged to group of the TP-rich salamis, when compared to Nitran D, though Nitran H did not satisfy CTP content.

The positions of labels on the loading plot also correspond with this observation (Figure 4). Nitran E, C, H and A are clustered together on the right side of the scatterpplot, because of similarities in attributes explained by 1st PC. However, Nitran D, due to its unlikeness, is separated from other salamis, on the left opposite side of the scatterplot.

The CTR coefficients indicated that Nitran H, J, I and C also marcantly contributed to variance in C, TF, TM and W, described by 2nd PC. Nitran H and A were those with the highest amount of C (4.08% and 4.16%, respectively), whereas Nitran C contained the lowest one (2.48%). But in turn, Nitran H and J had the lowest content of W (22.80% and 23.29%, respectively) and the highest one of TF (49.79% and 47.95%, respectively). Salami is one of the meat products that contain high fat content, usually up to 30% **(Pramualkijja et al., 2015)**.

The CTR values for 3rd PC showed that Nitran A was the poorest in TF (36.62%), but on the other hand the richest in C and CTP (4.16% and 17.94%, respectively) (Figure 5).

Nitran H, A, J and B contributed to variance in A, which was explained by 4th PC. Nitran H and B belonged to the group of low content of A, while Nitran A and J belonged to that one with the highest one (5.32% and 5.45%, respectively) (Figure 6).

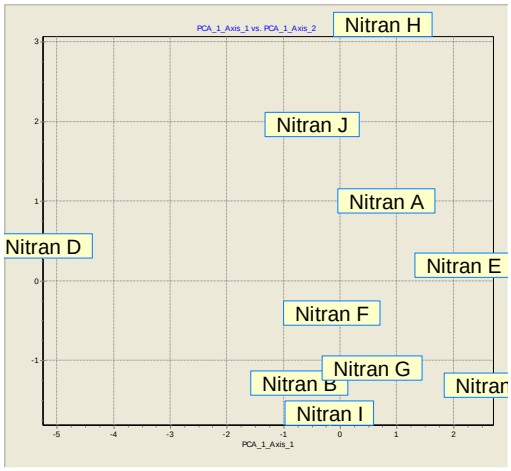

Figure 4 Loading plot - PC1 vs. PC2.

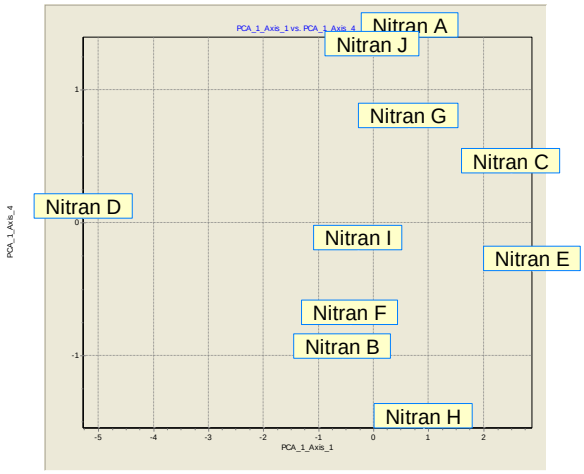

Figure 5 Loading plot - PC1 vs. PC3.

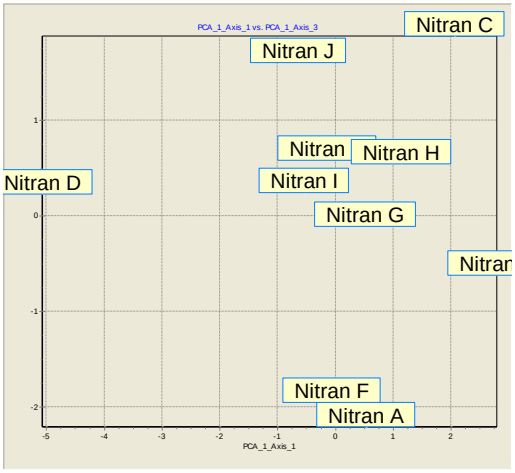

Figure 6 Loading plot - PC1 vs. PC4.

CONCLUSION

It can be concluded that the PCA has shown how chemical attributes of salamis are grouped in the independent sets. In both the 1st and the 2nd PCs, the P-CTP, TP, TM, TF, CTP and NaCl attributes had the highest loadings. In other words, these attributes explained the large part of observed variation in chemical composition among Nitran salamis, which make these attributes as a main predictor of salamis quality. The most distinct differences in these attributes were observed within a pair of Nitran salamis from manufacturer 2 (Nitran C and D). The sliced variant (Nitran D) had the lowest value of TP-associated attributes and the highest ones of TF, CTP and NaCl, even within all the salamis. Besides Nitran D, Nitran A, F and H also did not satisfy CTP content specified in the Decree of MASR and MHSR no. 1895/2004-100, which might indicate extra addition of connective tissue. The CTP content of Nitran B, C, E, G, I and J was in accordance with the decree, whereas Nitran C was that sample with the lowest one (10.74%). Some differences in other attributes were also observed within and among the all couples of salamis, which confirmed the uniqueness of each one. These differences were notable, but not so relevant compared to those described by 1st PC.

REFERENCES

AOAC 991.36:1996. *Fat (Crude) in Meat and Meat Products - Solvent Extraction (Submersion) Method.*

AOAC 2001.11:2005. *Protein (crude) in animal feed, Forage (plant tissue), Grain and oilseeds. Block digestion method using copper catalyst and steam distillation into boric acid.*

Bianchi, F., Cantoni, C., Careri, M., Chiesa, L., Musci, M., Pinna, A. 2007. Characterization of the aromatic profile for the authentication and differentiation of typical Italian dry-sausages. *Talanta*, vol. 72, no. 4, p. 1552-1563. http://dx.doi.org/10.1016/j.talanta.2007.02.019

Cevoli, C., Fabbri, A., Tabanelli, G., Montanari, C., Gardini, F., Lanciotti, R. and Guarnieri, A. 2014. Finite element model of salami ripening process and successive storage in package. *Journal of Food Engineering*, vol. 132, p. 14-20. http://dx.doi.org/10.1016/j.jfoodeng.2014.02.003

Corral, S., Salvador, A., Flores, M. 2013. Salt reduction in slow fermented sausages affects the generation of aroma active compounds. *Meat Science*, vol. 93, no. 3, p. 776-785. http://dx.doi.org/10.1016/j.meatsci.2012.11.040

da Silva, C., Spinelli, E., Rodrigues, S. 2015. Fast and sensitive collagen quantification by alkaline hydrolysis/hydroxyproline assay. *Food Chemistry*, vol. 173, p. 619-623. http://dx.doi.org/10.1016/j.foodchem.2014.10.073

Decree of the Ministry of Agriculture of the Slovak Republic and the Ministry of Health of the Slovak Republic of 18 August 2005 no. 1895/2004-100 establishing a chapter of the Food Codex of the Slovak Republic regulating meat products. Official publication 22/2005, 30.10.2005, p. 218-276.

Fabbri, A., Cevoli, C. 2015. 2D water transfer finite elements model of salami drying, based on real slice image and simplified geometry. *Journal of Food Engineering*, vol. 158, p. 73-79. http://dx.doi.org/10.1016/j.jfoodeng.2015.03.005

Fongaro, L., Alamprese, C., Casiraghi. E. 2015. Ripening of salami: Assessment of colour and aspect evolution using image analysis and multivariate image analysis. *Meat Science*, vol. 101, p. 73-77. http://dx.doi.org/110.1016/j.meatsci.2014.11.005

Herranz, B., Ordóñez, J. A., De La Hoz, L., Hierro, E., Soto, E., Cambero, M. I. 2008 Fatty acid composition of salami from different countries and their nutritional implications. International Journal of Food Sciences & Nutrition, vol. 59, no. 7-8, p. 607-618. http://dx.doi.org/10.1080/09513590701550270

ISO 936:1998. *Meat and meat products - Determination of total ash.*

ISO 1442:1997. *Meat and meat products - Determination of moisture content (Reference method).*

ISO 1841-1:1996. *Meat and meat products - Determination of chloride content - Part 1: Volhard method.*

ISO 3496:1994. *Meat and meat products - Determination of hydroxyproline content.*

Jerković, I., Kovačević, D., Šubarić, D., Marijanović. Z., Mastanjević, K., Suman, K. 2010. Authentication study of volatile flavour compounds composition in Slavonian traditional dry fermented salami "kulen" *Food Chemistry*, vol. 119, no. 2, p. 813-822. http://dx.doi.org/10.1016/j.foodchem.2009.07.024

Marino, R., Albenzio, M., della Malva, A., Muscio, A. and Sevi, A. 2015. Nutritional properties and consumer evaluation of donkey bresaola and salami: Comparison with conventional products. *Meat Science*, vol. 101, p. 19-24. http://dx.doi.org/10.1016/j.meatsci.2014.11.001

Martín-Sánchez, A. M., Chaves-López, C., Sendra, E., Sayas, E., Fenández-López, J., Pérez-Álvarez, J. Á. 2011. Lipolysis. proteolysis and sensory characteristics of a Spanish fermented dry-cured meat product (salchichón) with oregano essential oil used as surface mold inhibitor. *Meat Science*, vol. 89, no. 1, p. 35-44. http://dx.doi.org/10.1016/j.meatsci.2011.03.018

McLean, B. 1999. *Meat and meat products : the calculation of meat content, added water and connective tissue content from analytical data.* Gloucesteshire: Chipping Campdem. 51 p. ISBN 0905942175.

Papavergou, E., Savvaidis, I., Ambrosiadis, I. 2012. Levels of biogenic amines in retail market fermented meat products. *Food Chemistry*, vol. 135, no. 4, p. 2750-2755. http://dx.doi.org/10.1016/j.foodchem.2012.07.049

Pramualkijja, T., Pirak, T., Kerdsup, P. 2015. Effect of salt, rice bran oil and malva nut gum on chemical, physical and physico – Chemical properties of beef salt – Soluble protein and its application in low fat salami. *Food Hydrocolloids*, vol. 53, p. 303-310. http://dx.doi.org/10.1016/j.foodhyd.2015.03.004

Sentandreu, M., Sentandreu, E. 2014. Authenticity of meat products: Tools against fraud. *Food Research International*, vol. 60, p. 19-29. http://dx.doi.org/10.1016/j.foodres.2014.03.030

Tabanelli, G., Montanari, C., Grazia, L., Lanciotti, R., Gardini, F. 2013. Effects of a_w at packaging time and atmosphere composition on aroma profile, biogenic amine content and microbiological features of dry fermented sausages. *Meat Science*, vol. 94, no. 2, p. 177-186. http://dx.doi.org/10.1016/j.meatsci.2013.01.018

Van Schalkwyk, D. L., McMillin, K. V., Booyse, M., Witthuhn, R. C., Hoffman, L. C. 2011. Physico-chemical, microbiological, textural and sensory attributes of matured game salami produced from springbok (*Antidorcas marsupialis*), gemsbok (O*ryx gazella*), kudu (*Tragelaphus strepsiceros*) and zebra (*Equus burchelli*) harvested in Namibia. *Meat Science*, vol. 88, no. 1, p. 36-44. http://dx.doi.org/10.1016/j.meatsci.2010.11.028

Zajác, P., Čurlej, J., Barnová, M., Čapla, J. 2015. Analysis of texturometric properties of selected traditional and commercial sausage. *Potravinarstvo*, vol. 9, no. 1, p. 458-467. http://dx.doi.org/10.5219/473

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EVALUATION OF THE NUTRITIONAL QUALITY OF VEAL SUPPLEMENTED WITH ORGANIC SELENIUM AND ITS EFFECT ON SELENIUM STATUS OF PEOPLE

Klára Vavrišínová, Jana Mrázová, Ondřej Bučko, Petra Lenártová, Jana Moravčíková

ABSTRACT

In the first stage of our research we found out a higher content of selenium in the meat of calves of experimental group (with added the organic form of selenium to the feed mixture) compared to control group (fed without organic form of selenium). In the second stage of our research we focused on monitoring the impact of selenium enriched veal meat and on selenium concentration in blood serum and the selected biochemical parameters of lipid spectrum of the experimental group of volunteers. Ten people who were participating in the research were at the age range between 29 – 56 years. All the volunteers consumed veal meat enriched with organic selenium for 4 weeks. Before starting the experiment we took venous blood of the volunteers and this blood was considered as a control sample of selenium in blood serum of the experimental group. Selenium concentration in blood serum of the examined group was determined by an average of 58.31 ±5.36 μg.L^{-1} and none of them reached the optimal level of selenium. Consequently, we carried out the additional blood sampling after 2 and 4 weeks of the consumption of veal meat. There was registered a slight increasing of selenium status, whereas after the finishing the consumption, we determined the average selenium concentration in blood serum of the experimental group 60.73 ±4.05 μg.L^{-1}. The evaluation of lipid profile of the experimental group showed (after input blood sampling) higher values of total cholesterol level and lower levels of HDL cholesterol. This fact shows the higher risk of starting the cardiovascular diseases. Reported research results didn't show statistically significant changes of blood lipid spectrum of the experimental group. We concluded that the consumption of supplemented veal meat can positively affect the level of selenium in our body and thereby increase it can increase the protective effect against the influence of free radicals.

Keywords: organic selenium; supplementation of veal; veal quality; selenium status; lipid profile of poeple

INTRODUCTION

The importance of selenium was proved in 1957, when the presence of selenium was found in so-called factor 3 which is the prevention against necrosis in the liver of the rats. In 1976 many experiments showed obvious necessity of selenium for people, in spite of that, it was pointed on its negative effects in the 1940s of last century. Nowadays, an interest about selenium and its role and significance in food considerably increased because many researches point out the importance of this element for the health of people. In the past, we only knew its toxic effect on the organism but present studies focus on, that a lack of selenium can cause cardiovascular diseases and also oncogenous diseases **(Hegedus et al., 2007)**. Selenium belongs to the important antioxidants which improve defensive power of the organism and these antioxidants also protect some elements of food, mainly vitamins and food fats against unwilling oxidation. It is important essential mineral element which is important for the health of people and animals. The selenium together with the vitamin E positive influence on the technological characteristics (properties) of meat because of its antioxidants features **(Pavlata et al., 2002**). In food of animal origin the concentration of selenium is given by the nourishment of the animal or its content in feedstuff **(Lyons et al., 2007)**. According to several publications, the use of organic selenium in animal nutrition and consumption the products of these animals are accessible source of selenium in the human diet **(Fisinin et al., 2009)**. Mainly at red meat in this regard refers **Wiliams (2007)**, however probably its concentration is of the heavily influenced by nutrition. **Marounek et al., (2006)** in the experiment found out differences in the content of selenium in veal. In group experimental group (with selenium yeast) was content Se higher compared to the control group. In the different parts of world there are also different intake of selenium in people and animals. It is also regarded according to the selenium status, it means saturation of the organism by this microelement and its combinations. Selenium status depends on different factors such as absorption, food intake, excretion according to biological accessibility **(Ermidou-Pollete et al., 2005)**. The concentrations of selenium in blood plasma/serum of people in the European countries are in the scale between 60 – 111 μg.L^{-1}. Selenium status in Slovak population is in the low limits of this scale **(Combs, 2001)**. **Thomson (2004)** confirmed starting concentration of selenium in blood serum for protective effect against the influence of free radicals 100 - 200 μg.L^{-1}. Selenium has a strong antioxidant activity and participates in the system of conversion of aggressive oxidant products, transforms intracellular free radicals into less reactive or neutral elements **(Elasal et al., 2014)**. Increased intake of selenium decreases the risk of starting the cancer and

softens the progress of other pathological processes causing oxidative stress and an irritation **(Lukáč, 2007)**. Sufficient supplementation with selenium of the animals is important not only because of good health state and utility of the animals, but it can be increased in human population by higher content of selenium in the products. The features of organic form of selenium allow an effective transfer in foodweb. It is used in the world practise in modern approach of the production of so-called functional articles of food. The animal products can belong to this category and they are enriched by organic selenium **(Lagin et al., 2009)**.

The aim of work was evaluation physical and chemical patrameters of veal enriched of organic selenium and its effect on selenium status of people.

MATERIAL AND METHODOLOGY

Two groups of calves (10 +10 heads) to 150 kg of the body weight (at the same time, the same age and rearing condition) were reared for the purposes of the experiment. The difference between the groups was in the feeding after weaning to the end of experiment. The organic form of selenium was added to the feed mixture in the experimental group (selenium content per 1 kg of mixture was: E8 form 1.12 mg and 3b8.10 form 0.8 mg). The control group was fed without organic form of selenium.

There was analysed an effect of supplementation of veal meat on selenium status and we also examined chosen biochemical parameters of lipid spectrum of the consumers. In the experiment was selected group of people. People who participated in the research were represented by 5 women and 5 men at the age scale between 29 – 56 years, with the average age of the experimental group which was 46.3 ±8.34 years. The experimental group consisted of the healthy volunteers, without any healthy problems and pathologic changes in basic biochemical parameters in blood. All the members of the experimental group didn't use supplements before starting the clinical study, and not also during the realization of the research.

Referring to our experiment which was focused on monitoring the impact of supplementation of selenium to the feeding mixture for the calves, we gain meat from the MLT in the experimental groups of the animals. Meat which was enriched by selenium was canned in 1% salt brine in the airtight cans and sterilised them in the thermostatic pot.

Meat was sterilised for 3 hours and the temperature was 100 °C. The consumption of veal meat was done three times a week during 28 days individually. The amount of meat was 130 g.

The biochemical examination of blood tests before starting the consumption was carried out. The 1st blood sampling was determined total cholesterol level, a level of HDL-cholesterol, LDL-cholesterol level, triglycerides and concentration of selenium in blood of the experimental group of the volunteers. We did the 2nd (repeated) blood taking after two weeks of consumption of supplemented veal meat and the last (the 3rd) blood taking was done immediately after finishing the consumption. The samples of blood serum were stored in the fridge on the temperature 80 °C after their separation and consequently after de-freezing them we specified (estimated) biochemical parameters of blood serum of the experimental group.

Biochemical parameters of blood were defined by the estimation analyser (estimation device) Biolis 24i premium (Tokyo Boeki Medisys, Japan). Total cholesterol, triglycerides were determined by calorimetric method fy Randox CHOD-PAP and HDL cholesterol level, LDL cholesterol level were defined by direct method clearance fy Randox.

The atomic absorptive spectrometer made by the company Perkin-Elmer 4100ZL (Norwalk, CT, USA) was used for defining the concentration of selenium. This spectrometer has cross heating electrothermic atomizer (THGA, Part No.B050-4033). And this spectrometer was also used in connection with automatic feeder machine with the samples AS-70. We used corrector of Zemanovsky for the correction of the background. EDL (System 2) for Se (Perkin-Elmer) was used as a source of radiation, which was working in 260 mA. The wave length was 196.0 nm and width of the gap (crack) was 2.0 nm.

We evaluated gained data from the experiments by adequate biostatistical methods using applicative programmes. Statistical data processing was realised by algorithms which were found in the applications SAS in 9 and also by statistical functions in MS Excel. Data processing was also done by one factor analysis of dispersion using ANOVA.

RESULTS AND DISCUSSION

The Table 1 showes the higher content of selenium in the meat of calves of experimental group compared to control group. There was not found significant differences in the chemical composition between groups. Ing the meat of the control group was higher decrease in pH levels at 24 hours after slaughter. **Marounek et al., (2006)** in the experiment found out higher Se content also in the control of the experimental group (with the addition selenium yeast) and also in the control group, compared with our results.

By the determined significant differences in selenium

Table 1 The physical and chemical patrameters and selenium content of *m. longissimus thoracis et lumborum* (MLT).

Parameters	Control group $\bar{x}$ ±SD	Experimental group $\bar{x}$ ±SD	Significance
Se content (mg.kg^{-1})	0.064 ±0.003	0.101 ±0.006	+++
Protein content (g.100g^{-1})	22.510 ±0.467	22.880 ±0.798	-
IMF content (g.100g^{-1})	1.703 ±0.358	1.893 ±0.148	-
Water content (g.100g^{-1})	74.707 ±0.682	74.420 ±0.349	-
pH_1	6.313 ±0.152	6.377 ±0.037	-
pH_{24}	6.033 ±0.029	6.173 ±0.065	+

content between the control and the experimental group we can perform the second stage of the experiment. The veal from the experimental group was used in the second stage.

The impact of supplementation of veal meat with organic selenium on the concentration of selenium in blood of the experimental group

In the second stage of our research was focused on monitoring of veal meat enriched with organic selenium and its influence on the concentration of selenium in blood serum in the experimental group and we also examined chosen biochemical parameters of lipid spectrum of consumers.

The volunteers in the nutritional protocols where they wrote what kind of food they ate during the whole day. The nutritional software Alimenta version 4.3e was used to intake of selenium from food of the volunteers (the experimental group) during the days when they didn't eat veal meat enriched by selenium. Average daily taking of selenium in the group of ten people we registered an amount 131.34 μg.day^{-1}. The selenium intake of veal meat supplemented was 10 μg.100g^{-1}. According to The World Health Organization taking selenium per day moves between 50 to 200 μg.day^{-1} **(Rayman, 2012)**.

The first blood sampling in the experimental group before starting our research and we noticed that average concentration of selenium was 58.31 ±5.36 μg.L^{-1}, and we can say that none of the volunteers had an optimal level of the concentration of selenium in blood. The second blood sampling we did after two weeks of consumption of supplemented veal meat with organic selenium and we marked slight increasing of selenium in blood serum in average 59.99 ±4.16 μg.L^{-1}. The last blood taking was done after finishing the consumption of veal meat enriched by selenium. It was after four weeks and was also noticed increasing of the concentration of selenium in blood in average 60.73 ±4.05 μg.L^{-1}. The results didn't show evidentiary changes in concentration of selenium in blood serum of the experimental group. The results were made after short time of consuming of veal meat with selenium content. The particular concentrations of selenium in blood are shown in the figure 1 and table 2.

According to determined concentrations of selenium in blood is probably that the interval of the concentrations in monitored group, which is 49.6 – 67.8 μg.L^{-1}, is comparable with the results of the last study which was done in Slovakia by **Maďarič and Karabová (1998)**.

They determined concentrations of selenium in blood plasma of 1056 chosen people who were examined and they were from different parts of Slovakia. This concentration was in the range 46 – 77 μg.L^{-1}. Another similar research was done in Czech Republic by **Střitecká et al. (2009)**. In this study was experimental group of 386 healthy people and the concentration of selenium in their blood was in the range 52.9 – 73.43 μg.L^{-1}. This study also approved slight deficiency of selenium concentration similarly than it was shown in our experiment. Low levels of selenium in blood which were shown in above mentioned studies are connected with low saturation of selenium in soil.

The influence of supplementation of veal meat enriched with organic selenium on lipid profile of the experimental group

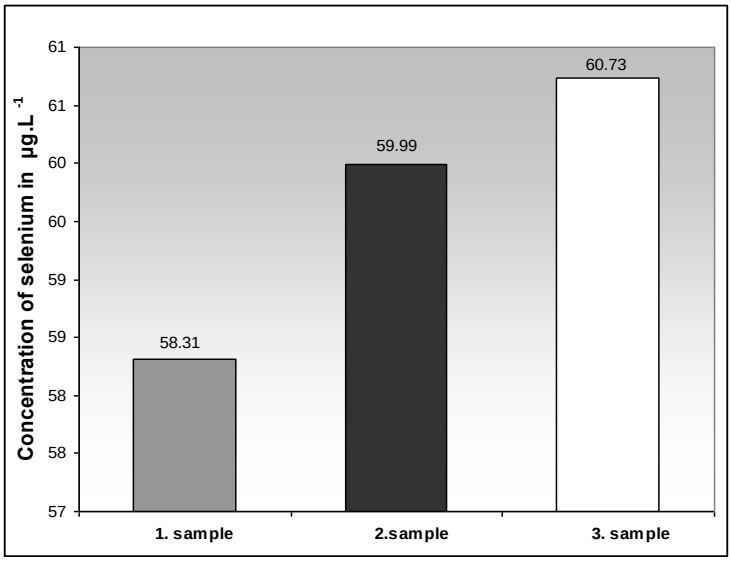

Figure 1 Comparing the concentration of selenium in blood serum of the experimental group during the realisation of the research.

Table 2 Selenium concentration in human blood serum.

Sex	1.sample μg.L^{-1}			2. sample μg.L^{-1}			3. sample μg.L^{-1}		
	$\bar{x}$	s	min-max	$\bar{x}$	s	min-max	$\bar{x}$	s	min-max
Men	61.28	3.72	58.8 – 67.8	62.30	2.75	58.8-65.4	60.88	3.91	56.7 – 66.9
Women	55.34	5.37	49.6 – 62.2	57.68	4.25	54.5-63.4	60.58	4.64	55.4 – 65.5
Total	58.31	5.36	49.6 – 67.8	59.99	4.16	54.5-65.4	60.73	4.05	55.4 – 66.9

significance $p \geq 0.05$.

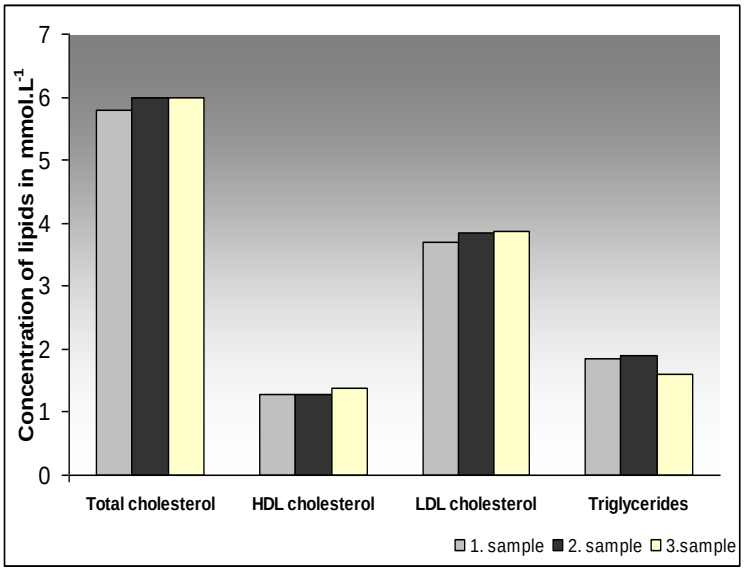

Figure 2 Comparing the concentration of lipids in blood of the experimental group.

Table 3 The lipid profile in human blood serum.

PARAMETERS	**1. SAMPLE** $\bar{x}$ **±SD**	**2. SAMPLE** $\bar{x}$ **±SD**	**3. SAMPLE** $\bar{x}$ **±SD**
Total cholesterol (mmol.L^{-1})	5.80 ±1.34	5.98 ±1.28	5.99 ±1.53
LDL cholesterol (mmol.L^{-1})	3.70 ±1.02	3.84 ±1.05	3.87 ±1.18
HDL cholesterol (mmol.L^{-1})	1.27 ±0.29	1.29 ±0.26	1.39 ±0.34
Triacylglycerols (mmol.L^{-1})	1.86 ±0.69	1.91 ±0.48	1.60 ±0.56

significance $p \geq 0.05$.

In evaluation of lipid profile of the volunteers we recorded increased values of total cholesterol which were seen in entry blood taking and these values were in average 5.80 ±1.34 mmol.L^{-1} and was found out low levels of HDL cholesterol in average 1.27 ±0.29 mmol.L^{-1} which in 30% of the volunteers points on higher risk of cardiovascular diseases. **Ferenčík et al., (2002)** states that the following parameters belong to the most important effects of supplementation of selenium: they are - decreasing of the risk of starting arteriosclerosis and cardiovascular diseases, stimulation of immune system, preventive effect against inflammatory diseases, decreasing of virulence of some viruses.

Opposite of results of **Ferenčík et al., (2002)** we didn´t found out a positive effect on concentration of total cholesterol in blood in the experimental group during the experiment, we can say that after short time of the consumption of veal meat enriched with organic selenium we found out that metabolism of lipids was better whereby the concentration of HDL cholesterol increased and the level of triglycerides slightly decreased. The results of our research didn't show statistically significant changes in lipid spectrum of the volunteers in the experimental group. The average values of the parameters of lipid profile are shown in the Figure 2 and Table 3.

The impact of selenium on lipid profile was examined on the experimental group of the animals. It was shown that the supplementation of selenium decreased the value of total cholesterol and also LDL cholesterol level and increased the value of HDL cholesterol level whereas the lack of selenium had an opposite effect.

It was found that an inactivation of synthesis of selenoproteins of the mice causes increasing concentration of cholesterol in plasma, increasing an amount of apolipoprotein E, improves gene expression for biosynthesis of cholesterol and decreases gene expression which is responsible for metabolism and transport of cholesterol. Relevancy of these studies connected with people is a questionable. It is supposed that the association between selenium status and the risk of starting the cardiovascular diseases depends on selenium status of monitored population **(Rayman, 2011)**.

CONCLUSION

According to our results was concluded that the application of organic selenium to feed mixture in the fattening process of the calves has the significance for effective transfer / transmission of essential microelement selenium to the foodweb. Integration of veal meat enriched with selenium to food of people leads to the increasing of selenium status of consumers and it can also lead to the protection of the cells of immune system against the damage during oxidative stress.

REFERENCES

Hegedus, O., Hegedusová, A., Šimková, S. 2007. *Selenium as biogenic element (In Slovak: Selén ako biogénny prvok).* Nitra : UKF, 2007. 75 p. ISBN 978-80-8094-168-0.

Combs, G. F. 2001. Selenium in global food systems. *British Journal of Nutrition*, vol. 85, no. 5, p. 517-547. http://dx.doi.org/10.1079/BJN2000280 PMid:11348568

Elassal, G., Samy, H., Said, M., Elbatrawy, S. 2014. Significance of selenium levels in non - small cell lung cancer patients. *Egyptian Journal of Chest Diseases and Tuberculosis*, vol. 63, no. 4, p. 1019-1023. http://dx.doi.org/10.1016/j.ejcdt.2014.07.012

Ermidou-Pollet, S., Silagyi, M., Pollet, S. 2005. Problems associated with the determination of trace element status and trace element requirements - a mini review. *Trace Element and Electrolytes*, vol. 22, no. 4, p. 105-113. http://dx.doi.org/10.5414/TEP22105

Ferenčík, M., Abringer, L. 2002. The effect of selenium and zinc on the immune system (In Slovak: Vplyv selénu a zinku na imunitný system). *Klinická imunológia a alergológia*, ed. 12, no. 2, p. 9-18.

Fisinin, V. I., Papazyan, T. T., Surai, P. F. 2009. Producing selenium-enriched eggs and meat to improve the selenium status of the general population. *Critical Reviews in Biotechnology*; vol. 29, no. 1, p. 18-28. http://dx.doi.org/10.1080/07388550802658030 PMid:19514900

Lagin, L., Bobček, B., Obtulovič, P., Mrázová, J., Bučko, O. 2009. Quality pork meat with added organic selenium compound to the feed for pigs (In Slovak: Kvalita bravčového mäsa s prídavkom organického selénu do kŕmnej zmesi ošípaných). *Acta fytotechnica et zootechnica*, special no., p. 337-341.

Lukáč, N., Massányi, P. 2007. Effect of trace elements in the immune system (In Slovak: Vplyv stopových prvkov na imunitný system). *Epidemiologie, mikrobiologie, imunologie*, vol. 56, no. 1, p. 3-9.

Lyons, M. P., Tapazyan, T. T., Surai, P. F. 2007. Selenium in Food Chain and Animal Nutrition: Lessons from Nature. *Asian-Australasian Journal of Animal Science*, vol. 20, no.7, p. 1135-1155. http://dx.doi.org/10.5713/ajas.2007.1135

Maďarič, A., Kadrabová, J. 1998. Selenium in food and the possibility of selenium supplementation in the Slovak population (In Slovak: Selén v potravinách a možnosť jeho suplementácie u slovenskej populácie). *Bulletin of Food Research*, vol. 37, no.1, p. 11-17.

Marounek, M. Skřivanová, E., Skřivanová, V. 2006. Selenium content and antioxidant status in tissues of veal calves fed a diet supplemented with selenium yeast. *Slovak Journal of Animal Science*, vol. 39, no.1-2, p. 51-54.

Pavlata, L., Pechová, A., Illek, J. 2002. Practical recommendations for the diagnosis of selenium deficiency in cattle in the Czech Republic (In Czech: Praktická doporučení pro diagnostiku karence selenu u skotu v České republice). *Veterinářství*, vol. 52, p. 170-173.

Rayman, M. P. 2011. Effect of Supplementation With High-Selenium Yeast on Plasma Lipids: A Randomized Trial. *Annals of Internal Medicine*, vol. 154, no. 10, p. 656-665. http://dx.doi.org/10.7326/0003-4819-154-10-201105170-00005 PMid:21576533

Rayman, M. P. 2012. Selenium and human healt. *The Lancet*, vol. 379, no. 9822, p.1256-1268. http://dx.doi.org/10.1016/S0140-6736(11)61452-9

Střítecká, H., Hlúbik, P., Nováková, J. 2009. Serum selenium in a group of 386 volunteers from the Czech Republic Rescue Fire Brigades. *Mediterranean Journal of Nutrition and Metabolism*, vol. 2, no 2, p. 133-138. http://dx.doi.org/10.1007/s12349-009-0049-0

Thomson, C. D. 2004. Assessment of requirements for selenium and adequacy of selenium status: a review. *Eururopean Journal of Clinical Nutr.*, vol. 58, no. 3, p. 391-402. http://dx.doi.org/10.1038/sj.ejcn.1601800 PMid:14985676

Williams, P. G. 2007. Nutritional composition of red meat. *Nutrition and Dietetics*, vol 64, Suppl. 4, p.113-119. http://dx.doi.org/10.1111/j.1747-0080.2007.00197.x

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INFLUENCE OF HARVEST DAY ON CHANGES IN MECHANICAL PROPERTIES OF GRAPE BERRIES

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ABSTRACT

Changes in the composition, physical and mechanical properties occur in grape berries during the ripening process, but the heterogeneity of the grapes harvested at different ripening stages affects the reliability of the results obtained. The characterization of the mechanical properties of grape berries seems to be an important parameter for understanding grape ripening. In this work, these changes were studied in seven grapevine varieties (*Riesling, Blaufränkisch, Pinot Noir, Cerason, Malverina, Laurot,* and *Hibernal*) harvested during six consecutive weeks. Mechanical behaviour was measured using compression and puncture tests using of TIRATEST 27025 testing machine. Skin mechanical properties were evaluated using a puncture test carried out on the equatorial side. The dependence of these properties on the chemical composition has been evaluated. These parameters of force/time curves were studied by puncture test: the berry skin break force, the needle displacement at the skin break and the berry skin break energy. The crushing force, the plate displacement at the crushing strength and the berry crushing energy were studied from force/time curves by compression test. Results of the puncture test shows that there the skin break strength and the acidity content are monotonic functions of the time. A comparison of different varieties from the point of the value of the crushing force was obtained by vertical and transversal loading. The crushing force is monotonically decreasing function of the harvesting time like the break force evaluated at the puncture test. The correlation between the skin break strength and the sugar content is significant namely for the varieties: *Hibernal, Riesling, Malverina,* and *Cerason*.

Keywords: grapes; acidity; sugars; texture; rupture

INTRODUCTION

Wine grapes undergo numerous physiological and biochemical changes during ripening inducing colour and texture changes **(Ribereau-Gayon et al., 2006; Coombe and McCarthy, 2000; Letaief et al., 2013; Le Moigne, 2008)**. During ripening, changes in the composition and structure of the cell wall as well as in the structure of the tissue, may determine the mechanical resistance and the texture of the fruit **(Abbott, 2004; Brummell et al., 2004; Hertog et al., 2004; Brummell et al., 2006; Deytieux-Belleau et al., 2008; Rolle et al., 2011)**. Grapes with low level of mechanical properties and damaged may be contaminated by fungi (e.g. *Penicillium expansum*) **(Tančinová et al., 2016)**. From this point of view the characterization of the mechanical properties of grape berries seems to be an important parameter for understanding grape ripening **(Doumouya et al., 2014; Carbajal-Ida et al., 2016; Fava et al., 2011)**. Previous studies applied the puncture test to characterize and compare the crunch texture of different table grapes **(Sato et al., 1997; Sato and Yamada, 2003)** and to follow the ripening process of white wine grapes such as Chardonnay and Riesling **(Lee and Bourne, 1980)**. These last authors showed that the mechanical properties of grape skin evolved during ripening and were significantly correlated with the °Brix for most grapes. Further work showed differences in mechanical properties of red wine grape varieties at a chosen harvest maturity level **(Letaief et al., 2008)** and differences in grape skin hardness **(Río Segade et al., 2008)**. However, there is no published work addressing the assessment of a mechanical method designed to monitor wine grapes ripening.

Preliminary research on the grape texture change showed that compression measurements were able to recognize veraison (a marker stage of berry development) earlier than a visual identification performed in the field, which is of particular importance for white grapes for which the colour change is slight **(Robin et al., 1997; Grotte et al., 2001)**. **Bernstein and Lustig (1985)** measured grape firmness and showed the relationship between turgor pressure and firmness. **Zouid et al., (2013)** show that the instrumental texture analysis can be very useful for to study the impact of the grapes heterogeneity according to sugar level on the physical and mechanical properties of *Cabernet Franc* grapes and to select the best instrumental parameters of the whole berries or of the skin linked with anthocyanins extractability. The next information on the instrumental texture analysis is presented by **Rolle et al., (2012)**.

The aim of this study was to define the best conditions to describe grape texture during ripening in order to obtain additional parameters that could be of benefit to ascertain

the quality of ripening grape berries, in addition to the physiological parameters commonly used such as the acidity and sugar content.

MATERIAL AND METHODOLOGY

All grapevine varieties under study were grown in the experimental vineyard of the aforementioned faculty. This vineyard is situated in the vineyard site called "V Mendeleu" (In Mendeleum) in the wine village Lednice (region South Moravia, Czech Republic). The spacing of plants was 2.2 x 1.0 m and the plants were trained using Guyot pruning with 10 eyes per vine. This vineyard was established in 1993 and all varieties were grafted on the rootstock 5C.

Within the framework of this study altogether 3 cultivars of *Vitis vinifera* L. – *Riesling, Blaufränkisch* and *Pinot Noir* were evaluated together with 4 interspecific varieties: *Cerason*, *Malverina*, *Laurot*, and *Hibernal*. These varieties are maintained and evaluated within the framework of a collection of genetic resources of grapevine. Berries were sampled using the method described by **Iland et al., (2004)**.

Berries were randomly picked once per week, during the maturation period (from September to October in 2015). For compression test has been chosen six different dates: September 4 (week 1), September 13 (week 2), September 22 (week 3), September 30 (week 4), October 7 (week 5), and October 13 (week 6). For puncture test has been chosen the same dates without September 4 (week 1).

Each day of the harvest the following parameters were evaluated total acids in grapes. Total acid was calculated as all acids determined by HPLC method and expressed as tartaric acid. Total sugar was the sum of glucose and fructose **(Katalinic et al., 2013)**. The detail description of this method of analysis is described in **Pavloušek and Kumšta (2011)** briefly.

Mechanical behaviour was measured using compression and puncture tests. These tests were performed using of TIRATEST 27025 (TIRA Maschinenbau GmbH, Germany) testing machine. Skin mechanical properties were evaluated using a puncture test carried out on the equatorial side. Tests were performed with a cylindrical needle probe of 0.56 mm in diameter at speed test of 10 $mm \cdot s^{-1}$. Force/time curves were analyzed and three parameters were studied: the berry skin break force F_{sk} in Newton, the needle displacement p_{sk} [mm]at the skin break and the berry skin break energy W_{sk} [J = N·mm], see Eq. (1). These tests have been conduced on the lateral side of the berry, positioned on the base of the texture analyser **(Brummell et al., 2004)**.

$$W_{sk} = \int_0^{p_{sk}} F_{sk}\, dp. \qquad (1)$$

Whole berry mechanical properties were assessed using a compression test. Berries were compressed both in the equatorial position (perpendicular to berry height *L*, [mm]) and vertically along of the berry symmetry axis. The compression velocity was also 10 $mm \cdot s^{-1}$. The following mechanical parameters have been measured: crushing force F_c [N], the plate displacement at the crushing strength p_c [mm] and the berry crushing energy W_c [J = N·mm], see Eq. (2). The crushing force is the compression force that is necessary to cause the skin break when the first grape juice is coming out **(Brummell et al., 2004)**.

$$W_c = \int_0^{p_c} F_c\, dp. \qquad (2)$$

The results obtained were statistically analysed using the statistical toolbox of software MATLAB version 7.12.0.635 (R2011a) (The MathWorks, MA, USA). Evaluated were the means and standard deviations using ANOVA with subsequent Tukey's test at significance levels of $p < 0.05$.

RESULTS AND DISCUSSION

In the Figure 1 an example of the experimental record force *F* vs displacement *p* is shown. The same qualitative features exhibited all experimental records. The force increases up to some maximum value corresponding to the skin break force F_{sk}. The force is non-linear function of the displacement. This is slightly different result than that obtained e.g. by **Maury et al., (2009)** and/or **Río Segade et al., (2011)**. In these papers the considered dependence was linear.

The berry skin break force F_{sk} for different wine varieties is displayed in the Figure 2. This force decreases with the time of the harvesting. It means this force exhibits a good correlation with the content of total acids, see Figure 3. This dependence is different for the different wine varieties. It means the value of this force cannot be used for the identification of single varieties.

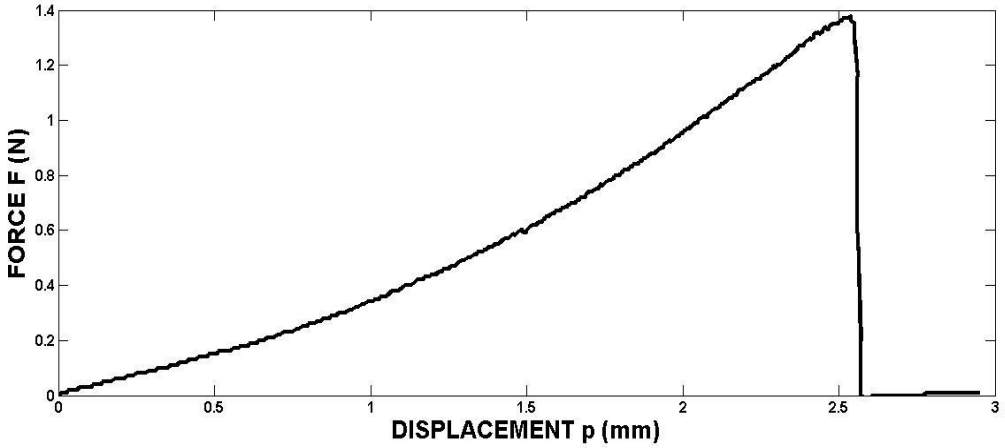

Figure 1 Example of the experimental record break force – displacement during the puncture test.

The dependence of the break force F_{sk} on the sugar content can be considered as a linear. The best correlation, i.e. higher than 0.85 have been observed for the following varieties: *Hibernal*, *Riesling*, *Malverina*, and *Cerason*. For the remaining grapevines the correlation coefficient was between 0.73 and 0.82. Nearly no correlation has been found between displacement at the skin break p_{sk} and the total sugars content. Very good correlation has been also found between the berry skin break energy W_{sk} and total content both of sugars and acids. Development of this energy during the harvest period is displayed in the Figure 4.

In the Figure 5 an example of the experimental record of the force F_c – displacement during the compression test is displayed. The qualitative features of this record are the same like in the case of the puncture test, see Figure 1. This conclusion is valid for both transversal and vertical tests and for all winegrape varieties.

The average values of the crushing force F_c for different wine varieties are displayed in the Figure 6.

This force F_c decreases with the time of the harvesting. Qualitatively the same dependence exhibits crushing force

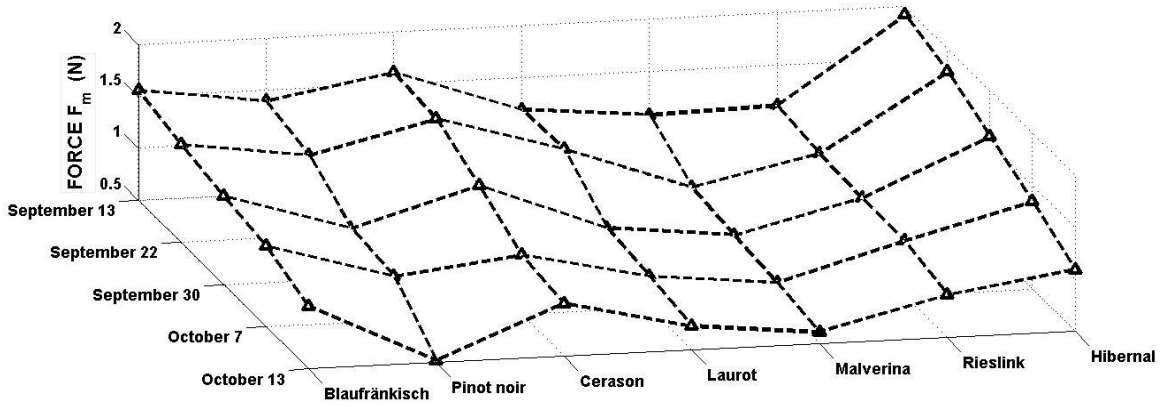

Figure 2 Skin break force evaluated from the puncture test.

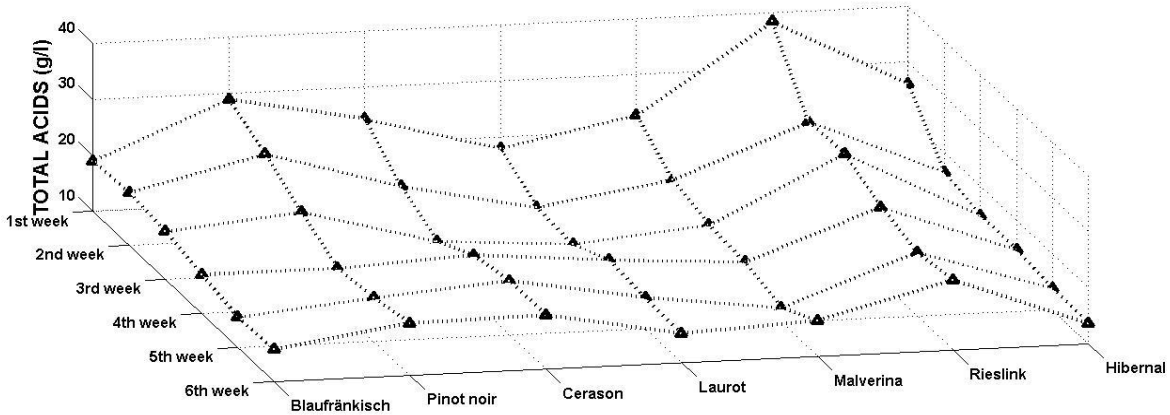

Figure 3 Content of total acids in grapes of tested varieties.

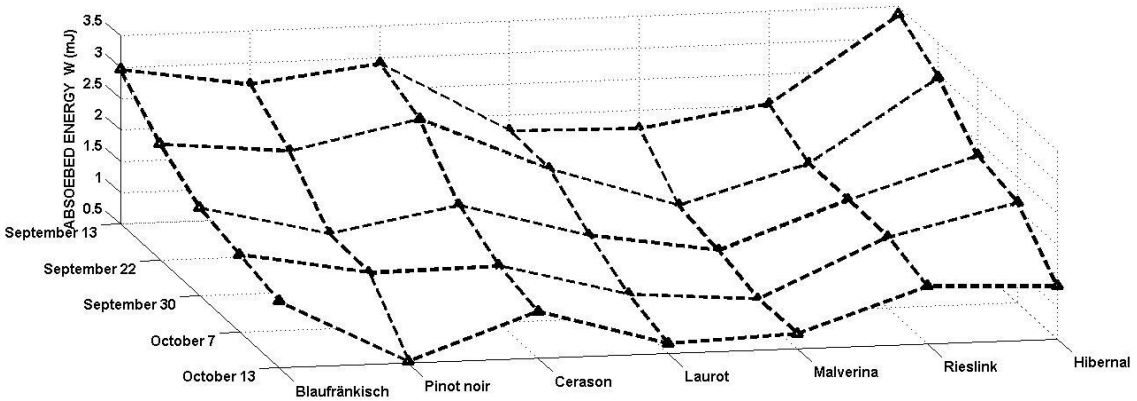

Figure 4 Berry skin break energy at the different days of the harvest.

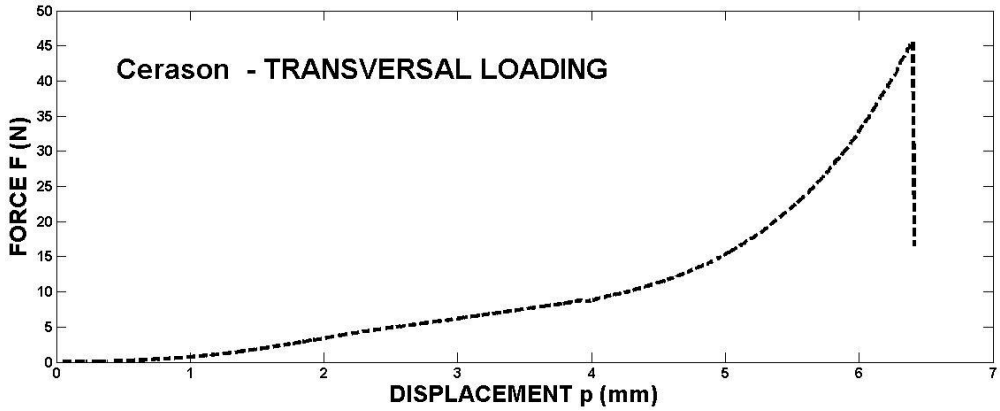

Figure 5 Example of the experimental record of the wine berry compression.

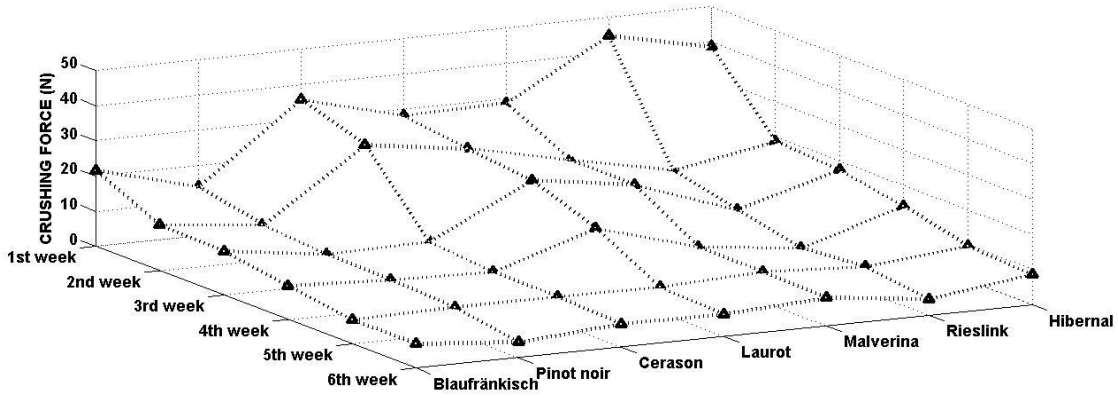

Figure 6 Average values of the crushing force during harvesting period – transversal compression.

obtained at the vertical loading. The differences between values of these force is described in the Table 1. In this Table 1 corresponds to the situation when the crushing force obtained during the transversal compression is higher than that obtained at the vertical compression. Zero corresponds to the opposite case.

It is evident that the crushing force corresponding to *Pinot Noir, Blaufränkisch* varieties evaluated at the lateral compression is higher than that evaluated at the vertical compression. The crushing force of remaining varieties does not exhibit this tendency. The crushing force is monotonically decreasing function of the harvesting time like the break force evaluated at the puncture test. If we perform a comparison of different varieties from the point of the value of the crushing force we obtain an arrangement given in the Table 2.

The minimum value of the crushing force exhibits *Pinot Noir* grapevine variety. The order of remaining varieties is different at different days of the harvesting. The arrangement made according to the crushing force evaluated at the lateral compression is different from that given in the Table 2, see Table 3.

The same arrangement according to the values of the break force evaluated at the puncture test is given in the Table 4.

The results are different from those obtained at the compression test. Qualitatively the same conclusions can be deduced from the values of the absorbed energy and from the values of the displacements at the crushing force. One can see that the critical values of the forces which describe the strength of the berry skin (puncture test) and the whole berry (compression test) gives a different order of grapevine varieties at different days of harvesting.

As it has been mentioned in the introduction, grape

Table 1 Comparison of crushing force for transversal and vertical compression.

Week	*Hibernal*	*Riesling*	*Malverina*	*Laurot*	*Cerason*	*Pinot Noir*	*Blaufränkisch*
1st	1	0	0	0	0	1	1
2nd	1	0	0	0	0	1	1
3rd	1	0	0	0	1	1	1
4th	1	1	1	0	1	1	1
5th	1	0	1	1	1	1	1
6th	0	1	0	1	1	1	1

Table 2 Order of grapevine varieties at the single data of their harvesting – vertical loading.

1st week	2nd week	3rd week	4th week	5th week	6th week
September 4	September 13	September 22	September 30	October 7	October 13
Riesling	*Riesling*	*Riesling*	*Riesling*	*Riesling*	*Riesling*
Malverina	*Malverina*	*Laurot*	*Laurot*	*Cerason*	*Hibernal*
Laurot	*Laurot*	*Malverina*	*Malverina*	*Malverina*	*Laurot*
Cerason	*Cerason*	*Pinot Noir*	*Pinot Noir*	*Laurot*	*Cerason*
Blaufränkisch	*Blaufränkisch*	*Blaufränkisch*	*Blaufränkisch*	*Pinot Noir*	*Malverina*
Hibernal	*Pinot Noir*	*Cerason*	*Cerason*	*Blaufränkisch*	*Pinot Noir*
Pinot Noir	*Hibernal*	*Hibernal*	*Hibernal*	*Hibernal*	*Blaufränkisch*

Table 3 Order of grapevine varieties at the single data of their harvesting – transversal loading.

1st week	2nd week	3rd week	4th week	5th week	6th week
September 4	September 13	September 22	September 30	October 7	October 13
Pinot Noir	*Pinot Noir*	*Pinot Noir*	*Riesling*	*Blaufränkisch*	*Pinot Noir*
Blaufränkisch	*Blaufränkisch*	*Cerason*	*Cerason*	*Riesling*	*Riesling*
Laurot	*Riesling*	*Riesling*	*Pinot Noir*	*Laurot*	*Laurot*
Malverina	*Hibernal*	*Blaufränkisch*	*Malverina*	*Cerason*	*Cerason*
Cerason	*Malverina*	*Hibernal*	*Blaufränkisch*	*Pinot Noir*	*Blaufränkisch*
Hibernal	*Laurot*	*Malverina*	*Hibernal*	*Malverina*	*Malverina*
Riesling	*Cerason*	*Laurot*	*Laurot*	*Hibernal*	*Hibernal*

Table 4 Order of grapevine varieties at the single data of their harvesting – puncture test.

2nd week	3rd week	4th week	5th week	6th week
September 13	September 22	September 30	October 7	October 13
Malverina	*Malverina*	*Malverina*	*Malverina*	*Pinot Noir*
Riesling	*Riesling*	*Laurot*	*Laurot*	*Malverina*
Laurot	*Laurot*	*Pinot Noir*	*Pinot Noir*	*Laurot*
Pinot Noir	*Pinot Noir*	*Riesling*	*Riesling*	*Riesling*
Blaufränkisch	*Blaufränkisch*	*Cerason*	*Cerason*	*Cerason*
Cerason	*Cerason*	*Blaufränkisch*	*Blaufränkisch*	*Hibernal*
Hibernal	*Hibernal*	*Hibernal*	*Hibernal*	*Blaufränkisch*

maturity is associated with changes in the composition and structure of the cell wall of skin and pulp as well as in the structure of the tissue. Therefore, the test conducted on whole berry, which assess the parameters such as crushing strength etc., is actually the best test to monitoring the ripeness, although the values of parameters measured can be affected by rainfalls **(Malheiro et al., 2011; Bonada et al. 2015)**. In this type of test, pulp and skin data are aggregate. On the contrary, by puncture test conduced with thin rounded probe only skin characteristics can be defined. Actually, the break skin force F_{sk} could be considered an important parameter to be monitored for the assessment of the anthocyanins extractability. It means both tests must be used for the evaluation of the berry softening during the maturation.

CONCLUSION

A detail study of the mechanical characteristics of seven winegrape varieties during ripening has been performed. Results of the puncture test shows that there the skin break strength and the acidity content are monotonic functions of the time. The correlation between the skin break strength and the sugar content is significant namely for the varieties: *Hibernal*, *Riesling*, *Malverina*, and *Cerason*. The correlation for the remaining varieties is weaker. Very similar results are valid for the parameters of the compression test. Results of these tests are dependent on the loading orientation. The effect of this parameter is different at different stage of the ripening. Generally the results obtained in this work approved some previous hypothesis that mechanical texture parameters were able to show differences between grapes having different ripening level. In order to support results performed up to now it is necessary to perform some additional experiments with different values of compression velocities and with different diameters of the cylindrical needle probe.

REFERENCES

Abbott, J. A. 2004. Textural Quality Assessment for Fresh Fruit and Vegetables. In Shadidi F. et al. *Quality of Fresh and Processed Food*. New York, USA : Springer, p. 265-279. ISBN 978-1-4613-4790-3. http://dx.doi.org/10.1007/978-1-4419-9090-7_19

Bernstein, Z., Lustig, I. 1985. Hydrostatic methods of measurement of firmness and turgor pressure of grape berries (*Vitis vinifera L.*). *Scientia Horticulturae*, vol. 25, no. 2, p. 129-136. http://dx.doi.org/10.1016/0304-4238(85)90084-6

Bonada, M., Sadras, V. O. 2015. Review: Critical appraisal of methods to investigate the effect of temperature on grapevine berry composition. *Australian Journal of Grape and Wine Research*, vol. 21, no. 1, p. 1-17. http://dx.doi.org/10.1111/ajgw.12102

Brummell, D. A. 2006. Cell wall disassembly in ripening fruit. *Functional Plant Biology*, vol. 33, no. 2, p. 103-119.

Brummell, D. A., Dal Cin, V., Crisosto, C. H., Labavitch, J. M. 2004. Cell wall metabolism during maturation, ripening and senescence of peach fruit. *Journal of Experimental Botany*, vol. 55, no. 405, p. 2029-2039. http://dx.doi.org/10.1071/FP05234

Carbajal-Ida, D., Maury, C., Salas, E., Siret, R., Mehinagic, E. 2016. Physico-chemical properties of botrytised *Chenin blanc* grapes to assess the extent of noble rot. *European Food Research and Technology*, vol. 242, no. 1, p. 117-126. http://dx.doi.org/10.1007/s00217-015-2523-x

Coombe, B. G., McCarthy, M. G. 2000. Dynamics of grape berry growth and physiology of ripening. *Australian Journal of Grape and Wine Research*, vol. 6, no. 2, p. 131-135. http://dx.doi.org/10.1111/j.1755-0238.2000.tb00171.x

Deytieux-Belleau, C., Vallet, A., Donèche, B., Geny, L. 2008. Pectin methylesterase and polygalacturonase in the developing grape skin. *Plant Physiology and Biochemistry*, vol. 46, no. 7, p. 638-646. http://dx.doi.org/10.1016/j.plaphy.2008.04.008

Doumouya, S., Lahaye, M., Maury, C., Siret, R. 2014. Physical and physiological heterogeneity within the grape bunch: Impact on mechanical properties during maturation. *American Journal of Enology and Viticulture*, vol. 65, no. 2, p. 170-178. http://dx.doi.org/10.5344/ajev.2014.13062

Fava, J., Hodara, K., Nieto, A., Guerrero, S., Alzamora, S. M.,Castro, M. A. 2011. Structure (micro, ultra, nano), color and mechanical properties of *Vitis labrusca* L. (grape berry) fruits treated by hydrogen peroxide, UV-C irradiation and ultrasound. *Food Research International*, vol. 44, no. 9, p. 2938-2948. http://dx.doi.org/10.1016/j.foodres.2011.06.053

Grotte, M., Cadot, Y., Poussier, A., Loonis, D., Piétri, E., Duprat, F., Barbeau, G. 2001. Determination of the maturity status of grape berry (*Vitis Vinifera*) from physical measurement : Methodology. *Journal International des Sciences de la Vigne et du Vin*, vol. 35, no. 2, p. 87-98.

Hertog, M. L. A. T. M., Ben-Arie, R., Róth, E., Nicolaï, B. M. 2004. Humidity and temperature effects on invasive and non-invasive firmness measures. *Postharvest Biology and Technology*, vol. 33, no. 1, p. 79-91. http://dx.doi.org/10.1016/j.postharvbio.2004.01.005

Iland, K., Wedekind, J., Wölk, J., Wagner, P. E., Strey, R. 2004. Homogeneous nucleation rates of 1-pentanol. *Journal of Chemical Physics*, vol. 121, no. 24, p. 12259-12264. http://dx.doi.org/10.1063/1.1809115

Katalinic, V., Mozina, S. S., Generalic, I., Skroza, D., Ljubenkov, I., Klancnik, A. 2013. Phenolic profile, antioxidant capacity, and antimicrobial activity of leaf extracts from six *Vitis vinifera* L. Varieties. *International Journal of Food Properties*, vol. 16, no. 1, p. 45-60. http://dx.doi.org/10.1080/10942912.2010.526274

Le Moigne, M., Maury, C., Bertrand, D., Jourjon, F. 2008. Sensory and instrumental characterisation of Cabernet Franc grapes according to ripening stages and growing location. *Food Quality and Preference*, vol. 19, no. 2, p. 220-231. http://dx.doi.org/10.1016/j.foodqual.2007.03.004

Lee, C. Y., Bourne, M. C. 1980. Changes in grape firmness during maturation. *Journal of Texture Studies*, vol. 11, no. 2, p. 163-172. http://dx.doi.org/10.1111/j.1745-4603.1980.tb00315.x

Letaief, H., Maury, C., Symoneaux, R., Siret, R. 2013. Sensory and instrumental texture measurements for assessing grape seed parameters during fruit development. *Journal of The Science of Food and Agriculture*, vol. 93, no. 10, p. 2531-2540. http://dx.doi.org/10.1002/jsfa.6071

Letaief, H., Rolle, L., Gerbi, V. 2008. Mechanical behavior of winegrapes under compression tests. *American Journal of Enology and Viticulture*, vol. 59, no. 3, p. 323-329.

Malheiro, A. C., Gonçalves, I. N., Fernandes-Silva, A. A., Silvestre, J. C., Conceição, N. S., Paço, T. A., Ferreira, M. I. 2011. Relationships between relative transpiration of grapevines and plant and soil water status in Portugal's Douro wine region. *Acta Horticulturae*, vol. 922, p. 261-267. http://dx.doi.org/10.17660/ActaHortic.2011.922.34

Maury, C., Madieta, E., Le Moigne, M., Mehinagic, E., Siret, R., Jourjon, F. 2009. Development of a mechanical texture test to evaluate the ripening process of Cabernet franc grapes. *Journal of Texture Studies*, vol. 40, no. 5, p. 511-535. http://dx.doi.org/10.1111/j.1745-4603.2009.00195.x

Pavloušek, P., Kumšta, M. 2011. Profiling of primary metabolites in grapes of interspecific grapevine varieties: Sugars and organic acids. *Czech Journal of Food Sciences*, vol. 29, no. 4, p. 361-372.

Ribereau-Gayon, P., Dubourdieu, D., Doneche, B., Lonvaud, A. 2006. *Handbook of Enology The Microbiology of Wine and Vinifications*. 2nd ed. New York, USA : Wiley. 497 p. ISBN 9780470010365. http://dx.doi.org/10.1002/0470010363.index

Río Segade, S., Orriols, I., Giacosa, S., Rolle, L. 2011. Instrumental texture analysis parameters as winegrapes varietal markers and ripeness predictors. *International Journal of Food Properties*, vol. 14, no. 6, p. 1318-1329. http://dx.doi.org/10.1080/10942911003650320

Río Segade, S., Rolle, L., Gerbi, V., Orriols, I. 2008. Phenolic ripeness assessment of grape skin by texture analysis. *Journal of Food Composition and Analysis*, vol. 21, no. 8, p. 644-649. http://dx.doi.org/10.1016/j.jfca.2008.06.003

Robin, J. P., Abbal, P., Salmon, J. M. 1997. Firmness and grape berry maturation. Definition of different rheological parameters during the ripening. *Journal International des Sciences de la Vigne et du Vin*, vol. 31, no. 3, p. 127-138.

Rolle, L., Gerbi, V., Schneider, A., Spanna, F., Río Segade, S. 2011. Varietal relationship between instrumental skin hardness and climate for grapevines (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry*, vol. 59, no. 19, p. 10624-10634. http://dx.doi.org/10.1021/jf203254k

Rolle, L., Siret, R., Segade, S. R. S., Maury, C., Gerbi, V., Jourjon, F. 2012. Instrumental texture analysis parameters as markers of table-grape and winegrape quality: A review. *American Journal of Enology and Viticulture*, vol. 63, no. 1, p. 11-28. http://dx.doi.org/10.5344/ajev.2011.11059

Sato, A., Yamada, M. 2003. Berry texture of table, wine, and dual-purpose grape cultivars quantified. *HortScience*, vol. 38, no. 4, p. 578-581.

Sato, A., Yamane, H., Hirakawa, N., Otobe, K., Yamada, M. 1997. Varietal differences in the texture of grape berries measured by penetration tests. *Vitis*, vol. 36, no. 1, p. 7-10.

Tančinová, D., Felšöciová, S., Rybárik, L., Mašková, Z. Císarová, M. 2015. Colonization of grapes berries and cider by potential producers of patulin. *Potravinarstvo*, vol. 9, no. 1, p. 138-142. http://dx.doi.org/10.5219/460

Zouid, I., Siret, R., Jourjon, F., Mehinagic, E., Rolle, L. 2013. Impact of grapes heterogeneity according to sugar level on both physical and mechanical berries properties and their anthocyanins extractability at harvest. *Journal of Texture Studies*, vol. 44, no. 2, p. 95-103. http://dx.doi.org/10.1111/jtxs.12001

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MicroRNA (miRNA) IN FOOD RESOURCES AND MEDICINAL PLANT

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ABSTRACT

MicroRNAs (miRNAs) are a class of 19 – 24 nucleotide long non-coding RNAs derived from hairpin precursors, regulating various biological, metabolic and developmental processes at the post-transcriptional level. Many of the known miRNAs are evolutionary conserved across diverse plant species and function in the regulatory control of fundamentally important biological processes. It is known that exogenous plant miRNAs specifically target approximately 30% of protein-coding genes in mammals. The research was focused to analyze the occurrence of selected families of miRNAs (miR156, miR168 and miR171) in less used species but nutritionally important plant food resources (flax and medlar) and medicinal plant (milk thistle). The analyses were done by two individual approaches, by (a) miRNA-based molecular markers - as a novel type of functional markers and (b) qualitative Real-Time PCR. The expression pattern of selected miRNAs was analyzed depending on various plant tissues and developmental stages. Results have confirmed the significance and reliability of novel type of markers based on miRNA molecules as well as the species-specific and tissues-specific expression patterns of plants miRNAs. Significant polymorphism profile of miR156b was detected in various flax tissues of genotypes varying in the content of alpha-linolenic acid. Conclusions indicate that the variable behavior of the miRNA molecules, depending on various factors, may reflect the variability of the gene expression regulation of the human genome. The exploitation of the background of miRNA functioning within different species and plant tissues will help us to understand the molecular machinery as well as the regulatory mechanisms involved in the expression of miRNAs in plants and consequently in human genome.

Keywords: miRNA; human nutrition; functional food; medicinal plant

INTRODUCTION

Recent findings show that genetic material in plant foods may survive digestion, circulate through our bodies and modulate our gene expression **(Hirschi, 2012)**. Exogenous plant microRNAs that are primarily acquired orally, through food intake, are present in the sera and tissue of various animals **(Zhang et al., 2012)**. Microvesicles (MVs) may encapsulate these miRNAs, because these small vesicles are shed from almost all cell types. Stable microRNAs in mammalian serum and plasma are actively secreted from tissues and cells and can serve as a novel class of biomarkers for diseases, and act as signaling molecules in intercellular communication **(Zhang et al., 2012)**. MicroRNAs (miRNAs) are small RNAs that can regulate target mRNAs by binding to their 3′-UTRs **(Singh et al., 2008)**, leading to either translation delay or mRNA degradation **(Erson-Bensan, 2014)**. A single miRNA can regulate many mRNA targets, and several miRNAs can regulate a single mRNA. All miRNAs have similar secondary hairpin structures, many of these are evolutionary conserved **(Zhang et al., 2006)**. The high conservation of miRNA sequences provides an opportunity to develop a novel type of molecular markers **(Fu et al., 2013; Yadav et al., 2014; Mondal and Ganie 2014; Ganie, Mondal, 2015)**.

miRNAs have been implicated in a number of diseases, and both miRNA inhibition and activation show great promise in the treatment of various types of cancer, and viral and metabolic diseases **(Singh et al., 2008)**.

Plants miRNAs play important roles in plant development and physiology, as well as tolerance to abiotic and biotic stresses **(Taylor et al., 2014)**. Expression of miRNAs in plants involves transcription from *MIRNA* loci by RNA polymerase II, multi-step processing of the primary transcripts, pri-miRNAs by the Dicer-like complex in plants and Drosha and Dicer in animals into precursors, pre-miRNAs with a characteristic hairpin structure **(Xie et al., 2010; Zhang et al., 2006)**. Then, pre-miRNA is further cleaved to a miRNA duplex (miRNA: miRNA*), a short double-stranded RNA (dsRNA) and a mature miRNA. Finally, mature miRNAs are predominantly incorporated in the in the RNA-induced silencing complex (RICS) **(Bartel, 2004)**.

The findings of **Zhang et al., (2012)** have demonstrated that exogenous plant miRNAs in food can regulate the expression of target genes in mammals. miR156a and miR168a are abundant in rice and the miR168a is one of the most highly enriched exogenous plant miRNAs in the sera of Chinese subjects. Functional studies demonstrated that MIR168a could decrease low-density lipoprotein (LDL) removal from mouse plasma.

Lukasik and Zielenkiewicz (2014) by *in silico* approach identified in mammalian breast milk exosomes the highest abundance levels yielded the ath-miR166a (ath, *Arabidopsis thaliana*), while in the porcine breast milk exosomes, the zma-miR168a, zma-miR156a (zma, *Zea mays*) and ath- miR166a.

Several miRNA families have multiple members within the same plant species. For instance, miR395 has 18 members in rice. Although they are conserved as mature miRNA sequences, the other parts of miRNA precursor differ widely, suggesting that the different members of the same miRNA family may evolve at different rates within the same plant species **(Zhang et al., 2006)**.

As the link between metabolism and major disease processes becomes more well-defined, the identification of key molecular targets is leading to new therapeutic strategies **(Palmer et al., 2014)**. Dietary interventions have been used to change metabolism and to potentially alter disease progression. Since microRNAs may fine tune many molecular processes, it is reasonable to assume that dietary alterations that induce miRNA changes will modulate these pathways. Many microRNA families have already been associated with various nutrient interventions. MiRNA represent a link between nutrient intake, obesity and insulin resistance, and disease **(Ali et al., 2011)**.

Within our research we are focused on the exploitation of microRNA as molecular markers of plant genome characterization and mapping their activity in different plant species of nutritional and pharmaceutical importance (*Linum usitatissimum, Messpilus germanica, Silybum marianum, Hedera helix* and *Ginkgo biloba,*), plant organs and tissues (flower buds, flowers, bolls, leaves, seeds) and developmental stages (flowers development, flowering, seed development). The abundance of mature miRNAs, which is linked to the expression of *MIRNA* genes, varies greatly among different miRNAs, tissue types or developmental stages, indicating the spatially and temporally regulated expression patterns of plant miRNAs **(Xie et al., 2010)**.

Understanding the function of miRNAs in the complex molecular network regulating the development and function of various cells and tissues will increases our knowledge about the potential role of miRNAs and their involvement in gene regulation **(Singh et al., 2008)**.

MATERIAL AND METHODOLOGY

Based on the type of plant biological material was the total genomic DNA extracted either commercial isolation kit or different isolation protocols **(Saghai-Maroof et al., 1984; Padmalantha and Prasad, 2006)**. The extracted DNA was quantified by the Implen NanoPhotometer®, and diluted to 70 ng.µl^{-1}. The primers for the miRNA-based markers were designed according to the mature miRNAs sequences, which are part of the miRNA precursors (pre-miRNA), originating from the miRNA database (http://www.mirbase.org/). The single forward primers and the universal miRNA reverse primer **(Kulcheski et al., 2010; Chen et al., 2005)** were combined to perform a marker assays. The effectiveness and species transferability of used primers was confirmed in previous studies **(Hlavačková et al., 2015; Ražná et al., 2015)**.

miRNA-marker assay

Polymorphism analyzes were applied for three flax genotypes of different alpha-linolenic acid content, genotype Amon (less than 3%), Raciol (30%) and Libra (more than 57%), 5 genotypes of milk thistle of various origins, 6 genotypes of medlar and one genotype of ivy.

The modification of miRNA-based markers assay was perfomed based on methodologies **Fu et al., (2013)** and **Yadav et al., (2014)**. PCR amplifications were perfomed in a 20-µl reaction mixture containing 70 ng of genomic DNA, 10 pmol.dm^{-3} of each primer, 2 units of DreamTaq

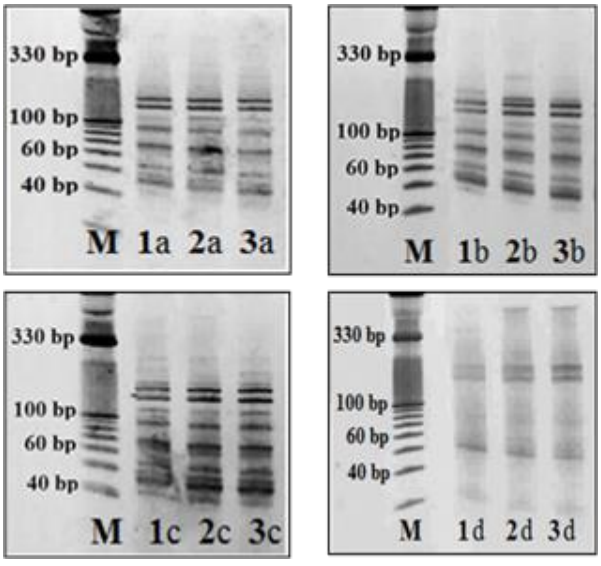

Figure 1 PCR amplification profiles generated with lus-miR168-F/miR-R markers across tissues of buds (a), flower petals (b), bolls (c), leaves (d) of flax genotypes. Legend: M- 10 bp DNA Ladder, genotypes: 1- Amon, 2- Libra, 3- Raciol.

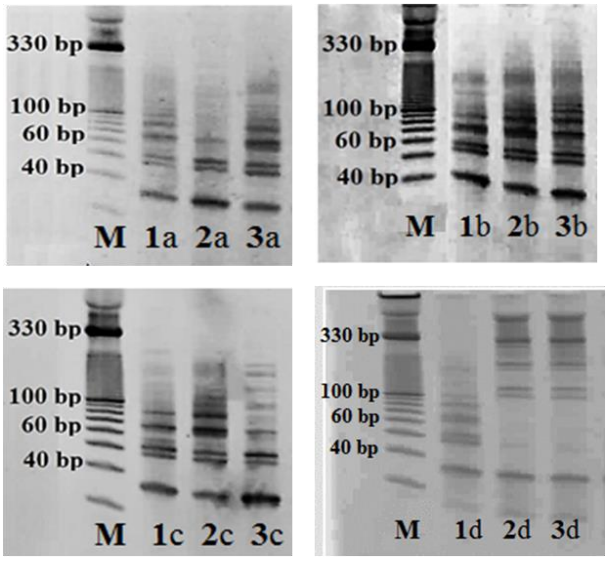

Figure 2 PCR amplification profiles generated with gm-miR156b-F/miR-R markers across tissues of buds (a), flower petals (b), bolls (c), leaves (d) of flax genotypes. Legend: M- 10 bp DNA Ladder, genotypes: 1- Amon, 2- Libra, 3- Raciol.

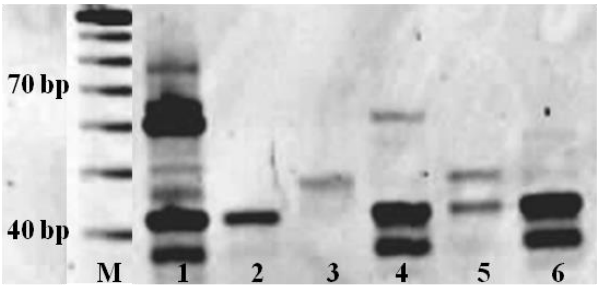

Figure 3 PCR amplification profiles generated with markers gm-miR156b/gm-miR171a of *Mespilus germanica* genotypes. Legend: M - 10 bp DNA Ladder, genotypes: 1 - Sz. Rozsa, 2 - Holandská Veľkoplodá, 3 - GR1, 4 - GR2, 5 - GR3, 6 - GR4.

DNA polymerase, 0.8 mmol.dm^{-3} dNTPs (Bioline) and 1 × DreamTaq Buffer (KCl, $(NH_4)_2SO_4$, 20 mmol.dm^{-3} $MgCl_2$). The PCR amplification programme used the 'touchdown' method as follows: initial denaturation at 94 °C for 5 min; 5 cycles of 30 s at 94 °C, 45 s at 64 °C (with a 1 °C decrease in annealing temperature per cycle), and 60 s at 72 °C; 30 cycles of 30 s at 94 °C, 45 s at 60 °C, and 60 s at 72 °C; and the final extension at 72 °C for 10 min. The samples were subsequently stored at 8 °C.

The PCR products were separated using 15% TBE-PAGE gels, running in 1 × TBE Running Buffer at a constant power 90 V, 25 mA for 120 min. The polyacrylamide gels were stained with the GelRed™ Nucleic Acid Gel stain and were visualized in the G-Box Syngene electrophoresis documentation system. For the recording of loci number and unique identification of fragments, the gels were analyzed by the GeneTools software (Syngene).

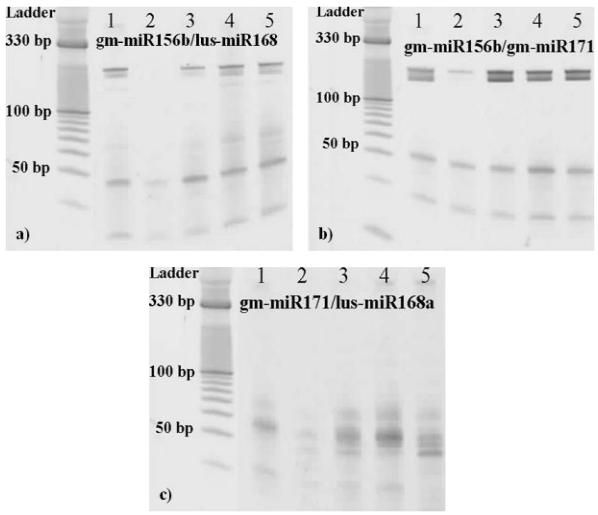

Figure 4 PCR amplification profiles generated with combination of markers: a) gm-miR156b/lus-miR168, b) gm-miR156b/gm-miR171a and c) gm-miR171a/lus-miR168 of *Silybum marianum* samples.
Legend: M - 10 bp DNA Ladder, genotypes: 1 - Silyb 1, 2 - Silyb 2, 3 - Mirel, 4 - Silma, 5 - sample of unknown origin.

miRNA expression analysis by qRT-PCR

The methodology of qRT-PCR analysis of miRNA was done based on **Barvkar et al., (2013)** and **Neutelings et al., (2012)** approach. For qRT-PCR analysis were used three genotypes of flax differing content of alpha-linolenic acid (Amon, raciol, Libra). From the 10-days old *in vitro* seedlings was isolated miRNA by PureLink miRNA Isolation Kit (Life Technologies). Consequently was miRNA diluted in 10 mM Tris-HCl, pH 7.0 in ratio 1:1 and quantified by NanoPhotometr (Implen). By means of the kit NCode™ miRNA First-Strand cDNA Synthesis and qRT-PCR (Invitrogen) was done miRNA polyadenylation and cDNA synthesis. qRT-PCR reactions were performed by SYBR® GreenER qPCR SuperMix Universal (Invitrogen) based on manufacturer instructions. cDNA was diluted in ratio 1:10. Two types of miRNA, gm-miR156b and lus-miR168 were analyzed. As a reference gene *UBE2* (Ubiquitin-conjugating enzymes E2) was selected **(Barvkar et al., 2013)**.

The conditions of qRT-PCR were as followed: 2 min incubation at 50 °C, 95 °C 10 min, 40 cycles of 95 °C 15 sec, 57 °C 60 sec and 95 °C 10 sec. Fluorescence reading of the PCR product took place after the analysis phase of the amplification and melting points were read for 30 seconds and the temperature rise of 0.5 °C. Analyzes were performed by CFX96 Real-Time detection system. Reactions were done in triplicates.

On the basis of the average value of threshold cycle of miRNA and reference gene *UBE2* value $2^{-\delta CT}$ **(Shi and Chiang, 2005)** was calculated.

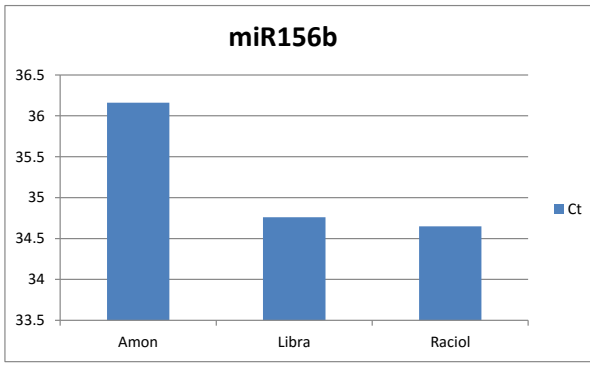

Figure 5 Comparison of gm-miR156b expression based on values of threshold cycle (C_T) in flax genotypes of different alpha-linolenic acid content.

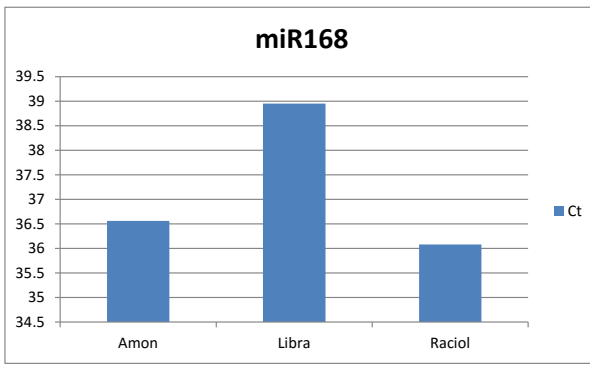

Figure 6 Comparison of lus-miR168 expression based on values of threshold cycle (C_T) in flax genotypes of different alpha-linolenic acid content.

RESULTS AND DISCUSSION

The aim of our research was mapping the abundance, polymorphism and activity of several conservative (miR156, miR168 and miR171) miRNAs in plants genome.

One of the extensively reviewed miRNA networks in plants includes the conservative miR156 family, which consists of 10 miRNAs (miR156a-j), and miRNA156a-f have identical nucleotide sequences (miRBase) **(Bari et al., 2013)**. miR156 family members are predicted to be associated with the mRNAs of genes encoding the DNa-binding proteins - squamosa promoter binding protein (SBP), transcription factor in monocot and dicots and F-box protein sequences **(Barvkar et al., 2013; Xie et al., 2010)**. SBS transcription factors regulated many developmental processes of plants. miR156 regulates processes at post-germinative stages, which is important for the transition to autotrophic growth, it regulates transition phase from the juvenile to adult stage **(Nonogaki, 2010)** and also play a critical role in reproductive phases such as shoot maturation **(Shikata et al., 2009)**. The study of **Kulcheski et al., (2010)** provided evidence that the expression stability of miR156b was the highest across the soybean tissue and applied stress conditions.

One of the target sequences of miR168 family are sequences of cytochromeP450 which is involved in a wide range of biosynthetic reactions, including fatty acid biosynthesis. The miR168 is also considered as the biomarker of plant stress response **(Bej and Basak 2014)**.

Target sequences of the miR171a are represented by HAMs genes **(Bari et al., 2013)** which belong to the GRAS transcription factor family. These genes play crucial roles in diverse fundamental processes of plant growth and development **(Huang et al., 2015)**.

Of the following figures (Figure 1, 2, 3 and 4) it is observed that molecular markers based on microRNA represent polymorphic and significant type of molecular markers. It is more apparent that the expression profile of miR156b, miR168 and miR171a is species specific and even tissue specific as confirmed by several studies (**Barvkar et al., 2013; Neutelings et al., 2012**). Tissue-specific expression of miRNA (Figure 1 and 2) also points to the different levels of miRNA activity in various stages of development of the plant organism. The same miRNA can be found in different abundance among tissue types or developmental stages, indicating the spatially and temporally regulated expression patterns of plants miRNAs **(Jones-Rhoades et al., 2006; Xie et al., 2010)**. From this point of view is quite significant polymorphism profile of miR156b in various flax tissues of genotypes varying in the content of alpha-linolenic acid (ALA). It can be observed visible difference among individual genotypes in regard to miRNA profile. Interesting is distinguished pattern of intermediate type of flax genotype Amon (less than 3% content of ALA) and other two oily genotypes with higher ALA content (Raciol 30%, Libra more than 57%). Detected polymorphism by miRNA-based molecular markers may indicate sequence changes in the miRNA loci, which consequently may change the regulation pattern of targeted genes **(Htwe et al., 2015; Fu et al., 2013)**.

Considering the indirect correlation between the abundance of miRNAs and the expression level of their target sequences **(Barvkar et al., 2013; Neutelings et al., 2012)** we can assume the spatially and temporally machinery of metabolic processes regulation as well as the expression patterns of plant miRNAs.

The effectiveness and reliability of miRNA molecular markers has been confirmed for medlar genotypes (Figure 3). Medlar as a source of new valuable compounds and their pharmacological properties, has gained a value in human consumption and commercial importance in recent years **(Rop et al., 2011)**. By the combination of miRNAs markers, miR156b and miR171a, was possible to distinguish almost all six genetic resources collected on the territory of Slovak Republic. This confirms the status that miRNA-based molecular markers comprise a novel functional molecular marker **(Yadav et al., 2014; Fu et al., 2013)**. miR171 potentially targets a beta-1,3 glycanase-like transcript. The corresponding enzyme is implicated in developmental as well as biotic and abiotic stress processes **(Roy Choudhury et al., 2010)**.

Silybum marianum (L.) Gaertn. or milk thistle is a medicinal plant of unique pharmaceutical properties. It is the most cultivated medicinal plant in Slovakia. In the years 2014-2015 it exceeded the growing area of 1000 hectares **(Habán et al., 2015)**.

Although monomorphic but miRNA-type specific microRNA profile can be observed in milk thistle genotypes of different origine (Silyb 1- Malanta, Slovak Republic, Silyb 2 - Šumperk, Czech Republic, Mirel - Brno, Czech Republic, Silma – Poland and sample of unknown origin) in two miRNA markers combination (Figure 4, a and b). The fingerprint profile amplified by primer pairs combinations ranged from 4 (gm-miR156b/gm-miR171a) to 7 (gm-miR156b/lus-miR168) miRNA loci per genotype. The another marker combination (Figure 4, c) has provided polymorphic miRNA loci pattern, even genotype specific. In comparison with previous two species, namely the flax genotypes, it can be stated that the abundance of analyzed types of miRNAs in milk thistle genome is not so significant although the studied miRNAs families represent conservative types of miRNA families. It seems that for the mapping of this genome will be required the application of species-specific miRNAs.

It is apparent that the research of food resources includes various approaches based on application of different types of molecular markers or molecular analyses **(Balážová et al., 2016; Gálová et al., 2015; Žiarovská et al., 2015)**.

Results based on qRT-PCR and evaluation of $2^{-\delta CT}$ value suggest significant difference in miR156b activity of Amon genotype (low content of ALA) in comparison to other two genotypes with medium and high content of alpha-linolenic acid (Figure 5). Within the miR168 expression analysis was the difference recorded between genotype Libra (high content of ALA) and other two genotypes Amon and Raciol (Figure 6).

The most of miRNA families, including miR156 and miR168, are characterized by negative correlation between miRNA expression and expression of their target sequences. It means, that if the expression of a specific miRNA increased, the activity of target sequences

regulated by this miRNA will be suppressed and vice versa.

The miR168 expression profile, from the above point of view, can indicate two possible explanations. As we mentioned before, most of the miRNA families have several target sequences, not excluding these two types of miRNAs. Significantly higher expression of miR168 in Libra genotype (57% ALA) points out downregulation of the target sequences, one of which is the cytochrome P450 involved in a wide range of biosynthetic reactions. It seems that the genome of this genotype mediates the production of miRNA168 increasingly over other genotypes. . It should be recalled that for the analysis were used 10-days old seedling *in vitro*. There is another explanation connected to reaction of the flax genome to stress factor presented by cultivation under *in vitro* conditions. miR168 as a stress biomarker molecule may indicate greater sensitivity of genotypes with high content of fatty acid to abiotic stress.

From the Figures 2d and Figure 5 can be observed similar pattern of miRNA expression in leaves tissues. miRNA loci profile generated by miR156b-F/miR-R markers and expression profile generated by qRT-PCR seems to show different behavior of miR156 in genotype Amon in comparison to oily genotypes Libra and Raciol. The answer might be found in the character of the major group of target sequences of miR156, what does mean the SBS transcription factors. It seems that in oily genotypes (Libra and Raciol) are, due to downregulation of miR156, its target sequences more active than in intermediate genotype (Amon), which may be associated with a plant structure of oily genotypes or indirectly with higher metabolism of fatty acids in those two genotypes. These results are confirmed by the research of **Nonogaki (2010)**. As the consequence of a decrease expression in miRNA levels is the increased accumulation of some of SPS transcripts (and proteins) which are necessary for the juvenile to adult transition in *Arabidopsis* seedlings.

The aim of the research was to highlight the broad spectrum of miRNA molecules behavior in various food resources, functional foods and medicinal plant. As was observed, different plants miRNAs accumulate at different levels depending on developmental stage or the plant tissues. It can be presumed that their regulation pattern of gene expression in human genome may be influenced also by several aspects of human metabolism and health conditions.

CONCLUSION

The aim of the research was to highlight the broad spectrum of regulatory impact activities of miRNA molecules in different plant species of nutritional and pharmaceutical uses. As has been recorded, the polymorphism and expression of analyzed gm-miR156, lus-miR168 and gm-miR171a is not only species- but also tissue- and developmentally-specific. It points out the fact that, depending on the type of food of plant origin (species, state of maturity, bio products or traditional agriculture), miRNA molecules can regulate the expression of genes of the human genome in many ways.

REFERENCES

Bari, A., Orazova, S., Ivashchenko, A. 2013. miR156- and miR171- binding sites in the protein-coding sequences of several plant genes. *BioMed Research International*, vol. 2013, p. 1-7.

Balážová, Ž., Petrovičová, L., Gálová, Z., Vivodík, M. 2016. Molecular characterisation of rye cultivars. *Potravinarstvo*, vol. 10, no. 1, p. 54-58. http://dx.doi.org/10.5219/522

Bartel, D. P. 2004. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell*, vol. 116, no. 2, p. 281-297. http://dx.doi.org/10.1016/S0092-8674(04)00045-5

Ali, A. S., Ali, S., Ahmad, A., Bao, B., Philip, P. A., Sarkar, F. H. 2011. Expression of microRNAs: potential molecular link between obesity, diabetes and cancer. *Obesity Review*, vol. 12, no. 12, p. 1050-1062. http://dx.doi.org/10.1111/j.1467-789X.2011.00906.x PMid:21767342

Barvkar, V. T., Pardeshi, V. C., Kale, S. M., Qiu, S., Rollins, M., Datla, R., Kadoo, N. Y. 2013. Genome-wide identification and characterization of microRNA genes and their targets in flax (*Linum usitatissimum*): Characterization of flax miRNA genes. Planta, vol. 237, no. 4, p. 1149-1161. http://dx.doi.org/10.1007/s00425-012-1833-5 PMid:23291876

Bej, S., Basak, J. 2014. MicroRNAs: The potential Biomarkers in Plant Stress Response. *American Journal of Plant Science*, vol. 5, no. 5, p. 748-759. http://dx.doi.org/10.4236/ajps.2014.55089

Erson-Bensan, A. E. 2014. Introduction to MicroRNAs in Biological Systems. In: Yousef, M. and Allmer, J. ed. (2014) *MiRNomics. MicroRNA Biology and Computational Analysis.* New York : Springer Science+Business Media, p. 1-14. ISBN 978-1-62703-747-1.

Fu, D., Ma, B., Mason, A. S., Xiao, M., Wei, L., An, Z. 2013. MicroRNA-based molecular markers: a novel PCR-based genotyping technique in *Brassica* species. *Plant Breeding*, vol. 132, no. 4, p. 375-381. http://dx.doi.org/10.1111/pbr.12069

Gálová, Z., Vivodík, M., Balážová, Ž., Kuťka-Hlozáková, T. 2015. Identification and differentiation of *Ricinus communis* L. using SSR markers. *Potravinarstvo*, vol. 9, no. 1, p. 556-561. http://dx.doi.org/10.5219/516

Ganie, S. A., Mondal, T. K. 2015. Genome-wide development of novel miRNA-based microsatellite markers of rice (*Oryza sativa*) for genotyping applications. *Molecular Breeding*, vol. 35, no. 51, p. 1-12. http://dx.doi.org/10.1007/s11032-015-0207-7

Habán, M., Luščáková, D., Kobidová, R., Habánová, M. 2015. Production and quality of milk thistle (*Silybum marianum* L. Gaertn.) cultivated during the vegetation periods 2012 - 2014 in a warm agri-climatic macroregion. Proceeding: Scientific conferences Banat´s university of agricultural sciences and veterinary medicine. Timişoara: Banat University of Agricultural Sciences and Veterinary Medicine, pp. 18. ISSN 2343-9459.

Hirschi, K. D. 2012. New foods for thought. *Trends in Plant Science*, vol. 17, no. 3, p. 123-125. http://dx.doi.org/10.1016/j.tplants.2012.01.004

Hlavačková, L., Ražná, K., Žiarovská, J., Bežo, M., Bjelková, M. 2015. Application of miRNA-based markers in flax genotypes characterisation. Proceeding: International Cooperation for the Future Agricultural Researches. Debrecen: University of Debrecen, pp. 37-40. ISBN 978-963-473-816-9.

Htwe, N. M. P. S., Luo Z. Q., Jin, L. G., Nadon, B., Wang, K. J., Qiu, L. J. 2015. Functional marker development of miR1511-InDel and allelic diversity within the genus *Glycine*. *MBC Genomics*, vol. 16, no. 467. http://dx.doi.org/10.1186/s12864-015-1665-3

Huang, W., Xian, Z., Kang, X., Tang, N., Li, Z. 2015. Genome-wide identification, phylogeny and expression analysis of GRAS gene family in tomato. *BMC Plant Biology*, vol. 15, no. 209, p. 1-18. http://dx.doi.org/10.1186/s12870-015-0590-6

Chen, C., Ridzon, D. A., Broomer, A. J., Zhou, Z., Lee, D. H., nquyen, J. T., Barbisin, M., Xu, N. L., Mahuvakar, V. R., Andersen, M. R., Lao, K. Q., Livak, K. J., Guegler, K. J. 2005. Real-time quantification of microRNAs by stem-loop RT-PCR. *Nucleic Acids Research*, vol. 33, no. 20, p. 1-9. http://dx.doi.org/10.1093/nar/gni178

Jones-Rhoades, M. W., Bartel, D. P., Bartel, B. 2006. MicroRNAs and Their Regulatory Roles in Plants. *Annual Review of Plant Biology*, vol. 57, p. 19-53. http://dx.doi.org/10.1146/annurev.arplant.57.032905.105218 PMid:16669754

Kulcheski, F. R., Marcelino-Guimaraes, F. C., Nepomuceno, A. L., Abdelnoor, R. V., Margis, R. 2010. The use of microRNAs as reference genes for quantitative polymerase chain reaction in soybean. *Analytical Biochemistry*, vol. 406, no. 2, p. 185-192. http://dx.doi.org/10.1016/j.ab.2010.07.020 PMid:20670612

Lukasik, A., Zielenkiewicz, P. 2014. *In silico* identification of plant miRNAs in mammalian breast milk exosomes - a small step forward? *PLOS one*, vol. 9, no. 6, e99963. http://dx.doi.org/10.1371/journal.pone.0099963

Mondal, T. K., Ganie, S. A. 2014. Identification and characterization of salt responsive miRNA-SSR markers in rice (*Oryza sativa*). *Gene*, vol. 535, no. 2, p. 204-209. http://dx.doi.org/10.1016/j.gene.2013.11.033 PMid:24315823

Neutelings, G., Fénart, S., Lucau-Danila, A., Hawkins, S. 2012. Identification and characterization of miRNAs and their potential targets in flax. *Journal of Plant Physiology*, vol. 169, no. 17, p. 1754-1766. http://dx.doi.org/10.1016/j.jplph.2012.06.011 PMid:22841625

Nonogaki, H. 2010. MicroRNA gene regulation cascades during early stages of plant development. *Plant and Cell Physiology*, vol. 51, no. 11, p. 1840-1846. http://dx.doi.org/10.1093/pcp/pcq154

Padmalatha, K., Prasad, M. 2006. Optimization of DNA isolation and PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India. *African Journal of Biotechnology*, vol. 5, no. 3, p. 230-234.

Palmer, J. D., Soule, B. P., Simone, B. A., Zaorsky, N. G. 2014. MicroRNA expression altered by diet: Can food be medicinal? *Ageing research reviews*, vol. 17, p. 16-24. http://dx.doi.org/10.1016/j.arr.2014.04.005

Ražná, K., Hlavačková, L., Bežo, M., Žiarovská, J., Habán, M., Sluková, Z., Pernišová, M. 2015. Application of the RAPD and miRNA markers in the genotyping of *Silybum marianum* (L.) Gaertn. *Acta phytotechnica et zootechnica*, vol. 18, no. 4, p. 83-89. http://dx.doi.org/10.15414/afz.2015.18.04.83-89

Rop, O., Sochor,J., Jurikova, T., Zitka, O., Skutkova, H., Mlcek, J., Salas, P., Krska, B., Babula, P., Adam, V., Kramarova, D., Beklova, M., Provaznik, I., Kizek, R. 2011. Effect of Five Different Stages of Ripening on Chemical Compounds in Medlar (*Mespilus germanica* L.). *Molecules*, vol. 16, no. 1, p. 74-91. http://dx.doi.org/10.3390/molecules16010074

Roy Choudhury, S., Roy, S., Singh, S. K., Sengupta, D. N. 2010. Molecular characterization and differential expression of ß-1,3-glucanase during ripening in banana fruit in rečsponse to ethylene, auxin, ABA, wounding, cold and light-dark cycles. *Plant Cell Reports*, vol. 29, no. 28, p. 813-828. http://dx.doi.org/10.1007/s00299-010-0866-0

Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, J. A., Allard, R. W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academic of Sciences*, vol. 81, no. 24, p. 8014-8018. http://dx.doi.org/10.1073/pnas.81.24.8014 PMid:6096873

Shi, R., Chiang V. L. 2005. Facile means for quantifying microRNA expression by real-time PCR. *Biotechniques*, vol. 39, no. 4, p. 519-525. http://dx.doi.org/10.2144/000112010 PMid:16235564

Shikata, M., Koyama, T., Mirsuda, N., Ohme-Takagi, M. 2009. Arabidopsis SBP-Box genes SPL10, SPL11 and SPL2 control morphological change in association with shoot maturation in the reproductive phase. Plant and Cell Physiology, vol. 50, no. 12, p. 2133-2145. http://dx.doi.org/10.1093/pcp/pcp148

Singh, S. K., Bhadra, M. P., Girschick, H. J., Bhadra, U. 2008. MicroRNAs - micro in size but macro in function. *FEBS Journal*, vol. 275, no. 20, p. 4929-4944. http://dx.doi.org/10.1111/j.1742-4658.2008.06624.x

Taylor, R. S., Tarver, J. E., Hiscok, S. J., Donoghue, P. C. J. 2014. Evolutionary history of plant microRNAs. *Trends in Plant Science*, vol. 19, no. 3, 2013, p. 175-182. http://dx.doi.org/10.1016/j.tplants.2013.11.008 PMid:24405820

Xie, Z., Khanna, K., Ruan, S. 2010. Expression of microRNAs and its regulation in plants. *Seminars in Cell and Developmental Biology*, vol. 21, no. 8, p. 790-797. http://dx.doi.org/10.1016/j.semcdb.2010.03.012 PMid:20403450 PMCid:PMC2939293

Yadav, C. B. Y., Muthamilarasan, M., Pandey, G., Prasad, M. 2014. Development of novel microRNA-based genetic markers in foxtail millet for genotyping applications in related grass species. *Molecular Breeding*, vol. 34, p. 2219-2224. http://dx.doi.org/10.1007/s11032-014-0137-9

Zhang, L., Hou, D., Chen, X., Li, D., Zhu, L., Zhang, Y. et al. 2012. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell Research*, vol. 22, p. 107-126. http://dx.doi.org/10.1038/cr.2011.158

Zhang, B., Pan, X., Cannon, Ch. H., Cobb, G. P., Anderson, T. A. 2006. Conservation and divergence of plant microRNA genes. *The Plant Journal*, vol. 46, no. 2, p. 243-259.

Žiarovská, J., Grygorieva, O., Zeleňáková, L., Bežo, M., Brindza, J. 2015. Identification of sweet chesnut pollen in bee pollen pellet using molecular analyses. *Potravinarstvo*, vol. 9, no. 1, p. 352-358. http://dx.doi.org/10.5219/497

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EFFECTS OF CROSS-LINKING MODIFICATION WITH PHOSPHORYL CHLORIDE ($POCL_3$) ON PYSIOCHEMICAL PROPERTIES OF BARLEY STARCH

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ABSTRACT

Chemical methods are one of the comon method in starch modification. This study aimed at investigating of cross-link affection of phosphoryl chloride with two different levels 0.5 and 1g.kg^{-1} in order to enhance funciotnal proeprties and physiochemical changes on extracted starch from barley variety Bahman which cultivates in Chahr-Mahal Bakhtiari Province of Iran. Obtained results indicated that cross-linking leads to reduce sweeling power of strach granuls compred to natural starch and the amount of reduciton increase via the substitituin level. Powerfull cross-linkingnetween starch chains casue more resistance of granules to seweeling which is increased by means of cross-linking dgree. Additioally, investigationresults from synersis revealed that releasing water percentage in cross-linked starches increase in comparison to natural starches and this amount depends onthe amount of cross-link surface with a significantly difference in (α <0.05). Gelatinization temperature in both levels negligibly increased by modification where in low level of cross-linking was more. Furthermoe evaluating gelation temperatures of both natural and cross-linked modified starches showed that addition of phosphate groups in starch and creating extra coovalent bonds make granues more compressed reulting in slight increase of To, Tp, Tcin barley starch. Icreasing of temperature observed more in less concentration of cross-links. Evaluation of viscosity changes also revealed that this modification depending on increasing the amount of Phosphoryl Chloride led to increasing peak temperature, diminish peak and setback viscosity. Result also exhibited that in morphological level, cross-link causes to incidence changes in particles' diameter size. The comparison of diameter average and frequency between natural starch and cross-links starch exhibited that in cross-linkd treatment with 0.5% phosphoryl chloride, increase in frequency of granules with diameter of 6 – 10μm and >20 μm obersced. While frequency of granules with diamater size of 2 – 6 μm and 10 – 20 μm has been reduced to 0 which create bigger granules.

Keywords: Barley; starch; modification; cross-linking; physiochemical properties

INTRODUCTION

Barley belongs to Poaceae and Hordeum species **(Sullivan et al., 2013)** uses more in malting, feeding animals, production of starch and ethanol as well **(Myllärinen et al., 1998)**. Starch is composed of two main constituents including amylose and amylopectin (72 – 87%). Starch is used as thickener, stabilizer, and gelling agent in food industries **(Dubois et al., 2001)**, but due to some restricting factors such as low thermal and cutting resistance **(Singh and Singh, 2005)** high tendency to staling and high synersis (**Yosif et al., 2012)** its application has limited in industries; however application can widen through modification **(Singh and Singh, 2005)**. First time, starch modification operated in year 1800 **(Kaur, Singh and Singh, 2006)**. Several targets define for development of functional properties such as strengthening the bonds, increase of thermal resistance, and increase of water binding capacity, emulsion stability and economic benefits **(Light, 1989)**. Cross-linking or intertwined starch is one of the conventional chemical modifications **(Zhao et al., 2012)**. Cross-linking factors include Sodium triphosphate (STM), Epihydrochlorine (EPI), phosphoril chloride (POCl3), and mixture of adipic acid, anhydride acetic and vinyl chloride **(Singh et al., 2007; Zhao et al., 2012)**. In this method, reaction factors react with starch hydroxyl groups **(Miyazaki et al., 2006)** which enhance through covalent or hydrogen bond inter and among granule molecules **(Singh et al., 2007; Ackar et al., 2010)**. Cross-linked starch strengthens versus heat, acid and cutting in comparison with natural starch **(Hung and Morita, 2005; Polnaya et al., 2013; Raina et al., 2007; Xiao et al, 2012)**. The target of this study is to investigate barley starch properties. Base on **Jun et al., (2003)** barley and corn starches use to microencapsulation of volatile compounds of flavor in meat industry **(Abbas et al., 2010)**.

MATERIALS AND METHODS

In present study, starch has extracted from barley Bahman variety which cultivated in Lordegan region, Chaharmahale Bakhtiari province of Iran. Initially 100 g of barley flour weighed with balance model Mark Sartoris AC 120 s, Germany and 0.0001 accuracy, mixed with 500 mL sodium hydroxide solution (0.005 – 0.025 M) and stirred at 25 °C for 30 min. Obtained mixture centrifuged with 1400 g (centrifuge Tehtnika, model 322-A, Slovenia), then sedimentation filter through a screen with mesh size 270 (50 μm). Permeated suspension neutralized with chloridric acid 1 M and recentrifuged, and over layer of starch separated with spatula remained sedimentation

dissolved in the water again and dissolving continues to reach the minimum amount of creamy layer on it (3 times). Final sedimentation dried in oven (model Mark Memmert UNB-400, Germany) at 40 °C for 24 hr **(Lim et al., 1992)**.

PRODUCTION OF CROSSLINKED STARCH

Regarding production of cross-linked starch, **Kaur et al., (2012)** method used. In this method, 15 g of starch weighed with balance model Marksaritus AC 120S, Germany AND 0.0001 accuracy, then mixed with 24ml water and 0.3 g sodium sulfate added to it, pH of obtained mixture(pH meter model Mark metrum 827, Switzerland used to measured pH) adjusted by sodium hydroxide solution (0.5M) at 25 °C. Phosphoryl chloride (0.5 and 1 g per starch kilo) added by micro-syringe and immediately container sealed. pH adjusted by chloridric acid (0.1 M) on 5.5 after 1hr. sedimentation washed by distilled water and filtered by vacuum filter and finally dried in oven Markmemmert model UNB-400, Germany) at 40 °C **(Kaur et al., 2012).**

DETERMINATION OF SWEELING POWER

Lich et al., (1959) method used to determine swelling power. Initially, 0.1 g sample base on dried weight weighed in lidded test tube and 10 mL water added to it. Tubes placed and shook in water bath (Mark hak model SWB-20, Germany and equipped with shaker with constant race) at 95 °C for 30 min, then cooled to ambient temperature and centrifuged in 2500 x g for 10 min. Supernatant accurately removed and tube containing sedimentation reweighed. Regarding equation 1 swelling power measured **(Leach et al., 1959)**.

Equation (1):

SP – Sweeling percent

$$SP = \frac{\textbf{final weight} - \textbf{weight of empty pipe}}{\textbf{starch weight}} \times \mathbf{100}$$

DETERMINATION OF SYNERSIS PERCENT

To determine synersis percent, **Gioti et al., (2006)** method was used. Starch suspension 5% w/w prepared and 30 min mixed in water bath (model Markmemert w3 B10), heated at 90 – 95 °C, and then quickly cooled to ambient temperature in cooling bath. Starch paste placed at 4 °C for 24 hr, centrifuged at 2700 x g for 15 min and measured released water reported as synersis percent **(Jyothi, Moorthy and Rajasekharan, 2006)**.

INVESTIGATION OF VISCOSITY CHANGES

To determine viscosity changes, Initially, a 8% w/w suspension of starch prepared in pH = 5, then viscosity changes measured by viscometer model Brookfield DV III, America in temperature range 40 – 93 °C, keeping at 93 °C and then reduces it from 93 °C to 40 °C **(Das et al., 2010)**. Determination of substitution degree of cross-link

Substitution degree defines as the number of hydroxyl group in each anhydrous glucose having the ability of derivation with replacing groups **(Yosif et al., 2012)**. To investigate of this factor in cross-link starch, measures based on **Ackar et al., (2010)** method, viscosity data and equation 2 **(Hung and Morita, 2005)**.

Equation (2):

DS – Degree of substitution

$$DS = \frac{\textit{viscosity peak of natural starch} - \textit{viscosity peak of modified starch}}{\textit{viscosity peak of natural starch}} \times \mathbf{100}$$

INVESTIGATION OF THERMAL PROPERTIES

To investigate thermal properties of barley starch **Bello-Perez et al., (2010)** method used. Differential scanning calorimeter used to conduct thermal parameters of starch. In this method, 2 mg starch base on dry weight weighed in aluminum container and 7 mL deionized water added to it, then container sealed, and placed in ambient temperature in order to uniformly distribution of water and homogenization of sample . Sample placed in DSC model F3-200 and heated with race of 10 °C.min^{-1} from 20 °C – 120 °C, and automatically present data including Onset (To), peak (Tp), conclusive (Tc) and ΔH **(Bello-Perez, et al., 2010)**.

INVESTIGATION OF MORPHOLOGICAL PROPERTIES

Electronic microscope model Markzayef used take images and **Blupers et al., (2010)** method with a few changes. Samples fix on a conductive stick and cover with a gold layer **(Bello-Perez, et al., 2010)**. Image proplus software used to analyze images.

Statistical analysis

Complete random design and Duncan test using software SPSS ver. 21th used to conduct statistical analysis of data (α <0.05).

RESULTS AND DISCUSSION

DEGREE OF CROSS LINKING

Table 1 shows that increase of cross linking, cross-linking degree increased. Obtained results were in agreement with **Xiao et al., (2012)**. on investigation of different concentration of epi hydrochlorine on rice starch where cross linking degree increased within increase of epihydrochlorine concentration (16) **Co et al., (2010)**. Reported that an increase 0 – 10% of cross-linking concentration on corn starch cross-linking degree increased **(Koo et al., 2010)**.

SWELL POWER

Comparison of obtained results of swelling power of control and modified samples have summarized in Table 2 revealed a significant difference. Creation of cross-links reduces swelling power of granules with respect to natural starch and this reduction increase by the amount of substitution level. These results were in agreement with **Kim and Yoo (2010)** about using POCl3 on sweet potato and **Majzoobi et al., (2012)** in investigation of wheat starch phosphorlization. It is thought that reduction of swelling power attributed to creation of intermolecular bridges by remained phosphorus after cross linking reaction **(Majzoobi et al., 2012)**.

Cross-linking develops hydrogen bonds among granules and restricts swelling during gelatinization **(Kim et al., 2010)** due to high concentration of cross-linking degree in

Table 1 degree of cross-linking related to control starch and modfied starches.

Treatment	Degree of cross-link percent (%)
Control	0
Cross-linked starch (0.5%)	32.30
Cross-linked starch (1%)	46.15

Table 2 the average of sweeling power related to related to control starch and modfied starches.

Control	Cross-linked starch (0.5%)	Cross-linked starch (1%)
$14.21^{b} \pm 0.66$	$10.05^{a} \pm 2.02$	$7.27^{a} \pm 0.27$

Table 3 the amount of synersis in control and cross-linked starches.

Control	Cross-linked starch (0.5%)	Cross-linked starch (1%)
$65.10^{a} \pm 2.90$	$74.80^{b} \pm 2.34$	$78.94^{b} \pm 6.92$

Table 4 viscosity measurement of control and cross-linked starch.

Starch type	Setback viscosity	Breaking viscosity	Peak viscosity	Pasting Temp. (°C)	peak Temp (°C)
Control starch	5.4	0.96	2.6	63.60	69.55
Cross-linked starch (0.5%)	0.16	1.72	1.76	65.38	72.55
Cross-linked starch (1%)	0.08	1.36	1.4	66.98	76.2

Table 5 Affection of modification amount of thermal properties of barley starch.

Treatment	T_0	T_P	T_C	T_C- T_0	H(J/g)Δ
Control starch	59.8	65.4	73.2	13.4	-0.3412
Cross-linked starch (0.5%)	61.8	66.4	73.8	12	-0.2822
Cross-linked starch (1%)	60.7	65.9	73.4	12.7	-0.2403

presence of more concentrations of cross-linking factor **(Kaur et al., 2012)**.

Choi and Ker (2004) believe that cross-linked starch granules have more resistance to time and temperature of heating. Strong links between starch chains leads to increase of granules' resistance to swelling i.e. by increasing cross-linking degree, resistance increases **(Yosif et al., 2012)**.

AFFECTION OF CROSS-LINKING ON SYNERSIS PERCENT

Comparison of data average in Table 3 shows a significant difference between the amount of released water in natural starch and modified starch. The amount of released water in intertwined starch has increased in comparison with natural starch, furthermore increased by increasing of cross-link factor. These results are in agreement with **Mirmoghtadaei et al., (2009)** on oat starch.

VISCOSITY

Table 4 depicts that heating in 40 – 93°C causes to increase of viscosity gradually. When starch heats in high amount of eater, granules swell, some parts of it dissolves and in form of suspension distributed in surrounded medium (continuous phase) and maximum of viscosity occurs in this point. Continuously, due to dispersion of starch molecule when temperature is constant at 93 °C viscosity decreased, then in temperature reduction from 93 °C to 43 °Conce again viscosity increases. It is thought that arrangement of amylose linear chains (those formerly dissolved as a result of heating and keeping in constant temperature) causes to create lots of cross-links within gel forming process **(Bello-Perez et al., 2010)**.

Investigation of obtained results exhibited that viscosity peak in cross-linked starch has reduced while temperature of viscosity peak increased. It is thought that increase of strong intermolecular bonds due to cross-linking process which results in swelling and decrease of viscosity peak. Besides by increasing of the cross-linking surface, viscosity peak showed more reduction and Peak temperature more increase.

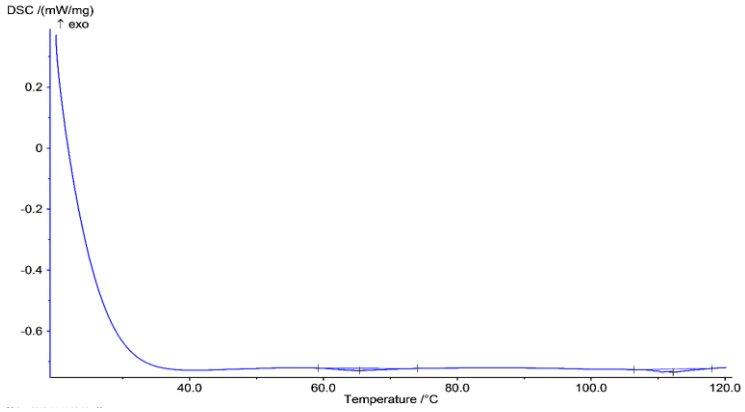

Figure 1 Thermal analysis curve of control starch.

THERMAL PROPERTIES

Analysis of obtained results of Table 5 including analysis of thermal curve related to natural barley starch (Figure 1), curves related to thermal analysis of cross-linked starch with 0.5% (Figure 2) and Figure 3 which shows cross-linked starch with 1% revealed that onset temperature (T0), peak temperature (Tp) and conclusive temperature (Tc) were 59.8 °C, 65.4 °C and 73.2 °C respectively. Obtained temperature were in the range measured by **Gujeral et al., (2013)** where the range of onset, peak and conclusive temperatures were 59.08 – 62 °C, 63.56 – 68.3 °C and 68.56 – 74.71 °C investigated respectively **(Gujral et al., 2013)**. Investigation of natural starch and cross-linked starch in present study suggested that this modification has increased negligibly To, Tp, and Tc of barley starch. These results were in agreement with **Majzoobi et al., (2012)** on wheat starch. Phosphates groups bond with starch molecules through covalent bonds and thus starch granules become more compressed, consequently followed by less molecule motivation and therefore gelatinization occurs in higher temperatures **(Carmona-Garcia et al., 2009)**.

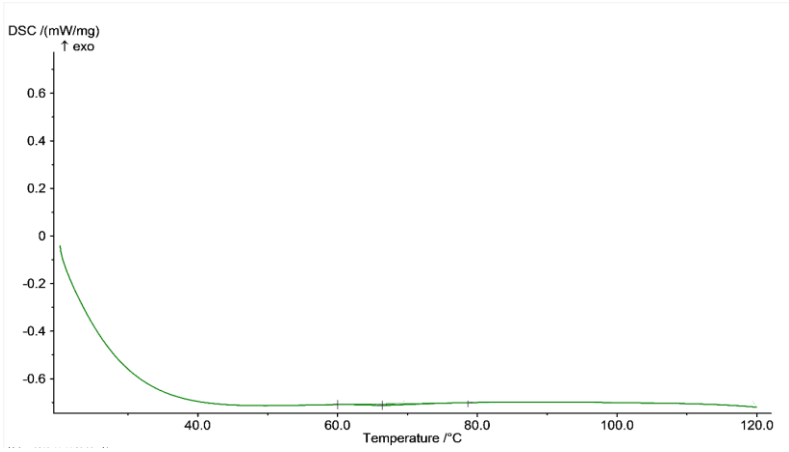

Figure 2 Thermal analysis curve of cross-linked starch (0.5%).

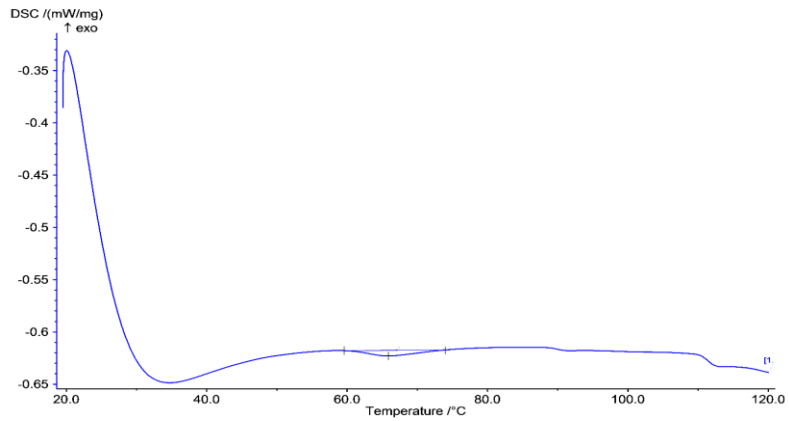

Figure 3 Thermal analysis curve of cross-linked starch (1%).

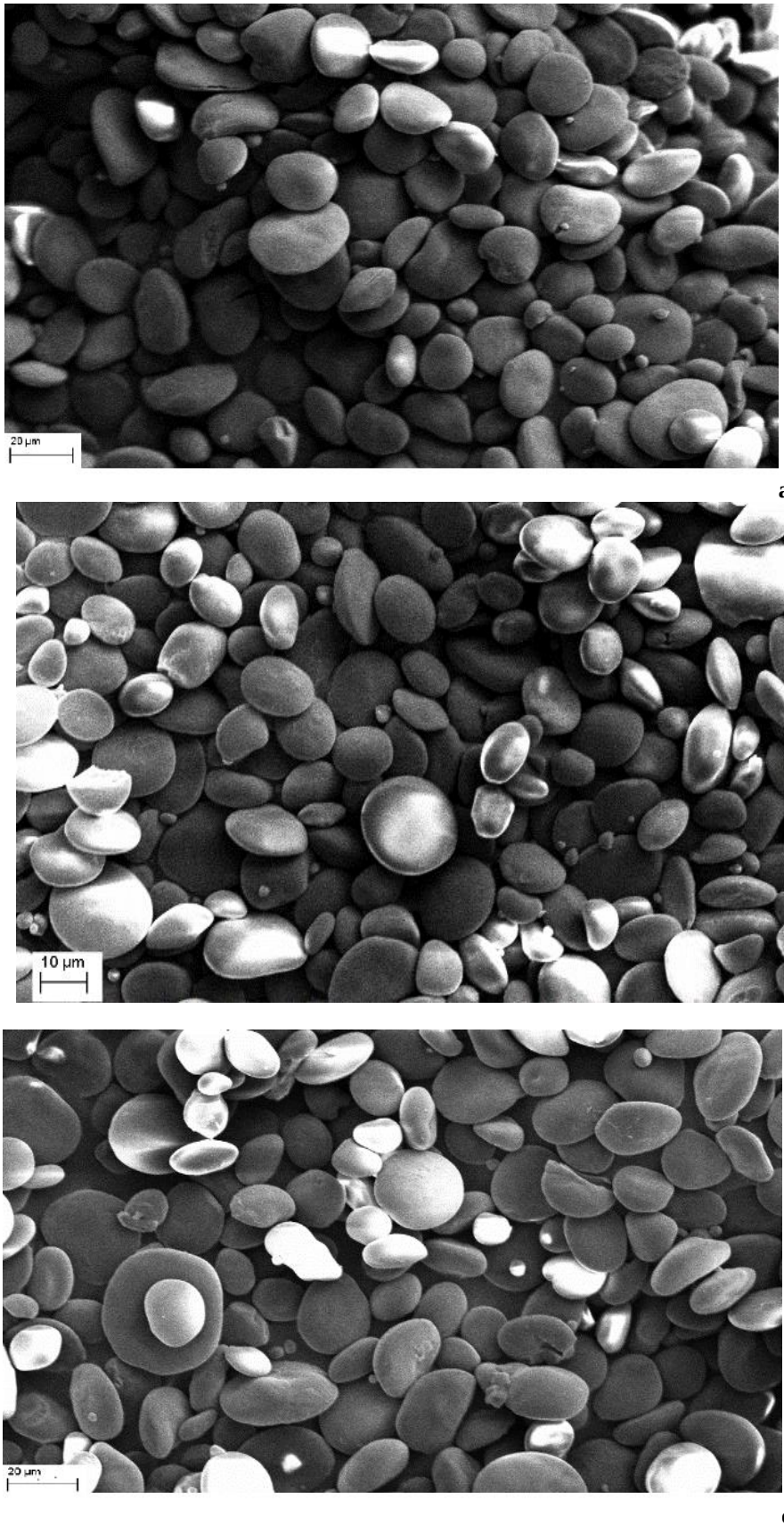

Figure 4 images of electronic microscope: a) control starch b) cross-linked starch (0.5%) c) cross-linked starch (1%) with zoom of 1.5 KX.

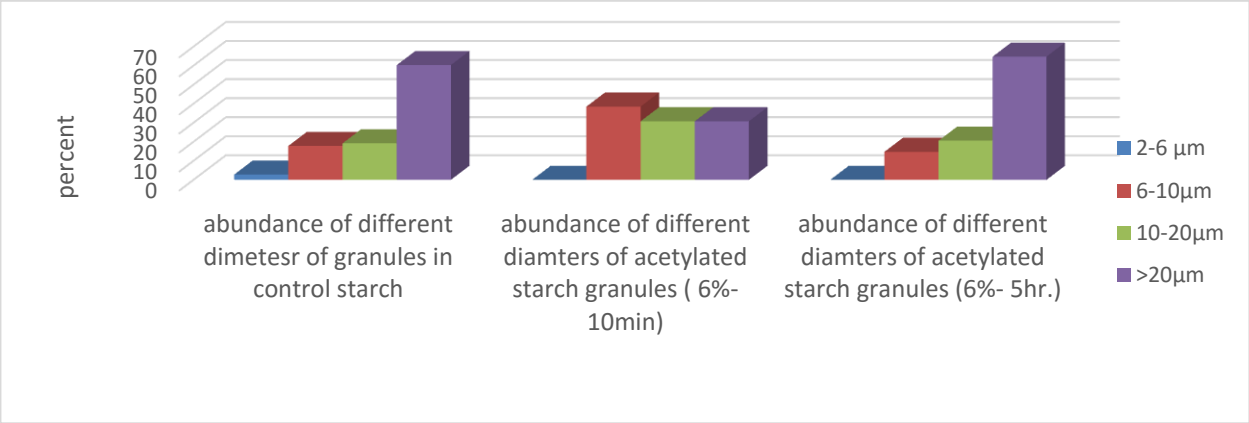

Figure 5 Investigations of abundant percent of granules' thickness in control and cross-linked starches.

Kim and Yoo (2010) found that in sweet potato, cross-linking with POCL3 creates no change in onset and conclusive temperatures. As an appropriate parameter of being crystal, gelatinization enthalpy point at damaging to molecule discipline due to breaking of hydrogen bonds **(Alvani et al., 2011)** after gelatinization. Low enthalpy introduces low stability of crystal structure **(Sing et al., 2006)**.

MORPHOLOGICAL PROEPRTIES

Comparison average of diameter and frequency (Figure 5) between image processing of natural starch and cross-linked starch (Figure 4) suggested that in crossed-link starch with 0.5% phosphoryl chloride, more granules with diameter of 20 µm and 6 – 10 µm observed.

While frequency of granules with diameter of 2 – 6 µm and 10 – 20 µm diminished. It is thought that aggregation of smaller granules and creation of larger granules is the reason of disappear of some size of granules. In cross-linked starch with 1% phosphoryl chloride, more reduction in granules with diameter of 20 µm observed, in addition that frequency of granules with 6 – 20 µm increased.

CONCLUSION

Starch modification creates novel properties in starch. Cross linking causes to increase of synersis and reduce of swelling so that has direct proportional with cross-linking factor (chloride phosphoryl). In comparison with control, Gelatinization temperature of modified starch increased. However no proportional trend observed through increase of cross linking. Furthermore the viscosity of cross-linked starch decreases with respect to control.

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REFERENCES

Abbas, K. A., Khalil, S. K., Hussin, A. S. M. 2010. Modified Starches and Their Usages in Selected Food Products. *Journal of Agricultural Science*, vol. 2, no. 2, p. 90-100. http://dx.doi.org/10.5539/jas.v2n2p90

Ackar, D., Babic, J., Subaric, D., Kopjar, M., Milicevic, B. 2010. Isolation of starch from two wheat varieties and their modification with epichlorohydrin. *Carbohydrate Polymer*, vol. 81, no. 1, p. 76-82. http://dx.doi.org/10.1016/j.carbpol.2010.01.058

Alvani, K., Qi, X. Tester, R, F. Snape, C, E. 2011. Physico-chemical properties of potato starches. *Food Chemistry*, vol. 125, no 3, p. 958-965. http://dx.doi.org/10.1016/j.foodchem.2010.09.088

Bello-Perez, L. A., Agama-Acevedo, E., Zamudio-Flores, P. B., Mendez-Montealvo, G., Rodriguez-Ambriz, S. L. 2010. Effect of low and high acetylation degree in the morphological, physicochemical and structural characteristics of barley starch. *Lwt-Food Science Technology*, vol. 43, no. 9, p. 1434-1440. http://dx.doi.org/10.1016/j.lwt.2010.04.003

Carmona-Garcia, R., Sanchez-Rivera, M. M., Mendez-Montealvo, G. 2009. Effect of the cross-linked reagent type on some morphological, physicochemical and functional characteristics of banana starch (*Musa paradisiace*). *Carbohydrate Polymer*, vol. 76, no. 1, p. 117-112. http://dx.doi.org/10.1016/j.carbpol.2008.09.029

Das, A. B., Singh, G., Singh, S., Riar, C. S. 2010. Effect of acetylation and dual modification on physico-chemical, rheological and morphological characteristics of sweet potato (*Ipomoea batatas*) starch. *Carbohydrate Polymer*, vol. 80, p. 725-732. http://dx.doi.org/10.1016/j.carbpol.2009.12.018

Dubois, I., Picton, L., Muller, G., Audibert- Hayet, A., Doublier, J. L. 2001. Structure/Rheological Properties Relations of Cross-linked Potato Starch Suspensions. *Journal of Applied Polymer Science*, vol. 81, no. 10, p. 2480-2489. http://dx.doi.org/10.1002/app.1690

Gujral, H. S., Sharma, P., Kaur, H., Singh, J. 2013. Physiochemical, pasting and thermal properties of starch isolated from different barley cultivars. *International Journal of Food propretés*, vol. 16, no. 7, 1494-1506. http://dx.doi.org/10.1080/10942912.2011.595863

Hung, P.V., Morita, N.2005. Effects of Granule Sizes on Physicochemical Properties of Cross-linked and Acetylated Wheat Starches. *Starch/Stärk.*, vol. 57, no. 9, p. 413-420. http://dx.doi.org/10.1002/star.200500417

Jyothi, A. N., Moorthy, S. N., Rajasekharan, K. N. 2006. Effect of Cross-linking with epichlorohydrin on the Properties of Cassava (Manihot esculenta Crantz) Starch. *Starch/Stärke*, vol. 58, no. 6, p. 292-299. http://dx.doi.org/10.1002/star.200500468

Kaur, L., Singh, J., Singh, N. 2006. Effect of cross-linking on some properties of potato (*Solanum tuberosum* L.) starches. *Journal of the Science of Food and Agriculture*, vol. 86, no. 12, p. 1945-1954. http://dx.doi.org/10.1002/jsfa.2568

Kaur, B., Ariffin, F., Bhat, R., Karim, A. A. 2012. Progress in starch modification in the last decade. *Food Hydrocolloids*, vol. 26, no. 2, p. 398-404. http://dx.doi.org/10.1016/j.foodhyd.2011.02.016

Kim, B. Y., Yoo, B. 2010. Effects of cross-linking on the rheological and thermal properties of sweet potato starch. *Starch/ Starke*, vol. 62, no. 11, p. 577-583. http://dx.doi.org/10.1002/star.201000035

Koo, S. H., Lee, K. Y., Lee, H. G. 2010. Effect of cross-linking on the physicochemical and physiological properties of corn starch. *Food Hydrocolloids*, vol. 24, no. 6-7, p. 619-625. http://dx.doi.org/10.1016/j.foodhyd.2010.02.009

Leach, H. W., Mccowen, L. D., Schoch, T. J. 1959. Structure of the starch granule: swelling and solubility patterns of various starches. *Cereal Science*, vol. 36, p. 534-544.

Lim, W. J., Liang, Y. T., Seib, P. A., Rao, C. S. 1992. Isolation of oat Starch from oat Flour. *Cereal Chemistry*, vol. 69, no. 3, p. 233-236.

M. Light, J. 1989. Modified Food Starches: Why, What, Where and How. In: Symposium on Modified Food Starches at AACC's74th Annual Meeting in Washington, DC, October 29-November 2.

Majzoobi, M., Sabery, B., Farahnaky, A., Karrila, T. T. 2012. Physicochemical properties of cross-linked-annealed wheat starch. *Iran Polymer Journal*, vol. 21, no. 8, p. 513-522. http://dx.doi.org/10.1007/s13726-012-0056-8

Miyazaki, M., Hung, P. V., Maeda, T., Morte, N. 2006. Recent advances in application of modified starches for breadmaking. *Trends in Food Science and Technology*, vol. 17, no. 11, p. 591-599. http://dx.doi.org/10.1016/j.tifs.2006.05.002

Myllärinen, P., Schulman, A. H., Salovaara, H. Poutanen, K. 1998. The Effect of Growth temperature on gelatinization properties of Barley Starch. *Acta agric. Scandinavica, Sect. B – Soil and Plant Science*, vol. 48, no. 2, p. 85-90. http://dx.doi.org/10.1080/09064719809362484

Polnaya, F. J., Haryadimarseno, D. W, Cahyanto, M. N. 2013. Effects of phosphorylation and cross-linking on the pasting properties and molecular structure of sago starch. *International Food Research Journal*, vol. 20, no. 4, p. 1609-1615. http://dx.doi.org/10.1016/j.foodchem.2009.02.006

Raina, C. S., Singh, S., Bawa, A. S., Saxena, D. C. 2007. A comparative study of Indian rice starches using different modification model solutions. *Food Science and Technology*, vol. 40, no. 5, p. 885-892. http://dx.doi.org/10.1016/j.lwt.2006.03.025

Singh, S. N., Singh, N. 2005. Characteristics of acetylated starches prepared using starches separated from different rice cultivars. *Journal of Food Engineering*, vol. 70, p. 117-127. http://dx.doi.org/10.1016/j.jfoodeng.2004.09.018

Singh, J., McCarthy, O J. Singh, H. 2006. Physico-chemical and morphological characteristics of New Zealand Taewa (Maori potato) starches. *Carbohydrate Polymers*, vol. 64, no. 4, p. 569-581. http://dx.doi.org/10.1016/j.carbpol.2005.11.013

Singh, J., Kaur, L., Mccarthy, O. J. 2007. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications. A review. *Food Hydrocolloids*, vol. 21, p. 1-22. http://dx.doi.org/10.1016/j.foodhyd.2006.02.006

Sullivan, P., Arendt, E., Gallagher, E. 2013. The increasing use of barley and barley by-products in the production of healthier baked goods. *Trends in Food Science and Technology*, vol. 29, no. 2, p. 124-134. http://dx.doi.org/10.1016/j.tifs.2012.10.005

Xiao, H., Lin, Q., Liu, G, Q. 2012. Effect of Cross-Linking and Enzymatic hydrolysis composite Modification on the Properties of Rice Starches. *Molecules*, vol. 17, no. 7, p. 8136-8146. http://dx.doi.org/10.3390/molecules17078136

Yosif, E. I., Gadallah, M. G. E., Sorour, A. M., 2011. Physico-chemical and rheological properties of modified corn starches and its effect on noodle quality. *Annals of Agriculture Science*, vol. 57, no. 1, p. 19-27. http://dx.doi.org/10.1016/j.aoas.2012.03.008

Zhao, J., Schols, H. A., Chen, Z., Jin, Z., Buwalda, P., Gruppen, H. 2012. Substituent distribution within cross-linked and hydroxypropylated sweet potato starch and potato starch. *Food Chemistry*, vol. 133, no. 4, p. 1333-1340. http://dx.doi.org/10.1016/j.foodchem.2012.02.021

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POSSIBILITIES OF MICROSCOPIC DETECTION OF ISOLATED PORCINE PROTEINS IN MODEL MEAT PRODUCTS

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ABSTRACT

In recent years, various protein additives intended for manufacture of meat products have increasing importance in the food industry. These ingredients include both, plant-origin as well as animal-origin proteins. Among animal proteins, blood plasma, milk protein or collagen are used most commonly. Collagen is obtained from pork, beef, and poultry or fish skin. Collagen does not contain all the essential amino acids, thus it is not a full protein in terms of essential amino acids supply for one's organism. However, it is rather rich in amino acids of glycine, hydroxyproline and proline which are almost absent in other proteins and their synthesis is very energy intensive. Collagen, which is added to the soft and small meat products in the form of isolated porcine protein, significantly affects the organoleptic properties of these products. This work focused on detection of isolated porcine protein in model meat products where detection of isolated porcine protein was verified by histological staining and light microscopy. Seven model meat products from poultry meat and 7 model meat products from beef and pork in the ratio of 1:1, which contained 2.5% concentration of various commercially produced isolated porcine proteins, were examined. These model meat products were histologically processed by means of cryosections and stained with hematoxylin-eosin staining, toluidine blue staining and Calleja. For the validation phase, Calleja was utilized. To determine the sensitivity and specificity, five model meat products containing the addition of isolated porcine protein and five model meat products free of it were used. The sensitivity was determined for isolated porcine protein at 1.00 and specificity was determined at 1.00. The detection limit of the method was at the level of 0.001% addition. Repeatability of the method was carried out using products with addition as well as without addition of isolated porcine protein and detection was repeated 10 times. Repeatability in both, positive and negative samples, for isolated porcine protein was determined at 100%. The results show that the histological processing of cryosections stained using Calleja is suitable for detecting isolated porcine protein in meat products.

Keywords: meat; collagen; isolated protein; light microscopy

INTRODUCTION

In recent years, development of a variety of functional ingredients for the most possible efficient optimization of the quality and texture of meat products is increasing in the meat industry. Functional raw-materials of animal origin include different animal derivatives of meat, skin and blood, as well as milk and egg products (collagen, gelatin, whey protein, casein, albumin, dehydrated beef protein, and other protein isolates) **(Petracci et al., 2013)**. Proteins play an important role in the functional and sensory properties in meat products. Vegetable proteins often give the product an atypical flavor, reduce the meaty taste, and many are representatives of allergens. Therefore, animal protein isolates as functional ingredients in meat industry are rising again **(Khiari et al., 2014)**. The basic reasons to use protein additives include: increasing nutritional value of meat products, improving technological properties of processed raw materials, improving sensory characteristics of finished products, and last but not least, there are also economic reasons **(Lat et al., 1984)**. In their study, **Prabhu (2004)** reports that isolated porcine protein containing 85% protein and 12% fat obtained from fresh pork trimmings by a technology at low temperature is able to attach as much water as up to four times its weight.

The nutritional value of proteins is then judged mainly according to the amino acids contained, in particular by the essential amino acids contained. Nutritional value of animal proteins is high, while value of plant proteins is considerably lower because they lack some essential amino acid (AAs) (lysine or sulfur-free AAs). Proteinaceous ingredients are produced with a protein content of 50 – 90% **(Straka and Malota, 2006)**. The most natural alternative to meat proteins are therefore animal proteins which are widely used in meat production. For some types of meat products, addition of animal proteins is necessary, e.g. gelatin applied in cooked meat products and specialties. The most common protein used in the production of ground meat products are blood proteins, milk proteins, egg proteins, and collagen proteins **(Budig and Mathauser, 2007)**. Collagen is a group of insoluble fibrous proteins found in all multicellular organisms. It is the most abundant protein in mammals,

amounting approximately to 25 – 30% of the total body protein. Collagen is a major component of skin, bones, cartilages, tendons, blood vessels, basement membranes and teeth **(Tarté, 2010)**. Native collagen consists of alpha 1 and alpha 2 chains, which differ in their order of amino acids. These chains form a triple helix called tropocollagen. It is approximately 256 nm long and it represents the basic unit of collagen. Secondary structure of collagen is then formed by a left-handed helix of an elongated type with thread pitch of 0.95 nm and individual peptides in 0.286 nm distance from each other. The tertiary structure of collagen consists of three chains that are intertwined and have a central axis. The resulting rod-shaped configuration is about 290 nm long with a diameter of 1.4 nm and it is called tropocollagen **(Khoshnoodi et al., 2006)**. For meat industry, collagen sources include skin, bones, entrails and skeletal muscle **(Bailey and Light, 1989)**. Collagen may be added to meat products as an ingredient (meat raw materials rich in collagen: skeletal muscle with connective tissue, pork skin) or in concentrated form as a direct additive. This can be produced from bones (bone collagen extract), pork skin, and skeletal muscle with connective tissue **(Gillett, 1987)**. Collagen of skeletal muscle can be concentrated mechanically or extracted by low temperature rendering followed by extrusion, dehydration, grinding, flaking, milling **(Gillett, 1987)**. These forms of collagen can significantly affect the processability and sensory properties of meat products.

MATERIAL AND METHODOLOGY

Manufacture of Model Meat Products

Model meat products were manufactured with the addition of various types of isolated porcine proteins. The used proteins included blood protein: pork plasma P; collagen-type proteins: PF, pork gel, Scancure DI 100, Scancure 95; combination of collagen-type proteins and pure muscle proteins: Scanpork D 80, Scanpork D 90 (Scanflavour, Denmark). The first set of model products was made of poultry meat with additions of 7 types of various isolated porcine proteins at concentration of 2.5%. The second set of model products was made of pork and beef in the ratio 1:1 with additions of 7 types of various proteins at concentration of 2.5%. Other ingredients to all model products included salt at a final concentration of 1.5% and 10 mL of water. Model samples were ground in a blender, then shaped and pressed in a ham mould in which they were boiled for 10 minutes after reaching 70 °C in its core, subsequently they were cooled down, cut into cubes and placed in a freezer. Next, cryosections were cut at the HM 550 cryostat (Germany, Microm). The sample was attached to a metal stip of the freezing microtome using Killik freezing medium. The stip was left in the freezer bar of the cryostat to solidify. The sample was thus ready for cutting, so it was attached to the holder and slicing started. 16 μm thick sections were transferred to specific Thermo Superfrost slides (Germany, Thermo scientific). 42 sections on 21 slides were made from each sample – each slide contained two sections. After adhering, the sections were stored at a cooling temperature until further processing.

Histological Staining

The sections were stained using differential and targeted staining. For each staining 3 slides containing 2 sections each were used. From differential stainings, hematoxylin-eosin and toluidine blue staining and targeted Calleja were applied. The prepared samples were examined using Leica DM 3000 microscope (Germany, Leica). Images were created using Leica DFC 295 camera (Germany, Leica) connected to a computer, by means of Xn Wiew software. Based on the results of this part of the experiment, the most suitable method to detect isolated porcine proteins was selected and pork protein called Scancure 95 by Scanflavour (Denmark) was chosen to be used for the production of model meat products in the second phase of the work, which was aimed at determining the detection limit of this method.

Samples for Determination of the Detection Limit

The following ingredients were selected for the second experiment: pork and beef in the ratio of 1:1 and isolated pork protein called Scancure 95 by Scanflavour. The following concentrations of samples were produced: 0.001%; 0.01%; 0.10%; 1.00%; 2.50%; 5.00%. Other ingredients to all model products included salt at a final concentration of 1.5% and 10 mL of water. Model samples were processed according to the procedure in the previous chapter. And they were stained by targeted Calleja.

RESULTS AND DISCUSSION

In the first step of the protocols, model meat products were manufactured from available commercially produced isolated porcine proteins. A combination of poultry, pork and beef meat was used as the basis of individual meat products in order to find the most suitable combination for the production of model meat products. Model meat products were manufactured with the addition of isolated porcine protein in concentration of 2.5%. This step resulted in the selection of the best detectable isolated porcine protein. Based on processing and examining samples of poultry meat and the combination of pork and beef, it was found that the raw material itself has no effect on the microscopic detection of isolated porcine protein. The raw material in particular affects processing of the samples and primarily it's cutting and staining. For this reason, the combination of pork and beef was used in the next phase.

The most widely used collagen protein (in terms of production of ground meat products) is collagen protein powder of pork origin obtained from pig skins. These are used primarily where there is a need to increase elasticity, improve slicability and cohesion. Stabilization of the product is achieved by a combination of collagen protein and other proteins of animal origin, e.g. blood plasma. Another property is a significant contribution to the reduction of syneresis in the finished meat products packaged in a vacuum or a modified atmosphere. Pork skins in the form of skin emulsion are used most commonly **(Budig and Mathauser, 2007)**. Functional properties of the added collagen used in meat products depend on the species and age of animal, anatomical sources, and extraction conditions. The potential use of collagen as an additive in meat products dates back to

approximately 1960 **(Tarté, 2010)**. From the created staining range applicable to detect collagen in practice, it was necessary to determine which ones are suitable to identify isolated porcine protein. They were verified by 2 differential and one targeted staining. From among differential staining, haematoxylin - eosin and toluidine blue stainings were used and, from among the targeted ones, Calleja was used (Figure 1). Targeted staining procedures were focused on the detection of collagen which should be distinguished from other structures in the product in each staining. All stainings applied are primarily intended for paraffin sections. However, our experiment showed that they are also suitable for cryosections. Cryosections were used because their examination is financially less demanding as well as less time consuming.

The first differential staining was hematoxylin - eosin. This staining is not too demanding on chemicals and time, but it does not belong among the simplest ones. It is highly effective in distinguishing, all the structures in the sample can be identified, even though they are only in various shades of red and blue. Isolated porcine protein is dark pink, sometimes up to crimson. An experienced examiner can recognize it because of its characteristic structure. Collagen protein has a net-like structure with cores forming different circular shapes in the margins. No sections were lost in this process due to floating away.

The second differential staining was toluidine blue. This method is neither demanding in terms of chemicals nor of time. Of all the applied stainings, this one is the easiest and quickest. It is very effective in terms of substances identification. Collagen protein is slightly purple and it is characterized by its typical structure. No cryosections were lost.

The targeted staining was performed according to Calleja. Calleja staining is relatively time-efficient. It is very effective in differentiation. There is a clear distinction in structures within the meat product. Collagen protein is stained kerosene or bluish-green and it is clearly distinguishable from other structures that are predominantly green (Figure 2). Another advantage is limited losses of sections. For these reasons, this staining method was selected as the most suitable for the detection of isolated porcine protein in meat products.

Method Validation

A graded series was produced in order to determine the detection limit. A combination of pork and beef in the ratio of 1:1 and isolated pork protein of Scancure 95 (Scanflavour, Denmark) were utilized. This isolated pork protein has a high content of collagen protein in its composition and thus it was the most suitable material for the microscopic detection. Scancure 95 is composed of 98% collagen protein and 1 – 2% pure muscle proteins. It is intended as an addition to smoked meat. The following concentrations were created: 0.001%; 0.01%; 0.10%; 1.00%; 2.5%; 5%. Of the above staining methods, Calleja was selected as the most suitable for the detection of isolated porcine protein because of the best color contrast between different structures in the meat product. Therefore, the following parameters were also established for this staining in order to validate this method: sensitivity and specificity, and repeatability. The method was validated as a qualitative method, i.e. a method to detect whether the analyte is present or absent. These methods are common in routine laboratory testing in particular as the first step for subsequent determination of the concentration of the investigated substance. They can therefore be ranked among screening methods. The advantage of its use is the reduction of costs as well as time **(Trullols et al., 2004)**. As with quantitative methods, user must be confident that the results of the qualitative method applied are suitable for the aimed purposes, which means that the method must be validated **(European Commission, 2002)**. Commonly selected methods for validation are based on quantitative methods, and there are also many validation procedures which are accepted by the supervisory authorities and professional community working in the field **(Trullols et al., 2004)**.

For detection of isolated porcine protein, the detection limit was determined at 0.001 % addition. Using isolated porcine proteins in meat products can be expected at about 10 % as suggested by a study **(Doerscher, et al., 2003)**. With regard to the optimum concentration applicable in meat production, the method is suitable for detection of isolated porcine proteins in meat products.

The evaluation criteria for qualitative methods inlcude also sensitivity and specificity **(European Food Safety Authority, 2004)**. For qualitative methods, sensitivity means the ability of a method to detect truly positive

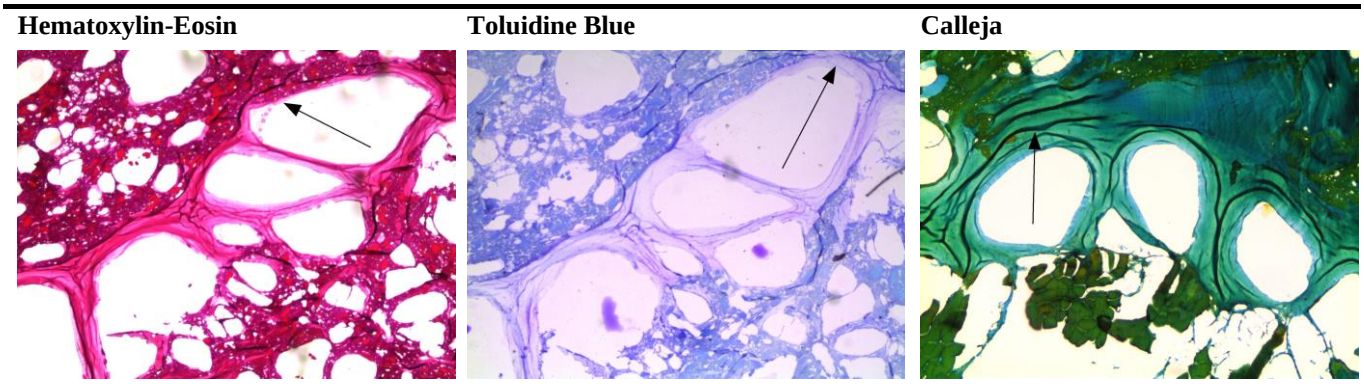

Figure 1 Isolated porcine protein in a model meat product (25 x magnification).

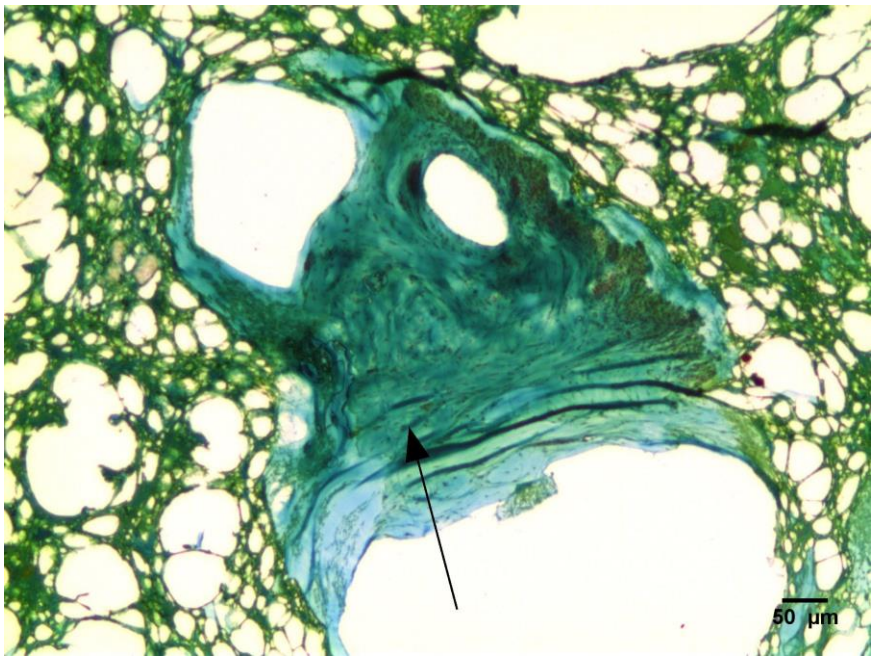

Figure 2 Isolated porcine protein (bluish green) in a model meat product (25 x magnification).

Table 1 Detection of sensitivity and specificity of the Calleja method.

Sample no.	1/2	1/3	1/4	1/5	1/6	2/2	2/3	2/4	2/5	2/6
Declaration	N	N	N	N	N	P	P	P	P	P
Examiner A	N	N	N	N	N	P	P	P	P	P
Examiner B	N	N	N	N	N	P	P	P	P	P

Note: Declaration – N (negative), P (positive)-addition of the isolated porcine protein in model sample.
Sample no: 1/2- 1/6: model sample without the isolated porcine protein.
Sample no: 2/2- 2/6: model sample with the isolated porcine protein.

samples as positive **(O'Rangers and Condon, 2000)**. The sensitivity rate is thus the probability for a given concentration, that the method will classify the test sample as positive **(Massart et al., 1997)**. In contrast, specificity is defined as the ability of a method to detect truly negative samples as negative **(O'Rangers and Condon, 2000)**. The specificity rate is thus the probability, for a given concentration, that the method will classify the test sample as negative **(Massart et al., 1997)**. For Calleja staining method, sensitivity was determined at 1.00 for isolated pork protein. Specificity was determined at 1.00 according to the protocols **(Trullols et al., 2004)** (Table 1). An optional parameter for qualitative methods is also method repeatability. For histological methods, this parameter is particularly suitable because cutting the samples, sample processing and staining can take place on different days. This parameter is recommended to exclude the role of the environment. Repeatability of the method was carried out using the product with addition as well as without addition of isolated porcine protein and it was performed in harmony with the protocols **(Suchánek, 1999)**. Evaluation was performed by two examiners and the measurement was repeated 10 times. There was a 100% match.

CONCLUSION

Besides dairy products and bread, meat and meat products belong to the basic foodstuffs needed for good human nutrition. Above all, however, they are consumed due to their organoleptic properties. Nevertheless, with regard to the pressure on meat products buying prices and special offers in retail chains, industrial manufacturers have to substitute a technologically substantial portion of binding meat either by cheaper meat raw materials of inferior quality or by suitable ingredients and additives in order to stabilize the product, which applies in particular to ground meat products. To achieve the desired properties in the finished meat product, manufacturers use a considerably cheaper source, namely collagen proteins in the form of isolated porcine proteins. Due to their very specific functional properties, collagen proteins are used in particular for products where it is necessary to increase their elasticity, improve slicability and cohesion. Application of collagen protein significantly contributes to the reduction of syneresis in finished meat products

packaged in a vacuum or a modified atmosphere. In combination with other animal proteins, such as blood plasma, collagen proteins are also used as ingredients that facilitate emulsification or stabilization of the ground meat products. The aim of this work was to detect isolated pork protein in model meat products using histological staining of cryosections and light microscopy. In the first part of this work, 7 model meat products from poultry meat and 7 model meat products from beef and pork in the ratio of 1:1, which contained 2.5% concentration of various commercially produced isolated porcine proteins, were examined. These model meat products were histologically processed by means of cryosections and stained with hematoxylin-eosin staining, toluidine blue staining and Calleja staining. The second part focused on validation of Calleja method, where model meat products with the addition of isolated porcine protein of Scancure 95 containing 98% of collagen protein and 1 – 2% of pure muscle protein were used. The sensitivity was determined for isolated porcine protein at 1.00 and specificity was determined at 1.00. The detection limit of the method was determined at 0.001% addition. Repeatability in both, positive and negative samples, for isolated porcine protein was determined at 100%. The results show that the histological processing of cryosections stained using Calleja is suitable for detecting isolated porcine protein in meat products.

REFERENCES

Bailey, A. J., Light, N. D. 1989. *Connective tissue in meat and meat products.* London: Elsevier Science Publishers Ltd, 355 p. ISBN-1 85166 284 7. http://dx.doi.org/10.1016/S0309-1740(03)00082-2

Budig, J. Mathauser, P. 2007. Technicko-technologické aspekty výroby díla mělněných masných výrobků v minulosti a v současnosti. *Maso*, vol. 4, p. 21.

Doerscher, D. R., Briggs, J. L., Lonergan, S. M. 2004. Effects of pork collagen on thermal and viscoelastic properties of purified porcine myofibrillar protein gels. *Meat Science*, vol. 66, no. 1, p. 181-188.

European Commision. Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of European Commission. L, 2002 vol. 221, p. 8-36.

Gillett, T. A. 1987. Gelatin gels. In*:* A. M. Pearson, et al. *Collagen as a food*. Van Nostrand Reinhold Company, New York, N.Y., p. 223-249.

Khiari, Z. 2014. Poultry protein isolate prepared using an acid solubilization/precipitation extraction influences the microstructure, the functionality and the consumer acceptability of a processed meat product. *Food Structure*, vol. 2, no. 1, p. 49-60. http://dx.doi.org/10.1016/j.foostr.2014.08.002

Khoshnoodi, J., Cartailler, J. P., Alvares, K., Veis, A., Hudson, G. B. 2006. Molecular Recognition in the Assembly of Collagens: Terminal Noncollagenous Domains Are Key Recognition Modules in the Formation of Triple Helical Protomers. *Journal of Biological Chemistry*, vol. 281, no. 50, p. 38117-38121. http://dx.doi.org/10.1074/jbc.R600025200 PMid:17082192

Lát, J., Beneš, J., Blanka, R., Gola, J., Hlavinka, J., Klíma, D., Mareček, S., Petříček, M., Pokorný, V., Rusz, J. 1976. *Technologie masa (Meat technology).* Praha: SNTL, 635 s. ISBN- 978-80-8069-861-4. PMid:941647

European Food Safety Authority. 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes. *The EFSA Journal*, vol. 32, p. 1-197.

Massart, D. L., Vandeginste, B. G. M., Buydens, L. M. C., De Jong, G. J., Lewi, P. J., Smeyers-Verbeke, J. 1997. Handbook of Chemometrics and Qualimetrics (Data Handling in Science and Technology), vol. 20, Elsevier, Amsterdam. ISBN- 978-0-444-82853-8.

O'rangers, J. J., Condon, R. J. 2000. Current Issues in Regulatory Chemistry, AOAC Int., Gaithersburg, Maryland, USA, p. 207.

Petracci, M., Bianchi, M., Mudalal, S., Cavani, C. 2013. Functional ingredients for poultry meat products. *Trends in Food Science & Technology*, vol. 33, no. 1, p. 27-39. http://dx.doi.org/10.1016/j.tifs.2013.06.004

Prabhu, G. A., Doerscher, D. R., Hull, D. H. 2004. Utilization of pork collagen protein in emulsified and whole muscle meat products. *Journal of Food Science*, vol. 69, p. C388-389. http://dx.doi.org/10.1111/j.1365-2621.2004.tb10703.x

Straka, I., Malota, L. 2006. *Chemické vyšetření masa: klasické laboratorní metody (Chemical determination of meat: classic laboratory methods)*. Tábor, Czech Republic : OSSIS, 94 s. ISBN-80-86659-09-7.

Suchánek, M. 1999. *Qualimetry 9. The appropriateness of the analytical methods for a particular purpose*. p. 61. ISBN-80-901868-7-4.

Tarté, R. 2010. *Ingredients in meat products: properties, functionality and applications*. Springer Science & Business Media, 129 p. ISBN-978-1-4419-2436-0. PMCid:PMC3317889

Trullols, E., Ruisanchez, I., Rius, F. X. 2004. Validation of qualitative analytical methods. *TrAC Trends in Analytical Chemistry*, vol. 23, no. 2, p. 137-145. http://dx.doi.org/10.1016/S0165-9936(04)00201-8

Vognarová, I., Vrchlabský, J. 1984. *Technologie masa (Meat technology).* 2 nd ed. Praha, Czech Republic : SNTL - Nakladatelství technické literatury. ISBN- 04-846-46.

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THE EFFECT OF STORAGE ON QUALITY OF HERBS GENUS *ORIGANUM*

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ABSTRACT

Herbs of *Origanum* genus are rich in essential oils and contain large amounts of phenols, lipids, fatty acids, flavonoids and anthocyanins. Antioxidant activity of these herbs depends on many factors, including the type herbs, post-harvest processing and subsequent processing. The aim of this study was therefore to confirm the hypothesis that the composition of oils of these two herbs of the *Origanum* genus depends on the post-harvest treatment of herbs and that the dried herb antioxidant activity is higher for fresh than that of frozen herbs. *Lamiaceae* family herbs: oregano (*Origanum vulgare* L.) and Greek oregano (*Origanum heracleoticum* L.) were planted and analyzed. Herb samples were extracted by hot demineralised water. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method was used for antioxidant activity assessment. The total phenolic content was determined spectrophotometrically by using Folin-Ciocalteu reagent. Steam distillation of essential oils was carried out via Clevenger Apparatus. The obtained essential oils were analysed by GC-MS technique. Results of tested fresh, dried and frozen herbs showed a considerable potential for quenching the free DPPH radical. Significantly higher antioxidant activity was found in dried herbs comparing to fresh and frozen, but only in case of values calculated per 100 g of the sample. However, the differences were not statistically significant after recalculation when expressed on dry matter content. There was no difference between fresh and frozen samples. The content of total phenols was significantly higher in dried than in frozen herbs in values recalculated per 100 g of sample. A strong correlation between the results of DPPH and TPC was found again only for values expressed per 100 g of the sample. Post-harvest treatment of herbs affects the composition of their essential oils. The dominant essential oil component of Greek oregano is carvacrol with a proportion of 60% or more. On the contrary, there is no such dominating component in oregano essential oil but there are more components with a share of 10 to 20%.

Keywords: Oregano; Greek oregano; DPPH method; total phenolics contents; essential oils composition

INTRODUCTION

Herbs of the *Origanum* genus are found mainly in the Mediterranean region and Asia. This genus is rich in essential oils where the quantity and quality vary considerably **(Asensio et al., 2015)**. Herbs of *Origanum* genus contain large amounts of phenols, lipids, fatty acids, flavonoids and anthocyanins **(Kintzios, 2002)**.

Oregano is a perennial plant of *Lamiaceae* family groving in Asia, Europe and North Africa. This aromatic herb is widely used in many world cuisines **(Teixeira et al., 2013)**, and in traditional medicine **(Kintzios, 2002)**. There are many studies describing the effect of oregano for food preservation **(Chorianopoulos et al., 2004; Chouliara et al., 2007; Carmo et al., 2008)**, as well as its antioxidant activity **(Sahin et al., 2004; Capecka et al., 2005; Ličina et al., 2013)**. Biologically active substances of oregano include phenols, phenolic acids, flavonoids, glycosides and their esters and steroids. According to **Crocoll et al., (2010)**, the dominant components of essential oils of this herb are monoterpenes and sesquiterpenes showing significant antioxidant activity **(Kinzitos et al., 2002; Grassmann, 2005; Beena et al., 2013)**. The main compounds with antifungal properties include thymol and carvacrol **(Rao et al., 2011)**.

Origanum vulgare L. has been used in Persian traditional medicine for its anti-inflamantory **(Javadian et al., 2016)** and other effects such as diuretics, stomachics, antineuralgics, antitussives and expectorans **(Afsharypour et al., 1997)**.

Greek oregano is widely used in gastronomy. Its typical flavour is produced by the presence of essential oils **(Stamenic et al., 2014)**. Antimicrobial **(De Martino et al., 2009; Govaris et al., 2010; Stamenic et al. 2014)**, antifungal **(Adam et al., 1998)** and antioxidant **(Kulisic et al., 2004; Zheng et al., 2009)** effects were described in this plant. **Charles (2013)** indicates that the most significant secondary metabolites in Greek oregano are tannins, resins, flavonoids, bitter substances, sterols, phenols, and essential oils. **Kikuzaki et al., (1989)** and **Koukoulitsa et al., (2006)** found a variety of phenolic compounds with antioxidant activity in Greek oregano such as rosmarinic acid and its derivatives, caffeic acid, protocatechuic acid and phenyl glucoside. In a report by **Zheng et al., (2009)**, the essential oil of Greek oregano consists of 78.28% of phenolic compounds carvacrol and thymol followed by γ-terpinene (5.54%) and p-cymene (7.35%). The antioxidant activity of essential oil of Greek

oregano and its use in the food industry was also discussed by **Kulisic et al., (2004)**.

Antioxidant activity of herbs depends on many factors, including the type herbs, the methods and conditions of cultivation, harvest, post-harvest processing and subsequent processing. The extraction method, the manner of extraction and the type of solvent used also affect the level of antioxidant activity **(Škrovánková et al., 2012)**. The effect of different growing conditions on the chemical composition and biological activity of the essential oil of oregano was studied **De Falco et al., (2013)**. **Kouřimská et al., (2014)** investigated the antioxidant activity of plants of the *Lamiaceae* family grown under organic and conventional conditions. **Ozkan et al., (2010) and Baranauskienė et al., (2013)** focused on the impact of harvest on essential oil composition and antioxidant activity of oregano. They found the highest antioxidant activity was found in herbs harvested during their flowering.

The water content in fresh *Lamiaceae* herbs is typically in the range of 75 – 80%. For the preservation of the herbs, it is necessary to reduce the amount of water to less than 15% **(Diaz-Maroto et al., 2002)**. Lowering the water activity inactivates the enzymes, which in its active form may be cause degradation of antioxidant ingredients of fresh herbs **(Hossain et al., 2010)**. Water activity may be reduced via different methods. The most commonly used method is drying. This method results in an increase in the content of some substances. Cell tissues of herbs are damaged during the drying process which leads to the release of phenolic compounds and increase of antioxidant activity. Changes in appearance and flavour are due to loss or development of volatile compounds because of oxidation and esterification reactions **(Hossain et al., 2010)**.

Air drying is the simplest and cheapest method of drying. Low temperature prevents the degradation of the active ingredients of herbs during this type of conservation method, but the drying is relatively slow and thus the metabolic processes may continue and cause changes in quality of herbs **(Keinänen and Julkunen-Tiitto, 1996)**. Slow loss of water can act as a stressor and the defensive mechanism of most plants is the production of phenolic compounds **(Dixon and Paiva, 1995; Hossain et al., 2010)** which may contribute to a higher antioxidant activity of dried herbs. **Hossain et al., (2010)** found significantly higher amounts of rosmarinic acid in the extracts of herbs dried at room temperature than in fresh samples. Higher amounts of carvacrol can be obtained during drying at lower temperatures (below 40 °C) **(Novák et al., 2011)**.

Freezing is another method to reduce the activity of water. Crystals are formed during freezing which causes destruction of plant cells enabling better extraction of the active substances **(Keinänen and Julkunen-Tiitto, 1996)**. **Tomsone and Kruma (2014)** considered this phenomenon as a possible explanation of higher phenol content of frozen herbs. **Chan et al., (2014)** argues that the effect of freezing and other methods on the antioxidant activity varies depending on the particular herb. **Tomsone and Kruma (2014)** investigated the effect of drying and freezing on the phenol content and antioxidant activity of lovage and horseradish. Both these parameters were highest in frozen herbs and therefore the authors evaluated this processing method as the most suitable for preserving the antioxidant activity and the content of phenols.

Different extraction methods can be employed for the isolation of antioxidants from herbs. Extraction from the solid phase into the liquid and steam distillation are frequently used methods. Extraction using non-toxic solvents, such as supercritical fluid extraction with carbon dioxide and subcritical water extraction are increasingly applied **(Rodríguez-Meizoso et al., 2006)**. **Škrovánková et al., (2012)** reported that polar solvents (ethanol, methanol, water, etc.) and non-polar (hexane, etc.) as the most commonly used extraction liquids. Different solvents for the isolation of antioxidative components were used for Greek oregano by **Tsimogiannis et al., (2006)**. The highest antioxidant activity determined by DPPH was found in diethyl ether and ethanol extract, the lowest in petroleum extract.

Although there is a lot of scientific literature focussed on the effects of drying or freezing on the antioxidant activity and composition of certain medicinal and aromatic herbs, there is still lack of a comprehensive study comparing these two conservation practices both at the same time in the case of oregano and Greek oregano. The aim of this study was therefore to confirm the hypothesis that the composition of oils of these two herbs of the *Origanum* genus depends on the post-harvest treatment of herbs and that the dried herb antioxidant activity is higher for fresh than that of frozen herbs.

MATERIAL AND METHODOLOGY

Herbs

Lamiaceae family herbs: oregano (*Origanum vulgare L.*) and Greek oregano (*Origanum heracleoticum L.*) were planted and analyzed. The seeds were sown on 20th April to the sunny, unfertilized plot of sandy loam medium soil in the Jirny locality (50° 6' 56'' N, 14° 41' 57'' E, district Prague-East). The seeds were purchased from Kiepenkerl company. Plant parts were harvested before flowering on 30th July and divided into three parts. One part was spread on papers in the laboratory and dried at 25 °C for one week. Another part was placed in plastic bags and frozen at -18 °C. The last third was analysed immediately.

Chemicals

All chemicals, methanol (Lachner, CR), sodium carbonate anhydrous (Lachner, CR), DPPH 2,2-difenyl-1-pikrylhydrazyl (Sigma Aldrich, USA), Folin & Ciocalteu´s phenol reagent (Merck, Germany), ascorbic acid (Penta, CR), Gallic acid (Sigma Aldrich, USA), n-hexane (Lachner, CR) and sodium sulphate anhydrous (Lachema, CR) were of analytical grade purity.

Determination of dry matter content

Balances with infrared dryer, Precisa HA 300 (Precisa Instruments, Swirzerland) were used for dry mater content determination. Samples of herbs (1 g) were ground and spread on aluminium foil and dried at a maximum temperature 105 °C to the constant weight (the weight difference less than 2 mg for 30 s). All samples were measured in triplicate and the average was calculated.

Herb extraction

Fresh herbs (6 g) or the equivalent amount of dried herbs (calculated from total dry matter of individual herbs) were taken for the preparation of water extracts. Herb samples were extracted twice by 50 mL of hot demineralised water in the ultrasonic bath for 10 min. Samples were then filtered into 100 mL volumetric flasks and filled up to the mark after cooling. The extracts were analysed on the same day.

Determination of antioxidant activity by the DPPH method

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method taken from **Adámková et al., (2015)** and **Chrpová et al., (2010)** was used for antioxidant activity assessment. The intensity of the violet DPPH radical solution was measured at 522 nm. As the reaction equilibrium is usually reached after two hours for most compounds, the absorbance of samples was measured after 1, 2 and 3 hours and its minimum was used for the antioxidant activity calculation. The method was calibrated with ascorbic acid and the results were expressed as equivalents of ascorbic acid per unit mass of sample.

Determination of total phenolic compounds (TPC)

The total phenolic content was determined spectrophotometrically (spectrophotometer UV-2900, Tsingtao Unicom-Optics Instruments Co., Ltd., China) at 760 nm by using Folin-Ciocalteu reagent. The method was previously reported by **Dorman et al., (2003)** and **Stratil et al., (2008)**. Results were expressed as the content of Gallic acid per unit mass of the sample.

Steam distillation of essential oils

Steam distillation of essential oils was carried out via Clevenger Apparatus (Wilmad-LabGlass, USA) for 4 hours **(Memarzadeh et al., 2015)**. The apparatus was also used for the determination of extracted volume of essential oil.

GC analysis of essential oils

The obtained essential oils (10 μL) were placed into the vials with 500 μL of n-hexane (with a few crystals of anhydrous sodium sulphate) and analysed by GC-MS using Agilent 7890A GC coupled to Agilent 5975C single-quadrupole mass detector equipped with a HP-5MS column (30 m × 0.25 mm ID, 0.25 μm film). The sample volume of 1 μL was injected in split mode (ratio 12:1) into the injector heated to 250 °C. The initial oven temperature was 60 °C (hold 3 min), ramp to 250 °C at 3 °C . min^{-1} (hold 10 min). Helium was used as carrier gas with the flow rate of 1 $mL.min^{-1}$. The MS analysis was carried out in full scan mode, the electron ionization energy was set at 70 eV. The analytes were identified according to their relative retention times and by the comparison of their mass spectra with the National Institute of Standards and Technology Library (NIST, USA). The results were calculated by area normalisation method.

Statistical evaluation

The data obtained were analysed using statistical software Statistica 12.0 (StatSoft Inc.). Analysis of variance (one-way ANOVA) was performed and the significant differences in the means were separated using the Scheffé's test. The data were expressed as an average of triplicates ± standard deviation. For all statistical tests, a 5% level of significance was used.

RESULTS AND DISCUSSION

Comparison of dry matter content, antioxidant activity and phenolics content

Dry matter content results of analysed herbs are presented in Table 1. The results correspond with the works of other authors, for example **Kouřimská et al., (2014) and Adámková et al., (2015)**. It can be seen that there are considerable losses of water content during drying compared to freezing.

Results of antioxidant activity of tested herbs analysed by DPPH method expressed in mg of ascorbic acid (AA) per 100 g of sample or per 100 g of dry matter are shown in

Table 1 Dry matter (DM) content of tested herbs in % (w/w).

Herb	DM (% w/w)		
	fresh	dried	frozen
Oregano (*Origanum vulgare L.*)	32.73 ±0.97	85.27 ±0.34	26.73 ±1.12
Greek oregano (*Origanum heracleoticum L.*)	24.04 ±1.41	83.62 ±0.12	21.35 ±0.96

Table 2 Antioxidant activity of tested herbs analysed by DPPH method expressed in mg of ascorbic acid (AA) per 100 g of sample or per 100 g of dry matter (DM).

Herb	DPPH (mg AA/100 g)	DPPH (mg AA/100 g DM))
Oregano – fresh	1156 ±160	3532 ±450
Oregano – dried	3572 ±481	4189 ±560
Oregano - frozen	1034 ±162	3867 ±607
Greek oregano - fresh	463 ±9	1926 ±39
Greek oregano - dried	2608 ±563	3119 ±674
Greek oregano - frozen	566 ±63	2652 ±294

Table 3 Total phenolics content (TPC) results of tested herbs expressed in mg of Gallic acid (GA) per 100 g of sample or per 100 g of dry matter (DM).

Herb	TPC (mg GA/100 g)	TPC (mg GA/100 g DM))
Oregano – fresh	1248 ±14	3812 ±44
Oregano – dried	2166 ±5	2540 ±6
Oregano - frozen	425 ±6	1588 ±23
Greek oregano - fresh	795 ±6	3309 ±27
Greek oregano - dried	3067 ±43	3668 ±52
Greek oregano - frozen	390 ±1	1825 ±5

Table 2. The results show that both herbs possess significant potential to quench the free radical DPPH which confirmed the work carried out by **Chrpová et al., (2010)**; **Ličina et al., (2013)** or **Skotti et al., (2014)**. Dried herbs had the highest antioxidant activity. The values of frozen and fresh herbs expressed per 100 g of sample were not very different. There can be seen, only a slight difference between fresh, dried and frozen samples of oregano expressed on dry matter content. When comparing oregano and Greek oregano there was always higher antioxidant activity in case of oregano samples.

Table 3 shows the total phenolics content of tested herbs analysed by Folin-Ciocalteu reagent and expressed in mg of Gallic acid (GA) per 100 g of sample or per 100 g of dry matter. It can be observed that fresh, dried and frozen herbs contain reasonable amounts of phenols, which also corresponds to the results of **Ivanova et al., (2005)** and **Skotti et al., (2014)**. The highest amount of phenols per 100 g of the plant was determined on the dried herbs, which correlates with the highest antioxidant activity. After recalculating the results based on dry matter content, fresh oregano samples had the highest phenol content. The lowest amount of total phenols showed frozen herbs even after recalculating to dry matter content. Higher phenol content in dried herbs corresponds with the conclusion of **Ahmad-Qasem et al., (2013)**, who found lower levels of phenols in the frozen sample of olive-tree than in the dried sample. The authors explained this lower content of phenols in a frozen sample as a result of the temporary inactivation of the enzymes during freezing.

Statistical comparison of the results of the fresh, dried and frozen herbs expressed per dry matter content did not show any significant differences in antioxidant activity including the content of total phenols (pDPPH = 0.6509, pTPC = 0.0731). However, significant differences were found comparing the values per 100 g of herbs for both antioxidant activity (p = 0.0339) and in the content of total phenols (p = 0.0266). A more detailed analysis using Scheffé test showed a statistically significant difference in antioxidant activity between fresh and dried herbs (p = 0.0498) and between frozen and dried herbs (p = 0.0493). In the case of TPC, there was only a statistically significant difference between frozen and dried herbs (p = 0.0290). A strong correlation (r = 0.83) was observed between the values of DPPH and TPC expressed per 100 g of herbs.

Higher antioxidant activity by DPPH method in the dried sample may be due to stress of plants accompanied by the formation of phenolic compounds. Also the disruption of tissues during drying could result in the release of phenolics and increasing their content in the extraction process. This is partly confirmed by the results of total phenols, where a strong correlation between the values of TPC and DPPH was found, but only for the results expressed per 100 g of herbs. A significant correlation between the total content of phenols and antioxidant

Table 4 GC-MS analysis of major components of essential oil extracted from oregano.

Component	Content in fresh (%)	Content in dried (%)	Content in frozen (%)
Sabinene	ND	4.30	3.49
β-Phellandrene	1.51	ND	ND
1-Octen-3-ol	0.23	3.09	3.32
β-Myrcene	0.95	ND	1.92
α-Terpinene	ND	2.38	2.36
(E)-β-Ocimene	5.77	5.44	ND
(Z)-β-Ocimene	6.86	ND	5.41
α-Pinen	ND	8.38	8.77
3-Carene	2.54	3.03	0.07
γ-Terpinene	12.20	16.40	15.47
1,6-Octadien-3-ol, 3,7-dimethyl-	0.33	1.71	0.80
Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-	5.38	3.36	3.59
Germacene D	10.16	24.44	29.18
Caryophyllene	14.39	5.07	3.86
α-Caryophyllene	2.10	0.77	ND
Cubebol	ND	6.60	5.14
β-Cubebene	16.82	ND	ND
β-Bisabolene	8.34	2.92	2.12

ND = not detected

Table 5 GC-MS analysis of major components of essential oil extracted from Greek oregano.

Component	Content in fresh (%)	Content in dried (%)	Content in frozen (%)
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	1.27	ND	1.76
Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-, didehydro deriv.	ND	1.42	ND
Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	ND	ND	1.23
β-Myrcene	1.47	1.73	1.92
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	1.20	0.09	0.08
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	ND	1.70	ND
Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl-	ND	ND	1.93
(E)-β-Ocimene	4.61	4.21	4.36
1,3,6-Octatriene, 3,7-dimethyl-, (E)-	0.97	1.41	1.70
γ-Terpinene	6.21	9.33	9.73
Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	0.97	ND	1.08
Phenol, 5-methyl-2-(1-methylethyl)-	ND	ND	7.19
Carvacrol	73.09	67.92	59.66
Caryophyllene	1.00	1.46	1.24

ND = not detected

activity of plants has been demonstrated in several studies **(Ivanova et al., 2005; Wojdyło et al., 2007; Chrpová, et al., 2010)**.

Higher antioxidant activity of frozen herbs (values expressed on dry matter) was found compared to fresh herbs. The reason could be damage of herb tissues due to the formation of crystals during freezing and subsequently increased release of secondary metabolites into the solvent **(Keinänen and Julkunen-Tiitto, 1996)**. It should be noted that the results and conclusions of studies investigating the effect of freezing on the antioxidant activity of plants are not always unambiguous and conclusive as mentioned for example by **Chan et al., (2014)**.

A statistically significant difference in antioxidant activity of fresh, dried and frozen herbs determined by DPPH in values calculated per 100 g of plant corresponds with the results of many studies **(Hossain et al., 2010; Kouřimská et al., 2014)**, but they also show a statistically significant difference even after recalculation to dry matter content. **Hossain et al., (2010)** explain the lower antioxidant activity of fresh herbs by the presence of active enzymes that may cause degradation of the antioxidants. Antioxidant activity determined by DPPH was in all cases higher in oregano samples then in Greek oregano samples, concurring with the findings of **Chrpová et al., (2010)**.

The highest content of total phenols was in most cases determined in samples of dried herbs, as reported by **Hossain et al., (2010)** and **Kouřimská et al., (2014)**. It is caused by the release of phenolic compounds and increase of antioxidant activity when the cell tissues of herbs are damaged during the drying process. Comparing the total phenolic content of fresh and dried herbs there was no statistically significant difference which is in line with **Adámková et al., (2015)**.

Comparison of extracted volume of essential oil and its composition

Extraction of the essential oils from the fresh, dried and frozen samples gave significantly higher yields from samples of Greek oregano (6.3, 14.8 and 8.2 $mL.kg^{-1}$ respectively) than for oregano (0.2, 3.2 and 1.7 $mL.kg^{-1}$ resp.). The lowest yield was in case of fresh samples. **Baranauskienė et al., (2013)** also found a higher yield at the Greek oregano compared with oregano and all samples that they harvested in different periods.

The main components of fresh oregano essential oil analysed by GC-MS were β-cubebene, caryophyllene, γ-terpinene and germacene D (Table 4). **Mockutë et al., (2004)** and **Şahin et al., (2004)** also determined germacene D and caryophyllene as the major components of oregano essential oil. The high content of γ-terpinene is consistent with results of **Ličina et al., (2013)**. Other studies found additional components which is influenced by many factors such as growing conditions, locality, time of harvest, extraction method etc. **(Ozkan et al., 2010; Tibaldi et al., 2011; De Falco et al., 2013; Kawase et al., 2013)**. Cubebol, sabinene and α-terpinene were found only in the essential oils of dried and frozen herbs, while β-cubebene and β-phellandrene were only in the samples of fresh oregano.

Carvacrol was the main component of Greek oregano essential oil samples (Table 5), its highest content was in the fresh sample. The second major component was γ-terpinene and (E)-β-ocimene. **Zheng et al., (2009)** and **Stefanakis et al., (2013)** also reported carvacrol and γ-terpinene as major compounds. The results show that the various post-harvest treatments cause the changes in the composition and the content of essential oils components. This corresponds to the studies of **Novák et al., (2011)** who described the changes in the composition of the Greek oregano essential oil treated by various types of drying.

Najafian (2014) observed different representation of ingredients of lemon balm essential oil as a result of the storage of herbs in the freezer, refrigerator and room temperature.

The smallest changes in the composition of essential oils were found during the storage of herbs in the freezer and refrigerator compared to storage at room temperature. The highest level of carvacrol was found in the essential oil of fresh oregano Greek, while the lowest amount was in the frozen sample. Reduced proportion of carvacrol was always associated with increased content of its precursor γ-terpinene. This phenomenon was also highlighted by **Novák et al., (2011)**. High concentration of carvacrol in the essential oil of Greek oregano is responsible for its antioxidant activity.

CONCLUSION

The antioxidant activity and the composition of essential oils of selected plants of the genus *Origanum* were affected by post-harvest treatment of plants. All fresh, dried and frozen herbs showed a considerable potential for quenching the free DPPH radical. Significantly higher antioxidant activity was found in dried herbs comparing to fresh and frozen, but only in case of values calculated per 100 g of the sample. However, the differences were not statistically significant after recalculation when expressed on dry matter content. There was no difference between fresh and frozen samples. The content of total phenols was significantly higher in dried than in frozen herbs in values recalculated per 100 g of sample. A strong correlation between the results of DPPH and TPC was found again only for values expressed per 100 g of the sample. Post-harvest treatment of herbs affects the composition of their essential oils. The dominant essential oil component of Greek oregano is carvacrol with a proportion of 60% or more. On the contrary, there is no such dominating component in oregano essential oil but there are more components with a share of 10 to 20%.

REFERENCES

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M. 1998. Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. *Journal of Agricultural and Food Chemistry,* vol. 46, no. 5, p. 1739-1745. http://dx.doi.org/10.1021/jf9708296

Adámková, A., Kouřimská, L., Kadlecová, B. 2015. The effect of drying on antioxidant activity of selected *Lamiaceae* herbs. *Potravinarstvo*, vol. 9, no. 1, p. 252-257. http://dx.doi.org/10.5219/474

Afsharypuor, S., Sajjadi, S. E., ErfanManesh, M. 1997. Volatile constituents of *Origanum vulgare* ssp viride (syn *O. heracleoticum*) from Iran. *Planta Medica*, vol. 63, no. 2, p. 179-180. http://dx.doi.org/10.1055/s-2006-957640

Ahmad-Qasem, M. H., Barrajón-Catalán, E., Micol, V., Mulet, A., García-Pérez, J. V. 2013. Influence of freezing and dehydration of olive leaves (var. Serrana) on extract composition and antioxidant potential. *Food Research International*, vol. 50, no. 1, p. 189-196. http://dx.doi.org/10.1016/j.foodres.2012.10.028

Asensio, C. M., Grosso, N. R., Juliani, H. R. 2015. Quality characters, chemical composition and biological activities of oregano (*Origanum* spp.) essential oils from Central and Southern Argentina. *Industrial Crops and Products*, vol. 63, p. 203-213. http://dx.doi.org/10.1016/j.indcrop.2014.09.056

Baranauskienė, R., Venskutonis, P. R., Dambrauskienė, E., Viškelis, P. 2013. Harvesting time influences the yield and oil composition of *Origanum vulgare* L. ssp. *vulgare* and ssp. *hirtum*. *Industrial Crops and Products*, vol. 49, p. 43-51. http://dx.doi.org/10.1016/j.indcrop.2013.04.024

Beena, Kumar, D., Rawat, D. S. 2013. Synthesis and antioxidant activity of thymol and carvacrol based Schiff bases. *Bioorganic*, vol. 23, no. 3, p. 641-645. http://dx.doi.org/10.1016/j.bmcl.2012.12.001

Capecka, E., Mareczek, A., Leja, M. 2005. Antioxidant activity of fresh and dry herbs of some *Lamiaceae* species. *Food Chemistry*, vol. 93, no. 2, p. 223-226. http://dx.doi.org/10.1016/j.foodchem.2004.09.020

Carmo, E. S., Lima, E. D., de Souza, E. L. 2008. The potential of *Origanum vulgare* L. (*Lamiaceae*) essential oil in inhibiting the growth of some food-related *Aspergillus* species. *Brazilian Journal of Microbiology*, vol. 39, no. 2, p. 362-367. http://dx.doi.org/10.1590/S1517-83822008000200030

Chan, E. W. C., Tan, Y. P., Chin, S. J., Gan, L. Y., Kang, K. X., Fong, C. H., Chang, H. Q., How, Y. C. 2014. Antioxidant properties of selected fresh and processed herbs and vegetables. *Free Radicals and Antioxidants*, vol. 4, no. 1, p. 39-46. http://dx.doi.org/10.5530/fra.2014.1.7

Charles, D. J. 2013. *Antioxidant properties of spices, herbs and other sources.* Springer. New York. ISBN: 978-146-1443-094.

Chorianopoulos, N., Kalpoutzakis, E., Aligiannis, N., Mitaku, S., Nychas, G. J., Haroutounian, S. A. 2004. Essential oils of *Satureja*, *Origanum*, and *Thymus* species: chemical composition and antibacterial activities against foodborne pathogens. *Journal of Agricultural and Food Chemistry,* vol. 52, no. 26, p. 8261-8267. http://dx.doi.org/10.1021/jf049113i

Chouliara, E., Karatapanis, A., Savvaidis, I. N., Kontominas, M. G. 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 degrees C. *Food Microbiology*, vol. 24, no. 6, p. 607-617. http://dx.doi.org/10.1016/j.fm.2006.12.005

Chrpová, D., Kouřimská, L., Gordon M. H., Heřmánková, V., Roubíčková, O., Pánek, J. 2010. Antioxidant activity of selected phenols and herbs used in diets for medical conditions. *Czech Journal of Food Science*, vol. 28, no. 4, p. 317-325. http://www.agriculturejournals.cz/publicFiles/26123.pdf

Crocoll, C., Asbach, J., Novak, J., Gershenzon, J., Degenhardt, J. 2010. Terpene synthases of oregano (*Origanum vulgare* L.) and their roles in the pathway and regulation of terpene biosynthesis. *Plant Molecular Biology*, vol. 73, no. 6, p. 587-603. http://dx.doi.org/10.1007/s11103-010-9636-1

De Falco, E., Mancini, E., Roscigno, G., Mignola, E., Taglialatela-Scafati, O., Senatore, F. 2013. Chemical composition and biological activity of essential oils of *Origanum vulgare* L. subsp. *vulgare* L. under different growth conditions. *Molecules*, vol. 18, no. 12, p. 14948-14960. http://dx.doi.org/10.3390/molecules181214948

De Martino, L., De Feo, V., Formisano, C., Mignola, E., Senatore, F. 2009. Chemical composition and antimicrobial activity of the essential oils from three chemotypes of *Origanum vulgare* L. ssp. hirtum (Link) letswaart growing wild in Campania (Southern Italy). *Molecules*, vol. 14, no. 8, p. 2735-2746. http://dx.doi.org/10.3390/molecules14082735

Díaz-Maroto, M., Pérez-Coello, M., Cabezudo, M. 2002. Effect of different drying methods on the volatile components of parsley (*Petroselinum crispum* L.). *European Food Research and Technology*, vol. 215, no. 3, p. 227-230. http://dx.doi.org/10.1007/s00217-002-0529-7

Dixon, R. A., Paiva, N. L. 1995. Stress induced phenylpropanoid metabolism. *Plant Cell*, vol. 7, no. 7, p. 1085-1097. http://dx.doi.org/10.1105/tpc.7.7.1085

Dorman, H. J. D., Peltoketo, A., Hiltunen, R., Tikkanen, M. J. 2003. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected *Lamiaceae* herbs. *Food Chemistry*, vol. 83, no. 2, p. 255-262. http://dx.doi.org/10.1016/S0308-8146(03)00088-8

Govaris, A., Solomakos, N., Pexara, A., Chatzopoulou, P. S. 2010. The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella Enteritidis* in minced sheep meat during refrigerated storage. *International Journal of Food Microbiology*, vol. 137, no. 2-3, p. 175-180. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.12.017

Grassmann, J. 2005. Terpenoids as plant antioxidants. *Plant Hormones*, vol. 72, p. 505-535. http://dx.doi.org/10.1016/S0083-6729(05)72015-X

Hossain, M. B., Barry-Ryan, C., Martin-Diana, A. B., Brunton, N. P. 2010. Effect of drying method on the antioxidant capacity of six *Lamiaceae* herbs. *Food Chemistry*, vol. 123, no. 1, p. 85-91. http://dx.doi.org/10.1016/j.foodchem.2010.04.003

Ivanova, D., Gerova, D., Chervenkov, T., Yankova, T. 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology*, vol. 96, no. 1, p. 145-150. http://dx.doi.org/10.1016/j.jep.2004.08.033

Javadian, S., Sabouni, F., Haghbeen, K. 2016. *Origanum vulgare* L. extracts versus thymol: An anti-inflammatory study on activated microglial and mixed glial cells. *Journal of Food Biochemistry*, vol. 40, no. 1, p. 100-108. http://dx.doi.org/10.1111/jfbc.12199

Kawase, K. Y. F., Mothé, C. G., Furtado, F. A., Vieira, G. L. 2013. Changes in essential oil of *Origanum* vulgare L. affected by different extraction methods. *International Journal of Research and Reviews in Applied Sciences*, vol. 14, no. 2, p. 238-247. http://www.arpapress.com/Volumes/Vol14Issue2/IJRRAS_14_2_04.pdf

Keinänen, M., Julkunen-Tiitto, R. 1996. Effect of sample preparation method on birch (*Betula pendula* Roth) leaf phenolics. *Journal of Agricultural and Food Chemistry*, vol. 44, no. 9, p. 2724-2727. http://dx.doi.org/10.1021/jf960168x

Kikuzaki, H., Nakatani, N. 1989. Structure of a new antioxidative phenolic acid from oregano (*Origanum vulgare* L.). *Agricultural and Biological Chemistry*, vol. 53, no. 2, p. 519-524. https://www.jstage.jst.go.jp/article/bbb1961/53/2/53_2_519/_pdf

Kintzios, S. E. 2002. *Oregano the Genera* Origanum *and* Lippia. CRC Press. London. ISBN: 02-032-2209-1.

Koukoulitsa, C., Karioti, A., Bergonzi, M. C., Pescitelli, G., Di Bari, L., Skaltsa, H. 2006. Polar constituents from the aerial parts of *Origanum vulgare* L. ssp. *hirtum* growing wild in Greece. *Journal of Agricultural and Food Chemistry*, vol. 54, no. 15, p. 5388-5392. http://dx.doi.org/10.1021/jf061477i

Kouřimská, L., Sabolová, M., Dvořáková, B., Roubíčková, I., Pánek, J., Nový, P. 2014. Antioxidant activity of *Lamiaceae* herbs grown under organic and conventional farming. *Scientia Agriculturae Bohemica*, vol. 45, no. 1, p. 19-25. http://www.sab.czu.cz/?r=5595&mp=sab.detail&sab=86#605

Kulisic, T., Radonic, A., Katalinic, V., Milos, M. 2004. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, vol. 85, no. 4, p. 633-640. http://dx.doi.org/10.1016/j.foodchem.2003.07.024

Ličina, B. Z., Stefanovic, O. D., Vasic, S. M., Radojevic, I. D., Dekic, M. S., Čomić, L. R. 2013. Biological activities of the extracts from wild growing *Origanum vulgare* L. *Food Control*, vol. 33, no. 2, p. 498-504. http://dx.doi.org/10.1016/j.foodcont.2013.03.020

Memarzadeh, S. M., Pirbalouti, A. G., AdibNejad, M. 2015. Chemical composition and yield of essential oils from Bakhtiari savory (*Satureja bachtiarica* Bunge.) under different extraction methods. *Industrial Crops and Products*, vol. 76, p. 809-816. http://dx.doi.org/10.1016/j.indcrop.2015.07.068

Mockutë, D., Bernotienë, G., Judžentienë, A. 2004. Chemical composition of essential oils of *Origanum vulgare* L. growing in Lithuania. *Biologija*, vol. 2004, no. 4, p. 44-49. http://www.lmaleidykla.lt/publ/1392-0146/2004/4/Bio044_049.pdf

Najafian, S. 2014. Storage conditions affect the essential oil composition of cultivated Balm Mint Herb (*Lamiaceae*) in Iran. *Industrial Crops and Products*, vol. 52, p. 575-581. http://dx.doi.org/10.1016/j.indcrop.2013.11.015

Novák, I., Sipos, L., Kókai, Z., Szabó, K., Pluhár, Z., Sárosi, S. 2011. Effect of the drying method on the composition of *Origanum vulgare* L. subsp. *hirtum* essential oil analysed by GC-MS and sensory profile method. *Acta Alimentaria*, vol. 40, p. 130-138. http://dx.doi.org/10.1556/AAlim.40.2011.Suppl.13

Ozkan, G., Baydar, H., Erbas, S. 2010. The influence of harvest time on essential oil composition, phenolic constituents and antioxidant properties of Turkish oregano (*Origanum onites* L.). *Journal of the Science of Food and Agriculture*, vol. 90, no. 2, p. 205-209. http://dx.doi.org/10.1002/jsfa.3788

Rao, G. V., Mukhopadhyay, T., Annamalai, T., Radhakrishnan, N., Sahoo, M. R. 2011. Chemical constituents and biological studies of *Origanum vulgare* Linn. *Pharmacognosy Research*, vol. 3, no. 2, p. 143-145.

Rodríguez-Meizoso, I., Marin, F. R., Herrero, M., Señorans, F. J., Reglero, G., Cifuentes, A., Ibáñez, E. 2006. Subcritical water extraction of nutraceuticals with antioxidant activity from oregano. Chemical and functional characterization. *Journal of Pharmaceutical and Biomedical Analysis*, vol. 41, no. 5, p. 1560-1565. http://dx.doi.org/10.1016/j.jpba.2006.01.018

Şahin, F., Güllüce, M., Daferera, D., Sökmen, A., Sökmen, M., Polissiou, M., Agar, G., Özer, H. 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. *vulgare* in the Eastern Anatolia region of Turkey. *Food Control*, vol. 15, no. 7, p. 549-557. http://dx.doi.org/10.1016/j.foodcont.2003.08.009

Skotti, E., Anastasaki, E., Kanellou, G., Polissiou, M., Tarantilis, P. A. 2014. Total phenolic content, antioxidant activity and toxicity of aqueous extracts from selected Greek medicinal and aromatic plants. *Industrial Crops and Products*, vol. 53, p. 46-54. http://dx.doi.org/10.1016/j.indcrop.2013.12.013

Stamenic, M., Vulic, J., Djilas, S., Misic, D., Tadic, V., Petrovic, S., Zizovic, I. 2014. Free-radical scavenging activity and antibacterial impact of Greek oregano isolates obtained by SFE. *Food Chemistry*, vol. 165, p. 307-315. http://dx.doi.org/10.1016/j.foodchem.2014.05.091

Stefanakis, M. K., Touloupakis, E., Anastasopoulos, E., Ghanotakis, D., Katerinopoulos, H. E., Makridis, P. 2013.

Antibacterial activity of essential oils from plants of the genus *Origanum*. *Food Control*, vol. 34, no. 2, p. 539-546. http://dx.doi.org/10.1016/j.foodcont.2013.05.024

Stratil, P., Kuban, V., Fojtova, J. 2008. Comparison of the phenolic content and total antioxidant activity in wines as determined by spectrophotometric methods. *Czech Journal of Food Sciences*, vol. 26, no. 4, p. 242-253. http://www.agriculturejournals.cz/publicFiles/01961.pdf

Škrovánková, S., Mišurcová, L., Machů, L. 2012. Antioxidant activity and protecting health effects of common medicinal plants. *Advances in Food and Nutrition Research*, vol. 67, p. 75-139. http://dx.doi.org/10.1016/B978-0-12-394598-3.00003-4

Teixeira, B., Marques, A., Ramos, C., Serrano, C., Maros, O. Neng, N. R., Nogueira, J. M. F, Saraiva, J. A., Nunes, M. L. 2013. Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil. *Journal of the Science of Food and Agriculture*, vol. 93, no. 11, p. 2707-2714. http://dx.doi.org/10.1002/jsfa.6089

Tibaldi, G., Fontana, E., Nicola, S. 2011. Growing conditions and postharvest management can affect the essential oil of *Origanum vulgare* L. ssp. *hirtum* (Link) letswaart. *Industrial Crops and Products*, vol. 34, no. 3, p. 1516-1522. http://dx.doi.org/10.1016/j.indcrop.2011.05.008

Tomsone, L., Kruma, Z. 2014. Influence of freezing and drying on the phenol content and antioxidant activity of horseradish and lovage [online]. [cit. 2015-01-07]. FoodBalt conference papers. Available at: http://llufb.llu.lv/conference/foodbalt/2014/FoodBalt_Proceedings_2014-192-197.pdf

Tsimogiannis, D., Stavrakaki, M., Oreopoulou, V. 2006. Isolation and characterisation of antioxidant components from oregano (*Origanum heracleoticum*). *International Journal of Food Science and Technology*, vol. 41, p. 39-48. http://dx.doi.org/10.1111/j.1365-2621.2006.01259.x

Wojdyło, A., Oszmiański, J., Czemerys, R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, vol. 105, no. 3, p. 940-949. http://dx.doi.org/10.1016/j.foodchem.2007.04.038

Zheng, Z. L., Tan, J. Y. W., Liu, H. Y., Zhou, X. H., Xiang, X. 2009. Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistence against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). *Aquaculture*, vol. 292, no. 3-4, p. 214-218. http://dx.doi.org/10.1016/j.aquaculture.2009.04.025

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RELATION BETWEEN SELECTED NUTRIENTS IN THE CHICKEN MEAT DEPENDING ON PHYTOGENIC FEED ADDITIVES

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ABSTRACT

The aim of study was to evaluate the relation between selected nutrients in the breast and thigh muscles after the application of different phytogenic additives in the diet of broiler chickens and between same indicators of meat disregarding additive and parts of carcass, from which muscles originate. We realized an *in vivo* experiment on the Zámostie Company poultry test station with deep litter breeding system. The experiment included 100 pcs of one-day-old hybrid chickens Cobb 500 divided into 2 groups (n = 50): the 1st experimental group with an application of feed additive from chestnut tree and lemon fruit extracts and the 2nd experimental group with an application of feed additive from citrus fruits extract. We used a cereal and soybean basal diet and we divided the fattening period into four phases: starter (1 – 10 days), grower I (11 – 20 days), grower II (21 – 28 days) and finisher (29 – 42 days). We applied a powder form feed mixtures. Nutritive value of feed mixtures was the same in each experimental group during the whole experiment and in accordance with the physiological needs of broiler chickens. We fed the 1st experimental group with a basal diet enriched by feed additive from chestnut tree and lemon fruit extracts (50 g/100 kg). As for the 2nd experimental group, we applied feed additive from citrus fruits extracts through the drinking water (100 mL/100 L). In the 2nd part of our experiment, we compared results obtained from two experimental groups with other four groups of diet. We applied other phytogenic additives to these four groups and we did not take into account the origin of the meat sample. We measured indicators of the chemical composition of protein, fat, water and cholesterol on a sample (50 g) of breast and thigh muscle without skin by the method of FT IR by use of the apparatus Nicolet 6700. Detected relations between nutrients of breast and thigh muscles were defined by correlation coefficient of $-0.6 \leq r \geq +0.6$. When additive with chestnut tree and lemon fruit extracts was used, we detected a negative correlation ($p \leq 0.01$) between protein and cholesterol of breast muscle. In thigh muscle, the negative correlation was observed between protein and energy ($p \leq 0.05$), protein and fat ($p \leq 0.01$) as well as fat and water. The only positive correlation was detected between protein and cholesterol of breast muscle ($p \leq 0.01$), with additive citrus fruits extract. When nutrition and parts of carcass, from which muscles originate, were disregarded, protein of meat increased, energy and fat decreased ($p \leq 0.001$). When fat of meat increased, energy increased ($p > 0.05$) as well, but water decreased ($p \leq 0.05$; $p \leq 0.001$).

Keywords: phytogenic feed additive; breast and thigh muscle; chicken meat; nutrient

INTRODUCTION

Poultry meat is an important source of proteins, but other constituents as fats have an important role in its composition, too. Manipulation in animal feeding **(Kennedy et al., 2005)** or *post mortem* manipulation of carcass body may affect meat quality. In recent years, products containing essential oils derived from several spices and herbs could be used in animal nutrition as feed additives to promote the growth. These phytogenic additives may have more than one mode of action, including improving feed intake and flavour, stimulating the secretion of digestive enzymes, increasing gastric and intestinal motility, endocrine stimulation, antimicrobial, anti-viral, anthelminthic and coccidiostat activities, immune stimulation, and anti-inflammatory and anti-oxidative activity and pigments **(Kirkpinar et al., 2011)**. Earlier published papers **(Smid and Gorris, 1999)** present that essential oils achieved positive performance in antibacterial *in vitro* studies. We need greater concentration of essential oil to achieve the same effect in foods. High levels of fat and/or protein in foodstuffs protect the bacteria from the action of the essential oil in some way **(Tassou, 1995)**.

This short literature review suggests that an application of phytogenic substances based on essential oils have some unanswered questions in food research.

The aim of our study was to evaluate the relation: a) between selected nutrients, energy and water of breast and thigh muscles regarding to application of two different phytogenic additives in the diet of broiler chickens; b) between the same nutrients, energy and water of chicken meat disregarding parts of carcass, from which muscles originate (breast and thigh muscles together).

MATERIAL AND METHODOLOGY

Experiment, broiler chickens, nutrition

We realized an *in vivo* experiment on the Zámostie Company poultry test station with deep litter breeding system. The experiment included 100 pcs of one-day-old hybrid chickens Cobb 500 divided into 2 groups (n = 50): the 1st experimental group with an application of feed additive from chestnut tree and lemon fruit extracts and the 2nd experimental group with an application of feed additive from citrus fruits extract. Phytogenic substances obtained from chestnut tree and lemon fruit extracts created a base of applied feed additive. The feed additive represents a mixture of taste compounds with supporting antioxidative, antimicrobial and cleansing effects in the digestive tract. Citrus fruits extract included extracts obtained from four species of citrus fruits: grapefruit (*Citrus paradisi*), tangerine (*Citrus reticulata blanco*), bergamot (*Citrus aurantium* ss. Bergamia) and sweet orange (*Citrus sinensis*). We used a cereal and soybean basal diet and we divided the fattening period into four phases: starter (1 – 10 days), grower I (11 – 20 days), grower II (21 – 28 days) and finisher (29 – 42 days). We applied a powder form of feed mixtures. Nutritive value of feed mixtures was the same in each experimental group during the whole experiment and in accordance with the physiological needs of broiler chickens. We fed the 1st experimental group with a basal diet enriched by feed additive from chestnut tree and lemon fruit extracts (50 g/100 kg). As for the 2nd experimental group, we applied feed additive from citrus fruits extracts through the drinking water (100 mL/100 L). In the 2nd part of our experiment, we compared results obtained from two experimental groups with other four groups of diet. We applied other phytogenic additives to these four groups and we did not take into account the origin of the meat sample.

Sample analyses

At the end of the experiment (day 42), we randomly selected 6 pcs from each group with an average live body weight of about 1800 g. We performed a slaughtering of chickens by human rapid cut of the carotid artery (*Ateria carotis communis*). We separated breast and thigh muscle from the carcass and we used them for chemical analysis. We measured indicators of the chemical composition of protein, fat, water and cholesterol on a sample (50.0 g) of breast and thigh muscle without skin by the method of FT IR by use of the apparatus Nicolet 6700. We performed a molecular spectroscopy for infrared spectrum of muscle homogenates analyses. The principle of this method is infrared absorption spectrum of the sample passes and there is a change from the rotary vibrating energy conditions of the molecule depending on the changes of the dipole moment of the molecule. Analytical output is the infrared spectrum, which is a graphical representation of the function of the energy dependence, mostly mentioned as a percentage of transmittance (T) or in units of absorbance (A) at a wavelength of the incident radiation. Permeability is defined as ratio of the radiation intensity which has passed through the sample (I) and of the emission intensity of emitted source (Io). Absorbance is defined as a decimal logarithm of 1/T.

Calculation of the energy value of meat according to the measured values of protein and fat, and the corresponding coefficients: 16.75 ×protein +37.68 ×fat (kJ/100 g).

Statistical analyses

We present our results in the form of mean, standard deviation, minimum and maximum values We used Scheffe´s test at the significance level of $\alpha = 0.05$ to compare a difference between groups. We used a Pearson´s correlation coefficient to reflect a degree of relation between two variables of selected chemical indicators of chicken breast muscle, thigh muscle and meat. Pearson´s r reflects the degree of linear relation between the two data sets. Its value is between -1 and +1. A value of +1 means, that there is a perfect positive linear relation between two data sets. A value of -1 means that there is a perfect negative linear relation and a value of 0 means, that there is no linear relation at all between data sets. We mainly focused our attention on the assessment of relation between two variables defined by correlation coefficient $-0.6 \leq r \geq +0.6$. We supplemented our results of correlation coefficient statistical significance at the significance level of $\alpha = 0.05$, 0.01 and 0.001. We used SAS statistical package (**SAS Institute, 1998**) to perform statistical analyses.

RESULTS AND DISCUSSION

Chemical composition of breast and thigh muscles in relation to feed additive and disregarding parts of carcass from which muscles originate (breast and thigh muscles together)

Chemical composition of breast and thigh muscles in relation to feed additive are shown in Table 1 and disregarding parts of carcass from which muscles originate (breast and thigh muscles together) in Table 2.

In two experimental groups, values of protein content of breast muscles are relatively similar 23.83 and 24.09 g/100 g, respectively. We observed slightly larger difference between the protein content of breast muscle compared to protein content of thigh muscle depending on the type of phytogenic substances in feed mixtures. We did not observe a statistically significant difference ($p > 0.05$) between groups. Similar values of protein content in breast muscles found **Haščík et al., (2012),** but in the hybrid combination of the chickens Ross 308.

We recorded the protein content of 22.73 g/100 g in the group with a feed additive from chestnut tree, and lemon fruit extracts compared with 22.15 g/100 g for the group with the feed additive from citrus fruits extract in the thigh muscles. We observed statistically significant difference ($p \leq 0.05$) between protein content of thigh muscle of the 1st group and the breast muscle of the 2nd group (Table 1). Table 2 presents that, the average protein content of chicken meat was 23.21 g/100 g disregarding parts of carcass, from which muscles originate. The type of phytogenic supplements did not affect the energy value of breast muscle and thigh muscle.

The energy values of breast samples were 435.69 kJ/100 g and 437.26 kJ/100 g in the 1st experimental group and the 2nd experimental group, respectively. Slightly lower energy value of broiler chicken breast muscles Cobb 500 found **Angelovičová and Semivanova (2013)**. We

Table 1 Nutrients, energy and water in the chicken breast and thigh muscles in relation to feed additive.

Variable	n	Breast muscle				Thigh muscle			
		Mean	*SD*	Min.	Max.	Mean	*SD*	Min.	Max.
Feed additive from chestnut tree and lemon fruit extracts									
Protein (g/100 g)	6	23.83	0.49	22.90	24.21	22.73	0.92	21.69	24.40
Energy (kJ/100 g)	6	435.69	6.03	428.72	444.50	458.13	15.82	438.17	474.04
Fat (g/100 g)	6	0.69	0.24	0.46	1.02	1.39	0.60	0.35	2.00
Water (g/100 g)	6	74.85	0.35	74.29	75.32	74.33	0.44	73.77	74.83
Cholesterol (g/100 g)	6	0.23	0.07	0.17	0.34	0.32	0.09	0.22	0.45
Feed additive from citrus fruits extract									
Protein (g/100 g)	6	24.09	0.42	23.57	24.63	22.15	0.49	21.58	22.92
Energy (kJ/100 g)	6	437.26	11.21	424.86	453.78	459.26	12.55	440.66	472.53
Fat (g/100 g)	6	0.69	0.42	0.39	1.50	1.21	0.07	1.09	1.29
Water (g/100 g)	6	74.73	0.24	74.39	75.07	74.09	0.56	73.62	74.92
Cholesterol (g/100 g)	6	0.25	0.11	0.12	0.36	0.36	0.06	0.31	0.44

Legend: n – the number of samples, *SD* – standard deviation, Min. – the minimum value, Max. – the maximum value.

Table 2 Nutrients, energy and water in the chicken meat disregarding parts of carcass from which muscles originate (breast and thigh muscles together).

Variable	n	Mean	*SD*	Min.	Max.
Protein (g/100 g)	72	23.21	0.91	21.58	24.71
Energy (kJ/100 g)	72	448.35	18.42	415.73	492.78
Fat (g/100 g)	72	1.05	0.49	0.21	2.10
Water (g/100 g)	72	74.46	0.59	72.52	75.32
Cholesterol (g/100 g)	72	0.29	0.08	0.10	0.45

Legend: n – the number of samples, *SD* – standard deviation, Min. – the minimum value, Max. – the maximum value.

detected almost the same energy value of thigh muscle in two experimental groups. The energy value of breast muscle and thigh muscle of two experimental groups was not statistically significant (p >0.05), while calculated energy value disregarding parts of carcass, from which muscle originates (Table 2) was 448.35 kJ/100 g.

We obtained interesting results of the fat content of breast muscles in our experiment. We detected the same value of 0.69 g/100 g in the group with feed additive from chestnut tree and lemon fruit extracts, and in the group with feed additive from citrus fruits extract. Fat content values of thigh muscle samples were higher than values of breast muscle samples. In the group with feed additive from chestnut tree and lemon fruit extracts, we detected an average value 1.39 g/100 g of fat in thigh muscle, and 1.21 g/100 g of fat in the group with feed additive from citrus fruits extracts. A difference of fat content was not statistically significant (p >0.05) between groups. The value of fat content in chicken meat was 1.5 g/100 g, when we disregarded the part of carcass, from which the muscle originates and feed additive. Moisture content results in breast samples were almost the same, i.e. 74.85 g/100 g and 74.73 g. /100 g in the 1st experimental group and the 2nd experimental group, respectively.

We did not find statistically significant difference (p >0.05) between experimental groups. Similar values of dry matter of chicken breast muscle of Cobb 500 found **Medveď and Angelovičová (2010).**

Other study with the same hybrid of chickens – Cobb 500 presents lower water content values of 73.73 and 73.29 g/100 g. Authors used commercial feed and they slaughtered chickens of experimental groups at the age of 42 days **(Suchý et al., 2002)**.

Our results of detected water content in the thigh samples were 74.33 g/100 g and 74.09 g/100 g in the 1st experimental group and 2nd experimental group, respectively. The conclusions of other studies **(Al-Sultan, 2003; Latshaw and Moritz, 2009)** confirmed decreased water content of the thigh muscles compared with breast muscle. We obtained same results. Water content of chicken meat was 74.46 g/100 g disregarding parts of carcass, from which the muscles originates and feed additive.

The image of meat and meat products is relatively negative due to their content of fat and saturated fatty acids, cholesterol, sodium and any other substances (e.g. nitrosamines) that somehow can be involved in most prevalent diseases of Western societies like cardiovascular diseases and *diabetes mellitus* **Micha et al., 2010)** and cancer **(Ferguson, 2010)**. Our results of cholesterol content were almost the same in relation to the type of used phytogenic substances. The cholesterol content was slightly increased in the group with feed additive from chestnut tree and lemon fruit extracts compared to group with feed additive from citrus fruits extract. We did not observe statistically significant difference between two experimental groups (p >0.05). The cholesterol content of breast muscles was 0.23 g/100 g in the 1st experimental group and 0.25 g/100 g in the 2nd experimental group. The cholesterol content of thigh muscles was 0.32 g/100 g (the 1st experimental group) and 0.36 g/100 g (the 2nd experimental group). The cholesterol content of chicken meat was 0.29 g/100 g when we disregarded parts of carcass from which the muscles originate.

Within this context, the poultry meat has maintained its identity and a higher value compared to other species for several reasons. Indeed, worldwide poultry meat production and consumption have increased rapidly and, in many parts of the world, it is assumed, per capita consumption of poultry meat will continue to grow

(Cavani, 2009). Relatively low and competitive prices compared to other meats, the absence of cultural or religious obstacles, and dietary and nutritional properties are the main factors explaining poultry meat's attractiveness **(Valceschini, 2006)**.

Relation between selected nutrients, energy and water of chicken breast and thigh muscles depending on feed additive and disregarding parts of carcass from which the muscles originate (breast and thigh muscles together)

A positive correlation coefficient indicates that an increase in the first variable would correspond to an increase in the second variable, thus implying a direct relation between the variables. A negative correlation indicates an inverse relation where as one variable increases, the second variable decreases.

Relation between selected nutrients, energy and water of chicken breast and thigh muscles in the 1st experimental group with feed additive from chestnut tree and lemon fruit extracts

Relation between selected nutrients, energy and water of chicken breast muscle in the 1st experimental group

Table 3 presents a correlation coefficient (r) between nutrients, energy and water of breast muscles in the 1st experimental group.

Table 3 Correlation coefficient (r) between nutrients, energy and water of breast muscles in the 1st experimental group.

Variable	Energy	Fat	Water	Cholesterol
Protein	-0.27	-0.42	0.60	-0.95^{++}
Energy		-0.12	-0.63	0.01
Fat			-0.47	0.43
Water				-0.36

Legend: Numerical data – the correlation coefficient (r) between two variables.
++: value with superscript mark is significantly different ($p \leq 0.01$).

Combined essential oils have additive, synergistic, and antagonistic effects **(Burt, 2004)**. Many commercial products on the market have one or more combined essential oils. Utilization of any feed additive is justified due to the larger beneficial effect compared to the cost of the product. In our experiment, feed additive from chestnut tree and lemon fruit extracts influenced the correlative relation between certain nutrients, energy and water of breast muscle. We detected the relation with correlation coefficient of $-0.6 \leq r \geq +0.6$ between protein and water, between protein and cholesterol and between energy and water. All relations with correlation coefficient value of $-0.6 \leq r \geq +0.6$ were not statistically significant. A positive correlation without statistically significant difference ($p > 0.05$) was between protein and water. We observe statistically significant difference ($p \leq 0.01$) between protein and cholesterol. When protein content of breast muscle increased, cholesterol content decreased. When energy value of breast muscle increased, water content decreased, without statistically significant differences ($p > 0.05$).

We did not find any literature information about effects of essential oils on relations between nutrients of chicken meat, parts of carcass, respectively. Essential oils have antimicrobial, antifungal and antioxidant effects. The effects of several type of essential oils, their combinations, or a combination with other substances might be related to the relations between nutrients of chicken meat, breast and thigh muscles. Citrus species of various origins have been assessed for their phenolic constituents and antioxidant activities **(Guimarães et al., 2009)**. Citrus fruits, citrus fruit extracts and citrus flavonoids exhibit a wide range of promising biological properties including anti-atherogenic, anti-inflammatory and antitumor activity, inhibition of blood clots and strong antioxidant activity **(Middleton and Kandaswami, 1994)**.

Extracts of citrus fruit (e.g. lemon, orange, and grape fruit) are among the most studied natural antimicrobials for food applications. Extracts of citrus fruit effectively decrease the growth of bacteria. Limonoids obtained from *Citrus limon* showed good antibacterial and antifungal activity **(Corbo et al., 2008)**. There are several citrus species, i.e. *Citrus limon* (lemon), *Citrus aurantium* (bitter orange), *Citrus limetta* (sweet lemon), *Citrus jambhiri* (rough lemon) and *Citrus paradise* (grapefruit) **(Al-Ani et al., 2009)**.

Three types of flavonoids occur in citrus fruit, i.e. flavanones (including 3-hydroxyflavanones), flavones (including 3-hydroxyflavones) and anthocyanins **(Horowitz and Gentili, 1977)**.

Eight tested limonoids, i.e. nomilin, limonin, deacetylnomilin, limonol, obacunone, deoxylimonin, isoobacunoic acid and ichangin stimulated the detoxifying enzyme, glutathione S-transferase **(Lam and Hasegawa, 1989)**. Glutathione S-transferase enzymes are one of the major enzyme systems responsible for the detoxification of xenobiotics **(Chasseaud, 1979)**.

Vitamin C and bioflavonoids. Bioflavonoids are a class of water-soluble plant pigments. Vitamin C-rich fruits and vegetables, especially citrus fruits, are often rich sources of bioflavonoids as well. Two small published studies examined the effect of bioflavonoids on the bioavailability of ascorbic acid. In one study, synthetic ascorbic acid given in a natural citrus extract containing bioflavonoids (in the ratio of bioflavonoids to ascorbic acid of 4:1), proteins, and carbohydrates, was more slowly absorbed and 35% more bioavailable than synthetic ascorbic acid alone, based on plasma levels of ascorbate over time and 24-hour urinary excretion of ascorbate. In the other study, there was no difference in the bio-availability of 500 mg of synthetic ascorbic acid and that of a commercially available vitamin C preparation with added bioflavonoids, where the ratio of bioflavonoids to ascorbic acid was 0.05:1 **(Higdon, 2001)**.

Relation between selected nutrients, energy and water of chicken thigh muscles in the 1st experimental group

Table 4 presents a correlation coefficient (r) between nutrients, energy and water of thigh muscles in the 1st experimental group.

Table 4 Correlation coefficient (r) between nutrients, energy and water of thigh muscles in the 1st experimental group.

Variable	Energy	Fat	Water	Cholesterol
Protein	-0.83+	-0.95++	0.70	-0.25
Energy		0.75	-0.64	0.35
Fat			-0.82+	0.10
Water				-0.15

Legend: Numerical data – the correlation coefficient (r) between two variables,
+, ++: value with superscript mark is significantly different ($p \leq 0.05$, $p \leq 0.01$).

Extracts of chestnut tissue display a strong antimicrobial activity against many plant pathogens, which is probably associated with antimicrobial compounds such as flavonol glycoside and several terpenoid substances **(Hao et al., 2012)**. The results presented in study **(Blaiotta et al., 2013)** indicate that chestnut extracts can greatly improve the tolerance of lactobacilli to simulated gastric and bile juice. Chestnut extracts exhibited a surprising effect in improving the tolerance to gastric transit of lactobacilli. The study confirmed that scoparone and scopoletin isolated from chestnut inner shell extract have antioxidant effects, and scopoletin has relatively higher antioxidant capacity than scoparone in an oxidative stress-induced in vitro system.

Chestnut inner shell extract including scoparone and scopoletin as main compounds has the ability to protect against damage due to oxidative stressors including tert-butyl hydroperoxide, carbon tetrachloride (CCl_4), and high-fat diet, by preventing reactive oxygen species generation, decrease of antioxidant enzyme activity, and inhibiting malondialdehydu production. The chestnut inner shell extract might be useful as a natural ingredient for the prevention of oxidative damage in liver cells and tissues **(Noh et al., 2010)**.

In our experiment, the influence of feed additive from chestnut tree and lemon fruit extracts on relation between certain nutrients, energy and water of the thigh muscles was higher compared to breast muscle. We detected a relation with correlation coefficient of $-0.6 \leq r \geq +0.6$ between protein and energy, protein and fat, protein and water, energy and fat, energy and water as well as fat and water. All these correlation relations were not statistically significant. When energy and fat of thigh muscles increased, protein content decreased. These relations were statistically significant ($p \leq 0.05$, $p \leq 0.01$, respectively). When fat content of the thigh muscles increased, water content decreased, with statistically significant difference ($p \leq 0.05$).

Many authors have concluded that essential oils exhibit greater antimicrobial activity than other major components taken together. This could means that either the minor components are critical to the antimicrobial activity or that synergistic effects may occur **(Burt, 2004)**. The major components reflect the biological properties of essential oils, but minor components can modulate their activity, for example the cell penetration, hydrophobicity and fixation on membranes **(Bakkali et al., 2008)**. The composition of essential oil results in interactions between the components that both qualitatively and quantitatively change their evaporation rates **(Saiyasombati and Kasting, 2003)**.

Relation between selected nutrients, energy and water of chicken breast and thigh muscles in the 2nd experimental group with feed additive from citrus fruits extract

Relation between selected nutrients, energy and water of chicken breast muscle in the 2nd experimental group

Table 5 presents a correlation coefficient (r) between nutrients, energy and water of breast muscles in the 2nd experimental group.

We could consider citrus essential oils as suitable alternatives to chemical additives for use in the food industry, attending to the needs for safety and satisfying the demand of consumers for natural components **(Viuda-Martos et al., 2008)**. Since the major component of citrus essential oils is limonene, the chemical, physical and biological properties of this compound greatly affect the properties of the citrus essential oils **(Bakkali et al., 2008)**. For this reason, we can find documented antimicrobial effect of citrus essential oils attributed to the essential oil or to limonene as well, as its main component. Biodegradation essential oils, and in particular limonene; mechanism by which essential oils inhibit anaerobic digestion is not yet understood **(Ruiz and Flotats, 2014)**.

Table 5 Correlation coefficient (r) between nutrients, energy and water of breast muscles in the 2nd experimental group.

Variable	Energy	Fat	Water	Cholesterol
Protein	-0.08	-0.51	-0.40	-0.50
Energy		-0.62	0.08	0.86+
Fat			0.37	-0.40
Water				0.32

Legend: Numerical data – the correlation coefficient (r) between two variables,
+: Value with superscript mark is significantly different ($p \leq 0.05$).

The antimicrobial activity of terpenes and terpenoids (cyclic hydrocarbons) is due mainly to their interaction with the cell membrane **(Bakkali et al., 2008)**. We detected an influence of feed additive from citrus fruits extract only on relations between energy and fat, as well as energy and cholesterol. A correlation coefficient of relation between energy and fat, as well as energy and cholesterol was $-0.6 \leq r \geq +0.6$. An interesting result is the relation between energy and fat. When fat content of breast muscles increased, energy content decreased. The difference was not statistically significant ($p > 0.05$). We detected statistically significant difference ($p \leq 0.01$) of positive correlation between energy and cholesterol.

Relation between selected nutrients, energy and water of chicken thigh muscles in the 2nd experimental group

Table 6 presents a correlation coefficient (r) between nutrients, energy and water of thigh muscles in the 2nd experimental group.

Table 6 Correlation coefficient (r) between nutrients, energy and water of thigh muscles in the 2nd experimental group.

Variable	Energy	Fat	Water	Cholesterol
Protein	-0.41	0.04	-0.27	-0.30
Energy		0.66	-0.25	0.42
Fat			0.05	-0.14
Water				-0.73

Legend: Numerical data – the correlation coefficient (r) between two variables.

The influence of feed additive from citrus fruits extract on relation between some nutrients, energy and water of thigh muscles was lower compared to the effects of feed additive from chestnut tree and lemon fruit extracts. We found a positive correlation with correlation coefficient of $-0.6 \leq r \geq +0.6$ between energy and fat of thigh muscles. The difference was not statistically significant ($p > 0.05$). Citrus essential oils are a complex mixture of volatile compounds that show, among other properties, antifungal activity by reducing or totally inhibiting fungal growth in a dose-response manner **(Sharma and Tripathi, 2006)**. This activity is a resalt of a single major compound or synergistic or antagonistic effect of various compounds **(Deba et al., 2007)**.

Relation between selected nutrients, energy and water of chicken meat disregarding parts of carcass, from which muscles originate

Table 7 presents a correlation coefficient (r) between nutrients, energy and water of chicken meat disregarding parts of carcass, from which muscles originate (breast and thigh muscles together).

Table 7 Correlation coefficient (r) between nutrients, energy and water of chicken meat disregarding parts of carcass from which the muscles originate (breast and thigh muscles together).

Variable	Energy	Fat	Water	Cholesterol
Protein	-0.64^{+++}	-0.75^{+++}	0.42^{+++}	-0.37^{+++}
Energy		0.70^{+++}	-0.67^{+++}	0.39^{+++}
Fat			-0.59^{+++}	0.26^{+}
Water				-0.30^{++}

Legend: Numerical data – the correlation coefficient (r) between two variables,
+, ++, +++: Value with superscript mark is significantly different ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$).

In our experiment, disregarding nutrition and parts of carcass, from which muscles originate, we found a statistically significant relation with correlation coefficient of $-0.6 \leq r \geq +0.6$ between protein and energy, protein and fat, as well as energy and fat, energy and water, fat and water of chicken meat. When protein content of chicken meat increased, energy content and fat content decreased with statistically significant differences ($p \leq 0.001$). These relations are relatively comparable to the relations between protein, energy and water of thigh muscles in the 1st experimental group. When fat content of chicken meat increased, energy content increased as well without statistically significant difference ($p > 0.05$), but water content decreased with statistically significant difference ($p \leq 0.001$). When energy content of chicken meat increased, water content decreased with statistically significant difference ($p \leq 0.001$). We observed a relation with correlation coefficient of $-0.6 \leq r \geq +0.6$ between cholesterol and protein, cholesterol and fat, cholesterol and energy, cholesterol and water, as well as protein and water. The differences were statistically significant ($p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$, respectively). These relations are relatively comparable to relations between cholesterol, energy and water of thigh muscles in the 1st experimental group.

CONCLUSION

We can confirm based on a statistical evaluation of the results of the experiment that:
a) broiler chicken nutrition is one of the major factors which must be taken into account in the production of chicken meat;
b) various additives of phytogenic substances in the feed mixtures for broiler chicken differently affected relations between protein and cholesterol in breast muscle, and between protein and energy, between protein and fat, and the fat and water in thigh muscle.

REFERENCES

Al-Ani, W. N., Al-Haliem, S. M., Tawfik, N. O. 2009. Evaluation of the antibacterial activity of citrus juices: an *in vitro* study. *Al-Rafidain Dental Journal*, vol. 10, no. 2, p. 376-382.

Al-Sultan, I. S. 2003. The Effect of *Curcuma longa* (Tumeric) on Overall Performance of Broiler Chickens. *International Journal of Poultry Science*, vol. 2, no. 5, p. 351-353. http://dx.doi.org/10.3923/ijps.2003.351.353

Angelovičová, M., Semivanová, M. 2013. The effect of iodine in production of broiler chickens and selected quality indicators of breast muscles. Potravinarstvo, vol. 7, no. 1, p. 111-119. http://dx.doi.org/10.5219/297

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. 2008. Biological effects of essential oils: a review. *Food and Chemical Toxicology*, vol. 46, no. 2, p. 446-475. http://dx.doi.org/10.1016/j.fct.2007.09.106 PMid:17996351

Blaiotta, G., La Gatta, B., Di Capua, M., Di Luccia, A., Coppola, R., Aponte, M. 2013. Effect of chestnut extract and chestnut fiber on viability of potential probiotic *Lactobacillus* strains under gastrointestinal tract conditions. *Food Microbiology*, vol. 36, no. 2, p. 161-169. http://dx.doi.org/10.1016/j.fm.2013.05.002 PMid:24010594

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods: a review. *International Journal of Food Microbiology*, vol. 94, no. 3, p. 223-253. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022 PMid:15246235

Cavani, C., Petracci, M., Trocino, A., Xiccato, G. 2009. Advances in research on poultry and rabbit meat quality. *Italian Journal of Animal Science*, vol. 8, suppl.2, p. 741-750. http://dx.doi.org/10.4081/ijas.2009.s2.741

Chasseaud, L. F. 1979. The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. *Advances in Cancer Research*, vol. 29, p. 175-274. http://dx.doi.org/10.1016/S0065-230X(08)60848-9

Corbo, M. R., Speranza, B., Filippone, A., Granatiero, S., Conte, A., Sinigaglia, M., Del Nobile, M. A. 2008. Study on the synergic effect of natural compounds on the microbial quality decay of packed fish hamburger. *The International Journal of Food Microbiology*, vol. 127, no. 3, p. 261-267. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.07.014 PMid:18804302

Deba, F., Dang Xuan, T., Yasuda, M., Tawata, S. 2007. Hrbicidal and fungicidal activities and identification of potential phytotoxins from *Bidens pilosa* L. var. radiata Scherff. *Weed Biology* and *Management*, vol. 7, no. 2, p. 77-83. http://dx.doi.org/10.1111/j.1445-6664.2007.00239.x

Ferguson, L. R. 2010. Meat and cancer. *Meat Science*, vol. 84, p. 308-313. http://dx.doi.org/10.1016/j.meatsci.2009.06.032 PMid:20374790

Guimarães, R., Barros, L., Barreira, J. C. M., Sousa, M. J., Carvalho, A. M., Ferreira, I. C. F. R. 2009. Targeting excessive free radicals with peels and juices of citrus fruits: grapefruit, lemon, lime and orange. *Food and Chemical Toxicology*, vol. 48, no. 1, p. 99-106. http://dx.doi.org/10.1016/j.fct.2009.09.022 PMid:19770018

Hao, J. J., Liu, H. W., Donis-Gonzalez, I. R., Lu, X. H., Jones, A. D., Fulbright, D. W. 2012. Antimicrobial activity of chestnut extracts for potential use in managing soilborne plant pathogens. *Plant Disease*, vol. 96, no. 3, p. 354-360. http://dx.doi.org/10.1094/PDIS-03-11-0169

Haščík, P., Elimam, I. O. E., Garlík, J., Kačániová, M., Bobko, M., Kňazovická, V., Vavrišinová, K., Arpášová, H., Bučko, O. 2012. Chemical composition of muscle after pollen application in nutrition of broiler chickens. *Potravinarstvo*, vol. 6, no. 2, p. 26-32. http://dx.doi.org/10.5219/190

Higdon, J. 2001. The Bioavailability of different forms of vitamin C. In *LPI Research Associate* [online], May 2001 [cit. 2005-11-05]. Available at: http://lpi.oregonstate.edu/ss01/bioavailability.html.

Horowitz, R. M., Gentili, B. 1977. Flavonoid Constituents of Citrus. In Nagy, S., Shaw, P. E., Veldhuis, M. K. *Citrus Science and Technology*. 1st ed. Westport, C. T. AVI Publishing Co., p. 397-426.

Kennedy, O. B., Stewart-Knox, B. J., Mitchell, P. C., Thurnham, D. I. 2005. Vitamin E supplementation, cereal feed type and consumer sensory perceptions of poultry meat quality. *British Journal of Nutrition*, vol. 93, no. 3, p. 333-338. http://dx.doi.org/10.1079/BJN20041336 PMid:15877872

Kirkpinar, F., Ünlü, H. B., Özdemir, G. 2011. Effects of oregano and garlic essential oils on performance, carcase, organ and blood characteristics and intestinal microflora of broilers. *Livestock Science*, vol. 137, no. 1-3, p. 219-225. http://dx.doi.org/10.1016/j.livsci.2010.11.010

Lam, L. K. T., Li, Y., Hasegawa, S. 1989. Effects of citrus limonoids on glutathione S-transferase activity in mice. *Journal of Agricultural and* Food Chemistry, vol. 37, p. 878-880. http://dx.doi.org/10.1021/jf00088a010

Latshaw, D. J., Moritz, S. J. 2009. The partitioning of metabolizable energy by broiler chickens. *Poultry Science*, vo. 88, no. 1, p. 98-105. http://dx.doi.org/10.3382/ps.2008-00161 PMid:19096063

Medveď, J., Angelovičová, M. 2010. Protein and fat in breast breast muscules of broilers in application welfare principles in practical conditions. *Potravinarstvo*, vol. 4, no. 3, p. 50-52. http://dx.doi.org/10.5219/66

Micha, R., Wallace, S. K., Mozaffarian, D. 2010. Red and processed meat consumption and risk of incident coronary heart disease, stroke and diabetes mellitus. A systematic review and meta-analysis. *Circulation*, vol. 121, no. 21, p. 2271-2283. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.924977 PMid:20479151

Middleton, E., Kandaswami, C. 1994. Potential health-promoting properties of Citrus flavonoids. *Food Technology*, vol. 48, no. 11, p. 115-120.

Noh, J., Gang, G., Kim, I., Yang, K., Hwang, J., Lee, H., Oh, W., Song, K., Lee, Ch. 2010. Antioxidant effects of the chestnut (*Castanea crenata*) inner shell extract in t-BHP-treated HepG2 cells, and CCl_4, and high-fat diet-treated mice. *Food and Chemical Toxicology*, vol. 48, no. 11, p. 3177-3183. http://dx.doi.org/10.1016/j.fct.2010.08.018 PMid:20732376

Ruiz, B., Flotats, X. 2014. Citrus essential oils and their influence on the anaerobic digestion process: an overview. *Waste Manage*, vol. 34, no. 11, p. 2063-2079. http://dx.doi.org/10.1016/j.wasman.2014.06.026 PMid:25081855

Saiyasombati, P., Kasting, G. B. 2003. Two-stage kinetic analysis of fragrance evaporation and absorption from the skin. *International Journal of Cosmetic Science*, vol. 25, no. 5, p. 235-243. http://dx.doi.org/10.1046/j.1467-2494.2003.00193.x PMid:18494906

SAS Institute. 1998. StatView. Version 5.0. SAS Institute, Cary, N. C.

Sharma, N., Tripathi, A. 2006. Effects of *Citrus sinensis* (L.) Osbeck epecarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiological Research*, vol. 163, no. 3, p. 337-344. http://dx.doi.org/10.1016/j.micres.2006.06.009 PMid:16870411

Smid, E. J., Gorris, L. G. M. 1999. Natural antimicrobials for food preservation. In Rahman, M. S. *Handbook of Food Preservation*. New York: Marcel Dekker, p. 285-308.

Suchý, P., Jelínek, P., Straková, E., Hucl, J. 2002. Chemical composition of muscles of hybrid broiler chickens during prolonged feeding**.** *Czech Journal of Animal Science*, vol. 47, no. 12, p. 511-518.

Tassou, C., Drosinos, E. H., Nychas, G. J. E. 1995. Effects of essential oil from mint (*Mentha piperita*) on *Salmonella enteritidis* and *Listeria monocytogenes* in model food systems at 4 jC and 10 jC. *Journal* of *Applied Bacteriology*, vol. 78, no. 6, p. 593-600.

Valceschini, E. 2006. Poultry Meat Trends and Consumer Attitudes. In *Proceedings of the 12th European Poultry Conference [CD]*. Verona, Italy, Verona, Italy: Verona Fiere.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Pérez-Álvarez, J. 2008. Antifungal activity of lemon (Citrus lemon L.), mandarin (Citrus reticulata L.), grapefruit (Citrus paradisi L.) and orange (Citrus sinensis L.) essential oils. Journal of Food Safety, vol. 28, no. 4, p. 567-576.

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EFFECT OF DIET SUPPLEMENTED WITH PROPOLIS EXTRACT AND PROBIOTIC ADDITIVES ON PERFORMANCE, CARCASS CHARACTERISTICS AND MEAT COMPOSITION OF BROILER CHICKENS

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ABSTRACT

The present research focused on the effects of propolis extract and probiotic preparation based on *Lactobacillus fermentum* (1×10^9 CFU per 1 g of bearing medium) on performance, carcass characteristics and meat composition of broiler chickens. The experiment was performed with 360 one day-old Ross 308 broiler chicks of mixed sex. The chicks were randomly allocated into 3 groups (n = 120 pcs chicks per group), namely, control (C) and experimental (E1, E2). Each group consisted of 3 replicated pens with 40 broiler chickens per pen. The experiment employed a randomized design, and dietary treatments were as follows: 1. basal diet with no supplementation as control (group C), 2. basal diet plus 400 mg propolis extract per 1 kg of feed mixture (group E1), 3. basal diet plus 3.3 g probiotic preparation added to drinking water (group E2). Besides, the groups were kept under the same conditions. Fattening period lasted for 42 days. Feed mixtures were produced without any antibiotic preparations and coccidiostats. As regards performance of broilers, all the investigated parameters were improved after addition of the supplements, especially after probiotic supplementation. However, neither propolis extract nor probiotic in diet of broiler chickens had any significant effect ($p \geq 0.05$) on performance. Meat composition was evaluated as proximate composition (dry matter, crude protein, fat and ash), cholesterol content and energy value in the most valuable parts of chicken meat (breast and thigh muscles). The statistically significant results ($p \leq 0.05$) were attained in fat, ash and cholesterol content, as well as energy value in both breast and thigh muscles after the propolis supplementation. To sum up, the present study demonstrated the promising potential of propolis extract and probiotic to enhance the performance, carcass characteristics and meat composition under conditions of the experiment with, however, statistical significance of results in a few parameters.

Keywords: performance; meat; chicken; propolis; probiotic

INTRODUCTION

Chickens are the most popular amongst different poultry species worldwide. Owing to their relatively low fat and cholesterol contents, chicken meat is considered a healthy animal food. Moreover, chicken continues to be the cheapest among all types of meat consumed in the world and its consumption is expected to increase by 34% by 2018 **(Umaya Suganthi, 2014; Petrová et al., 2015)**. Modern intensive chicken production has achieved phenomenal gains in the efficient and economical production of high quality and safe chicken meat. The use of feed additives has been an important part of achieving this success **(Hashemi et al., 2012)**.

For several decades, antibiotics have been widely used in the chicken diet **(Goodarzi and Nanekarani, 2014)**. However, the use of dietary antibiotics have resulted in controversial problems such as development of antibiotic resistant bacteria and drug residue in the final products which can be harmful to consumers **(Goodarzi et al., 2014)**. As a result, additives such as probiotics and natural substances such as propolis have received increased attention as possible antibiotic growth promoter substitutions in chicken diet **(Haščík et al., 2012; Daneshmand et al., 2015)**.

Propolis is a resinous material elaborated by bees, through the recollection of the exudates from different plant species **(Valenzuela-Barra et al., 2015)** and is used in construction and adaptation of their hives. It possesses many pharmacological activities, such as anti-inflammatory, antibiotic, antiviral and immunostimulant **(Fan et al., 2013)**.

In many studies conducted on propolis, many positive effects like increase in feed intake, body weight, flavonoid content, taste improvement, antioxidant and antimicrobial properties have been reported **(Tatlı Seven et al., 2008)**. The properties of propolis are based on its rich flavonoid, phenolic acid and terpenoid contents **(Seven et al., 2012)**.

An alternative approach to subtherapeutic antibiotics in chicken diet is also the use of probiotic microorganisms **(Alkhalf et al., 2010)**. Probiotics are live, non-pathogenic bacteria that contribute to the health and balance of the intestinal tract **(Giannenas et al., 2012)**. The most important advantage of a probiotic is that it neither has any residues in animal production nor exerts any antibiotic

resistance by consumption **(Alkhalf et al., 2010)**. Several studies showed that dietary supplementation of lactic acid bacteria (e.g. *Lactobacillus*) improve the performance and feed conversion **(Taklimi et al., 2012; Bai et al., 2013)**, stimulate immune response and increase bone strength of broiler chickens. The enhanced growth with probiotics may be partly attributed to the colonisation of the gastrointestinal tract of the chicks, which improved the digestion of essential nutrients **(Khaksefidi and Rahimi, 2005)**.

This study was designed to investigate the effects of dietary addition of propolis extract and probiotic preparation based on *Lactobacillus fermentum* on performance, carcass characteristics and meat composition of Ross 308 broiler chickens.

MATERIAL AND METHODOLOGY

Chickens and dietary treatments

The experiment was carried out in test poultry station of Slovak University of Agriculture in Nitra. A total of 360 one day-old broiler chicks of mixed sex (Ross 308) were randomly divided into 3 groups, namely, control (C) and experimental (E1, E2). Each group consisted of 3 replicated pens with 40 broiler chickens per pen. The experiment employed a randomized design, and dietary treatments were as follows: 1. basal diet as control (group C), 2. basal diet plus 400 mg propolis extract per 1 kg of feed mixture (group E1), 3. basal diet plus 3.3 g probiotic preparation added to drinking water (group E2). Besides, the groups were kept under the same conditions.

The experiment lasted for 42 days. The broiler chickens were reared on breed litter (wood shavings), in a temperature-controlled room; ambient temperature in test poultry station was maintained at 33 °C during the first week and gradually decreased by 2 °C, and finally fixed at 19 °C thereafter. Throughout the entire experimental period, the chickens had *ad libitum* access to feed and water, and were kept under constant light regime.

Table 1 lists the basal diet formulated according to nutrient requirements of broilers. The broiler chickens were fed a starter diet from 0 to 21st day and grower diet from 22nd to 42nd day. The feed mixtures both starter and grower were produced without any antibiotics and coccidiostats.

Propolis had origin in the Slovak Republic. The extract was prepared from minced propolis in the conditions of the 80% ethanol in the 500 cm^3 flasks, according to **Krell (1996)**. Determination of phenolic compounds, namely the phenolic acids (caffeic acid, *p*-coumaric acid, ferulic acid, cinnamic acid) and flavonoids (routines, quercetin, kaempferol, apigenin, tectochrysin) in propolis extract (Table 2) was performed using an Agilent 1200 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a degasser, an autosampler and a diode array detector (DAD).

In the experiment, the probiotic preparation based on *Lactobacillus fermentum* (1×10^9 CFU per 1 g of bearing medium) was used.

Slaughter and measurements

At 42 days of age, chickens were weighed and slaughtered at the experimental slaughterhouse of Slovak University of Agriculture in Nitra.

After evisceration, the carcasses were kept at approximately 18 °C for 1 h *post mortem* and thereafter longitudinally divided into two parts. After that, the half-carcasses and giblets were weighed and stored at 4 °C until 24 h *post mortem*. The right half-carcasses were used in order to determinate the parameters as described below, whereas the left half-carcasses were assigned to different analysis. All the weight measurements were performed using the precision balance Kern 440 (Kern & Sohn, Germany) with accuracy of 0.01 g. The carcass yield was calculated by dividing carcass weight with giblets and abdominal fat weight by live body weight.

The chemical analysis of chicken meat (breast muscle without skin, thigh muscle with skin and subcutaneous fat) was performed using an Infratec 1265 Meat Analyzer. The cholesterol content of chicken meat was determined by spectrophotometric method according to **Horňáková et al., (1974)**. The energy value (kJ/100 g) was calculated through the conversion factors for fat and protein **(Strmiska et al., 1988)**.

Statistical analysis

The data processing was performed using a statistical program Statgraphics Plus Version 5.1 (AV Trading Umex, Dresden, Germany). For the determination of significant difference between the tested groups, analysis of variance (ANOVA) was used.

RESULTS AND DISCUSSION

The effects of propolis and probiotic supplementation on performance and carcass characteristics of Ross 308 broiler chickens are shown in Table 3. Live body weight of broilers did not differ statistically between the control and experimental groups ($p \geq 0.05$). Similarly, no differences ($p \geq 0.05$) were found between the groups in carcass weight, giblets weight and carcass yield.

Yet, effect of the supplementation has shown to be favourable since the chickens fed diet containing the propolis extract (2316.9 g) and probiotic preparation (2335 g) had higher live body weight than control chickens (2270.2 g).

The results of the study for performance and carcass characteristics of broiler chickens are in general agreement to those of previous studies where the inclusion of propolis in chicken diet also resulted in slight effect on meat performance.

Tatlı Seven et al., (2008) found higher body weight of chickens fed a diet supplemented with 0.5, 1 and 3 g propolis extract per 1 kg of feed mixture (1975 – 2010 g) than that in control (1940 g).

Shalmany and Shivazad (2006) showed that propolis extract in levels 200 and 250 mg.kg^{-1} has positive effect on growth performance of chickens due to improved weight gain and feed efficiency compared with chickens fed a basal diet.

Table 1 Composition of basal diet and nutrient content.

Ingredients (%)	Starter (HYD-01) (day of age 1 – 21)	Grower (HYD-02) (day of age 22 – 42)
Wheat	35.00	35.00
Maize	35.00	40.00
Soybean meal (48% N)	21.30	18.70
Fish meal (71% N)	3.80	2.00
Dried blood	1.25	1.25
Ground limestone	1.00	1.05
Monocalcium phosphate	1.00	0.70
Fodder salt	0.10	0.15
Sodium bicarbonate	0.15	0.20
Lysine	0.05	0.07
Methionine	0.15	0.22
Palm kernel oil Bergafat	0.70	0.16
Premix Euromix BR 0.5%*	0.50	0.50
Nutrient content ($g.kg^{-1}$)		
Crude protein	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
Linoleic acid	13.51	14.19
ME_N ($MJ.kg^{-1}$)	12.02	12.03

* active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 20 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; D-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1 200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 20 000 mg; folic acid 400 mg; biotin 40 mg; kobalamin 8.0 mg; choline 100 000 mg; betaine 50 000 mg; Mn 20 000 mg; Zn 16 000 mg; Fe 14 000 mg; Cu 2 400 mg; Co 80 mg; I 200 mg; Se 50 mg.

Positive effects of propolis were also observed in the study of **Biavatti et al., (2003)**, where effects of propolis extract, the *Alternanthera brasiliana* extract and lindseed oil as alternative feed additives were evaluated. The researches have suggested the additives in diet of broiler chickens due to improved broiler performance in the same way (similar body weight ($p \geq 0.05$) among the treatments that was higher than that in the control).

In another study **(Ziaran et al., 2005)**, body weight of chickens (47 day-old) fed a diet containing different levels of propolis (oil extract) was not affected when compared to those fed a diet containing no supplement (1916.64 – 1935.67 g vs. 1912.08 g).

Similar to the present findings, **Haščík et al., (2014)** demonstrated that propolis extract (200, 300, 400 $mg.kg^{-1}$) added in feed mixture increased the body weight of broiler chickens (2354.6 – 2382.9 g). However, no major effects on chicken growth performance were observed (2272.89 g in control group).

In contrast, **Açıkgöz et al., (2005)** reported significant decrease in body weight of male broilers after propolis supplementation (powder). The body weight of chickens fed diet containing propolis powder ranged from 2061 to 2229 g compared with that in control group (2302 g). In the study, pine originated propolis, which is characterized by strict genuine odour, volatile compounds and a bitter taste, was used. Because of these specific characteristics, broilers might reject the feed mixture that results in adverse effects on growth performance.

In the study of **Daneshmand et al., (2015)**, the body weight of broiler chickens (42 day-old) fed a diet containing 200 $mg.kg^{-1}$ propolis extract (2395 g) was also lower compared with that in the control (2433 g). On the contrary, probiotic preparation (0.45 $g.kg^{-1}$ of feed mixture) containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum* and

Table 2 Concentration of analysed phenolic compounds in propolis extract.

Compound	RT[1] (min)	Concentration ($mg.g^{-1}$)
Caffeic acid	8.48	4.976 ±2.049
p-Coumaric acid	12.83	9.826 ±8.232
Ferulic acid	14.00	7.436 ±6.710
Cinnamic acid	26.47	0.367 ±0.182
Routines	22.33	4.578 ±1.714
Quercetin	29.59	2.963 ±0.762
Kaempferol	32.93	2.503 ±0.502
Apigenin	33.69	3.970 ±2.181
Tectochrysin	37.00	7.523 ±3.959

[1]RT – retention time

Enterococcus faecium used in the same study increased the body weight of experimental chickens (2527 g). However, there was no significant increase ($p \geq 0.05$). Moreover, there was investigated the effects of propolis in combination with the probiotics (0.20 and 0.45 $g.kg^{-1}$ of feed mixture, respectively). Although the combination did not significantly affect performance, the body weight of broiler chickens receiving a combination of these additives was higher than that in control. It may reflect synergetic and complementary effects between the additives in diet of broiler chickens.

As far as the probiotics are concerned, there is considerable variation in published studies that evaluate the effect of probiotic strains on performance of broiler chickens.

There are conflicting reports on the effects of application of probiotics because the response of broiler chickens to probiotics can be affected by different factors such as the duration and method of probiotic feeding, dose and nature of the administered strains and their persistence, variation in the physiological state of the chicken, the actual microbiota balance in the gut of the chicken, as well as the sex and age of chickens **(Aliakbarpour et al., 2012)**.

In the present study, body weight was increased in probiotic-supplemented group compared with that in control and propolis-supplemented group (Table 3), but no significant difference was detected ($p \geq 0.05$).

Many studies have confirmed the positive effect of probiotics on meat performance of broiler chickens. In the study of **Apata (2008)**, addition of probiotic preparation based on *Lactobacillus bulgaricus* to the basal diet (20, 40, 60 and 80 $mg.kg^{-1}$) resulted in improved performance of broiler chickens (35 day-old). Among the dietary treatments, 60 $mg.kg^{-1}$ probiotic preparation elicited the best performance of broiler chickens.

Similar results were observed in the previous study of **Zulkifli et al., (2000)**, who reported that dietary supplementation with *Lactobacillus* cultures improves the performance of chickens.

The significant increase ($p \leq 0.05$) in body weight was demonstrated also by **Ahmed et al., (2014)**, who investigated the effects of *Bacillus amyloliquefaciens* probiotic on growth performance of broiler chickens fed for 35 days. Increasing concentration of probiotic had positive linear effect on the body weight of broilers, with the highest values being observed in broilers offered 20 $g.kg^{-1}$ probiotic.

On the contrary, **Ghasemi et al., (2014)** observed the significant increase ($p \leq 0.05$) in body weight of male broilers only after synbiotic supplementation (probiotic in combination with prebiotic). In the study, the basal diet supplemented with 1 $g.kg^{-1}$ probiotic (combination of *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Enterococcus faecium*) did not result in significant effects on body weight of chickens compared to the control. The findings indicate that after probiotics + prebiotics supplementation may be achieved much better effects on performance of broilers.

Table 3 Effect of propolis extract and probiotic on performance and carcass characteristics of broiler chickens.

Parameter	Group	x	*SD*	SEM	CV (%)
Live body weight (g)	C	2270.20	107.88	34.11	4.75
	E1	2316.90	106.12	33.56	4.58
	E2	2335.00	107.37	33.96	4.60
Carcass weight (g)	C	1629.80	73.64	23.29	4.56
	E1	1669.10	102.48	32.41	6.14
	E2	1674.00	99.54	31.48	5.95
Giblets weight (g)	C	152.08	19.83	6.27	13.04
	E1	155.64	11.53	3.45	7.41
	E2	161.21	12.26	3.88	7.61
Carcass yield (%)	C	78.54	1.41	0.45	1.80
	E1	78.31	1.18	0.37	1.50
	E2	78.58	1.50	0.47	1.91
Abdominal fat (g)	C	22.14^{a}	4.77	1.51	21.54
	E1	21.85^{b}	6.48	2.05	26.66
	E2	24.70^{ab}	7.59	2.40	30.74
Liver (g)	C	40.91	4.63	1.46	11.31
	E1	40.61	5.46	1.73	13.44
	E2	44.50	7.09	2.24	15.93
Gizzard (g)	C	26.00	5.62	1.78	21.62
	E1	25.09	3.30	1.04	13.15
	E2	25.40	4.82	1.52	18.96
Heart (g)	C	10.72	1.10	0.35	10.25
	E1	10.88	1.49	0.47	13.67
	E2	10.77	1.73	0.55	16.10

Legend: C – control group; E1, E2 – experimental groups; x – arithmetic mean; *SD* – standard deviation; SEM – standard error of mean; CV – coefficient of variation; a, b – means with different superscripts within a column differ significantly ($p \leq 0.05$).

The positive effect of probiotic supplementation ($p \leq 0.05$) was reported in the study of **Aliakbarpour et al., (2012)**. The researches demonstrated that supplementation of either *Bacillus subtilis* as the mono-strain probiotic or *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* as the multi-strain probiotic in the feed mixture has the same potent stimulatory effects on broiler performance. Mono-strain probiotic fed broilers (2672.23 g), as well as multi-strain probiotic fed broilers (2664.92 g), had after 42 days of fattening higher body weight compared with control chickens (2608.99 g).

In the study of **Naseem et al., (2012)**, probiotic supplementation in two different doses (50 and 150 g per 1 ton of feed mixture) resulted in higher ($p \leq 0.05$) and similar body weight of broiler chickens (2141 g and 2120.3 g, respectively) compared with control chickens fed a basal diet (1962.1 g). The probiotic preparation consisted of *Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus salivarius*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida pintolopessii*.

In another study, **Khaksefidi and Rahimi (2005)** also found significant increase ($p \leq 0.05$) in live body weight of chickens. On the one hand, the body weight of chickens in the experimental group (1700 g) at the end of fattening (42 days) was higher than that in the control (1620 g), but on the other hand it was markedly lower than that in the present study. The probiotic preparation used in the study of **Khaksefidi and Rahimi (2005)** consisted of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Aspergillus oryzae*, *Streptococcus faecium* and *Torulopsis* spp. and was fed at 100 $mg.kg^{-1}$ diet. The different results may be thus caused by the dosage and strain of probiotics.

Alkhalf et al., (2010) reported that administration of probiotic (*Pediococcus acidilactici*) in chickens appeared to have noticable effect ($p \leq 0.05$) on final body weight of broiler chickens, which was as low as that in the study of **Khaksefidi and Rahimi (2005)**. Chickens fed on probiotic levels 1 and 0.8 $g.kg^{-1}$ diet (1863.6 and 1844 g, respectively) exhibited higher body weight than control chickens (1661.31 g).

The beneficial effect of probiotic supplementation on chicken diet in terms of increased body weight (2372.50 vs. 1997.5 g) was also observed in the study of **Kabir et al., (2004)**. The probiotic preparation consisted of *Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus, Enterococcus aecium, Aspergillus oryzae* and *Candida pintolopessi.* It is important to note that broilers were administered the probiotic by drinking water application (consistent with present study).

Promising effect of probiotic ($p \leq 0.05$) as alternative for antibiotics was demonstrated by **Ghahri et al., (2013)**. They used the same probiotic preparation that was used in the study of **Kabir et al., (2004)**. The probiotic (applied into feed mixture) in two different doses increased live body weight of chickens (2475.13 and 2491 g) compared with that of the control group (2243.09 g). The most significant effect ($p \leq 0.05$) was, however, observed in synbiotic-supplemented group compared with that of other groups, which is in agreement with the results of **Ghasemi et al., (2014)**.

Contrary to the above-mentioned studies, no significant effect was observed in the study of **Brzóska et al., (2012)** and **Swiatkiewicz et al., (2014)**, whereas **Ritzi et al., (2014)** found even the negative effect of probiotic supplementation (*Bifidobacterium animalis* subs. *animalis*, *Lactobacillus salivarius* subs. *salivarius* and *Enterococcus faecium*) on performance of broiler chickens.

Regarding carcass yield, neither supplementation of the diet with propolis extract (78.31%), nor the probiotic preparation (78.31%) had any effect on carcass yield of broiler chickens compared to the control (78.54%). Yet, carcass yield of chickens in the present study was higher in comparison to other studies.

Our carcass yield results are consistent with those of **Tatlı Seven et al., (2008)** (76 – 77% vs. 75%), slightly lower were observed in study of **Attia et al., (2014)** (72.1% vs. 68.9%).

Also, **Swiatkiewicz et al., (2014)** reported similar carcass yield, which was, however, not affected when chickens were fed a probiotic bacteria (*Lactobacillus salivarius*) (74.89 vs. 75.53%).

Daneshmand et al., (2015) found much lower carcass yield, 62.77% in the probiotic-supplemented group, 62.86% in the propolis-supplemented group, and 62.93% in probiotic + propolis-supplemented group, that was, however, still higher than that in control (61.9%).

The effects of propolis extract and probiotic supplementation on composition, cholesterol content and energy value of meat of Ross 308 broiler chickens are shown in Table 4. It is evident that the parameters were not absolutely affected by dietary propolis extract and probiotic supplementation.

The results for meat samples of chickens fed the diet with propolis extract and probiotic were similar to those fed the basal diet, which is consistent with results of some experiments where various supplements were used. However, the significant changes ($p \leq 0.05$) were observed in some parameters.

As has been shown by our study, propolis supplementation was the most favourable among the groups, namely as for fat content in both breast (0.93 $g.100\ g^{-1}$) and thigh (9.62 $g.100\ g^{-1}$) muscles, the ash content in both breast (1.19 $g.100\ g^{-1}$) and thigh (1.05 $g.100\ g^{-1}$) muscles, the cholesterol content in breast muscle (86.42 $mg.100\ g^{-1}$), and the energy value in both breast (408.99 $kJ.100\ g^{-1}$) and thigh (664.8 $kJ.100\ g^{-1}$) muscles. Besides, the propolis-supplemented group showed low crude protein content in both breast (22.33 $g.100\ g^{-1}$) and thigh (18.05 $g.100\ g^{-1}$) muscles when compared with the other groups. As regards the probiotic-supplemented group, there was negative effect on the fat content (1.11 $g.100\ g^{-1}$), as well as the cholesterol content (92.17 $mg.100\ g^{-1}$), and the energy value (415.62 $kJ.100\ g^{-1}$) in breast muscle observed. It is noteworthy that the cholesterol content depends mainly on the type of muscle not the diet.

Regarding the meat composition of broiler chickens, some researchers have observed significant positive effects of natural feed supplements, whereas others reported no effect on the meat composition.

Table 4 Effect of propolis extract and probiotic on proximate composition, cholesterol content and energy value of chicken meat.

Parameter	Group	x	*SD*	SEM	CV (%)
		Breast muscle			
Dry matter (g.100 g^{-1})	C	25.11	0.24	0.07	0.95
	E1	24.94	0.39	0.11	1.55
	E2	25.05	0.38	0.11	1.50
Crude protein (g.100 g^{-1})	C	22.52	0.40	0.11	1.76
	E1	22.33	0.58	0.17	2.61
	E2	22.32	0.28	0.08	1.25
Fat (g.100 g^{-1})	C	1.01^{ab}	1.13	1.04	13.02
	E1	0.93^{a}	0.10	0.03	11.28
	E2	1.11^{b}	0.12	0.03	10.66
Ash (g.100 g^{-1})	C	1.18^{ab}	0.03	$8.7.10^{-3}$	2.56
	E1	1.19^{a}	$9.85.10^{-3}$	$2.84.10^{-3}$	0.83
	E2	1.17^{b}	0.01	$4.14.10^{-3}$	1.22
Cholesterol (mg.100 g^{-1})	C	87.06	8.86	3.62	10.18
	E1	86.42	4.37	1.78	5.05
	E2	92.17	4.59	1.87	4.98
Energy value (kJ.100 g^{-1})	C	415.46^{a}	6.10	1.76	1.47
	E1	408.99^{b}	7.17	2.07	1.75
	E2	415.62^{a}	6.85	1.98	1.65
		Thigh muscle			
Dry matter (g.100 g^{-1})	C	29.50	1.37	0.40	4.65
	E1	29.22	0.40	0.11	1.37
	E2	29.10	0.60	0.17	2.05
Crude protein (g.100 g^{-1})	C	18.48^{a}	0.21	0.06	1.17
	E1	18.05^{b}	0.34	0.10	1.88
	E2	18.06^{b}	0.21	0.06	1.16
Fat (g.100 g^{-1})	C	9.81	1.43	0.41	14.54
	E1	9.62	0.40	0.11	4.16
	E2	9.80	0.78	0.22	7.92
Ash (g.100 g^{-1})	C	1.02^{a}	0.02	$6.38.10^{-3}$	2.16
	E1	1.05^{b}	$9.84.10^{-3}$	$2.84.10^{-3}$	0.94
	E2	1.02^{a}	0.02	$6.66.10^{-3}$	2.27
Cholesterol (mg.100 g^{-1})	C	121.25	7.50	3.06	6.19
	E1	118.68	7.68	3.14	6.47
	E2	113.08	10.70	4.37	9.47
Energy value (kJ.100 g^{-1})	C	679.44	54.45	15.72	8.01
	E1	664.80	13.43	3.88	2.02
	E2	671.89	28.34	8.18	4.22

Legend: C – control group; E1, E2 – experimental groups; x – arithmetic mean; *SD* – standard deviation; SEM – standard error of mean; CV – coefficient of variation; a, b – means with different superscripts within a column differ significa ($p \leq 0.05$).

In the study of **Hossain et al., (2014)**, addition of 0.5% fermented water plantain (*Alisma canaliculatum*) increased the crude protein content in both breast and thigh muscles (24.99 and 23.19%, respectively) compared with the control (24.42 and 21.65%, respectively).

The results coincide with the findings of **Skřivan et al., (2012)**, who reported the highest protein content and the lowest fat content in the thigh muscle of broilers fed a diet with vitamin C (720 and 218 g.kg^{-1} of dry mater, respectively) and broilers fed a diet with selenite (724 and 216 g.kg^{-1} of dry mater, respectively). The results are similar to those in the present study (when converting into g/100 g).

Ahmed et al., (2015) found significantly higher crude protein content ($p \leq 0.05$) in the group of broilers fed a diet supplemented with pomegranate in breast (28.55%), as well as thigh muscle (23.44%) than that in non-supplemented group (26.21 and 22.18%, respectively). Moreover, there was a significant decrease ($p \leq 0.05$) in cholesterol content of breast muscle in the pomegranate-supplemented group (62.8 mg.100 g^{-1}) compared with the control (77.44 mg.100 g^{-1}).

On the contrary, **Swiatkiewicz et al., (2014)** noted no effect on the composition of breast muscle after probiotic supplementation, whereby the probiotic-supplemented

group has shown the values very similar to the other groups, with a crude protein content of 23.5%.

Also, the probiotic supplementation in the study of **Haščík et al., (2011)** did not influence the composition of chicken meat significantly despite the slight positive effect in the probiotic-supplemented groups when compared with the control. The researchers have obtained the results similar to those in the present study.

To sum up the previous studies concerning the composition of chicken meat, there is a positive effect on fat content after natural feed additives observed in most of them, while the effect on protein content is not so noticeable.

CONCLUSION

The results of our study demonstrated that none of the experimental supplements (propolis extract and probiotic preparation based on *Lactobacillus fermentum*) caused a significant changes ($p \geq 0.05$) in performance and carcass characteristics of Ross 308 broiler chickens. However, the data have shown positive effect of propolis extract and probiotic due to the higher values of all the investigated parameters (especially in probiotic-supplemented group) than those in the control. The positive fact highlights the importance of evaluating the administration level of supplements in order to maximize the efficacy. As far as proximate composition, cholesterol content and energy value are concerned, there was a significant change ($p \leq 0.05$) in fat, ash and cholesterol content, as well as energy value in both breast and thigh muscles after the propolis supplementation. On the contrary, the probiotic supplementation was rather adverse for meat composition. Therefore, we assume that probiotic supplementation is more applicable for the performance and carcass characteristics, whereas the propolis supplementation is more applicable for meat composition of Ross 308 broiler chickens. Overall, further studies are needed to investigate the effect of the supplements.

REFERENCES

Açikgöz, Z., Yücel, B., Altan, Ö. 2005. The effects of propolis supplementation on broiler performance and feed digestibility. *Archiv Für Geflügelkunde*, vol. 69, no. 3, p. 117-122.

Ahmed, S. T., Islam, M. M., Bostami, A. B. M. R., Mun, H., Kim, Y., Yang. C. 2015. Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (*Punica granatum* L.) by-products. *Food Chemistry*, vol. 188, p. 481-488. http://dx.doi.org/10.1016/j.foodchem.2015.04.140

Ahmed, S. T., Islam, M. M., Mun, H., Sim, H., Kim, Y., Yang, C. 2014. Effects of *Bacillus amyloliquefaciens* as a probiotic strain on growth performance, cecal microflora, and fecal noxious gas emissions of broiler chickens. *Poultry Science*, vol. 93, no. 8, p. 1963-1971. http://dx.doi.org/10.3382/ps.2013-03718

Aliakbarpour, H. R., Chamani, M., Rahimi, G., Sadeghi, A. A., Qujeq, D. 2012. The *Bacillus subtilis* and Lactic Acid Bacteria Probiotics Influences Intestinal Mucin Gene Expression, Histomorphology and Growth Performance in Broilers. *Asian-Australasian Journal of Animal Sciences*, vol. 25, no. 9, p. 1285-1293. http://dx.doi.org/10.5713/ajas.2012.12110

Alkhalf, A., Alhaj, M., Al-Homidan, I. 2010. Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. *Saudi Journal of Biological Sciences*, vol. 17, no. 3, p. 219-225. http://dx.doi.org/10.1016/j.sjbs.2010.04.005

Apata, D. F. 2008. Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus*. *Journal of the Science of Food and Agriculture*, vol. 88, no. 7, p. 1253-1258. http://dx.doi.org/10.1002/jsfa.3214

Attia, Y. A., Abd Al-Hamid, A. E., Ibrahim, M. S., Al-Harthi, M. A., Bovera, F., Elnaggar, A. S. 2014. Productive performance, biochemical and hematological traits of broiler chickens supplemented with propolis, bee pollen, and mannan oligosaccharides continuously or intermittently. *Livestock Science*, vol. 164, p. 87-95. http://dx.doi.org/10.1016/j.livsci.2014.03.005

Bai, S. P., Wu, A. M., Sing, X. M., Lei, Y., Bai, J., Zhang, K. Y., Chio, J. S. 2013. Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. *Poultry Science*, vol. 92, no. 3, p. 663-670. http://dx.doi.org/10.3382/ps.2012-02813

Biavatti, M. W., Bellaver, M. H., Volpato, L., Costa, C., Bellaver, C. 2003. Preliminary studies of alternative feed additives for broilers: *Alternanthera brasiliana* extract, propolis extract and linseed oil. *Brazilian Journal of Poultry Science*, vol. 5, no. 2, p. 147-151. http://dx.doi.org/10.1590/s1516-635x2003000200009

Brzóska, F. A., Śliwiński, B. A., Stecka, K. B. 2012. Effect of *Lactococcus lactis* vs. *Lactobacillus* spp. bacteria on chicken body weight, mortality, feed conversion and carcass quality. *Annals of Animal Science*, vol. 12, no. 4, p. 549-559. http://dx.doi.org/10.2478/v10220-012-0046-y

Daneshmand, A., Sadeghi, G. H., Karimi, A., Vaziry, A., Ibrahim, S. A. 2015. Evaluating complementary effects of ethanol extract of propolis with the probiotic on growth performance, immune response and serum metabolites in male broiler chickens. *Livestock Science*, vol. 178, p. 195-201. http://dx.doi.org/10.1016/j.livsci.2015.04.012

Fan, Y., Lu, Y., Wang, D., Liu, J., Song, X., Zhang, W., Zhao, X., Nguyen, L., Hu, Y. 2013. Effect of epimedium polysaccharide-propolis flavone immunopotentiator on immunosuppression induced by cyclophosphamide in chickens. *Cellular Immunology*, vol. 281, no. 1, p. 37-43. http://dx.doi.org/10.1016/j.cellimm.2013.01.008

Ghahri, H., Toloei, T., Soleimani, B. 2013. Efficacy of Antibiotic, Probiotic, Prebiotic and Synbiotic on Growth Performance, Organ Weights, Intestinal Histomorphology and Immune Response in Broiler Chickens. *Global Journal of Animal Scientific Research*, vol. 1, no. 1, p. 25-41.

Ghasemi, H. A., Kasani, N., Taherpour, K. 2014. Effects of black cumin seed (*Nigella sativa* L.), a probiotic, a prebiotic and a synbiotic on growth performance, immune response and blood characteristics of male broilers. *Livestock Science*, vol. 164, p. 128-134. http://dx.doi.org/10.1016/j.livsci.2014.03.014

Giannenas, I., Papadopoulos, E., Tsalie, E., Triantafillou, E., Henikl, S., Teichmann, K., Tontis, D. 2012. Assessment of dietary supplementation with probiotics on performance, intestinal morphology and microflora of chickens infected with *Eimeria tenella*. *Veterinary Parasitology*, vol. 188, no. 1-2, p. 31-40. http://dx.doi.org/10.1016/j.vetpar.2012.02.017

Goodarzi, M., Nanekarani, S. 2014. Effect of Onion Extract in Drink Water on Performance and Carcass Traits in Broiler Chickens. *IERI Procedia 8: International Conference on*

Agricultural and Biosystem Engineering, p. 107-112. http://dx.doi.org/10.1016/j.ieri.2014.09.018

Goodarzi, M., Nanekarani, S., Landy, N. 2014. Effect of dietary supplementation with onion (*Allium cepa* L.) on performance, carcass traits and intestinal microflora composition in broiler chickens. *Asian Pacific Journal of Tropical Disease*, vol. 4, p. S297-S301. http://dx.doi.org/10.1016/s2222-1808(14)60459-x

Hashemi, S. R., Zulkifli, I., Davoodi, H., Zunita, Z., Ebrahimi, M. 2012. Growth performance, intestinal microflora, plasma fatty acid profile in broiler chickens fed herbal plant (*Euphorbia hirta*) and mix of acidifiers. *Animal Feed Science and Technology*, vol. 178, no. 3-4, p. 167-174. http://dx.doi.org/10.1016/j.anifeedsci.2012.09.006

Haščík, P., Elimam, I. O. E., Garlík, J., Kačániová, M., Bobko, M., Kňazovická, V., Vavrišinová, K., Arpášová, H., Bučko, O. 2012. Chemical composition of muscle after pollen application in nutrition of broiler chickens. *Potravinarstvo*, vol. 6, no. 2, p. 26-32. http://dx.doi.org/10.5219/190

Haščík, P., Kačániová, M., Bobko, M., Pochop, J., Mihok, M., Arpášová, H. 2011. Effect of probiotic preparation for chemical composition of meat cocks different combinations of hybrid chicks. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 59, no. 5, p. 83-93. http://dx.doi.org/10.11118/actaun201159050083

Haščík, P., Kročko, M., Garlík, J., Elimam, I., Kačániová, M., Bobko, M., Arpášová, H., Vavrišinová, K., Bučko, O. 2014. The effect of propolis extract in the diet of chickens Ross 308 on their performance. *Journal of Central European Agriculture*, vol. 15, no. 4, p. 133-146. http://dx.doi.org/10.5513/jcea01/15.4.1521

Horňáková, M., Chromý, V., Heyrovský, A. 1974. Spektrofotometrické stanovení cholesterolu. *Biochem. Clin. Bohem.*, vol. 3, p. 27-34.

Hossain, M. E., Yang, C. J. 2014. Effect of fermented water plantain on growth performance, meat composition, oxidative stability, and fatty acid composition of broiler. *Livestock Science*, vol. 162, p. 168-177. http://dx.doi.org/10.1016/j.livsci.2014.01.016

Kabir, S. M. L., Rahman, M. M., Rahman, M. B., Rahman, M. M., Ahmed, S. U. 2004. The Dynamics of Probiotics on Growth Performance and Immune Response in Broilers. *International Journal of Poultry Science*, vol. 3, no. 5, p. 361-364. http://dx.doi.org/10.3923/ijps.2004.361.364

Khaksefidi, A., Rahimi, S. H. 2005. Effect of Probiotic Inclusion in the Diet of Broiler Chickens on Performance, Feed Efficiency and Carcass Quality. *Asian-Australasian Journal of Animal Sciences*, vol. 18, no. 8, p. 1153-1156. http://dx.doi.org/10.5713/ajas.2005.1153

Krell, R. 1996. *Value-added products from bee keeping*. FAO. Rome (IT). 395 p.

Naseem, S., Rahman, S. U., Shafee, M., Sheikh, A. A., Khan, A. 2012. Immunomodulatory and Growth-Promoting Effect of a Probiotic Supplemented in the Feed of Broiler Chicks Vaccinated Against Infectious Bursal Disease. *Brazilian Journal of Poultry Science*, vol. 14, no. 2, p. 109-113. http://dx.doi.org/10.1590/s1516-635x2012000200004

Petrová, J., Terentjeva, M., Puchalski, C., Hutková, J., Kántor, A., Mellen, M., Čuboň, J., Haščík, P., Kluz, M., Kordiaka, R., Kunová, S., Kačániová, M. 2015. Application of lavender and rosemary essential oils improvement of the microbiological quality of chicken quarters. *Potravinarstvo*, vol. 9, no. 2, p. 530-537. http://dx.doi.org/10.5219/550

Ritzi, M. M., Abdelrahman, W., Mohnl, M., Dalloul, R. A. 2014. Effects of probiotics and application methods on performance and response of broiler chickens to an *Eimeria* challenge. *Poultry Science*, vol. 93, no. 11, p. 2772-2778. http://dx.doi.org/10.3382/ps.2014-04207

Seven, I., Aksu, T., Tatli Seven, P. 2012. The effects of propolis and vitamin C supplemented feed on performance, nutrient utilization and carcass characteristics in broilers exposed to lead. *Livestock Science*, vol. 148, no. 1-2, p. 10-15. http://dx.doi.org/10.1016/j.livsci.2012.05.001

Shalmany, S. K., Shivazad, M. 2006. The effect of diet propolis supplementation on Ross broiler chicks performance. *International Journal of Poultry Science*, vol. 5, no. 1, p. 84-88. http://dx.doi.org/10.3923/ijps.2006.84.88

Skřivan, M., Marounek, M., Englmaierová, M., Skřivanová, E. 2012. Influence of dietary vitamin C and selenium, alone and in combination, on the composition and oxidative stability of meat of broilers. *Food Chemistry*, vol. 130, no. 3, p. 660-664. http://dx.doi.org/10.1016/j.foodchem.2011.07.103

Strmiska, F., Holčíková, K., Simonová, E., Mrázová, M., Hodeková, J., Vojtaššáková, A., Pristašová. M., Strmiska, J., Strmisková, G., Krupařová, M., Gola, J., Papajová, H., Mareš, J., Kostkanová, E., Ehrenhaft, B. 1988. Foodstuff tables for food materials (in Slovak). Výskumný ústav potravinársky, Bratislava (SK), p. 10-12.

Swiatkiewicz, S., Arczewska-Wlosek, A., Jozefiak, D. 2014. Feed enzymes, probiotic, or chitosan can improve the nutritional efficacy of broiler chicken diets containing a high level of distillers dried grains with soluble. *Livestock Science*, vol. 163, p. 110-119. http://dx.doi.org/10.1016/j.livsci.2014.03.001

Taklimi, S. M. S. M., Lotfollahian, H., Shahne, A. Z., Mirzaei, F., Alinejad, A. 2012. Study on efficacy of probiotic in broiler chickens diet. *Agricultural Sciences*, vol. 3, no. 1, p. 5-8. http://dx.doi.org/10.4236/as.2012.31002

Tatlı Seven, P., Seven, I., Yilmaz, M., Şimşek, G. Ü. 2008. The effect of Turkish propolis on growth and carcass characteristics in broilers under heat stress. *Animal Feed Science and Technology*, vol. 146, no. 1-2, p. 137-148. http://dx.doi.org/10.1016/j.anifeedsci.2007.11.003

Umaya Suganthi, R. 2014. The Uniqueness of Immunocompetence and Meat Quality of Native Chickens: A Specialized Review. *World Journal of Pharmacy and Pharmaceutical Sciences*, vol. 3, no. 2, p. 2576-2588.

Valenzuela-Barra, G., Castro, C., Figueroa, C., Barriga, A., Silva, X., De Las Heras, B., Hortelano, S., Delporte, C. 2015. Anti-inflammatory activity and phenolic profile of propolis from two locations in Región Metropolitana de Santiago, Chile. *Journal of Ethnopharmacology*, vol. 168, p. 37-44. http://dx.doi.org/10.1016/j.jep.2015.03.050

Ziaran, H. R., Rahmani, H. R., Pourreza, J. 2005. Effect of dietary oil extract of propolis on immune response and broiler performance. *Pakistan Journal of Biological Sciences*, vol. 8, no. 10, p. 1485-1490. http://dx.doi.org/10.3923/pjbs.2005.1485.1490

Zulkifli, I., Abdullah, N., Azrin, N. M., Ho, Y. W. 2000. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. *British Poultry Science*, vol. 41, no. 5, p. 593-597. http://dx.doi.org/10.1080/713654979

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THE HEAVY METALS CONTENT IN WILD GROWING MUSHROOMS FROM BURDENED SPIŠ AREA

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ABSTRACT

In this work, we evaluated the rate of entry of heavy metals into the edible parts of wild mushrooms, from central Spiš area. The area is characterized by extremely high content of heavy metals particularly mercury in abiotic and biotic components of ecosystems. The toxicity of heavy metals is well known and described. Known is also the ability of fungi to accumulate contaminants from substrates in which mushrooms grow. We have collected commonly consumed species of mushrooms (*Russula vesca., Macrolepiota procera, Lycoperdon pyriforme, Lecinum piceinum, Boletus reticulatus*). Sampling was conducted for two years 2012 and 2013. The samples taken mushrooms and substrates on which to grow, we determined heavy metal content (Cd, Pb, Cu), including total mercury content modified by atomic absorption spectrometry (AMA - 254). In the substrate, we determined the humus content and pH value. The heavy metal content in soils were evaluated according to Law no. 220/2004 Z.z The exceedance limit values of Cd, Pb, Cu and Hg was recorded. Most significantly the respective limit was recorded in soil samples in the case of mercury. The determined concentration Hg was 39.01 $mg.kg^{-1}$. From the results, we evaluated the degree of ability to bioaccumulate heavy metals different kinds of fungi. We also evaluated the health safety of the consumption of these fungi on the comparison with the limit values provided in the food code of SR. We recorded a high rate of accumulation of mercury in the species Boletus reticulatus and Macrolepiota procera. For these types we recorded the most significant than allowed concentrations of mercury in mushrooms. The highest recorded concentration reached 17.64 $mg.kg^{-1}$ Hg in fresh matter. The limit value was exceeded also in the case of copper. We do not recommend to increased consumption of wild mushrooms in the reference area.

Keywords: Mushrooms; heavy metals; soil; food chain; mercury; midle Spiš

INTRODUCTION

The area of middle Spiš is significantly burden by heavy metal in content of soils **Hronec et al., (2008)**. In addition to air pollution, there are other sources of pollution. They came from the extraction of minerals, their modification and processing. From the iron manufactory in Rudňany were emitted into air 120 tons of mercury. These data prepared by measuring groups of Research Institute of Mineral (now Research Institute of Geotechnics SAS) in Košice. These amounts have been challenged by State Inspection of Slovak Republik Based on the balance of atmospheric emission of mercury they calculated, that in the environment received 40 tons Hg per year. It means this is the highest source of mercury pollution in Europe **Závodský, (1991)**. In this area, the specific sources of pollution endogenous geochemical anomalies, especially in the area of Rudňany, Poráč, Gelnice, Slovinky and Krompachy **(Čurlík and Šefčík, 1999)**. In these days, the highest producer of air pollution is town Krompachy, where Kovohuty Krompachy and iron foundry Slovak energy manufactory (SEZ) produce 90% of total emissions (Hronec et al. 2008). Toxic metals can enter the human body by consumption of contaminated food crops, water or inhalation of dust and cause damage to the organism, its toxic efects (**Mahmoode et al. 2013; Timoracká et al., 2011)**. Most dangerous elements in terms of their content in the soil to a possible accumulation of plants in this area appear Hg, Cd, Pb, and Cu. Toxic effect of Hg and its compounds is largely the reaction of Hg ion with SH-groups of biomolecules with subsequent changes in the permeability of cell membranes and damage cellular enzymes. Mercury has the ability to accumulate in the human body and leads to toxic manifestations of brain damage and peripheral nerves **Zahir et al., (2005)**. All compounds of cadmium are toxic. Cd has a high acute toxicity tests and certified short current (short-term) inhalation of high levels may result in the human body resulting in lung damage. It is highly toxic, causing inhibition of sulfhydryl enzymes in particular. Binds in the liver, but also affects the metabolism of carbohydrates and inhibits insulin secretion **Godt et al., (2006)**. Depending on the amount of exposure, lead can adversely affect the nervous system, kidneys, the immune system, reproductive and developmental systems and the cardiovascular system. Its toxic effects vary from subtle changes in neurocognitive function in low-level exposures to a potentially fatal encephalopathy in acute lead poisoning **Gillis et al., (2012)**. Copper is one of the essential elements for humans, but many copper compounds are potentially toxic. Excessive intake of copper is manifested

neurological disorders **(Shaligram and Campbel, 2013)**. As risky part of the food chain can be considered by **Rieder (2011), Kalač (2010)** edible mushrooms due to their properties accumulation characteristics to heavy metals. The ability of fungi to accumulate in their fruiting body of heavy metals relates to the content of heavy metal in the soil, its link to the soil structure (fulvo and humic acids, clay particles etc.) soil pH, as well as the way of nutrition of the species of fungi. It was found significantly higher accumulation of heavy metals in certain species of fungi **(Falandysz and Gucia, 2008; Melgar et al., 2009)**. Heavy metals concentrations in mushrooms are also considerably higher than those in agricultural crop plants, vegetables and fruits. This suggests that mushrooms possess a very effective mechanism that enables them readily to concentrate certain heavy metals from the ecosystem, compared to green plants growing in similar conditions. According to the mechanism by which some heavy metals are accumulated is somewhat obscure although it seems to be associated with a chelation reaction with sulfhydryl groups of protein and especially with methionine. Thus considerable effort has been focused to evaluate the possible risk to human health from the consumption of mushrooms with regard to their heavy metal content **Paraskevi et al., (2007)**. Mushrooms are a popular part of the menu of the population and in particular mushroom pickers are therefore described as a vulnerable population in terms of income food of heavy metals **Ostos et al., (2015)**.

MATERIAL AND METHODOLOGY

Collection and processing of samples

Samples of fungi (n = 20) were collected, then cleaned of soil, and allowed to dry at 45 °C in a tray drier.

Samples of the substrates (n = 20) were taken from the same sites as samples of fungi, we left the air-dry and the final drying (50 °C) the samples were sifted through a sieve (particle diameter = 2 μm). The samples were collected during 2012 and 2013. Mushrooms species were collected: *Russula vesca*, *Macrolepiota procera*, *Lycoperdon pyriforme*, *Lecinum piceinum*, *Boletus reticulatus*.

The water content of the samples was determined by the moisture analyzer DLB 160-3A (Kern, Germany). Homogenized mushroom samples (1.000 g) were mineralized in a closed system of microwave digestion using Mars X-Press 5 (CEM Corp., USA) in a mixture of 5 cm^3 HNO_3 (Suprapur, Merck, Germany) and 5 cm^3 deionized water (0.054 $mS.cm^{-1}$) from Simplicity 185 (Millipore, UK). Digestive conditions for the applied microwave system comprised heating to 160 °C for 15 minutes and keeping it constant for 10 minutes. A blank sample was treated in the same way. The digested substances were subsequently filtered through a quantitative filter paper Filtrak 390 (Munktell, Germany) and filled up with deionized water to a volume of 50 cm^3.

Analytical procedure

Metal determinations were performed in a Varian AA240Z (Varian, Australia) atomic absorption spectrometer with Zeeman background correction. The graphite furnace technique was used for the determination of Cd and Pb, whereas the flame AAS Varian AA240FS (Varian, Australia) was used for the determination of Cu (detection limits for FAAS: 2.0 $mg.kg^{-1}$), and GF-AAS: 10.0 and 10.0 $ng.kg^{-1}$ for Cd and Pb, respectively). The total content of Hg was determined in the homogenized dried samples of mushrooms (0.005 – 0.01 g) using a cold-vapor AAS analyzer AMA 254 (Altec, Czech Republic).

We conducting determination of organic carbon and humus in the soil according to Turin and the modifications by Nikitin.

Soil organic carbon is oxidized with oxygen in chromo-sulfuric mixture. The amount of oxygen consumed in the oxidation is determined by the difference consumed and unconsumed chromo-sulfuric mixture. Humus content was found by calculation according to the following of relations:

$$C_{ox} = \frac{(a-b)\times 0{,}03\times 1{,}17\times f_{mohr}}{n}$$

(a - b) = difference between sample and blank test

f_{mohr} = accurate substance concentration of Mohr salt

n = sample weight in g

humus content = $c_{ox} \times 1{,}724$ [%]

c_{ox} = percentage of oxidizable carbon

1,724 = conversion factor to humus content in the soil sample.

Determination of active reactions in soil (pH/H_2O)

Twenty grams of soil samples were taken. Subsequently 50 ml of H_2O was added. The suspension was allowed 10 minutes to shake by shaker Heidolphpromax 1020 at a frequency of 180 oscilation per minute. After shaking and settled solution we filtered suspension through filter paper *FILTRAK* 390. After filtering the suspension, the pH of the filtrate was measured on the pH meter Metrohm 691, we calibration aparat to two buffers at pH 4 and 7. The resulting values were subtracted from pH meter display with two decimal places.

Bioavailability calculation (Baf): the heavy metal content in mushroom (fresh veight) / heavy metal content in substrat.

RESULTS AND DISCUSSION

The contents of heavy metals (Table 1) in substrates were evaluated according to the Annex. 2 of the Slovak decree no. 220/2004 col. Type of soil samples from the point of delivery 1 was sandy-loam, loam. Soils are predominantly slightly acidic. The humus content is very high. The minimum content of Cd was 0.03 $mg.kg^{-1}$ and maximum content of Cd was was 6.07 $mg.kg^{-1}$. The limit value from Cd in soil exceeded 47% of the samples. Pb minimum in soil we recorded 0,10 $mg.kg^{-1}$ and maximum in value172.50 $mg.kg^{-1}$. Only one sample exceeded the limit value from Pb in soil. Copper minimum in soil was 0.04 $mg.kg^{-1}$ and maximum 145.20 this sample exceeded the limit value from copper in soil 2.42 times. Minimum of mercury content was 0.24 $mg.kg^{-1}$ and maximum was 39.01 $mg.kg^{-1}$. In our study 19 from 20 samples exceeded the limit value for Hg in soil. The soils in middle Spiš area are extremely contaminated with mercury.

Extremely contamined soil by mercury, for which they were collected Wild mushrooms were also recorded in the

Spanish province of Asturias, where the values of Hg concentrations in the soil reach values 1 – 895 mg.kg^{-1}, is also contamination of anthropogenic origin **(Ordóñez et al., 2013)**.

Contents of the monitored heavy metals in mushroom samples varied at different intervals depending on the mushroom species (Table 2). Mushrooms are generally considered extensive accumulators of heavy metals. The level of the transfer of heavy metals into fruiting bodies is affected by a large number of factors, such as mushroom species, chemical parameters of the substrate (substrate composition, heavy metals content, pH, humus content), the age of the mycelium and, probably, the interval between fructification events. **Kalač, 2010**. Content of heavy metals in samples of mushrooms were evaluated according to the Food codex of the Slovak republic (decree of the Ministry of Agriculture and the Ministry of Health no. 608/3/2004-100 of 15 March 2004).

Cadmium

Minimum concentration of cadmium we recorded in sample *Boletus reticulatus* 0.027 mg.kg^{-1} and maximum in (0.48 mg.kg^{-1}) sample *Russula vesca.* No sample exceeded the limit value. The highest mean content we determined in species *Russula vesca*. Biovailability declined from species: *Boletus reticulatus* > *Russula vesca* > *Lycoperdon pyriforme* > *Lecinum piceinum* > *Macrolepiota procera*.

High content of Cadmium (4.27 mg.kg^{-1}) in *Russula* species determined in work from Turkia **(Tüzen, 2003)**.

Lead

The minimum concentration of lead we recorded in sample Boletus reticulatus 0.01 mg.kg^{-1} and maximum in sample Macrolepiota procera 0.51 mg.kg^{-1}.

The highest mean content of lead we determined in species Macrolepiota procera. Analysed samples do not exceed the limit value. Biovailability declined from species: Boletus reticulatus > Lycoperdon pyriforme > Macrolepiota procera > Russula vesca > Lecinum piceinum. High level of lead in species Macrolepiota procera determined also García et al., 2008.

Copper

Minimum content of copper we determined in sample *Lecinum piceinum* 1.61 mg.kg^{-1}. Maximum content (35.86 mg.kg^{-1}) we determined in sample *Lycoperdon pyriforme*, this sample exceede the limit value 3,58 times. The highest mean content we determined in species *Lycoperdon pyriforme.* Three samples exceeded the limit value (*Macrolepiota procera* (2) and *Lycoperdon pyriforme*). Biovailability declined from species: *Boletus reticulatus* > *Macrolepiota procera* > *Russula vesca* > *Lycoperdon pyriforme* > *Lecinum piceinum.* Comparable Cu concentration in mushrooms also mentioned **Kalač 2010.**

Mercury

Minimum content of mercury we determined in sample *Lecinum piceinum* (0.009 mg.kg^{-1}) and maximum mercury content (17.64 mg.kg^{-1}) we determined in sample *Macrolepiota procera* this sample exceeded the limit value 70.56 times.

High mean content we determined in species *Macrolepiota procera* (10.91 mg.kg^{-1}). 90% of samples exceeded the limit value from mercury in wild growing mushrooms. Bioavailability declined from species: *Macrolepiota procera* > *Lycoperdon pyriforme* > *Boletus reticulatus* > *Lecinum piceinum* > *Russula vesca.*

Table 1 Substrate parameters from mushrooms sampling.

Mushroom species	Substrate parameter					
	pH (H_2O)	Humus content %	Cd (mg.kg^{-1}) *LV (0.7)	Pb (mg.kg^{-1}) *LV (70)	Cu (mg.kg^{-1}) *LV (60)	T-Hg (mg.kg^{-1}) *LV (0.5)
Russula vesca						
range	4.44 – 7.29	2.48 –12.28	0.09 – 4.29	0.20 – 61.00	0.71 – 17.80	6.07 – 39.01
mean	5.93	7.70	1.72	25.64	10.33	20.06
SD	1.40	3.74	1.99	30.34	10.55	13.88
Macrolepiota procera						
range	3.73 – 6.18	1.75 – 7.32	0.04 – 4.10	0.10 – 64.50	0.08 – 32.30	0,50 – 18.83
mean	4.72	4.53	2.57	41.32	16.52	10.20
SD	1.15	2.36	1.83	30.05	13.17	7.65
Lycoperdon pyriforme						
range	4.63 – 6.17	2.42 – 6.60	0.03 – 6.07	0.13 – 39.70	0.14 – 145.20	1.26 – 10.66
mean	5.55	4.28	2.05	13.36	48.48	6.39
SD	0.71	1.72	3.48	20.80	83.76	4.75
Lecinum piceinum						
range	4.30 – 6.38	5.33 –14.70	0.09 – 3.44	0.48 – 172.50	0.19 – 37.50	0.24 – 19.30
mean	4.92	7.88	1.39	49.52	11.98	6.98
SD	0.89	3.94	1.59	8.20	17.55	8.66
Boletus reticulatus						
range	4.16 – 5.10	6.29 –10.05	0.14 – 0.14	0.31 – 2.14	0.04 – 0.23	2.33 – 36.35
mean	4.63	8.17	0.14	1.23	0.13	19.34
SD	0.66	2.65	0.00	1.29	0.13	24.05

Note: SD – standard deviation, * LV – limit value (Annex. 2 of the Slovak decree no. 220/2004 Col., Cd, Pb and Cu after aqua regia extraction).

Table 2 The heavy metals content in mushrooms samples (fresh matter).

Mushrooms species	Heavy metals content in fresh matter (mg.kg^{-1})			
	Cd (mg.kg^{-1}) *LV(1.0)	Pb (mg.kg^{-1}) *LV(1.0)	Cu (mg.kg^{-1}) *LV(10.0)	T – Hg (mg.kg^{-1}) *LV(0.25)
Russula vesca				
range	0.05 – 0.48	0.02 – 0.11	3.66 – 6.12	1.65 – 3.54
mean	0.18	0.06	4.65	2.33
SD	0.20	0.03	1.04	0.83
Macrolepiota procera				
range	0.08 – 0.16	0.16 – 0.51	3.09 – 20.93	1.24 – 17.64
mean	0.10	0.37	11.46	10.91
SD	0.03	0.16	9.03	7.14
Lycoperdon pyriforme				
range	0.08 – 0.21	0.12 – 0.26	7.71 – 35.86	0.56 – 6.96
mean	0.13	0.20	17.10	3.61
SD	0.06	0.07	16.24	3.20
Lecinum piceinum				
range	0.03 – 0.11	0.03 – 0.11	1.61 – 7.14	0.009 – 2.86
mean	0.06	0.06	3.28	1.32
SD	0.03	0.03	2.58	1.49
Boletus reticulatus				
range	0.027 – 0.10	0.01 – 0.07	1.78 – 2.10	1.62 – 14.35
mean	0.06	0.04	1.94	7.99
SD	0.05	0.04	0.22	9.00

Note: SD – standard deviation, * LV – limit value (Decree of the Ministry of Agriculture and the Ministry of Health of the Slovak republic no. 608/3/2004-100 of 15 March 2004).

Table 3 Pearson corelation coeficients between heavy metals conntent in mushrooms and substrates

corelation	Cd (mushrooms)	Pb (mushrooms)	Cu (mushrooms)	Hg (mushrooms)	Cd (substrates)	Pb (substrates)	Cu (substrates)	Hg (substrates)
Cd (mushroom)								
Pb (mushrooms)	0.03							
Cu (mushroom)	0.16	0.27						
Hg (mushroom)	0.015	0.67*	0.09					
Cd (substrates)	0.27	0.44	0.47	0.18				
Pb (substrates)	0.17	0.21	0.08	-0.04	0.65*			
Cu (substrates)	0.29	0.22	0.82*	0.04	0.76*	0.35		
Hg (substrates)	-0.10	-0.08	-0.14	0.41	-0.22	-0.34	-0.16	1

Note: * p <0.05

Contamination of wild mushroom species (*Boletus* and *Agaricus*) with mercury dealt **Melgar et al. (2009)**, with the highest concentration of mercury found in species of the genus *Boletus* and *Agaricus* in the range of 2.0 to 6.9 mg.kg $^{-1}$. The lowest concentrations reported in species of the genus Fistulina 0.22 mg.kg^{-1}. Bioavailability was also recorded the highest in the species of the genus *Boletus* and *Macrolepiota*. The observed area was northwest Spain. Their findings are comparable to our resultes.

We demonstrated high correlation (Table 3) between content of lead and mercury in mushrooms. We found high correlation between copper content in mushrooms and substrates on which they grow. We found high correlation between the cadmium content and lead, copper content in substrates.

CONCLUSION

Mushrooms as a popular culinary raw material is in our country a source of heavy metals, particularly mercury, in the food chain. This is due to their ability to accumulate well heavy metals from substrates on which they grow. This is confirmed by their biovailability. Mushrooms are therefore a real risk to human health following exposure to heavy metals in the target area. We don´t have known data about year-round consumption of mushrooms for better risk assessment. It would be appropriate to continue to complement those data and thus continue in this direction of research.

REFERENCES

Čurlík, J., Ševčík, P. 1999. Geologický atlas Slovenskej republiky – pôdy. Záverečná správa. (Geological atlas of the Slovak Republik - soils. Final report). *Bratislava: MŽP SK, VÚPOP*, 137 p.

Falandysz, J. Gucia, M. 2008. Bioconcentration factors of mercury by Parasol Mushroom (*Macrolepiota procera*). *Environ Geochem Health*, vol. 30, p. 121-125. http://dx.doi.org/10.1007/s10653-008-9133-5 PMid:18239997

Garcia, M. A., Alonso, J., Melgar, M. J. 2009. Lead in edible mushrooms: Levels and bioaccumulation factors. *Journal of Hazardous Materials*, vol. 167, p. 777-783. http://dx.doi.org/10.1016/j.jhazmat.2009.01.058 PMid:19217716

Gillis, B., S., Arbieva, Z., Gavin, I., M. 2012. Analysis of lead toxicity in human cells. *BMC Genomics*, vol. 13, 344 p. http://dx.doi.org/10.1186/1471-2164-13-344 PMid:22839698

Godt, J., Scheidig, f., Grosse-Siestrup, Ch., Esche, V., Brandenburg, P., Reich, A., Groneberg, D. A. 2006. The toxicity of cadmium and resulting hazards for human health. *Journal of Occupational Medicine and Toxicology*. vol. 1. http://dx.doi.org/10.1186/1745-6673-1-22 PMid:16961932

Hronec, O., Vilček, J., Tóth, T., Andrejovský, P., Adamišin, P., Andrejovská, A., Daňová, M., Huttmanová, E., Vilimová, M., Škultéty, P., Juhszová, M. 2008. Ťažké kovy v pôdach a rastlinách Rudniansko-gelnickej zaťaženej oblasti. (Heavy metals in soils and plants in Rudniansko-gelnicka burdened area) *Acta regionalia et enviromentalica*, vol. 1, p. 24-28.

Kalač, P. 2010. Trace element contents in European species of wild growing edible mushrooms: A review for the period 2000 – 2009. *Food Chemistry*, vol. 122, p. 2-15. http://dx.doi.org/10.1016/j.foodchem.2010.02.045

Mahmoode, A., Malik, R. 2013 Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian Journal of Chemistry*, vol. 7, p. 543-549. http://dx.doi.org/10.1016/j.arabjc.2013.07.002

Melgar, M. J., Alonso, J., García, M. A. 2009. Mercury in edible mushrooms and underlying soil: Bioconcentration factors and toxicological risk, *Science of the Total Environment*, vol. 407, no. 20, p. 5328-5334. http://dx.doi.org/10.1016/j.scitotenv.2009.07.001

Ordóñez, A., Álvarez, R., Loredo, J. 2013. Asturian mercury mining district (Spain) and the environment: a review. *Environmental Science and Pollution Research International*, vol. 20, no. 11, p. 7490-7508. http://dx.doi.org/10.1007/s11356-013-1663-4 PMid:23589252

Ostos, C., Pérez-Rodríguez, F., Arroyo, B. M., Moreno-Rojas, R. 2015. Study of mercury content in wild edible mushrooms and its contribution to the Provisional Tolerable Weekly Intake in Spain. *Journal of Food Composition and Analysis*, vol. 37, p. 136-142. http://dx.doi.org/10.1016/j.jfca.2014.04.014

Paraskevi, K. O., Panayotis, G. V., Evangelos, K. P., Kyriakos, A. R. 2007. Determination of metal content in wild edible mushroom species from regions of Greece, *Journal of Food Composition and Analysis*, vol. 20, p. 480-486 http://dx.doi.org/10.1016/j.jfca.2007.02.008

Rieder, S. R., Brunner, I., Horvat, M., Jacobs, A., Frey, B. 2011. Accumulation of mercury and methylmercury by mushrooms and earthworms from forest soils. *Environmental Pollution* vol. 159, no. 10, p. 2861-2869. http://dx.doi.org/10.1016/j.envpol.2011.04.040

Shaligram, S., Campbell, A. 2013. Toxicity of copper salts is dependent on solubility profile and cell type tested. *Toxicology in Vitro*, vol. 27, p. 844-851. http://dx.doi.org/10.1016/j.tiv.2012.12.026 PMid:23287045

Timoracká, M., Vollmannová, A., Ismael, D. 2011. Minerals, trace elements and flavonoids content in white and coloured kidney bean. *Potravinarstvo*, vol. 5, no. 1, p. 56-60. http://dx.doi.org/10.5219/116

Tüzen, M. 2003. Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry. *Microchemical Journal*, vol. 74, p. 289-297. http://dx.doi.org/10.1016/S0026-265X(03)00035-3

Zahir, F., Rizwi, S. J., Haq, S. K., Khan, R. H. 2005. Low dose mercury toxicity and human health. *Environmental Toxicology and Pharmacology*. vol. 20, p. 351-360. http://dx.doi.org/10.1016/j.etap.2005.03.007 PMid:21783611

Závodský, D. 1991. *Ochrana ovzdušia, (Protection of Atmosfere)*, Bratislava : SHMÚ. p. 85-87.

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DIAGNOSTICS OF SUBTROPICAL PLANTS FUNCTIONAL STATE BY CLUSTER ANALYSIS

Oksana Belous, Valentina Malyarovskaya, Kristina Klemeshova

ABSTRACT

The article presents an application example of statistical methods for data analysis on diagnosis of the adaptive capacity of subtropical plants varieties. We depicted selection indicators and basic physiological parameters that were defined as diagnostic. We used evaluation on a set of parameters of water regime, there are: determination of water deficit of the leaves, determining the fractional composition of water and detection parameters of the concentration of cell sap (CCS) (for tea culture flushes). These settings are characterized by high liability and high responsiveness to the effects of many abiotic factors that determined the particular care in the selection of plant material for analysis and consideration of the impact on sustainability. On the basis of the experimental data calculated the coefficients of pair correlation between climatic factors and used physiological indicators. The result was a selection of physiological and biochemical indicators proposed to assess the adaptability and included in the basis of methodical recommendations on diagnostics of the functional state of the studied cultures. Analysis of complex studies involving a large number of indicators is quite difficult, especially does not allow to quickly identify the similarity of new varieties for their adaptive responses to adverse factors, and, therefore, to set general requirements to conditions of cultivation. Use of cluster analysis suggests that in the analysis of only quantitative data; define a set of variables used to assess varieties (and the more sampling, the more accurate the clustering will happen), be sure to ascertain the measure of similarity (or difference) between objects. It is shown that the identification of diagnostic features, which are subjected to statistical processing, impact the accuracy of the varieties classification. Selection in result of the mono-clusters analysis (variety tea Kolhida; hazelnut Lombardsky red; variety kiwi Monty and Hydrangea forma rosea) shown as a helpful tool to detect drastically different varieties.

Keywords: subtropical crops; cluster analysis; diagnostics; adaptability; water regime; pigments; enzymes activity

INTRODUCTION

Recently in Sochi, the importation of tropical and subtropical plants has increased, in the fruit and ornamental areas. These crops are deservedly popular among the local residents, especially given the existence of the municipal program "Development within territories of municipal formation the city-resort Sochi» **(Resolution of the Sochi city administration, 2014)**. At the same time, in light of recent political events and the imposition of sanctions, Russia is actively carrying out activities on import substitution, which implies the saturation of the farmer's market local adapted varieties, process of restoring tea plantations is in full swing **(Resolution of the Russian Federation Government, 2012; Decree of the Russian Federation President, 2014)**. The subtropics of Krasnodar region are in undoubted interest and as well as a year-round family resort direction, therefore, the issues of urban green space in settlements are required as a must. In this regard, of paramount importance in the development of horticulture in Russia is the development of a scientifically-based selection of crops and use of better varieties and garden forms that meet modern requirements and the most adapted to the conditions of damp subtropics.

However, often in the cultivation of new varieties, we have to face failure, because of the classical approach to plant care carried out by analogy with well known established varieties. At the same time, it is known that the choice of crop conditions is individual and depends on the varietal characteristics. All this leads to the need for a thorough, comprehensive research not only by each culture but by each class. Research is often lengthy; require large analysis, which in connection with the use of phenological observations and the large number of morphological, anatomical, physiological indicators, sometimes is difficult. Thus, before researchers there is a question about the search for more rapid methods of assessment introduced new material.

In this regard, in the laboratory of biotechnology, plants physiology and biochemistry in recent years conducted a comprehensive study of adaptive reactions of various subtropical and tropical crops (tea, kiwi, hazelnut, gidrangea, weigela, etc.) aimed at the search of diagnostic criteria for their evaluation.

For a number of crops already established diagnostic indicators, and established scales for assessment of drought resistance varieties and crops such as tea, kiwi, gidrangeya, and weigela **(Belous, 2009; Malyarovskaya and Belous, 2012; Klemeshova and Belous, 2013; Malyarovskaya and Belous, 2015)**. However, attracting new assortment leads to more specific research in respect

of obtaining a large number of indicators. As is known, methods of multivariate analysis are effective quantitative tools for the study of fundamental processes described by a large number of characteristics. Cluster analysis most clearly reflects the characteristics of a multivariate analysis in classification and its use allows you to quickly organize the extensive material available in the laboratory. In this case, combining objects into clusters so that way, similar classes maximally got into one class, and objects from different classes would maximally differ from each other.

MATERIAL AND METHODOLOGY

As objects of researches were made by different varieties of the following plants: tea (*Camellia sinensis* L); filbert (*Corylus pontica* C. Koch); kiwi (*Actinidia deliciosa*); gidrangeya large-leaved (*Hydrangea macrophylla* (Thunb.) Ser.); weigela (*Weigela* X *Wagnera*). The accounting of plants resistance to adverse climate conditions was determined visually (in balls) by Technique of the State grades researches **(Technique, 1968)** and methods of laboratory and field assessment of water regime parameters: water deficiency (for a drought resistance assessment) **(Pochinok, 1976)**; the concentration of cell sup (CCS) (for heat tolerance testing) **(Filippov, 1975)**; express diagnostics of change parameters of leaf blade (for a drought resistance assessment) **(Goncharova, 2005)**; activity of enzyme catalase - gasometrical method **(Gunar, 1972)**; thickness of leaf was determined by field turgor meter, coefficient of heat resistant - method express diagnosing **(Kushnirenko, et al., 1986)**.

When processing the data and evaluating the results of the research used the statistical software package STATGRAPHICS Centurion XV standard mathematical software package MS Excel XP. To construct the dendrograms and partition the varieties into homogeneous the adaptability of the group used clustering k-average, or «nearest neighbor».

RESULTS AND DISCUSSION

The establishment of the indicator organ that can serve as a reliable diagnostic authority in the evaluation of the adaptability of cultures was the paramount importance. In the result of conducted research we have established that when long diagnosis culture of the kiwi should consider the existence of tiers in plants (to take away the leaves from the middle tier) and the location of leaves in relation to inflorescences and fruits; for the diagnosis of plant resistance gidrangea and weigela gives a more accurate physiologically formed the third, starting from the terminal of a leaf bud; while the research on tea should be selected physiologically Mature leaves that are after the so-called «fishy».

The following moment was the selection of diagnostic indicators to assess the sustainability of the studied cultures. In the order of the country's scientific institutions developed different methods of diagnosis of plant resistance, recommending for practical use a variety of techniques for evaluation of resistance to extreme factors **(Tsukanov, 2007; Goncharova, 2011)**. Their analysis showed that the entire diversity of ways to diagnose plant resistance lies with a small number of general principles of evaluation based on views on the adaptation mechanisms of plants to stresses **(Tsukanova, 2007; Goncharova, 2011)**.

As a rule, when determining the resistance of the varieties we used two or three well-known varieties (grown in the area), clearly differing from each other in terms of resistance to a specific type of stress: highly resistant, moderately resistant and unstable. However, variety, highly resistant to extreme factors, but not with great productivity potential (realized only in optimal conditions) and gives the highest absolute yield. Most often, the introduction of such varieties in production is recognized as inappropriate; however, it retains its value for breeding as a genetic source of high resistance to stress, which does not preclude his selection as recommended.

We also took into account the fact that the sustainability of any plant organism changes in ontogenesis: it is low at a young age, then gradually increases. From this overall biological patterns should be that a comparative assessment of crops for resistance to stress factors is possible only on the basis of the same age.

Since the main disadvantage of our subtropical zone is the uneven distribution of rainfall, with recurrent drought periods and high temperatures, often accompanied by hair dryers, the main focus of the research was done on plant resistance to drought. In particular, we used evaluation on a set of parameters of water regime. These settings are characterized by high liability and high responsiveness to the effects of many abiotic factors that determined the particular care in the selection of plant material for analysis and consideration of the impact on sustainability. Methods of using identified indicators that are closely related to water status of crops, are: determination of water deficit of the leaves, determining the fractional composition of water and detection parameters of the concentration of cell sap (CCS) (for tea culture flushes).

To confirm the validity of the studies conducted statistical processing of experimental data summarizing and averaging of results and involvement analysis of variance, according to the methodical instructions on conducting field experiments specific to perennial crops.

As a result, on the basis of the experimental data calculated the coefficients of pair correlation between climatic factors and used physiological indicators. The result was a selection of physiological and biochemical indicators proposed to assess the adaptability and included in the basis of methodical recommendations on diagnostics of the functional state of the studied cultures **(Ryndin at all 2014)**. The tea culture is established that a significant relationship exists between enzyme activity – temperature and activity of the enzyme – solar insulation; the culture of hazelnuts dependence between temperature and physiological indicators of above average or even high, the strong correlation observed between temperature and amount of carotenoids (r = 0. 98), the temperature rise causes a decrease in the synthesis of chlorophylls (r = -0. 73) and indicators related to water regime: the water content (r = -0. 81), water-holding capacity (r = -0. 83) and the amount of free water (r = -0. 78); revealed close correlations between the indicators of water status weigela (water deficit, the concentration of cell sap (CCS) and the activity of the enzyme catalase **(Belous, 2008; Belous, Ryndin and Pritula, 2009; Klemeshova and Belous**

2011; Kozhevnikova, 2014; Malyarovskaya and Belous 2015).

However, as already mentioned, the analysis of complex studies involving a large number of indicators is quite difficult, especially does not allow to quickly identify the similarity of new varieties for their adaptive responses to adverse factors, and, therefore, to set general requirements to conditions of cultivation. In this case, for the separation of the studied cultivars on the most similar in agility classes (clusters), it is desirable to connect the cluster analysis, which is the most effective way to solve this problem.

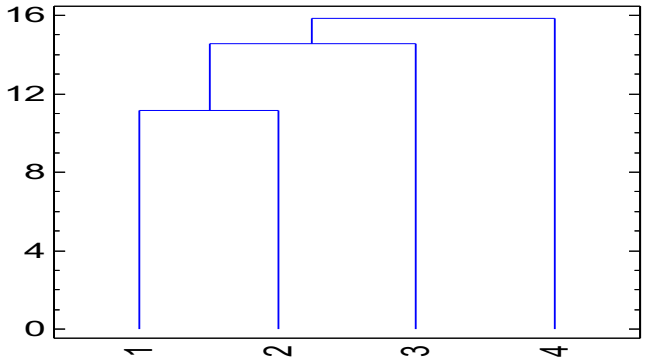
As known, cluster analysis is a multivariate statistical procedure that performs the data collection that contains information about the sample objects, and then marshalling them into homogeneous groups **(Gorsky and Orlov, 2002; Bessokirny, 2003; Savvina, 2013)**. Thus, the objective of cluster analysis is that the newly introduced varieties appear to refer to one of the already defined classes.

Use of cluster analysis suggests that in the analysis of only quantitative data; define a set of variables used to assess varieties (and the more sampling, the more accurate the clustering will happen), be sure to ascertain the measure of similarity (or difference) between objects. In addition, it is necessary that the sample should be homogeneous (not to contain «emission») and the distribution of indicators should be close to normal **(Acopov at all 2013; Sidorenko, 2001)**.

Cluster analysis of the tea plants allowed determining similar to the adaptive potential of varieties. In close clusters are of local plant populations, varieties, and Kimyn and Karatum (Figure 1). Moreover, the more similar the sustainability of local plant populations (sufficiently adapted to growing conditions) and grade Karatum (anthocyanin pigment flushes which is an indirect confirmation of its stability). At the same time the cultivar Kolkhida is located in a separate cluster, as a highly variable grade. High temperature instantly results in drying of young flushes, which are unsuitable for the collection and production of the drink.

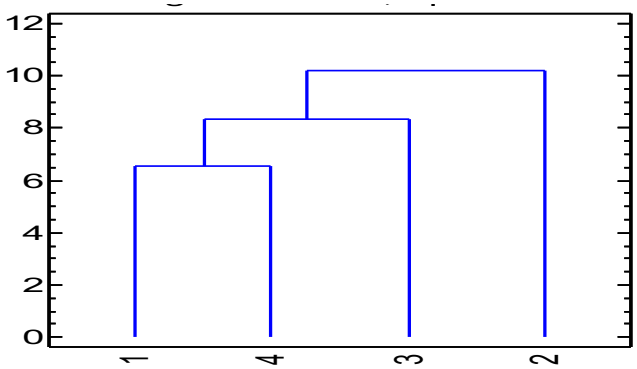
According to the results of the cluster analysis were divided into classes that demonstrate their adaptive capacity, these varieties of hazelnut, as the Cherkessky -2, Lombardsky red, President and Futkurami (Figure 2).

As can be seen from the dendrogram 2 varieties Cherkessky -2, President and Futkurami form a group similar in terms of resistance to stress factors, while variety Lombardsky red according to the degree of adaptability differs from the others. At the same time, considering the combined group of varieties within this cluster are the President that differ in their adaptive potential of varieties Cherkessky -2 and Futkurami.



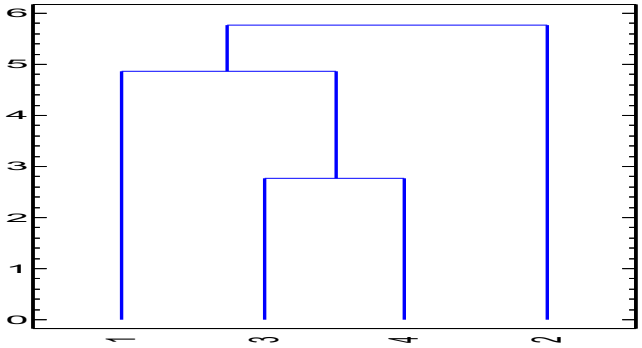
1 – Karatum, 2 – local plants, 3 – Kimyn and 4 – Kolhida

Figure 1 Dendrogramm of tea plants.



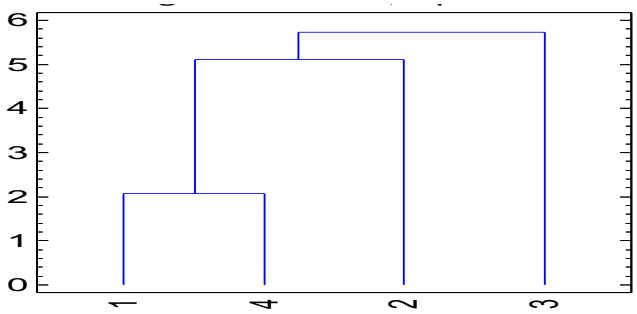
1 – Cherkessky-2, 2 – Lombardsky red, 3 – President and 4 – Futkurami

Figure 2 Dendrogramm of varieties filbert.



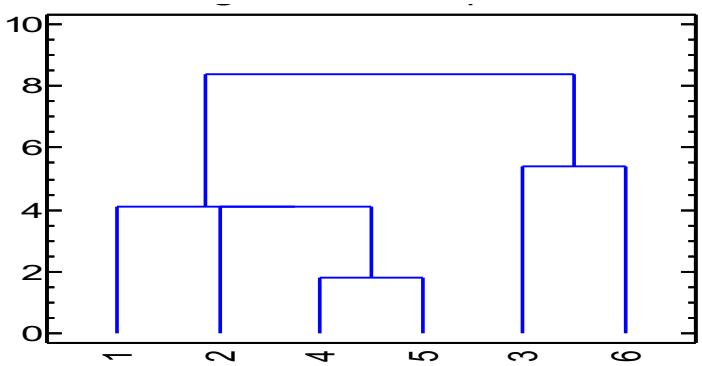
1 – Hayward, 2 – Monty, 3 – Allisson and 4 – Bruno

Figure 3 Dendrogramm of varieties kiwi.



1 – Sester Teresa, 2 – Bichon, 3 – f. rosea and 4 – Draps Wonder

Figure 4 Dendrogramm of varieties gidrangeya.



1 - Eva Ratke, 2 - Gustav Male, 3 – Avgusta, 4 – Arleqin, 5 - Mon Blanc, 6 - Variegata

Figure 5 Dendrogramm of varieties weigela.

As in previous stat analysis using the method of «Nearest neighbor» we managed to get the explanation for the greater stability of the Monti varieties compared to other varieties of kiwi (Figure 3). As can be seen from the dendrogram 3, this variety occupies a position in a separate cluster, which causes the difference of metabolic reactions, including the action of stress factors.

Similarly, the distribution of the studied varieties hydrangea large groups, characterized by similar adaptive potential (Figure 4). As can be seen from the dendrogram 17 variety Draps Wonder, be determined by us on the basic physiological indicators as the most responsive, reasonably is a separate cluster, variety Sester Teresa close to it in terms of resistance to hydrothermal factors, occupying a total cluster.

While form rosea is unstable, rapidly losing its decorative qualities when exposed to high temperatures and lack of water availability (Figure 4). It is not surprising, since form rosea genotypic and phenotypic different from other varieties.

According to the results of the cluster analysis all the studied varieties weigela were divided into the following groups that demonstrate their adaptive capacity (Figure 5).

As can be seen from the dendrogram 5 varieties of Gustav Male, and Arleqin form a cluster, characterized by high resistance to stress factors, while the varieties Avgusta and Variegata according to the degree of adaptability differ greatly, being unstable and leaving a separate group. At the same time, variety Eva Ratke as

melatonin, stands in its phenotypic characteristics closer to the group Gustav Male and Arleqin.

CONCLUSION

Thus, it is possible to establish the fact that the cluster analysis reliably and sufficiently illustrates the analyzed material, which gives the opportunity to use it in classification purposes. Moreover, this method is confirmed by the findings on sustainability of cultures that we have done on the results of physiological and biochemical tests. In the end, we propose a two-stage analysis to select the most informative features and classification of the studied cultivars, where the first phase involves a correlation analysis, the second step - cluster analysis. In this case, the value of the correlation coefficient affects the accuracy of further classification. The resulting dendrogram can be used for inter cluster distance quickly assess the differences in functional state of the species and its place in the classification of resistance to stress factors. In addition, the selection in the result of the analysis of mono clusters (as in the case of dendrograms: 1 - variety tea Kolkhida, 2 – hazelnut Lombardsky red; 3 – variety kiwi Monty and 4 – hydrangea forma rosea is a good tool to detect drastically different varieties.

REFERENCES

Decree of the Russian Federation Government. 14.07.2012 N 717 "About the State program of development of agriculture and regulation of markets of agricultural products, raw materials and food for 2013 - 2020 years".

Decree of the Sochi administration. 17.02.2014 N 245 (an edition of 23.07.2014) "On approval of the municipal program of Sochi "the development of territories of municipal formation the city-resort Sochi" for 2014 - 2016 years".

Decree of the Russian Federation President. 06.08.2014 N 560 "On applying certain special economic measures to ensure security of the Russian Federation".

Brown, D. R., Southern, L. L. 1985. Effect of citric acid and ascorbic acids on performance and intestinal pH of chicks. *Poultry Science*, vol. 64, no. 7, p. 1399-1401. http://dx.doi.org/10.3382/ps.0641399 PMid:4022914

Acopov, A. C., Moskovtsev, A. A., Dolenko, S. A., Savina, G. D. 2013. Cluster analysis in medico-and-biological researches. *Pathological Physiology and Experimental Therapy,* vol. 59, no. 4, p. 84-96.

Belous, O. G. 2008. Diagnostics of stability of tea plants to stress factors. *Bulletin of Russian Academy of Agrarian Sciences*, vol. 6, p. 41-43.

Belous, O. G. 2009. *Biological features of tea culture in the conditions of humid subtropics of Russia.* Ph.D. Thesis: Sochi, Russia : Kuban State Agrarian University, 314 p.

Belous, O. G., Ryndin, A. V., Pritula, Z. V., 2009. *Methodical recommendations about application of diagnostic indicators of stability of plants of tea to a stress factors.* Sochi, Russia : GNU VNIITsiSK, 24 p.

Bessokirny, G. P. 2003. *The discriminant analysis for selection of informative variables. Statistical methods and analysis of data.* no. 16, p. 25.

Filippov, L. A. 1975. Refractometer method and principles of a diagnostics of terms of watering of tea plantations. In: *The water regime and an irrigation of fruit and subtropical crops in mountain conditions.* Instructions. Sochi, Russia : NIIGSiTs, vol. 21, p. 102-122.

Goncharova, E. A. 2005. *Water status of cultural plants and its diagnostics*. Edited by academician V. A. Dragavtsev. SPb.: VIR, 112 p.

Goncharova, E. A. 2011. Strategy of diagnostics and forecast of resistance of agricultural plants to weather and climatic anomalies. *Agricultural Biology*. vol. 15, no. 1, p. 24-31.

Gorsky, V. G., Orlov, A. I. 2002. Mathematical methods of research: results and prospects. *Factory Laboratory*. vol. 68, no. 1, p. 108-112.

Gunar, I. I., 1972. Activity of enzyme catalase - gasometrical method. In: Workshop on physiology of plants. Moskow, Russia : Colos, 168 p.

Kushnirenko, M. D., Kurchatov, G. I, Shtefyrtse, A. A., Pecherskaya, S. N., Bashtovaya, S. I., Kryukov, E. V., 1986. *Express method of diagnostics of heat tolerance and terms of watering of plants.* Kishinev, Russia : Shtiintsa. 38 p.

Klemeshova, K. V., Belous, O. G., 2011. Diagnostics of a functional condition of kiwi-fruit. *Bulletin of Russian Academy of Agrarian Sciences*. no. 4, p. 21-23. ISSN 0869-3730

Klemeshova, K. V., Belous, O. G. 2013. *High-quality diagnostics of functional condition of Aktinidiya sweet. Main abiotic stressor, diagnostic indicators and technique of diagnostics.* Saarbrücken, Germany : LAP Lambert Academic Publishing. 52 p. ISBN: 978-3-659-44008-3

Malyarovskaya, V. I., Belous, O. G., 2012. Methodical recommendations about an assessment of drought resistance of a gidrangea large-leaved *(Hydrangea macrophylla* Ser.). *Subtropical and Decorative Gardening*. vol. 47, p. 228-246.

Malyarovskaya, V. I., Belous, O. G. 2015. Methodical recommendations on use of physiology-and-biochemical parameters for an assessment of stability of weigela (Weigela × wagnera L. H. Bailey) in the conditions of the Black Sea coast of Krasnodar region. *Subtropical and Decorative Gardening*. vol. 52, p. 107-125.

Pochinok, H. K. 1976. *Methods of the biochemical analysis of plants.* Kiev, Ukraine : Naukova dumka, p. 318-320.

Ryndin, A. V., Belous, O. G., Malyarovskaya, V. I., Pritula, Z. V., Abilfazova, Yu. S., Kozhevnikova, A. M. 2014. Use of physiology-and-biochemical methods for identification of mechanisms of adaptation subtropical, southern fruit and decorative crops in conditions subtropics of Russia. *Agricultural Biology*, no. 3, p. 40-48. http://dx.doi.org/10.15389/agrobiology.2014.3.40eng

Savvina, E. A., 2013. Diagnostics of invariant conditions of systems by method of the cluster analysis in flour-grinding production. *Bulletin of the Voronezh State University of Engineering Technologies*. no. 1, p. 68-72.

Sidorenko, E. A. 2001. *Information description and diagnostics of a condition of hierarchically organized systems*. Ph.D. Thesis: Voronezh, Russia : Voronezh State University of Engineering Technology, 192 p.

Tsukanova, E. M. 2007. *Express diagnostics of a condition of plants and increase of efficiency of the production technology of fruits and berries.* Ph.D. Thesis: Michurinsk, Russia : State Agrarian University, 368 p.

Technique of the state assay grades of crops. 1968. Moscow, Russia : Colos, p. 14-221.

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^{137}Cs MONITORING IN THE MEAT OF WILD BOAR POPULATION IN SLOVAKIA

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ABSTRACT

Currently, due to the elapsed time and the nature of the Chernobyl accident, the only artificial radionuclide present in the soil is ^{137}Cs, with a physical half-life conversion of 30.17 years. The ^{137}Cs is quickly integrated into a biological cycle, similar to potassium. Generally, radionuclides are characterized by their mobility in soil. Contamination of materials and food by radionuclides represent a serious problem and has a negative impact on human health. The threat of international terrorism and the inability to forestall the impact of natural disasters on nuclear energetic (Fukushima accident), are also reasons for continuous monitoring of food safety. According screening measurement performed in European countries, high radioactivity levels were reported in the wild boars muscles from Sumava (Czech Republic). Seasonal fluctuation of ^{137}Cs activity in the wild boar meat samples was observed in the forests on the southern Rhineland. Monitoring of ^{137}Cs activity in the wild boar meat samples in the hunting grounds in Slovakia was initiated based on the reports on exceeding limits of the content of radiocaesium in the meat of wild boar from the surrounding countries. The aim of this study was to determine the ^{137}Cs post Chernobyl contamination of wild boars population in different hunting districts of Slovakia during 2013 – 2014. A total of 60 thigh muscle samples from wild boars of different age categories (4 months – 2 years) were evaluated. ^{137}Cs activity was measured by gamma spectrometry (Canberra). Despite the fact Slovakia is closer to Chernobyl as Czech Republic and Germany, the ^{137}Cs activity measured was very low and far below the permitted limit. The highest radiocaesium activity level measured in muscle was 37.2 Bq.kg^{-1} ±4.7%. Wild boar originated from Zlate Moravce district. The measurement results show, that ^{137}Cs contamination levels of game in Slovakia are low. Radiocaesium activity in examined samples was very low and therefore consumption of wild boar meat does not represent a health risk problem.

Keywords: wild boar; contamination; radiocaesium; Slovakia

INTRODUCTION

In the terrestrial environment of Europe the contamination by anthropogenic radionuclides comes from two different sources. The first was the global atmospheric fallout, which appeared after the start of intensive atmospheric nuclear weapons tests in the 50-ies of the last century, and has been observed for a long time after their completion in 1963.

From the radio-ecological perspective are relevant only the long-living components of nuclear weapons tests (^{90}Sr, ^{137}Cs, ^{238}Pu, ^{239}Pu). The most significant long-lived contaminant was the cesium ^{137}Cs isotope **(Högberg, 2013)**. The level of contamination in a certain area was dependent on the latitude and a long-term weather situation, particularly precipitation **(Csupka et al., 1978)**. Currently, the ^{137}Cs from this source, is already largely immobilized in the clay fraction of the soil, with limited access for plant roots **(Nimis, 1996)**.

The second source of the terrestrial contamination was the fallout after the nuclear reactor accident at Chernobyl in 1986. The Chernobyl nuclear accident on April 26, 1986, caused the release of radioactive caesium in the amount up to 3.8 x 10^{16} Bq. The ratio of ^{137}Cs to ^{134}Cs long-term radionuclides released was approximately 2:1 **(UNSCEAR, 1988)**. The radioactive cloud passed three times over the continent. On some territories with intensive rainfalls an increased soil contamination by large amounts of radionuclides was observed. Among most affected parts of Europe, except for Ukraine, Belarus and Russia, belongs despite the distance Norway, where surface activity values reached up to 500 kBq.m^{-2} **(Pedersen et al., 1998)**.

Currently, due to the elapsed time and the nature of the Chernobyl accident, the only artificial radionuclide present in the soil is ^{137}Cs, with a physical half-life conversion of 30.17 years **(Krolak et al., 2010)**.

High mobility of radiocaesium gradually decreased after its deposition to the soil. Differences in mobility were observed especially in the non-cultivated meadow and forest soils, and were associated with the soil depth. In mineral soils by increasing depth, the amount of ^{137}Cs accessible by plants may rise, however it depends on many factors and circumstances **(Schimmack and Bunzl, 1996)**. In the Central Europe, the most contaminated animal product by Chernobyl accident was the meat of game **(Sprem et al. 2013)**.

Based on information from the neighboring countries on exceeding limits of radiocaesium content in wild boar meat, monitoring of the hunting grounds in Slovakia was initiated.

MATERIAL AND METHODOLOGY

Thigh muscle samples of 60 wild boars (*Sus scrofa*) of different age categories (4 month – 2 years) collected from different hunting districts in Slovakia were evaluated in the period of 2013 – 2014. Thigh muscle samples of 500g collected from wild boars weighing from 15 up to 35 kg and originated from 26 hunting grounds were used in the study.

^{137}Cs activity was measured by gamma spectrometry (Canberra) consisting of a Ge detector (GC 3520: 20% effectiveness, 2.0 keV resolution) and a multichannel analyzer (Desktop Inspector) in 450 mL geometry Marinelli beakers and 200 mL PE bottle. Results of mass activity Bq.kg^{-1} were determined by a 2000 Genie (Canberra) and Gamvin (Envinet) software systems. It is further stated the % of relative standard uncertainty for each sample. All geometres including the gamma-spectrometric system were certified in the Czech Metrological Institute.

RESULTS AND DISCUSSION

The highest radiocaesium activity level measured in muscle was 37.2 Bq.kg^{-1} ±4.7%. Wild boar (Table 1) sample originated from Zlate Moravce district. In 8 muscle samples the highest value was less than 3.6 Bq.kg^{-1} ±2.2%. In all other muscle samples, the radiocaesium activity levels were below the minimum detectable activity.

Due to the fact that Scandinavia was heavily contaminated by Chernobyl radiocaesium fallout, within the food chain considerable attention was paid to elk **(Palo et al., 2003)** and reindeer meat **(Skuterud et al., 2004)**. Similar attention was paid also to marine organisms (seafood), such as crab from the north of Ireland **(Copplestone et al., 2004)**. In the Central Europe, the most contaminated animal product by Chernobyl accident, was the meat of game **(Sprem et al., 2013; Škrkal et al., 2015)**. Relatively higher activity of ^{137}Cs in the wild animals is based on the mosaic pattern of contamination area after the Chernobyl accident, in the way the wild animals searches and acquires food (especially wild boars), and in a significantly greater mobility and persistence of radiocaesium in the forest ecosystems, compared to intensively used agricultural land **(Vaaramaa et al., 2009)**. The ^{137}Cs is quickly integrated into a biological cycle, similar to potassium. Generally, radionuclides are characterized by their mobility in soil **(Gadd, 1996)**. Analysis has shown, that the diffusion coefficient of radionuclides in the soil is affected by the soil moisture, presence of chemical homologs, which determine the capacity of the exchange process in the soil, soil acidity, soil humus content and temperature.

Fungi, as one of the most important constituents of forest ecosystem are capable to accumulate a significant amount of radionuclides including ^{137}Cs **(Škrkal et al., 2013; Guillen and Baeza, 2014)**. It is due to their heterotrophic metabolism, significantly different from green plants, and dependence on the supply of final organic compounds **(Yoshida and Muramatsu, 1994)**.

Some species of fungi, including edible ceps (*Boletaceae family*) growing in deciduous forests of Central Europe, are not only bio indicators of environmental contamination by radiocesium (and heavy metals as well), but do to their consumption represent a potential hygiene and health risks problem **(Linkov et al., 2000; Dvořák et al., 2006)**. Radiocesium distribution in different mushroom body parts was uneven, nevertheless higher activity was determined in the mushroom caps **(Mukhopadhyay et al., 2007)**. Ability to accumulate radionuclides from the environment differs among different species of ceps (*Boletaceae family*). This corresponds with the finding, that increased accumulation of ^{137}Cs in bay bolete (*Xerocomus badius, syn. Boletus badius*) is mainly due to the presence of a pigment norbadion A, present in the mushroom cap **(Aumann et al., 1989)**. It is assumed that, as in higher plants, the radiocesium activity in fungi is associated with the growth phase, and the total radiocesium activity decreases with the gradual growth. Mushrooms samples collected in coniferous forests, compared to deciduous forests, are characterized by a high content of radionuclides **(Čipáková, 2004)**.

Table 1 Radiocaesium activity levels in muscles.

Hunting districts, sample data	^{137}Cs (Bq.kg^{-1})	^{40}K (Bq.kg^{-1})
Uhrinc (Kosice) Juvenile female, 1 year, 35 kg	0.60 ±27.6%	81.2 ±4.8%
Jablonov n/T (Roznava) Juvenile female, 1 year, 35 kg	2.03 ±10.0%	47.4 ±4.8%
Rakos, Camovce II (Lucenec) Squeaker female, 6 month, 17 kg	1.1 ±16.6%	51.2 ±2.6%
Vidoslav, Camovce II (Lucenec) Squeaker female, 4.5 month, 15 kg	0.9 ±23.8%	73.0 ±2.6%
Sklens (Turcianske Teplice) Squeaker male, 5 month, 15 kg	0.8 ±33.0%	77.0 ±4.8%
Smolnik (Gelnica) Juvenile female, 1 year, 30 kg	1.5 ±15.5%	75.7 ±4.8%
Lovce (Zlate Moravce) Wild boar female, 2 years, 35 kg	3.6 ±2.2%	63.6 ±2.2%
Topolcianky (Zlate Moravce) Wild boar female, 15 month, 25 kg	37.2 ±4.7%	57.9 ±4.8%
Velky Krtis (Velky Krtis) Squeaker female, 8 month, 20 kg	0.47 ±34.5%	96.8 ±4.8%

Results of the radiocesium mass activity from different areas of the Czech and Slovak Republic in 2000 – 2004, were published by **Dvořák et al., (2006)**. The highest ^{137}Cs activity of 6,263 Bq.kg^{-1} dry weight (measured by gamma spectrometry method) was found in the *Xerocomus badius* from the Old Ransko area (Czech-Moravian Highlands). The highest measured ^{137}Cs level in Slovakia was 966 Bq.kg^{-1} dry weight (*Suillus luteus*), in the area of Senica. However, when converted to fresh weight this value do not exceed permitted limit. For comparison, the ^{137}Cs activity in the sample of mixed dried ceps (*Boletaceae family*) coming from the 30 km Chernobyl border zone, was 6,000 Bq.kg^{-1} dry weight. Results for the dried mushrooms show, that currently there is no ^{137}Cs activity dependency related to the distance from the place of radioactive accident or the location altitude. These results also indicate significantly lower values of ^{137}Cs activity in the Slovak Republic compared to the Czech Republic, despite the fact Slovakia is closer to Ukraine. Explanations should be sought in the airborne radioactive cloud movement through the various parts of Europe after the Chernobyl disaster. In the fresh mushrooms collected in the French Alps in 1999 – 2002, the value of the ^{137}Cs activity ranged from 273 – 1,165 Bq.kg^{-1} **(Pourcelot et al., 2003)**.

After a gradual decline of the radiocaesium (^{137}Cs) activity in the game muscles in the 1990s, unexpected activity increase occurred after the floods in North-East Moravia in 1997. In the meat of wild boars, the activity exceeded the hygienic limit set for EU countries (600 Bq.kg^{-1}), especially in the age category up to 1 year. Since 2000 the ^{137}Cs activity was reduced back to the level before the flood **(Obzina, 2002)**. Seasonal fluctuation of ^{137}Cs activity in the wild boar meat samples was observed in the forests on the southern Rhineland. The stomach contents were examined as well. A positive correlation (0.66) for the activity of the stomach contents and the activity of muscle was found, but the stomach contents were usually less contaminated compared to muscles; the median of the stomach content was 22 Bq.kg^{-1}, the maximum 1,749 Bq.kg^{-1}, while the median of muscle was 129 Bq.kg^{-1}, and the maximum 5,573 Bq.kg^{-1}. No difference in the specific activities of female and male muscles was proved **(Hohmann and Huckschlag, 2005)**. From 1998 to 2008, 656 samples from the wild boars were analysed in the district of Ravensburg (southern Germany). The activity was variable from less than 5 up to 8,266 Bq.kg^{-1}, depending on the season, weather conditions and the associated changes in dietary habits and food availability **(Semizhon et al., 2009)**. High radioactivity levels (up to 10,699 Bq.kg^{-1}) were reported in the wild boars muscles from Sumava (Czech Republic) **(Latini, 2011)**. In 2012, the highest measured value was 14,252 Bq.kg^{-1} **(Kouba et al., 2013)**. In Croatia, some locations were monitored during the years 2000 and 2002 **(Vilic et al., 2005)**; radiocesium in boar muscles ranged from 0.4 to 611.5 Bq.kg^{-1}. The highest muscle contamination was observed in autumn. The authors interpreted this fact as a result of higher mushroom intake by boars at that time. Our muscle samples were collected during different annual periods, however no significant seasonal differences in the radiocaesium activity we observed. This was probably due to low radiocaesium activity in fungi in the observed area, and thus subsequently in our samples as well.

The **Recommendation of the European Commission (2003)** highlights the existence of limit exceeding activity of radiocaesium in the meat of game, and call the Member States to act, in order to protect consumers. Member states should implement steps to ensure that the limits set by **Directive no. 737/90 / EEC (2000)** for placing the game meat, wild berries, mushrooms, and predatory lake fish to the market, are respected. At the same time it recommends to warn the inhabitants of affected regions on the health risks resulting from contaminated food consumption. Member States are asked to prepare for the European Commission and other EU Member States a feedback on the implementation of this Directive.

Wild boar muscles contamination is mainly due to the consumption of the underground fruiting bodies of the mushroom genus of *Elaphomyces sp.* (*E.granulatus* - deer truffle, hart's truffles) **(Hohmann and Huckschlag, 2005; Dvořák et al., 2010)**. The highest ^{137}Cs specific activity of 4,743 Bq.kg^{-1} was detected in the mushroom fruiting bodies in the area of Šabrava, while the other components of the food chain of feral pigs do not exceed few tens of Bq.kg^{-1} **(Dvořák et al., 2010)**. The results of stomach content analysis showed the considerable importance of additional feeding and beechnuts in the food of boars while rootlets and sprouts were less important, and the importance of animal components was minimal. Mushrooms were not identified in any stomach, probably due to their relatively high digestibility in boars. Furthermore, the following food components of boars manifested the ^{137}Cs specific activities higher then MDA in the samples tested: 16 Bq.kg^{-1} in meadow earthworms, 200 Bq.kg^{-1} in rootlets at the Šabrava location, and finally 4,743 Bq.kg^{-1} and 2,858 Bq.kg^{-1} in *Elaphomyces granulatus* fruiting bodies. The fruiting bodies were not found in the boar stomach as other mushrooms, however, they were found in the marginal areas around the rooted locations. We may assume that this mushroom with a vegetation season from September to April is searched and consumed by boars, similarly as e.g. the truffles in France. For example, considering the high activity of 4,743 Bq.kg^{-1} measured in one sample and taking into account the high mushroom digestibility in boars, the specific activity of chyme can be estimated to about 237 Bq.kg^{-1} only due to the intake of the mushroom when 5% intake in 1 kg of the stomach content was taken into account. For monogastric animals, the radiocaesium resorption can reach up to 100% while excretion is about 25%, which would mean a daily growth of specific activities of 21 Bq.kg^{-1} in the net muscle weight of 25 kg (for a boar life weight of 100 kg and food consumption of 3 kg daily). However, such chyme usually does not consist only of the fruiting bodies of the above mentioned mushroom and other components with the specific activities below MDA. The following example based on our results (muscle sample with the specific activities of 4,743 Bq.kg^{-1}, soil elements 173 Bq.kg^{-1}, rootlets 200 Bq.kg^{-1}, earthworms 16 Bq.kg^{-1}, all at the Šabrava location) shows the importance of other ^{137}Cs sources. The proved presence of soil, earthworms, rootlets and other components increases the ^{137}Cs activity in the stomach content. In case of food consumption of 3 kg.day^{-1} at the

Šabrava location with the individual contributions, i.e. 5% fruiting body of *Elaphomyces granulatus* (total 711 Bq), 5% soil elements (26 Bq), 20% rootlets (120 Bq), 2% earthworms (1 Bq) and other components with the specific activities below MDA, the total activity of the stomach content would be 858 $Bq.day^{-1}$. For the stomach content of 1 kg, this means the specific activity of 286 $Bq.kg^{-1}$. The daily increase of the specific activity in muscle would be 26 $Bq.kg^{-1}$. However, the increase due to other components in the food chain is only 5 $Bq.kg^{-1}$, i.e. an increase by 25%. *Elaphomyces granulatus* is mainly found in soils with high content of humus, especially in sandy pine and spruce forests. It grows under the ground and appears on the ground surface usually due to wild game (wild boar) activity. The main period of the fungi growth is from September to April. This is the time when animals have limited access to food sources. In Slovakia, the occurrence of this fungus is very rare, and is mainly present in protected landscape areas, where hunting is not allowed. Compared to the situation in livestock, limited possibility of protective measures implementation represents the biggest problem of ^{137}Cs contamination in game. The only possible solution remains to check each hunted animal individually. One possibility of preventing the game contamination is the supplementary feeding.

The use of alternative food sources as prevention, may result in reduced consumption of natural food, including *Elaphomyces granulatus*, to cover organism energy needs. Slovakia has so far monitored very limited number of hunted animals. The value of ^{137}Cs samples activity collected are still very low **(Beňová et al., 2014)**, what is consistent with our results.

CONCLUSION

In recent years, the wild game meat is more frequently present on menus of variety of restaurants and is becoming very popular in consumers. Despite the fact Slovakia is closer to Chernobyl as Czech Republic and Germany, the ^{137}Cs activity measured was very low and far below the permitted limit. The measurement results show, that ^{137}Cs contamination levels of game in Slovakia are so low, that they do not represent an increased health risk for humans.

REFERENCES

Aumann, D. C., Clooth, G., Steffan, B., Steglich, W. 1989. Komplexierung von Caesium-137 durch die Hutfarbstoffe des Maronenröhrlings (*Xerocomus badius*). *Angewandte Chemie,* vol. 101, no. 4, p. 495-496. http://dx.doi.org/10.1002/ange.19891010429

Beňová, K., Dvořák, P., Tomko, M., Čurlík, J., Lazar, P. 2014: Radiocaesium in the meat in Slovakia. In *Hygiena Alimentorum XXXV*. Košice: UVLF, 2014, p. 168-170. ISBN 978-80-8077-401-1.

Commission Recommendation 2003/120/EC, On the protection and information of the public with regard to exposure resulting from the continued radioactive cesium contamination of certain wild food products as a consequence of the accident at the Chernobyl nuclear power station, Official Journal of the European Union. L47, 21.2.2003, p. 53-55.

Copplestone, D., Jackson, D., Hartnoll, R. G., Johnson, M. S., McDonald, P., Wood, N. 2004: Seasonal variation in activity concentrations of Tc-99 and Cs-137 in the edible meat fraction of crabs and lobsters from the central Irish Sea. *Journal of Environmental Radioactivity*, vol. 73, p. 29-48. http://dx.doi.org/10.1016/j.jenvrad.2003.06.001 PMid:15001294

Council Regulation 616/2000/EC, Council Regulation of 20th March 2000 amending Regulation (EEC) No 737/90 on the conditions governing imports of agricultural products originating in third countries following the accident at the Chernobyl nuclear power station, Official Journal of the European Union. L75, 20.3.2000, p. 1.

Csupka, Š., Carach, J., Petrášová, M. 1978. *Environmental Radioactivity in Slovakia / Czechoslovakia in 1961–1975*. Regional Public Health Authority, Bratislava, 226 p.

Čipáková, A. 2004. Cs – 137 content in mushrooms from localities in eastern Slovakia. *Nucleonika*, vol. 49, suppl. 1, p. 25-29.

Dvořák, P., Kunová, V., Beňová, K., Ohera, M. 2006. Radiocesium in mushrooms from selected locations in the Czech Republic and the Slovak Republic. *Radiation Environmental Biophysics*, vol. 45, no. 2, p. 145-151. http://dx.doi.org/10.1007/s00411-006-0044-5 PMid:16628432

Dvořák, P., Snášel, P., Beňová, K. 2010. Transfer of radiocesium into wild boar meat. *Acta Veterinaria Brno*, vol. 79, p. 85-91. http://dx.doi.org/10.2754/avb201079S9S085

Gadd, G. M. 1996. Influence of Microorganisms on the Environmental Fate of Radionuclides, *Endeavour*, vol. 20, no. 4, p. 150-156. http://dx.doi.org/10.1016/S0160-9327(96)10021-1

Guillen, J., Baeza, A. 2014. Radioactivity in mushrooms: A health hazard? *Food Chemistry*, vol. 154, p. 14-25. http://dx.doi.org/10.1016/j.foodchem.2013.12.083 PMid:24518310

Hohmann, U., Huckschlag, D. 2005. Investigations on the radiocesium of wild boar (*Sus scrofa*) meat in Rhineland – Palatine: a stomach content analysis. *European Journal of Wildlife Research*, vol. 51, no. 4, p. 263-270. http://dx.doi.org/10.1007/s10344-005-0108-x

Högberg, L. 2013. Root Causes and Impacts of Severe Accidents at Large Nuclear Power Plants. *A Journal of the Human Environment*, vol. 42, no. 3, p. 267-284.

Kouba, F., Cipínová, E., Drápal, J., Hanzal, V., Malena, M., Vernerová, K. 2013. The radioactivity monitoring of wild boars in the South Bohemian Region. *Mäso*, vol. 3, p. 151-154.

Krolak, E., Kwapulinski, J., Fischer, A. 2010. (137)Cs and (40)K isotopes in forest and wasteland soils in a selected region of eastern Poland 20 years after the Chernobyl accident. *Radiation And Environmental Biophysics*, vol. 49, no. 2, p. 229-237. http://dx.doi.org/10.1007/s00411-010-0265-5 PMid:20076963

Latini, T. 2011. The presence of radioactivity in the feral pigs. *Mäso*, vol. 5, p. 24-26.

Linkov, I., Yoshida, S., Steiner, M. 2000. Fungi contaminated by radionuclides. Critical review of approaches to modeling. In *Proceedings of the 10th International Congress of the International Radiation Protection Association*, Hiroshima, p. 1-10.

Mukhopadhyay, B., Nag, M., Laskar S., Lahiri S. 2007. Accumulation of radiocesium by *Pleurotus citrinopileatus* species of edible mushroom. *Journal of Radioanalytical and Nuclear Chemistry*, vol. 273, no. 2, p. 415-418. http://dx.doi.org/10.1007/s10967-007-6875-y

Nimis, P. L. 1996. Radiocesium in Plants of Forest Ecosystems. *Studia Geobotanica*, vol. 15, p. 3-49.

Obzina, J. 2002. *Presence of radiocesium the game meat from the districts Šumperk and Jeseník in-between 1992-2002*. Postgraduate certificate, Brno: University of Veterinary Medicine and Pharmacy. 35 p.

Palo, R. T., White, N., Danell, K. 2003. Spatial and temporal variations of Cs-137 in moose *Alces alces* and transfer to man in northern Sweden. *Widlife Biology*, vol. 9, p. 207-212.

Pedersen, Ch. S., Nyb, S., Varskog, P. 1998. Seasonal variation in radiocesium concentration in Willow Ptarmigan and Rock Ptarmigan in Central Norway after the Chernobyl Fallout. *Journal of Environmental Radioactivity*, vol. 41, p. 65-81. http://dx.doi.org/10.1016/S0265-931X(97)00095-7

Pourcelot, L., Renaud, P., Louvat, D., Gurriaran, R., Richon, P. 2003. Influence of hot spots on cesium-137 contamination of an alpine food chain and doses associated with it, Environnement. *Risques et Santé*, vol. 2, no. 2, p. 112-120.

Semizhon, T., Putyrskaya, V., Zibold, G., Klemt, E. 2009. Time-dependency of the ^{137}Cs contamination of wild boar from a region in Southern Germany in the years 1998 to 2008. *Journal of Environmental Radioactivity*, vol. 100, no. 11, p. 988-992. http://dx.doi.org/10.1016/j.jenvrad.2009.06.023 PMid:19616349

Schimmack, W., Bunzl, K. 1996. Mobility of Chernobyl – Derived Radiocesium in the Soil, Proceesings of the International Symposium on Radioecology. In: *Mitteilungen Osterreichische Bodenkundliche Gesellschaft*. Vienna : International symposium on radioecology 1996, vol. 53, p. 11-18. ISSN 0029-893X.

Skuterud, L., Pederson, O., Staaland, H., Roed, K. H., Salbu, B., Liken, A., Hove, K. 2004. Absorption, retention and tissue distribution of radiocaesium in reindeer: effects of diet and radiocaesium source. *Radiation and Environmental Biophysics*, vol. 43, no. 4, p. 293-301. http://dx.doi.org/10.1007/s00411-004-0257-4 PMid:15459770

Sprem N., Babic I., Barisic D., Barisic D. 2013. Concentration of ^{137}Cs and ^{40}K in meat of omnivore and herbivore game species in mountain forest ecosystems of Gorski Kotar, Croatia. *Journal of Radioanalytical and Nuclear Chemistry*, vol. 298, no. 1, p. 513-517. http://dx.doi.org/10.1007/s10967-013-2475-1

Škrkal, J., Rulik, P., Fantinova, K., Burianová, J., Helebrant, J. 2013. Long-term Cs-137 activity monitoring of mushrooms in forest ecosystems of the Czech Republic. *Radiation Protection Dosimetry*, vol. 157, no. 4, p. 579-584. http://dx.doi.org/10.1093/rpd/nct172 PMid:23838098

UNSCEAR. 1988. *Sources, Effects and Risks of Ionizing Radiation*, New York: UN. 647 p., ISBN 92-1-142143-8.

Vaaramaa, K., Solatie, D., Aro, L. 2009. Distribution of Pb-210 and Po-210 concentrations in wild berries and mushrooms in boreal forest ecosystems. *Science Of The Total Environment*, vol. 408, no. 1, p. 84-91. http://dx.doi.org/10.1016/j.scitotenv.2009.09.005 PMid:19800659

Vilic M., Barisic D., Kraljevic P., Lulic S. 2005. 137Cs concentration in meat of wild boars (*Sus scrofa*) in Croatia a decade and after the Chernobyl accident. *Journal of Environmental Radioactivity*, vol. 81, no. 1, p. 55-62. http://dx.doi.org/10.1016/j.jenvrad.2004.12.001 PMid:15748661

Yoshida, S., Muramatsu, Y. 1994. Accumulation of radiocesium in basidiomycetes collected from Japanese forests. *Science of the Total Environment*, vol. 157, p. 197-205. http://dx.doi.org/10.1016/0048-9697(94)90580-0

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THE EFFECT OF FEEDING MILK THISTLE SEED CAKES ON QUALITY INDICATORS OF BROILER CHICKENS MEAT

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ABSTRACT

This study was conducted to evaluate the effect of feeding milk thistle *(Silybum marianum L.)* seed cakes at dose 5% and 15% in feed mixture on quality indicators of broiler chickens meat. The used milk thistle seed cakes contained 3.73% of flavonolignans and 129.83 mg.kg^{-1} of cyanidin-3-glucoside. A 150 cockerels of Ross 308 were divided into three equal groups. The chickens were fattened on conventional deep litter system. The experimental groups received feed mixtures containing 5% of milk thistle seed cakes (MT5), 15% of milk thistle seed cakes (MT15) and third group was control – without milk thistle seed cakes (C). The trial lasted 37 days. At the end of trial was observed significant higher average weight of chickens (2,320.31 g) in control group. Compare to that the experimental group MT5 achieved significant lower mean bodyweight 2,166.69 g. From the perspective of fattening was decreased growth of chickens where a higher percentage of milk thistle seed cakes (MT15). The group MT15 was up to 420 g lower slaughter weight compared to the control group. This was probably due to the higher content of fiber in the feed. At the end of experiment 15 birds were selected randomly from each group, weighed and slaughtered. Feathers were removed and chickens were eviscerated. Carcass yield was calculated for each group like as percentage of live weight. The MT5 and MT15 group had significantly higher breast meat tenderness that the control group. Initial pH1 was highest in group with its middle addition of milk thistle seed cakes (MT5). Significant differences were not observed between control and group MT15. Breast meat was rated as the best in parameter flavour in control and MT15 group. The thigh meat was evaluated significantly best for colour parameter in MT15 group. Fibreness was rated as the finest in MT15 group. The addition of milk thistle seed cakes do not worsened sensory characteristic of breast or thigh meat of broilers and reflects optimal sensory quality traits.

Keywords: *Silybum marianum L.*; meat quality; growth; texture; colour; flavour

INTRODUCTION

Milk thistle *(Silybum marianum* L.; Asteraceae) have been used for almost 2 000 years as a natural treatment for the liver diseases **(Ding et al., 2001)**. The seeds of milk thistle contain flavonoids (anthocyanins) and flavonolignans (silymarin) in an amount of 1.5 – 3% **(Opletal and Šimerda, 2015)**.

Flavonoids are widespread in nature, including the anthocyanins commonly found in dark-coloured fruits and vegetables. Anthocyanins are produced by plants as secondary metabolites to protect against environmental stress factors and fungal infections **(Chalker-Scott, 1999)**. And they also promote health status **(Wallace, 2011; Pojer et al., 2013)**. Phenolic compounds, mainly anthocyanins have antioxidant and anti-inflammatory activities **(Jung et al., 2014)**. This anthocyanin, cyanidin-3-glucoside (CG) respectively, have been reported to be bioavailable **(Miyazawa et al., 1999)**. CG decreased obesity and circulating triglycerides in an in vivo study **(Wei et al., 2011)**. In vitro, CG decreased inflammation in isolated vascular endothelial cells and monocytes and produced an insulin-like effect in human adipocytes **(Luo et al., 2012; Scazzocchio et al., 2011)**.

The main active substances occurring in milk thistle are flavonolignans, which are hepatoprotective substances. The mixture of silydianin (10%), silychristin (20%), silybin A and silybin B (50 – 60%), isosilybin A and isosilybin B is known as silymarin **(Opletal and Skřivanová, 2010; Ding et al., 2001; Zahid and Durrani 2007; Comelli et al., 2007)**. Silymarin complex exhibits chemopreventive activity against chemical, viral, bacterial and fungal toxins, inhibits lipid peroxidation, and stabilizes the cell membranes of the liver parenchyma **(Opletal and Skřivanová, 2010)**. Various trials showed that silymarin addition in diet or silymarin administration increased productive and reproductive performances and improved livestock health status of animals **(Tedesco, 2001)**.

Many works investigate the effect of herbs addition to feed mixtures for broiler chickens and their influence to the meat quality **(Haščík et al., 2015; Bobko et al., 2009)**.

The rapid growth of modern broilers hybrid and toxic substances in the feed mixtures can lead to metabolic and oxidative stress. It can worsen feed conversion ratio, growth parameters and it can affect the quality of chicken meat **(Erdogan et al., 2005; Carreras et al., 2004)**. The consumers have very high requirements on their food. It

must be natural, healthy, quality, safe and, on top of that, it should have pleasant appearance, texture, odour and taste **(Drobná et al., 2006)**.

This study was conducted to evaluate the effect of feeding milk thistle seed cakes at dose 5% and 15% in feed mixture on quality indicators of broiler chickens meat.

MATERIAL AND METHODOLOGY

Growth performance, body and chemical composition

The used milk thistle seed cakes contained 3.73% of flavonolignans and 129.83 mg.kg^{-1} of cyanidin-3-glucoside. Table 1 shows chemical composition of used milk thistle seed cakes.

Table 1 Chemical composition of milk thistle seed cakes.

Dry matter (%)	100
Gross energy (MJ.kg^{-1})	17.44
Crude protein (%)	18.65
Crude fat (%)	8.66
Crude fibre (%)	25.13
Crude ash (%)	5.84

The experiment was performed with cockerels of Ross 308 hybrid (n = 150) which were fattened on conventional deep litter system. Wood shavings were used as bedding material. The trial was conducted from day 12 to day 37 of chick's age. Room temperature and humidity were controlled. Lighting system was 16 hours light and 8 hours dark. Cockerels were divided into three equal groups. The two experimental groups received feed mixtures containing 5% and 15% of milk thistle seed cakes (groups MT5 and MT15, respectively). The third group was without milk thistle seed cakes (Control group). The rations were calculated according to the Recommended nutrient content in poultry diets and nutritive value of feeds for poultry **(Zelenka et al., 2007)**. The composition of feed mixtures is shown in Table 2.

The chickens were fed *ad-libitum*. Health status was evaluated daily and live weight measured every week during the trial. Body weight gain was measured individually.

At the end of experiment fifteen birds were selected randomly from each group, weighed and slaughtered. Feathers were removed and chickens were eviscerated. Carcass yield was calculated. The breast muscle and leg muscle were deboned and weighed in these selected chickens. These values were calculated by the percentage of live weight.

Texture, colour and pH of meat

The tenderness of the fillets was determined through the application of the Meullenet–Owens razor shear (MORS) test, using a texture analyzer (Model TA-XT2*Plus*, Texture Technologies, Scarsdale, N.Y., U.S.A.) as described by **Meullenet et al., (2004)** and **Cavitt et al., (2005)** during which Razor Blade Shear Force (N) were recorded. Tests using the MORS blade are conducted on whole intact right fillets with 5 replicates. The sharp blade was replaced every 80 measurements for optimum shearing performance. Test Settings: test speed 10 mm.s^{-1}, distance 20 mm.

Table 2 Composition of feed mixture (g.kg^{-1}).

Component	C	MT5	MT15
Wheat	378.2	271.8	269
Corn	247	282.4	251
Milk thistle seed cakes	0	50	150
Soybean meal	105	120	128
Soybean extruded	190	190	78
Rapeseed oil	20	30	40
Wheat gluten	18.8	15.2	40
Premix*	30	30	30
Monocalciumphosphate	7	6.5	7
Limestone milled	4	4	5
L-lysine	0	0	2
Chemical composition (per kg of diet)			
Dry matter (%)	100	100	100
Gross energy (MJ)	18.59	18.83	19.07
Crude protein (%)	21.41	21.73	22.43
Crude fat (%)	7.60	9.60	8.96
Crude fibre (%)	2.81	4.07	7.03
Crude ash (%)	5.96	5.84	6.65

* Premix contains (per kg): lysine 60 g; methionine 75 g; threonine 34 g; calcium 200 g; phosphorus 65 g; sodium 42 g; copper 500 mg; iron 2,500 mg; zinc 3,400 mg; manganese 4,000 mg; cobalt 7 mg; iodine 30 mg; selenium 6 mg; tocopherol 450,000 mg; calciferol 166,700 IU; tocoferol 1,500 mg; vit K 350 mg; B_1 140 mg; B_2 230 mg; B_6 200 mg; B_{12} 1,000 mg; biotin 7 mg; niaciamid 1,200 mg; folic acid 57 mg, calcium pantothenate 450 mg; choline chloride 6,000 mg; salinomycin sodium 2,333 mg.

Colour measurement was performed by CIE L*a*b* colour space. L* (lightness), a*(redness) and b* (yellowness) values from the breast muscle sample surface on the dorsal side were measured using a Spectrophotometer CM-3500d (Konica Minolta Sensing Inc., Osaka, Japan) in SCE mode (specular component excluded), angle 8°, 8 mm slit. Each sample was measured at three places 1-hour *post-mortem*. Average value was taken as the final result. ΔE^*_{ab} **(CIE, 2007)** was calculated according next formulas **(Valous et al., 2009)**:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

$$\Delta L^* = L^*_{control} - L^*_{group}$$

$$\Delta a^* = a^*_{control} - a^*_{group}$$

$$\Delta b^* = b^*_{control} - b^*_{group}$$

The samples was measured using pH meter Portavo 907 Multi (Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany) with a needle-type electrode (SE104N; Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany) immediately (inicial pH, abbreviation pH1) after chicken´s slaughter and 1 hour *post-mortem* (abbreviation pH2).

Sensory analysis

Sensory analysis of breast and thigh muscle samples was evaluated by 10 panellists in special sensory laboratory (Department of Food Technology, MENDELU) according ISO 8589. Each sample (breast and thigh) was packed into plastic case and frozen (freezer, -18 °C). After two weeks was thawed (cold storage room, 4 °C) and boiled in convection oven (200 °C, 60% humidity, 1 hour). Professional evaluation group was represented by a panel of trained panellists under ISO 8586-1. We used a graphic non-structured scale (100 mm) to compare experimental group of descriptors (odour, colour, fibreness, chewiness, juiciness, flavour, fatty taste) with control group.

Statistical analysis

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). We used one-way analysis (ANOVA). To ensure evidential differences Scheffe´s test was applied and p <0.05 was regarded as statistically significant difference.

RESULTS AND DISCUSSION

Growth performance, body and chemical composition

At the end of trial was observed significant (p <0.05) higher average weight of chickens (2,320.31 g) in control group. Compare to that the experimental group MT5 achieved significant lower mean bodyweight 2,166.69 g. The significant lowest mean bodyweight was achieved in the group MT15 with value 1,988.78 g at the end of trial (Table 3).

Table 3 Live weight at the day of slaughter (g).

Group	Mean ±standard error
C	2,320.31 ±29.24[c]
MT5	2,166.69 ±36.43[b]
MT15	1,988.78 ±30.09[a]

[a,b,c] – different letters are statistically significant differences (p <0.05).

According to the technological procedure for ROSS 308, the average body weight of cockerels would be 2,493 g at 37 days of age **(Aviagen Group, 2014)**. This is much closer to the value of the control group (2,320 g) in our trial. **Suchý et al., (2008)** in their experiment observed then the addition of 0.2% and 1% *Sylibum Marianum* seed cakes caused a decrease in the weight gain. **Gawel et al., (2003)** found an increase in the slaughter weight in broilers when supplied silymarin.

From the perspective of fattening was decreased growth of chickens where a higher percentage of milk thistle seed cakes (MT15). The group MT15 was up to 420 g lower slaughter weight compared to the control group, which was probably due to the higher content of fiber in the feed.

Table 4 present the carcass yield parameters of chickens. The carcass yield was not show significant (p >0.05) differences. Carcass yield stated in the technological procedure for ROSS 308 **(Aviagen Group, 2014)** is 72.08% for 2,200 g live weight. The highest carcass yield showed the control group with a value 72.09%. It is comparable with technological procedure for ROSS 308 **(Aviagen Group, 2014)**.

The higher breast meat yield was found in the group 5% of milk thistle seed cakes (22.11 ±0.42% SE). The differences among groups were not statistically significant (p >0.05). The manual for hybrid Ross 308 **(Aviagen Group, 2014)** is stated similar percentage of breast muscle

Table 4 Body composition (%).

Group	n	Carcass	Breast meat	Leg meat
		Mean ±standard error		
C	15	72.09 ±1.05	21.62 ±0.63	14.84 ±0.33
MT5	15	71.44 ±0.95	22.11 ±0.42	14.77 ±0.28
MT15	15	70.51 ±0.75	20.70 ±0.49	15.21 ±0.37

Differences between groups are not signifficant (p >0.05).

Table 5 Chemical analysis of breast and thigh (%).

			C	MT5	MT15
		n	Mean ±standard error		
Dry Matter	Breast meat	6	23.97 ±0.62	24.20 ±0.25	23.56 ±0.54
	Leg meat		24.62 ±0.37	24.12 ±0.29	23.95 ±0.18
Crude Protein	Breast meat	6	20.94 ±0.77	21.39 ±0.28	21.68 ±0.57
	Leg meat		18.68 ±0.19	18.46 ±0.22	18.89 ±0.31
Crude Fat	Breast meat	6	1.24 ±0.19	1.10 ±0.11	0.96 ±0.15
	Leg meat		4.17 ±0.25	4.31 ±0.28	3.88 ±0.34

Differences between groups are not signifficant (p >0.05).

Table 6 Effect of addition milk thistle seed cakes into feed on texture, pH and colour of breast meat (means ±SE).

Parameter	Control	MT5	MT15
Razor Blade Shear [N]	11.23 ±0.47[a]	8.94 ±0.27[b]	9.52 ±0.36[b]
L*	62.00 ±2.20[a]	61.25 ±1.82[a]	62.58 ±2.07[a]
a*	4.42 ±0.35[a]	4.86 ±0.71[a]	5.16 ±0.87[a]
b*	11.33 ±0.44[a]	13.19 ±0.62[b]	13.51 ±0.27[b]
ΔE^*_{ab}	0[a]	2.06[a]	2.38[a]
pH1	6.40 ±0.08[a]	6.64 ±0.09[b]	6.47 ±0.12[a]
pH2	6.12 ±0.10[a]	6.38 ±0.11[b]	6.33 ±0.20[ab]

pH1 values were measured just after slaughter in breast, likewise pH2 values were measured after 1 hour post-mortem.
ΔE^*_{ab} is compared with control group.
[a,b] Means in a row within effect with no common superscript differ significantly (p <0.05).

of body weight to our results.

The highest non-significant difference (p >0.05) in thigh meat yield was observed in the MT15 group (15.21 ±0.37%) compared to the experimental groups. The manual for the hybrid Ross 308 **(Aviagen Group, 2014)** indicates a yield of leg meat 16.03% for 2,200 g of live weight.

Schiavone et al., (2007) performed an experiment with the addition of silymarin into feed mixture for broilers chickens. They found that the control group (without silymarin) achieved significantly highest carcass yield 75.04%. The breast muscle was reached highest weight (29.62%) in group with addition of 40 ppm of silymarin. And the leg muscle reached the highest yield (29.34%) in the group with addition of 80 ppm of silymarin. The lipid content of breast (1.19%) and thigh (3.81%) muscle was affected (p <0.05) by silymarin supplementation, and the lowest amount of lipid content was observed in group with 40 ppm of silymarin.

The chemical composition of breast and thigh muscles is shown in the Table 5. Differences between groups are not significant (p >0.05).

Chemical composition of breast and thight muscles of chickens was not found statistically significant differences. The breast meat of MT15 group contain the most of protein and minimum of fat. The nutrient composition of leg muscle is comparable across all three groups.

Table 7 Sensory analysis of breast meat (mm).

Group	C	MT5	MT15
	Mean ±standard error		
Sensory trait n	60	60	60
Odour	63.97 ±2.75[a]	70.80 ±1.75[a]	69.27 ±1.60[a]
Colour	73.18 ±1.45[a]	75.50 ±1.71[a]	77.40 ±1.39[a]
Fibreness	55.18 ±2.61[a]	56.43 ±2.38[a]	56.97 ±2.06[a]
Chewiness	62.75 ±2.51[a]	59.78 ±3.05[a]	58.60 ±2.20[a]
Juiciness	51.22 ±2.77[a]	44.03 ±3.08[a]	47.33 ±2.07[a]
Flavour	74.00 ±1.50[b]	65.02 ±3.02[a]	73.97 ±1.73[b]
Fatty taste	78.77 ±2.09[a]	81.08 ±1.39[a]	83.40 ±1.06[a]

[a,b] – different letters on one line - statistically significant differences (p <0.05).

Table 8 Sensory analysis of thigh meat (mm).

Group	C	MT5	MT15
	Mean ±standard error		
Sensory trait n	60	60	60
Odour	70.98 ±1.94[a]	69.07 ±1.92[a]	72.02 ±1.42[a]
Colour	50.08 ±1.19[a]	53.05 ±1.51[ab]	57.72 ±1.52[b]
Fibreness	56.67 ±1.39[a]	61.58 ±1.46[b]	65.55 ±1.24[b]
Chewiness	64.83 ±1.59[a]	65.47 ±1.60[a]	66.13 ±1.35[a]
Juiciness	66.90 ±1.97[a]	64.30 ±1.58[a]	67.70 ±1.54[a]
Flavour	74.25 ±1.87[a]	69.42 ±2.16[a]	72.78 ±1.72[a]
Fatty taste	76.35 ±2.56[a]	77.15 ±2.62[a]	79.13 ±2.21[a]

[a,b] – different letters on one line - statistically significant differences (p <0.05).

Texture, colour and pH of meat

The Razor Blade Shear Force results (n = 30) are shown in Table 7. The MT5 and MT15 groups had significantly (p <0.05) higher breast meat tenderness that the control group. Presented in Table 6.

The colour change is not significant in all coordinates (lightness L*, a* and b*), see Table 6. There were no significant differences between all three groups. However, compared with the control group, the yellowness (b*) was higher in both experimental groups. The total colour change (ΔE^*_{ab} from 1.5 to 3.0) is clearly perceptible but not yet discordant and it is acceptable for consumers **(Saláková, 2012)**.

The pH values from control group and groups with addition of *Silybum marianum* into feed is illustrated in Table 6. Initial pH1 was highest (p <0.05) in group with its middle addition of milk thistle seed cakes (MT5). Significant differences were not observed between control and group MT15. The highest pH decrease was noticed in control group breasts. pH2 values measured after 1-hour post mortem is obviously higher in MT5 and MT15 than control group. Between control and MT5 group was significant difference (p <0.05). Some authors **(Zhang et al., 2011, Salami et al., 2015)** confirm the slower post mortal process of muscle acidification on the grounds of various feeding supplementation, but still with acceptable sensory traits.

Sensory analysis

Breast meat was rated as the best in parameter flavour in control and MT15 group (p <0.05). The odour parameter was the best evaluated in MT5 group. Chewiness and juiciness were the best rated in the control group. The fibreness parameter was best rated in the group with the highest addition of milk thistle seed cakes (MT15). This data was not significant (p >0.05) (Table 7).

Some people prefer to consume leg meat of chickens, because it´s more fatty and therefore contains more of flavour substances **(Komprda et al., 2002)**. Table 8 shown sensory analyses of thigh meat. The leg meat was evaluated significantly (p <0.05) best for colour parameter in MT15 group. Fibreness was rated as the finest in MT15 group (p <0.05). The most typical flavour of chicken meat was evaluated in the control group and the chewiness parameter was the best in MT15. There are no significant differences.

Overall assessment of sensory analysis of breast and leg meat shows that the flavour was the best evaluated in the control group. The color and fibrous parameters of meat were the best in the MT15 group. The fatty taste was the lowest in the control group.

Cook and Homer (1996) classified chewiness, juiciness and flavour intensity as the important sensory traits in sensory analyses. This claim is also confirms **Poste et al., (1996)** who advise that flavour is one of the most important sensory traits.

CONCLUSION

The MT5 and MT15 group had significantly (p <0.05) higher breast meat tenderness that the control group. Overall, the total colour change of meat is not significant differences between all three groups. Initial pH1 was highest (p <0.05) in group with its middle addition of milk thistle seed cakes (MT5). Significant differences were not observed between control and group MT15. Breast meat was rated as the best in parameter flavour in control and MT15 group (p <0.05). The leg meat was evaluated significantly (p <0.05) best for colour parameter in MT15 group. Fibreness was rated as the finest in MT15 group (p <0.05).

In this study, the presence of milk thistle seed cakes at dose 5% and 15% in feed mixture were evaluated. The addition of this do not worsened sensory characteristic of breast or leg meat of broilers and reflects optimal sensory quality traits.

REFERENCES

Aviagen Group, 2014. Technological procedure for broiler Ross [online]. Aviagen Group 2014. [cit. 2015-12-02]. Available at: http://en.aviagen.com/ross-308.

Bobko, M., Lagin, L., Angelovičová, M., Bobková, A., Haščík, P. 2009. Vplyv prídavku fytoaditív na kvalitu kuracieho mäsa (Effect of phyto-additives addition on the chicken meat quality). *Potravinarstvo*, vol. 3, no. 2, p. 3-7.

Carreras, I., Castellari, M., Garcia Regueiro, J. A., Guerrero, L., Esteve-Garcia, E., Sarraga, C. 2004. Influence of enrofloxacin administration and alpha-tocopheryl acetate supplemented diets on oxidative stability of broiler tissues. *Poultry Science*, vol. 83, no. 5, p. 796-802. http://dx.doi.org/10.1093/ps/83.5.796 PMid:15141838

Cavitt, L. C., Meullenet, J. F., Xiong, R., Owens, C. M. 2005. The relationship of razor blade shear, Allo-Kramer shear, Warner-Bratzler shear and sensory tests to changes in tenderness of broiler breast fillets. *Journal of Muscle Foods*, vol. 16, no. 3, p. 223-242. http://dx.doi.org/10.1111/j.1745-4573.2005.00001.x

CIE. 2007. Colorimetry, part 4: CIE 1976 L*a*b* Colour space. CIE Central Bureau, Vienna, CIE DS 014-4.3/E:2007, 8 p.

Cook, G. L., Homer, D. B. 1996. The effect of number of sensory assessments on the accuracy of treatment comparisons in meat quality trials. *Food quality and Preference*, vol. 7, no. 2, p. 95-99. http://dx.doi.org/10.1016/0950-3293(95)00041-0

Comelli, M. C., Mengs, U., Prosdocimi, M., Schneider, C. 2007. Toward the definition of the mechanism of action of silymarin: Activities related to cellular protection from toxic damage induced by chemotherapy. *Integrative Cancer Therapies*, vol. 6, no. 2, p. 120-129. http://dx.doi.org/10.1177/1534735407302349 PMid:17548791

Ding, T. M., Tian, S. J., Zhang, Z. X., Gu, D. Z., Chen, Y. F., Shi, Y. H., Sun, Z. P. 2001. Determination of active component in silymarin by RP-LC and LC/MS. *Journal of Pharmaceutical and Biomedical Analysis*, vol. 26, no. 1, p. 155-161. http://dx.doi.org/10.1016/S0731-7085(01)00364-8

Drobná, Z., Zelenka, J., Mrkvicová, E., Kladroba, D. 2006. Influence of dietary lineseed and sunflower oil on sensory characteristics of rainbow trout (*Oncorhynchus mykiss*). *Czech Journal of Animal Science*, vol. 51, no. 11, p. 475-482.

Erdogan, Z., Erdogan, S., Celik, S., Unlu, A. 2005. Effects of ascorbic acid on cadmium-induced oxidative stress and performance of broilers. *Biological Trace Element Research*, vol. 104, no. 1, p. 19-31. http://dx.doi.org/10.1385/BTER:104:1:019

Gawel, A., Kotonski, B., Madej, J. A., Mazurkiewicz, M. 2003. Effect of silimarin on chicken and turkey broilers' rearing and the production indices of reproduction hen flocks. *Medycyna Weterynaryjna*, vol. 59, no. 6, p. 517-520.

Haščík, P., Trembecká, L., Bobko, M., Čuboň, J., Bučko, O., Tkáčová, J. 2015. Evaluation of meat quality after application of different feed additives in diet of broiler chickens. *Potravinarstvo*, vol. 9 no. 1, p. 174-182. http://dx.doi.org/10.5219/429

Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology*, vol. 70, no. 1, p. 1-9. http://dx.doi.org/10.1111/j.1751-1097.1999.tb01944.x

Jung, H., Kwak, H.-K., Hwang, K. T. 2014. Antioxidant and anti-inflammatory activities of cyanidin-3-glucoside and cyanidin-3-rutinoside in hydrogen peroxide and lipopolysaccharide treated RAW264.7 cells (830.23). *The Journal of the Federation of American Societies for Experimental Biology*, vol. 28, no. 1, p. 823-830.

Komprda, T., Zelenka, J., Drobná, Z., Jarošová, A., Fajmonová, E. 2003. Sensory quality of meat of turkeys fed the diet with sunflower, linseed or fish oil. *Archiv für Geflügelkunde*, vol. 67, no. 5, p. 225-230.

Luo, X., Fang, S., Xiao, Y., Song, F., Zou, T., Wang, M, Xia, M., Ling, W. 2012. Cyanidin-3- glucoside suppresses TNF-α-induced cell proliferation through the repression of Nox activator 1 in mouse vascular smooth muscle cells: involvement of the STAT3 signaling. *Molecular and Cellular Biochemistry*, vol. 362, no. 1, p. 211-218. http://dx.doi.org/10.1007/s11010-011-1144-3 PMid:22120492

Meullenet, J. F., Jonville, E., Grezes, D., Owens, C. M. 2004. Prediction of the texture of cooked poultry pectoralis major muscles by near-infrared reflectance analysis of raw meat. *Journal of Texture Studies*, vol. 35, no. 6, p. 573-585. http://dx.doi.org/10.1111/j.1745-4603.2004.35510.x

Miyazawa, T., Nakagawa, K., Kudo, M., Muraishi, K., Someya, K. 1999. Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, into rats and humans. *Journal of Agricultural and Food Chemistry*, vol. 47, no. 3, p. 1083-1091. http://dx.doi.org/10.1021/jf9809582 PMid:10552420

Opletal, L., Skřivanová, V. 2010. Přírodní látky a jejich biologická aktivita. Praha: Karolinum, p. 140-142. ISBN 978-80-246-1801-2.

Opletal, L., Šimerda, B. 2015. Flavanoidy ve výživě zvířat [online] s.a. [cit. 2015-12-05]. Avaliable at: http://www.vuzv.cz/sites/Flavanoidy(2).pdf.

Pojer, E., Mattivi, F., Johnson, D., Stockley, C. S. 2013. The case for anthocyanin consumption to promote human health: A review. *Comprehensive Reviews in Food Science and Food Safety*, vol. 12, no. 5, p. 483-508. http://dx.doi.org/10.1111/1541-4337.12024

Poste, L. M., Butler, G., Cave, N. A., Burrows, V. D. 1996. Sensory analysis of meat from broiler chickens fed diets containing hulless oats (Avena nuda). *Canadian Journal of Animal Science*, vol. 76, no. 3, p. 313-319.

Saláková, A. 2012. Instrumental measurement of texture and color of meat and meat products. *Maso International Brno*, vol. 2, no. 2, p. 107-114.

Salami, S. A., Majoka, M. A., Saha, S., Garber, A., Gabarrou, J. F. 2015. Efficacy of dietary antioxidants on broiler oxidative stress, performance and meat quality: science and market. *Avian Biology Research*, vol. 8, no. 2, p. 65-78.

Scazzocchio, B., Vari, R., Filesi, C., D'Archivio, M., Santangelo, C., Giovannini, C., Iacovelli, A., Silecchia, G., Li Volti, G., Galvano, F., Masella, R. 2011. Cyanidin-3-O-β-glucoside and protocatechuic acid exert insulin-like effects by upregulating PPAR activity in human omental adipocytes. *Diabetes*, vol. 60, no. 9, p. 2234-2244. http://dx.doi.org/10.2337/db10-1461 PMid:21788573

Schiavone, A., Righi, F., Quarantelli, A., Bruni, R., Serventi, P., Fusari, A. 2007. Use of Silybum marianum fruit extract in broiler chicken nutrition: influence on performance and meat quality. *Journal of animal physiology and animal nutrition*, vol. 91, no. 5-6, p. 256-262. http://dx.doi.org/10.1111/j.1439-0396.2007.00701.x PMid:17516949

Suchý, P., Straková, E., Kummer, V., Herzig, I., Písaříková, V., Blechová, R., Mašková, J. 2008. Hepatoprotective effects of milk thistle (Silybum marianum) seed cakes during the chicken broiler fattening. *Acta Veterinaria Brno*, vol. 77, no. 1, p. 31-38. http://dx.doi.org/10.2754/avb200877010031

Tedesco, D. 2001. The potentiality of herbs and plants extracts as feed additives in livestock production. *Zootecnica e Nutrizione Animale*, vol. 27, no. 3-4, p. 111-133.

Valous, N. A., Mendoza, F., Sun, D. W., Allen, P. 2009. Colour calibration of a laboratory computer vision system for quality evaluation of pre-sliced hams. *Meat Science*, vol. 81, no. 1, p. 132-141. http://dx.doi.org/10.1016/j.meatsci.2008.07.009 PMid:22063973

Wallace, T. C. 2011. Anthocyanins in cardiovascular disease. *Advances in Nutrition*, vol. 2, p. 1-7. http://dx.doi.org/10.3945/an.110.000042 PMid:22211184

Wei, X., Wang, D., Yang, Y., Xia, M., Li, D., Li, G., Zhu, Y., Xiao, Y. Ling, W. 2011. Cyanidin-3-O-β-glucoside improves obesity and triglyceride metabolism in KK-Ay mice by regulating lipoprotein lipase activity. *Journal of the Science of Food and Agriculture*, vol. 91, no. 6, p. 1006-1013. http://dx.doi.org/10.1002/jsfa.4275 PMid:21360538

Zahid, R., Durrani, F. R. 2007. *Biochemical, hematological, immunological and growth promotant role of feed added Milk Thistle (Silybum marianum) in broiler chicks* : M.Sc (Hons) thesis.Ssubmitted to NWFP Agricultural University Peshawar, Pakistan.

Zhang, W. G., Xiao, S., Lee, E. J., Ahn, D. U. 2011. Consumption of Oxidized Oil Increases Oxidative Stress in Broilers and Affects the Quality of Breast Meat. *Journal Of Agricultural And Food Chemistry*, vol. 59, no. 3, p. 969-974. http://dx.doi.org/10.1021/jf102918z PMid:21192702

Zelenka, J., Heger, J., Zeman, L. 2007. Recommended nutrient content in poultry diets and nutritive value of feeds for poultry (in Czech). Brno: MZLU. 77 p. ISBN 978-80-7375-091-6.

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DETERMINATION OF ASCORBIC ACID IN PHARMACEUTICAL PREPARATION AND FRUIT JUICE USING MODIFIED CARBON PASTE ELECTRODE

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ABSTRACT

Acrobic acid is key substance in the human metabolism and the rapid and accurate determination in food is of a great interest. Ascorbic acid is an electroactive compound, however poorly responded on the bare carbon paste electrodes. In this paper, brilliant cresyl blue and multi-walled carbon nanotubes were used for the modification of carbon paste electrode. Brilliant cresyl blue acts as a mediator improving the transition of electrons, whereas multiwalled carbon nanotubes increased the surface of the electrode. Both brilliant cresyl blue and multiwalled carbon nanotubes were added directly to the composite material. The electrochemical behavior of modified electode was determined in electrolyte at various pH, and the effect of the scan rate was also performed. It was shown that the electrochemical process on the surface of the modified carbon paste electrode was diffusion-controlled. The resulted modified carbon paste electrode showed a good electrocatalytic activity towards the oxidation of ascorbic acid at a reduced overpotential of +100 mV descreasing the risk of interferences. A linear response of the ascorbic acid oxidation current measured by the amperometry in the range of 0.1 – 350 μmol.L^{-1} was obtained applying the sensor for the standard solution. The limit of detection and limit of quantification was found to be 0.05 and 0.15 μmol.L^{-1}, respectively. The novel method was applied for the determination of ascorbic acid in pharmaceutical vitamin preparation and fruit juice, and the results were in good agreement with the standard HPLC method. The presented modification of carbon paste electrode is suitable for the fast, sensitive and very accurate determination of ascorbic acid in fruit juices and pharmaceutical preparation.

Keywords: ascorbic acid; carbon nanotubes; amperometry; brilliant cresyl blue

INTRODUCTION

Ascorbic acid (AA) is a significant vitamin in the diet of humans which prevents scurvy and takes part in several biological reactions **(Oguntibeju 2008)**. In recent studies, it has been found that ascorbic acid may be used as a supporting agent for the treatment of cancer **(Du et al., 2012)** and seems to be interesting in research of Alzheimer's disease **(Bowman 2012)**. However, AA cannot be synthesized by humans and must be supplied from various natural and prepared foods, drugs and physiological fluids, fruit juices, soft drinks and vegetables. For that reason, the determination of AA is very important for biological and agro-industry. At present, vitamin C is determined using widely different techniques including colorimetric and titrimetric measurement, UV spectrophotometry, as well as fluorimetric, chromatographic and other spectroscopic methods **(Ötleş and Karaibrahimoglu, 2012)**. The most common method for analysis of vitamin C is HPLC which is more accurate, selective and sensitive than other methods mention above. However, HPLC methods require specific equipments which are very expensive, difficult in monitoring and generally time-consuming. A need has arisen for a fast, sensitive and inexpensive method for the detection of AA. Recently, a portable strip used for the rapid determination of ascorbic acid has been described **(Kudrnáčová a Kouřimská, 2015)**. The electrochemical determination is one of the approaches as was described in literature **(Skrovankova et al., 2015; Pisoschi et al., 2014)**. Research and development in amperometric sensors for the determination of AA based on the carbon paste composite material have gained increasing importance in the last few years for their advantageous properties as analytical tools, namely the easy of application, lower cost, providing direct, sensitive and fast detection of AA, in comparison with well-established, lab-based methods **(Weng et al., 2013; Li et al., 2011; Huang et al., 2014; Heli and Sattarahmedy 2015; Chang et al., 2014)**. The carbon paste electrodes have attractive advantages, such as simple preparation, low-cost implementation, renewability, low background current, and wide potential window **(Švancara et al., 2012)**. Carbon nanotubes (CNTs) modified CPE have been applied in many studies due to the unique properties of CNTs such as large active surface area, high electronic conductivity, anti-fouling capability and their ability to reduce over potential **(Jacobs et al., 2010; Huang et al., 2014; Bijad et al., 2014)**.

Brilliant cresyl blue (BCB) is a cationic quinine-imide dye with a planar and rigid structure which has been proven to possess promising properties as a redox catalyst. BCB can absorb strongly on the electrode surfaces and these chemically modified electrodes have been used for the determination of various organic compounds **(Lin et al., 2012; Ding et al., 2016; Shaikh et al., 2013)**. The

reduction of BCB by ascorbic acid has been described **(Ulusoy et al., 2011)** therefore we chose this organic dye as a suitable redox mediator for amperometric determination of ascorbic acid.

MATERIAL AND METHODOLOGY

Reagents and equipment

All the reagents were purchased from Sigma-Aldrich (Czech Republic). Deionized water was used in this study (G ≤0.055 μS). Dissolved oxygen was removed from all the solutions by purging with argon for 5 min (purity 99.99%, Linde Technoplyn, Prague, Czech Republic).

A solution of ascorbic acid (10^{-2} mol.L^{-1}) was daily prepared in deionized water and was kept in a dark bottle during the experiments. Briton Robinson (0.04 M, B-R) buffer solution containing 0.1 M KCl was used as a supporting electrolyte.

A three electrode system consisting of CPEs (working), Ag/AgCl/3.0 M KCl (reference) and platinum wire (counter electrode) connected to PalmSens (Ivium Technologies, Netherland) was used for electrochemical measurement. The surfaces of CPE were regenerated by renewing and polishing them on wet filter paper before each measurement.

Preparation of CPE

Both bare and modified electrodes were prepared by mixing of 0.5 g of graphite powder 5.5 – 7.0 μm (CR-5, Maziva Týn n. L., s.r.o., Czech Republic) with 130 μL of mineral oil (M5904, Sigma-Aldrich, Germany). The modified CPE electrode contained 1.0% of multiwalled carbon nanotubes (40 – 60 nm, Shenzhen NanoTech Port Co., China) (MWCNTs), and 3.0% of brilliant cresyl blue powder (BCB). The resulting paste was packed into the Teflon piston holder (3.0 mm inner diameter) (**Švancara and Metelka 2000)**. The resistance of the composite material was always ≤15.0 Ω.

Electrochemical procedure

The effect of modifiers in carbon paste was investigated using cyclic voltammetry in 0.04 M Britton-Robinson buffer solution (B-R) at the pH 5.0 containing 0.1 M KCl at a scan rate of 50 mV.s^{-1} in the range of potentials from -400 mV to +1000 mV. The influence of pH between 3.0 and 9.0 was also investigated at the scan rate of 50 mV.s^{-1} in the same potential window. In order to study the effect of the scan rate on the peak potentials and peak currents, the cyclic voltammograms of the BCB-MWCNTs/CPE were recorded at different scan rates in the potentials ranged from -300 to 800 mV in B-R buffer solution at the pH 5.0 containing 0.1 M KCl.

The amperometric detection of ascorbic acid based on its electrocatalytic oxidation was studied by using BCB-MWCNTs/CPE. Aliquots of a stock solution of AA were added to the supporting electrolyte solution (B-R buffer solution at the pH 5.0 plus 0.1M KCl) after the background current reached a steady state value at an applied potential +100 mV.

Sample preparation

The proposed method was applied for the determination of AA in pharmaceutical sample (tablet, Celaskon 250 mg, Zentiva, Czech Republic) and fruit juice sample (Toma Juice Multivitamin, Pepsico CZ s.r.o., Czech Republic). The tablet (15 mg) was dissolved in deionized water, transferred to a 250 mL volumetric flask, and then was diluted by deionized water until the mark. Juice sample was used directly without any treatment. The amount of ascorbic acid was also determined using HPLC/UV (LC-10AD, Shimadzu Co., Japan) equipped with column LiChrospher RP-18e (250 × 4 mm, 5 μm). A mixture of 250 μL of dilute sample and 50 μL of internal standard (isoascorbic acid in metaphosphoric acid) was vigorously shaken following by centrifugation (13.000 rpm, 5 min). The supernatant (5 μL) was injected into the mobile phase (sodium phosphate/phosphoric acid, pH 2.0, flow rate 1 mL.min^{-1}) with detection wavelength at 263 nm.

Student´s t-test was used for determination of statistical differences between results at the probability level $p = 0.05$ (Origin Pro v. 9, OriginLab Corp., USA).

RESULTS AND DISCUSSION

Electrochemical behavior of BCB-MWCNTs/CPE

As can be seen from Figure 1, an anodic and cathodic peak was observed at +125 mV and +75 mV, respectively. The formal potential of the redox process was +100 mV, and a peak-to-peak separation was +50 mV. The reverse-to-forward current peak ration was approximately unity, which reflects the reversible electrochemical behavior of BCB.

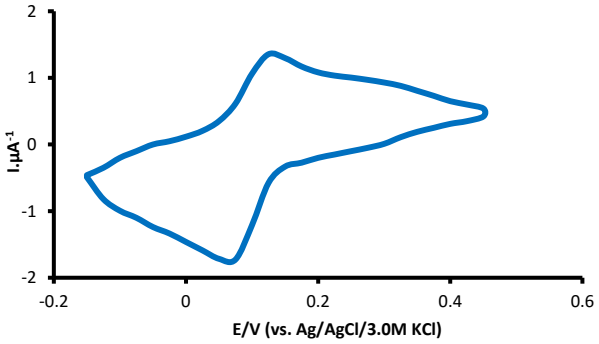

Figure 1 Cyclic voltammograms of BCB-MWCNTs/CPE in the absence of ascorbic acid in B-R buffer solution (pH 5.0) containing 0.1M KCl at the scan rate 50 mV.s^{-1}. Potential range from -150 to +450 mV, potential step 25 mV.

The electrochemical behavior of BCB-MWCNTs/CPE was investigated by recording of cyclic voltammograms in a B-R buffer solution at the pH 3.0 containing 0.1M KCl at various scan rates. The anodic (I_{pa}) and cathodic (I_{pc}) peak currents were proportional to the scan rates (ν) in the range from 10 to 100 mV.s^{-1}. The equations and the regression coefficients were found to be: $I_{pa} = 0.11v^{1/2} + 0.02$ ($R^2 = 0.998$) and $I_{pc} = -0.11v^{1/2} + 0.04$ ($R^2 = 0.994$), respectively.

These results indicate that the electrochemical process on the BCB-MWCNTs/CPE is diffusion-controlled. The shifting of the potentials was not observed between 10 and 100 mV.s^{-1}.

In order to study the effect of pH on the electrochemical behavior of BCB-MWCNTs/CPE, B-R buffer with the pH ranged from 3.0 to 9.0 as a supporting electrolyte was used

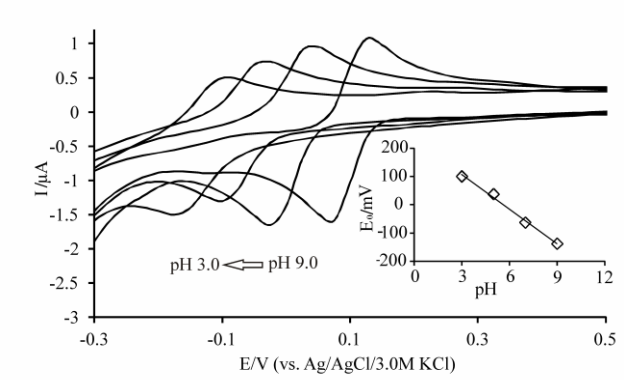

Figure 2 Cyclic voltammograms of BCB-MWCNTs/CPE in B-R buffer solutions (pH 3.0, 5.0, 7.0 and 9.0) containing 0.1M KCl, scan rate 50 mV.s^{-1}, potential range from -500 to +900 mV. Inset: Effect of formal potential (E_0) of BCB-MWCNTs/CPE on the pH.

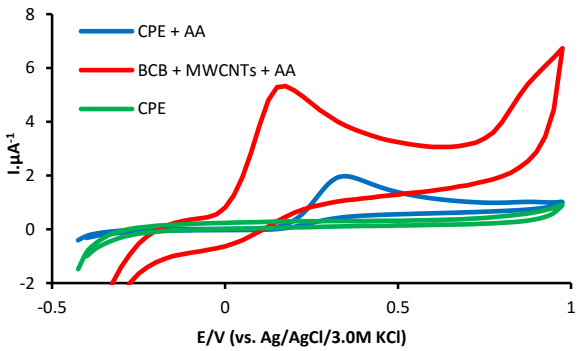

Figure 3 Cyclic voltammograms of BCB-MWCNTs/CPE and CPE in the presence of 250 μmol.L^{-1} of ascorbic acid in B-R buffer (pH 5.0) containing 0.1M KCl, scan rate 50 mV.s^{-1}, potential step 25 mV. Bare CPE (green) in buffer solution served as control.

for determination of cyclic voltammograms of BCB-MWCNTs/CPE at the scan rate of 50 mV.s^{-1}. It was seen that the pH significantly influenced the behavior of BCB-MWCNTs/CPE, since the shape and the position of the peak for redox pair was better in acidic conditions (3.0 and 5.0) in comparison with those obtained in the pH 7.0 and 9.0 (Figure 2).

The inset in Fig. 2 shows the formal potential (E^0) of the BCB-MWCNTs/CPE plotted against pH in the range from 3.0 to 9.0. It shows a slope -40.63 mV.pH^{-1}, which is close to that given by the Nernstian equation for equal number of electrons and protons transfer process. Moreover, the both oxidation and reduction currents proportionally decreased with increasing pH. The electrochemical behavior has been documented in electrodeposited BSB film on the surface of multi-walled carbon nanotubes modified glassy carbon electrode **(Lin et al., 2012)**.

Ascorbic acid oxidation at BCB-MWCNTs/CPE

Cyclic voltammograms of BCB-MWCNTs/CPE were recorded both in the absence and presence of ascorbic acid in order to study the electrocatalytic activity of modify electrode towards AA oxidation. As shown in Figure 3, anodic and cathodic peaks were obtained at +125 and 75 mV, respectively. Oxidation of AA in the bare CPE and BCB-MWCNTs/CPE gave peak potentials at about 350 and 150 mV, respectively. The overpotential for AA oxidation was found to shift by about 200 mV.

The modification of CPE by each modifier separately was also investigated. The oxidation peak potential of AA using CPE with 1.0% of MWCNTs shifted to more negative potentials by about +50 mV with increasing (p *<0.05*) of oxidation current of AA to 3.06 ±0.02 μA in comparison with that obtained in bare CPE (2.13 ±0.02 μA). On the other hand, the separate addition of 3.0% of BCB to CPE resulted in similar oxidation current of AA (2.91 ±0.05 μA) but more progressive shift of the peak potential to +175 mV was observed. It confirms that MWCNTs just increased the electroactive surface area of the CPE electrode (with slight increase of overpotential) whereas BCB acts as a redox mediator. This result clearly indicates that BCB-MWCNTs/CPE in B-R buffer solution (pH 5.0) containing 0.1M KCl exhibited a significant supporting electrocatalytic activity towards AA oxidation. In the study of **Zhang et al., (2013)**, the film of poly(bromcresol purple) at glassy carbon electrode also showed good electrocatalytic effect towards AA oxidation with reduced the oxidation overpotential for about 240 mV with increasing current. In view of oxidation current and the targeted analyte (AA), pH 5.0 was chosen as the best buffer since it offered a relatively high oxidation current and a low oxidation potential.

Various values of applied potential from -50 to +400 mV were used for the amperometric detection of AA. The oxidation current of AA exhibited steep increase from -50 to 100 mV. An applied potential is considered suitable when it offers relatively high oxidation current and low oxidation peak potential. The applied potential +100 mV was chosen for the amperometric determination of AA at BCB-MWCNTs/CPE since it gave the highest current (4.86 ±0.01 μA) compared with that obtained from the lower applied potentials. Besides, using low applied potential can avoid the interferences of some compounds from the matrices. Figure 4 shows the current-time curves for the amperometric responses at various concentration of AA.

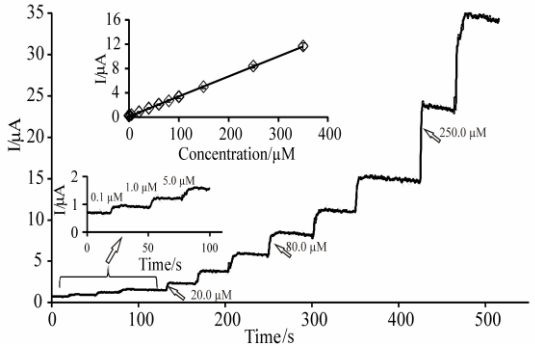

Figure 4 Amperometric current-time curves of AA with various concentrations (μM) using BCB-MWCNTs/CPE. Inlet: the plot of maximum oxidation current vs. concentration of AA. B-R buffer solution at pH 5.0, constant potential +100 mV, stirring speed 400 rpm. The vertical bars represent standard deviation (n = *6*).

Table 1 Comparison of carbon paste electrode (CPE) based electrochemical sensors for amperometric determination of ascorbic acid.

Electrode modification	Applied potential (mV)	LOD ($\mu mol.L^{-1}$)	Dynamic range ($\mu mol.L^{-1}$)	Reference
AF-MWCNT/EPI[1]	+420	4.1	NA	**Huang et al., 2014**
Gold decorated SiO_2@PANI[2] core-shell microsphere	+400	3.8	150.0-8000.0	**Weng et al., 2013**
Graphene doped CPE	+310	0.07	0.1-106.0	**Li et al., 2011**
Graphene oxide CoHCF[3] nanocomposite	+430	0.29	2.5-62.5	**Heli and Sattarahmady 2015**
trans-PEPACC[4]	+450	2.27	0-550	**Chang et al., 2014**
BCB/MWCNT[5]	+100	0.05	0.1-350.0	This work

[1] amino-functionalized multi-walled carbon nanotube electroactive polyamide, [2] polyaniline, [3] cobalt hexycyanoferrate. [4] photoactive and electroactive azo-based polyimide/amino-functionalized multiwalled carbon nanotubes, [5] briliant cresyl blue/muti-walled carbon nanotube.

Table 2 Ascorbic acid content ($mg.L^{-1}$) in real sample using BCB-MWCNTs/CPE and standard HPLC method (n = 20).

Sample	Amperometric method	HPLC method
Juice	120.1 ±5.2*	120.5 ±4.7
Tablet	25.8 ±0.5	25.2 ±0.4

* standard deviation

A linear relationship between the AA concentration and the peak oxidation current was obtained over the concentration range $1 \times 10^{-7} – 3.5 \times 10^{-4}$ $mol.L^{-1}$ (Figure 4, inlet) with equation:

$$I_{pa}\ (\mu A) = 0.033 \times c\ (\mu mol.L^{-1}) + 0.166\ (R^2 = 0.993)$$

The repeatability of the method was investigated by amperometric measurements of 5 $\mu mol.L^{-1}$ and 300 $\mu mol.L^{-1}$ of AA (n = 20) and the relative standard deviation (RSD) was found to be 4.5% and 5.2%, respectively. The limit of detection (LOD) using the equation $LOD = 3s_b/m$, where s_b is the standard deviation of the blank response and m is the slope of the calibration plot, was found to be 5.0×10^{-8} $mol.L^{-1}$. The limit of quantification (LOQ) using the equation $LOQ = 10s_b/m$ was found to be 1.5×10^{-7} $mol.L^{-1}$ (signal/noise = 10). Various modified carbon paste electrodes used for ascorbic acid determination were compared (Table 1). Among amperometric sensors based on CPE, our modification allowed the detection of AA at lower applied potential avoiding the interference species. BCB-MWCNTs/CPE electrochemical sensor is proven to be extremely sensitive, simple renewable and easy in preparation and storage.

After 45 days of storage at room temperature, the amperometric responses of AA at the concentration of 5.0 $\mu mol.L^{-1}$ was not significantly ($p > 0.05$) different in comparison with those obtained using the freshly prepared electrode. The low detection limit is an advantageous character of BCB-MWCNTs/CPE compared with the most CPE modified electrodes mentioned in Table 1. BCB-MWCNTs/CPE is also applicable for the analysis of AA in fruit juices and pharmaceutical preparations. As described in Table 2, the contents of AA in real samples did not differ from those determined by HPLC method.

CONCLUSION

This study demonstrated that BCB-MWCNTs/CPE showed significant electrocatalytic activity towards the oxidation of AA. It was observed that the oxidation peak potential of AA shifted from +375 mV at CPE to +100 mV at BCB-MWCNTs/CPE together with increasing of the oxidation current. The electrocatalytic activity of BCB-MWCNTs/CPE was investigated to detect AA by amperometry with a good linearity and sensitivity. We may conclude that BCB-MWCNTs/CPE represents a steady electrode material for electrocatalytic oxidation of AA.

REFERENCES

Bijad, M., Karimi-Malch, M., Khalilzadech, M. A. 2013. Application of ZNo/CNTs nanocomposite ionic liquid paste electrode as a sensitive voltammetric sensor for determination of ascorbic acid in food samples. *Food Analytical Methods*, vol. 6, p. 1639-1647. http://dx.doi.org/10.1007/s12161-013-9585-9

Bowman, G. L. 2012. Ascorbic acid, congitive function, and Alzhaimer´s disease: a current review and future direction. *Biofactors*, vol. 38, no. 2, p. 114-122. http://dx.doi.org/10.1002/biof.1002 PMid:22419527

Chang, K. C., Chu, C. M., Chang, C. H., Cheng, H. T., Hsu, S. C., Lan, C. C., Chen, H. H., Peng, Y. Y., Yeh, J. M. 2014. Photoisomerization of electroactive polyimide/multiwalled carbon nanotube composites on the effect of electrochemical sensing for ascorbic acid. *Polymer International*, vol. 64, no. 3, p. 373-382. http://dx.doi.org/10.1002/pi.4797

Ding, M., Yhou, Y., Liang, X., Zou, H., Wang, Z., Wang, M., Ma, J. 2016. An Electrochemical Sensor Based On Graphene/Poly(Brilliant Cresyl Blue) Nanocomposite For Determination Of Epinephrine. *Journal of Electroanalytical Chemistry*, vol. 763, p. 25-31. http://dx.doi.org/10.1016/j.jelechem.2015.12.040

Du, J., Cullen, J. J., Buettner, G. R. 2012. Ascorbic acid: Chemistry, biology and the treatment of cancer. *Biochimica et Biophysica Acta (BBA) – Reviews on Cancer*, vol. 1826, no. 2, p. 443-457.

Heli, H., Sattarahmedy N. 2015. Amperometric determination of ascorbic acid in pharmaceutical formulations by a reduced graphene oxide-cobalt hexacyanoferrate nanocomposite. *Iranian Journal of Pharmaceutical Research*, vol. 14, no. 2, p. 453-463. PMid:25901152

Huang, T. C., Yeh, L. C., Huang, H. Y., Nian, Z. Y., Yeh, Y. C., Chou, Y. C., Yeh, J. M., Tsai, M. H. 2014. The use of a

carbon paste electrode mixed with multiwalled carbon nanotube/electroactive polyamide composites as an electrode for sensing ascorbic acid. *Polymer Chemistry*, vol. 5, p. 630-637. http://dx.doi.org/10.1039/C3PY00787A

Jacobs, C. B., Peairs, M. J., Venton, B. J. 2010. Review: Carbon nanotube based electrochemical sensors for biomolecules. *Analytica Chimica Acta*, vol. 662, no. 2, p. 105-127. http://dx.doi.org/10.1016/j.aca.2010.01.009 PMid:20171310

Kudrnáčová, E., Kouřimská L. 2015. Qualitative parameters of non-traditional types of vegetables – determination of nitrates and ascorbic acid content. *Potravinarstvo*, vol. 9, no. 1, p. 237-241. http://dx.doi.org/10.5219/466

Li, F., Li. J., Feng, Y., Yang, L., Du, Z. 2011. Electrochemical behavior of graphene doped carbon paste electrode and its application for sensitive determination of ascorbic acid. *Sensors and Actuators B: Chemical*, vol. 157, no. 1, p. 110-114. http://dx.doi.org/10.1016/j.snb.2011.03.033

Lin, K. C., Huang, J. Y., Chen, S. M. 2012. Poly(brilliant cresyl blue) electrodeposited in multi-walled carbon nanotubes modified electrode and its application for persulfate determination. *International Journal of Electrochemical Science*, vol. 7, p. 9161-9173.

Oguntibeju, O. O. 2008. The biochemical, physiological and therapeutic roles of ascorbic acid. *African Journal of Biotechnology*, vol. 7, no. 25, p.4700-4705.

Ötleş, S., Karaibrahimoglu, S. 2012. Analysis of vitamins for health, pharmaceutical, and food sciences. In Ötleş, S. *Methods of Analysis of Food Components and Additives*. Boca Raton, Florida, CRC Press, p. 211-230. ISBN: 978-1-4398-1552-6.

Pisoschi, A. M., Pop, A., Serban, A. I., Fafaneata, C. 2014. Electrochemical methods for ascorbic acid determination. *Electrochimica Acta*, vol. 121, p. 443-460. http://dx.doi.org/10.1016/j.electacta.2013.12.127

Shaikh, A. A., Saha, S. K., Bakshi, P. K., Hussain, A., Ahammad, A. J. S. 2013. Poly(Brilliant Cresyl Blue)-Modified Electrode For Highly Sensitive And Simultaneous Determination Of Hydroquinone And Catechol. *Journal of the Electrochemical Society*, vol. 160, no. 4, p. B37-B42. http://dx.doi.org/10.1149/2.008304jes

Skrovankova, S., Mlcek, J., Sochor, J., Baron, M., Kynicky, J., Jurikova, T. 2015. Determination of ascorbic acid by electrochemical techniques and other methods. *International Journal of Electrochemical Science*, vol. 10, p. 2421-2431.

Švancara, I., Kalcher, K., Walcarius, A., Vytras, K. 2012. *Electroanalysis with carbon paste electrodes*. BOCA RATON, FL: CRC Press. ISBN: 978-1-4398-3019-2.

Švancara, I., Metelka, R. 2000. Piston-driven carbon paste holders for electrochemical measurements. In Vytřas, K., Kalcher, K. *Sensing in Electroanalysis*, vol. 1. Pardubice: University of Pardubice, p. 7- 8. ISBN: 80-7194-831-4.

Ulusoy, H. I., Gürkan, R., Akçay, M. 2011. Kinetic spectrophotometric determination of trace copper(II) ions by their catalytic effect on the reduction of brilliant cresyl blue by ascorbic acid. *Turkish Journal of Chemistry*, vol. 35, p. 599-612.

Weng, C. J., Chen, Y. L., Chien, C. L., Hsu, S. C., Jhuo, Y. S., Yeh, J. M., Dai, C. F. 2013. Preparation of gold decorated SiO2@polyaniline core–shell microspheres and application as a sensor for ascorbic acid. *Electrochimica Acta*, vol. 95, p. 162-169. http://dx.doi.org/10.1016/j.electacta.2013.01.150

Zhang, R., Liu, S., Wang, L., Yang, G. 2013. Electroanalysis of ascorbic acid using poly(bromcresol purple) film modified glassy carbon electrode. *Measurement*, vol. 46, no. 3, p. 1089-1093. http://dx.doi.org/10.1016/j.measurement.2012.11.007

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ADSORPTIVE STRIPPING VOLTAMMETRY IN LIPOPHILIC VITAMINS DETERMINATION

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ABSTRACT

The aim of this contribution was to check if adsorptive stripping differential pulse voltammetry (AdSDPV) is suitable tool for sensitive simultenous electrochemical detection of lipophilic vitamins. Retinol (vitamin A_1), cholecalciferol (vitamin D_3), *α*-tocopherol (vitamin E) and phylloquinone (vitamin K_1) were selected as representatives. All electrochemical measurements were performed in two separate steps due to the lipophilic character of the analytes. In the first step, an accumulation of lipophilic vitamin on the surface of glassy carbon electrode (GCE) was done by immersing working electrode into the aqueous-acetonitrile solutions (50%, *v/v*) of each vitamin (50.0 μmol.L^{-1}) at 400 rpm for 5 min. In the second one, differential pulse voltammetry of accumulated vitamins was performed in 0.01 mol.L^{-1} acetate (pH 4.5) buffer at potential step (E_{step}) 5 mV, potential of amplitude (E_{ampl}) 25 mV, interval time (t) 0.1 s and scan rate (v) 50 mV.s^{-1}. It was observed that electrochemical behaviour of lipophilic vitamins adsorbed on surface of solid GCE in the aqueous electrolyte was very similar to those performed in organic/aqueous electrolyte in literature. Due to reversible electrochemical behaviour of vitamin K_1 (phylloquinone/phyllohydroquinone redox couple), it was possible to detect all lipophilic vitamins only in one analysis. Observed values of peak potentials (E_p) were sufficiently different for their recognition which was confirmed by the analysis of real sample. The results obtained in this study showed that simultaneous determination of some lipophilic vitamins is possible requiring further optimization study. For this reason, it is necessary to understand this work as an initial step in simultaneous determination of lipophilic vitamins without application of any chromatographic technique.

Keywords: lipophilic vitamin; glassy carbon electrode; adsorptive voltammetry; margarine analysis

INTRODUCTION

It is known that lipophilic vitamins are nonpolar organic compounds essential for proper functioning of the human metabolism which have to be received through diet **(Cockburn, 2003)**. Thus, their detection and quantification in different kinds of samples in a great importance in nutrition, medicine, cosmetics and food technology **(Gonnet et al., 2010)**. Unfortunately, analysis of lipophilic vitamins is quite complicated and time consuming due to their lipophilic character. The main disadvetage is the use of organic solvents.

The determination of lipophilic vitamins is not practically possible without using chromatographic techniques, especially by high performance liquid chromatography (HPLC) followed by extraction of lipophilic vitamins into organic solvent. It is necessary to remind that HPLC analysis of fats may take up to several hours.

All lipophilic vitamins contain conjugated system of double bonds in their structures, therefore a normal-phased HPLC with combination of UV detection is common way of their determination **(Kamal-Eldin et al., 2000)**. Moreover, they were also determined in human serum by reversed-phase HPLC with electrochemical detection **(Wang et al., 2001)**.

Generally, lipophilic vitamins are classified in four main groups **(Webster, 2012)**. In our experiment, the most biologically active forms (all-trans-retinol; vitamin A_1, cholecalciferol; vitamin D3, α-tocopherol, vitamin E and phylloquinone, vitamin K_1) were selected as standards to explore if an adsorptive stripping differential pulse voltammetry (AdSDPV) is suitable electrochemical method for their sensitive simultanenous detection in model sample and selected margarine.

Adsorptive stripping voltammetry (AdSV) is similar to anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV) with the preconcentration step being not controlled by electrolysis **(Wang, 1990)**. In our case, the preconcentration step is controlled by adsorption of analytes on solid glassy carbon electrode (GCE). Their electrochemical detection was performed by differential pulse voltammetry (DPV) which is the most commonly used electrochemical technique for simultaneous determinations **(Baranowska et al., 2008)**.

For comparison, the declared contents of lipophilic vitamins in selected traditional Czech margarines are shown in Table 1. Contents of all present lipophilic vitamins were only copied from nutrition facts of corresponding labels. Additionally, it was observed that tested margarines always contained a mixture of several plant (palm, sunflower and rapeseed) oils whose volume ratios were not surprisingly listed.

Table 1 Declared contents of lipophilic vitamins in several traditional Czech margarines.

Margarines (types)	*Vitamin A (μg/100g)*	*Vitamin D (μg/ 100g)*	*Vitamin E (mg/ 100g)*
Flora light	*800*	*7.5*	*10*
Flora gold	*800*	*7.5*	*—*
Flora original	*800*	*7.5*	*14*
Flora pro-active	*800*	*7.5*	*11*
Perla plus vitamíny	*800*	*7.5*	*—*
Perla tip	*800*	*7.5*	*—*
Rama classic	*800*	*7.5*	*9.2*
Stella	*800*	*3.5*	*—*

It is evident that concentration levels of present lipophilic vitamins are mutually very different (~10 mg E, ~1 mg A and ~0.01 mg D). Therefore, it can be assumed that simultaneous electrochemical determination of lipophilic vitamins in real samples, especially in margarines, remains a challenge for further scientific research.

MATERIAL AND METHODOLOGY

Standards of lipophilic vitamins

Vitamin A_1 as retinol (crystalline), vitamin E as (+)-*α*-tocopherol (from vegetable oil; 1000 $IU.g^{-1}$), vitamin K_1 as phylloquinone (viscous liquid) and acetonitrile (ACN) of HPLC purity (99.8%) were purchased from Sigma Aldrich (Vieanna, Austria). Vitamin D_3 as cholecalciferol (40×10^6 $IU.g^{-1}$; crystalline) was obtained from Merk (Darmstadt, Germany).

Instrumentation

All electrochemical measurements were carried out at conventional three-electrode system consisting solid GCE with surface diameter 2 mm from, Ag/AgCl and 3.0 $mol.L^{-1}$ KCl as salt bridge (reference) and platinum wire (auxiliary) electrode which were together connected to potentiostat Autolab PGSTAT101 from Metrohm (Prague, Czech Republic) which is also compatible with software Nova (Prague, Czech Republic).

Pretreatment of glassy carbon electrode

Surface of solid GCE was renovated by polishing pad with presence of wet Al_2O_3 powder for 30 s. After subsequent rinsing of the surface by distilled water, the GCE was ready for new electrochemical experiment.

Sample preparation

The sample preparation is consisted only by dissolving of 2 g margarine type "Perla plus vitamíny" from UNILEVER ČR, spol. s r.o. (Prague, Czech Republic) in pure ACN and filled to the mark of 50 mL volumetric flask.

Procedure

Adsorptive stripping voltammetry of lipophilic vitamins was performed in two separate steps. In the first step, the analytes adsorption on GCE surface was done by immersing working electrode in aqueous-acetonitrile solutions (50% content of ACN) containing 50 $\mu mol.L^{-1}$ of each vitamin 10 min at 400 rpm. In second one, repettive CV of accumulated lipophilic vitamins in 0.01 $mol.L^{-1}$ acetate (pH 4.5) buffer was done to examine their electrochemical behaviours at potential step (E_{step}) 5 mV, scan rate (v) 50 $mV.s^{-1}$ and fivecycles repetition.

Analogically, DPV of accumulated vitamins (100 $\mu mol.L^{-1}$ of each vitamin in 25% ACN at 400 rpm for 10 min) was performed in 0.01 $mol.L^{-1}$ acetate (pH 4.5) buffer with deposition potential (E_{dep}) –0.6 V for 120s, potential step (E_{step}) 5 mV, potential of amplitude (E_{ampl}) 25 mV, interval time (t) 0.1 s and scan rate (v) 50 $mV.s^{-1}$.

RESULTS AND DISCUSSION

Cyclic voltammetry of accumulated vitamins

Electrochemistry of retinol (vitamin A) film deposited on GCE surface

Vitamin A1 deposited on surface GCE provided only one sensitive oxidation peak at +0.708 V whose current response dramatically decreased with the number of cycles. For demonstration, typical repetitive CV of vitamin A1 accumulated at GCE in acetate buffer is shown in Figure 1.

Similar electrochemical behaviour was observed at GCE in a methanol/acetate (pH 5.0) buffer at scan rate 50 $mV.s^{-1}$ **(Wring et al., 1988)** which corresponds to irreversible electrochemical oxidation of retinol to the retinaldehyde with participation of two protons and electrons **(Ziyatdinova et al., 2010)**.

However, it is important to note that a background

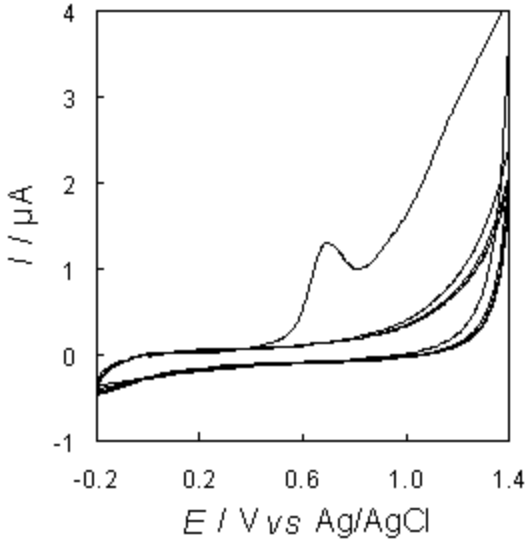

Figure 1 Repetitive cyclic voltammetry of vitamin A_1 (50.0 $\mu mol.L^{-1}$) in 0.01 $mol.L^{-1}$ acetate (pH 4.5) buffer at v = 50 $mV.s^{-1}$.

current increased after oxidation of vitamin A1, probably due to adsorption of the oxidized products. Unfortunately, this phenomenon can negatively affect an electrochemical detection of other lipophilic vitamins which could be oxidized at higher values of potential than present vitamin A1.

Electrochemistry of cholecalciferol (vitamin D_3) film deposited on GCE surface

Electrochemically similar behaviour as in previous situation was observed also for vitamin D3 which also provided only one oxidation peak at +1.032 V which was not visible under following repetitions. According to obtained cyclic voltammogram shown in Figure 2, the oxidation process of cholecalciferol appeared to be irreversible. In fact, the same electrochemical behaviour has been obtained at GCE in in a methanol/acetate (pH 6.0) buffer at scan rate 50 $mV.s^{-1}$ **(Hart et al., 1992)**.

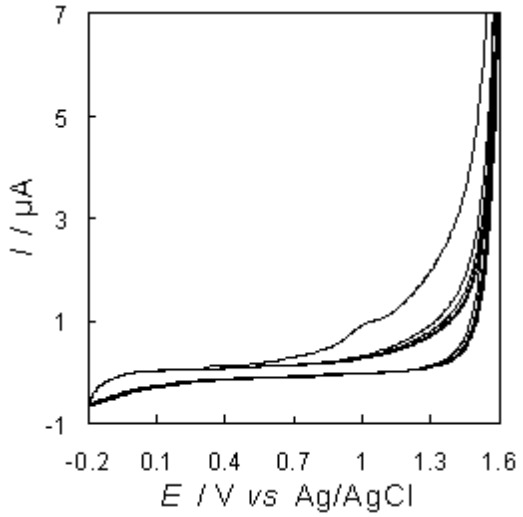

Figure 2 Repetitive cyclic voltammetry of vitamin D_3 (50.0 $\mu mol.L^{-1}$) in 0.01 $mol.L^{-1}$ acetate (pH 4.5) buffer at v = 50 $mV.s^{-1}$.

Electrochemistry of α-tocopherol (vitamin E) film deposited on GCE surface

Thin layer electrochemistry of α-tocopherol (α-TOH), known as the most active form of vitamin E in aqueous electrolytes was investigated resulting in formation of lipid multilayer **(Yao et al., 2009)** or modification of carbon paste **(Kim and Kusuda, 1994)**. Electrochemical behaviour of α-TOH deposited on surface of solid GCE in aqueous electrolytes was also published by our research group **(Sýs et al., 2016)**.

Electrochemistry of phylloquinone (vitamin K_1) film deposited on GCE surface

In comparison to previous measurements, cyclic voltammetry of vitamin K_1 always began with cathodic scan due to content of quinone unit in its structure **(Wang et al., 1994)**. Thus, the electrochemical behaviour of vitamin K_1 was very similar to redox couple quinone/hydroquinone. According to Figure 3, the vitamin K_1 provided typical two reversible electrochemical peaks at –0.325 and –0.006 V. Moreover, another sensitive cathodic peak was observed at –0.832 V.

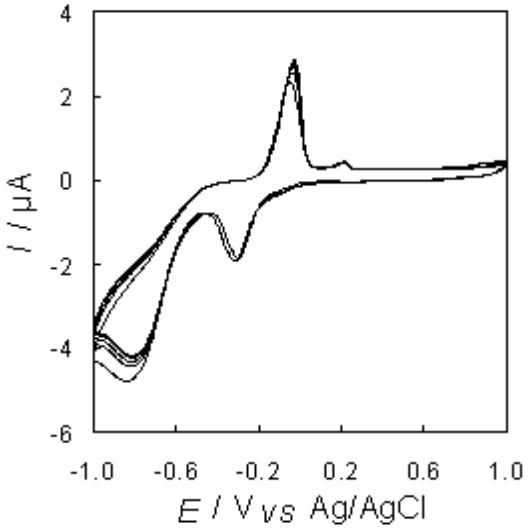

Figure 3 Repetitive cyclic voltammetry of vitamin K_1 (50.0 $\mu mol.L^{-1}$) in 0.01 $mol.L^{-1}$ acetate (pH 4.5) buffer at v = 50 $mV.s^{-1}$.

Simultenous differential pulse voltammetry of accumulated vitamins

Form the previous section, it states that only vitamin K_1 can not be electrochemically oxidized because it usually occurs in its oxidation form as naphthoquinone with long alkyl chain. Based on this finding, it was necessary to reduce the phylloquinone to phyllohydroquinone with participation of two protons and electrons. Additional lipophilic vitamins accumulated together with phylloquinone on surface of working electrode were present in their corresponding reduction forms. Therefore, applying of deposition potential –0.6 V for 120 s did not cause any electrochemical changes of these vitamins (A_1, D_3 and E).

Only after electrochemical reduction of vitamin K_1, anodic DPV can be used for simultaneous electrochemical detection of all presented lipophilic vitamins in potential window from –0.6 to +1.4 V. The evidence that all selected lipophilic vitamins can be determined together in one analysis is demonstrated in Figure 4. Moreover, it shows that distance of individual voltammetric peak was satisfactory for their sufficient resolution without using any chromatographic technique due to sufficiently different values of the appropriate peak potentials.

It is very important to realize that the electrochemical method presented in this contribution has not been optimized yet. It can be assumed that whole optimization will be very time consuming because it is always based on the finding the optimal working conditions to obtain high sensitivity such as selection of suitable electrode material, organic solvent and many others.

For example, an amount of deposited analytes on solid electrode material is limited by surface area. Therefore, it is obvious that linearity range of developed analytical method will be very narrow and the sensitivity will be completely dependent on the time of accumulation. The

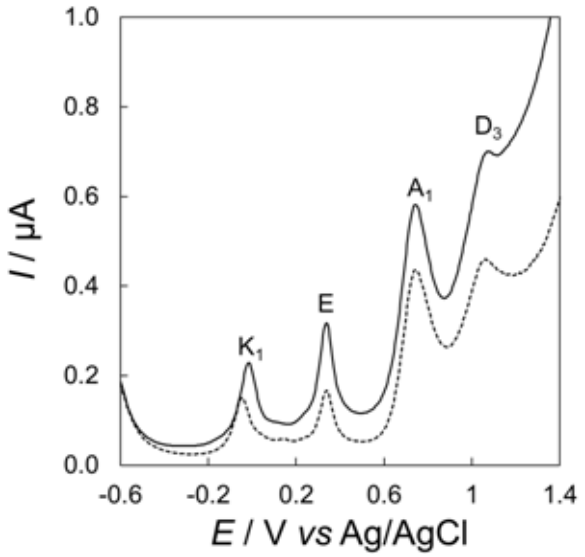

Figure 4 Simultaneous adsorptive stripping voltammetry of lipophilic vitamins deposited on GCE surface from their 100 µmol.L^{-1} solution containing 25% ACN at 400 rpm for 10 min; then detected by DPV in 0.01 mol.L^{-1} acetate (pH 4.5) buffer at E_{dep} = –0.6 V at 120 s, E_{step} = 5 mV, E_{ampl} = 0.025 V, v = 25 mV.s^{-1} (dashed line) and v = 50 mV.s^{-1} (solid line).

solution can be found in using of suitable kind of carbon paste electrode (CPE) which can be classified from physical point of view as a dispersion of solid carbon powder particles in a viscous lipophilic binder **(Švancara et al., 1996)**. In this case, the amount of accumulated analyte is controlled by corresponding extraction equilibrium.

According to publication **(Žabčíková and Červenka, 2015)**, carbon paste can be prepared from plant oils which are commonly used in technology of margarines. Thus, it can be another way how lipophilic vitamins also could be electrochemically detected.

Using carbon nanomaterials offers another possibility. Especially, carbon nanotubes (CNTs) immobilized on some carbon-based electrode material usually cause dramatical increasing of electrode surface due to their specific physical properties **(Volder et al., 2013)**.

Analysis of margarine

Analysis of margarine (Perla plus vitamíny) was only based on qualitative determination of present lipophilic vitamins. Therefore, any sophisticated statistical treatment was not necessary to use. Values of peak potentials are usually presented as arithmetic mean ($\bar{x}$) of minimally five repetitions (n) and corresponding standard deviations (σ) were less than 2% due to polishing of electrode surface after each measurement.

In this case, 2.5 mL sample solution was added into the 7.5 mL pure water in order to obtain 25% ACN in total volume. After that, GCE was immersed into resulting solution and deposition occurred at 400 rpm for 20 min. Obtained voltammograms of accumulated analytes performed in acetate buffer is shown in Figure 5.

It is interesting that only vitamin K_1 and vitamin E were qualitatively determined in the sample of margarine, although these vitamins were not listed on the product label. An explanation lies in the basic ingredients of all margarines. From physical point of view, they can be defined as emulsions of water in edible plant oil which are natural resources of these lipophilic vitamins **(Piironen et al., 1997)**. According to manufacturer, the analyzed margarine contains sunflower and rapeseed oils.

Mentioned rapeseed oil usually contains relatively high amounts of oleic and linoleic acids **(Frančáková et al., 2015)** which are very important like lipophilic vitamins. It is maybe reason why these compounds beneficial for health are very often abused in commercials.

It is clear from Figure 5 that second peak at +0.332 V (anodic oxidation of vitamin E) is not symmetric like oxidation peak of vitamin K_1 at –0.080 V. It is necessary to remember that vitamin E is not chemical individual but group of eight isomers known as tocopherols **(Gliszczyńska-Świgło1 et al., 2007)** which have similar electrochemical properties. Therefore it is quite possible that not only α-TOH was present in the sample of the margarine.

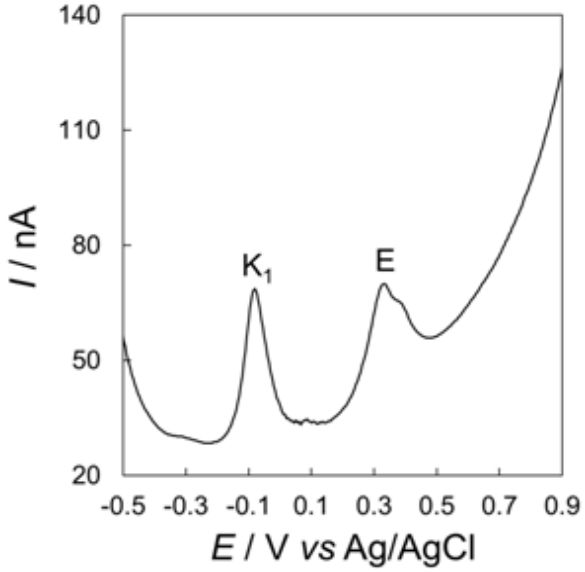

Figure 5 Qualitative determinations of lipophilic vitamins in margarine (Perla plus vitamíny) by adsorptive stripping voltammetry of at solid GCE.

CONCLUSION

According to our experimental results, it may be concluded that simultaneous qualitative determination of lipophilic vitamins is possible using adsorptive stripping differential pulse voltammetry. Unfortunately, it is clear that deposition of analytes on solid glassy carbon electrode and their following electrochemical detection does not provide satisfactory sensitivity, especially, in determination of vitamin D_3. However, it can be assumed that the sensitivity to all lipophilic vitamins can be improved using carbon nanomaterials or heterogeneous carbon materials which are known as carbon pastes. It is necessary to understand this work as an initial step in simultaneous determination of lipophilic vitamins.

REFERENCES

Baranowska, I., Markowski, P., Gerle, A., Baranowski, J. 2008. Determination of selected drugs in human urine by differential pulse voltammetry technique. *Bioelectrochemistry*, vol. 73, no. 1, p. 5-10. http://dx.doi.org/doi:10.1016/j.bioelechem.2008.04.022

Cockburn, F. 2003. Role of infant dietary long-chain polyunsaturated fatty acids, liposoluble vitamins, cholesterol and lecithin on psychomotor development. *Acta Paediatrica Supplement*, vol. 92 no. s442, p. 19-33. http://dx.doi.org/doi:10.1111/j.1651-2227.2003.tb00660.x

Frančáková, H., Ivanišová, E., Dráb, Š., Krajčovič, T., Tokár, M., Mareček, J., Musilová J. 2015. Composition of fatty acids in selected vegetable oils. *Potravinarstvo*, vol. 9, no. 1, p 538-542. http://dx.doi.org/doi:10.5219/556

Gliszczyńska-Świgło1, A., Sikorska, E., Khmelinskii, I., Sikorski, M. 2007. Tocopherols content in edible plant oils. *Polish Journal of Food and Nutrition Sciences*, vol. 57, no. 4(A), p. 157-161.

Gonnet, M., Lethuaut, L., Boury, F. 2010. New trends in encapsulation of liposoluble vitamins. *Journal of Controlled Release*, vol. 146, no. 3, p. 276-290. http://dx.doi.org/doi:10.1016/j.jconrel.2010.01.037

Hart, J. P., Norman, M. D., Christopher, J. L. 1992. Voltammetric behaviour of vitamins D_2 and D_3 at a glassy carbon electrode and their determination in pharmaceutical products by using liquid chromatography with amperometric detection. *Analyst*, vol. 117, no. 9, p. 1441-1445. http://dx.doi.org/doi:10.1039/AN9921701441

Kamal-Eldin, A., Görgena, S., Pettersson, J., Lampi, A. M. 2000. Normal-phase high-performance liquid chromatography of tocopherols and tocotrienols: Comparison of different chromatographic columns. *Journal of Chromatography A*, vol. 881, no. 1-2, p. 217-227. http://dx.doi.org/doi:10.1016/S0021-9673(99)01346-1

Kim, O. S., Kusuda, K. 1994. Electrochemical behaviour of α-tocopherol in a thin film of Nujol, a model of adipose tissue. *Bioelectrochemistry and Bioenergetics*, vol. 33, no. 1, p. 61-65. http://dx.doi.org/doi:10.1016/0302-4598(94)87033-0

Piironen, V., Koivu, T., Tammisalo, O., Mattila, P. 1997. Determination of phylloquinone in oils, margarines and butter by high-performance liquid chromatography with electrochemical detection. *Food Chemistry*, vol. 59, no. 3, p. 473-480. http://dx.doi.org/doi:10.1016/S0308-8146(96)00288-9

Švancara, I., Hvízdalová, M., Vytřas, K., Kalcher, K., Novotný, R. 1996. A microscopic study on carbon paste electrodes. *Electroanalysis*, vol. 8, no. 1, p. 61-65. http://dx.doi.org/doi:10.1002/elan.1140080113

Sýs, M., Stočes, M., Metelka, R., Vytřas, K. 2016. Electrochemical properties of a-tocopherol in aqueous electrolytes after its previous extraction into the glassy carbon paste from aqueous-acetonic mixture. *Monatshefte für Chemie*, vol. 147, p. 31-38. http://dx.doi.org/doi:10.1007/s00706-015-1620-7

Volder, M. F. L., Tawfick, S. H., Baughman, R. H., Hart, A. J. 2013. Carbon nanotubes: Present and future commercial applications. *Science*, vol. 339, no. 6119, p. 535-539. http://dx.doi.org/doi:10.1126/science.1222453

Wang, J. 1990. Recent advances in stripping analysis. *Fresenius Journal of Analytical Chemistry*, vol. 337, no. 5, p. 508-511. http://dx.doi.org/10.1007/BF00322854

Wang, L. H., Wang, J. F. 2001. Determination of retinoids in human serum, tocopherol and retinyl acetate in pharmaceuticals by RP-LC with electrochemical detection. *Journal of Pharmaceutical and Biomedical Analysis*, vol. 25, no. 5-6, p. 785-793.

Wang, L. Z., Ma, C. S., Zhang, X. L., Xu, L. 1994. Determination of vitamin K_3 by cathodic stripping voltammetry. *Microchemical Journal*, vol. 50, no. 1, p. 101-105. http://dx.doi.org/doi:10.1006/mchj.1994.1064

Webster, R. D. 2012. Voltammetry of the liposoluble vitamins (A, D, E and K) in organic solvents. *The Chemical Record*, vol. 12, no. 1, p. 188-200. http://dx.doi.org/10.1002/tcr.201100005 PMid:22121121

Wring, S. A., Hart, J. P., Knight, D. W. 1988. Voltammetric behaviour of all-trans-retinol (vitamin A_1) at a glassy carbon electrode and its determination in human serum using high-performance liquid chromatography with electrochemical detection. *Analyst*, vol. 113, no. 12, p. 1785-1789. http://dx.doi.org/doi:10.1039/an9881301785

Yao, W. W., Peng, H. M., Webster, R. D. 2009. Electrochemistry of α-tocopherol (vitamin E) and *α*-tocopherol quinone films deposited on electrode surfaces in the presence and absence of lipid multilayers. *Journal of Physical Chemistry C*, vol. 113, p. 21805-21814. http://dx.doi.org/doi:10.1021/jp9079124

Žabčíková, S., Červenka, L. 2015. Modified carbon paste electrode as a tool for the evaluation of oxidative stability of rapeseed oil. *Potravinarstvo*, vol. 9, no. 1, p. 347-351. http://dx.doi.org/doi:10.5219/432

Ziyatdinova, G., Giniyatova, E., Budnikov, H. 2010. Cyclic voltammetry of retinol in surfactant media and its application for the analysis of real samples. *Electroanalysis*, vol. 22, no. 22, p. 2708-2713. http://dx.doi.org/doi:10.1002/elan.201000358

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INFLUENCE OF TYPE AND SHELF-LIFE ON TWO BRANDS COMPLEMENTARY FOOD IN COLOR, VITAMINS, AND SENSORY EVALUATION

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ABSTRACT

The aim of our study was to measure the color by system CIELAB, sensory analysis, and determination of vitamins in children vegetable complementary feeding (carrot, vegetable mix) with the option to extend shelf life from eighteen to twenty-one months. Complementary children food was obtained from private factory in the Czech Republic. In this research there were used only carrot and vegetable mix samples. To determine the color changes by system CIELAB and determination of vitamins, samples of mash were analyzed before filling into jars and sterilization, and then immediately after sterilization. Further analyzes were performed for twenty-one months, with run of every three months (p <0.05). The comparison of color CIELAB parameter L* (lightness) for two process steps: raw mash and sterilized mash; there were significant differences when processing (p <0.0001, r^2 = 0.9983). Mainly, the parameter L* (Lightness) showed statistically significant differences in carrot and garden mix (p <0.05). β-carotenes such as provitamin A, is in food of plant origin stable substance in the absence of air. Storing time had significant influence on contain of β-carotenes, the mean content during twenty-one months was 0.862 mg.100g^{-1} (p <0.05, r^2 = 0.2300). There were no significant differences in dark storing (p >0.05, r^2 = 0.1097). The sensory evaluation showed statistical differences in all descriptors (color saturation, uniformity of color, consistency and homogeneity) (p <0.05) in course of months of storage time and storage conditions (daylight-dark). The results can be recommended to manufacturers, extending the period of minimum shelf life of the required three months to twenty-one months due to instability as characteristics of color and textural properties which were obtained.

Keywords: carrot; vegetable; colorimetry; storing; baby food; sensory analysis; vitamins

INTRODUCTION

The period of transition from exclusive breastfeeding to family foods, referred to as complementary feeding, covers a child from 6 – 23 months of age. Complementary feeding embraces all solid and liquid foods other than breast milk or infant formula and follow-on formula **(ESPGHAN, 2008)**. Malnutrition in young children can be prevented by feeding them with enough nutritious and safe complementary foods. The Association of United Kingdom Dietitians (BDA) and its Department of Health (DH) guidelines recommend the introduction of complementary feeding at around six months **(BDA, 2013)**. Furthermore BDA guidelines correspond with WHO (World Health Organization) statement. Most infants are developmentally ready for other foods at about 6 months. During the period of complementary feeding, children are at high risk of undernutrition **(WHO, 2009)**.

Complementary foods are known as weaning foods, which are semi-solid or solid foods **(Bond et al., 2005)**. Complementary food should be thick enough so that it stays on a spoon and does not drop off. Generally, such food is thicker or more solid with more energy- and nutrient- rather dense than thin. Malnutrition is common problem in infants, between age of 6 – 8, 9 – 11, and 12 – 23 months should intake than 200, 300, and 550 kcal per day, respectively **(WHO, 2009; Bronský et al., 2014)**. Home complementary food can hold lower amount of energy than processed food, with unstable content of minerals and vitamins. Infants should be fed by some fortified food. Fortified food-based products meant to be added to other foods or eaten alone to improve macronutrient, micronutrient, and vitamin intake **(Thurnham, 2013)**. On the other hand, if commercially prepared foods are used to increase micronutrient intake, their packaging instructions should clearly show purpose of feeding (age, increasing of micro-, macronutrient, etc.).

In the Czech Republic and Germany, complementary feeding usually starts with a single vegetable mash, (mostly carrot), or single fruit puree. Especially, fresh vegetables and fruits offer a high potential of taste and flavor variety and therefore the opportunity to get used to the taste of vegetable and fruits early in life depending on the season. On the other hand, commercial complementary food products provide a broader range of taste, flavors, and texture **(Foterek, Hilbig and Alexy, 2015; Bronský et al. 2014; MZČR, 2015)**.

In the past, baby foods were carefully prepared at homes. However, modern lifestyles have led to the commercialization of ready-made complementary food. Currently commercial baby jars have become an important part of baby food due changes in lifestyle. People do not have enough time for homemade alternatives of baby food

and they also tend to increase consumption of ready-cooked foods **(WHO, 2009; Mir-Marqués, 2015)**.

In general, complementary foods are made of fruit, vegetable, and meat from different animals, such as pork **(Nebot et al., 2014)**, chicken, beef, rabbit, calf, turkey, or tuna meat. Complementary food is defined as all semi-solid, pureed or mashed foods. Commercial complementary food is defined as all industrially processed, pre-packed foods in the jars or packets. Homemade complementary food is defined as all home prepared semi-solid, pureed or mashed food **(Foterek, Hilbig and Alexy, 2015)**. Complementary food can be classified in couple of groups, for example according to nutrient content, color, shape, texture, and consistency **(Rodriguez-Oliveros, Bisogni, Frongillo, 2014)**.

In recent years, commercial complementary foods have become an important part of baby dairy food. An increasing number of mothers feed their infants by processed complementary food in jars or plastic pots. The assortment of products offered has grown significantly. The processed complementary food is standardized rather than homemade complementary food, mainly incontent of vitamins, minerals, proteins, lipids, and carbohydrates **(Foterek, Hilbig and Alexy, 2015)**. Despite the benefits of infant complementary food as a major source of food for infants, the presence of contaminants, such as heavy metals may pose health risks to children **(Pandelova et al., 2012)**. Infants are exposed to daily intake of established by PTWI (Provisional Tolerable Weekly Intake) receive in complementary food. In addition, the amount of contaminants as lead, mercury and others in the European Union basket of complementary food descended in last decades **(Agostoni, Brunser, 2007; EFSA, 2012a; EFSA, 2012b)**.

Commission Directive 2006/125/EC (as amended) "on processed cereal-based foods and baby foods for infants and young children" gives an account of essential composition for baby foods, for infants and young children like protein, fat, sodium, vitamins, and minerals. Further information about specific maximum residue of pesticides or metabolites of pesticides in processed complementary food is in the annex VI. In scientific field, there are studies about the content of minerals, vitamins, contaminants **(Melø et al., 2008; Carbonell-Barrachina, 2012; Pandelova et al. 2012, Juan et al., 2014; Mir-Marqués 2014)** further researches are focused on e.g. mycotoxins, tetracyclines, acryl-amid etc.

In children diets a major role play basic characteristics of raw material (texture, pigments, etc.) that react with electromagnetic radiation in the visible spectrumand give the response to evaluators **(Figura, Teixeira, 2007)**. In food products, it was found around 10,000 inhalants **(Berger, 1995)**. Functional element which predominates is the main indicator of intra- and intermolecular interactions in the food system and its amount, gives resulting values for appearance, color, aroma, taste, and texture **(McGorrin, 2006; Stępniewski, Grundas 2013)**. On the other hand, if it uses objective measurements, the result may not be the overall color effect, therefore, still uses classical sensory analysis **(Pomerancz, Meloan, 1994)**.

The aim of our study was to measure the color by system CIELAB, sensory analysis and determination of vitamins in children vegetable complementary feeding with the option to extend shelf life of 18 to 21 months.

MATERIAL AND METHODOLOGY

Material

Complementary children feeding were obtained from private factory in Czech Republic. In this research there were used two kinds as carrot and vegetable mix samples.

Composition of samples was:

Carrot: carrot (70%), water, rice flour, citric concentrate. Nutrition per 100 g: energy 180 kJ, 0.3g of fat, carbohydrates 0.1 g, and protein 4.5 g.

Vegetable mix: potato purée (water, spray potato flocs, emulsifier: mono- and diglycerides of fatty acids), water, garden pea, carrot, spinach, leek, vegetable oil, rice flour, citric concentrate. Total vegetable content is 64%. Nutrition per 100 g: energy 287 kJ, 2.2 g of fat, carbohydrates 10.4 g, protein 1.7 g.

Methodology

To determine the color changes by system CIELAB and determination of vitamins, samples were analyzed before filling into jars and sterilization, and then immediately after sterilization. Further analyzes were performed for 21 months every 3 months. Sensory analysis was performed immediately after production – sterilization, and then again 21 months, in a run of every 3 months. The last determining of all parameters were already 3 months after the expiry date of minimum shelf life.

Color

Color of the complementary food samples were determined as reflectance values based on the L*a*b* system (lightness, redness, yellowness) using a spectrophotometer CM-3500d (Konica Minolta, Osaka, Japan) containing an integrated spectral component, a D65 illuminator and a 10° observer. Samples were measured in Petri dish with flat surface at room temperature with SCE. The L*a*b* values were determined in duplicate; the average value from these three determinations was used in the statistical evaluation.

Determination of β-carotene

5 g of homogenous samples were placed to a vial with volume of 40 mL and added 10 mL of metanolic KOH solution (KOH p.a., Merck, Czech Republic; Methanol for HPLC, Merci, Czech Republic), shaked 60 min at 350 RPM in Vortex. Next, adding of 10 mL deionization water and 3 mL of hexan p.a. (Merck, Czech Republic), vial was placed into the shaker for 10 min and 350 RPM. To 5 mL of vial was moved the top surface of hexan take out by micropipette and evaporated in blow of nitrogenous. To vial there was added next 3 mL of hexan and placed to shaker for 10 min. Upper surface was placed out and put to previous 5 mL of hexan, the content of vial was dried by nitrogenous, this steps were used triplicate. Into vial there was added 0.5 mL of methanol and 0.5 mL of dichlormethan (Merck, Czech Republic), solution was mixed in Vortex. Ready samples were place for HPLC analysis. Standard of β-carotene for HPLC was solution of 20 mg β-carotene (Fluka, Czech Republic) in 10 mL of methanol and it was shaken in Vortex and calibration

curve was papered. For HPLC analysis Shimadzu set with column Exlipse XDB-C8, 50 x 4.6 mm, 1.8 µm, 55 °C was used, mobile phase methanol-water, gradient of methanol 0 min 90%, 2 min 90%, 3 min 95%, 6 min 100%, 13 min 100%, 14 min 90%, 15 min 90%, flow of mobile phase 0.8 $mL.min^{-1}$, analysis was resulted in 15 minutes. The volume of samples 20 µL, UV/VIS detector at 450 nm was measured. Results were obtained by programme Chemstation.

Sensory analysis

Sensory evaluation of two samples of complementary food was performed immediately after the production, and for storage with minimum durability of 18 months every 3 months and 3 months after the storage by 10 trained members in sensory laboratory equipped according to ISO 8589. Determination of color saturation, uniformity of color, homogeneity and consistency were evaluated using a continuous unstructurated scale (100 mm) without references. There were evaluated 4 descriptors: color saturation, uniformity of color, consistency and homogeneity.

Statistical evaluation

Panel data were collected by Excel and tested with one-way analysis of variance (ANOVA, Statistica 12) by means of Duncan's test (p <0.05) for multiple comparisons.

RESULTS AND DISCUSSION

When comparing color CIELAB parameter L* (lightness) for two process steps: raw mash and preserved mash; there were significant differences between them p <0.0001, r^2 = 0.9983. L* parameter of carrot mash showed direct decrease of lightness. On the other hand, parameter a* (redness) and b* (yellowness) were saturated in color p <0.0001, r^2 = 0.9995, and p <0.0001, r^2 = 0.9850, respectively. The samples garden mix appeared before heat treatment and after as color stable for human eye. However, the significant statistical differences were calculated for all CIELAB parameters L*a*b* p <0.05 they showed significant differences of processing raw mash compared preserved mash which was filled in to jars. Lightness tendency were decreasing in the parameter L* in both samples; regardless, redness and yellowness parameters a* and b* were more saturated after-processing in both cases.

Majority of vegetable-based complementary food is carrot and mixes of carrot with other vegetable and other products, these similary tendencies are in the Middle Europe such is Germany **(Mesch et al. 2014)**. Studied carrot samples were measured in following storing conditions: room temperature with daylight storing. The samples were observed in raw mash, after preservation and every 3 months to "use before" and furthermore 3 months for last measurement run. The main differences between raw and preserved mash were carried out previously, the advance measurement shows significant differences between storing periods. In most cases, in parameter L* there were calculated statistically significant differences p <0.05. The lightness has changed with advance storing, however, no significant trend was observed. Variability in lightness was observed in raw mash, over L* 52, but after

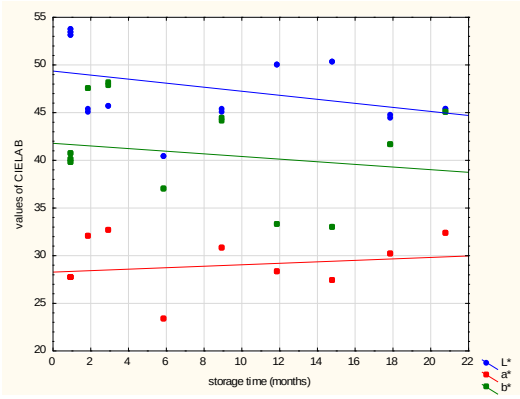

Figure 1 Monitoring changes color co-ordinates L*a*b* in relation to the length of storage in daylight for carrot samples.

heat treatment decreased to L* 44, for instance, high differences were measured during shelf-life (Figure 1).

Significant differences p <0.05 were observed in a* parameter. This parameter was relatively stable because the variation of a* was not as high as in L* parameter. The raw mash was less saturated before preservation, on the other hand saturation of a* parameter was variable in advanced storing. In redness parameter b* there were calculated significant difference in most cases. Regardless, for preservation and 3 months, 12 and 15 months, two pairs of homogenous groups p <0.05 were carried out. For instance, could be obtained non-significant data in food where the shelf-life is only one month **(Kročko et al., 2015)**.

Carrot samples were stored in the dark and in room temperature and the measure runs were the same as carrot storing in the daylight. Significant differences (p <0.05) were calculated for these samples in most cases. However, in this kind of storing there were observed more homogenous groups in parameter L*. Similar tendencies in variability of parameter L* ware observed with advance storing (Figure 2). In comparison of two storing mode were investigated no high differences. The color stability was no stable with advance storing, but for dark storing were calculated more homogenous groups in all parameters L*a*b*.

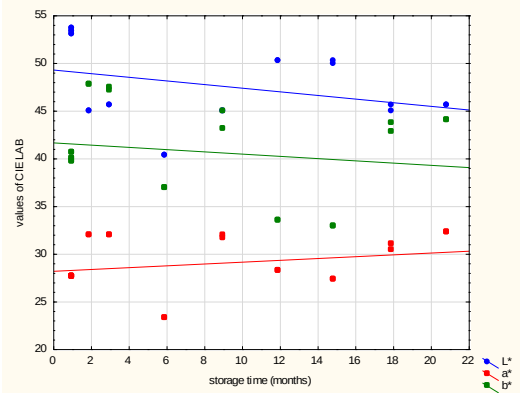

Figure 2 Monitoring changes color co-ordinates L*a*b* in relation to the length of storage in dark for the samples carrot.

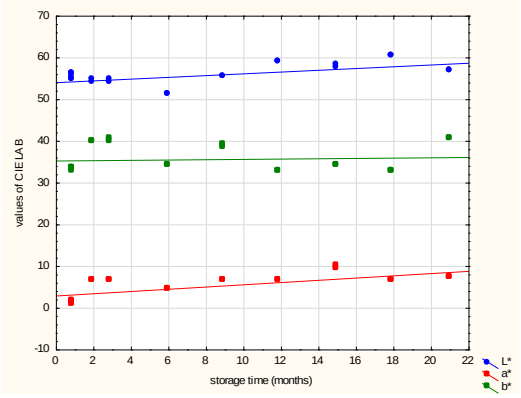

Figure 3 Monitoring changes color co-ordinates L*a*b* in relation to the length of storage in daylight for the samples vegetable mix.

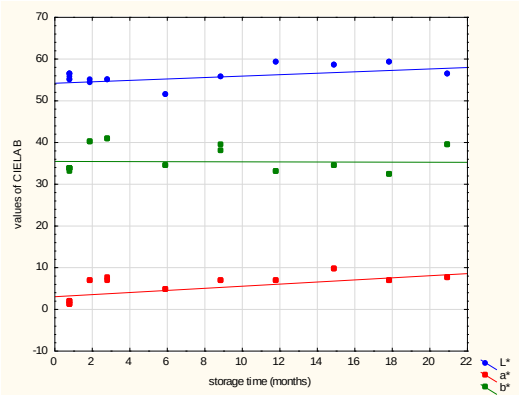

Figure 4 Monitoring changes color co-ordinates L*a*b* in relation to the length of storage in dark for the samples vegetable mix.

Furthermore, samples which called garden mix were investigated in same parameters as previous carrot mash. Lightness for daylight and dark storing had similar behavior, where lightness tendency in both were decreasing till 6 months and for the rest of runs were increasing (Figure 3 and Figure 4). On the other hand, for statistical analysis were calculated in most cases paramount significance differences p <0.05, except for preservation and 3th month and for raw and nine months for both types of storing. For instance, in dark were calculated next homogenous group for twelve and eighteen months.

For parameter a* (daylight storage) were calculated the most homogenous groups p <0.05 for preservation, 3, 9, 12, and 18 months. On the other hand, this trend was not investigated for dark storage, where were two main groups preserved, 9, and 21 months and 9, 21, and 3 months, respectively.

B-carotene

β-carotene such as provitamin A is in food of plant origin stable substance in the absence of air. During food preservation, it can isomerize to neocarotenes (still counting to vitamin A) which are less intensely colored **(Velíšek, 2014)**. It was not corresponded with our obtained data for carrot, because the color on redness axis a* was higher after preservation than before; and contain of β-carotene decrease from 0.186 to 0.015 mg.100g^{-1}, and the redness increase from L* 27 to 31, respectively. Storing time had significant influence to contain of β-carotenes, the mean content during 21 months was 0.862 mg.100g^{-1}, in the case of light storing there were calculated statistical differences p <0.05, r^2 = 0,2300. Samples of vegetable mix mean contains 0.135 mg.100g^{-1} of provitamin β-carotene and the statistically significant differences were calculated in light storing p <0.05, r^2 = 0.2300; on the other hand, no significant differences were obtained in dark storing p >0.05, r^2 = 0.1097. The obtain results shows nonsignificant connection between contain of β-carotene to redness parameter a* p <0.05.

Sensory evaluation

After assessing the results of sensory analysis, it was found that the length and type of storage affect the color changes of children complementary food and is perceptible to the human senses.

Carrot: When the sensory analysis of samples carrot compared to the daylight-dark were in the first 9thmonths of storage found statistically significant differences between the samples. In the ninth month of storage (p <0.05), as well as in 15th (p <0.01), there was a change in the descriptor consistency.At 18th months, again there were no statistically significant differences between the samples stored in daylight and in darkness. In the last month of monitoring, three months after the expiry date of minimum durability, changes have occurred only in the descriptor uniformity of color (p <0.05). From the results we can conclude that storage of the product carrot is only minimally affected when exposed to daylight.

The relative proportions of the samples were stored in daylight for 21st months (Figure 5). At color saturation occurred two homogeneous groups were created. The biggest differences that separated the groups were between 12th and 15th months of storage. The inferior results were recorded in the 21st months of storage, so three months after the expiry date of minimum durability. This change was highly significant (p <0.0001, r^2 = 0.3978). At descriptor uniformity of color, occurred to separation of homogeneous groups between 12th and 15th months of storage. Again, a statistically significant difference between all groups was evaluated with the last assessment in the 21st months (p <0.0001, r^2 = 0.3892). For descriptors consistency and homogeneity, the results were very inconsistent and have failed to form strictly according to

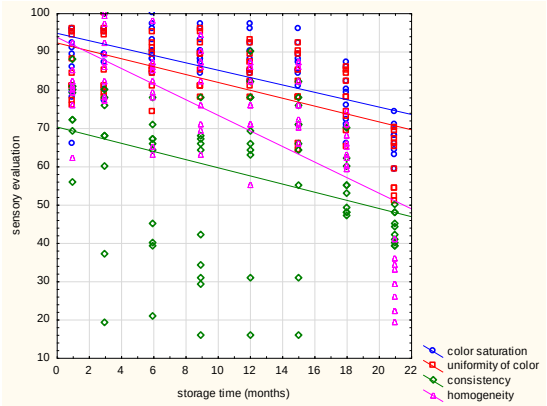

Figure 5 Dependence changes observed in sensory quality descriptors on the duration of storage of complementary food carrot for children in daylight.

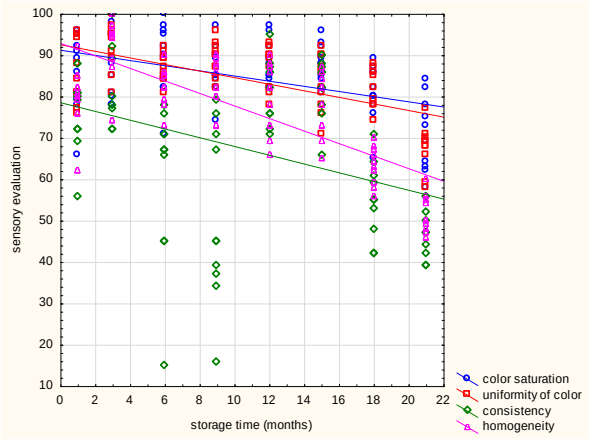

Figure 6 Dependence changes observed in sensory quality descriptors on the duration of storage of complementary food carrot for children in dark.

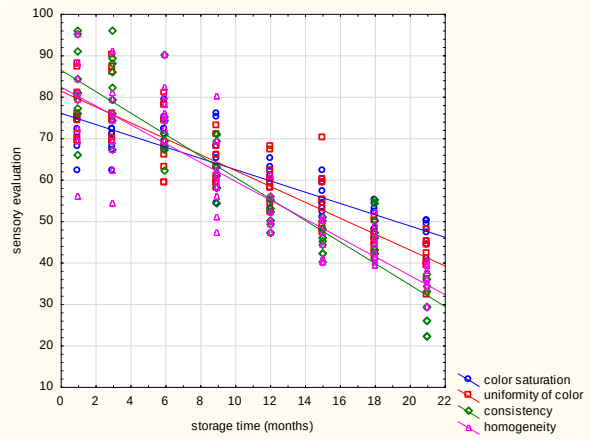

Figure 7 Dependence changes observed in sensory quality descriptors on the duration of storage of complementary food vegetable mix for children in daylight.

the length of storage. Even when descriptor consistency (p <0.001, r^2 = 0.1316) and a descriptor of homogeneity (p <0.0001, r^2 = 0.5027) showed a statistically significant difference, it can not be said that it was quite adequate to the storage time.

For samples stored in the dark (Figure 6), it was found that the color saturation created two homogeneous groups, from1st to 15th, respectively 18th month, with a fluctuation in the sixth month. Evaluation of the 21st month was quite different from the others (p <0.0001, r^2 = 0.1989). The same tendency was observed also in the descriptor uniformity of color (p <0.0001, r^2 = 0.3657), consistency (p <0.001, r^2 = 0.1393), and homogeneity (p <0.0001, r^2 = 0.5387). In the last mentioned descriptor a significant improvement occurred in the 3rd month, not only when stored in the dark, but also in the light.

Vegetable mix: By comparing storage at daylight-dark there was found no influence on color change after 3 months. Changes occurred at the rising trend in following months. After 6 months of storage there were changes (p <0.05) in the descriptor uniformity of color and after 9 months there were noticeable changes in color saturation (p <0.05) and uniformity of color (p <0.01). After 12 months of storage, there were found statistically significant differences between samples (p <0.01) for all measured descriptors –color saturation, uniformity of color, consistency, and. Fifteen months of storage significantly influenced the evaluation. There were found statistically significant differences between light and dark with descriptors color saturation (p <0.01), the uniformity of color (p <0.001) and homogeneity (p <0.001). When assessing influence of storage at samples after 18 and 21 months, there were statistically significant differences at all investigated descriptors (p <0.05). For descriptors of color uniformity in the 18th month and color saturation descriptors, consistency and homogeneity was detected very high, with statistical significance difference (p <0.001) between the samples stored in the light and in the dark.

In the samples storage by 21 months in daylight were found relatively significant differences (p <0.0001). There was a significant decline in sensory quality, particularly towards the end of the storage period (Figure 7). At descriptor color saturation (r^2 = 0.7872), there were created two homogeneous groups, one to 6 months of storage, and the second one from 9th to 21th months of storage. The most significant changes in sensory quality of descriptor for color saturation were between 6th to 9thmonths of storage. Further significant degradation of color saturation occurred between 18th and 21thmonths of storage. At descriptor uniformity of color there were established two homogenous groups, one from 1st to 15th months storage; and the second one from18th to 21stmonths. The most important changes were at 6th months and then between 15th and 18th months (r^2 = 0.8213). For consistency there was found the largest difference between 9th and 12th months, leading to their deterioration (r^2 = 0.8603). Significant changes also occurred in the descriptor of homogenity (r^2 = 0.7732). Two separate homogeneous groups were obtained and separated by 6thto 9th months. The biggest changes were occurred between 9th and 12th months of storage. At two last mentioned descriptors the samples had only low sensory evaluation after 21 months of storage.

Samples storage in the dark there were found significant differences (p <0.0001) between months again (Figure 8). Differences during storage time were not so significant such as during storage in the daylight. At descriptor color

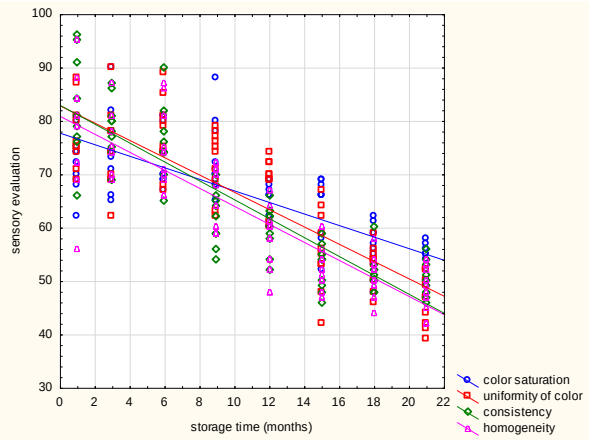

Figure 8 Dependence changes observed in sensory quality descriptors on the duration of storage of complementary food vegetable mix for children in dark.

saturation there were found two homogeneous groups, and the most significant changes occurred between 12th and 15th months of storage (r^2 = 0.5974). In another reference descriptor uniformity of color, the most significant changes were between 9th and 12th months and 12th and 15th months of storage. The 21st month exceeded by its value to other phases of evaluation (r^2 = 0.7179). Descriptor consistency recorded the most significant deterioration in sensory quality between the 12th and 15th month (r^2 = 0.7915). However, very important sensory quality deterioration was also in the 9th month. The same evaluation is also evident in the descriptor homogeneity (r^2 = 0.7552).

Generally, the color of complementary food has not so much investigated; some papers were issued **(Palazón et al., 2009)**. The objective measurement of color is not so common but sensory evaluation is wide used. In last decade, the scope of researches is on food safety and product quality such as: content of vitamins, minerals, etc. **(Bosh et al., 2013; Mir-Marqués et al., 2015; Melø et al., 2008; Mesh et al., 2014)**. Sensory quality and changes in the descriptor are affected by materials, processing, and foodstuffs **(Trejo Arayaa et al., 2009; Berger et al., 2008)**.

CONCLUSION

There were found significant differences in all monitored descriptors by sensory evaluation in storage time for samples in daylight and dark in 21 months. The samples were evaluated every 3 months. The paramount statistically differences were carried out in daylight storage and in last third of sensory evaluation in all descriptors from 15th to 21st months. The obtained data shows the same results as a CIELAB. The samples from dark were more stable than daylight storage. On the other hand, the storage time had significant influence to complementary food in both storing conditions. β-carotene was affected by storage time and there was found significant differences. Sensory analysis plays an important role in the selection of food in general, but especially for infant food. From the results of objective measurement, were found that the color and storage conditions (light/dark) statistically varied over time. However, consumer preferred changes obtained by sensory analysis to select and purchase food before changes detected by measuring CIELAB.

REFERENCES

Agostoni, C., Brunser, O., 2007. *Issues in complementary feeding*. Basel: Nestlé Nutrition workshop series, Karger. ISBN: 978–3–8055–8283–4.

Agostoni, C., Decsi, T., Fewtrell, M., Goulet, O., Kolacek, S., Koletzko, B., Michaelsen, K., Moreno, L., Puntis, J., Rigo, J., Shamir, R., Szajewska, H., Turck, D., van Goudoever, J. (2008). Complementary Feeding: A Commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, vol. 4, no. 1, p. 99-110. http://dx.doi.org/10.1097/01.mpg.0000304464.60788.bd PMid:18162844

Berger, R. G. 1995. *Aroma biotechnology*, Berlin: Springer-Verlag. ISBN 978-3-642-79373-8. http://dx.doi.org/10.1002/food.19960400130

Berger, M., Küchler, T., Maaßen, A., Busch-Stockfisch, M., Steinhart, H. 2008. Correlations of carotene with sensory attributes in capot sunder differents to rage conditions. *Food Chemistry*. vol. 106, no. 1, p. 235-240.

BDA. 2015. Complementary Feeding: Introduction of Solid Food to an Infants Diet. [online] s.a. [cit.2016-01-16] Available at: https://www.bda.uk.com/publications/professional/complementary_feeding_weaning, The British Dietetic Association.

Bond, B., Fernandez, D., Van der Jagt, D., Williams, M., Huang, Y., Chuang, L., Millson, M., Andrews, R., Glew, R. 2005. Fatty acid, amino acid and trace mineral analysis of three complementary foods from Jos, Nigeria. *Journal of Food Composition and Analysis*, vol. 18, no. 7, p. 675-690.

Bronský, J., Bělohlávková, S., Burianová, I., Frühauf, P., Fuchs, M., Kotalová, R., Malý, J., Mydlilová, A., Nevoral, J., Pozler, O., Sýkora, J. 2004. Doporučení pracovní skupiny dětské gastroenterology a výživy ČPS pro výživu kojenců a batolat (Recommendation of Working Group of Pediatric Gastroenterology and Nutrition CPS for infants and toddlers). *Česko-Slovenská Pedaitrie*, vol. 69, suppl. 1, p. 1-48.

Carbonell-Barrachina, Á., Wu, X., Ramírez-Gandolfo, A., Norton, G., Burló, F., Deacon, C., Meharg, A. 2012. Inorganic arsenic contents in rice-based infant foods from Spain, UK, China and USA. *Environmental Pollution*, vol. 163, p. 77-83. http://dx.doi.org/10.1016/j.foodchem.2014.11.078

Foterek, K., Hilbig, A., Alexy, U. 2015. Associations between commercial complementary food consumption and fruit and vegetable intake in children. Results of the DONALD study. *Appetite*, vol. 85, p. 84-90.

EFSA, 2012a. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food [online] s.a. [cit.2015-01-16] Available at: http://www.efsa.europa.eu/en/efsajournal/pub/2985.htm

EFSA, 2012b. Lead dietary exposure in the European population [online] s.a. [cit.2015-01-16] Available at: http://www.efsa.europa.eu/en/efsajournal/pub/2831.htm

Figura, L., Teixeira, A. 2007. *Food physics physical properties - measurement and application.* Berlin: Springer Berlin Heidelberg. ISBN 9783540341949.

Juan, C., Raiola, A., Mañes, J., Ritieni, A. 2014. Presence of mycotoxin in commercial infant formulas and baby fous from Italian market. *Food Control*, vol. 39, p. 227-236. http://dx.doi.org/10.1016/j.foodcont.2013.10.036

Kročko, M., Ďurík, M., Bučko, O., Tkáčová, J., Čanigová, M., Ducková, V. 2015. Effect of rosemary in combination with yeast extract on microbiology quality, oxidative stability and color of non-fermented cooked salami "Inovec". *Potravinarstvo*, vol. 9, no. 1, p. 160-165. http://dx.doi.org/doi:10.5219/464

McGorrin, R., J. 2006. Food Analysis Techniques: Introduction. In *Encyclopedia of Analytical Chemistry*. http://dx.doi.org/10.1002/9780470027318.a1001

Ministerstvo zdravotnictví České Republiky, Pracovní skupina Ministersva zdravotnictví. Doporučení k zavádění komplementární výživy příkrmu u kojenců (The Ministry of Health of the Czech Republic, The working group of the Ministry of Health. Recommendations for the introduction of complementary feeding supplementary food in infants), 2013. [online] s.a. [cit. 2015-01-08] Available at: http://www.mzcr.cz

Mir-Marqués, A., González-Masó, A., Cervera, M., de la Guardia, M. 2015. Mineral profile of Spanish commercial baby food. *Food Chemistry*, vol. 172, p. 238-244. http://dx.doi.org/10.1016/j.foodchem.2014.09.074

Nebot, C., Guarddon, M., Seco, F., Iglesias, A., Miranda, J., Franco, C., Cepeda, A. 2014. Monitoring the presence of

residues of tetracyclines in baby food samples by HPLC-MS/MS. *Food Control*, vol. 46, p. 495-501. http://dx.doi.org/10.1016/j.foodcont.2014.05.042

Rodriguez-Oliveros, M., Bisogni, C., Frongillo, E. 2014. Knowledge about food classification systems and value attributes provides insight for understanding complementary food choices in Mexican working mothers. *Appetite*, vol. 83, p. 144-152. http://dx.doi.org/10.1016/j.appet.2014.08.022

Melø, R., Gellein, K., Evje, L., Syversen, T., 2008. Minerals and traceelements in commercial infant food. *Food and Chemical Toxicology*, vol. 46, no. 10, p. 3339-3342.

Mesch, C., M., Stimming, M., Foterek, K., Hilbig, A., Ute, A., Kersting, M., Libuda, L. 2014. Food variety in commercial and homemade complementary meals for infants in Germany. Market survey and dietary practice. *Appetite*, vol. 76, p. 113-119.

Palazón, M., A., Pérez-Conesa, D., Abellán, P., ROS, G., Romero, F., Vidal, M., L. 2009. Determination of shelf-life of homogenized apple-based beikost storage at different temperatures using Weibull hazard model. *LWT - Food Science and Technology*, vol. 42, no. 1, p. 319-326. http://dx.doi.org/10.1016/j.lwt.2008.03.011

Pandelova, M., Lopez, W., L., Michalke, B., Schramm, K.-W. 2012. Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: Comparison of assessed infant intakes with the present safety limits for minerals and trace elements. *Journal of Food Composition and Analysis*, vol. 27, no. 2, p. 120-127. http://dx.doi.org/10.1016/j.jfca.2012.04.011

Pomeranz, Y., Meloan C., E. 1994. *Food analysis: theory and practice*. 3rd ed. New York: Chapman and Hall, 778 p. ISBN 0412065916.

Stepniewski, A., Grundas, S., 2013. *Advances in agrophysical researc*h. Rijeka: InTech. 408 p. ISBN 978-953-51-1184-9 http://dx.doi.org/10.5772/52372

Thurnham, D. I., 2013. Adequate Nutrient Intakes for Infancy. [onlline] s.a. [cit.2015-08-06] Available at: http://www.sightandlife.org

VELÍŠEK, J. 2014. The chemistry of food. Chichester, England: Wiley. 1124 p. ISBN 978-1-118-38381-0.

WHO, 2009. Infant and young child feeding: model chapter for textbooks for medical students and allied health professionals. Geneva: World Health Organization. 99 p. ISBN 9789241597494.

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THE EFFECT OF REDUCED ZINC LEVELS ON PERFORMANCE PARAMETERS OF BROILER CHICKENS

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ABSTRACT

The experiment was conducted to determine the effect of reduced supplemental zinc levels on broiler growth and carcass yield. A total of 160 male broiler chicks (Ross 308) divided into four groups were allotted to 16 cages with 10 birds per cage in each of group and kept in a temperature-controlled room. During the trial, chicks were ad libitum access to feed and water. The experiment started at 11 days of broiler age and chicks were fattened up to 35 days of age. It consisted of 4 dietary treatments with 4 replications per treatment. A corn-wheat-soybean meal basal diet containing 25.84 mg $Zn.kg^{-1}$ was formulated and zinc levels of 120, 40 or 20 $mg.kg^{-1}$ was supplied as zinc oxide to give four dietary treatments. At the end of the feeding trial, 24 birds from each group were randomly selected, slaughtered and carcass evaluation was performed. The results show that different levels of zinc had no significant effect on body weight of broilers or feed consumption ratio. These parameters increased by decreasing zinc levels from 120 to 20 mg $Zn.kg^{-1}$ similarly as the carcass yield, percentages of breast meat and leg meat, but differences between these groups were not significant. In case of relative liver weight and zinc concentration in liver there were significant difference (p <0.05) between group given supplemented zinc of 40 $mg.kg^{-1}$ and group without zinc supplementation and 120 $mg.kg^{-1}$ and 40 $mg.kg^{-1}$, respectively. No signs of disorders such as loss of appetite, growth depression or abnormalities of the skin was appeared in chicks. It seems that reduced supplemented zinc levels from 120 to 20 $mg.kg^{-1}$ (total Zn 153.13 $mg.kg^{-1}$ to 45.28 $mg.kg^{-1}$ respectively) not influenced growth performance parameters of broilers fed corn-wheat-soybean meal diet.

Keywords: broiler; zinc level; zinc oxide; carcass yield; liver

INTRODUCTION

Zinc (Zn) is an essential trace mineral, cofactor of more than 200 enzymes **(Nair and Choudhury, 2013)** involved in protein synthesis, carbohydrate metabolism and many other biochemical reactions, affects all cellular functions, especially growth and development of organism **(Ao et al., 2011)**. In all species, zinc is necessary for growth, immune system and disease resistance. Deprivation of zinc is characterized by loss of appetite, growth depression, abnormalities of the skin or outgrowths (hair, wool, feathers, hoof, horn) and reproductive disorders **(Suttle, 2010)**. Deficiency of zinc in chicks can cause decreased growth, frizzled feathers, shortened and thickened legs or enlarged hocks **(Nielsen, 2012)**.

The National Research Council (1994) estimates the dietary zinc requirement for broilers as 40 $mg.kg^{-1}$. The requirement of nutrients is usually defined as the minimum dietary concentration required for animal performance. A diet much higher in zinc (60 – 100 $mg.kg^{-1}$) apparently is needed to prevent disorders such as frizzled feathers in poultry **(Underwood and Suttle, 1999)**.

Feeds are routinely supplemented with zinc, because feed materials are either too low in zinc or availability of zinc is inadequate to cover the requirements. Zinc is added to the diets in inorganic sources (usually zinc oxide, zinc sulphate, zinc chloride) or in organic forms complexed to amino acids, proteins, or carbohydrates. The nutritional value of mineral sources depends on the composition of the diet, concentration in the feed, interactions with other mineral elements, and the bioavailability of the element to the chicks **(Star et al., 2012)**. The most commonly used sources of zinc are the oxide (ZnO). Fear of zinc deprivation causes exceed NRC (1994) recommendation, but if the requirement is markedly exceeded, additional zinc is not absorbed or endogenously secreted, but passes the gut and ends up in the manure **(EFSA, 2014)**. Manure from broilers fed high zinc levels spread on fields may enrich soil and drainage water with zinc and zinc contamination can affect quantity and quality of humus and lead to reduced crop yields.

The potential problem of high zinc in manure leaded to a recommendation by the Scientific Committee for Animal Nutrition (SCAN) to reduce zinc levels in feeds, followed by Regulation (EC) No 1334/2003 to decrease the maximum total zinc contents in complete feed for all animals. Maximum authorised total zinc content for poultry is 150 $mg.kg^{-1}$ complete feed. In 2014, EFSA posted a study „Scientific Opinion on the potential reduction of the currently authorised maximum zinc content in complete feed“ and the FEEDAP Panel proposed new maximum content of total zinc in complete feed for poultry (except turkeys for fattening) at the level of 100 mg $Zn.kg^{-1}$. EFSA expected that the reduction of maximum zinc contents in complete feed (from 150 to

100 mg Zn.kg^{-1} for broiler chickens) ensure health, welfare and productivity of food-producing animals as well as reduction of zinc emissions from animal production of about 20% in case of the application in feeding practices without affect consumer safety. The reduction of currently authorised maximum total zinc content in feeds would decrease the zinc load in the environment, but it is necessary to check the effect of reduced zinc levels on animal health and performance.

MATERIAL AND METHODOLOGY

Experimental birds, diets and treatments

A total of 160 7-d-old broiler chicks (Ross 308) were allotted to 16 balance cages with 10 birds per cage. The chicks had free access to feed and water throughout feeding trial. The lighting regime was 18 hours light and 6 hours dark. Birds were marked by wing tags and housed in a room that had a temperature set according to Management Handbook for broilers Ross 308. Temperature and relative humidity was recorded every day.

The experiment started at 11 days of broiler age and chicks were fattened up to 35 days of age. It consisted of 4 dietary treatments with 4 replications per treatment. As shown in Table 1, the basal diet was formulated to meet or exceed NRC (1994) nutritional requirements except zinc, with using Zn-low mineral premix containing minimum amount of zinc, so basal diet contained 25.84 mg Zn.kg^{-1} and it was added 120 mg of zinc.kg^{-1} (Zn 120) to achieve overall 153 mg Zn.kg^{-1}, (150 mg Zn.kg^{-1} is currently the maximum authorised total zinc contents for poultry). In other groups was added 40 (Zn 40) and 20 (Zn 20) mg of zinc.kg^{-1} and one group was without zinc supplementation. The source of added zinc was zinc oxide (ZnO). Total content of zinc in the diets were analysed (Table 2).

Feed consumption was noticed every day. Body weight of each chicks was measured on the digital scales at the start of experiment (11 d of age), then twice a week in the morning before feeding and at the final day (35 d of age) before slaughter.

Table 1 Composition of the basal diet fed from d 11 of age to 35 d of age.

Ingredients	%
Maize	34
Wheat	31.5
Soybean meal	26
Sunflower oil	4
Vitamin-mineral premix [1]	2
Experimental Zn-premix[2]	2
Chromium oxide	0.5
Nutrient composition	
ME_N (MJ.kg^{-1})	12.69
Crude protein	20.66
Ether extract	5.89
Crude fibre	3.14
Ash	5.53
Lysine	1.19
Methionine	0.58
Calcium	0.97
Non-phytate P	0.30
Zinc (mg)	25.84

[1]Supplied per kilogram of premix: lysine 101.65 g, methionine 135.63 g, threonine 51.22 g, calcium 200 g, phosphorus 98.19 g, natrium 62.89 g, sulphur 0.39 g, chlorine 119.69 g, copper 752.5 mg, iron 3768.6 mg, zinc 44.73 mg, manganese 6046.07 mg, cobalt 11 mg, iodine 47.95 mg, selenium 8.96 mg, vitamin A 680000 IU, vitamin D 250000 IU, vitamin E 2250 mg, K_3 74.8 mg, B_1 206.44 mg, B_2 344 mg, B_6 300.44 mg, B_{12} 1999.2 mg, biotin 11 mg, niacinamid 1793.4 mg, calcium pantothenate 676.2mg, folic acid 82.8 mg, cholinechlorid 9000 mg.

[2]Content different levels of Zn according to the dietary treatments.

Table 2 Dietary treatments.

Group	Supplement level of zinc (mg.kg^{-1})	Total content of zinc in the diet (mg.kg^{-1})
Zn 120	120	153.13
Zn 40	40	71.96
Zn 20	20	45.28
non-supplement	0	25.84

Evaluation of carcass quality

At the end of the experiment (35 d of age), 96 broilers (24 birds from each treatment) were selected, weighed and slaughtered by cervical cutting. Carcasses and livers were weighed, breast and leg meat were cut, skinned and percentages of live body weight were calculated.

Statistical analysis

Data has been processed by Microsoft Excel (USA) and STATISTICA.CZ, version 12.0 (CZ). The results were expressed as mean ±standard deviation (SD). It was used one-way analysis (ANOVA). Sheffe´s test was applied to defined statistical differences and differences between groups were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The correct environment and brooding conditions should be managed to meet all nutritional and physiological requirements to support body-weight gain throughout the growing period **(Nevrkla et al., 2015)**. Zinc oxide is commonly used source of zinc added as a supplement to poultry diets. The advantage of inorganic zinc sources is lower price, so inorganic zinc sources are still preferred than organic ones.

The effects of supplemental zinc level on slaughter weight and carcass yield are shown in Table 3.

Against non-supplement diet, slaughter weight was improved by Zn supplement of 20 mg.kg^{-1}, total dietary Zn of 45.28 mg.kg^{-1}. That agree with recommendation by **NRC (1994)** that a total dietary Zn concentration of about 40 mg.kg^{-1} is necessary to achieve normal growth in chicks. However, these parameters decreased by increasing zinc contents to 120 mg.kg^{-1} (total Zn 153.13 mg.kg^{-1}). **Mohanna and Nys (1999, In: Huang et al., 2007)**

Table 3 Effects of supplemental zinc levels on slaughter weight (g) and carcass weight (g).

Group	Slaughter weight	Carcass weight
Zn 120	2052.7 ±275.2	1505.1 ±229.5
Zn 40	2041.0 ±330.3	1499.4 ±253.2
Zn 20	2126.5 ±328.2	1573.8 ±263.2
non-supplement	1972.1 ±270.6	1438.5 ±213.7

No significant differences at a level of p <0.05.

reported that body weight gain increased with the dietary Zn supplementation of 25 mg.kg^{-1} (45 mg.kg^{-1} total dietary Zn), when chicks were fed a diet supplemented with Zn (added as Zn sulfate) at 0, 10 or 25 mg.kg^{-1}.

Jahanian et al., (2008) observed that this parameter was not affected by added zinc during wk 1 to 5, but in contrast with our results, in their trial taken 42 days, daily feed intake and weight gains decreased by decreasing Zn level from 120 to 40 mg.kg^{-1}. On the other hand, weight gain could be influenced by many other factors **(Nevrkla et al., 2014)**. **Haščík et al., (2010)** noted average saughter weight 2086 g at the age of 40 d of broiler cockerels fed commercial feed mixture and carcass weight 1475.20 g. **Liptaiová et al., (2010)** attained an average slaughter weight of unsexed broiler chickens 1651 g at 38 days of age and carcass weight 1124.17 g.

Carcass yield parameters are expressed as a percentages of live body weight measured at the day of slaughter (35 day of age). Breast meat and leg meat was weighed without skin. As shown in Table 4, the best efficiency of carcass (73.9%) and its parts, breast meat (21.3%) and leg meat (19.4%) was found in group Zn 20 and these parameters decreased with increasing Zn levels up to 120 mg Zn.kg^{-1}.

Nevertheless reduced zinc levels had no significant effect on carcass yield. Similar to our opinion, **Jahanian et al., (2008)** referred no influence of dietary zinc supplementation on carcass parameters. In their study, the highest percentage of carcass (68.64%) and breast meat (21.00%) was noticed in broilers given diet with added 80 mg Zn.kg^{-1} and relative carcass weight decreased by 120, followed 40 mg Zn.kg^{-1}, whereas breast yield decreased by 40, followed 120 mg Zn.kg^{-1}.

Our results show that zinc supplementation did not affect carcass yield, but affected relative weight of livers. Relative liver weights (see Table 5) is expressed as a percentage of live body weight measured at the day of slaughter (35 day of age). There was significant difference (p <0.05) between group Zn 40 and chicks fed non-supplemented diet.

Table 4 Effects of varying supplemental zinc levels on carcass yield (% of live body weight).

Group	Carcass	Breast meat	Leg meat
Zn 120	73.2 ±0.4	20.7 ±0.5	19.1 ±0.2
Zn 40	73.4 ±0.3	21.0 ±0.3	19.1 ±0.2
Zn 20	73.9 ±0.4	21.3 ±0.4	19.4 ±0.2
non-supplement	72.8 ±0.3	21.3 ±0.3	19.2 ±0.2

No significant differences at a level of p <0.05.

Table 5 Effect of dietary zinc on livers.

Group	Relative liver weights (% of live body weight)	Zinc concentration (mg) in 1000g of liver
Zn 120	2.13 ±0.19 ^{ab}	24.4 ±2.2 ^{a}
Zn 40	1.96 ±0.26 ^{a}	26.9 ±2.2 ^{b}
Zn 20	1.99 ±0.26 ^{ab}	25.8 ±2.3 ^{ab}
non-supplement	2.19 ±0.31^{b}	25.14 ±3.3 ^{ab}

Different letters ^{a,b} in the columns indicate significant differences at a level of p <0.05.

The heaviest livers were assigned to chicks fed on diet supplemented by 120 mg Zn.kg^{-1} (43.69), but relative to live body weight, non-supplemented group was the highest value (2.19), followed group Zn 120 (2.13).

Zinc concentration in liver calculated to 1000 g of liver is shown in Table 5. Dietary Reference Values have been established for zinc as 7 – 11 mg.day^{-1} for adult males and 6 – 9 mg.kg^{-1} for adult females. Tissues and products of animal origin participate in about 40 – 50% of total zinc intake. Based on collected data by **EFSA (2014)**, reduction in dietary zinc from 150 mg Zn.kg^{-1} to requirements do not affect zinc concentration in animal tissues so expect no concern about consumers' safety.

CONCLUSION

In this experiment, reduced zinc levels were evaluated for their effects on the growth performance of broiler chicks from 11 days up to 35 days of their age. Dietary zinc level had no significant effect on body weight or carcass yield. Only relative liver weight and zinc concentration in liver, there were significant differences (p <0.05). No signs of disorders such as loss of appetite, growth depression or abnormalities of the skin was appeared in chicks. It seems that reduced supplemented zinc levels from 120 to 20 mg.kg^{-1} (total Zn 153.13 mg.kg^{-1} to 45.28 mg.kg^{-1} respectively) not influenced growth performance parameters of broilers fed corn-wheat-soybean meal diet and incline to the proposal by EFSA to reduce total maximum zinc content in complete feed for broiler chicken, even so it will be necessary to examine interaction with other minerals before formulating feed with reduced zinc content.

REFERENCES

Ammerman, C. B., Henry, P. R., Miles, R. D. 1998. Supplemental organically-bound mineral compounds in livestock nutrition. In Garnsworthy, P. C. Wiseman, *Recent advances in animal nutrition*. Nottingham, UK: Nottingham University Press, p. 67-91.

Anonymous. 2014. Technological procedure for broiler Ross [online]. Aviagen Group [cit. 2015-09-15]. Available at: http://en.aviagen.com/ross-308.

Ao, T., Pierce, J. L., Pescatore, A. J., Cantor, A. H., Dawson, K. A., Ford, M. J., Paul, M. 2011. Effects of feeding different concentration and forms of zinc on the performance and tissue mineral status of broiler chicks. *British Poultry Science*, vol. 52, no. 4, p. 466-471.

http://dx.doi.org/10.1080/00071668.2011.588198
PMid:21919574

Cao, J., Henry, P. R., Guo, R., Holwerda, R. A., Toth, J. P., Littell, R. C., Miles, R. D., Ammerman, C. B. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. *Journal of Animal Science*, vol. 78, no. 8, p. 2039-2054. PMid:10947086

Commission Regulation (EC) No 1334/2003 of 25 July 2003amending the conditions for authorisation of a number of additives in feeding stuffs belonging to the group of trace elements. OJ L 187, 26.7. 2003, p. 11.

EFSA, 2014. Scientific Opinion on the potential reduction of the currently authorised maximum zinc content in complete feed. *EFSA Journal 2014*, vol. 12, no. 5, p. 3668-3745.

Haščík, P., Melich, M., Kačániová, M., Pál, G., Mihok, M., Čuboň, J., Mellen, M., Vavrišinová, K. 2010. The influence of propolis application to meat utility on Ross 308 broiler chickens. *Potravinarstvo*, vol. 4, no. 2, p. 29-34. http://dx.doi.org/10.5219/48

Huang, Y. L., Lu, L., Luo X. G., Liu B. 2007. An optimal dietary zinc level of broiler chicks fed a cornsoybean meal diet. *Poultry Science*, vol. 86, no. 12, p. 2582-2589. http://dx.doi.org/10.3382/ps.2007-00088
PMid:18029804

Jahanian, R., Moghaddam, H. N., Rezaei, A. 2008. Improved broiler chick performance by dietary supplementation of organic zinc sources. *Asian-Australasian Journal of Animal*, vol. 21, no. 9, p. 1348-1354. http://dx.doi.org/10.5713/ajas.2008.70699

Liptaiová, D., Angelovičová, M., Močár, K., Štofan, D. 2010. The effect of cinnamomi aetheroleum used per os on fat content in broilers meat. *Potravinarstvo*, vol. 4, no. 2, p. 45-49. http://dx.doi.org/10.5219/51

Nair, K. M., Choudhury, D. R. 2013. Zinc nutrition in health and diseases. *Journal of SAT Agricultural Research*, vol. 11, p. 1-6.

Nielsen, F. H. 2012. History of zinc in agriculture. *Advances in Nutrition*, vol. 3, no. 6, p. 783-789. http://dx.doi.org/10.3945/an.112.002881
PMid:23153732

Nevrkla, P., Čechová, M., Hadaš, Z. 2014. Use of repopulation for optimizing sow reproductive performance and piglet loss. *Acta Veterinaria Brno*. vol. 83, no. 4, p. 321-325. http://dx.doi.org/10.2754/avb201483040321

Nevrkla, P., Hadaš, Z. 2015. Effect of housing of lactating sows on their reproductive performance and losses of piglets from birth to weaning. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 63, no. 1, p. 95-100. http://dx.doi.org/10.11118/actaun201563010095

NRC (National Research Council), 1994. *Nutrient Requirements of Poultry*. 9th revised edition. National Academy Press, Washington, DC, USA.

Star, L., van der Klis, J. D, Rapp, C., Ward, T. L. 2012. Bioavailability of organic and inorganic zinc sources in male broilers. *Poultry Science*, vol. 91, no. 12, p. 3115-3120. http://dx.doi.org/10.3382/ps.2012-02314
PMid:23155021

Suttle, N. F. 2010. Zinc. In *Mineral nutrition of livestock*. 4th ed. Cambridge, UK: CABI, p. 426-458. ISBN 9781845934729.
http://dx.doi.org/10.1079/9781845934729.0426

Underwood, E. J., Suttle, N.F. 1999. Zinc. In *Mineral nutrition of livestock.* 3rd ed. New York, USA: CABI, p. 477-512. http://dx.doi.org/10.1079/9780851991283.0477

Wedekind, K. J., Baker, D. H. 1990. Zinc bioavailability in feed-grade sources of zinc. *Journal of Animal Science*, vol. 68, no. 3, p. 684-689. PMid:2318732

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QUALITY ASSESSMENT OF JUICE PREPARED FROM DIFFERENT VARIETIES OF CURRANT (*Ribes* L.)

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ABSTRACT

In the Slovak Republic currants are traditionally grown species of small fruits mainly in house gardens. Although currently their area is very small compared to the other types of fruit. We can see the importance of growing this genus (*Ribes* L.) in its good adaptability to climate conditions, in small growing demands and in stable production of nutritionally highly valuable fruit. Currant berries as well as fresh currant juice are characterized by the presence of whole complex of antioxidant active substances. The aim of this study was to evaluate the nutritional quality of currant juice prepared from various species and varieties of genus *Ribes* (L.). based on the content of their total polyphenols, anthocyanin dyes and antioxidant activity. In work we used varieties Blanka, Primus, Viktória, Heinemannova neskorá, Red Lake, Treny, Jonkheer van Tets, Fertödi, Titania, Triton and Öjebyn. Contents of evaluated components were assessed spectrophotometrically. Total polyphenol content of monitored samples determined by the Folin-Ciocalteu method reached values from 1897.43 mg GAE.dm^{-3} DM to 3712.21 mg GAE.dm^{-3} DM. The highest one was in juice from variety Primus and the lowest from variety Blanka. In white varieties of currant, the presence of anthocyanin dyes was immeasurable. In varieties of red and black currant anthocyanin dye content achieved values from 1947.64 mg.dm^{-3} DM (Jonkheer van Tets) to 4161.07 mg.dm^{-3} DM (Heinemannova neskorá). The antioxidant activity determined by the FOMO method reached values from 4130.42 mg AA.dm^{-3} DM to 6571.69 mg AA.dm^{-3} DM. We recorded the highest antioxidant activity in juice of variety Fertödi and the lowest of variety Primus.

Keywords: currant; juice; anthocyanin; total polyphenols; antioxidant activity

INTRODUCTION

Members of the genus *Ribes* L. are mostly bushes naturally occurring in wild or cultivated in gardens and orchards in different mild climate areas of the world. Fruits are a rich source of vitamin C and other health promoting substances such as organic acids, pectin, micronutrients and trace elements **(Mattila et al., 2011)**. Berry fruit is an important source of various biologically active compounds with interesting physiological effects. **Puuppone et al., (2015)** state that small fruit is rich in fiber, vitamins, minerals, anthocyanins, and especially in various phenolic compounds. **Szajdek and Borowska (2008)** present in their work that main representatives of biologically active compounds of berry fruit are particularly vitamin C and polyphenols, such as anthocyanins, phenolic acids, flavanols, flavonols and tannins. **Battino et al., (2009)** state that berry fruit is characterized by a high content and wide variety of phenolic compounds, which differ in structure and molecular weight. The phenolic compounds present in currants are benzoic acid and cinnamic acid derivatives, tannins, stilbenes and flavonoids such as anthocyanins, flavonols and flavanols catechins. Their concentration is usually higher in the skin and just beneath it, than in the central part of the fruit. **Pinto et al., (2007)** found that most represented phenolic acids in berry fruit are cinnamic and benzoic acid derivatives, which predominantly occur in the form of esters and glycosides. To the benzoic acid derivatives present in currants belong p-hydroxybenzoic acid, salicylic acid, gallic acid and ellagic acid. From the cinnamic acid derivatives, the authors confirmed the presence of coumaric acid, caffeic acid and ferulic acid in currants. **Szajdek and Borowska (2008)** state that in the black currant a high p-coumaric and caffeic acid content was detected.

Goleniowski et al., (2013) report positive effect of phenolic compounds in the prevention of many civilization diseases such as coronary heart disease, stroke and cancer. Anthocyanins belong to the most important flavonoids occurring in black and red currant. Anthocyanins in currants are in the form of mono-, di- or triglycosides, wherein the glycoside residues are typically substituted at C3, or less frequently at C5 or C7 position. The most predominant sugars in anthocyanin molecule are glucose, galactose, rhamnose, arabinose and rutinose. Anthocyanin glycoside residues are often acylated by acids, mostly by p-coumaric, caffeic or ferulic acid, and less often by p-hydroxybenzoic, malonic or acetic acid **(Sójka, Król, 2009)**. Anthocyanins have many biologically significant features and the main attention is paid to their antioxidant activity. It is well known that they play an important role in the prevention of degenerative neuronal disorders,

cardiovascular diseases, cancer and diabetes **(Lee et al., 2013)**.

From the stilbene group **Szajdek and Borowska (2008)** pointed to the presence of trans-resveratrol in red currant berries. Trans-resveratrol is a phenolic compound produced by plants in response to stress conditions, e.g. climate variability, exposure to ozone, sun radiation or heavy metals presence in soil. The pharmaceutical effects of trans-resveratrol include antioxidant and anti-inflammatory activity, as well as inhibition of LDL cholesterol oxidation, platelet aggregation and growth of various tumor cells **(Fei, 2015)**.

The chemical composition of berry fruit is highly variable depending on the variety, site of cultivation, on ripening, harvesting and storage conditions **(Talcott, 2007)**. **Battino et al., (2009)** state that the content of phenolic compounds in the berry fruits is determined by a number of factors such as species, cultivation method, region, weather conditions, maturity, harvesting, storage time and conditions. The authors further submit that fruits that grow in the cold northern climate with a short growing season, without fertilizers and pesticides, has higher polyphenol content than the same varieties grown in milder climate.

The aim of the work was to compare the quality of different types and varieties of currant processed to currant juice. We focused mainly on the determination of total polyphenols, antioxidant activity and anthocyanin dyes.

MATERIAL AND METHODOLOGY

In the work we evaluated 11 varieties of white, red and black currants. From the white group were varieties Blanka, Primus and Viktória, from the red group Heinemannova neskorá, Red Lake, Treny and Jonkheer van Tets and from the black group Fertödi, Titania, Triton and Öjebyn. The fruits were grown in Botanical garden of Slovak University of Agriculture in Nitra and collected at the stage of consumer maturity. Growing area, according to agro-climatic characteristics is included into very hot region and very dry sub-region with an average annual temperature of 9.5 °C and average annual rainfall of 584.5 mm. According to the soil characteristics, it is a heavy Gleyic Fluvisol formed on alluvial uncalcareous and calcareous sediments. In order to obtain the juice from currants, we used the screw press machine. There were used the whole currant bunches and during the pressing, peelings and seeds were removed.

Total polyphenol content was analyzed by the Folin-Ciocalteu method, whose principle is the reaction of Folin-Ciocalteu reagent with reducing substances to form a blue complex. The blue coloring intensity is proportional to the polyphenol content. We performed the evaluation with a spectrophotometer UV-VIS Jenway, at a wavelength of 700 nm and the content of total polyphenols is expressed as equivalent of gallic acid in mg GAE. dm^{-3} **(Singleton and Rossi, 1965)**.

Anthocyanin dye content was determined by spectrophotometry. The samples were extracted in ethanol with addition of 0.01% HCl. Repeated dye extraction until complete sample decolorization was carried out by heat. Anthocyanin dye content was investigated by measuring the absorbance on spectrophotometer UV-VIS Jenway at the wavelength selected by the dominant anthocyanin present in a given kind of fruit.

Antioxidant activity was determined by the FOMO method **(Prieto et al., 1999).** The principle of method is the reduction of Mo (VI) to Mo (V) by the action of reducing substances in the phosphorus presence. Coloring intensity of the resulting green phosphomolybdate complex is measured spectrophotometrically at a wavelength of 695 nm. Reducing ability of the compounds is expressed as the equivalent amount of ascorbic acid (AA), which is required to achieve the same reduction effect.

Results of analyzes were processed by statistical package Statistica 8.0 (StatSoft Inc., Tulsa, USA). Differences between the samples were monitored by Fisher's LSD test.

RESULTS AND DISCUSSION

High quality of black and red currant berries was for a long time evaluated only on the basis of the sugar, organic acids and vitamin C content. We know that the high nutritional quality of berries corresponds to the wide complex of compounds, often referred to as a phenol compounds, and these, together with vitamins, dyes and minerals, take part in forming the fruit antioxidant activity **(Nour et al., 2011)**.

In our work, we mainly focused on determination of total polyphenol content in samples of currant juices. We found out that the highest polyphenol content was in juice of red currant variety Titania with value 694.65 mg $GAE.dm^{-3}$ and the lowest content in juice of white currant variety Blanka with value 178.17 mg $GAE.dm^{-3}$. Total polyphenol content in currant juice samples was decreasing in the order Titania >Triton >Fertödi >Öjebyn >Primus >Treny >RedLake >Viktória >Jonkheer van Tets >Heinemannova neskorá >Blanka. After the total polyphenol content conversion to dry matter, the highest content showed juice of variety Primus (3712.21 mg $GAE.dm^{-3}$ DM) and the lowest of variety Blanka (1897.43 mg $GAE.dm^{-3}$ DM) (Table 1).

Table 1 Varietal differences in the total polyphenol content of currant juices.

Variety	mg $GAE.dm^{-3}$	mg $GAE.dm^{-3}$ DM
Blanka	178.17	1897.43^{a}
Heinemannova neskorá	189.03	1903.63^{a}
Viktória	225.360	1946.11^{b}
Jonkheer van Tets	220.12	2349.16^{c}
Red Lake	262.44	2688.92^{d}
Öjebyn	466.59	2903.29^{e}
Treny	282.66	3092.59^{f}
Triton	558.32	3184.93^{g}
Titania	694.65	3656.04^{h}
Fertödi	508.13	3658.25^{h}
Primus	390.15	3712.21^{i}

Note: $^{a-i}$ Means with the same letter are not significantly different from each other (Fisher's LSD test, $p > 0.05$); DM – dry matter.

By Fisher's LSD test we observed mutual differences between the currant juice samples in total polyphenol

content. Statistically significant (p <0.01) highest polyphenol content was detected in the juice of white currant variety Primus, which was followed by the black currant juice varieties of Fertödi, Triton and Titania. There was not detected any statistically significant difference (p >0.05) between the variety of Fertödi and Triton. From juices made from red currant, the highest polyphenol content was found in the variety Treny. The lowest polyphenol content from the black currant group was found in the juice from variety Öjebyn. Middle polyphenol content was found in the juice sample from red currant varieties Red Lake and Jonkheer van Tets and in the juice from white currant variety Victoria. The lowest polyphenol content was in the juice samples from red variety Heinemannova neskorá and in the juice of white variety Blanka.

Total polyphenol content in selected varieties of raspberries, blackberries and currants grown in Hungary was observed by **Dénes et al., (2011)**. The highest content of polyphenols with an average value of 533 mg.100g^{-1} recorded authors in the black currant and blackberries with value 379 mg.100g^{-1}. Average content of polyphenols detected in white currant varieties was 333 mg.100g^{-1} and 192 mg.100g^{-1} in red ones. Results of the above-mentioned authors also correspond with our findings. **Sójka a Król (2009)** used Folin-Ciocalteu method to determine total polyphenol content in the black currant marcs and reached values ranged from 2189.6 to 2285.6 mg.100g^{-1}. From that we can conclude that solid fruit components have higher content of polyphenolic substances than currant juice.

Nótin et al., (2011) investigated, what is the effect of drying temperature on the content of currant polyphenols. They used a black currant variety Titania. The samples were dried in a vacuum at 40, 50 and 60 °C, until moisture content below 10%. By exploring was found that the smallest changes in the polyphenol content were obtained by drying at 50 °C. Larger losses were observed when the black currants were dried at a temperature above 60 °C or below 40 °C, but for a longer time.

The aim of work of **Mitić et al., (2011)** was to assess the quality of dried red currants of Random variety grown in different regions of Serbia. The chemical composition can be highly variable depending on the growing region, what was also confirmed by this study. The authors found that the polyphenol content of red currants from the Beograd region with values from 3.96 to 12.68 mg GAE.g^{-1} was higher than in the currants from the Niska Banja region with values from 3.47 to 7.46 mg GAE.g^{-1}.

Anthocyanin dyes are responsible for a wide range of red to violet fruits and vegetables coloration. The obtained results have confirmed this statement too. In the juices from white currant varieties Blanka, Primus and Viktória was found an undetectable presence of anthocyanin dyes (Table 2). On the basis of detected values we can state that the highest content of anthocyanin dyes was in juices from black currant varieties and specifically in juice of variety Titania with value 599.13 mg.dm^{-3}. The lowest levels of anthocyanin dyes were found in the juice of red currant variety Jonkheer van Tets with value 182.49 mg.dm^{-3}. Samples of currant juices can be ordered by the decreasing anthocyanin dye levels in fresh mass as follows Titania >Triton >Öjebyn >Fertödi >Heinemannova neskorá >Red Lake >Treny >Jonkheer van Tets. After the conversion of anthocyanin content to dry matter was their highest content detected in juice of variety Heinemannova neskorá (4161.07 mg.dm^{-3} DM) and the lowest in juice of variety Jonkheer van Tets (1947.64 mg.dm^{-3} DM) (Table 2).

Table 2 Varietal differences in the anthocyanin dye content of currant juices.

Variety	mg.dm^{-3}	mg.dm^{-3} DM
Jonkheer van Tets	182.49	1947.64^{a}
Red Lake	244.47	2504.85^{a}
Öjebyn	444.18	2764.06^{bc}
Fertödi	423.52	3049.13^{cd}
Triton	537.15	3064.19^{cd}
Titania	599.13	3153.33^{d}
Treny	303.00	3315.20^{d}
Heinemannova neskorá	413.19	4161.07^{e}

Note: $^{a-e}$ Means with the same letter are not significantly different from each other (Fisher's LSD test, p >0.05); DM - dry matter.

Samples were mutually compared using Fisher's test. The highest content of dyes was found in juice of red variety Heinemannova neskorá, which statistically significantly differed (p <0.01) from the other currant juice samples. The second highest content was detected in juice of red currant variety Treny, which statistically significantly did not differ in anthocyanin content from juice sample prepared from black currant variety Titania. Black currant samples created 3 consecutive homogeneous groups from *d* to *bc*. The highest content was found in juice of black currant variety Titania, which statistically significantly did not differ from varieties Triton and Fertödi, but differed from Öjebyn sample, where we found the lowest content of anthocyanins. Juices from varieties Triton, Fertödi and Öjebyn statistically significantly did not differ in the anthocyanin content among themselves. We found the lowest dye content in juices of red currant varieties Jonkheer van Tets and Red Lake.

Koponen et al., (2008) investigated the content of anthocyanins in black currants and found out that their content in unprocessed currants is higher than the content after their processing. The total anthocyanin concentration in unprocessed currants was 3170 mg.kg^{-1} and after processing into currant juice concentration decreased to 2790 mg.kg^{-1}. Enzymatic modification of currant juice led to an increase in total anthocyanin concentration to values from 2870 to 3330 mg.kg^{-1}, which are similar to those in unprocessed currants.

Dénes et al., (2011) monitored the concentration of anthocyanins in selected varieties of currant, blackberries and raspberries grown in Hungary. The highest anthocyanin values were found in black currants at the level of 3540 mg.kg^{-1} DM, which is the value very similar to our results. Anthocyanin content at the level of 1450 mg.kg^{-1} DM found authors in blackberries and at level 4190 mg.kg^{-1} DM in a red currant variety, what is similar to our sample Heinemannova neskorá.

Mikkelsen and Poll (2002) state in their work that in the production process of black currant juice was maintained about 75% of anthocyanin content.

Rubinskien et al., (2005) were determining the anthocyanin content in the 9 varieties of black currant grown in Lithuania. The highest content of anthocyanins was recorded in juice produced from Kupoliniai variety with obtained value of 195.6 mg.L^{-1} and the lowest content was in Ben Lomond variety with a value of 119.9 mg.L^{-1}.

Määttä et al., (2001) investigated the content of anthocyanins in black (Öjebyn), red (Red Dutch) and white (White Dutch) currant varieties. Black currant had very high anthocyanin content up to 3011 mg.kg^{-1}, while the red currant variety only 1770 mg.kg^{-1}. The presence of anthocyanins was not confirmed in white currants. As mentioned earlier, anthocyanins are responsible for the typical black and red pigments of relevant currants, whereas white currants lack their presence.

Koponen et al., (2008) observed the effect of pectolytic enzymes addition on the content of anthocyanins in juice from blueberries and black currants. The authors discovered that by the use of pectolytic preparations, the anthocyanin content increased up to 83% in blueberry juice and to 58% in black currant juice compared to the control containing no enzymatic preparation.

Currants belong to the fruits with highly positive health effects, also due to the whole complex of substances with antioxidant effects.

Based on the obtained results, we can say that the highest antioxidant activity from our samples reached the juice of black currant variety Titania (1167.61 mg AA.dm^{-3}) and on the contrary, the lowest reached white currant variety Blanka (417.10 mg AA.dm^{-3}). On the basis of a decreasing antioxidant activity, the monitored juices can be ranked as follows Titania >Fertödi >Öjebyn >Triton >Jonkheer van Tets >Heinemannova neskorá >Red Lake >Viktória >Treny >Primus >Blanka. By the antioxidant activity content conversion to dry matter was the highest antioxidant activity detected in juice of Fertödi variety (6571.69 mg AA.dm^{-3} DM) and the lowest in juice of variety Primus (4130.42 mg AA.dm^{-3} DM).

When evaluating differences in antioxidant activity between the juices by a Fisher´s LSD test, we found that in the monitored quality indicators are the smallest relative differences among samples right in the juice antioxidant activity. Whereas the juice samples were divided into 9 homogeneous groups when evaluating the polyphenols and to 6 homogeneous groups (without white currants evaluation) when evaluating anthocyanins, they were divided into 5 homogeneous groups when assessing the antioxidant activity (Table 3).

Juices from varieties Titania and Fertödi had the highest statistically significant antioxidant activity, in which they did not differ. They statistically significantly differed only from the juices of white varieties Primus, Victoria and Blanka. Juices from white varieties had the lowest antioxidant activity, but they statistically significantly differed just from juices of varieties Titania, Fertödi a Jonkheer van Tets. To the balanced group of juices with similar antioxidant activity belong juices of red varieties Heinemannova neskorá, Red Lake and Treny and juices of black varieties Öjebyn and Triton, among which we did not find statistically significant differences in antioxidant activity.

Using the FRAP method, **Borges et al., (2010)** investigated the antioxidant capacity in samples of black and red currants, blueberries, raspberries and cranberries. Authors observed the highest measured values of antioxidant capacity in black currants 51.6 μmol $Fe^{2+}.g^{-1}$, then in blueberries 30.0 μmol $Fe^{2+}.g^{-1}$ and raspberries 27.7 μmol $Fe^{2+}.g^{-1}$. Lower antioxidant activity was found in red currants 24.6 μmol $Fe^{2+}.g^{-1}$ and cranberries 18.6 μmol $Fe^{2+}.g^{-1}$.

Table 3 Varietal differences in the antioxidant activity of currant juices.

Variety	mg AA.dm^{-3}	mg AA.dm^{-3} DM
Primus	434,11	4130,42^{a}
Viktória	502,98	4343,50^{a}
Blanka	417,10	4441,98^{ab}
Treny	458,00	5011,00^{abc}
Triton	879,48	5016,97^{abc}
Red Lake	517,33	5300,49^{abc}
Heinemannova neskorá	531,73	5354,83^{abc}
Öjebyn	891,34	5546,6^{abc}
Jonkheer van Tets	567,61	6057,74^{bc}
Titania	1167,61	6145,32^{c}
Fertödi	612,81	6571,69^{c}

Note: $^{a-c}$ Means with the same letter are not significantly different from each other (Fisher's LSD test, p >0.05); DM - dry matter.

Namiesnik et al., (2013) focused in their work on the determination of antioxidant capacity in selected types of berries, while they used gooseberries, cranberries and blueberries. The analysis was realized using the FRAP method. The highest values of total antioxidant capacity authors found in blueberries 94.10 μM TE.g^{-1}. The antioxidant capacity of cranberries and gooseberries was 26.97 μM TE.g^{-1} and 6.51 μM TE.g^{-1}.

Kendir and Köroğlu (2015) observed antioxidant activity of several species of the genus *Ribes* - *Ribes alpinum, R. anatolica, R. biebersteinii, R. multiflorum, R. nigrum, R. orientale, R. rubrum and R. uva-crispa*, growing wild in Turkey. The authors prepared water and methanol extracts of plant leaves and shoots and found that *Ribes orientale* reached the highest antioxidant activity among the assessed species.

Moyer et al., (2002) investigated correlation dependences between the antioxidant activity, the content of total polyphenols, and anthocyanin dyes in 107 genotypes of the genera *Vaccinium* L., *Rubus* L. a *Ribes* L., while found that the antioxidant activity correlates in the fruit more significantly with polyphenol content than with the anthocyanin dyes content.

CONCLUSION

Currant are an important raw material for the production of fruit juices, soft drinks and last but not least, of fruit wines. All these products are characterized by good organoleptic properties and beneficial effects on consumer health due to the presence of active antioxidant substances of different types. The aim of this work was to evaluate the content of total polyphenols, anthocyanin dyes and antioxidant activity in the juice from selected varieties of white, red and black currant. The value of total polyphenols in the currant juice ranged from 1897.43 mg. dm^{-3} DM (Blanka)

to 3712.21 mg. dm^{-3} DM (Primus). In the white varieties of currant was immeasurable amount of anthocyanin dyes. In the assessed juices from red and black currants was their content from 1947.64 mg. dm^{-3} DM (Jonkheer van Tets) to 4161.07 mg. dm^{-3} DM (Heinemannova neskorá). Among the evaluated indicators were the smallest differences between the samples in the indicator of antioxidant activity. The highest antioxidant activity reached juice sample of Fertödi variety (6571.69 mg AA.dm^{-3} DM) and the lowest sample of variety Primus (4130.62 mg AA.dm^{-3} DM).

REFERENCES

Battino, M., Beekwilder, J., Denoyes, R., Laimer, M., McDougall, G., Mezzetti, B. 2009. Bioactive compounds in berries relevant to human health. *Nutriton Reviews*, vol. 67, no. 1, p. 145-150. http://dx.doi.org/10.1111/j.1753-4887.2009.00178.x

Borges, G., Degeneve, A., Mullen, W., Crozier, A. 2010. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. *Journal of Agricultural and Food Chemistry*, vol. 58, no. 7, p. 3901-3909. http://dx.doi.org/10.1021/jf902263n

Dénes, F., Lugasi, A., Hóvari, J., Kádar, G. 2011. Phenolics in raspberry, blackberry and currant cultivars grown in Hungary. *Acta Alimentaria*, vol. 40, no. 1, p. 52-64. http://dx.doi.org/10.1556/AAlim.40.2011.1.8

Fei, M., Peiwu, L., Zhang, Q., Yu, L., Zhang, L. 2015. Rapid determination of trans-resveratrol in vegetable oils using magnetic hydrophilic multi-walled carbon nanotubes as adsorbents followed by liquid chromatography-tandem mass spectrometry. *Food Chemistry*, vol. 178, no. 1, p. 259-266. http://dx.doi.org/10.1016/j.foodchem.2015.01.021

Goleniowski, M., Bonfill, M., Cusido, R., Palazón, J. 2013. *Natural Products*. Berlin: Springer Heidelberg, 2013, p. 1951-1973. ISBN 978-3-642-22143-9. http://dx.doi.org/10.1007/978-3-642-22144-6_64

Kendir, G., Köroğlu, A. 2015. In vitro Antioxidant Effect of the Leaf and Branch Extracts of *Ribes* L. Species in Turkey. *International Journal of Pharmaceutical Science Research*, vol. 2, no. 108.

Koponen, J., Buchert, J., Poutanen, K. S., Törrönen, R. 2008. Effect of pectinolytic juice production on the extractability and fate of bilberry and black currant anthocyanins. *European Food Research Technology*, vol. 227, no. 2, p. 485-494. http://dx.doi.org/10.1007/s00217-007-0745-2

Lee, D. J., Lee, H., Lee, S. H., Lee, Ch. Y., Kim, D. 2013. Effects of Jam Processing on Anthocyanins and Antioxidant Capacities of *Rubus coreanus* Miquel Berry. *Food Science Biotechnology*, vol. 22, no. 6, p. 1607-1612.

Ma, F., Li., P., Zhang, Q., Yu, L., Zhang, L. 2015. Rapid determination of trans-resveratrol in vegetable oils using magnetic hydrophilic multi-walled carbon nanotubes as adsorbents followed by liquid chromatography-tandem mass spectrometry. *Food Chemistry*, vol. 178, no. 1, p. 259-266. http://dx.doi.org/10.1016/j.foodchem.2015.01.021

Mattila, P. H., Hellström, J., McDougall, G., Pihlava, J. M., Tiirikka, T., Karjalainen, R. O. 2011. Polyphenol and vitamin C content in European commercial black currant juice products. *Food Chemistry*, vol. 127, no. 3, p. 1216-1223. http://dx.doi.org/10.1016/j.foodchem.2011.01.129

Määttä, K., Kamal-Eldin, A., Törrönen, R. 2001. Phenolic compounds in berries of black, red, green, and white currants (Ribes sp.). Antioxidants and Redox Signaling, vol. 3, no. 6, p. 81-93. ISSN 1523-0864. http://dx.doi.org/10.1089/152308601317203521

Mikkelsen, B., Poll, L. 2002. Decomposition and transformation of aroma compounds and anthocyanins during black currant (*Ribes nigrum* L.) juice processing. *Journal of Food Science*, vol. 67, no. 9, p. 3447–3455. http://dx.doi.org/10.1111/j.1365-2621.2002.tb09604.x

Mitić, M., Obradović, M., Kostić, D., Micić, R., Paunović, D. 2011. Phenolic Profile and Antioxidant Capacities of Dried Red Currant from Serbia, Extracted with Different Solvent. *Food Science Biotechnology*, vol. 20, no. 6, p. 1625-1631. http://dx.doi.org/10.1007/s10068-011-0224-1

Moyer, R. J., Hummer, K. E., Finn, CH. E., Frei, B., Wrolstad, E. R. 2002. Anthocyanins, Phenolics, and Antioxidant Capacity in Diverse Small Fruits: *Vaccinium, Rubus*, and *Ribes. Journal of Agriculture and Food Chemistry*, vol. 50, no. 3, p. 519-525. http://dx.doi.org/10.1021/jf011062r

Namiesnik, J., Vearasilp, K., Kupska, M., Ham, K. S., Kang, S., Park, Y. K., Baraschd D., Nenirovski A., Gorinstein S. 2013. Antioxidant activities and bioactive components in some berries. *European Food Research and Technology*, vol. 237, no. 5, p. 819-829. http://dx.doi.org/10.1007/s00217-013-2041-7

Nour, V., Tradafir, I., Ionica, M. E. 2011. Ascorbic acid, anthocyanins, organic acids and mineral content of some black and red currant cultivars. *Cambridge Journals*, vol. 66, no. 5, p. 353-362. http://dx.doi.org/10.1051/fruits/2011049

Nótin, B., Stégér, M., Juhász, R., Jakab, D., Monspart-Sényi, J., Barta, J. 2011. Changes of phenolic compounds in black currant during vacuum drying process. *Acta Alimentaria*, vol. 40, no. 1, p. 120-129. http://dx.doi.org/10.1556/AAlim.40.2011.Suppl.12

Puupponen, R., Nohynek, L., Alakomi, H., Oksman, K. M. 2005. Bioactive berry compounds – novel tools against human pathogens. *Applied Microbiology and Biotechnology*, vol. 67, no. 1, p. 8-18. http://dx.doi.org/10.1007/s00253-004-1817-x

Pinto, M., Lajolo, F., Genovese, M. 2007. Bioactive compouds and antioxidant capacity of strawberry jams. *Plant Foods for Human Nutrition*, vol. 62, no. 3, p. 127-131. PMID:17701363

Prieto, P., Pineda, M., Aguilar., M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, vol. 269, p. 337-341. http://dx.doi.org/10.1006/abio.1999.4019 PMid:10222007

Rubinskiene, M., Viskelis, P., Jasutiene, I., Viskeliene, R., Bobinas, C. 2005. Impact of various factors on the composition and stability of black currant anthocyanins. *Food Research International*, vol. 38, no. 8, p. 867-871.

Singleton, V. L., Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic – phosphotun gstic acid reagents. *American Journal of Enology and Agriculture*, vol. 14, no. 3. p. 144-158.

Sójka, M., Król, B. 2009. Composition of industrial seedless black currant pomace. *European Food Research and Technology*, vol. 228, no. 4, p. 597-605. http://dx.doi.org/10.1007/s00217-008-0968-x

Talcott, S. T. 2007. *Berry Fruit Value-Added Products for Health Promotion*. USA: CRC Press, 2007, p. 51-72. ISBN 978-1-4200-0614-8. http://dx.doi.org/10.1201/9781420006148.ch2

Szajdek, A., Borowska, E. J. 2008. Bioactive Compounds and Health-Promoting Propertiesof Berry Fruits: A Review. *Plant Foods for Human Nutrition,* vol. 63, no. 4, p. 147-156. http://dx.doi.org/10.1007/s11130-008-0097-5

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PREVALENCE OF PATHOGENIC *YERSINIA ENTEROCOLITICA* IN MINCED MEAT, PIG TONGUES AND HEARTS AT THE RETAIL LEVEL IN THE CZECH REPUBLIC DETECTED BY REAL TIME PCR

Alena Lorencova, Michal Slany

ABSTRACT

Yersiniosis is the third most frequently reported zoonosis in the European Union and *Yersinia enterocolitica* is the most common species causing human infections. Pigs are assumed to be the main reservoir of human pathogenic *Y. enterocolitica* with the presence of bacteria mainly in the tonsils and intestinal content. Undercooked pork and pork products have been suggested as the primary source of human yersiniosis. Nevertheless, data on the prevalence of pathogenic *Y. enterocolitica* in foodstuffs including pork products are very limited. A molecular based method (real time PCR) targeting the *ompF* gene (detection of *Yersinia* genus) and the *ail* gene (a chromosomally located virulence marker of *Y. enterocolitica*) was used to determine the prevalence of pathogenic *Y. enterocolitica* in minced meat and edible pork offal at the retail level in the Czech Republic. A total of 50 pig tongues, 50 pig hearts, and 93 samples of minced meat containing pork were purchased at nine retail outlets in Brno. High detection rates of *Yersinia* spp. were found in all types of samples (pig tongues, 80.0%; pig hearts, 40.0%; and minced meat, 55.9%). The highest prevalence of pathogenic *Y. enterocolitica* was found in pig tongues (40.0%), followed by pig hearts (18.0%) and minced meat samples (17.2%). Although from the point of view of food safety the merely molecular detection of DNA of the pathogenic bacteria could represent a false positive result, our results indicate the presence of pathogenic *Y. enterocolitica* in raw pork products at the retail level in the Czech Republic, which may pose a risk of consumer infection. Sufficient heat treatment and prevention of cross-contamination during preparation of food in the kitchen should be recommended.

Keywords: *Yersinia enterocolitica*; *ail* gene; *ompF* gene; real time PCR; pork products; retail; zoonosis

INTRODUCTION

In the European Union, yersiniosis was the third most frequently reported zoonosis in 2014, despite the significantly decreasing trend between 2008 and 2014. *Yersinia enterocolitica* was the most common species reported, having been isolated as the causative agent from 97.7% of the confirmed cases **(EFSA and ECDC, 2015)**. Clinical manifestations of human infection are usually fever, enterocolitis, pseudoappendicitis, and mesenteric lymphadenitis with diarrhoea, vomiting, and abdominal pain. Post-infection complications such as reactive arthritis or erythema nodosum can also emerge **(Galindo et al., 2011)**.

Pigs have been considered to be the primary reservoir for the pathogenic *Y. enterocolitica* that has been isolated especially from tonsils, tongues, or throats, and to a lower extent from faeces, which all can be a source of contamination for other parts of the carcasses during slaughter procedures **(Fredriksson-Ahomaa et al., 2000; Fredriksson-Ahomaa et al., 2001a; 2001b; Simonova et al., 2008; Van Damme et al., 2015)**. The European Food Safety Authority (EFSA) considers *Y. enterocolitica* as one of the most relevant biological hazards in the context of meat inspection of swine **(EFSA, 2011)**.

Eating of raw or undercooked pork and pork products (especially minced meat) has been strongly associated with human yersiniosis **(Tauxe et al., 1987; Grahek-Ogden et al., 2007)**. **Fosse et al., (2008)** estimated that 77.3% of clinical cases of yersiniosis in humans are connected with the consumption of pork and the same genotypes of *Y. enterocolitica* strains isolated from slaughterhouse environments, pork products in retail outlets and patients with yersiniosis support this hypothesis **(Fredriksson-Ahomaa et al., 2001a)**. Nevertheless, at present, there is no harmonised surveillance of pathogenic *Yersinia* in food and animals in the EU **(EFSA and ECDC, 2015)**.

Y. enterocolitica is a ubiquitous microorganism. However, not all strains recovered from food and environmental samples are pathogenic. From the point of view of health hazard, it is necessary to distinguish between pathogenic and non-pathogenic variants **(Fredriksson-Ahomaa and Korkeala, 2003; EFSA and ECDC, 2015)**. The use of traditional culture methods may lead to underestimation of pathogenic *Y. enterocolitica* in clinical, food and environmental samples. Pathogenic yersinia have seldom been isolated from pork or other foods except for edible pig offal, because of their usually small numbers in the samples, limited sensitivity of the culture media without the ability to distinguish between pathogenic and non-pathogenic strains and subsequent overgrowth of target organisms by background flora **(Fredriksson-Ahomaa and Korkeala, 2003;**

Laukkanen-Ninios et al., 2014; EFSA and ECDC, 2015).

More rapid, sensitive and specific DNA-based methods have provided a better estimation of the occurrence of pathogenic *Y. enterocolitica* in naturally contaminated samples even when the pathogen is initially present in low numbers **(Fredriksson-Ahomaa et al., 1999 and 2001c; Fredriksson-Ahomaa and Korkeala, 2003; Messelhäusser et al., 2011; Laukkanen-Ninios et al., 2014)**. However, subsequent isolation of *Y. enterocolitica* strains is needed for further strain characterization (especially for information on the biotype and serotype) and to assess its public health significance **(EFSA and ECDC, 2015)**.

The virulence of *Y. enterocolitica* results from a complex of plasmid- and chromosomally encoded genes. Because of easy loss of the virulence plasmid during laboratory handling, chromosomally located genes are more reliable target genes for PCR assays **(Fredriksson-Ahomaa and Korkeala, 2003; Galindo et al., 2011)**. The chromosomally located *ail* gene (attachment and invasion locus) is an essential virulence factor in strains of *Yersinia* spp. **(Miller et al., 1989)** and it is the most frequently used target to detect human pathogenic *Y. enterocolitica* **(Miller et al., 1989; Fredriksson-Ahomaa and Korkeala, 2003)**. An enrichment step prior to PCR is recommended to increase sensitivity and probability of detecting viable cells; false-positive results due to dead cells can be avoided **(Lambertz et al., 2007)**.

According to the EFSA report **(EFSA and ECDC, 2015)**, not enough information is available about the prevalence of human pathogenic *Y. enterocolitica* in foodstuffs at the retail level. The aim of this pilot study was to survey the prevalence of pathogenic *Y. enterocolitica* in edible pork offal (tongues and hearts) and minced meat at the retail level in the Czech Republic in order to estimate the risk of consumer infection via products of porcine origin.

MATERIAL AND METHODOLOGY

During the period of Juny 2014 to August 2015, a total of 50 pig tongues, 50 pig hearts, and 93 samples of individually packaged minced meat (50 pure pork and 43 mixed with 15 to 50% beef) were purchased from nine different butcher shops and supermarkets in Brno, Czech Republic. The samples were transported to the laboratory under refrigeration and were analysed immediately. A 25 g portion of each sample was cut into small pieces and homogenized in 225 mL PSB (phosphate buffered saline with sorbitol and bile salts, HiMedia, India) in a stomacher blender for 2 min and enriched at 25 °C for 18 – 20 h.

One millilitre of the enriched culture was centrifuged at 14,000 g for 5 min. The pellet was used for DNA isolation using the DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany) slightly modified to include mechanical homogenization with zirconia/silica beads (0.2 mm) in a MagNALyser instrument (Roche, Mannheim, Germany). An aliquot (5 μL) of extracted DNA was used as a template for home-made triplex real time PCR (qPCR) assay able to detect genus *Yersinia* (*ompF* gene) **(Stenkova et al. 2008**) and differentiate pathogenic *Y. enterocolitica* strains (*ail* gene) **(Lambertz et al., 2008)**. The previously published internal amplification control was used in the assay to eliminate false negative samples **(Slana et al., 2008)**.

RESULTS AND DISCUSSION

Based on a qualitative risk assessment of foodborne hazards associated with chilled pork carcasses, *Y. enterocolitica* was considered of high relevance **(EFSA, 2011)**. However, pigs are mostly asymptomatic carriers of pathogenic *Y. enterocolitica* without any signs of illness or macroscopic lesions. Thus, routine meat inspection practices cannot reveal infected pigs or contaminated carcasses and their products can enter the food chain.

In the Czech Republic, a growing number of cases of human yersiniosis were recorded in recent years **(Dr. Cestmir Benes, The National Institute of Public Health, Czech Republic, personal communication)**. However, no systematic monitoring of the occurrence of pathogenic *Yersinia* spp. in animals and in food is performed. In previous studies, pathogenic *Y. enterocolitica* has been recovered from smears from pig tongues (from 1.1 to 27%), tonsils (7.5%), rectal content (7.4%), and skin surface (2.8%) obtained from different slaughterhouses in the Czech Republic **(Aldová and Švandová, 1984; Aldová et al., 1990; Vázlerová and Steinhauserová, 2006; Simonova et al., 2008)**. However, no information is available about the presence of pathogenic *Y. enterocolitica* in pork products at the retail level.

In the present study, we have found a high prevalence of *Yersinia* spp. in all three types of collected samples (Figure 1) with the highest contamination level in pig tongues (80.0%), followed by minced meat (55.9%) and pig hearts (40.0%). However, as was shown previously, the majority of *Yersinia* isolates obtained from food and environmental samples are non-pathogenic without any clinical importance **(Fredriksson-Ahomaa and Korkeala, 2003)**.

This fact is in accordance with our results because the detection rate of pathogenic *Y. enterocolitica* in the examined samples was considerably lower compared with the contamination by *Yersinia* in general (Figure 1). The highest positivity was found in pig tongues (40.0%) and then in hearts (18.0%) and minced meat samples (17.2%). Using PCR methods, even higher contamination rates with pathogenic *Y. enterocolitica* in pig tongues at the retail level were detected in previous European studies: 44.9% in Bavaria (Germany) **(Messelhäusser et al., 2011)**, 83% **(Fredriksson-Ahomaa et al., 2001c)** and 92% **(Fredriksson-Ahomaa et al., 1999)** in Finland. Pathogenic *Y. enterocolitica* was found also in 50% of pig hearts from retail shops in Finland, however, only a small number of samples (n = 8) were investigated **(Fredriksson-Ahomaa et al., 2001c)**. In the abovementioned studies, the prevalence of pathogenic *Y. enterocolitica* was found to be higher with PCR than with culture methods, which indicates a higher sensitivity of the PCR method for detection of pathogenic *Y. enterocolitica* in naturally contaminated samples.

The high prevalence of *Y. enterocolitica* in pig tongues and other offal might be caused by cross-contamination by tonsil tissue during the slaughtering process **(Fredriksson-Ahomaa et al., 2000; 2001b; Messelhäusser et al., 2011)**. Preventing contamination completely is practically impossible because the tonsils are removed and hung together with tongue, liver, lung and heart on a hook after

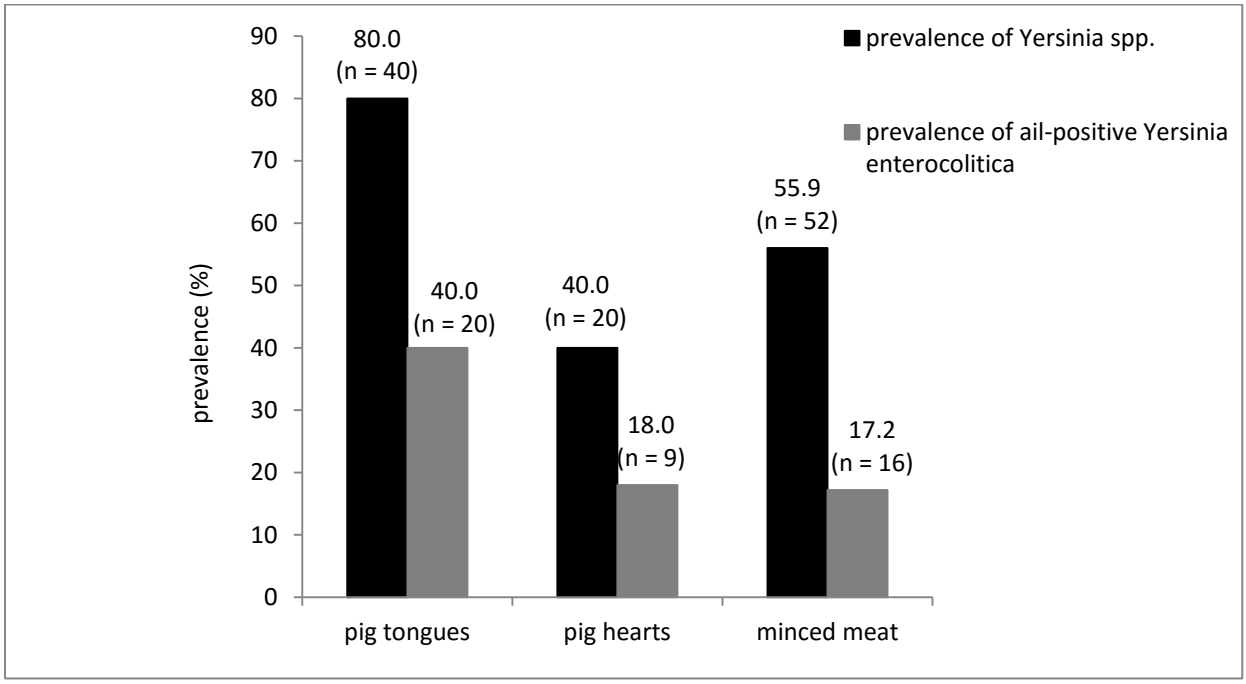

Figure 1 Prevalence of *Yersinia* spp. and pathogenic (*ail*-positive) *Yersinia enterocolitica* in pig tongues (n = 50), hearts (n = 50) and minced meat samples (n = 93) at the retail level using the real time PCR.

evisceration. The highest isolation rate (51%), in comparison to other raw pork products, of pathogenic *Y. enterocolitica* was also found in edible offal of slaughter pigs in southern Germany **(Bucher et al., 2008)**.

Van Damme et al., (2015) found that the initial presence of *Y. enterocolitica* in tonsils and/or in faeces of pigs at slaughter was significantly associated with carcass contamination and the findings of the same genotypes in tonsils, offal, and in minced pork support the assumption that tonsils are the primary source of contamination with pathogenic *Y. enterocolitica* at the slaughterhouse level **(Fredriksson-Ahomaa et al., 2000; Fredriksson-Ahomaa et al., 2001b)**. The blood of infected animals and rinse water can be another source of these bacteria for edible parts of the carcass and pathogenic strains were isolated also from the environment and from the air in a pig slaughterhouse **(Fredriksson-Ahomaa et al., 2000)**. Thus, the cross-contamination of carcases and offal of subsequently slaughtered non-infected pigs can also occur.

Minced meat represents another raw pork product with high risk of contamination by pathogenic *Y. enterocolitica*. Eating of raw ground pork and ground mixed meat was found to be strongly associated with human infection in Belgium **(Tauxe et al., 1987)**. In our study, 17.2% of individually packaged minced meat samples were found to contain pathogenic *Y. enterocolitica* using qPCR. Similarly, raw minced meat samples containing pork collected at the retail level in Finland **(Fredriksson-Ahomaa et al., 1999)** and in Sweden **(Lambertz et al., 2007)** were found to be relatively highly contaminated with pathogenic *Y. enterocolitica* using PCR methods (25% and 35% of samples, respectively). In the United States, 133 (38%) of 350 ground pork samples were found to be contaminated by pathogenic (*ail*-positive) *Y. enterocolitica* using qPCR assay. All samples investigated in that study were culture negative, which indicated only a low contamination level. Pathogenic *Y. enterocolitica* was also detected in 39% of cut meat samples (n = 155) collected at meat cutting plants before mincing in Finland studied with PCR **(Laukkanen–Ninios et al., 2014)**. On the other hand, in Germany, pathogenic *Y. enterocolitica* was detected only in 5 (4.9%) from 102 samples of pork minced meat using qPCR assay targeting the *ail* gene **(Messelhäusser et al., 2011)**.

In shops and then in kitchens *Y. enterocolitica* can easily contaminate other foods thorough direct contact with contaminated raw pork and edible offal or via contaminated hands or equipment during handling and preparation **(Fredriksson-Ahomaa et al., 2001a; 2001b)**. In our study, the detection of genomic equivalents of pathogenic *Y. enterocolitica* in pig tongues and/or hearts collected on the same day and in the same shop could be also the result of cross-contamination of offal stored together before sale. Moreover, because of the psychrotrophic character of *Y. enterocolitica*, these bacteria can persist and multiply in raw material and food during storage at refrigeration temperatures. This is of significant concern from the point of view of food safety and public health.

Sufficient heat treatment of raw meat and offal should eliminate *Y. enterocolitica*, but as was shown, bacteria can survive in core of the product, especially in foods high in fat content (e.g. some minced meat products) which can protect bacteria against the effect of high temperature **(Grahek-Ogden et al., 2007)**. Furthermore, subsequent cross-contamination of the heat-treated products can occur if good hygiene procedures are not followed.

The detection of only the DNA of the pathogenic bacteria in food could be dismissed as irrelevant with respect to food safety because of the possible presence of dead or damaged bacterial cells or free DNA alone **(Lambertz et al., 2007)**. However, if isolation of the pathogen is

difficult, as in the case of pathogenic *Y. enterocolitica*, positive PCR results indicate that it is present and this should be considered as a potential health hazard. In addition, an enrichment step prior to qPCR used in our study should increase the possibility of detecting only live bacteria **(Lambertz et al., 2007)**. Even if the bacteria were initially present in low numbers, their ability to survive and multiply in refrigerated meat and meat products increases the potential risk of infection to consumers.

CONCLUSION

Results of this pilot study indicate that raw pork products can be an important source of pathogenic *Y. enterocolitica* at retail level in the Czech Republic. Measures should be taken to prevent contamination of pig carcasses and edible offal during slaughter and good hygiene practices including proper cooking and prevention of cross-contamination at the household level should be adopted in order to minimize the spread of pathogenic yersiniae and risk of human infection. Further study is needed to obtain *Y. enterocolitica* isolates from the PCR-positive samples for their further characterisation in order to get important epidemiological information.

REFERENCES

Aldová, E., Švandová, E. 1984. *Yersinia enterocolitica* 03 findings on porcine tongues in comparison with yersiniosis incidence in man in Czechoslovakia. *Journal of Hygiene, Epidemiology, Microbiology and Immunology*, vol. 28, no. 3, p. 319-329. PMid:6491274

Aldová, E., Švandová, E., Votýpka, J., Šourek, J. 1990. Comparative-study of culture methods to detect *Yersinia enterocolitica* serogroup O3 on swine tongues. *Zentralblatt für Bakteriologie*, vol. 272, no. 3, p. 306-312. http://dx.doi.org/10.1016/S0934-8840(11)80032-0

Boyapalle, S., Wesley, I. V., Hurd, H. S., Reddy, P. G. 2001. Comparison of culture, multiplex, and 5' nuclease polymerase chain reaction assays for the rapid detection of *Yersinia enterocolitica* in swine and pork products. *Journal of Food Protection*, vol. 64, no. 9, p. 306-312.

Bucher, M., Meyer, C., Grötzbach, B., Wacheck, S., Stolle, A., Fredriksson-Ahomaa, M. 2008. Epidemiological data on pathogenic *Yersinia enterocolitica* in Southern Germany during 2000-2006. *Foodborne Pathogens and Disease*, vol. 5, no. 3, p. 273-280. http://dx.doi.org/10.1089/fpd.2007.0076 PMid:18564908

EFSA, 2011. EFSA panels on biological hazards (BIOHAZ), on contaminants in the food chain (CONTAM), and on animal health and welfare (AHAW). Scientific opinion on the public health hazards to be covered by inspection of meat (swine). *EFSA Journal 2011*, vol. 9, no. 10, 198 p.

EFSA and ECDC. 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA Journal 2015*, vol. 13, no. 12, 191 p.

Fosse, J., Seegers, H., Magras, C. 2008. Foodborne zoonoses due to meat: a quantitative approach for a comparative risk assessment applied to pig slaughtering in Europe. *Veterinary Research*, vol. 39, no. 1, p. 1-16. http://dx.doi.org/10.1051/vetres:2007039 PMid:18073088

Fredriksson-Ahomaa, M., Hielm, S., Korkeala, H. 1999. High prevalence of yadA-positive *Yersinia enterocolitica* in pig tongues and minced meat at the retail level in Finland. *Journal of Food Protection*, vol. 62, no. 2, p. 123-127. PMid:10030629

Fredriksson-Ahomaa, M., Korte, T., Korkeala, H. 2000. Contamination of carcasses, offals, and the environment with yadA-positive *Yersinia enterocolitica* in a pig slaughterhouse. *Journal of Food Protection*, vol. 63, no. 1, p. 31-35. PMid:10643766

Fredriksson-Ahomaa, M., Hallanvuo, S., Korte, T., Siitonen, A., Korkeala, H. 2001a. Correspondence of genotypes of sporadic *Yersinia enterocolitica* bioserotype 4/O:3 strains from human and porcine sources. *Epidemiology and Infection*, vol. 127, no. 1, p. 37-47. http://dx.doi.org/10.1017/S0950268801005611 PMid:11561973

Fredriksson-Ahomaa, M., Bucher, M., Hank, C., Stolle, A., Korkeala, H. 2001b. High prevalence of *Yersinia enterocolitica* 4:O3 on pig offal in southern Germany: A slaughtering technique problem. *Systematic and Applied Microbiology*, vol. 24, no. 3, p. 457-463. http://dx.doi.org/10.1078/0723-2020-00055 PMid:11822684

Fredriksson-Ahomaa, M., Lyhs, U., Korte, T., Korkeala, H. 2001c. Prevalence of pathogenic *Yersinia enterocolitica* in food samples at retail level in Finland. *Archiv für Lebensmittelhygiene*, vol. 52, no. 3, p. 66-68.

Fredriksson-Ahomaa, M., Korkeala, H. 2003. Low occurrence of pathogenic *Yersinia enterocolitica* in clinical, food, and environmental samples: a methodological problem. *Clinical Microbiology Reviews*, vol. 16, no. 2, p. 220-229. http://dx.doi.org/10.1128/CMR.16.2.220-229.2003 PMid:12692095

Galindo, C. L., Rosenzweig, J. A., Kirtley, M. L., Chopra, A. K. 2011. Pathogenesis of *Y. enterocolitica* and Y. pseudotuberculosis in human yersiniosis. *Journal of Pathogens*, vol. 2011, p. 1-16. http://dx.doi.org/10.4061/2011/182051 PMid:22567322

Grahek-Ogden, D., Schimmer, B., Cudjoe, K. S., Nygård, K., Kapperud, G. 2007. Outbreak of *Yersinia enterocolitica* serogroup O:9 infection and processed pork, Norway. *Emerging Infectious Diseases*, vol. 13, no. 5, p. 754-756. http://dx.doi.org/10.3201/eid1305.061062 PMid:17553258

Lambertz, S. T., Granath, K., Fredriksson-Ahomaa, M., Johansson, K. E., Danielsson-Tham, M. L. 2007. Evaluation of a combined culture and PCR method (NMKL-163A) for detection of presumptive pathogenic *Yersinia enterocolitica* in pork products. *Journal of Food Protection*, vol. 70, no. 2, p. 335-340. PMid:17340866

Lambertz, S. T., Nilsson, C., Hallanvuo, S., Lindblad, M. 2008. Real-time PCR method for detection of pathogenic *Yersinia enterocolitica* in food. *Applied and Environmental Microbiology*, vol. 74, no. 19, p. 6060-6067. http://dx.doi.org/10.1128/AEM.00405-08 PMid:18708521

Laukkanen-Ninios, R., Fredriksson-Ahomaa, M., Maijala, R., Korkeala, H. 2014. High prevalence of pathogenic *Yersinia enterocolitica* in pig cheeks. *Food Microbiology*, vol. 43, p. 50-52. http://dx.doi.org/10.1016/j.fm.2014.04.016 PMid:24929882

Messelhäusser, U., Kämpf, P., Colditz, J., Bauer, H., Schreiner, H., Höller, C., Busch, U. 2011. Qualitative and quantitative detection of human pathogenic *Yersinia enterocolitica* in different food matrices at retail level in Bavaria. *Foodborne Pathogens and Disease*, vol. 8, no. 1, p.

39-44. http://dx.doi.org/10.1089/fpd.2010.0589 PMid:21034270

Miller, V. L., Farmer, J. J. 3rd, Hill, W. E., Falkow, S. 1989. The ail locus is found uniquely in *Yersinia enterocolitica* serotypes commonly associated with disease. *Infection and Immunity*, vol. 57, no. 1, p. 121-131. PMid:2642465

Simonova, J., Borilova, G., Steinhauserova, I. 2008. Occurrence of pathogenic strains of *Yersinia enterocolitica* in pigs and their antimicrobial resistance. *Bulletin of the Veterinary Institute in Pulawy*, vol. 52, no. 1, p. 39-43.

Slana, I., Kralik, P., Kralova, A., Pavlik, I. 2008. On-farm spread of Mycobacterium avium subsp. paratuberculosis in raw milk studied by IS900 and F57 competitive real time quantitative PCR and culture examination. *International Journal of Food Microbiology*, vol. 128, no. 2, p. 250-257. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.08.013 PMid:18824269

Stenkova, A. M., Isaeva, M. P., Rasskazov, V. A. 2008. Development of a multiplex PCR procedure for detection of *Yersinia* genus with identification of pathogenic species (*Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*). *Moleculer Genetics, Microbiology and Virology*, vol. 23, no. 3, p. 119-125. http://dx.doi.org/10.3103/S0891416808030038

Tauxe, R. V., Vandepitte, J., Wauters, G., Martin, S. M., Goossens, V., De Mol, P., Van Noyen, R., Thiers, G. 1987. *Yersinia enterocolitica* infections and pork - the missing link. *Lancet*, vol. 1, no. 8542, p. 1129-1132. http://dx.doi.org/10.1016/S0140-6736(87)91683-7

Van Damme, I., Berkvens, D., Vanantwerpena, G., Baré, J., Houf, K., Wautersa, G., De Zutteral, L. 2015. Contamination of freshly slaughtered pig carcasses with enteropathogenic *Yersinia* spp.: Distribution, quantification and identification of risk factors. *International Journal of Food Microbiology*, vol. 204, p. 33-40. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.03.016 PMid:25835198

Vázlerová, M., Steinhauserová, I. 2006. The comparison of the methods for the identification of pathogenic serotypes and biotypes of *Yersinia enterocolitica*: Microbiological methods and PCR. *Czech Journal of Food Sciences*, vol. 24, no. 5, p. 217-222.

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FLAX – EVALUATION OF COMPOSITE FLOUR AND USING IN CEREAL PRODUCTS

Marie Hrušková, Ivan Švec

ABSTRACT

Two types of yellow and brown linseed, differing in granulation, were tested in form of wheat flour composites (additions 2.5% and 5.0%) by using the Farinograph, the Extensigraph and the Rapid Visco Analyser (RVA) apparatuses. Additions of brown and yellow flax fibre significantly affected Falling Number and Zeleny test values. Curves of farinograph were differed according to flax fibre type – finer flax (better terminology) granulation meant somewhat stronger negative changes in dough stability and dough softening degree. Results of extensigraph test demonstrated changes in dough elasticity and extensibility due to lowering of gluten protein content. Appearance of the RVA profiles was verifiably different, reflecting diverse wheat and flax polysaccharides, added dietary fibre type and its granulation. Due to that, bread volume and shape was lowered up to one-half in case of golden flax composites. Similar tendencies with smaller negative influence caused the brown linseed. Fibre from flax is used for technical (textile) use, but linseed dietary fibre addition affected quality of laboratory prepared cut-off biscuits and dried pasta differently, showing a dependence on the fibre type, granulation as well as addition level. Sensory profiles of all mentioned product types were acceptable.

Keywords: brown and yellow linseed; granulation; rheology; bread; biscuits; pasta

INTRODUCTION

Flax (*Linum usitatissimum* L.) is old utility plant originated in Asia. Slim stem and light-blue flowers could distinguish the plant. Its fruit is boll containing tiny brown seeds. There exist linseed varieties (e.g. Amon, Raciol) with lighter seed, named as "yellow flax".

Fibre flax is appraised according to flax thread characteristics, which exists into two forms for industrial processing. Between world producers belong France, Belgium and Netherlands, in the Czech Republic is not planted from the year 2010.

Linseed is planted for seeds and oil production, for both food and industrial usage. According to Catalogue of oil plant varieties (ÚKZÚZ Brno, 2015), four linseed varieties sorted in groups with low, high and usual linoleic-to-alpha-linolenic acid ratio were tested. Between varieties with medium content of these acids belongs the Czech variety Raciol, registered in year 2011.

Flax seed is, with respect to chemical composition, recommended into curative diets. Between brown and yellow coloured seeds, nutrition difference is not verifiable, but consumers prefer yellow seeds owing to somewhat intensive nutty-butter by-taste. Seed composition is typical by high oil content (40%), dietary fibre (28%) and proteins (21%). Further known constituents are minerals (4%) and non-starch polysaccharides (6%) as lignans, hemicelluloses and phenolics (**Fitzpatric, 2008; Bernacchia et al., 2014**; **Ding et al., 2014; Nitrayová et al. 2014**). Flax oil is the most favourable nutrition component, rich in omega-3 unsaturated fatty acids with short chain. Also content of alpha-linolenic acid is substantial **(Cunnane et al., 1993)**. For commercial purposes, limited stability of these oil components is discussed, which have approved health benefits in lifestyle diseases prevention (**Denmark-Wahnefriend, 2006)**. Employing of flax seed in cereal products is limited by specific structure characteristic. Owing to hard cover layers, it could be used as decorative material for spreading of special bread types. In cases of wholemeal bread, golden flax seeds are used more frequently. When it is involved into bread recipe, technological process has to be adapted in terms of so-called wort, which owing to time period and water temperature ensures sufficient water sorption and seeds softening. For such purpose, brown flax is preferred, because of difference in crumb colour profile compared to product based on wheat flour only.

Flax dietary fibre is commercial food supplement gained as by-product during oil pressing or extraction, sold in a dry powder form. Walramcom company, flax fibre producer from the New Zeeland, present mean nutritional values for the supplement from brown and golden flax: saccharides 2.4%, proteins 32.0% and total fat 16.6% (of which 13% unsaturated fatty acids). According to production method, majority mass portion presents dietary fibre (TDF 45.2%, IDF 37.9% and SDF 7.3%). Proteins have a non-gluten nature, and thus they are safe for coeliac patients. Further, flax fibre is a good source of constituents with high anti-oxidant activity, especially lignans (e.g. secoisolariciresinol diglucoside) and vitamin E. For flax lignans, a specific function in prevention cancer diseases of breast and prostate. **Budwig (2011)** presented their

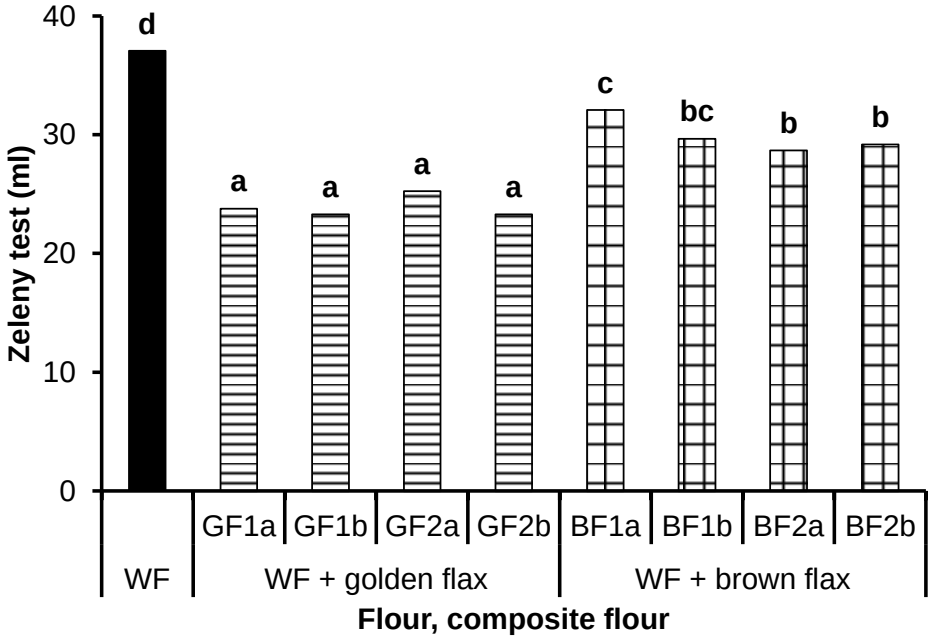

Figure 1 Zeleny test values for wheat flour (WF) and flour composites. For samples coding, see Table 1.

content 75 – 800 times higher compared to other vegetable and pulses. Owing to low presence of saccharides, flax fibre is appropriate for diabetics and sportsmen. Plant proteins may successfully enriched daily diet of vegetarians and vegans. Recommended consumption is equal to 13 g as additive into oat flakes, yogurts or soups. In terms of gluten-free material, it could serve as a base of bread, pancakes, pies etc. Flax fibre has a potential to be used as recipe component of sweet bread e.g. muffins **(Chetana et al., 2010)**, gluten-free products and pastas **(Kishk et al., 2011; Hrušková a Švec, 2013)**.

The pilot study was aimed at characteristics comparison of several commercial types of flax fibre, using them in form of composite flour during non-fermented and fermented dough properties testing. Verifying technological potential and consumer quality of these flour composites, leavened bread, cookies and pasta were manufactured in a laboratory scale.

MATERIAL AND METHODOLOGY

Preparation of flour composites

Semi-bright fine wheat flour (WF) was produced from wheat harvested in year 2015 by industrial mill Delta Prague. According to Falling number (392 s) and Zeleny sedimentation values (392 s and 37 ml, respectively), quality of the basic material is medium with lower amylases activity. The company Walramcom (New Zeeland) produced flax fibre (FF) samples, and they represent grounded flax seeds press cake after oil extraction (GF1, GF2 – golden flax fibre with granulation 0-300 μm and 500-700 μm, respectively; BF1, BF2 – brown flax fibre with granulation 300-500 μm and 500-700 μm, respectively). According to nutritional label, dietary fibre content is comparable in all tested ff samples. Flour composites involve 2.5% or 5.0% of ff on flour base (samples coding GF1a and GF1b, etc.).

Technological quality of flour composites

Technological features of wf and flour composites are described by Zeleny test (ČSN ISO 5529) and Falling number (ČSN ISO 3093). Non-fermented dough properties were determined by using of farinograph and extensigraph brabender (Germany), following the international norms (ČSN ISO 55 30-1, 55 30-2, respectively). Behaviour of suspension flour-water was recorded on the RVA 4500 equipment (Perten Instruments, Sweden; AACC method 76-21). According to internal procedures of the UCT Prague, rheological parameters of fermented dough was measured, using fermentograph SJA (Sweden),

Table 1 Falling number for wheat flour (WF) and flour composites.

Flour, flour composite	WF	GF1a	GF1b	GF2a	GF2b	BF1a	BF1b	BF2a	BF2b
Falling number* (s)	392 bc	349 ab	319 a	305 a	330 ab	442 c	438 c	426 c	456 c

GF1, GF2 - golden flax fibre with granulation 0-300 μm and 500-700 μm, respectively.
BF1, BF2 - brown flax fibre with granulation 300-500 μm and 500-700 μm, respectively.
Example of sample coding: GF1a, GF1b - flour composites containing 2.5% or 5.0% of golden flax fibre, respectively.
* a-c: row means described by the same letter are not significantly different (p = 95%).

Table 2 Farinograph characteristics of non-fermented dough from wheat flour (WF) and flour composites.

Flour, flour composite	Water absorption* (%)	Dough development time* (min)	Dough softening degree* (FU)
WF	67.8 a	3.15 bc	50 b
GF1a	70.3 b	2.00 a	30 ab
GF1b	72.5 d	2.50 ab	30 ab
GF2a	70.0 b	2.50 ab	20 a
GF2b	72.0 cd	4.00 cd	10 a
BF1a	71.0 bc	3.00 abc	30 ab
BF1b	71.9 cd	3.50 bcd	10 a
BF2a	70.3 b	3.50 bcd	15 a
BF2b	72.3 d	4.50 d	10 a

GF1, GF2 - golden flax fibre with granulation 0-300 μm and 500-700 μm, respectively.
BF1, BF2 - brown flax fibre with granulation 300-500 μm and 500-700 μm, respectively.
Example of sample coding: GF1a, GF1b - flour composites containing 2.5% or 5.0% of golden flax fibre, respectively.
FU – farinograph unit.
* a-d: column means described by the same letter are not significantly different (p = 95%).

maturograph and oven rise recorder (OTG) Brabender (Germany). From prepared wheat-flax fibre composites, bread, cut-off biscuits and pasta were manufactured, following further internal methods ended by quality evaluation.

Statistical evaluation of ff effect

Influence of FF type and dosage level was evaluated it terms of variation of selected dough rheological features and final products (Tukey test, p = 95%). Aimed on determination of quality features dominant for bread, biscuits and pasta, Principal Components Analysis (PCA) was used. In cases PCA of bread, biscuits and pasta, the datasets were analogous to ensure the comparability of the analysis findings – two analytical features, three farinograph and two extensigraph ones, a pair of the pasting characteristics and foursome of the product quality attributes immanent to the product type.

RESULTS AND DISCUSSION

Evaluation of technological quality of flour composites

According to Zeleny test values, bakery quality of proteins could be considered as lower compared to WF (Figure 1). Addition of GF caused a decrease in a higher extent (about 30%) in relation to BF influence (-18%) and WF control. As could be noticed in Figure 1, neither addition level nor FF granulation did not trigger significant differences.

In wheat flour sample, amylases activity as the Falling number was estimated about ca 25% lower than optimum for standard bakery processing is (250 s). With respect to variability in the FF type (GF vs. BF), granulation (2 types) as well as addition level (2.5% vs. 5.0%), flax fibre contributed to Falling number change softly. An insignificant lowering and increase was observed for GF and BF, respectively, with weak impact of addition level.

Evaluation of non-fermented dough features

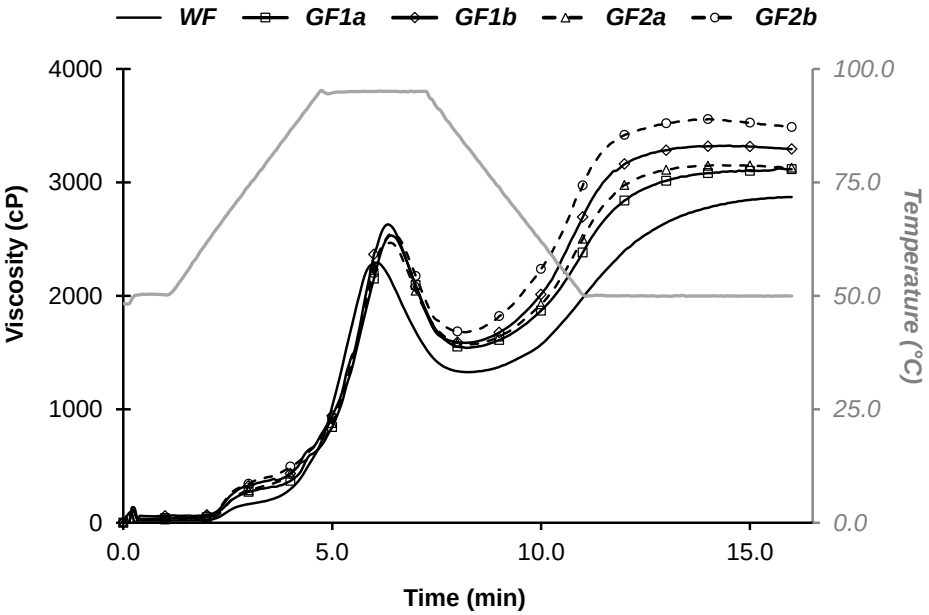

Figure 2 RVA profiles of wheat flour and selected flour composites. For samples coding, see Table 1.

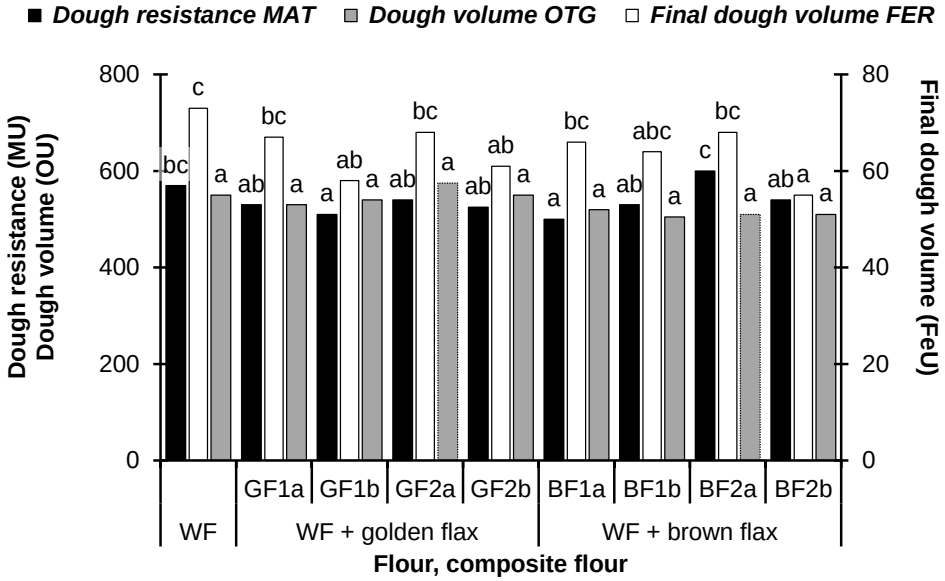

Figure 3 Characteristics of fermented dough from wheat flour and flour composites. For samples coding, see Table 1.

In case of standard farinograph proof, rheological properties of non-fermented dough prepared from flour composites are characterised by water absorption increase about 3% – 7%. Higher values were determined for 5% addition and BF of coarser granulation. Dough development time was shortened by the GF effect (from 3 min to 2 min), and reversal trend was observed for the BF (prolongation about 25%). Preparation of composite dough variants did not leave the line of standard process, and the values of dough softening degree point to higher resistance to overmixing (Table 2); on the farinograms, the second maxima were identified for more enhanced samples, which discussed **Xu et al. (2014)**.

According to extensigraph characteristics, change in dough viscoelastic properties implies the elasticity-to-extensibility ratio determined after 60 min of dough resting, which simulates II. phase of fermentation process during wheat bread manufacturing. The ratio increased from 2.18 (WF control) about 10% – 70% reflecting FF type and granulation of GF and BF (data not shown). In composite dough, diluted gluten protein contributed to lowering of bakery quality of tested dough samples; extensigraph energy decreased seriously by incorporation of finer GF (about 15%), while impact of coarser GF addition was softer (decrease ca 10%).

Both BF counterparts rather increased dough bakery quality, and effect of granulation was indefinite. RVA profile course describing viscous behaviour of wheat suspension could be designated as standard, values of the Peak Viscosity and Final Viscosity corresponds to presumed using for bread manufacturing. As illustrates Figure 2, suspensions of flour composites differed from the control in earlier gelatinisation beginning, reflecting the FF type and granulation. Between golden and brown flax, measured differences correspond to diverse representation of polysaccharide fractions, and they

Table 3 Features of bread prepared from wheat flour and flour composites.

Flour, flour composite	Specific bread volume** (ml/100g)	Bread shape*, ** (-)	Crumb penetration** (mm)
WF	334 d	0.61 a	14.3 f
GF1a	285 cd	0.54 ab	9.5 d
GF1b	242 abc	0.41 a	5.8 a
GF2a	283 cd	0.53 ab	10.4 e
GF2b	182 a	0.42 a	6.2 b
BF1a	205 ab	0.51 ab	6.2 ab
BF1b	268 bcd	0.55 ab	8.0 c
BF2a	197 ab	0.44 ab	6.6 b
BF2b	246 abc	0.56 ab	9.1 d

GF1, GF2 - golden flax fibre with granulation 0-300 μm and 500-700 μm, respectively.
BF1, BF2 - brown flax fibre with granulation 300-500 μm and 500-700 μm, respectively.
Example of sample coding: GF1a, GF1b - flour composites containing 2.5% or 5.0% of golden flax fibre, respectively.
* Height-to-diameter ratio.
** a-d: column means described by the same letter are not significantly different (p = 95%).

Table 4 Features of biscuits prepared from wheat flour and flour composites

Flour, flour composite	Specific biscuit volume** (ml/100 g)	Spread ratio*, ** (-)	Sensory profile** (-)
WF	165 ab	4.35 b	12.0 ab
GF1a	172 b	4.41 b	11.5 ab
GF1b	134 a	4.81 b	12.5 b
GF2a	167 ab	4.53 b	11.0 a
GF2b	147 ab	4.13 b	11.0 a
BF1a	143 ab	1.98 a	11.5 ab
BF1b	145 ab	1.73 a	11.5 ab
BF2a	149 ab	2.07 a	11.5 ab
BF2b	155 ab	2.05 a	11.5 ab

GF1, GF2 - golden flax fibre with granulation 0-300 μm and 500-700 μm, respectively.
BF1, BF2 - brown flax fibre with granulation 300-500 μm and 500-700 μm, respectively.
Example of sample coding: GF1a, GF1b - flour composites containing 2.5% or 5.0% of golden flax fibre, respectively.
* Diameter-to-height ratio.
** a-d: column means described by the same letter are not significantly different (p = 95%).

demonstrated a reversal tendency than published **Mueller et al. (2010)**. Influence of three factors on RVA features are discussed by **Švec and Hrušková (2016)**.

Evaluation of fermented dough features

With the help of three laboratory apparatuses, fermentograph, maturograph and OTG, three phases called fermentation, proofing and first stage of baking are simulated, allowing to describe fermented dough behaviour and estimation of technological potential of flour composites for operational application. Gained readings allow to evaluate 12 parameters, from which three are substantial for comparison with wheat dough – final dough volume (fermentograph), dough resistance (maturograph) and dough volume (OTG test). As shown Figure 3, type, granulation as well as FF type affected these features, but measurement accuracy did not allow distinguishing composite dough items from wheat control.

There could be noticed, that final dough volume depends more on FF addition level than its type. With exception of BF2a composite dough, dough resistance of other samples reached comparable level to wheat one. Bread volume in the third phase of fermentation predicts specific bread volume, and soft increase as result of GF enhancement was observed. Our results agree with effect of FF addition (6%) described by **Xu et al., (2014)**.

Evaluation of wheat bread features

Simulating a straight-dough process in a bakery, a baking trial was conducted according to the internal method (Babiaková, 2015). Quality assessment of laboratory prepared bread demonstrated a negative relationship between GF dosage and specific bread volume. For 5% addition of GF1 and GF2, a decrease reached almost one-third compared to control (242 ml.100 g^{-1} and 182 ml.100 g^{-1}, respectively, against 334 ml.100 g^{-1}). Buns vaulting became lower as FF portion in bread recipe increased; height-to-diameter ratio decrease was significant in cases of higher enhancement. Quantifying sensorial score, the change was confirmed, too – crumb of fortified bread samples were tougher and thus less tasty. By BF addition, consumer quality lowering of wheat bread

Table 5 Features of cooked pasta prepared from wheat flour and flour composites

Flour, flour composite	Absorption* (%)	Swelling index* (-)	Sediment* (ml)
WF	159.2 c	1.45 a	120 abcd
GF1a	134.0 ab	1.52 a	96 ab
GF1b	131.6 ab	1.58 a	96 ab
GF2a	147.2 bc	1.56 a	104 abc
GF2b	135.6 ab	1.58 a	80 a
BF1a	126.0 a	1.32 a	160 d
BF1b	135.2 ab	1.35 a	126 bcd
BF2a	136.8 ab	1.40 a	144 cd
BF2b	132.8 ab	1.50 a	80 a

GF1, GF2 - golden flax fibre with granulation 0-300 μm and 500-700 μm, respectively.
BF1, BF2 - brown flax fibre with granulation 300-500 μm and 500-700 μm, respectively.
Example of sample coding: GF1a, GF1b - flour composites containing 2.5% or 5.0% of golden flax fibre, respectively.
* a-b: column means described by the same letter are not significantly different (p = 95%).

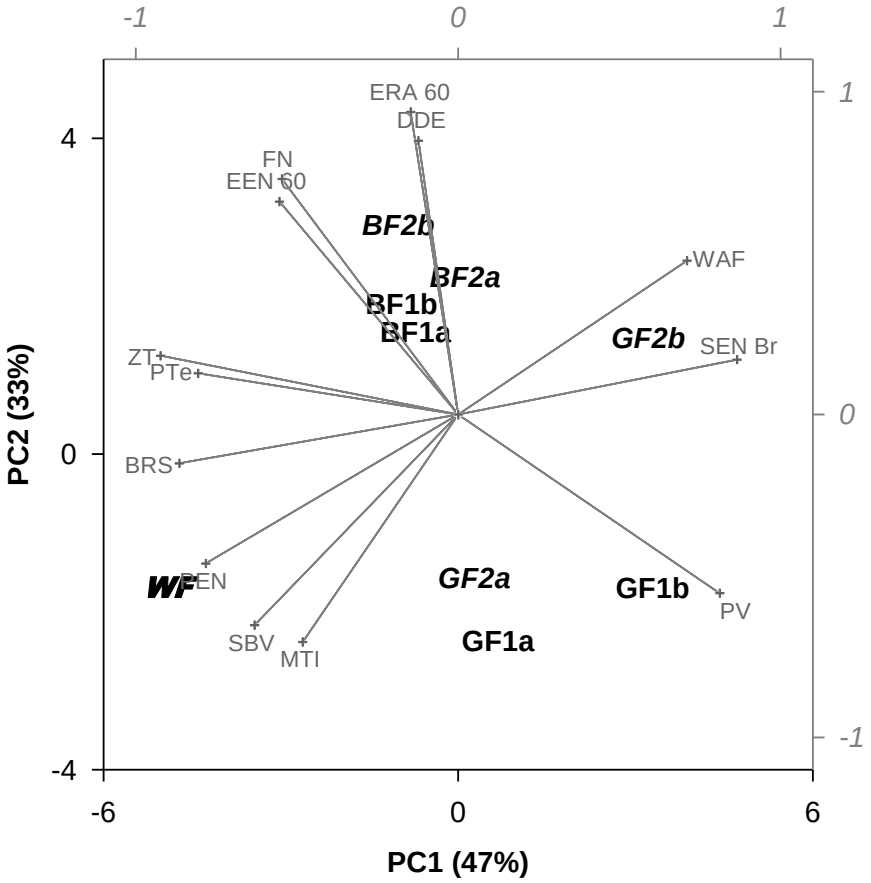

Figure 4 Principal component (PC) analysis of flax fibre effect on dough and bread technological quality. FN – Falling number, ZT – Zeleny test; WAB – water absorption, DDE – dough development time, MTI – mixing tolerance index (dough softening degree); ERA 60, EEN 60 – extensigraph elasticity-to-extensibility ratio and energy, respectively (dough resting 60 min); PTe – pasting temperature, PV – peak viscosity; SBV – specific bread volume, BRS – bread shape (height-to- diameter ratio, PEN – crumb penetration, SEN Br – bread sensory profile. For samples coding, see Table 1.

was not so dramatic, higher dosage of BF1 and BF2 (i.e. 5.0%) lowered bread volume to 268 ml.100 g^{-1} and 246 ml.100 g^{-1}, respectively; that finding correspond to conclusion in paper of **Marpalle et al. (2014)**. Samples containing 5.0% of both BF reached significantly higher volumes than their less enhanced counterparts did. Besides, shape and crumb texture of buns with BF obtained a score closer to the WF standard (Table 3).

Multivariate statistics explained 80% of data variability; 47% was covered by principal component PC1 and 33% by PC2 (Figure 4). Within PC1 x PC2 area, conjoining of observed dough and bread features as well as tested samples has a relation to their dependence rate on the FF effect. As was discussed, impact of GF and BF addition was verifiably different – golden flax fibre additions influenced bread quality less negatively. Within PC1 x PC2 area, position of GF1a, GF1b and GF2a samples are obviously closer to WF one, confirming lower level of the dough softening degree (MTI) and reversely higher values of the specific bread volume and crumb penetration. Within the biplot, there could be noticed a significant role of bread sensory score in bread recipe discrimination (explored from 75% by PC1, and from 3% by PC2).

Evaluation of cut-off biscuit features

Cut-off biscuits are characteristic by manufacturing technique, i.e. by cutting-off from dough plate of calibrated thickness, and within the Czech assortment of long-life confectionery, they represent ca 20%. The mentioned internal method (Hrušková and Švec, 2015) operates with seven quality characteristics, of which three (specific volume of baked biscuits, shape as spread ratio (diameter-to-height ratio) and sensory profile allow to compare different recipe variants. For biscuits containing GF, 2.5% such fibre had a positive influence on the evaluated features; the impact of GF granulation was less provable. Brown flax fibre caused a soft specific volume decrease (about approx. 10%), and biscuits shape spread into approx. a half scale (Table 4). Sensorial profiles of GF and BF biscuits did not deviate from the wheat control one. Yellow linseed contributed to attractive yellow shade of biscuit surface, and brownish one in case of BF. Coarser flax dietary fibre (granulation over 500 μm) was visually detectable as darker dots in biscuit crust, especially in case of biscuits containing brown seeds FF.

PCA results of biscuits quality, based on the analogous data set for composite flour and dough behaviour as in case of bread, shown comparable dependences as illustrated in Figure 4 (biplot not shown). The first two PC

explored also 80% of data variability, and the dominant features for biscuits recipe distinguishing maintained the Zeleny test, the extensigraph energy, both RVA pasting features and the specific volume together with the biscuit shape (spread ratio). The product sensory score depended mainly on the PC3 (80%), PC1-PC2 pair explained 18% of the scatter only.

Evaluation of dried pasta features

Employing a laboratory pasta line, dried (elbow shape) pasta was prepared, following the internal method of Vítová (2009). The link compose of pasta press Korgold TR 70 (Korngold, Austria), pre-drier Sun P+ and drying kiln Sun 405/2 (Mezos, Czech Republic), simulating process in factory. Prepared pasta is evaluated during pressing and in raw – dried – cooked forms by 12 characteristics in total. According to selected three ones (absorption, swelling index, sediment height), basic comparison of different pasta types could be carried out. Pressing of FF-fortified pasta passed off in standard way, pasta surface temperature did not overcome recommendedlevel 40 °C. Neither raw nor dried elbow pasta did not demonstrate an excessive shape deformation. After drying, pasta colour corresponded with FF type and addition level – GF contributed to lighter and BF to pleasant darker shade in surface colour of basic product. For all pasta variants, optimal cooking time reached a standard duration (8.00 min), but decrease in absorption, about 7.5% to 20.0% was evaluated. Swelling index, which characterises ability to absorb boiling water, was higher for samples enrichen by GF without impact of granulation and addition level. For pasta involving finer BF (300-500 μm), a lowering of the feature was observed. Sediment volume express a mass extracted during cooking into salt water, determined as a height of turbidity after 1 hour of standing. The feature has a relation to polysaccharides components in recipe, and measured values indicate a dependence on the FF addition level and its granulation.

Pasta quality features appointed to the dough one did not changed the data variation seriously – the PC1 explained 47% and the PC2 32% of the data scatter (Figure 5). Absorption of cooked pasta corresponds strongly with mechanical properties of proteins and polysaccharides in non-fermented dough, and swelling index with Peak viscosity on the RVA curve. Pasta samples containing either GF or BF were statistically different similarly to bread and biscuit items; within the biplot, pasta consumer quality belonged to the dominant quality features (57% and 29% of variation covered under PC1 and PC2, respectively).

CONCLUSION

Owing to chemical composition, flax seed and dietary fibre have a potential to be used in food industry. Dietary fibre gained from seeds of common flax is declared as

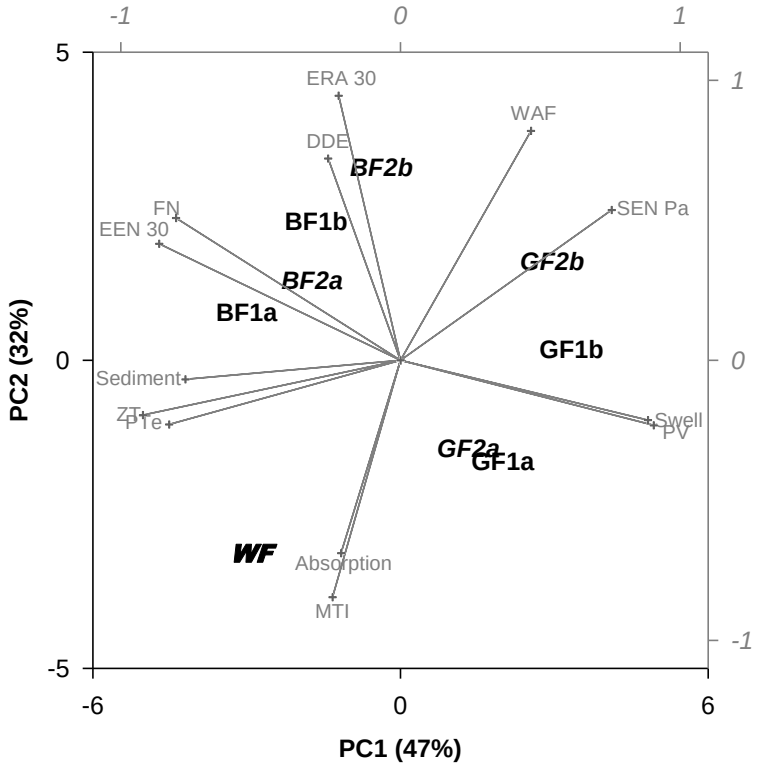

Figure 5 Principal component (PC) analysis of flax fibre effect on dough and pasta technological quality. FN – Falling number, ZT – Zeleny test; WAB – water absorption, DDE – dough development time, MTI – mixing tolerance index (dough softening degree); ERA 30, EEN 30 – extensigraph elasticity-to-extensibility ratio and energy, respectively (dough resting 30 min); PTe – pasting temperature, PV – peak viscosity; Absorption – amount of water absorbed by pasta, Swell – Swelling ratio, Sediment – height of sediment after pasta cooking, SEN Pa – pasta sensory profile. For samples coding, see Table 1.

source of plant proteins, lipids of valuable constitution and non-starch polysaccharides. Added into wheat flour, flax dietary fibre changed its technological and rheological behaviour in correspondence with flax fibre type (yellow/brown flax), fibre granulation as well as addition level. Compared to wheat flour, Zeleny test values demonstrated a lowering of protein quality and of Falling number soft increase, although the changes extent was significantly different between neither the fibre types nor both granulations. Wheat flour enrichment was verifiably reflected in water absorption rise and in the RVA features scatter; the recorded viscosity parameters differentiated flour composites containing yellow and brown flax dietary fibre. Rheological characteristics of fermented composite dough variants were less affected compared to wheat control as well as in relation to flax dietary fibre types, because observed differences were close to measurement accuracy of the internal methods. Bread buns containing flax dietary fibre was characterised by lower specific volume and less vaulted shape. According to objective quality features, shape and crumb of bread prepared from flour composite with fibre from yellow flax seeds were closer to wheat bread control characteristics. Towards to cut-off biscuit attributes, flax fibre from yellow seeds had stronger positive effect than the brown one. Enrichment of dried wheat pasta recipe did not affect its standard shape, both in raw and dried stage; after cooking, golden flax counterparts scored better. During sensory tests, all cereal product enhanced by flax fibre were described as acceptable for common consumers. In relation to tested recipe composition, a higher technological potential was observed for commercial fibre gained from yellow flax seeds, produced by the company Walramcom, the New Zeeland.

REFERENCES

Babiaková, B. 2015. *Characteristics of composite mixture containing non-traditional seeds*: master thesis. [In Czech]. Prague, Czech Republic: University of Chemistry and Technology Prague. 99 p.

Bernacchia, R., Preti, R., Vinci, G. 2014. Chemical composition and health benefits of flaxseed. *Austin Journal of Nutrition and Food Science*, vol. 2, no. 8, id 1045, 9 p.

Budwig, J. 2011. *The Budwig cancer and coronary hearth disease prevention diet*. 1st ed., LOS ANGELES, USA: Freedom Press, 215 p.

Chetana, Sudha, M. L., Begum, K., Ramasarma, P. R. 2010. Nutritional characteristics of linseed/flaxseed (*Linum usitatissimum*) and its application in muffin making. *Journal of Texture Studies*, vol. 41, no. 4, p. 563-578. http://dx.doi.org/10.1111/j.1745-4603.2010.00242.x

Cunnane, S. C., Ganuli, S., Menard, C., Wolever, T. M., Jenkins, D. J. 1993. High alfa-linolenic acid flaxseed: Some nutritional properties in humans. *British Journal of Nutrition*, vol. 69, no. 2, p. 443-453.

Denmark-Wahnefriend, W. 2006. Flaxseed and prostate cancer: demon seed or seed of salvation? *Seminars in Preventive and Alternative Medicine*, vol. 2, no. 4, p. 205-207.

Ding, H. H., Cui, S. W., Goff, H. D., Wang, Q., Chen, J., Han, N. F. 2014. Soluble polysaccharides from flaxseed kernel as a new source of dietary fibres: Extraction and physicochemical characterization. *Food Research International*, vol. 56, p. 166-173. http://dx.doi.org/10.1016/j.foodres.2013.12.005

Fitzpatric, C. K. 2008. Stabilizing flax for food application. *Cereal Food World*, vol. 53, no. 5, p. 280-282. http://dx.doi.org/10.1094/CFW-53-5-0280

Fitzpatric M., Švec I. 2015. Cookie making potential of composite flour containing wheat, barley and hemp. *Czech Journal of Food Science*, vol. 33, no. 6, 2015, p. 545-555. http://dx.doi.org/10.17221/9/2015-CJFS

Hrušková, M., Švec, I., Jurinová, I. 2013. Flax fibre – additions influence Lněná vláknina – vliv přídavků na kvalitu pšeničné mouky, *Pekař a cukrář*, vol. 2, p. 82-87.

Kishk, Y. F. M., Elsheshetawy, H. E., Mahmoud, E. A. M. 2011. Influence of isolated flaxseed mucilage as a non-starch polysaccharide on noodle quality. *International Journal of Food Science and Technology*, vol. 46, no. 3, p. 661-668. http://dx.doi.org/10.1111/j.1365-2621.2010.02547.x

Marpalle, P., Sonawane, S. K, Shalini Subhash Arya 2014. Effect of flaxseed flour addition on physicochemical and sensory properties of functional bread. *LWT – Food Science and Technology*, vol. 58, no. 2, p. 614.619. http://dx.doi.org/10.1016/j.lwt.2014.04.003

Mueller, K., Eisner, P., Yoshie-Stark, Y., Nakada, R., Kirchhoff, E. 2010. Functional properties and chemical composition of fractionated brown and yellow linseed meal (*Linum usitatissimum* L.). *Journal of Food Engineering*, vol. 98, no. 4, p. 453-460. http://dx.doi.org/10.1016/j.jfoodeng.2010.01.028

Nitrayová, S., Brestenský, M., Heger, J., Patráš, P., Rafay, J., Sirotkin, A. 2014. Amino acids and fatty acids profile of chia (*Salvia hispanica* L.) and flax (*Linum usitatissimum* L.) seed. *Potravinarstvo*, vol. 8, no. 1, p. 72-76. http://dx.doi.org/10.5219/332

Švec, I., Hrušková, M. 2016. Statistical description of RVA profiles of composite blends with flax fibre, In Proceedings of the Ingrovy dny 2016, MU Brno, 7 p.

UKZUZ Brno, 2015. *Seznam doporučených odrůd – Olejniny (Catalogue of oil plant varieties)*. 1st ed., Brno, Česká republika: Vydavatelství ÚKZÚZ Brno, 132 p.

Vítová, M. 2007. *Pasta laboratory test*: bachelor thesis [In Czech]. Prague, Czech Republic: University of Chemistry and Technology Prague, 74 p.

Xu, Y., Hall III, C. A., Manthey, F. A. 2014. Effect of flaxseed flour on rheologial properties of wheat flour dough and on bread characteristics. *Journal of Food Research*, vol. 3, no. 6, p. 83-91. http://dx.doi.org/10.5539/jfr.v3n6p83

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EFFECT OF SPICES COMMERCIAL MIXTURE WITH GLUCONO-DELTA-LACTONE ON THE QUALITY OF FERMENTED DRY-CURED SAUSAGES

Miroslav Kročko, Margita Čanigová, Viera Ducková, Ondřej Bučko

ABSTRACT

The main fermented meat products are fermented sausages in which lactic acid bacteria (LAB) are the essential agents of the ripening process. Their application as starter organisms ensures the dominance of the starter during the whole ripening process. However, when no starter cultures are used, direct addition of acids like a glucono-delta-lactone (GdL) is preferred. The goal of this study was to determine the influence of commercial spices mixtur (containing GdL) on selected technological parameters of fermented dry-cured sausages – Danube sausage in comparison with currently available conventional spices. Comparison was evaluated also with addition of starter cultures. Determinations of technological (value of pH, water activity, color) and microbiological properties (count of *Lactobacillus* spp., *Enterobacteriaceae* family, yeasts and moulds) were realized after 24 hours, 5 and 30 days. The sensory analysis of sausages was carried out after 30 days of ripening process. In sausages with the addition of commercial spice mixture in combination with starter culture were determined the lowest values of pH and a_w at the end of ripening process (30 days). Bacteria of *Enterobacteriaceae* family were occurred in the samples with the addition of currently available conventional spices at the beginning of ripening, but after 5 days of ripening were bacteria of this family not detected. The counts of yeasts in analyzed samples were not detected. Counts of LAB at the end of ripening proccess (30 days) were lower in coparison with result obtained after 5 days; however their count was comparable with count determined at the beginig of the ripening. Our results show, that the combination of starter culture and commercial spice mixture containig GdL may cause excesive sour taste and sensory defect of dry fermented meat products.

Keywords: GdL; dry-fermented sausages; starter culture; colour; sensory evaluation

INTRODUCTION

Nowdays, fermented sausage production can be considered more than a method of preservation – as, instead, a process of transformation, diversification which is strongly linked to culture and tradition of individual countries **(Fernández el al., 2000, Liu et al., 2011)**.

Fermented meat is produced with the addition of microbes when different condiments are mixed together with meat. The microbiota involved in the fermenting process is diverse and complex, and closely related to the ripening technique. Lactic acid bacteria (LAB) are usually present in high hygienic quality raw meat at low amounts and dominate the fermentation later **(Tu, et al., 2010)**. Their presence effectively prevents harmful bacteria growth and controls the fermentation processes. During the fermentation, acids and alcohols are produced, leading to a decrease of pH **(Xu, et al., 2008)**. *Lactobacillus* species are the most prevalent microorganisms in dry fermented sausages, and their use as starter cultures is widespread **(Hammes et al., 1990)**. Even though lactic acid bacteria are known as weak lipolytic and proteolytic organisms **(Johansson et al., 1994).**

In many cases, particularly when no starter cultures are used, direct addition of acids is preferred in order to assure pH lowering within a very short time. Common organic acids are used for this purpose, mainly lactic and citric acids, as well as their sodium and potassium salts, which show much less ability to lower pH values. Besides these, an acid-related molecule may also be used: glucono-delta-lactone (GdL) **(Toldrá, 2007)**.

GdL is a Generally Recognized as Safe (GRAS) substance and is a weak acid, which converts to gluconic acid in water and slowly dissociates into hydrogen ions with time **(Chang et al., 2009)**. After all, GdL slowly hydrolyzes to gluconic acid with a resulting reduction in pH, which finally causes the residual nitrite reduction **(Juncher et al., 2000)**. However, GdL does not control the indigenous flora, and consequently, using only GdL might result in post-acidification giving fault fermentation and sensory drawbacks **(Andersen and Cislaghi, 2007)**. Using a combination of starter culture and spices has resulted in changes in certain microbial properties, change in free fatty acids and effectively in product lowers the pH and a_w **(Zhao et al., 2011)**. Therefore, the aim of this study was to evaluate the effect of commercial spice mixture containing GdL in combination with starter culture on physical, microbiological and sensory characteristics of fermented sausages during ripening.

MATERIAL AND METHODOLOGY

Occurrence of GdL in spice mixture was determined by electrophoretic analyzer (Villa Labeco, Slovakia)

Pork and beef lean meat in ratio 2 : 1 in combination with back pork fat (30%) were trimmed and cured (2.0% salt and 0.01% nitrite). Then the cured trimmed meat mix was divided into four equal parts. Currently available conventional spices paprika (100 $g.kg^{-1}$), pepper (4 $g.kg^{-1}$), caraway (4 $g.kg^{-1}$), garlic (10 $g.kg^{-1}$), spicy paprika (4 $g.kg^{-1}$) were added to the first (C) and second parts (CC) of sausage mixture. Commercial spice mixture designed for meat processing plants contain GdL, was added to the third (M) and fourth part (MC) of sausage mixture. Added conventional spices and commercial spice mixture came from the same manufacture company and were sold under the same brand. Furthermore, second part (CC) and fourth part (MC) of sausage mixture contains starter culture Lyocarni SHI-59 (Clerici Sacco, Italy) in amount 0.2 $g.kg^{-1}$. Each part of sausage mixture was separately minced (4mm blade) and filled into 34 mm natural sausage casings, smoked and ripened in climatic chamber for 30 days.

Determinations of technological and microbiological properties were realized after 24 hours, 5 and 30 days.

Determination of pH value:

The pH value of Danube sausages was measured using a Gryf 209 (Gryf HB, Czech Republic) apparatus during whole period of ripening.

Determination of color: Color spaces L*, a*, b* was determined by CM 2600D spectrophotometer (Konica Minolta, Germany) after homogenization of samples. Color on the surface of homogenized sausages was measured with SCE (Specular Component Excluded).

Determination of a_w was determined by Testo 645 (Germany).

Microbioological examination

The samples of sausages (5 g) were taken after specified storage periods and homogenized in saline for 30 second by apparatus Heidolph DIAX 900 (*Heidolph*, Germany). The samples for enumeration of mesophilic bacteria (MBC) were cultured on selective diagnostic plate count agar (*Biokar Diagnostic*, France) at temperature 30 ±1 °C for 72 hours. The samples without addition of starter cultures (M and C) for enumeration of indigenous lactic acid bacteria count (LAB) were cultured on MRS agar (*Himedia*, India) at temperature 30 ±1 °C for 5 days.

The samples with addition of starter cultures (CC and MC) for enumeration of lactic acid bacteria count of genus *Lactobacillus* (LAB) were cultured on MRS agar (*Himedia*, India) at temperature 37 ±1 °C for 5 days in anaerobic conditions (Anaerogen, Oxoid UK). Count of *Enterobacteriaceae* family (ETB) was determined on VRBG agar (*Himedia*, India) at temperature 37 °C after 24 hours of cultivation. Count of yeasts and moulds were determined on DRBC and DG18 agar (Merck, Germany) at temperature 25 °C after 5 days.

Sensory evaluation

The sensory analysis of sausages was carried out after 30 days of ripening process. Samples of sausages before and after heat treatment (heating in 80°C water, while in core of sausages reached the temperature 70 °C for 10 minutes) were evaluated by a 6-member semi-trained panel of laboratory co-workers. Panelists evaluate, color, aroma, taste on 5-point hedonic scale where 1 (the worst) and 5 (the best) were the extremes of each characteristic.

RESULTS AND DISCUSSION

The following 4 types of sausages were evaluated:

- sample C – sausages with addition of conventional spices,
- sample M – sausages with addition of commercial spice mixture (with GdL),
-sample CC – sausages with addition of conventional spices and starter culture,
-sample MC – sausages with addition of commercial spice mixture (with GdL) and starter culture.

The lowest values of pH in samples of fermented sausages were determined after 5 days of ripening. The lowest pH values (5.25) after 30 days of ripening were determined in the samples of M and MC fermented sausages (Figure 1). According to Slovak decree no. 1895/2004-100, the both samples containing commercial spices mixture (M and MC) may be included into subgroups with pH value below 5.5, and mark them as fermented products. The products C and MC were classified according to pH and a_w value to subgroups dried (pH 5.5 to 6.2). The higher pH value of the sample C may be due to indigenous lactobacilli with the low acidifying ability **(Casaburi et al., 2007)**.

Continuous decline of the water activity was observed during whole ripening process. The most significant decrease of water activity was found in products M. The water activity after 24 hours in all samples was

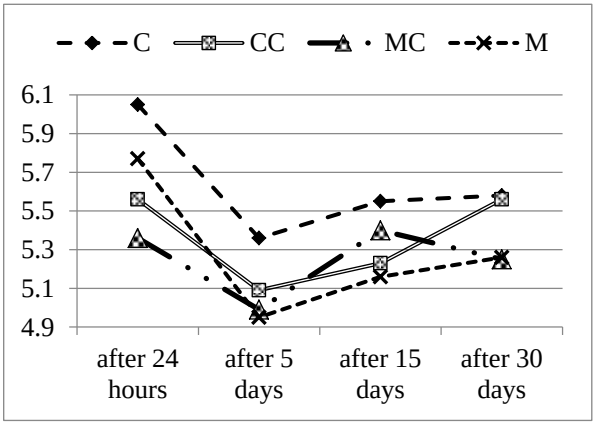

Figure 1 Changes of pH values during ripening of Danube sausages.

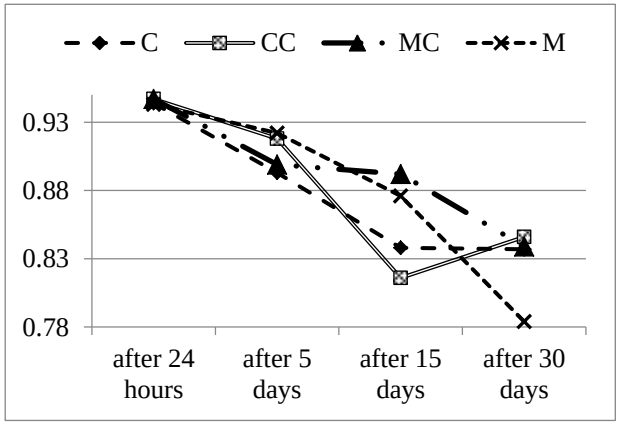

Figure 2 Changes of a_w values during ripening of Danube sausages.

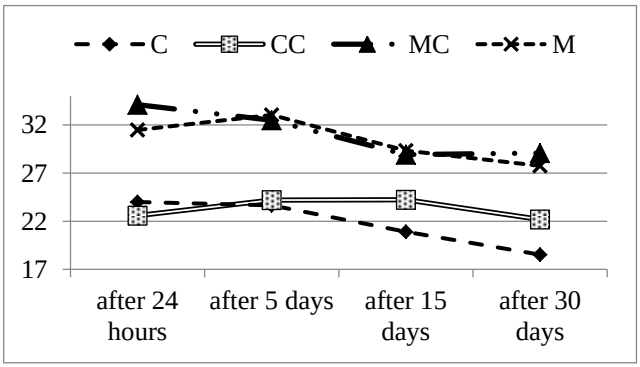

Figure 3 Intensity of red color (a*) during ripening of fermented sausages.

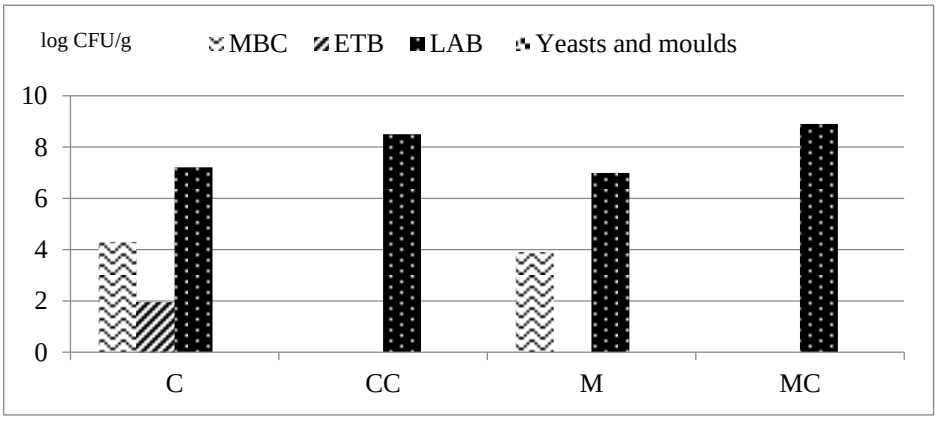

Figure 4 Occurrence of microorganisms in fermented sausages after 24 hours of ripening.

characterized by values close to the level which is an average value of 0.945 (Figure 2).

Intensity of red color (a *) during the whole period of ripening was determined in fermented sausages with the addition of commercial spice mixture, regardless of the addition of starter culture (CM and MC). These results are in contrary with previous studies **(Hong et al., 2008; Hong and Chin 2010)** noted increasing the GdL level significantly decreased the a* value (redness) of the fermented meat products.

The cause is probably due to the addition of colorant cochineal also called carmine (E 120) in a commercial spice mixture.

The addition of starter culture to the products with the addition of currently available conventional spices (CC) also increased the intensity of the red color (Figure 3).

In products with only the addition of currently available conventional spice (C) was found after 24 hours the number of microorganisms of the family *Enterobacteriaceae* 1.94 log CFU.g^{-1} (Figure 4). During the next period of maturation were not bacteria of this family detected. The reason for the occurrence of the family *Enterobacteriaceae* could be contamination of traditional spices used in manufacture.

In the all samples of fermented sausages count of LAB up to 5 days of ripening increased. Counts of LAB at the end of ripening proccess (30 days) were lower in coparison with result obtained after 5 days; however their count was comparable with count determined at the beginig of the ripening. Addition of commercial spice mixture which contains GdL had no effect on the count of LAB in the samples of fermented sausages during whole period of ripening proccess.

The rapid growth of LAB in the initial stage of fermentation is beneficial in reducing the pH of the fermented product and inhibits undesirable bacteria such as microorganisms of the family *Enterobacteriaceae* **(Essid and Hassouna, 2013)**.

Occurrences of yeasts in fermented meat products were not detected. According to **Fernández-López et al. (2008)** the number of yeast in fermented meat products stored 30 days is usual. Yeasts are characterized by lipolithic activity of secondary importance and can also contribute to the formation of an organoleptic profile of the final products. The sensory evaluation of fermented sausages was in raw state (uncooked), which was supposed to evoke the same possibilities for evaluating as consumers in stores. The highest values for appearance were after 30 days of ripening assigned to both samples of fermented sausages with addition of starter cultures (CC, MC). The products with added conventional spices (C) and commercial spice mixtures (M) without addition of starter cultures were characterized by strongly wrinkled surface and yellowish fat grains. The characterized intensity of red color in sliced samples was determined in sausages with added commercial spice mixtures (M, MC). Fermented sausages produced with currently available conventional spices (C), were characterized by a weaker shade color after red paprika. In the final assessment of raw sausages without heat treatment were the highest values for overall

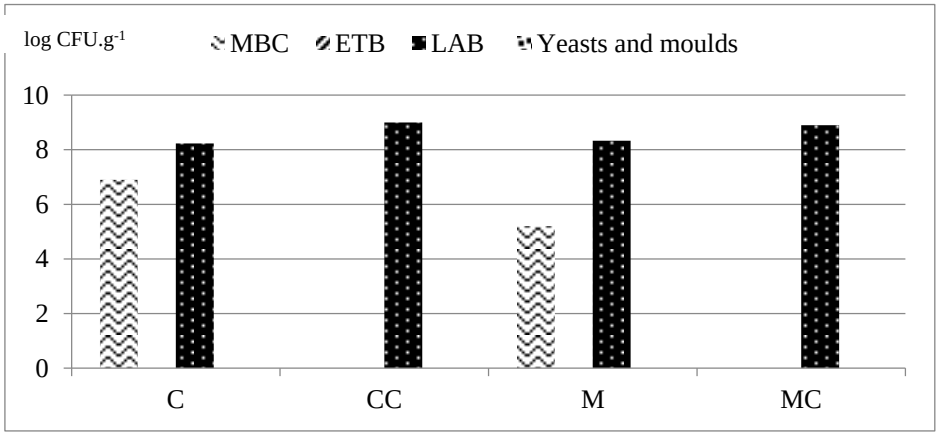

Figure 5 Occurrence of microorganisms in fermented sausages after 5 days of ripening.

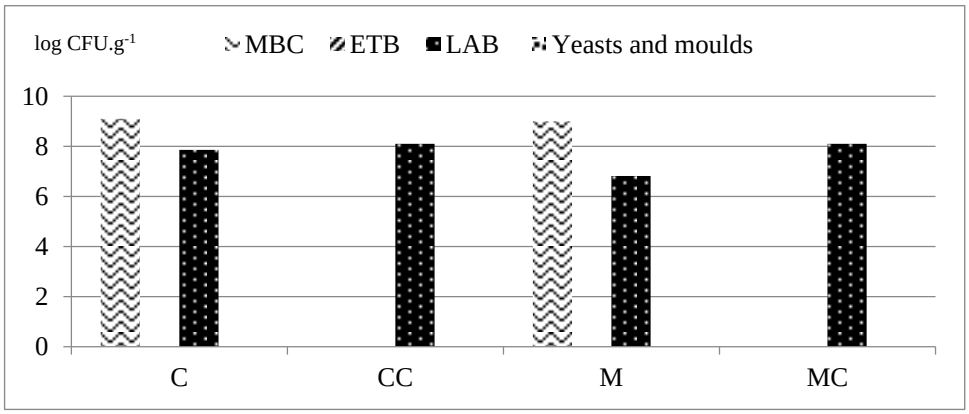

Figure 6 Occurrence of microorganisms in fermented sausages after 30 days of ripening.

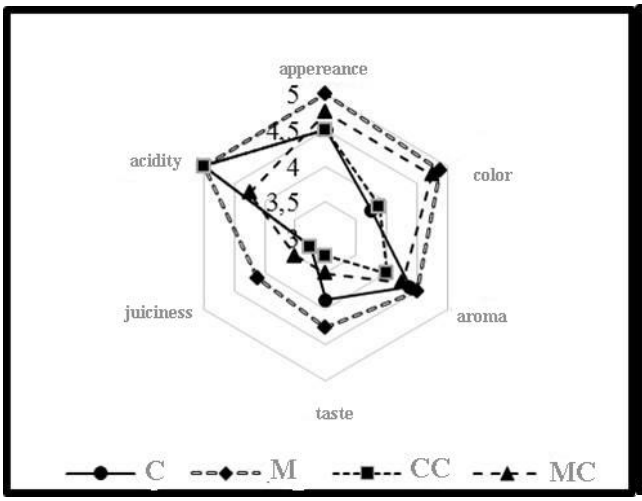

Figure 7 Sensory analyses of fermented sausages before heat treatment.

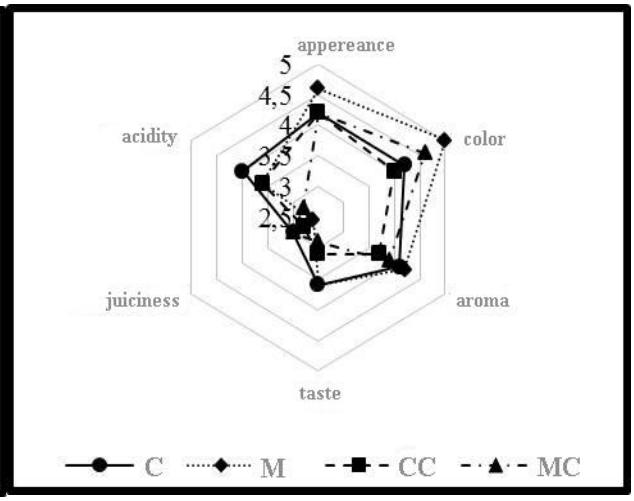

Figure 8 Sensory analyses of fermented sausages after heat treatment.

acceptability assigned to the samples with the addition of commercial spice mixture with combination of starter culture (MC).

The highest intensity of characteristic odor evaluated after heat treatment was found in products using currently available conventional spices (C) and commercial spice mixtures (M) without addition of starter cultures. On the other hand sausages with commercial spice mixture and starter culture (MC) due to sour taste reached the lowest values for overall taste acceptability. Increased sour taste of these products was probably caused by combining of commercial spice mixture containing GdL and starter culture. Also **Schillinger and Luecke, (1989)** and **Feiner (2006)** reported that application of GdL might negatively influence the taste (metallic off-flavour and bitterness), texture (grittiness), and color (paleness) of the salami relative to the amount of GdL added. Furthermore, high levels of GdL might promote growth of peroxide-forming lactobacilli resulting in rancidity and further color problems.

CONCLUSION

Composition of commercial spice mixtures designated for the manufacture of meat products is currently considered as know-how of manufacturing companies. Therefore, the packaging of these spice products does not provide the exact composition. It is necessary to label on the package of commercial spice mixtures the presence and quantity of GdL, especially in production where is probably posibility to use starter cultures. Our results show, that the combination of starter culture and commercial spice mixture containig GdL may cause excesive sour taste and sensory defect of dry fermented meat products.

REFERENCES

Andersen, L., Cislaghi, S. 2007. Comparison between using starter culture, GdL, and a combination o both in the production of salami. *Biotechnology in Animal Husbandry*, vol. 23, no. 5-6, p. 283-290.

Casaburi, A., Aristoy, M. C., Cavella, S., Di Monaco, R., Ercolini, D., Toldrá, F., Villani, F. 2007. Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures. *Meat Science*, vol. 76, no 2, p. 295-307. http://dx.doi.org/10.1016/j.meatsci.2006.11.011

Essid, I., Hassouna, M. 2013. Effect of inoculation of selected *Staphylococcus xylosus* and *Lactobacillus plantarum* strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage. *Food Control*, vol. 32, no. 2, p. 707-714. http://dx.doi.org/10.1016/j.foodcont.2013.02.003

Fernández-López, J., Sendra, E., Sayas-Barberá, E., Navarro, C., Pérez-Alvarez, J. A. 2008. Physico-chemical and microbiological profiles of „salchichón“ (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Science*, vol. 80, no. 2, p. 410-417. http://dx.doi.org/10.1016/j.meatsci.2008.01.010

Feiner, G. 2006, *Meat products handbook*. Cambridge : Woodhead Publishing, England, 672 p. ISBN 978-1-84569-050-2.

Fernández, L., Ordóñez, J. A., Bruna, J. M., Herranz, B., De La Hoz, L. 2000. Accelerated ripening of dry fermented sausages. *Trends in food science and technology*, vol. 11, no. 6, p. 201-209. http://dx.doi.org/10.1016/S0924-2244(00)00077-7

Hammes, W. P., Bantleon, A., Min, S. 1990. Lactic acid bacteria in meat fermentation. *FEMS Microbiology Reviews*, vol. 87, p. 165-174. http://dx.doi.org/10.1016/0378-1097(90)90704-T

Hong, G. P., Chin, K. B. 2010. Evaluation of sodium alginate and glucono-d-lactone levels on the cold-set gelation of porcine myofibrillar proteins at different salt concentrations. *Meat Science*. vol. 85, no. 2, p. 201-209. http://dx.doi.org/10.1016/j.meatsci.2009.12.026

Hong, G. P., Ko, S. H., Choi, M. J., Min, S. G. 2008. Effect of glucono-δ-lactone and j-carrageenan combined with high pressure treatment on the physico-chemical properties of restructured pork. *Meat Science*, vol. 79, no. 2, p. 236-243. http://dx.doi.org/10.1016/j.meatsci.2007.09.007

Chang, Y. H., Su, H. J., Shiau, S. Y. 2009. Rheological and textural characteristics of black soybean touhua (soft soybean curd) prepared with glucono-d-lactone. *Food Chemistry*, vol. 115, no. 2, p. 585-591. http://dx.doi.org/10.1016/j.foodchem.2008.12.056

Johansson, G., Berdagué, J. L., Larsson, M., Tran, N., Borch, E. 1994. Lipolysis, proteolysis and formation of volatile components during ripening of a fermented sausage with *Pediococcus pentosaceous* and *Staphylococcus xylosus* as starter cultures. *Meat Science*, vol. 38, p. 203-218. http://dx.doi.org/10.1016/0309-1740(94)90110-4

Juncher, D., Vestergaard, C. S., Jensen, J. S., Weber, C. J., Bertelsen, G., Skibsted, L. H. 2000. Effects of chemical hurdles on microbiological and oxidative stability of a cooked cured emulsion type meat product. *Meat Science*, vol. 55, no. 4, p. 483-491. http://dx.doi.org/10.1016/S0309-1740(00)00011-5

Liu, S., Han, Y., Zhou, Z. J. 2011. Lactic acid bacteria in traditional fermented Chinese foods. *Food Research International*, vol. 44, no. 3, p. 643-651. http://dx.doi.org/10.1016/j.foodres.2010.12.034

Schillinger, U., Luecke, F. K. 1989, Influence of pH on the drying pattern in dry sausages. *Fleischwirtschaft*, vol. 69, no. 5, p. 879-882.

Toldrá, F. 2007. *Handbook of fermented meat and poultry*. Iowa : Blackwell Publishing, USA. 555 p. ISBN 978-0-8138-1477-3.

Tu, R. J., Wu, H. Y., Lock, Y. S., Chen, M. J. 2010. Evaluation of microbial dynamics during the ripening of a traditional Taiwanese naturally fermented ham. *Food Microbiology*, vol. 27, no. 4, p. 460-467. http://dx.doi.org/10.1016/j.fm.2009.12.011

Xu, H. Q., Wang, W. W., Jiang, Y. S., Wang, Z. J. 2008. Study on fermentation properties of lactic acid bacteria isolated from traditional fermented meat products. *Science and Technology of Food Industry (Chinese)*, vol. 1, no. 29, p. 88-92.

Zhao, L., Jin, Y., Ma, C., Song, H., Li, H., Wang, Z., Xiao, S. 2011. Physico-chemical characteristics and free fatty acid composition of dry fermented mutton sausages as affected by the use of various combinations of starter cultures and spices. *Meat Science*, vol. 88, no. 4, p. 761-766. http://dx.doi.org/10.1016/j.meatsci.2011.03.010

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QUERCETIN-INDUCED CHANGES IN FEMORAL BONE MICROSTRUCTURE OF ADULT MALE RABBITS

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ABSTRACT

Flavonoids are a group of plant metabolites with antioxidant effects. One of the most abundant flavonoids in the human diet is quercetin. It is found widely in fruits, vegetables and has a lot of beneficial effects on human health. Quercetin has a positive pharmacological effect on bone metabolism and it prevents the organism against bone loss. However, its impact on the size of basic structural units of the compact bone is still unknown. Therefore, the aim of present study was to investigate the impact of the quercetin on femoral bone microstructure in 5-month-old male rabbits. Five rabbits of Californian broiler line were randomly divided into two groups. In the experimental group (E group; n=3), animals were intramuscularly injected with quercetin at dose 1000 $\mu g.kg^{-1}$ body weight (bw) for 90 days, 3 times per week. Two rabbits without quercetin administration served as a control group (C group). According to our results, intramuscular application of quercetin had an insignificant effect on cortical bone thickness in male rabbits. In these rabbits, changes in qualitative histological characteristics were present in the middle part of the compacta, where primary vascular longitudinal bone tissue was present and expanded there from the periosteum. Also, a lower number of secondary osteons was found in these animals. From the histomorphometrical point of view, significantly decreased sizes of primary osteons' vascular canals and secondary osteons (p <0.05) were found in rabbits administered by quercetin. Our findings indicate that subchronic administration of quercetin at the dose used in our study had considerable impact on both qualitative and quantitative histological characteristics of the compact bone in adult male rabbits.

Keywords: quercetin; femoral bone; histomorphometry; rabbit

INTRODUCTION

Flavonoids are a group of natural polyphenolic substances which consists of two aromatic rings linked by 3 carbons, usually in an oxygenated heterocycle ring **(Liu, 2004)**. These aromatic secondary plant metabolites have been recognized as important bioactive compounds due to their physiological and pharmacological role, and their health benefits **(Hooper and Cassidy, 2006)**. Fruits and vegetables, tea, and cocoa are rich natural sources of flavonoids **(Chen et al., 2010; Egert and Rimbach, 2011; Sekeroğlu and Sekeroğlu, 2012)**. In human diet, one of the most important vegetable with rich content of antioxidant polyphenols is onion. The results by **Kavalcová et al. (2015)** showed that a higher content of polyphenols and thus, a higher antioxidant activity have more colorful varieties of onions. According to **Danihelová and Šturdík (2011)**, average daily intake of flavonoids is strongly dependent on individual, country and culture usages. It is approximately in the range of 150 to 300 mg/day

In nature, flavonoids are most frequently found as conjugates in glycosylated or esterified forms; however, they can occur as aglycones, especially as a result of the effects of food processing **(Aggarwal and Heber, 2014)**. Many flavonoids are shown to have antioxidative activity, free-radical scavenging capacity, coronary heart disease prevention, and anticancer activity **(Yao et al., 2004)**. Their antioxidant capacity is associated with the presence of series of structural characteristics (most probably related to the phenolic hydroxyl groups attached to the ring structure) that allow them to quelate ions of transition metals such as Fe^{2+}, Cu^{2+}, or Zn^{2+} and to catalyze the electron transport **(Braun et al., 2011)**. Moreover, they are able to inhibit lipid peroxidation and platelet aggregation and improve increased capillary permeability and fragility **(Hubbard et al., 2004; Cirico and Omaye, 2006)**.

In the recent past, dietary supplements of flavonoids, as their alternative sources, have become increasingly popular. However, it is important to point out that natural sources of flavonoids contain a complex mixture of secondary plant metabolites and not only flavonoids *per se* **(Crassidy et al., 2011)**. This complex mixture cannot be simply exchanged by single purified substances as dietary supplements. Therefore, it is very essential to evaluate possible adverse effects of purified flavonoids as dietary supplements on human health. Indeed, there is growing evidence that purified flavonoids given in high doses may affect trace element, folate, and vitamin C status. Also, they can exhibit antithyroid and goitrogenic activities **(Egert and Rimbach, 2011)**.

One of the most widely distributed flavonoid in plants is quercetin (3, 3', 4', 5, 7-pentahydroxyflavone; **Liang et al., 2011**). Quercetin is found in many common foods including apples, tea, onions, nuts, berries, cauliflower, cabbage and many other foods **(Lakhanpal and Rai, 2007)**. The normal intake of quercetin is less than 5-40 mg/day. However, people who eat the peel of food with high amounts of quercetin may consume 200-500 mg/day **(Harwood et al., 2007)**. Only 30-50% of ingested quercetin is absorbed, the rest passes through gastro-intestinal tract **(Ross and Kasum, 2002)**.

Quercetin has a broad range of significant health promoting properties **(Agullo et al. 1997; Verhoeyen et al., 2002; Boots et al., 2007)**. According to several authors **(Formica and Regelson, 1995; Manach et al., 1996; Boik, 2001; Satyanarayana et al., 2001; Brookes et al., 2002; Davis et al., 2009; Wein et al., 2013; Wu et al., 2014; Forte et al., 2016)** quercetin has cardioprotective, anticarcinogenogenic, antioxidant, anti-inflammatory, antibacterial and antiapoptic properties. It facilitates apoptosis of tumor cells, in part through depression of an endogenous cytoprotective molecule, heat shock protein 70 **(Hosokawa et al., 1990)**. As well, quercetin may inhibit apoptosis in some nontumorigenic cells. For example, quercetin inhibits hydrogen peroxide (H_2O_2) induced apoptosis of mesangial cells, fibroblasts and epithelial cells **(Ishikawa and Kitamura, 2000)**.

This flavonoid also disposes reactive oxygen species (ROS) and reactive nitrogen species (RNS) scavenging activity **(Heijnen et al., 2001; Nickel et al., 2011; Dehghan and Khoshkam, 2012)** under *in vitro* and *in vivo* conditions **(Choi et al., 2001; Nabavi et al., 2012)**. Therefore, it has often been associated with the reduced risk of oxidative-stress related chronic diseases such as coronary heart disease, stroke and diabetes **(Skibola and Smith, 2000)**.

On the other hand, quercetin has potentially toxic effects, including its mutagenicity, prooxidant activity, mitochondrial toxicity, and inhibition of key enzymes involved in hormone metabolism **(Okamoto, 2005; Zhang et al., 2009)**. **Dunnick and Hailey (1992)** reported that high doses of quercetin over several years might result in the formation of tumors in the kidney of rats. The results by **Rise et al. (2006)** showed that quercetin can modulate ovarian functions by interfering with cell steroidogenic activity and angiogenic activity. Quercetin can also be a potential neurotoxic substance **(Jakubowicz-Gil et al., 2008)**. According to **Robaszkiewicz et al. (2007)**, quercetin-induced antioxidant or prooxidant effects are largely relates to its dose given to biological system. At concentrations > 50 μM, quercetin is able to participate in the oxidation of NADPH in liver cells, shifting the cellular conditions to a more oxidized states **(Buss et al., 2005).**

Regarding the bone, quercetin has a positive pharmacological effect on bone metabolism and it prevents the organism against bone loss **(Boots et al., 2007; Sharan et al., 2011)**. It inhibits mRNA expression of osteoclast-related genes and osteoclast differentiation, thereby reduces bone resorption **(Guo et al., 2012)**.

The studies by **Notoya et al. (2004)** and **Wattel et al. (2004)** revealed that inhibitory potential of quercetin on *in vitro* osteoclastic differentiation is connected via a mechanism involving NF kappa B and activator protein 1 (AP-1). Also, increased alkaline phosphatase activity in MG-63 osteoblasts followed by quercetin application was demonstrated **(Robaszkiewicz et al., 2007)**. **Zhou and Lin (2014)** reported that quercetin could enhance the osteogenic differentiation of adipose-derived stem cells (ASCs) and osteoblastic MC3T3-E1 cells and inhibit osteoclastogenesis in RAW 264.7 cells. Moreover, it could stimulate Osterix (Osx), BMP-2, Runx2, OCN, OPN, COL1 and ALP gene expression in ASCs, and increase bone sialoprotein (BSP) and OCN gene expression in osteoblastic MC3T3-E1 cells **(Kim et al., 2006; Satué et al., 2013)**. However, the effect of quercetin on osteoblast function is contradictory **(Yamaguchi and Weitzmann, 2011)**. According to **Prouillet et al. (2004)** it stimulates proliferation and differentiation of rat calvarial osteoblasts and MG-63 osteoblast-like cells. **Braun et al. (2011)** have found protective effect of quercetin on primary human osteoblasts against the toxic influence of cigarette smoke. This fact indicates that a dietary supplementation with quercetin could improve bone structure, skeletal integrity, and even fracture healing in smokers. On the contrary to above findings, **Kanno et al. (2004)** mention that quercetin induces apoptosis of MC3T3-E1 mouse calvarial osteoblasts. **Notoya et al. (2003)** found that it inhibited not only the proliferation but also the differentiation and mineralization of of rat calvarial osteoblast-like cells (ROB cells; **Hagiwara et al., 1996; Partridge et al., 1981)**. Quercetin-induced apoptosis (through a mitochondria-dependent mechanism involving ERK activation) and inhibition of migration (through activation of ERK and p38 pathways) of osteoblasts were also showed in the research by **Nam et al. (2008)**.

The impact of quercetin on histomorphometry of basic structural units of the compact bone is still unknown. Therefore, the aim of our study was to investigate the effect of intramuscular application of quercetin on femoral bone microstructure in adult male rabbits.

MATERIAL AND METHODOLOGY

Our research was carried out on five male rabbits of meat line M91, maternal albinotic line (crossbreed New Zealand White, Buskat rabbit, French Silver) and paternal acromalictic line (crossbreed Nitra's rabbit, Californian rabbit, Big Light Silver) of approximately 5 months of age, with a body weight 4.00 ±0.5 kg. Animals were obtained from an experimental farm of the Animal Production Research Centre in Nitra (Slovak Republic) and were housed in individual flat-deck wire cages. The animals were maintained under constant conditions of light (12-h light/12-h dark), temperature (20-24 °C) and humidity (55% ±10%), with access to food (feed mixture) and drinking water *ad libitum*. The rabbits were randomly assigned into two groups. In the first group (E group; n=3), quercetin was applied intramuscularly in the concentration of 1000 $\mu g.kg^{-1}$bw 3 times per week, for 90 days. The dose of quercetin (reflecting the constant exposure of animals to quercetin in rabbit feed) was chosen based on the literature data **(Choi and Li, 2005; Knab et al., 2011; Lesniak-Walentyn et al., 2013)**. Two rabbits without quercetin application served as a control group (C group). Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures were approved by the State Veterinary and Food Institute

of Slovak Republic, no. 3398/11-221/3 and ethics committee.

At the end of experimental period (after 90 days), all rabbits were killed and their femora were used for histological analyses. Thin sections from femora were prepared according to the methodology of **Martiniaková et al. (2008)**. The qualitative histological characteristics of compact bone were determined according to the internationally accepted classification systems of **Enlow and Brown (1956) and de Ricqlés et al. (1991),** who classified bone tissue into three broad categories: primary vascular tissue, non-vascular tissue and Haversian bone tissue. The quantitative (histomorphometrical) variables were assessed using the software Motic Images Plus 2.0 ML (Motic China Group Co., Ltd.). We measured area, perimeter, and minimum and maximum diameters of primary osteons' vascular canals, Haversian canals, and secondary osteons in all fields (i.e., anterior, posterior, medial and lateral) of the thin sections. The diaphyseal cortical bone thickness was also measured by Motic Images Plus 2.0 ML software. Twenty random areas were selected, and average thickness was calculated for each femur.

Statistical analysis was performed using SPSS 8.0 software. All data were expressed as mean ±standard deviation (SD). The unpaired t-test was used for establishing statistical significance (p <0.05) between both groups of rabbits.

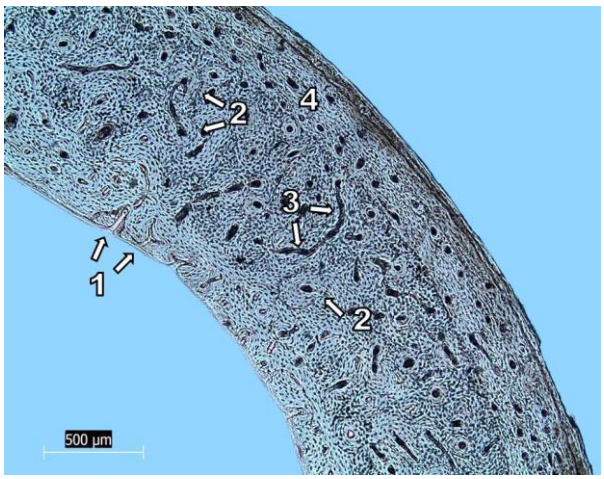
RESULTS AND DISCUSSION

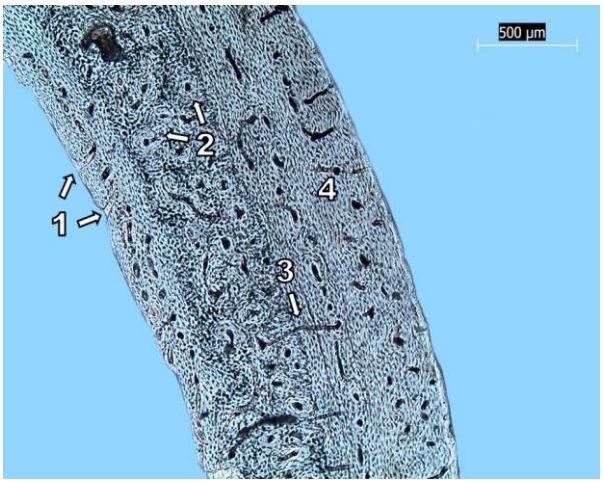
Our results showed an insignificant effect of quercetin on cortical bone thickness in male rabbits (1035.56 ±159.42 μm and 1025.06 ±209.09 μm in rabbits from E and C groups, respectively).

Compact bone microstructure in rabbits from C group (Fig.1) was formed near endosteal bone surfaces by primary vascular radial, irregular Haversian and/or dense Haversian bone tissues. The middle part of the compact bone considered of a layer of irregular and/or dense Haversian bone tissues. Secondary osteons were often connected with Volkmann's canals. The periosteal bone surface mostly consisted of primary vascular longitudinal bone tissue; irregular Haversian bone tissue was observed only in anterior side. These findings are consistent with the results of several authors **(Enlow and Brown, 1958; Martiniaková et al., 2003; Martiniaková et al., 2006)**.

In rabbits from E group, endosteal bone surface was composted by primary vascular radial and irregular Haversian bone tissues. Primary vascular longitudinal bone tissue was in some areas (anterior and posterior) near endosteal surface completely resorbed. The rabbits intramuscularly administered by quercetin had fewer secondary osteons in the middle part of *substantia compacta* because primary vascular longitudinal bone tissue expanded into this part of bone from periosteum. The periosteal border was formed only by primary vascular longitudinal bone tissue (Fig. 2).

Intramuscular administration of quercetin caused evident alterations in femoral bone microstructure of male rabbits. A lower number of secondary osteons in the middle parts of the *substantia compacta* could be associated with accelerated bone resorption. ROS have been reported to play a crucial role in the process of bone resorption **(Halliwell et al., 1992; Yang et al., 1998)**. During this process, osteoclasts produce large amounts of ROS, and their excessive accumulation inhibits bone formation and stimulates further bone resorption **(Baek et al., 2010)**. Quercetin has been described as the protector against ROS RNS **(Nickel et al., 2011; Kovacevic and Matulic, 2013)**. However, quercetin has the potential to produce ROS at high doses **(Rahman et al., 1992)**. In the process of scavenging reactive species, quercetin may be converted into potentially harmful oxidation products or subjected to *in vitro* oxidative degradation resulting in the formation of an ortho-quinone and the subsequent production of ROS (i.e., superoxide and hydrogen peroxide; **Boots et al., 2003**). The resultant prooxidant properties of quercetin are responsible for its mutagenic and cytotoxic effects **(Sahu**





1 - primary vascular radial bone tissue, 2 - several secondary osteons form irregular Haversian bone tissue,
3 - Volkmann's canals, 4 - primary vascular longitudinal bone tissue

Figure 1 Microstructure of femoral bone in rabbits from control (C) group.

Figure 2 Microstructure of femoral bone in rabbits from experimental (E) group.

Table 1 Data on vascular canals of primary osteons in male rabbits from C and E groups.

Rabbit's group	N	Area (μm^2)	Perimeter (μm)	Max. diameter (μm)	Min. diameter (μm)
C	80	344.68 ±50.07	66.43 ±4.78	11.48 ±0.99	9.60 ±0.98
E	120	317.35 ±51.82	63.80 ±5.11	11.10 ±1.00	9.13 ±1.00
t-test		***p* <0.05**	***p* <0.05**	**NS**	***p* <0.05**

Note: N: number of measured structures; NS: non-significant changes.

Table 2 Data on Haversian canals in male rabbits from C and E groups.

Rabbit's group	N	Area (μm^2)	Perimeter (μm)	Max. diameter (μm)	Min. diameter (μm)
C	80	322.15 ±65.07	64.25 ±6.53	11.13 ±1.35	9.20 ±1.19
E	120	301.32 ±56.49	62.20 ±6.06	10.82 ±1.37	8.90 ±0.98
t-test		**NS**	**NS**	**NS**	**NS**

Note: N: number of measured structures; NS: non-significant changes.

Table 3 Data on secondary osteons in male rabbits from C and E groups.

Rabbit's group	N	Area (μm^2)	Perimeter (μm)	Max. diameter (μm)	Min. diameter (μm)
C	80	5979.63 ±2816.19	273.19 ±60.51	47.97 ±11.30	38.12 ±9.18
E	120	4629.72 ±1888.92	244.67 ±45.93	43.81 ±8.79	32.86 ±8.00
t-test		***p* <0.05**	***p* <0.05**	**NS**	***p* <0.05**

Note: N: number of measured structures; NS: non-significant changes.

and Washington, 1991; Soria et al., 2010). So, it can indicate toxicity in that case, such as an induction of apoptosis of osteoblasts **(Notoya et al., 2003; Spencer et al., 2003; Son et al., 2008)**.

From histomorphometrical point of view, 200 vascular canals of primary osteons, 200 Haversian canals, and 200 secondary osteons were measured in rabbits from E and C groups. The results are summarized in Tables 1, 2 and 3. The values for measured parameters (area, perimeter, maximum and minimum diameters) of primary osteons' vascular canals and secondary osteons (except for their maximum diameters) were significantly lower (p <0.05) in rabbits from E group. The values from parameters of Haversian canals did not differ between both groups of rabbits. The primary osteons' vascular canals constriction in rabbits from E group can be related to the adverse effect ofquercetin on blood vessels, which provide bone nutrition **(Greenlee and Dunnell, 2010)**. According to **Pries et al. (2005)**, blood vessels are able to adapt its structure (vascular remodeling) as a response to continuous functional changes. *In vitro* studies **(Leikert et al., 2002; Huisman et al., 2004; Wallerath et al., 2005; Jackson and Venema, 2006; Schmitt and Dirsch, 2009)** suggest an adverse effect of quercetin on the enzyme nitric oxidesynthase (eNOS) expression and endothelial nitric oxide (NO) production. NO acts as a potential vasodilator,and itcontributes to the migration and growth of endothelial cells necessary for initiation of angiogenesis *in vivo* **(Carmeliet, 2000; Jackson and Venema, 2006)**. We suppose that alterations in the size of primary osteons' vascular canals in rabbits from E and C groups are connected with an adverse effect of quercetin on the expression of eNOS.

We assume that significant differences (p <0.05) in the size of secondary osteons between rabbits from E and C groups may be associated with the destruction of collagen fibers which are present in the osteons **(Dylevský, 2007)**. **Kang et al. (2001)** found that quercetin significantly inhibited collagens I and III expression and had a growth-inhibitory effect on keloid-derived fibroblasts. The adverse impact of various concentrations of quercetin (20, 40, and 80 $\mu mol.l^{-1}$) on human fibroblasts examined **Stipcevic et al. (2006).** According to these authors, the administration of the highest dose of quercetin leads to significantly decreased collagen concentration (more than 50%) in fibroblasts. We supports that similar effect could also been observed in osteoblasts.

CONCLUSION

The study indicates that intramuscular application of quercetin at dose 1000 $\mu g.kg^{-1}$ bw for 90 days, 3 times per week caused significant changes in qualitative and quantitative histological characteristics of the compact bone tissue in male rabbits. Rabbits exposed to quercetin had a lower number of secondary osteons in the middle part of the *substantia compacta*, and disposed thicker layer of primary vascular longitudinal bone tissue (periost and middle part of the bone). Histomorphometrical evaluations

showed significantly decreased sizes of primary osteons' vascular canals and secondary osteons in males from the E group. Our article provides initial information of the impact of quercetin on femoral bone microstructure in experimental animals.

REFERENCES

Aggarwal, B. B., Heber, D. 2014. *Immunonutrition: Interactions of diet, genetics, and inflammation*. Boca Raton: CRC Press. p. 53-84. ISBN 9781466503854.

Agullo, G., Gamet-Payrastre, L., Manenti, S., Viala, C., Remesy, C., Chap, H., Payrastre, B. 1997. Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition. *Biochem Pharmacol.*, vol. 53, p. 1649-1657. http://dx.doi.org/10.1016/S0006-2952(97)82453-7

Baek, K. H., Oh, K. W., Lee, W. Y., Lee, S. S., Kim, M. K., Kwon, H. S., Rhee, E. J., Han, J. H., Song, K. H., Cha, B. Y., Lee, K. W., Kang, M. I. 2010. Association of oxidative stress with postmenopausal osteoporosis and the effect of hydrogen peroxide on osteoclast formation in human bone marrow cell cultures. *Calcif Tissue Int*., vol. 87, p. 226-235. http://dx.doi.org/10.1007/s00223-010-9393-9

Boik, J. 2001. *Natural Compounds in Cancer Therapy*. Oregon Medical Press, Princeton, Minnesota. p. 251-259. ISBN 0-9648280-1-4.

Boots, A. W., Li, H., Schins, R. P., Duffin, R., Heemskerk, J. W., Bast, A., Haenen, G. R. 2007. The quercetin paradox. *Toxicol. Appl. Pharmacol.*, vol. 222, p. 89-96. http://dx.doi.org/10.1016/j.taap.2007.04.004 PMID: 17537471

Braun, K. F., Ehnert, S., Freude, T., Egaña, J. T., Schenck, T. L., Buchholz, A., Schmitt, A., Siebenlist, S., Schyschka, L., Neumaier, M., Stöckle, U., Nussler, A. K. 2011. Quercetin protects primary human osteoblasts exposed to cigarette smoke through activation of the antioxidative enzymes HO-1 and SOD-1. *Scientific World Journal*, vol. 11, p. 2348-2357. http://dx.doi.org/10.1100%2F2011%2F471426

Brookes, P. S., Digerness, S. B., Parks, D. A., Darley-Usmar, V. 2002. Mitochondrial function in response to cardiac ischemia-reperfusion after oral treatment with quercetin. *Free Radic. Biol. Med*., vol. 32, p. 1220-1228. http://dx.doi.org/10.1016/S0891-5849(02)00839-0 PMID: 12031906

Buss, G. D., Constantin, J., de Lima, L. C., Teodoro, G. R., Comar, J. F., Ishii-Iwamoto, E. L., Bracht, A. 2005. The action of quercetin on the mitochondrial NADH to NAD(+) ratio in the isolated perfused rat liver. *Planta Med*., vol. 71, p. 1118-1122. http://dx.doi.org/10.1055/s-2005-873174

Carmeliet, P. 2000. Mechanisms of angiogenesis and arteriogenesis. *Nat. Med*., vol. 6, p. 389-395. http://dx.doi.org/10.1038/74651 PMID: 10742145

Cirico, T. L., Omaye, S. T. 2005. Additive or synergetic effects of phenolic compounds on human low density lipoprotein oxidation. *Food Chem. Toxicol*., vol. 44, p. 510-516. http://dx.doi.org/10.1016/j.fct.2005.08.025

Cassidy, A., O'Reilly, E. J., Kay, C., Sampson, L., Franz M., Forman, J. P., Curhan, G., Rimm, E. B. 2011. Habitual intake of flavonoid subclasses and incident hypertension in adults. *Am. J. Clin. Nutr*., vol. 93, p. 338-347. http://dx.doi.org/10.3945%2Fajcn.110.006783

Danihelová, M., Šturdík, E. 2011. Flavonoid natural sources and their importance in the human diet. *Potravinarstvo*, vol. 5, p. 12-24. http://dx.doi.org/10.5219/160

Davis, J. M., Murphy, E. A., Carmichael, M. D., Davis, B. 2009. Quercetin increases brain andmuscle mitochondrial biogenesis and exercise tolerance. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., vol. 296, p. R1071–R1077. http://dx.doi.org/10.1152/ajpregu.90925.2008

de Ricqlés A. J., Meunier, F. J., Castanet, J., Francillon-Vieillot, H. 1991. *Comparative microstructure of bone. Bone 3, Bone Matrix and Bone Specific Products*. Hall BK. Bocca Raton: CRC Press, p. 1-78. ISBN 0-8493-8823-6.

Dehghan, G., Khoshkam, Z. 2012. Tin(II)–quercetin complex: Synthesis, spectral characterisation and antioxidant activity. *Food Chem*., vol. 131, p. 422-426. http://dx.doi.org/10.1016/j.foodchem.2011.08.074

Dylevský, I. 2007. *General kinesiology (Obecná kineziologie*, In Czech*)*. Praha: Grada Publishing, 192 p. ISBN 978-80-247-1649-7.

Egert, S., Rimbach, G. 2011. Which sources of flavonoids: complex diets or dietary supplements? *Adv. Nutr*., vol. 2, p. 8-14. http://dx.doi.org/10.3945/

Enlow, D. H., Brown, S. O. 1956. A comparative histological study of fossil and recent bone tissue. Part I. *Tex. J. Sci*., vol. 8, p. 405-412.

Enlow, D. H., Brown, S. O. 1958. A comparative histological study of fossil and recent bone tissue. Part III. *Tex. J. Sci*., vol. 10, p. 187-230.

Formica, J. V., Regelson, W. 1995. Review of the biology of Quercetin and related bioflavonoids. *Food Chem Toxicol.*, vol. 33, p. 1061-1080. http://dx.doi.org/10.1016/0278-6915(95)00077-1 PMID: 8847003

Forte, L., Torricelli, P., Boanini, E., Gazzano, M., Rubini, K., Fini, M., Bigi, A. 2016. Antioxidant and bone repair properties of quercetin-functionalized hydroxyapatite: An in vitro osteoblast-osteoclast-endothelial cell co-culture study. *Acta Biomater*., vol. 1, p. 298-308. http://dx.doi.org/10.1016/j.actbio.2015.12.013

Greenlee, D. M., Dunnell, R. C. 2010. Identification of fragmentary bone from the Pacific. *J. Arch. Sci.*, vol. 37, p. 957-970. http://dx.doi.org/10.1016/j.jas.2009.11.029

Guo, C., Hou, G. Q., Li, X. D., Xia, X., Liu, D. X., Huang, D. Y., Du, S. X. 2012. Quercetin triggers apoptosis of lipopolysaccharide (LPS)-induced osteoclasts and inhibits bone resorption in RAW264.7 cells. *Cell Physiol. Biochem*. vol. 30, p. 123-136. PMID: 22759961

Hagiwara, H., Inoue, A., Yamaguchi, A., Yokose, S., Furuya, M., Tanaka, S., Hirose, S. 1996. cGMP produced in response to ANP and CNP regulates proliferation and differentiation of osteoblastic cells. *Am. J. Physiol*., vol. 270, p. C1311-C1318. PMID: 8967430

Halliwell, B., Gutteridge, J. M., Cross, C. E. 1992. Free radicals, antioxidants, and human disease: where are we now? *J. Lab. Med*., vol. 119, p. 598-620. PMID: 1593209

Harwood, M., Danielewska-Nikiel, B., Borzelleca, J. F., Flamm, G. W., Williams, G. M., Lines, T. C. 2007. A critical review of the data related to the safety of quercetin and lack of evidence of in vitro toxicity including lack of genotoxic/carcinogenic properties. *Food Chem. Toxicol*., vol. 45, no. 11, p. 2179-2205. http://dx.doi.org/10.1016/j.fct.2007.05.015

Heijnen, C. G., Haenen, G. R., van Acker, F. A., van der Vijgh, W. J., Bast, A. 2001. Flavonoids as peroxynitrite scavengers: the role of the hydroxyl groups. *Toxicol In Vitro*, vol. 15, p. 3-6. http://dx.doi.org/10.1016/S0887-2333(00)00053-9 PMID: 11259863

Hooper, L., Cassidy, A. 2006. A review of the health care potential of bioactive compouds. *J. Sci. Food Agric*., vol. 86, p. 1805-1813. http://dx.doi.org/10.1002/jsfa.2599

Hosokawa, N., Hirayoshi, K., Nakai, A., Hosokawa, Y., Marui, N., Yoshida, M., Sakai, T., Ninoshino, H., Aoike, A., Kawai, K., Nagata, K. 1990. Flavonoids inhibit the expression of heat shock proteins. *Cell Struct Funct*., vol. 15, p. 393-401. http://doi.org/10.1247/csf.15.393

Hubbard, G. P., Wolffram, S., Lovegrove, J. A., Gibbins, J. M. 2004. Ingestion of quercetin inhibits platelen aggregation and essential components of the collagen-stimulated platelet activation pathway in humans. *J. Thromb. Haemost*., vol. 2, p. 2138-2145. http://dx.doi.org/10.1111/j.1538-7836.2004.01067.x

Chen, C., Zhou, J., Ji, C. 2010. Quercetin: a potential drug to reverse multidrug resistance. *Life Sci.*, vol., 87, p. 333-338. http://dx.doi.org/10.1016/j.lfs.2010.07.004PMID: 20637779

Choi, J. A., Kim, J. Y., Lee, J. Y., Kang, C. M., Kwon, H. J., Yoo, Y. D., Kim, T. W., Lee, Y. S., Lee, S. J. 2001. Induction of cell cycle arrest and apoptosis in human breast cancer cells by quercetin. *Int. J. Oncol*., vol. 19, p. 837-844. http://dx.doi.org/10.3892/ijo.19.4.837

Choi, J. S., Li, X. 2005. Enhanced diltiazem biovailability after oral administration of diltiazem with quercetin to rabbits. *Int. J. Pharm*., vol. 297, no. 1-2, p. 1-8. http://dx.doi.org/10.1016/j.ijpharm.2004.12.004

Ishikawa, Y., Kitamura M. 2000. Anti-apoptotic effect of quercetin: Intervention in the JNK- and ERK-mediated apoptotic pathways. *Kidney Int*., vol. 58, p. 1078-1087. http://dx.doi.org/10.1046/j.1523-1755.2000.00265.x

Jackson, S. J., Venema, R. C. 2006. Quercetin inhibits eNOS, microtubule polymerization, and mitotic progression in bovine aortic endothelial cells. *J. Nutr*., vol. 136, p. 1178-1184. PMID: 16614401

Jakubowicz-Gil, J., Rzeski, W., Zdzisinska, B., Dobrowolski, P., Gawron, A. 2008. Cell death and neuronal arborization upon quercetin treatment in rat neurons. *Acta Neurobiol Exp. (Wars)*, vol. 68, p. 139-146. PMID: 18511950

Kang, L. P., Qi, L. H., Zhang, J. P., Shi, N., Zhang, M., Wu, T. M., Chen, J. 2001. Effect of genistein and quercetin on proliferation, collagen synthesis, and type I procollagen mRNA levels of rat hepatic stellate cells. *Acta Pharmacol Sin.*, vol. 22, p. 793-796. PMID: 11749858

Kanno S., Hirano S., Kayama F. 2004. Effects of phytoestrogens and environmentalestrogens on osteoblastic differentiation in MC3T3-E1cells. *Toxicol*., vol. 196, p. 137-145. http://dx.doi.org/10.1016/j.tox.2003.12.002

Kavalcová, P., Bystrická, J., Trebichalský, P., Kopernická, M., Hrstková, M., Lenková, M. 2015. Content of total polyphenols and antioxidant activity in selected varieties of onion (*Allium cepa* L.). *Potravinarstvo*, vol. 9, p. 494-500. http://dx.doi.org/10.5219/524

Kim, Y. J., Bae, Y. C., Suh, K. T., Jung, J. S. 2006. Quercetin, a flavonoid, inhibits proliferation and increases osteogenic differentiation in human adipose stromal cells. *Biochem. Pharmacol*., vol. 72, p. 1268-1278. http://dx.doi.org/10.1016/j.bcp.2006.08.021

Knab, A. M., Shanely, R. A., Jin, F., Austin, M. D., Sha, W., Nieman, D. C., 2011. Quercetin with vitamin C and niacin does not affect body mass or composition. *Appl. Physiol. Nutr. Metab*., vol. 36, p. 331-338. http://dx.doi.org/10.1139/h11-015 PMID: 21574787

Kovacevic, G., Matulic, A. 2013. Effect of quercetin on the green hydra (*Hydra viridissima* Pallas, 1766). *Int. J. Biol*., vol. 5, p. 57- 63. http://dx.doi.org/10.5539/ijb.v5n3p57

Lakhanpal, P., Kumar, P. 2007. Quercetin: A versatile flavonoid. *Int. J. Med. Update*, vol. 2, p. 22-37. http://dx.doi.org/10.4314/ijmu.v2i2.39851

Leikert, J. F., Räthel, T. R., Wohlfart, P., Cheynier, V., Vollmar, A. M., Dirsch, V. M. 2002. Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. *Circulation*, vol. 106, p. 1614-1617. http://dx.doi.org/10.1161/01.CIR.0000034445.31543.43 PMID:12270851

Lesniak-Walentyn, A., Kolesarova, A., Medvedova, M., Maruniakova, N., Capcarova, M., Kalafova, A., Hrabia, A., Sirotkin, A. V. 2013. Proliferation and apoptosis in the rabbit ovary after administration of T-2 toxin and quercetin. *J. Animal and Feed Sciences*, vol. 22, p. 264-271. http://dx.doi.org/10.1016/j.repbio.2012.11.043

Liang, W., Luo, Z., Ge, S., Li, M., Du, J., Yang, M., Yan, M., Ye, Z., Luo, Z. 2011. Oral administration of quercetin inhibits bone loss in rat model of diabetic osteopenia. *Eur. J. Pharmacol*., vol. 670, no. 1, p. 317-324. http://dx.doi.org/10.1016/j.ejphar.2011.08.014

Liu, R. H. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J. Nutr*., vol. 134, p. 3479S-3485S. PMID: 15570057

Manach, C., Regerat, F., Texier, O. 1996. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. *Nutr. Res.*, vol. 16, no. 3, p. 517-534. http://dx.doi.org/10.1016/0271-5317(96)00032-2

Martiniaková, M., Vondráková, M., Fabiš, M. 2003. Investigation of the microscopic structure of rabbits compact bone tissue. *Scripta medica (Brno)*, vol. 76, p. 215-220.

Martiniaková, M., Grosskopf, B., Omelka, R., Vondráková, M., Bauerová, M. 2006. Differences in bone microstructure of mammalian skeletons: use of a discriminant function analysis for species identification. *J. Forensic Sci*., vol. 51, p. 1235-1239. http://dx.doi.org/10.1111/j.1556-4029.2006.00260.x PMID: 17199608

Martiniaková, M., Omelka, R., Grosskopf, B., Sirotkin, A. V., Chrenek, P. 2008. Sex-related variation in compact bone microstructure of the femoral diaphysis in juvenile rabbits. *Acta Vet. Scand*., vol. 50, p. 15. http://dx.doi.org/10.1186/1751-0147-50-15 PMID: 18522730

Nabavi, S.M., Nabavi, S.F., Eslami, S., Moghaddam, A.H. 2012. In vivo protective effects ofquercetin against sodium fluoride-induced oxidative stress in the hepatic tissue. *Food Chem*., vol. 132, no. 2, p. 931-935. http://dx.doi.org/10.1016/j.foodchem.2011.11.070

Nam, T. W., Yoo, C. I., Kim, H. T., Kwon, C. H., Park, J. Y., Kim, Y. K. 2008. The flavonoid quercetin induces apoptosis and inhibits migration through a MAPK-dependent mechanism in osteoblasts. *J. Bone Miner. Metab*., vol. 26, p. 551-560. http://dx.doi.org/10.1007/s00774-008-0864-2

Nickel, T., Hanssen, H., Sisic, Z., Pfeiler, S., Summo, C., Schmauss, D., Hoster, E., Weis, M. 2011. Immunoregulatory effects of the flavonol quercetin in vitro and in vivo. *Eur. J. Nutr*., vol. 50, p. 163-172. http://dx.doi.org/ 10.1007/s00394-010-0125-8 PMID: 20652710

Notoya, M., Tsukamoto, Y., Nishimura, H., Woo, J. T., Nagai, K., Lee, I. S., Hagiwara, H. 2004. Quercetin, a flavonoid, inhibits the proliferation, differentiation, and mineralization of osteoblasts in vitro. *Eur. J. Pharmacol*., vol. 485, p. 89-96. http://dx.doi.org/10.1016/j.ejphar.2003.11.058

Okamoto T. 2005. Safety of quercetin for clinical application (Review). Int J Mol Med., vol. 16, p. 275-278. http://dx.doi.org/10.3892/ijmm.16.2.275

Partridge, N. C., Alcorn, D., Michelangeli, V. P., Kemp, B. E., Ryan, G. B., Martin, T. J. 1981. Functional properties of hormonally responsive cultured normal and malignant rat

osteoblastic cells. *Endocrinology*, vol. 108, p. 213-219. http://dx.doi.org/10.1210/endo-108-1-213

Pries, A. R., Reglin, B., Secomb, T. W. 2005. Remodeling of blood vessels: responses of diameter and wall thickness to hemodynamic and metabolic stimuli. *Hypertension*, vol. 46, p. 725-731. http://dx.doi.org/10.1161/01.HYP.0000184428.16429.be

Prouillet, C., Mazièreb, J. C., Mazièreb, C. 2004. Stimultatory effect of naturaly occurring flavonols quercetin and kaempferol on alkaline phosphatase activity in MG-63 human osteoblasts through EKR and estrogen receptor pathway. *Biochem. Pharmacol*, vol. 67, p. 1307-1313. http://dx.doi.org/10.1016/j.bcp.2003.11.009

Rahman, A., Fazal, F., Greensill, J., Ainley, K., Parish, J. H., Hadi, S. M., 1992. Strand scission in DNA induced by dietary flavonoids: role of Cu(I) and oxygen free radicals and biological consequences of scission. *Mol, Cell Biochem*., vol. 111, p. 3-9. PMID: 1588940

Rice, S., Mason, H. D., Whitehead, S. A. 2006. Phytoestrogens and their low dose combinations inhibit mRNA expression and activity of aromatase in human granulosa-luteal cells. *J, Steroid Biochem. Moll Biol*., vol. 101, p. 216-225. http://dx.doi.org/10.1016/j.jsbmb.2006.06.021

Robaszkiewicz, A., Balcerczyk, A., Bartosz, G. 2007. Antioxidative and prooxidative effects of quercetin on A549 cells. *Cell Bioll Int*., vol. 31, p. 1245-1250. http://dx.doi.org/10.1016/j.cellbi.2007.04.009

Ross, J. A., Kasum, C. M. 2002. Dietary flavonoids: bioavailability, metabolic effects and safety. *Annul Revl Nutr*., vol. 22, p. 19-34. http://dx.doi.org/10.1146/annurev.nutr.22.111401.144957

Sahu, S. C., Washington, M. C. 1991. Quercetin-induced lipid peroxidationand DNA damage in isolated rat-liver nuclei. *Cancer Lett*., vol. 58, p. 75-79. http://dx.doi.org/10.1016/0304-3835(91)90026-E

Satué, M., Arriero, M. del M., Monjo, M., Ramis, J. M. 2013. Quercitrin and taxifolin stimulate osteoblast differentiation in MC3T3-E1 cells and inhibit osteoclastogenesis in RAW 264.7 cells. *Biochem. Pharmacol*., vol. 86, no. 10, p. 1476-1486. http://dx.doi.org/10.1016/j.bcp.2013.09.009

Satyanarayana, P. S., Singh, D., Chopra, K. 2001. Quercetin, a bioflavonoid, protects again oxidative stress-related renal dysfunction by cyclosporine in rats. *Methods Find. Exp. Clin. Pharmacol*., vol. 23, p. 175-181. http://dx.doi.org/10.1358/mf.2001.23.4.634641 PMID: 11676225

Schmitt, C. A., Dirsch, V. M. 2009. Modulation of endothelial nitric oxide by plant-derived products. *Nitric Oxide*, vol. 21, p. 77-91. http://dox.doi.org/10.1016/j.niox.2009.05.006 PMID: 19497380

Şekeroğlu, Z. A., Şekeroğlu, V. 2012. Effects of Viscum album L. extract and quercetin on methotrexate-induced cyto-genotoxicity in mouse bone marrow cells. *Mutat Res*., vol. 746, no. 1, p. 56-59. http://dx.doi.org/10.1016/j.mrgentox.2012.02.012

Sharan, K., Mishra, J. S., Swarnkar, G., Siddiqui, J. A., Khan, K., Kumari, R., Rawat, P., Maurya, R., Sanyal, S., Chattopadhyay, N. 2011. A novel quercetin analogue from a medicinal plant promotes peak bone mass achievement and bone healing after injury and exerts an anabolic effect on osteoporotic bone: the role of aryl hydrocarbon receptor as a mediator of osteogenic action. *J. Bone Miner. Res*., vol. 26, p. 2096-2111. http://dx.doi.org/10.1002/jbmr.434 PMID: 21638315

Skibola, C. F., Smith, M. T. 2000. Potential health impacts of excessive flavonoid intake. *Free Radic. Biol. Med*., vol. 29, p. 375-383. http://dx.doi.org/10.1016/S0891-5849(00)00304-X

Son, Y. O., Kook, S. H., Choi, K. C., Jang, Y. S., Choi, Y. S., Jeon, Y. M., Kim, J. G., Hwang, H. S., Lee, J. C. 2008. Quercetin accelerates TNF-α-induced apoptosis of MC3T3-E1 osteoblastic cells through caspase-dependent and JNK-mediated pathways. *Eur. J. Phramacol.*, vol. 579, p. 26-33. http://dx.doi.org/10.1016/j.ejphar.2007.10.003 PMID: 17988664

Soria, E. A., Eynard, A. R., Bongiovanni, G. A. 2010. Cytoprotective effects ofsilymarin on epithelial cells against arsenic-induced apoptosis incontrast with quercetin cytotoxicity. *Life Sci*., vol. 87, p. 309-315. http://dx.doi.org/10.1016/j.lfs.2010.07.007

Spencer, J. P., Kuhnle, G. G., Williams, R. J., Rice-Evans, C. 2003. Intracellular metabolism and bioactivity of quercetin and its in vivo metabolites. *Biochem J*., vol. 15, p. 173-181. http://dx.doi.org/10.1042/bj20021972 PMID: 12578560

Stipcevic, T., Piljac, J., Vanden Berghe, D. 2006. Effect of different flavonoids on collagen synthesis in human fibroblasts. *Plant Foods Hum. Nutr.*, vol. 61, p. 29-34. http://dx.doi.org/10.1007/s11130-006-0006-8 PMID: 16642409

Verhoeyen, M. E., Bovy, A., Collins, G., Muir, S., Robinson, S., Vos, C., Colliver, S. 2002. Increasing antioxidant levels in tomatoes through modification of the flavonoid biosynthetic pathway. *J. Exp. Bot*., vol. 53, p. 2099-2106. http://dx.doi.org/10.1093/jxb/erf044

Wallerath, T., Li, H., Gödtel-Ambrust, U., Schwarz, P. M., Förstermann, U. 2005. A blend of polyphenolic compounds explains the stimulatory effect of red wine on human endothelial NO synthase. *Nitric Oxide*, vol. 12, p. 97-104. http://dx.doi.org/10.1016/j.niox.2004.12.004 PMID: 15740983

Wattel, A., Kamel, S., Prouillet, C., Petit, J. P., Lorget, F., Offord, E., Brazier, M. 2004. Flavonoid quercetin decreases osteoclastic differentiation induced by RANKL via a mechanism involving NFκB and AP-1. *J. Cell Biochem*., vol. 92, p. 285-295. http://dx.doi.org/10.1002/jcb.20071

Wein, S., Schrader, E., Rimbach, G., Wolffram, S. 2013. Oral quercetin supplementation lowers plasma sICAM-1 concentrations in female db/db mice. *Pharmacol. Pharm.*, vol. 4, p. 77-83. http://dx-doi.org/10.4236/pp.2013.41011

Wu, Q. H., Wang, X., Yang, W., Nüssler, A. K., Xiong, L. Y., Kuča, K., Dohnal, V., Zhang, X. J., Yuan, Z. H. 2014. Oxidative stress-mediated cytotoxicity and metabolism of T-2 toxin and deoxynivalenol in animals and humans: an update. *Arch. Toxicol*., vol. 88, p. 1309-1326. http:/dx.doi.org/10.1007%2Fs00204-014-1280-0

Yamaguchi, M., Weitzmann, M. N. 2011. Quercetin, a potent suppressor of NF-κB and Smad activation in osteoblasts. *Int. J. Mol. Med*., vol. 28, p. 521-525. http://dx.doi.org/10.3892/ijmm.2011.749

Yang, S., Ries, W., Key, Jr L. L. 1998. Nicotinamide adenine dinucleotide phosphate oxidase in the formation of superoxide in osteoclasts. *Calcif Tissue Int*., vol. 63, p. 346-350. PMID: 9744995

Yao, L. H., Jiang, Y. M., Shi, J., Tomás-Barberán, F. A., Datta, N., Singanusong, R., Chen, S. S. 2004. Flavonoids in food and their health benefits. *Plant Foods Hum. Nutr*., vol. 59, p. 113-122. PMID: 15678717

Zhou, C., Lin, Y. 2014. Osteogenic differentiation of adipose-derived stem cells promoted by quercetin. *Cell Prolif*., vol. 47, p. 124-132. http://dx.doi.org/10.1111/cpr.12097

Zhang, Q., Zhao, X., H., Wang, Z. J. 2009. Cytotoxicity of flavones and flavonols to a human esophageal squamous cell carcinoma cell line (Kyse-510) by induction of G2/M arrest and apoptosis. *Toxicol. In Vitro*, vol. 23, p. 797-807. http://dx.doi.org/10.1016/j.tiv.2009.04.007

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ASSESSMENT OF DNA QUALITY IN PROCESSED TUNA MUSCLE TISSUES

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ABSTRACT

Authentication of tuna fish products is necessary to assure consumers of accurate labelling of food products. The quality of species specific DNA crucially affects the efficiency of amplification during the subsequent PCR. The problem in DNA detection in canned products lies in the possibility of the fragmentation of DNA during the processing technologies and the use of ingredients (oil, salt, spice), that may inhibit the PCR reaction. In this study three DNA extraction methods were compared: DNeasy Blood and Tissue Kit, DNeasy mericon Food Kit and Chemagic DNA tissue 10 Kit. The quantity and quality of DNA were evaluated by measuring DNA concentration and ratios A260/A280. Several parameters were estimated: the effect of whole and mechanically treated muscle, sterilization procedure used in canned process (high temperature in combination with high pressure) and addition of raw materials. The highest DNA concentrations were observed in non-processed muscle that is not influenced by the sterilization process. Canned whole muscle demonstrated lower DNA yield, and furthermore, the mechanical treatment (canned ground) resulted in lower values of DNA concentration that was registered by using all three types of DNA extraction kits. DNeasy mericon Food Kit produced DNA of higher concentration in non-processed sample, Chemagic DNA tissue 10 Kit delivered higher DNA yields than kits DNeasy Blood and Tissue Kit and DNeasy mericon Food Kit in canned samples, although the purity was lower, but still within the range 1.7 – 2.0. DNA was considered to be satisfactorily pure in all three types of samples and using all three types of DNA isolation. In case of the samples enriched of ingredients and treated with sterilization process as whole or ground muscle Chemagic DNA tissue 10 Kit produced in all samples (whole and ground muscle) the highest values of DNA concentration, but almost all values of A260/A280 were lower than 1.7. Therefore DNeasy mericon Food Kit appears to be a favorite one, in all samples with whole muscle gives higher values of DNA concentrations than DNeasy Blood and Tissue Kit. Addition of ingredients influenced the DNA yield in terms of decreasing in samples containing vinegar and lemon, but some of the ingredients resulted surprisingly in higher yield of DNA. This was not consistent in whole and ground muscle, and the differences were described also among particular kits. The impact of ingredients was not conclusively approved and their importance to the suitability of extracted DNA for PCR amplification is needed to be discussed in further analysis.

Keywords: canned product; fish food; DNA extraction; PCR; *Thunnus albacares*

INTRODUCTION

Fish species identification gains attention due to the commercialization of fish through filleted, salted, smoked or canned fish products. Tuna fish belong among the most economically important fishery resources because are typically used to manufacture canned products, the main format for marketing of these species **(Espineira et al., 2009)**. Different quality and price of tuna species can lead the manufacturers to the tendency to highlight the quality of fish products. From that reason the substitution or mixing of valuable fish by less valuable ones may occur. The Council Regulation (EC) **No. 1536/92** laying down common marketing standards for preserved tuna and bonito states specific rules for the tuna marketing. The species belonging to tuna and bonito are named in the annex of this Regulation. Below is determined, that the trade description on the prepackaging of preserved tuna or bonito shall state the type of fish (tuna or bonito). The identification of tuna and bonito species according to their morphological features is possible only in whole or lightly processed fish. In processed products such as filleted or canned fish the morphological characteristics are removed, hence analytical methods as an important tool for species identification must be used. Analytical methods are focused mainly on protein or DNA molecule, which are extracted from the fish tissues. Due to the protein denaturation caused by heating or canning (high temperature in combination with high pressure) process **(Mackie et al., 1999)**, DNA is more suitable molecular marker for fish species authentication, because it is more resistant to the thermal treatment. Indeed DNA is also degraded into smaller fragments during the thermal process but these are still detectable. **Ram et al. (1996)** claim, that the canning process degrades DNA to fewer than 123 bp in length. Moreover DNA is largely independent of tissue source, age, or sample damage **(Bossier, 1999; Lockley and Bardsley, 2000)**. Nevertheless the fragment size is limited factor for the

subsequent PCR reaction that is based on the selective amplification of specific region of DNA using oligonucleotides **(Lockley and Bardsley, 2000)**. PCR **(Bartlett and Davidson, 1991; Bottero et al., 1997; Dalmasso et al., 2006)** and its modification – PCR-RFLP **(Takeyama et al., 2001, Pardo and Pérez-Villareal, 2004, Lin et al., 2005, Lin and Hwang, 2007)**, PCR-SSCP **(Rehbein et al., 1999; Colombo et al., 2005)**, real-time PCR **(Lopez and Pardo, 2005)** or PCR-ELISA **(Santaclara et al., 2015)** represents crucial approaches available for tuna fish species identification. PCR analysis comprises of DNA extraction from the sample, PCR and electrophoresis, or alternatively other detection system for the final results evaluation. The critical step is extraction of high quality DNA in great enough quantities from the heterogeneous food matrices. In view of the fact, that raw material for the final product manufacture comes under different effect during the manufacturing process (high temperature, high pressure, addition of ingredient, etc.), which considerable influences the quality of DNA **(Chapela et al., 2007, Besbes et al., 2011, Cawthorn et al., 2011)**, it is required for every type of food products to apply and optimize particular DNA isolation procedure. In addition ingredients and other substances presented in food products may work as PCR inhibitors, substances that may negative affect the sensitivity of PCR reaction. Or in another case, the DNA may be stimulated due to the ingredients.

Primary requirement of this study is to find out, how far is DNA influenced by the technological processes using in food industry (mechanical treatment, high temperature, high pressure, addition of ingredients) in model canned samples from the muscle tissue of yellowfin tuna (*Thunnus albacares*) and how the subsequent sample preparation and DNA extraction procedure can affect its qualitative and quantitative parameters.

MATERIAL AND METHODOLOGY

Samples preparation

The samples of tuna fish were prepared from the muscle tissue of yellowfin tuna (*Thunnus albacares*), that was purchased in the Czech market as frozen steak. Its species identity was confirmed via sequencing of the partial sequence of cytochrome *b* gene (Seqme, Hradec Kralove, Czech Republic). Besides non-processed muscle tissue two types of tuna samples that were made under similar conditions used in cans production were prepared - canning of solid piece of muscle (whole) and canning of

Table 1 List of ingredients.

	Raw food	[g]	Whole muscle / Mechanically modified muscle 27 g	
1	Raw muscle	-	42	
2	Sunflower oil	15	42	
3	Olive oil	15	42	
4	Soy sauce	15	42	
5	Brine	5%	42	
6	Alcohol vinegar	10	37	
7	Wine vinegar	10	37	
8	Apple cider vinegar	10	37	
9	Lemon + juice	4,5	31,5	
10	Tomato puree	20	47	**Total [g]**
11	Chili spice	1	28	
12	Oregano	0,5	27,5	
13	Fresh garlic	2	29	
14	Garlic spice	1	28	
15	Onion	5	32	
16	Corn	10	37	
17	Pea	10	37	
18	Bean	15	42	
19	Carrot	10	37	
20	Tomatoes	10	37	
21	White + green pepper	5+5	37	
22	Black olives	10	37	
23	Fresh chili pepper	5	32	

mechanically processed muscle (ground). Mechanic treatment was provided using the cutter setting in two rotations. Furthermore the sets of the samples comprising whole/ground muscle enriched of the selective ingredients were mixed thoroughly and placed into the autoclavable glass vessels with caps – the amount and composition is described in Table 1. The proportions were assessed according to the real composition described on the packaging of tuna fish products occurring on the commercial market. The samples were subjected to the sterilization in autoclave (Systec V95); sterilization conditions included the temperature 121 °C and pressure 200 kPa for 15 min. These samples were prepared in laboratories of the Department of Meat Hygiene and Technology (University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic).

DNA isolation

The DNA was extracted in duplicate using three commercial available kits based on the column system (DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) - kit A and DNeasy mericon Food (Qiagen) – kit B) and magnetic separation using magnetic particles (Chemagic DNA Tissue 10 Kit (Chemagen, Baesweiler, Germany) – kit C). Pretreatment of the samples 2 - 8 (Table 1) was performed according to **Chapela et al. (2007)**; oil, lipids or other substances were removed from canned muscle by soaking it in the mixture of chloroform/methanol/water (1:2:0.8) overnight. The extraction procedures were performed according to the protocol supplied by the manufacturer. Sample weight was 10 mg in kit A and C, and 200 mg in kit B, proteolysis was carried out overnight in all types of the extraction protocols.

The assessment of DNA quality

The quality of extracted DNA was compared by measurement the concentration and purity using a UV spectrophotometer (NanoDrop™ 1000, Thermo Scientific). DNA extracts were quantified by measuring the absorbance at 260 nm (A260). DNA purities were estimated by calculating the A260/A280 ratios. Samples calculated to have A260/A280 ratios of 1.7 – 2.0 were assumed to be pure samples, free from protein and other contamination. Every sample was measured three times. The instrument calibration was performed using the Elution Buffer. Measurement was done at room temperature and sufficient mixing of all samples.

Species identification via sequencing of cytochrome b gene

For the species identification of yellowfin tuna (*Thunnus albacares*) in frozen fish the amplification of 569 bp fragment of cytochrome *b* gene using primer pair L14735 and BRmod (Espineira et al., 2009) was used. The PCR protocol consisted of initial denaturation step at 95 °C/3 min, following by 35 cycles including denaturation at 95 °C/30 s, annealing at 60 °C/30 s and extension at 72 °C/30 s, and terminated by final extension at 72 °C/3 min.

RESULTS AND DISCUSSION

In canned products DNA is considered to be damaged, exposure to heat, physical or chemical treatment that can affect the quality and quantity of DNA, presumably the fragmentation of DNA molecule. To choose an optimal extraction procedure several factors have to be taken into account. DNA should contain as little as possible proteins, RNA, organic compounds or any other PCR inhibitors. The DNA concentration and purity were determined spectrophotometrically by measuring the DNA absorbance and A260/A280 ratios. The DNA was considered to be satisfactorily pure when the ratios of the A260 to A280 were within the range of 1.7 – 2.0. Contamination of DNA with proteins usually reduces the A260 to A280 ratio to values lower than 1.7 **(Cawthorn et al., 2011)**. High 260/280 purity ratios are not necessarily indicative of a problem. Residual impurities carried over from the DNA extraction procedure, such as phenol or ethanol, are also reported to reduce the A260 to A280 ratio. Furthermore residual chemical contamination from nucleic acid extraction procedures may result in an overestimation of the nucleic acid concentration.

The main task was to find out whether non-processed and processed muscle tissue (from *Thunnus albacares*) has the difference between the concentration and purity of DNA. Another parameter was to follow up the effect of the addition of ingredients mainly used in canned tuna products. And also to evaluate the efficiency of the three commercial kits used for the DNA isolation. The first group of analyzed samples include sample prepared from non-processed muscle without any further technological processes (frozen muscle), sample prepared from whole muscle undergoing the sterilization process and sample prepared from mechanically treated (ground) muscle undergoing the sterilization process. The comparison of the DNA concentration and DNA purity is shown in Figure 1. The highest DNA concentrations were observed in non-processed muscle that is not influenced by the sterilization process. The sample with canned whole muscle demonstrated lower DNA yield, and furthermore, the mechanical treatment resulted in even lower values of DNA concentration that was registered by using all three

Table 2 Group rate of values A260/A280.

Ratio A260/A280	Kit A	Kit A	Kit B	Kit B	Kit C	Kit C
	W	G	W	G	W	G
<1.7	1	4	11	4	22	22
1.7 – 2.0	11	13	12	17	1	1
>2	11	6	0	2	0	0

Note: W – whole, G – ground.

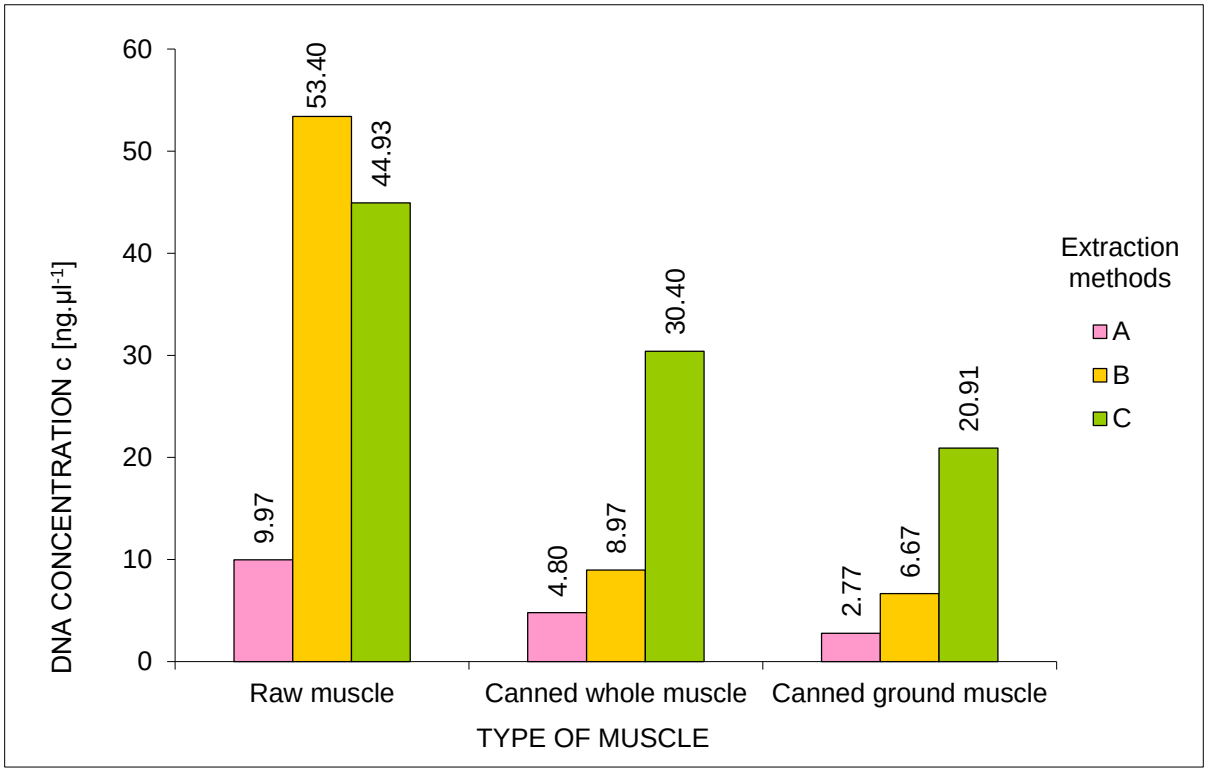

Figure 1 Determination of DNA concentration (type of muscle).

types of DNA extraction kits. Kit B produced DNA of higher concentration in non-processed sample, kit C delivered higher DNA yields than kit A and B, although the purity was lower, but still within the range 1.7 – 2.0. In the case of DNA purities, DNA was considered to be satisfactorily pure in all three types of samples and using all three types of DNA isolation.

The second group consisted of 23 samples prepared from the whole or ground muscle tissue and enriched with the ingredients (22 with ingredients and 1 muscle without ingredients). For the comparison of the samples of whole and ground canned muscle tissue (regardless of the effect of the ingredients) the Wilcoxon matched-pairs signed rank test was used and for the comparison of the efficiency of the particular extraction kits Friedman test + Dunn post-hoc test (non-parametric ANOVA) was used. The frequency values of A260/A280 in groups $A < 1.7$ / $1.7 \leq A \leq 2.0$ / $A > 2.0$ were estimated with χ^2 independence test and Fisher exact test.

Statistically significant differences in DNA concentration between the whole and ground muscle were found in the case of kits A (p <0.01; Wilcoxon test) and B (p <0.05; Wilcoxon test). While in kit A the values of DNA concentrations in most of the samples with whole muscle were lower than in the samples with ground muscle, in kit B it was conversely. In kit C statistically significant difference between whole and ground muscle was not proved. In kit A probably the chemical substances used during the extraction procedure could cause more efficient permeation to the ground muscle in comparison with whole muscle, but this was not observed in sample 1 (whole and ground muscle without ingredients). In case of the samples with whole muscle we managed to prove that among the kits there is statistically significant difference (p <0.01; Friedman test) in DNA concentration. Following testing demonstrated that statistically significant difference is evident between all pairs of kits (p <0.01; Dunn test), while the highest values of DNA concentration is presented with kit C, the lowest in kit A. In case of the samples with ground muscle we managed to prove that among the kits there is statistically significant difference (p <0.01; Friedman test). Following testing showed up that statistically significant difference is only between kit A and C, B and C (p <0.01; Dunn test), while the highest values of DNA concentration is produced by the kit C. Between kit A and B the statistically significant difference was not observed.

Statistically significant difference of A260/A280 between whole and ground muscle was observed only in kit B (p <0.01; Wilcoxon test), while highest values of A260/A280 was reached in samples with ground muscle. In case of the samples with whole muscle we managed to prove that among the kits there is statistically significant difference (p <0.01; Friedman test). Following testing demonstrated that statistically significant difference is evident between all pairs of kits (p <0.01; Dunn test), while the highest values of A260/A280 is presented by kit A, the lowest in kit C (except the samples 1 always under the limit 1.7). In case of the samples with ground muscle we managed to prove that among the kits there is statistically significant difference (p <0.01; Friedman test). Following testing showed up that statistically significant difference is only between kit A and C, B and C (p <0.01; Dunn test), while the lowest values A260/A280 is produced by the kit C. Between kit A and B the statistically significant difference was not observed.

χ^2 independence test confirms that there is association (p <0.01) between the distribution of A260/A280 ratios and kit resp. the type of the sample (whole/ground muscle). In case of the type of the sample the highest statistically significant difference (p <0.05) was detected in kit B (Table 2).

The effect of ingredients mixed together with muscle reveal the differences among particular kits and also among whole and ground muscle. According **to Chapela et al. (2007)** lower amount of DNA can be caused by the presence of brine, this finding could be explain by a washing out effect used in the extraction procedure. The decreasing effect of brine on DNA yield was observed only in kit C. In kits A and B the concentrations of DNA were even higher in comparison with the sample without brine. Other ingredients vinegar and lemon are substances

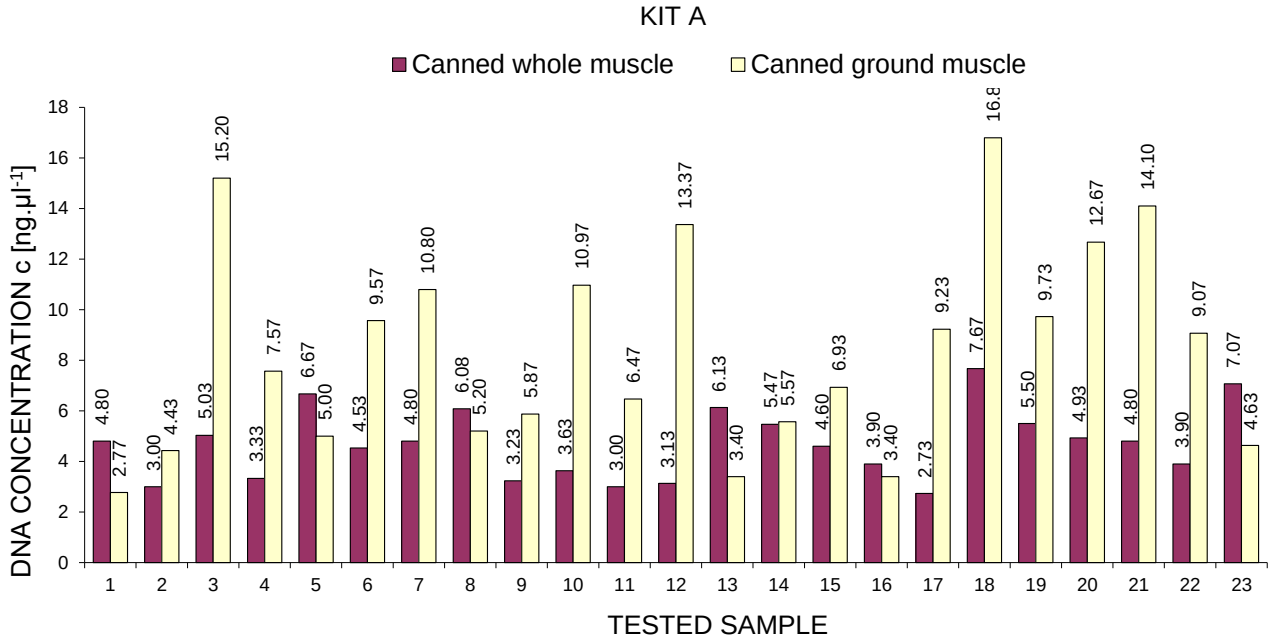

Figure 2 Comparison of DNA concentration of whole and ground muscle determined by kit A.

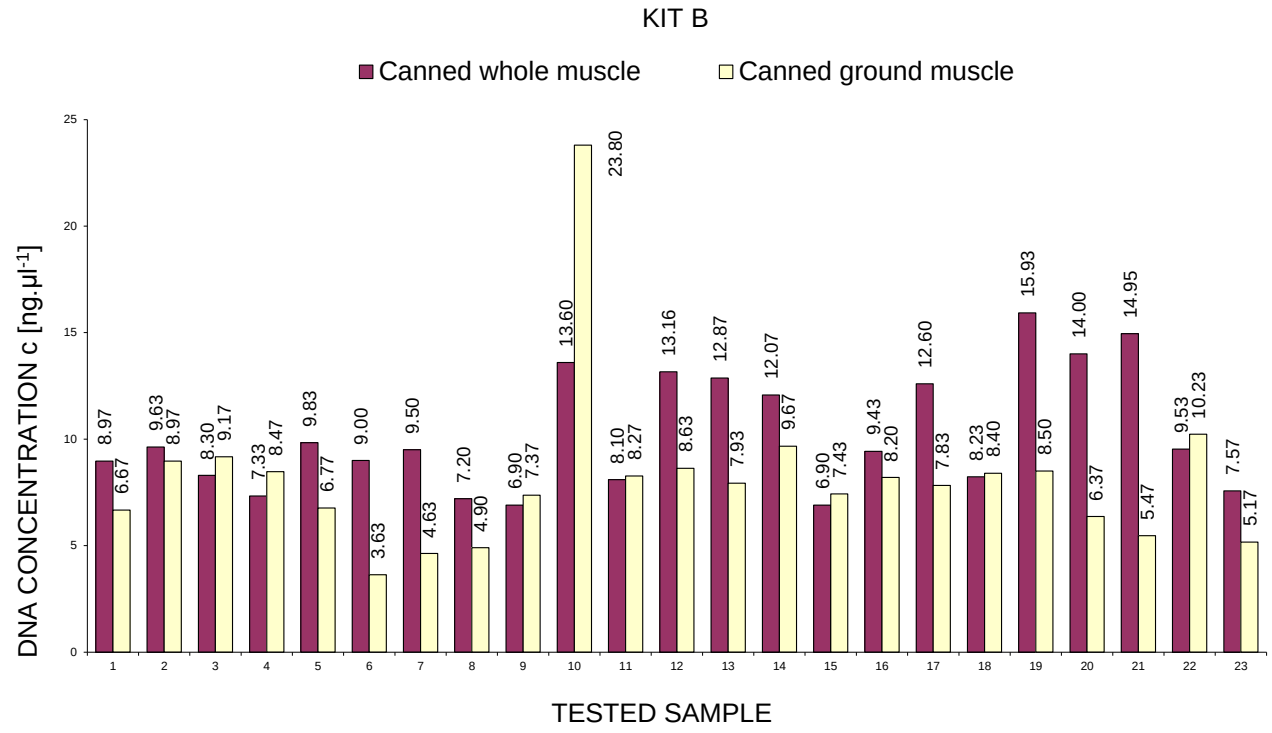

Figure 3 Comparison of DNA concentration of whole and ground muscle determined by kit B.

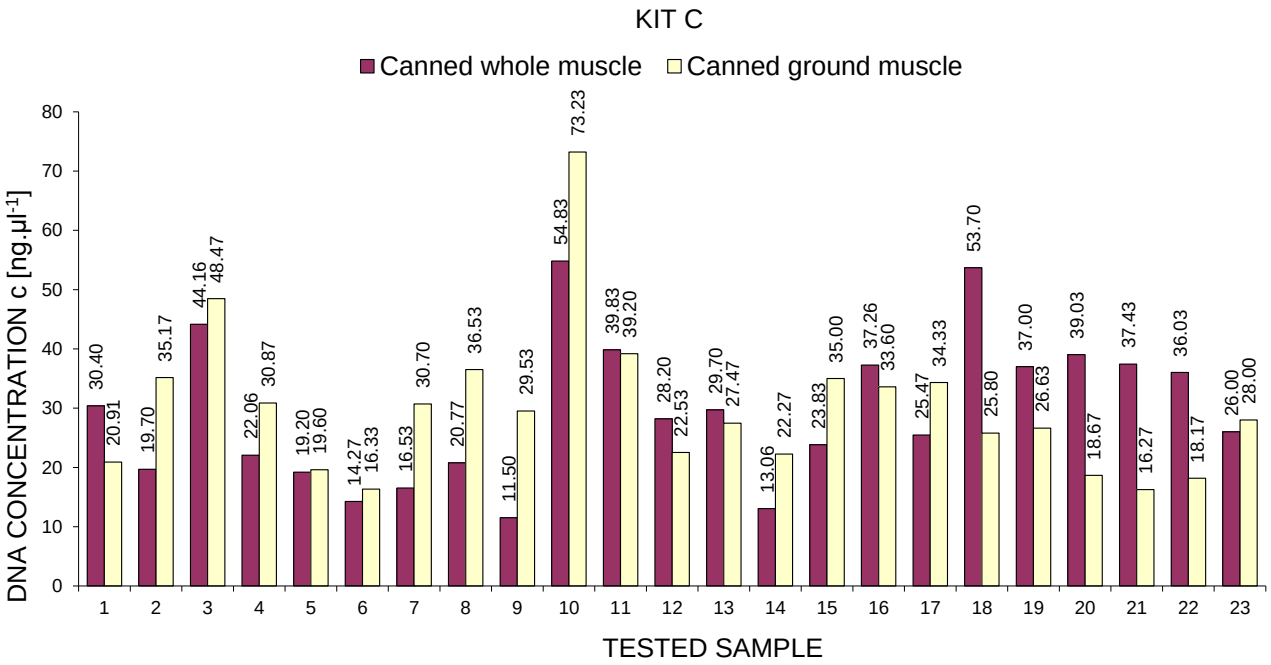

Figure 4 Comparison of DNA concentration of whole and ground muscle determined by kit C.

that are known as a low pH media which could be the reason of DNA degradation **(Bauer et al., 2003)**. In both kit B and C the decreasing effect on DNA yield caused by the presence of vinegar and lemon was observed. In kit A this was observed only in lemon and in case of ground muscle this effect was not demonstrated (Figures 2 – 4). Onion contains quercetin that belongs to the flavonoids and that can inhibit the protein kinase C activity (PKC) and mitogen activated protein kinase 1 (MEK). Therefore it appears to decrease the DNA yield in the samples containing onion **(Lee et al., 2008).** This was observed in whole muscle samples containing onion consistently in all three types of kits. In contrary the presence of ingredients in some samples resulted in better DNA yield. The color extracted from the samples containing particular ingredients (carrots in all three kits, tomato puree, chili, oregano, tomatoes, green pepper or black olives in kit B or C) could cause the higher values of absorbance which could misinterpret obtained results **(Chapela et al., 2007)**. Unexpectedly in kit A the lowest DNA yield was estimated in sample containing pea, the highest value of DNA concentration was assessed in sample containing bean. Although both are legumes their effect was completely contradictory. The quality assessed by the ratios A260/A280 were decreased (A260/A280 <1.7) in samples containing brine and vinegar in both kits A and B, in kit C every sample resulted in ratios lower than 1.7. Although purity ratios are important indicators of sample quality, the best indicator is functionality in the following PCR amplification. There are occasions when the purity ratios are within expected limits, but there is a problem with the sample. Accordingly the presence of ingredients may negative influence the subsequent PCR amplification, when they could inhibit the DNA polymerase activity in PCR **(Di Pinto et al., 2007)** and decrease its sensitivity. The impact of ingredients was not conclusively approved and their connotation to the suitability of extracted DNA for PCR amplification is needed to be discussed in further analysis.

CONCLUSION

The quality of DNA affect the efficiency of amplification during the subsequent PCR reaction. The results of this analysis revealed variability of particular extraction procedures in assessment of DNA quality and quantity in tuna muscle tissue treated with different modifications. The highest DNA concentrations were observed in non-processed muscle, whole canned muscle demonstrated lower DNA yield, and canned ground muscle resulted in even lower values of DNA concentration that was registered by using all three types of DNA extraction kits. Kit B produced DNA of higher concentration in non-processed sample, kit C delivered higher DNA yields in canned whole and ground muscle than kit A and B, although the purity was lower, but still within the range 1.7 – 2.0. In the case of DNA purities, DNA was considered to be satisfactorily pure in all three types of samples and using all three types of DNA isolation. Comparing the parameters of whole and ground canned muscle tissue with the content of ingredients, kit C produced in all samples with whole and ground muscle the highest values of DNA concentration, but almost all values of A260/A280 were lower than 1.7. Kit B in all samples with whole muscle gives higher values of DNA concentrations than kit A, in samples with ground muscle this assumed in almost all samples, so it appears to be a good choice for the DNA isolation from canned whole muscle with ingredients. The effect of ingredients mixed together with muscle reveal the differences in terms of

decreasing but also raising the DNA yield among particular kits and also among whole and ground muscle. Nevertheless the presence of ingredients may negative affect the subsequent PCR amplification, which will be the subject of further comparative analysis.

REFERENCES

Bartlett, S. E., Davidson, W. S. 1991. Identification of *Thunnus* tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome b genes. *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 48, p. 309-317. http://dx.doi.org/10.1139/f91-043

Bauer, T., Weller, P., Hammes, W.P., Hertel, C. (2003). The effect of processing parameters on DNA degradation in food. *European Food Research Technology*, vol. 217, p. 338-343. http://dx.doi.org/10.1007/s00217-003-0743-y

Bossier, P. 1999. Authentication of seafood products by DNA patterns. *Journal of Food Science*, vol. 64, p. 189-193. http://dx.doi.org/10.1111/j.1365-2621.1999.tb15862.x

Bottero, M. T., Dalmasso, A., Cappelletti, M., Secchi, C., Civera, T. 1997. Differentiation of five tuna species by a multiplex primer-extension assay. *Journal of Biotechnology*, vol. 129, no. 3, p. 575-580. http://dx.doi.org/10.1016/j.jbiotec.2007.01.032 PMid:17353060

Besbes, N., Fattouch, S., Sadok, S. 2011. Comparison of methods in the recovery and amplificability of DNA from fresh and processed sardine and anchovy muscle tissue. *Food Chemistry*, vol. 129, p. 665-671. http://dx.doi.org/10.1016/j.foodchem.2011.05.002

Cawthorn, D., Steinman, H. A., Witthuhn, R. C. 2011. Comparative study of different methods for the extraction of DNA from fish species commercially available in South Africa. *Food Control*, vol. 22, p. 231-244. http://dx.doi.org/10.1016/j.foodcont.2010.07.003

Chapela, M. J., Sotelo, C. G., Péez-Martin, R. I., Pardo, M. A., Pérez-Villareal, B., Gilardi, P., Riese, J. 2007. Comparison of DNA extraction methods from muscle of canned tuna for species identification. *Food Control*, vol. 18, p. 1211-1215. http://dx.doi.org/10.5219/189

Colombo, F., Mangiagalli, G., Renon, P. 2005. Identification of tuna species by computer-assisted and cluster analysis of PCR-SSCP electrophoretic patterns. *Food Control*, vol. 16, p. 51-53. http://dx.doi.org/10.1016/j.foodcont.2003.11.006

Dalmasso, A., Civera, T., Bottero, M. T. 2006. Biomolecular approaches for the identification of tuna species. *Veterinary Research Communications*, vol. 30, p. 179-181. http://dx.doi.org/10.1007/s11259-006-0035-7

Di Pinto, A., Forte, V. T., Guastadisegni, M.C., Martino, C., Schena, F.P., Tantillo, G. 2007. A comparison of DNA extraction methods for food analysis. *Food Control*, vol. 18, p. 76-80. http://dx.doi.org/10.1016/j.foodcont.2005.08.011

Espineira, M., Gonzalez-Lavín, N., Vieites, J. M., Santaclara, F. J. 2009. Development of a method for the identification of scombroid and common substitute species in seafood products by FINS. *Food Chemistry*, vol. 117, p. 698-704. http://dx.doi.org/10.1016/j.foodchem.2009.04.087

Lee, K. W., Kang, N. J., Heo, Y. S., Rogozin, E. A., Pugiliese, A., Hwang, M. K., Bowden, G. T., Bode, A. M., Lee, H. J., Dong, Z. 2008. Raf and MEK Protein kinases are direct molecular targets for the chemopreventive effect of quercetin, a major flavonol in red wine. *Cancer Research*, vol. 68, p. 946-955. http://dx.doi.org/10.1158/0008-5472.CAN-07-3140 PMid:18245498

Lin, W. F., Shiau, C. Y., Hwang, D. F. 2005. Identification of four *Thunnus* tuna species using mitochondrial cytochrome b gene sequence and PCR-RFLP analysis. *Journal of Food and Drug Analysis*, vol. 13, p. 382-387.

Lin, W. F. and Hwang, D. F. 2007. Application of PCR-RFLP analysis on species identification of canned tuna. *Food Control*, vol. 18, p. 1050-1057. http://dx.doi.org/10.1016/j.foodcont.2006.07.001

Lockley, A. K.; Bardsley, R. G. 2000. DNA-based methods for food authentication. *Trends in Food Science and Technology*, vol. 11, no. 2, p. 67-77. http://dx.doi.org/10.1016/S0924-2244(00)00049-2

Lopez, I. and Pardo, M. A. 2005. Application of relative quantification TaqMan real-time polymerase chain reaction technology for the identification and quantification of *Thunnus alalunga* and *Thunnus albacares*. *Journal of Agricultural and Food Chemistry*, vol. 53, p. 4554-4560. http://dx.doi.org/10.1021/jf0500841 PMid:15913324

Mackie, I. M., Pryde, S. E., Gonzales-Sotelo, C., Medina, I., Peréz-Martín, R., Quinteiro, J., Rey-Mandez M., Rehbein, H. 1990. Challenges in the identification of species of canned fish. *Trends in Food Science and Technology*, vol. 10, p. 9-14. http://dx.doi.org/10.1016/S0924-2244(99)00013-8

Pardo, M. A., Pérez-Villareal, B. 2004. Identification of commercial canned tuna species by restriction site analysis of mitochondrial DNA products obtained by nested primer PCR. *Food Chemistry*, vol. 86, p. 143-150. http://dx.doi.org/10.1016/j.foodchem.2003.09.024

Ram, J. L., Ram, M. L., Baidoun, F. F. 1996. Authentication of canned tuna and bonito by sequence and restriction site analysis of polymerase chain reaction products of mitochondrial DNA. *Journal of Agricultural and Food Chemistry*, vol. 44, p. 2460-2467. http://dx.doi.org/10.1021/jf950822t

Regulation (EC) No 1536/92 of the European Parliament and of the Council of 9 June 1992 laying down common marketing standards for preserved tuna and bonito. OJ L 163, 17.6.1992, p. 1-4.

Rehbein, H., Mackie, I. M., Pryde, S., Gonzales-Sotelo, C., Medina, I., Perez-Martin, R., Quinteiro, J., Rey-Mendez, M. 1999. Fish species identification in canned tuna by PCR-SSCP: validation by a collaborative study and investigation of intra-species variability of the DNA-patterns. *Food Chemistry*, vol. 64, p. 263-268. http://dx.doi.org/10.1016/S0308-8146(98)00125-3

Santaclara, F. J., Velasco, A., Pérez-Martin, R. I., Quinteiro, J., Rey-Méndez, M., Pardo, M. A., Jimenez, E., Sotelo, C. G. 2015. Development of a multiplex PCR-ELISA method for the genetic authentication of *Thunnus* species and *Katsuwonus pelamis* in food products. *Analytical Methods*, vol. 180, p. 9-16. http://dx.doi.org/10.1016/j.foodchem.2014.11.076

Takeyama, H., Chow, S., Tsuzuki, H., Matsunaga, T. 2001. Mitochondrial DNA sequence variation within and between tuna *Thunnus* species and its application to species identification. *Journal of Fish Biology*, vol. 58, p. 1646-1657. http://dx.doi.org/10.1111/j.1095-8649.2001.tb02319.x

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THE COMPARISON OF BIOLOGICAL ACTIVITY OF CHOCOLATES MADE BY DIFFERENT TECHNOLOGICAL PROCEDURES

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ABSTRACT

Chocolate is one of the most consumed delicacies in the world. Nowadays, raw chocolates without vanilla or allergens are getting more attention. The aim of this study was to evaluate and compare the biological activity of different types of chocolate – cold processed chocolate and chocolate made by traditional way. Total content of fat, crude fibre, polyphenols, flavonoids, phenolic acids and metylxantines – theobromine and caffeine was evaluated. The antioxidant activity was determined by a method using DPPH radical, reducing power method and phosphomolybdenum method. Both evaluated chocolates had similar content of fat and crude fibre, but sample of chocolate made by traditional way probably due to the higher content of cocoa mass had almost two times higher content of total polyphenols, flavonoids and phenolic acids as cold processed chocolate. Also the content of theobromine and caffeine was slightly higher in chocolate made by traditional way. This sample had the highest antioxidant activity – 93.68 mg TEAC.g^{-1} determined by phosphomolybdenum method, while in the sample of chocolate made by cold processed way was measeured value 50.82 mg TEAC.g^{-1}. Similarly, reducing power of chocolate made by traditional way was almost two times higher, but antioxidant activity determined with DPPH method was similar in both samples (3.58 and 3.62 mg TEAC.g^{-1}). The antioxidants and metylxantines in chocolates determine its potential to be a significant source of biologicaly active compounds with favorable effects to human health. It can be concluded in this study, that chocolate produced by conventional production technology can have more health-promoting ingredients reserved, but more extensive researches are still needed.

Keywords: chocolate; antioxidant activity; polyphenols; flavonoids; theobromine; caffeine

INTRODUCTION

In recent decades, nutrition research has focused on the investigation of bioactive dietary flavonoids, widely found in many plant-based foods and beverages, in order to elucidate their beneficial properties to human health. Cocoa (*Theobroma cacao* L.) and chocolate products appear to be one of the most promising foods due to their high polyphenol content, which evidently highlights the link with health-promoting properties **(Alañón et al., 2016)**.

Worldwide consumption of chocolate and cocoa-containing products increased by 10% from 2002 to 2010, which might be attributed to consumer economic enhancement and increasing knowledge of potential health benefits derived from cocoa constituents. Chocolate and cocoa-containing products are a good source of non-nutrient bioactive polyphenols with potential health benefits including reduced risk of cardiovascular disease and prebiotic activity **(Hu et al., 2016)**.

Chocolate and cocoa products are a rich source of flavonoids. Flavanoids, naturally occurring polyphenolic compounds present in plant-based foods, represent up to 20% of the compounds present in cocoa beans. Flavanols, and in particular epicatechin, are a subgroup of flavonoids, and are the most common cocoa flavonoids. High levels of flavanols are also found in tea, red wine, and fruits such as grapes and apples. In addition to cocoa flavonols, other psychoactive components of chocolate include the methylxanthines (caffeine and theobromine), both of which have been associated with improving alertness and cognitive function. One hundred grams of dark chocolate contain approximately 100 mg of flavanols, while 100 g of unsweetened cocoa powder without meythlxanthines can contain up to 250 mg of flavanols **(Crichton et al., 2016)**.

Chocolate represents functional properties due to its high level of flavonoid content, namely catechins and procyanidins, and beneficial impacts of chocolate consumption on human health. However, consumers are becoming more demanding in food market and they would like to have more options to choose from than ever before. Therefore, manufacturers desire to broaden their product ranges such as having organic chocolate, high-cocoa polyphenol-rich chocolate, probiotic chocolate, and prebiotic chocolate rather than ordinary chocolate. It was also showed that dark chocolate ensured a high probiotic survival rate **(Gültekin-Özgüven et al., 2016)**.

Therefore the aim of this study was to determine the biological activity of selected types of commerially available chocolates and evaluate their antioxidant activity, amount of total polyphenols, flavonoids, phenolic acids and main methylxantines – theobromine and caffeine.

MATERIAL AND METHODOLOGY

Biological material

The chocolates evaluated in this study were purchased from local market and signed as sample 1 and 2. Sample 1 was italian raw chocolate named „*Cioccolatino biologico 75% cacao con zucchero da fiori di palma da cocco*“, produced in town Modica, in Sicily (Italy). It contains 75% of cooca solids and the temperature throughout the process never exceeds 45 °C. Sample 2 was slovakian traditionally processed chocolate named „*Bean to Bar Dark 78%*“, with 78% cocoa solids. Both samples were made only from cocoa mass and coconut flower sugar and both were organic and distributed by same company.

Chemicals

All chemicals were analytical grade and were purchased from CentralChem (Slovakia) and Sigma Aldrich (USA).

Sample preparation

Samples were homogenized in a mortar and then defatted with petroleum ether. Defatted sample – 0.25 g of was extracted with 20 mL of 80% ethanol for 2 hours in a shaker (GFL 3005, Germany). After centrifugation at 4000 RPM (Rotofix 32A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, total polyphenols, flavonoids, phenolic acids, theobromine and caffeine – each analysis was carried out in ten replicates).

Determination of fat content

Fat content was determined by Fat extractor Ancom XT15 (ANKOM Technology, New York, USA) with the methodology recommended by the producer. The sample (1.5 g, W_1) was weighted to special filter bag (XT4, Ancom, USA) and dried for 3 hours in an oven (WTB, Binder, Germany) at 102 °C to remove moisture prior to the extraction. Samples were placed in a desiccant pouch for 15 minutes and after re-weighted (W_2) and extracted 60 minutes at 90 °C with petroleum ether. After process samples were removed and dried in an oven at 102 °C for 30 minutes, placed in desiccant pouch and re-weighted (W_3). The analysis was carried out in duplicate. Fat content was calculated by following formula:

$$\text{Fat content (\%)} = \frac{100 \times (W2 - W3)}{W1}$$

Determination of crude fiber content

Dietary fiber content was determinated with Ancom 200 Fiber analyzer (ANKOM Technology, New York, USA), with methodology recommended by the producer. One gram (W_2) of the sample was weighted to special filter bag (W_1 – bag tare weight; F57, Ancom, USA). Samples were defatted with petroleum ether, air-dried and placed to analyzer. 2000 mL of 0.1 M sulphuric acid was added and samples were hydrolyzed 45 minutes at 100 °C, after this process samples were washed with hot distilled water 3 times. Then 2000 mL of 0.1 M potassium hydroxide was added and samples were hydrolyzed 45 minutes at 100 °C, after this process were washed with hot distilled water 3 times. Water was gently pressed from the bags and bags were soaked in acetone for 5 minutes, removed, air-dried and placed in the oven at 102 °C (WTB, Binder, Germany) for 2 hours. After cooled to room temperature, bags were re-weigted and ashed in pre-weighetd crucibles for 2 hours at 550 °C for 2 hours. Ashed crucibles were weighted to calculate the loss of weight of organic matter (W_3). The analysis was carried out in duplicate. Crude fiber content was calculated by following formula:

$$\text{Crude fiber (\%)} = \frac{100 \times [W3 - (W1 \times C1)]}{W2}$$

C_1 – ash corrected blank bag factor (running average of loss of weight on ignition of blank bag/original blank bag)

Total polyphenol content

Total polyphenol content of chocolate extracts was measured by the method of **Singleton and Rossi, (1965)** using Folin-Ciocalteu reagent. A 0.1 mL of each sample extract was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate and 8.8 mL of distilled water. After 30 minutes in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25 – 250 $mg.L^{-1}$; $r^2 = 0.9978$) was used as the standard and the results were expressed in $mg.g^{-1}$ gallic acid equivalents.

Total flavonoid content

Total flavonoids were determined using the modified method of **Willett, (2002)**. A 0.5 mL of sample extract was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M sodium acetate and 4.3 mL of distilled water. After 30 minutes in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.01 – 0.5 $mg.L^{-1}$; $r^2 = 0.9977$) was used as the standard and the results were expressed in $mg.g^{-1}$ quercetin equivalents.

Total phenolic acids content

Total phenolic acids content was determined using method of **Farmakopea Polska, (1999)**. A 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent (10% $NaNO_2$ + 10% Na_2MoO_4), 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1-200 $mg.L^{-1}$, r^2=0.9996) was used as a standard and the results were expressed in $mg.g^{-1}$ caffeic acid equivalents.

Antioxidant activity

Radical scavenging activity

Radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(Sánchéz-Moreno et al., 1998)**. The extract (0.4 mL) was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). After 10 minutes in darkness, absorbance of the sample extract was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10 – 100 $mg.L^{-1}$; $r^2 = 0.9881$) was used

as the standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Reducing power

Reducing power of samples was determined by the method of **Oyaizu, (1986)**. One mililiter of sample extract was mixed with 5 mL PBS (phosphate buffer with pH 6.6) and 5 mL of 1% potassium ferricyanide (w/v) in the test tube. Mixture was stirred thoroughly and heated in water bath for 20 minutes at 50 °C. After cooling, 5 mL of 10% trichloroacetic acid was added. 5 mL of mixture was pipetted into the test tube and mixed with 5 mL of distilled water and 1 mL of 0.1% (w/v) ferric chloride solution. Absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Reducing power was expressed in $mg.g^{-1}$ Trolox equivalents, using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10 – 100 $mg.L^{-1}$; $r^2 = 0.9974$) as the standard and results were expressed in $mg.g^{-1}$ Trolox equivalents.

Phosphomolybdenum method

Phosphomolybdic method was determined by a method of **Prieto et al. (1999)**. Monopotassium phosphate (2.8 mL, 0.1 M, w/v) was mixed with sulfuric acid (6 mL, 1 M), ammonium molybdate (0.4 mL, 0.1 M, w/v), distilled water (0.8 mL) and 1 mL of sample extract. Test tubes were mixed thoroughly and heated in water bath for 120 minutes at 90 °C. After cooling, absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Antioxidant activity was expressed in $mg.g^{-1}$ Trolox equivalents (10 – 1000 $mg.L^{-1}$, r^2=0.9975).

Methyxantines content determination by HPLC-DAD method

Theobromine and caffeine content was determined using separation gradient method RP-HPLC/UV-DAD by Agilent 1260 Infinity high performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany). Separation was achieved on a Purosphere reverse phase C18 column (4 mm × 250 mm × 5 μm) (Merck, KGaA, Darmstadt, Germany). The mobile phase consisted of HPLC methanol (B) and 0.1% formic acid in HPLC water (C). The following gradient program was employed: 0 – 2 min. isocratic elution (20% B and 80% C), 2 – 15 min. linear gradient elution (40% B and 60% C), and 40% B and 60% C 15 – 20 min. The flow rate was 1 $mL.min^{-1}$. Column oven temperature was set up to 25 °C and the samples were kept at 4 °C in the Peltier sample manager. The DAD signal was conducted at 210 – 400 nm with preferred wavelength 330 nm for quantitative purposes with data acquisition rate of 5 Hz.

Statistical analysis

The basic statistical analyzes were realized in SAS programming packages (THE SAS SYSTEM V 9.2.). Correlation coefficients were calculated by CORR analysis and it was also used t-test **(SAS, 2009)**.

RESULTS AND DISCUSSION

Fat content

Dark chocolate can be considered as products with an important nutritional density, because of their richness in carbohydrates and fats. Cocoa butter is considered the most important cocoa by-product, due to its physical (rheology and texture) and chemical characteristics, and also organoleptic qualities. The prevalence of saturated fatty acids over unsaturated fatty acids is considered to be negative from the nutritional point of view. For many years, saturated fatty acids whose chain length is C12:0 – C16:0 have been considered to provoke atherosclerosis, and to be associated with cardiovascular disease. Thus, because of its high SFA content, chocolate is often postulated as having a hypercholesterolemic effect. However, it has been suggested in recent clinical trials that stearic acid (C18:0), a non-cholesterolemic and atherogenic type of dietary saturated fat, is neutral. These trials have shown that chocolate consumption has neutral effects on serum total cholesterol and LDL-cholesterol, as neither lowers HDL-cholesterol **(Torres-Moreno et al., 2015)**.

Fat content of both evaluated samples was similar. Cold processed chocolate contained 35.24 ±0.01% of fat, while chocolate made by traditional way contained 37.60 ±1.47% of fat. The manufacturer of the chocolate made by traditional way maintains on the package that the chocolate contains 41 grams of fat per 100 g of chocolate, which correlates with our results. On the package of cold processed chocolate there was no data about fat content. **Rezende at al., (2015)** showed that increasing the cocoa butter content enhanced the acceptance score of chocolates. This relationship is widely accepted in chocolate production, as cocoa butter provides rheological properties that promote proper hardness, mouth feel and swallow.

Although evidence in the literature suggests that chocolate consumption may have beneficial effects on health, it must be noted that chocolate has a high total fat and sugar content; in consequence, daily consumption of large amounts of chocolate may increase weight in the long term. That is why scientific evidence suggests that chocolate consumption should be considered in the context of a healthy diet, and dark chocolate must be consumed in moderate amounts (20 – 25 g per day) **(Machálková et al., 2016; Torres-Moreno et al., 2015)**.

Crude fiber content

The health benefits of dietary fiber have long been appreciated. Higher intakes of dietary fiber are linked to less cardiovascular disease and fiber plays a role in gut health. Higher intakes of fiber are linked to lower body weights **(Slavin, 2013)**. The recommended dietary reference intake of total fiber is 25 g per day for young women and 38 g pred day for young men; however, a usual intake of dietary fiber in the US is only about 15 g per day **(Jurasová et al., 2011; Brownawell et al., 2012)**.

Content of cruder fiber was in both samples on the similar level – 0.561 ±0.01% for cold precessed chocolate and 0.535 ±0.02% for chocolate made by traditional way. Also **Torres-Moreno et al., (2015)** reported that chocolate contains less than 2% of fibre. Thus, chocolate

Table 1 Content of biologically active compounds in chocolate samples.

	TPC (mgGAE.g^{-1})	TFC (mgQE.g^{-1})	TPA (mgCAE.g^{-1})	DPPH (mgTEAC.g^{-1})	RP (mgTEAC.g^{-1})
Sample 1	8.14 ±0.98	0.34 ±0.08	9.47 ±0.91	3.62 ±0.09	31.39 ±1.14
Sample 2	16.37 ±2.07	0.74 ±0.02	16.16 ±0.63	3.58 ±0.03	55.91 ±0.95

TPC – total polyphenol content; TFC – total flavonoid content; TPA – total phenolic acid content; RP – reducing power; GAE – gallic acid equivalent; QE – quercetin equivalent; CAE – caffeic acid equivalent; TEAC – Trolox equivalent antioxidant capacity; ±standard deviation; sample 1 – cold processed chocolate; sample 2 – chocolated meda by traditional way.

consumption does not contribute significantly to protein and dietary fibre intake.

Erdem et al., (2014) showed that dietary fiber addition didn't show negative effects, such as off-flavor, unwanted aroma or taste, on color and organoleptic properties of chocolate samples. Among fibers, maltodextrin and lemon fiber addition had positive effects on the sensory characteristics.

Total polyphenol content

Latest studies have shown that chocolate was not only a simple blend of fat and sugar, but also a rich source of flavonoids and polyphenols which shows high antioxidant activities **(Erdem et al., 2014)**. Main groups of polyphenols are the catechins (37%), procyanidins (58%) and anthocyanins (4%). The polyphenols in cocoa beans contribute to about 12 – 18% of the dry weight of the whole bean **(Bordiga et al., 2015)**. Furthermore, these non-nutrient bioactive compounds have potential health benefits including reduced risk of cardiovascular disease and prebiotic activity **(Hu et al., 2016)**.

Total polyphenol content (TPC) of samples is shown in Table 1. Chocolate made by traditional way had almost double polyphenol content compared to cold processed chocolated. Similar results for dark chocolates produced in Serbia reported **Todorovic et al., (2015)**, where TPC varied from 7.21 ±0.49 to 12.65 ±0.45 mg GAE.g^{-1} for chocolates with 60 – 75% of cooca content.

Our results are also with accordance to **Hu et al., (2015)** results, which determined TPC 27.34 ±0.20 mg GAE.g^{-1} in defatted sample of 70% Peru origin chocolate, while our 78% Peru origin chocolate made by traditional way had similar amount – 26.23 ±2.07 mg GAE.g^{-1} in defatted sample.

Bordiga et al., (2015) reported that both processing conditions (fermentation and drying) and the chocolate-making practices influence the polyphenolic content in the final products. **Hu et al., (2015)** explained that manufacturers use a variety of cacao cultivars as well as processing and storage parameters, and all of these impact antioxidant capacity and phenolic profiles of the final products.

Total flavonoid content

Flavonoids are an important class of plant pigments, naturally found in fruit and vegetables. This class of naturally occurring polyphenolic compounds which cannot be synthesized by humans possesses a series of biological properties, acting on biological systems as antioxidants. They act as antiviral, antiinflammatory, and antitumoral agents, affecting capillary permeability and acting as exogenous antioxidants. Flavonoids present in the diet are directly linked to the prevention of arthrosclerosis, as various studies show that the reduction of total blood cholesterol levels and the antioxidant effect lead to lower risks of arthrosclerosis, teratogenicity, and coronary disease **(Calado et al., 2015)**.

Total flavonoid content (TFC) of chocolate made by traditional way was again much higher than cold processed chocolate (Table 1). **Calado et al., (2015)** showed, that bitter chocolate can have higher flavonoid content compared to some kinds of cooked vegetables and advises, that people who don't like foods like broccoli or eggplant (daily dose between 20 and 26 g) could eat more chocolate (daily dose only 8 g).

The cocoa bean is one of the richest sources of flavanols, but the art is to preserve these wholesome components as much as possible in the final consumable products. The negative impact of the manufacturing process of chocolate and cocoa powder products on the flavanol content should not be underestimated **(Paoletti et al., 2012)**.

Total phenolic acids content

Phenolic acids are secondary plant metabolites widely spread throughout the plant kingdom. Chemically, phenolic acids are hydroxylated derivatives of benzoic, cinnamic, phenylacetic and phenylpropanoic acids. In nature, hundreds of phenolic acids have been identified. They are most abundant in coffee, tea and especially in berries. Recent interest in phenolic acids stems from their potential protective role against oxidative stress, inflammation, diabetes and cancer in experimental studies **(Zamora-Ros et al., 2013)**.

Protocatechuic acid is a hydroxybenzoic acid that can be found in many foods and it is also the most important phenolic acid (69.16%) found in cocoa liquor. It has been reported to have several physiological functions including antioxidant, antibacterial activity, antimutagenic activity, antitumour activity, and anticancer effects. Coumaric acids, hydroxy derivatives of cinnamic acid, are another important group of phenolic compounds found in cocoa (2.65%) with antioxidant and antigenotoxic properties **(Zhou et al., 2015)**. Content of total phenolic acids (TPA) in chocolate samples is shown in Table 1. Chocolate made by traditional way again showed almost two times higher value with compare to cold processed chocolate. To date, limited data exist on the quantitative intake of phenolic acids **(Zamora-Ros et al., 2013)**.

Antioxidant activity

Radical scavenging activity

The antioxidant activity of cocoa powder is well known, but changes occur during the lifetime of the bean, and activity often depends on its processing. The content of polyphenols can vary greatly depending on the source of beans, primary and secondary processing conditions, and packaging and processing of chocolate making. Due to these factors, the ratio and types of polyphenols found in cocoa beans, as well as their activity, are unlikely to be the same as those found in the finished products **(Vertuani et al., 2014)**.

Cold processed chocolate has higher only radical scavenging activity determined using DPPH radical (Table 1). But if the product contains more cocoa butter, antioxidant capacity values would be lower than products with the same cacao content but more cacao solids. Because of the complexity of chocolate, many components, other than phenols are present that could influence the results in different ways **(Hu et al., 2016)**.

High antioxidant activity also reported **Vertuani et al., (2014)**, who showed that it is not only the amount of cocoa used that is important, but also the quality and provenience affect the product properties. In general, for organic chocolates, by increasing the percentage of cocoa, the amount of total polyphenols enhances and the antioxidant capacity of the product increases in proportion.

Reducing power

Antioxidant properties are known to be concomitant with the development of reducing power. Reductones can react directly with peroxides and can also prevent peroxide formation by reacting with certain precursors **(Kim et al., 2013)**.

Values for reducing power (RP) expressed as trolox equivalent antioxidant capacity (TEAC) are shown in Table 1. To compare it with results of other authors, RP of samples was expressed in absorbance at 700 nm (A_{700}), too. A_{700} of cold processed chocolate (1.04 ±0.03 nm) is almost 3 times higher than A_{700} of broccoli (0.30 ±0.00 nm) determined by **Kim et al., (2013)**. A_{700} of chocolate made by traditional way (1.85 ±0.03 nm) is higher than broccoli or cold processed chocolate or even leafy green vegetable *Aralia elata* (A_{700} 1.20 ±0.00 nm) consumed in Asia **(Kim et al., 2013)**. Reducing power and total flavonoids in evaluated chocolates showed a high correlation (r^2 = 0.972, p <0.05), similarly like reducing power and total phenolic acid content (r^2 = 0.982, p <0.05), implying that the phenolic compounds were major contributors to the observed antioxidant activity.

Phosphomolybdenum method

In the presence of antioxidant compounds, Mo (VI) is reduced to Mo (V) and forms a green colored complex of phosphomolybdenum (V) that gives absorbance at 700 nm. The method is based upon the spectrophotometric quantitation of total antioxidant capacity **(Prieto et al., 1999)**. Antioxidant capacity of cold processed chocolate was 50.82 ±3.69 mg TEAC.g^{-1}, while chocolate made by traditional way showed almost double the reducing power – 93.68 ±10.41 mg TEAC.g^{-1}. **Ibrić and Ćavar, (2014)** determined total antioxidant activity using molybdenum reduction method with catechin standard as IC_{50}, which is the concentration of extract to reduce 50% of molybdenum cation. IC_{50} of dark chocolate was 2.54 ±0.23 mg.mL^{-1}. There are also no previously published data concerning evaluation of antioxidant activity of cocoa products using this method.

Similarly like in reducing power, phosphomolybdenum method and total flavonoids and phenolic acids in evaluated chocolates showed a high correlation (r^2 = 0.964; r^2 = 0.979, p <0.05).

Methylxanthines content

Cocoa is the major natural source of the theobromine. Compared with coffee and tea, cocoa and chocolate products have much lower content of caffeine and only traces of theophylline. The level of methylxanthines in cocoa beans depends on varietal type and is influenced by the fermentation process. Besides psycho-pharmacological effects, methylxanthines of cocoa products particularly theobromine, and to a lesser extent caffeine, may have a role in lowering plasma glucose **(Todorovic et al., 2015)**.

As expected, theobromine was the predominant compound among methylxanthines. It content in cold processed chocolate was 22.095 ±0.058 mg.g^{-1} and slightly higher in chocolate made by traditional way (27.763 ±0.009 mg.g^{-1}). Content of caffeine was much lower, 1.326 ±0.002 mg.g^{-1} in cold processed chocolate and 1.758 ±0.005 mg.g^{-1} in chocolate made by traditional way, respectively. Theobromine/caffeine ratio was 16:1 for both samples.

Bordiga et al., (2015) and **Todorovic et al., (2015)** determined lower amount of these metylxantines in dark chocolates containing 40 – 75% cocoa solids. Theobromine/caffeine ratio was lower in chocolates containing more cocoa solids. The content of methylxanthines and the theobromine/caffeine ratio vary depending on the cocoa genotype **(Aprotosoaie et al., 2016)**.

Obtained values for methylxantines in cocoa products show that potential physiological effects of chocolate mainly come from theobromine, with only small contribution of caffeine. From literature is know that 50 g of dark chocolate can have sufficient quantity of theobromine to produce neurophysiological effects **(Todorovic et al., 2015)**. The main pharmacological activities include: central nervous system stimulation, cardiovascular and metabolic effects, bronhodilatation, diuresis, gastric secretion stimulation, and, in high doses, the stimulation of skeletal muscles Methylxanthines, mainly caffeine, enhance physical and intellectual performance, mitigate fatigue, and cause a feeling of alertness. Cocoa products represent only a small part of the human diet and the concentration of methylxanthine is low, so these products do not normally pose a risk to human health. Furthermore, theobromine appears to be even safer for humans than caffeine **(Aprotosoaie et al., 2016)**.

CONCLUSION

On the basis of the above findings we can conclude, that high percentage dark chocolate is in general very good source of biologicaly active compounds, but not only

antioxidants, but also fats, crude fiber or psychologically active compounds. Our results confirmed that traditional process of chocolate making probably due to the higher content of cocoa mass can preserve more nutritionally significant components than processes with low temperatures. Even if high quality, organic chocolates have interesting biological characteristic, much higher caloric value of this delicacies should not be forgotten. Since there is lack of data for this issue, especially raw chocolates, it is necessary to expand the array of samples and performed analyzes to confirm or refute our conclusions. Results in this work can be an important tool for next scientific works and for food producers.

REFERENCES

Alañón, M. E., Castle, S. M., Siswanto, P. J., Cifuentes-Gómez, T., Spencer, J. P. E. 2016. Assesment of flavanol stereoisomers and caffeine and theobromine content in commercial chocolates. *Food Chemistry*, vol. 208, p. 177-184. http://dx.doi.org/10.1016/j.foodchem.2016.03.116 PMid:27132838

Aprotosoaie, A. C., Luca, S. V., Miron, A. 2016. Flavor Chemistry of Cocoa and Cocoa Products – An Overview. *Comprehensive Reviews in Food Science and Food Safety*, vol. 15, no. 1, p. 73-91. http://dx.doi.org/10.1111/1541-4337.12180

Bordiga, M., Locatelli, M., Travaglia F., Coïson, J. D., Mazza, G., Arlorio, M. 2015. Evaluation of the effect of processing on cocoa polyphenols: antiradical activity, anthocyanins and procyanidins profiling from raw beans to chocolate. *International Journal of Food Science & Technology*, vol. 50, no. 3, p. 840-848. http://dx.doi.org/10.1111/ijfs.12760

Brownawell, A. M., Caers, W., Gibson, G. R., Kendall, C. W. C., Lewis, K. D. 2012. Prebiotics and the Health Benefits of Fiber: Current Regulatory Status, Future Research, and Goals. *The Journal of Nutrition*, vol. 142, no. 5, p. 962-974. http://dx.doi.org/10.3945/jn.112.158147 PMid:22457389

Calado, J. C. P., Albertão, P. A., De Oliveira, E. A., Letra, M. H. S., Sawaya, A. C. H. F., Marcucci, M. C. 2015. Flavonoid Contents and Antioxidant Activity in Fruit, Vegetables and Other Types of Food. *Agricultural Sciences*, vol. 6, no. 4, p. 426-435. http://dx.doi.org/10.4236/as.2015.64042

Crichton, G. E., Elias, M. F., Alkerwi, A. 2016. Chocolate intake is associated with better cognitive function: The Maine-Syracuse Longitudinal Study. *Appetite*, vol. 100, p. 126-132. http://dx.doi.org/10.1016/j.appet.2016.02.010 PMid:26873453

Erdem, Ö., Gültekin-Özgüvena, M., Berktaşa, I., Erşana, S., Tunaa, H. E., Karadağa, A., Özçelika, B., Güneşa, G., Cuttingb, S. M. 2014. Development of a novel synbiotic dark chocolate enriched with *Bacillus indicus* HU36, maltodextrin and lemon fiber: Optimization by response surface methodology. *LWT – Food Science and Technology*, vol. 56, no. 1, p. 187-193. http://dx.doi.org/10.1016/j.lwt.2013.10.020

Farmakopea Polska, V. I. 1999. Wyd. PTFarm. Warszawa, p. 880-881.

Gültekin-Özgüven, M., Karadag, A., Duman, S., Özkal, B., Özçelik, B. 2016. Fortification of dark chocolate with spray dried mulberry (*Morus nigra*) waste extract encapsulated in chitosan-coated liposomes and bioaccesability studies. *Food Chemistry*, vol. 201, p. 205-212. http://dx.doi.org/10.1016/j.foodchem.2016.01.091 PMid:26868567

Hu, Y., Pan, Z. J., Liao, W., Li, J., Gruget, P., Kitts, D. D., Lu, X. 2016. Determination of antioxidant capacity and phenolic content of chocolate by attenuated total reflectance-Fourier transformed-infrared spectroscopy. *Food Chemistry*, vol. 202, p. 254-261. http://dx.doi.org/10.1016/j.foodchem.2016.01.130 PMid:26920292

Ibrić, A., Ćavar, S. 2014. Phenolic Compounds and Antioxidant Activity of Cocoa and Chocolate Products. *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*, vol. 42, p. 37-40.

Jurasová, M., Kohajdová, Z., Karovičová, J. 2011. Dietary fiber: definition, sources and extraction. *Potravinarstvo*, vol. 5, no. 2, p. 22-26. http://dx.doi.org/10.5219/125

Kim, S. J., Cho, A. R., Han, J. 2013. Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food Control*, vol. 29, no. 1, p. 112-120. http://dx.doi.org/10.1016/j.foodcont.2012.05.060

Machálková, L., Hřivna, L., Nedomová, Š., Jůzl, M. 2015. The effect of storage temperature on the quality and formation of blooming defects in chocolates. *Potravinarstvo*, vol. 9, no. 1, 39-47. http://dx.doi.org/10.5219/425

Oyaizu, M. 1986. Studies on products of browning reaction-antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese journal of nutrition*, vol. 44, no. 6, p. 307-314.

Paoletti, R., Poli, A., Conti, A., Visiolo, F. 2012. *Chocolate and Health*. Milan: Springer-Verlag, 156 p. ISBN 978-88-470-2038-2.

Prieto, P., Pineda, M., Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, vol. 269, no. 2, p. 337-341. http://dx.doi.org/10.1006/abio.1999.4019 PMid:10222007

Rezende, N. V., Benassi, M. T., Vissotto, F. Z., Augusto, P. P. C., Grossmann, M. V. E. 2015. Effects of fat replacement and fibre addition on the texture, sensory acceptance and structure of sucrose-free chocolate. *International Journal of Food Science & Technology*, vol. 50, no. 6, p. 1413-1420. http://dx.doi.org/10.1111/ijfs.12791

Sánchés-Moreno, C., Larrauri, A., Saura-Calixto, F. 1998. A procedure to measure the antioxidant efficiency of polyphenols. *Journal of the Science of Food and Agriculture*, vol. 76, no. 2, p. 270-276. http://dx.doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9

Singleton, V. L., Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Agricultural*, vol. 16, p. 144-158.

Slavin, J. 2013. Fiber and Prebiotics: Mechanisms and Health Benefits. *Nutrients*, vol. 5, no. 4, p. 1417-1435. http://dx.doi.org/10.3390/nu5041417 PMid:23609775

Todorovic, V., Radojcic Redovnikovic, I., Todorovic, Z., Jankovic, G., Dodevska, M., Sobajic, S. 2015. Polyphenols, methylxantines, and antioxidant capacity od chocolates produced in Serbia. *Journal of Food Composition and Analysis*, vol. 41, no., p. 137-143.

Torres-Moreno, M., Torrescasana, E., Salas-Salvadó, J., Blanch, C. 2015. Nutritional composition and fatty acids

profile in cocoa beans and chocolates with different geographical origin and processing conditions. *Food Chemistry*, vol. 166, no. 1, p. 125-132. http://dx.doi.org/10.1016/j.foodchem.2014.05.141 PMid:25053037

Vertuani, S., Scalambra, E., Vittorio, T., Bino, A., Malisardi, G., Baldisserotto, A., Manfredini, S. 2014. Evaluation of Antiradical Activity of Different Cocoa and Chocolate Products: Relation with Lipid and Protein Composition. *Journal of Medicinal Food*, vol. 17, no. 4, p. 512-516. http://dx.doi.org/10.1089/jmf.2013.0110

Zamora-Ros, R., Rothwell, J. A., Scalbert, A., Knaze, V., Romieu, I., Slimani, N., Fagherazzi, G., Perquier, F., Touillaud, M., Molina-Montes, E., Huerta, J. M., Barricarte, A., Amiano, P., Menéndez, V., Tumino, R., de Magistris, M. S., Palli, D., Ricceri, F., Sieri, S., Crowe, F. L., Khaw, K. T., Wareham, N. J., Grote, V., Li, K., Boeing, H., Förster, J., Trichopoulou, A., Benetou, V., Tsiotas, K., Bueno-de-Mesquita, H. B., Ros, M., Peeters, P. H., Tjønneland, A., Halkjær, J., Overvad, K., Ericson, U., Wallström, P., Johansson, I., Landberg, R., Weiderpass, E., Engeset, D., Skeie, G., Wark, P., Riboli, E., González, C. A. 2013. Dietary intakes and food sources of phenolic acids in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *British Journal of Nutrition*, vol. 110, no. 8, p. 1500-1511. http://dx.doi.org/10.1017/S0007114513000688 PMid:23507418

Willett, W. C. 2002. Balancing life-style and genomics research for disease prevention. *Science*, vol. 292, no. 5568, p. 695-698. http://dx.doi.org/10.1126/science.1071055 PMid:11976443

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CELIAC DISEASE: THE SITUATION ON THE SLOVAK MARKET

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ABSTRACT

Celiac disease, also known as celiac sprue, non-tropical sprue, idiopathic sprue, idiopathic steatorrhoea and gluten-sensitive enteropathy, is a serious genetic autoimmune disease, which damages the villi of the small intestine and interferes with absorption of nutrients from food. The latest researches show that while in the 1970s the prevalence of celiac disease in the world was 0.03%, in the present years the estimated prevalence is 1%. In average, the prevalence of celiac disease in the Western countries is close to 1:100. The celiac disease occurs more often in the case of women than of men, at a ratio of 2.8:1. The aim of the present paper was to bring few information about the celiac disease, highlight the increasing number of celiacs, as well as to determine the Slovak celiacs opinion about the situation on Slovak market and their consumer behaviour on the market of gluten free products. As research methods, there have been used the methods of survey and structured questionnaire consisting of 22 questions. The total number of respondents was 130 randomly selected celiacs from all over the Slovak republic. For a deeper analysis of the obtained results, there have been set out four assumptions and ten hypotheses, which have been tested with the use of Pearson´s chi-square test, Mann-Whitney U-Test and Cramer´s contingency coefficient. The results of the present paper show, that despite the fact that few of our findings are pleasing – almost 52% of our respondents stay that the labelling of gluten free products is sufficient, over 74% of respondents think that they have enough information about the availability of gluten free products and more than 89% of respondents think that the present scope of range of gluten free products is better as before; there are still some shortcomings, which has to be reduced or eliminated – only less than 7% of respondents think that the price of gluten free products is adequate, over 45% of respondents use this possibility of granting a monetary contribution for compensation of increased expenses on a special diet, almost 65% of respondents think that the scope of range of gluten free products is in the Slovak market insufficient, 53% of respondents think that the availability of gluten free products in the Slovak market is inadequate and only 48% of respondents prefer the domestic producers of gluten free products.

Keywords: celiac disease; gluten free product; gluten free diet; consumer behaviour; Slovak republic

INTRODUCTION

Celiac disease is a life-long gluten sensitive autoimmune disease of the small intestine affecting genetically susceptible individuals **(Gujral et al., 2012)**. In general we can say, that it can affect both – adults but also children and that it is not **(URL 1, 2015)**:

- simply a food allergy (IgE) – wheat allergies are rare among adults; in children, wheat allergies affect 0.04 – 0.05% of population,
- an idiosyncratic reaction to food proteins (mediated by IgE);
- typified by a rapid histamine-type reaction (such as bronchospasm, urticaria, etc.);
- an intolerance which is a non-immune system response to food.

*„When people with celiac disease eat foods or use products containing gluten, their immune system responds by damaging or destroying villi – the tiny, fingerlike projections on the inner lining of the small intestine. Villi normally absorb nutrients from food and pass the nutrients through the walls of the small intestine and into the bloodstream. Without healthy villi, people can become malnourished, no matter how much food they eat.“***(URL 2, 2015)**.

The first description of the disease derives from the 2nd century BC, when Aretaeus from Cappadocia described a patient with chronic diarrhea and failure to thrive and called it koiliakos, which was in 1856, translated by Francis Adams into English as “coeliac” **(Villanaci et al, 2011)**. The more detailed description was given by Samuel Gee (a paediatrician at the London Hospital of Saint Bartolommeo) in 1880 **(Ciclitira et al., 2005; Walker et al., 2010)** and the link between the consumption of gluten and the symptoms of the illness was demonstrated during the World War II **(Abdulkarim et al., 2003)**.

The classical symptoms of the disease are diarrhea, abdominal pain and weight loss **(Olén et al., 2011)**. Besides the classic symptoms there are appearing also new once, which are the result of insufficient absorption of important nutritional components in the digestion process. They are represented by osteoporosis, joint pain, chronic fatigue, skin lesions, anemia etc. **(Fasano, 2009)**.

Facts about celiac disease (National Foundation for Celiac Awareness, 2015; URL 3, 2015; URL 4, 2011)

- celiac disease is not a food allergy, it is an autoimmune disease, which damages the villi of the small intestine and interferes with absorption of nutrients from food , and which can never be "outgrown",
- celiac disease is a hereditary condition, which means it is passed through families – if one family member has celiac disease, other family members, especially 1st degree relatives (parents, brothers and sisters, or the children of people who have been diagnosed), should always be tested,
- it occurs in 3.9 – 12.3% of people with Diabetes Type 1, in 5 – 12% of people with Down syndrome, Turner syndrome and other auto-immune conditions, in 20% of people with collagenous colitis, in 4.5% of first degree relatives of people with the same disease,
- gluten is essentially toxic to people with celiac disease and gluten sensitivity,
- an estimated 1 in 133 Americans, or about 1% of the population, has celiac disease,
- celiac disease can affect men and women across all ages and races,
- it is estimated that 83% of Americans who have this disease are undiagnosed or misdiagnosed with other conditions,
- 6 – 10 years is the average time a person waits to be correctly diagnosed,
- 5 – 22% of celiacs have an immediate family member (1st degree relative) who also has celiac disease,
- celiac disease can lead to a number of other disorders including infertility, reduced bone density, neurological disorders, some cancers, and other autoimmune diseases
- the cause of disease is not known,
- over 90% of celiacs are undiagnosed or misdiagnosed,
- celiac disease declined during the bread shortages of the Second World War but climbed again after the war,
- half of all people with celiac do not show any symptoms,
- September 13th is a Celiac Awareness Day,
- some products, like lipstick, toothpaste and vitamins, use gluten for processing but because it is not food, does not need to be labelled,
- alcohol made with gluten-containing grains is gluten-free because the distillation process removes the gluten protein.

MATERIAL AND METHODOLOGY

The aim of the present paper was to bring few information about the celiac disease, highlight the increasing number of celiacs, as well as to determine the Slovak celiacs opinion about the situation on Slovak market and their consumer behaviour on the market of gluten free products.

In order to achieve the aim, as research methods, there have been used the methods of survey and structured questionnaire consisting of 22 questions formulated as closed, so that respondents (total number of respondents was 130 randomly selected celiacs, Table 1) had to choose one, alternatively several options.

Table 1 Characteristics of respondents.

Category of respondents	**Number**
Male	10
Female	120
Place of residence	**Number**
City	86
Village	44
Age structure	**Number**
15 – 19 years	11
20 – 25 years	22
26 – 35 years	47
36 – 49 years	38
50 and more years	12
Education structure	**Number**
Primary education	4
Secondary education without A level	9
Secondary education with A level	59
Higher professional education	7
Higher education	51
Net family income	**Number**
Up to 500 €	23
501 − 1.000 €	56
1.001 – 1.500 €	31
1.501 € and more	20
Region	**Number**
BanskáBystrica	20
Bratislava	34
Košice	4
Nitra	22
Prešov	4
Trenčín	18
Trnava	9
Žilina	19

Note: Source: Results of the research.

In consideration of lack of information about the exact number of celiacs living in Slovak republic (the estimated number of celiacs living in Slovak republic in the year 2014 was between 0.5 and 1% of the total population of Slovak republic **(URL 5, 2014)**, we tried to ensure the representativeness of the results by the random selection and geographic diversification of our respondents (celiacs).

The questionnaire was evaluated with the use of contingency tables, which were prepared by Excel, under which they were subsequently developed graphic representations.

For a deeper analysis of the obtained results, there have been set out the following assumptions:

1. Assumption no. 1 – we assume that most of our respondents are women.

2. Assumption no. 2 – we assume that most of our respondents have celiac disease diagnosed from 0 to 5 years.
3. Assumption no. 3 – we assume that most of our respondents use a so called possibility of granting a monetary contribution for compensation of increased expenses on a special diet.
4. Assumption no. 4 – we assume that most of our respondents think that the scope of range of gluten free products in the Slovak market is insufficient.

And the following and hypothesis:

1. H_{01} – there does not exist the dependence between the frequency of purchase of gluten free products and the respondent´s place of living.
   H_{11} – there exists the dependence between the frequency of purchase of gluten free products and the respondent´s place of living.
2. H_{02} – there does not exist the dependence between the place of purchase of gluten free products and the respondent´s age.
   H_{12} – there exists the dependence between the place of purchase of gluten free products and the respondent´s age.
3. H_{03} – there does not exist the dependence between the decisive criteria in the purchase of gluten free products and the respondent´s age.
   H_{13} – there exists the dependence between the decisive criteria in the purchase of gluten free products and the respondent´s age.
4. H_{04} – there does not exist the dependence between the decisive criteria in the purchase of gluten free products and the respondent´s level of education.
   H_{14} – there exists the dependence between the decisive criteria in the purchase of gluten free products and the respondent´s level of education.
5. H_{05} – there does not exist the dependence between the respondent´s opinion on the adequacy of available information and the region from which the respondent comes from.
   H_{15} – there exists the dependence between the respondent´s opinion on the adequacy of available information and the region from which the respondent comes from.
6. H_{06} – there does not exist the dependence between the respondent´s opinion on the scope of gluten free products on the Slovak market and the region from which the respondent comes from.
   H_{16} – there exists the dependence between the respondent´s opinion on the scope of gluten free products on the Slovak market and the region from which the respondent comes from.
7. H_{07} – there does not exist the dependence between the respondent´s opinion on the scope of gluten free products in his region and the region from which the respondent comes from.
   H_{17} – there exists the dependence between the respondent´s opinion on the scope of gluten free products in his region and the region from which the respondent comes from.
8. H_{08} – there does not exist the dependence between the respondent´s opinion on the availability of gluten free products on the Slovak market and the region from which the respondent comes from.
   H_{18} – there exists the dependence between the respondent´s opinion on the availability of gluten free products on the Slovak market and the region from which the respondent comes from.
9. H_{09} – there does not exist the dependence between the respondent´s opinion on the availability of gluten free products in his region and the region from which the respondent comes from.
   H_{19} – there exists the dependence between the respondent´s opinion on the availability of gluten free products in his region and the region from which the respondent comes from.
10. H_{010} – there does not exist the dependence between buying gluten free products abroad and the region from which the respondent comes from.
    H_{110} – there exists the dependence between buying gluten free products abroad and the region from which the respondent comes from.

To test the dependence respectively the independence between the tested variables there were used the tests of Pearson´s chi-square test, Mann-Whitney U-Test and Cramer´s contingency coefficient.

RESULTS AND DISCUSSION

As it was mentioned before, celiac disease is an autoimmune disease affecting mainly the small intestine, induced by an intolerance of proteins of wheat, barley, rye and oats. The intolerance refers to the mixture of proteins of cereal grain, which are commonly named and known as *gluten* – gluten is a protein found in wheat, rye, and barley, but it can be found also in other products like medicines, vitamins and supplements, lip balm, and even the glue on stamps and envelopes **(URL 6, 2015)**.

*"Someoftheglutenquantitiescouldbehazardousforsensitivepeopleasceliatics and allergic to gluten"***(Mati, et al., 2012)**. The causes of the intolerance can be several **(Bergendiová, 2012)**:

- the abnormal activation of own immune system to the presence of gluten in the food (so called hypersensitivity),
- the disorder of metabolism and of the activity of enzymes degrading the gluten,
- genetic factors.

The *symptoms* of celiac disease are a broad nature theme and therefore we have to remember that even in the case of celiac disease, each human is an individual case and example. There are patients in whom the celiac disease was manifested by other gastroenterology or other health problems and complications, which other patients may not have. It is also important to note that celiac disease may also occur in other than gastroenterology problems **(Hes et al., 2014)**.

Nevertheless the fact, that the *prognosis* of the disease is very good **(Frič, 2008)**, there still does not exist a test that could be universally accepted as a standard for the diagnosis. The early diagnosis and lifelong adherence to a so called *gluten free diet* causes that the complications caused by the illness are scarce and the life expectancy of celiacs does not significantly differ to the other populations **(Košičiarová et al., 2015)**.

Celiac disease diagnosis,prevalanceandtreatment

Pekárková et al., (2009) indicate four basic forms of celiac disease diagnosis:

1. *laboratory diagnosis* – the American College of Gastroenterology recommends, that antibody testing, especially immunoglobulin A anti-tissue transglutaminase antibody (IgA TTG), is the best first test for the diagnosis of celiac disease **(Goebel et al, 2015)**. The first step in the diagnosis is the investigation of patient´s blood count and prothrombin time when it comes to the quest for anemia, thrombocytosis, and coagulation disorders. Then, the serological tests are done, where the serological markers of celiac disease are investigated,
2. *endoscopy* – the procedure takes a little less than 30 minutes and is used for adults, sedatives and local anesthetic. Children are usually putted under general anaesthesia. During the biopsy, the gastroenterologist inserts a small tube with a camera through the digestive tract to the small intestine **(URL 7, 2015)**,
3. *histological examination* – samples sent for histological examination shall be assessed by histological scoring, in which is used so called Marsh classification,
4. *radio diagnostic methods* – in celiac disease are mainly used in the differential diagnosis, and therefore the exclusion of other diseases, such as maldigestive syndrome (characteristic for example with inflammation, cancer or cirrhosis) and malabsorption syndrome (important is to distinguish between the primary and secondary malabsorption syndrome).

Despite the fact, that the technology is still developing, scientists and doctors know more about the illness and that there are few possibilities how to diagnose the disease, the number of people sensitive on gluten is still increasing. While in the 1970s the *prevalence* of celiac disease in the world was 0.03% **(Lohi et al, 2007)**, in the present years the estimated prevalence is about 1% **(Košičiarová et al., 2015)**. In average, the prevalence of celiac disease in the Western countries is close to 1 :100 **(Gujral et al., 2012)**. The celiac disease occurs more often in the case of women than of men, at a ratio of 2.8 :1 **(Thomas et al., 2009)**. Heritability of celiac disease is autosomaly dominant with incomplete penetration. In the case of first degree relatives the celiac disease occurs in 8 – 18%, in the case of identical twins at 70% **(Prokopová, 2008)**.

The exact and estimated prevalence of celiac disease in USA, Europe and Slovak republic in the year 2014 is alarming. While in the year 2012 the prevalence of celiac dinase in USA was 0.71% (1 in 141) **(Rubio-Tapia et al, 2012)**, in the year 2014 the prevalence was 0.75% (1 in 133) **(URL 8, 2014)**, what represents an increase in 0.04%. Unfortunately, the situation in the case of Europe and Slovak republic, is wronger – while in the year 2010 and 2012 the prevalence of celiac disease in the Europe and in Slovak republic was for about 0.5% **(Mustalahti et al., 2010)** and 0.2% (1 in 404) **(Kabátová, 2014)** in the year 2014 it is estimated that the prevalence in Europe was exactly 1% **(URL 9, 2015)** and in Slovak republic between 0.5 and 1% **(URL 5, 2014)**, what represents an increase for about 0.5 – 0.8% in only two or four years. However, it must be also mentioned that lots of celiacs are still not diagnosed, what means, that the situation in the field of celiac disease can be much more wrong.

The most commonly used illustration of celiac disease forms is so called *celiac iceberg*, which is also shown on the Figure 1 and which can be very briefly described as follows – the tip of the iceberg is represented by the relatively small number of the world's population whose gross presentation of clinical symptoms often leads to the diagnosis of celiac disease. This is the classical case of celiac disease characterized by – gastrointestinal symptoms, malabsorption and malnourishment (it is confirmed with the "gold standard" of an intestinal biopsy).The middle part of the iceberg is largely invisible to classical clinical diagnosis, but not to modern serological screening methods in the form of antibody testing. This middle part is composed of asymptomatic and latent celiac disease as well as "out of the intestine" varieties of wheat intolerance. The base of the iceberg represents approximately 20 – 30% of the world's population (those who have been found to carry the HLA-DQ locus of genetic susceptibility to celiac disease on chromosome 6) **(Sayer, 2005)**.

The only possibility how to treat the disease is a so called *gluten free diet*, which means to exclude all the food, which contains wheat, rye and barley **(Suchá at al., 2015)**.

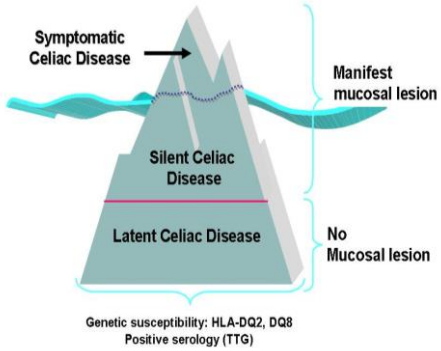

Figure 1 Iceberg image**.**

Source: Guandalini. Exploring the Iceberg.2009, p. 1.

The principles of gluten free diet can be described as follows **(URL 10, 2015)**:

1. avoid all the foods made from wheat, rye and barley,
2. avoid oats – soma celiacs can tolerate them, but the long term safety of oats in celiacs is unknown and some oat preparations can be contamined with wheat, thus it is better to avoid oats,
3. pay attention to processed food, which may contain gluten (e.g. canned soups, salad dressing, ice cream, candy bars, instant coffee, luncheon meats and processed or canned meats, ketchup and mustard, yogurt, pasta),
4. beware of tablets, capsules and vitamin preparations, which contain gluten,
5. avoid beer, but wine, brandy, whiskey and other alcohols without barley are good in moderation,
6. avoid milk and other dairy products, which contain lactose – with successful treatment, dairy products can be often reintroduced slowly into the diet later,

7. consult dietitians and national celiac disease societies for lists of gluten free food; read the food and product labels before buying or consuming any product,
8. because of the fact, that celiacs who have severe malabsorption can develop vitamin and mineral deficiencies, vitamin and mineral supplements are important.

Significant histological changes will, in keeping with the gluten-free diet, appear in three months and in two years of diet the celiacs will become practically asymptomatic **(Anderson, 2008)**. The main problem in the treatment is the non-compliance with the diet, which occurs in 50 – 80% of patients. Patients still continue in eating the food containing gluten due to lack of motivation or information. The key is the motivation of the patient, the doctor's approach and the cooperation with gastroenterologist or a registered dietitian who have the expertise in the gluten-free diet **(Hybenová at al., 2013)**.

Among the new treatments, which are nowadays tested and the research is concerned on them, are included the *genetic modification of the wheat* by which the gluten has to be removed, as well as *new medicine and vaccines*, which could prevent the damage to the intestine by the gluten **(URL 11, 2012)**.

Results of own research

With the aim to determine the Slovak celiacs opinion about the situation on Slovak market and their consumer behaviour on the market of gluten free products a structurized questionnaire survey was conducted in November 2015. The total number of respondents was 130 randomly selected celiacs from all over the Slovak republic.

From the Table 1 is clear, that the main groups of respondents were represented by women (92% of respondents – the assumption no. 1 was true), people living in the city (66% of respondents), people with the age between 26 and 35 years (36% of respondents), people with secondary education with A level (46% of respondents), people with net family income between 501 and 1.000 € and people from the Bratislava region (36% of respondents).

Up to the results of our own research we can say that most of our respondents have the celiac disease diagnosed from 0 to 5 years (68.46% of respondents – the assumption no. 2 was true), the most often symptoms up to which they have realized, that they are celiacs are diarrhea, abdominal pain, anemia, and allergy (rash and redness of the meal), which are also the typical symptoms of celiac disease **(URL 12, 2014),** most of our respondents did not have problems with the transition to a gluten free food (79.23% of respondents) and those who had the problems mentioned, that these problems were exactly – sadness, financial side of the illness, aversion to gluten free meals, need to learn how to cook without gluten, need to read all the information on the product and lack of choice (between the available products).

Despite the fact, that a few of our respondents have mentioned, that the situation with gluten free products and information about the celiac disease, resp. gluten free products on the Slovak market was before 10 to 15 years not very good, nowadays the situation is much more better – most of our respondents stay that the labelling of gluten free products is sufficient (51.54% of respondents), they have enough information about the availability of gluten free products (74.62% of respondents) and they think that the present scope of range of gluten free products is better as before (89.23% of respondents).

Unfortunately, there is still one drawback of gluten free products and that is their price – 93.08% of respondents mentioned that up to their opinion, the price of gluten free products is inadequate (Figure 2).

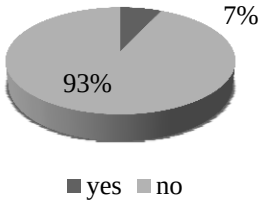

Figure 2 Adequacy of the price**.**
Source: Results of the research.

Because the Government of the Slovak Republic realizes, that this is a very huge problem, they offer (cross the Office of Labour, Social Affairs and Family) a so called possibility of granting a monetary contribution for compensation of increased expenses on a special diet. *"Health insurance companies are covering gluten-free products approximately from 60% of the price (flour, pasta, raw material) to 5 – 30% (ready baked bread, additional gluten free cookies)"* **(Rimarova, 2013)**. In spite of that, that most of our respondents, exactly 82.31% of respondents, know about this possibility, only 45.38% of them also use it (the assumption no. 3 was not true) and 46.15% of them are not satisfied with its height.

Because of the need to realize how are Slovak celiacs satisfied with the scope of range of gluten free products, as well as with their availability, in the questionnaire, there were formulated also the questions connected to these issues. Up to their evaluation we can say, that in these questions the Slovak market has still some reserves – most of our respondents (64.62% of them and 57.69% of them – the assumption no. 4 was true) think that the scope of range of gluten free products in the Slovak market, as well as in their region is insufficient and most of them also think (53.08% and 57.69% of respondents) that their availability in the Slovak market, as well as in their region is inadequate.

As it can be seen from the figure above, the most important criterion in the purchase of gluten free products is their quality (51.54% of respondents). This is, why we have been interested not just in the detection of the frequency of the gluten free product´s purchase, but also in

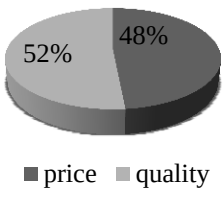

Figure 3 The decisive criterion in the purchase of gluten free products.
Source: Results of the research.

the place of their purchase (Figure 4), preference of domestic producers of gluten free products, mostly preferred labels of them, usage of the possibility to buy them abroad, as well as the reasons leading to their purchase abroad.

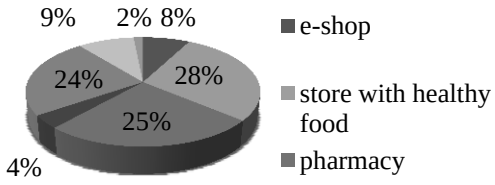

Figure 4 Place of purchase of gluten free products.
Source: Results of the research.

Up to the results of our questionnaire we can say, that most of our respondents buy gluten free products for few times in a week (39.23% of respondents), they buy them mostly in stores with healthy food, pharmacy and supermarket (28.46%, 25.38% and 23.85% of respondents), they do not exactly prefer the domestic producers of gluten free products (52.31% of respondents), they prefer mostly the labels of Schär, Novalim and Schnitzer (42.31%, 36.23% and 10.2% of respondents; Figure 5), they use the possibility to buy them abroad only randomly (63.85% of respondents do not use this possibility at all) and they use it only in the case of neighbouring countries because of the better quality and price of these products (81.11% and 79.3% of those respondents who use this possibility).

The above mentioned results correspond to some extant

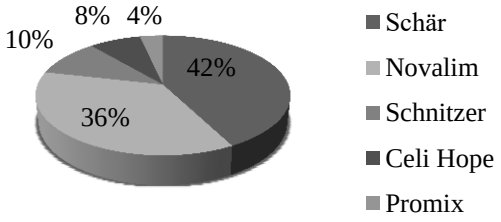

Figure 5 Mostly preferred labels of gluten free products.
Source: Results of the research.

to the results of research conducted by **Hes et al.,(2014)** on the sample of 289 randomly selected Slovak celiacs, where the authors have found out that the most important criterion in the purchase of gluten free products is their freshness and quality (57% of respondents), resp. the price (23% of respondents); that most of Slovak celiacs buy gluten free products for once or few times in a week (44% and 27% of respondents); most of them (45% of respondents) prefer to purchase gluten-free products in specialized stores, supermarkets (18% of respondents), hypermarkets (18% of respondents)and in pharmacies (10% of respondents) and that they prefer mostly the labels of Schär, Novalim and Pečivárne Liptovský Hrádok (96.19%, 28.35% and 23.18% of respondents); resp. with the results of research conducted by **Košičiarová et al., (2015)**on the sample of 506 randomly selected Slovak celiacs, where the authors have found out that the most important criterion in the purchase of gluten free products is their freshness and quality (56.56% of respondents), price level (21.06% of respondents) and their range width (14.04% of respondents), that most of Slovak celiacs buy gluten free products in specialized shops (52.17% of respondents), hypermarkets (18.77% of respondents) and supermarkets (17.98% of respondents) and that most of them are not satisfied with the range width of the gluten free products (69.96% of respondents). Unfortunately, there are no more researches with which we could compare our results, because our research is very specific and in the area of Slovak republic there was not implemented a similar research. This disadvantage brings with it also additional advantage in the form of the possibility of implementing other similar studies, resp. other studies focused on the situation of Slovak celiacs.

Evaluationoftheformulatedhypotheses

Connected with few of above evaluated questions, there have appeared also the questions of the dependence resp. indepence between few variables. This is, why in the part Material and Methodology were formulated ten different hypotheses, which have been tested with the use of Pearson´s chi-square test, Mann-Whitney U-Test and Cramer´s contingency coefficient; and which evaluation is following one:

1. H_{01} – there does not exist the dependence between the frequency of purchase of gluten free products and the respondent´s place of living – accepted.
2. H_{02} – there does not exist the dependence between the place of purchase of gluten free products and the respondent´s age – accepted.
3. H_{03} – there does not exist the dependence between the decisive criteria in the purchase of gluten free products and the respondent´s age – accepted.
4. H_{04} – there does not exist the dependence between the decisive criteria in the purchase of gluten free products and the respondent´s level of education – accepted.
5. H_{15} – there exists the dependence between the respondent´s opinion on the adequacy of available information and the region from which the respondent comes from – accepted.
6. H_{06} – there does not exist the dependence between the respondent´s opinion on the scope of gluten free products on the Slovak market and the region from which the respondent comes from – accepted.
7. H_{07} – there does not exist the dependence between the respondent´s opinion on the scope of gluten free products in his region and the region from which the respondent comes from – accepted.
8. H_{08} – there does not exist the dependence between the respondent´s opinion on the availability of gluten free products on the Slovak market and the region from which the respondent comes from – accepted.
9. H_{09} – there does not exist the dependence between the respondent´s opinion on the availability of gluten free products in his region and the region from which the respondent comes from – accepted.

H_{110} – there exists the dependence between buying gluten free products abroad and the region from which the respondent comes from – accepted.

CONCLUSION

Up to the results of our own research, which was conducted on the sample of 130 randomly selected celiacs from different parts of Slovak republic, we can conclude, that while the situation with the information and availability of gluten free products on the Slovak market is nowadays on a better level as it was before, there are still some drawbacks, which must be reduced – still too high price of gluten free products (only 6.92% of respondents think that the price is adequate), lack of people who use so called possibility of granting a monetary contribution for compensation of increased expenses on a special diet (only 45.39% of respondents use this possibility), insufficient scope of range of gluten free products in the Slovak market (almost 65% of respondents), insufficient scope of range of gluten free products in the respondent´s region (more than 58% of respondents), inadequate availability of gluten free products in the Slovak market (53% of respondents), inadequate availability of gluten free products in the respondent´s region (almost 58% of respondents) and small preference of domestic producers of gluten free products (only 47.69% of respondents prefer them).

Because of the need to execute a deeper analysis of the obtained results, as well as to determine the dependence between variables, four assumptions and ten hypotheses have been formulated and finally tested. Up to their evaluation we can say, that only three assumptions were true – the assumption no. 1, which has said that most of our respondents are women (exactly 92% of our respondents were women, which confirms also the statement that the celiac disease occurs more often in the case of women than of men); the assumption no. 2, which has said that most of our respondents have celiac disease diagnosed from 0 to 5 years (exactly 68.46% of our respondents, which confirms our previous notes about the increasing number of celiacs in the last few years); and the assumption no. 4, which has said that most of our respondents think that the scope of range of gluten free products in the Slovak market is insufficient (exactly 64.62% of our respondents, which is a very interesting result because exactly 89.23% of our respondents stated in an another questions, that the present scope of range of gluten free products is better as it was before); and only two hypotheses have been confirmed. These hypotheses show, that between the respondent´s opinion on the adequacy of available information and the region from which he/she comes from, there exists a small, but statistically significant dependence (the result of Cramer´s contingency coefficient was equal to 0.10085, what can be interpreted as a weak dependence, and the result of Mann-Whitney´s U-Test was − the U-value was 12.5, the critical value of U at $p \leq 0.05$ was 13, which means, that the result is at $p \leq 0.05$significant) and between buying gluten free products abroad and the region from which the respondent comes from there exists also some dependence, but it is statistically not significant (up to the results of Pearson´s chi-square test, the H_0 hypothesis must be on the level of significance 5% rejected and adopted must be its alternative H_1 hypothesis talking about the dependence between tested variables (TC = 18.988 >CV = 14.067), the result of Cramer´s contingency coefficient was equal to 0.04242, what can be interpreted as almost none dependence, and the result of Mann-Whitney´s U-Test was − the U-value was 24, the critical value of U at $p \leq 0.05$ was 13, which means that, the result is at $p \leq 0.05$not significant).

Based on the results of our research, we can propose the following recommendations for not just the Ministry of Health of Slovak Republic, but also for the Office of Labour, Social Affairs and Family, doctors as well as producers, suppliers and sellers of gluten free products:

- to increase the level, but also the promotion of so called possibility of granting a monetary contribution for compensation of increased expenses on a special diet – most of celiacs know about this possibility, but they do not use it, because they think that it is still very small and insufficient, as well as they say, that it is also difficult to obtain it,
- this is why it is needed also to reduce the bureaucracy and difficulty of its obtaining,
- to support the producers of gluten free products so that they could decrease their price,
- to organize free tasting of gluten free products,
- to increase their quality – exactly in the case of their taste,
- to organize free courses how to buy gluten free products, where to buy them, how to cook for celiacs, how to change to gluten free diet,
- to create separate corners with gluten free products, which will be visible for celiacs and where they could find also some added information about them, etc.

REFERENCES

Abdulkarim, A. S., Murray, J. A. 2003. Review article: the diagnosis coeliac disease. *Aliment Pharmacology and Therapeutics*, vol. 17, no. 8, p. 987-995. http://dx.doi.org/10.1046/j.1365-2036.2003.01442.x PMid:12694080

Anderson, R. P. 2008. Coeliac disease: current approach and future prospects. *Internal Medicine Journal*, vol. 38, no. 10, p. 790-799. http://dx.doi.org/10.1111/j.1445-5994.2008.01741.x PMid:19143879

Bergendiová, K. 2012. CELIAKIA – choroba, o ktorejaninemusítevedieť [online] s.a. [cit. 2015-12-23] Available at: http://abysportnebolel.sk/zdravie/celiakia/.

Catassi, C., Yachha, SK. 2008. The global village of celiac disease. In: Fasano, A., Troncone, R., Branski, D. Frontiers in celiac disease. *Pediatric and Adolescent Medicine*, vol. 2, p. 23-31. http://dx.doi.org/10.1159/000128610

Ciclitira, P. J., Johnson, M. W., Dewar, D. H., Ellis, H. J. 2005. The pathodenesis of coeliac disease.*Molecular Aspects of Medicine*, vol. 26, no. 6, p. 421-458. http://dx.doi.org/10.1016/j.mam.2005.05.001 PMid:16125764

Dubé, C., Rostom, A., Sy, R., Cranney, A., Saloojee, N., Garritty, C., Sampson, M., Zhang, L., Yazdi, F., Mamaladze, V., Pan, I., Macneil, J., Mack, D., Patel, D., Moher, D. 2005. The prevalence of celiac disease in average-risk and at-risk Western European populations: a systematic review. *Gastroenterology*, vol. 128, no. 4, p. 57-67. http://dx.doi.org/10.1053/j.gastro.2005.02.014

Fasano, A. 2009.Surprises from Celiac Disease.*Scientific American*, vol. 301, no. 2, p. 54-61. http://dx.doi.org/10.1038/scientificamerican0809-54 PMid:19634568

Fasano, A., Catassi, C. 2001. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology*, vol. 120, no. 3, p. 636-651. http://dx.doi.org/10.1053/gast.2001.22123 PMid:11179241

Frič, P. 2008. Celiakie – celosvětováchorobamnohatváří.*Česká a SlovenskáGastroenterologie a Hepatologie*, vol. 62, no. 4, p. 187-189.

Goebel, S. U. 2015. Celiac Disease (Sprue). [online]s.a. [cit.2015-12-23] Available at: http://emedicine.medscape.com/article/ 171805-overview.

Guandalini, S. 2009. Exploring the Iceberg.*Impact. A publication of the University of Chicago Celiac Disease Center*, vol. 8, no. 4, p. 1-4.

Gujral, N., Freeman, H. J., Thomson, A. B. R. 2012. Celiac disease: Prevalence, diagnosis, pathogenesis and treatment. *World Journal of Gastroenterology*, vol. 18, no. 42, p. 6036-6059. http://dx.doi.org/10.3748/wjg.v18.i42.6036 PMid:23155333

Hes, A., Nagyová, Ľ., Regnerová, M., Kleinová, K., Sedliaková, I. 2014.*Maloobchod s bezlepkovýmipotravinami v České a Slovenskérepublice*.1. vyd. Praha :Powerprint. 60 p. ISBN 978-80-87994-11-5.

How is Celiac DIseaseDIagnosed? 2015. [online] s.a. [cit.2015-12-23] Available at: https://www.csaceliacs.org/diagnosis_of_celiac_disease.jsp.

Hybenová, E., Štofirová, J., Mikulajová, A. 2013.Celiac disease and gluten-free diet. *Potravinarsvto*, vol. 7, no. 1, p. 95-100. http://dx.doi.org/10.5219/276

Kabátová, J. 2014, Epidemiology of Celiac Disease in Slovakia: Life Conditions of Celiac Disease Patients in Slovakia. *International Journal of Celiac Disease*, vol. 2, no. 2, p. 38-39. http://dx.doi.org/10.12691/ijcd-2-2-1

Košičiarová, I., Nagyová, Ľ., Rybanská, J., Holienčinová, M. 2015. Celiac Disease: Consumer behaviour in the field of gluten free products in the case of Slovak republic. In *ICABR 2015*. Brno: Mendel university, p. 452-467. ISBN 978-80-7509-328-8.

Lohi, S., Mustalahti, K., Kaukinen, K., Laurila, K., Collin, P., RIssanen, H., Lohi, O., Bravi, E., Gasparin, M., Reunanen, A., Mäaki, M. 2007. Increasing prevalence of coeliac disease over time.*Alimentary Pharmacology and Therapeutics*, vol. 26, no. 9, p. 1217-1225. http://dx.doi.org/10.1111/j.1365-2036.2007.03502.x PMid:17944736

Mati, M., Staruch, L. 2012. Monitoring of a gluten content in selected meat products from three biggest meat producers in Slovakia. *Potravinarstvo*, vol. 6, no. 1, p. 30-33. http://dx.doi.org/10.5219/167

Mustalahti, K., Catassi, C., Reunanen, A., Fabiani, E., Heier, M., McMillan, S., Murray, L., Metzger, M. H., Gasparin, M., Bravi, E., Mäki, M. 2010.The prevalence of celiac disease in Europe Results of a centralized international mass screening project. *Annals of Medicine*, vol. 42, no. 8, p. 587-595. http://dx.doi.org/10.3109/07853890.2010.505931 PMid:21070098

National Foundation for Celiac Awareness, 2015. [online] s.a. [cit.2015-12-22] Available at: http://www.celiaccentral.org/ celiac-disease/facts-and-figures/.

Olén, O., Askling, J., Ludviqsson, J. F., Ekbom, A., Smedby, K. E. 2011. Coeliac disease characteristics, compliance to a gluten free diet and risk of lymphoma by subtype. *Digestive and Liver Disease*, vol. 43, 2011, no. 11, p. 862-868.

Pekárková, B., Pekárek, B., Kabátová, J. 2009. Racionálnadiagnostika a liečba celiakie. Štandardný diagnostický a terapeutický postup. *Metodický list racionálnej farmakoterapie*, vol. 13, no. 1-2, p. 1-8.

Prokopová, L. 2008. Celiakie – Co má vědět ambulantní internista. *Interní Medicina pro praxi*, vol. 10, no. 5, p. 233-239.

Rimarova, K. (2013). Celiac Diseases – Global Demographic Context and Slovakia. *International Journal of Celiac Disease*, vol. 1, no. 1, p. 17-18. http://dx.doi.org/10.12691/ijcd-1-1-7

Rubio-Tapia, A., Ludvigsson, J. F., Brantner, T. L., Murray, J. A., Everhart, J. E. 2012. *American Journal of Gastroenterology*, vol. 107, no. 10, p. 1538-1544. http://dx.doi.org/10.1038/ajg.2012.219 PMid:22850429

Sayer, J. 2005. The Dark Side of Wheat – New Perspectives On Celiac Disease and Wheat Intolerance [online] s.a. [cit.2015-12-20] Available at: http://www.greenmedinfo.com/page/ dark-side-wheat-new-perspectives-celiac-disease-wheat-intolerance-sayer-ji.

Suchá, V., Jurgoš, Ľ., Dostálová, K., Móricová, Š. 2015. Aktuálnypohľadnaceliaku. *Solen*, vol. 12, no. 1, p. 22-28.

Thomas, H. J., Ahmad, T., Rajaguru, C., Barnardo, M., Warren, B. F., Jewell, D. P. 2009. Contribution of histological, serological, and genetic factors to the clinical heterogeneity of adult-onset coeliac disease.*Scandinavian Journal of Gastroenterology*, vol. 44, no. 9, p. 1076-1083. http://dx.doi.org/10.1080/00365520903100473 PMid:19593686

URL 1: What is Celiac Disease? 2015. [online] s.a. [cit.2015-12-22] Available at:https://www.csaceliacs.org/celiac_disease_defined.jsp

URL 2: NIH National Institute of diabetes and digestive and kidney disease, 2015. [online] s.a. [cit.2015-12-21] Available at: http://www.niddk.nih.gov/health-information/health-topics/digestive-diseases/celiac-disease/Pages/facts.aspx

URL 3: Celiac Support Association, 2015. [online] s.a. [cit.2015-12-21] Available at: https://www.csaceliacs.org/celiacdiseasefacts.jsp

URL 4: Did you know? Random facts about celiac and gluten. 2011. [online] 2011-10-11 [cit.2015-12-23] Available at: http://glutendude.com/gluten/did-you-know-random-facts-about-celiac-and-gluten/

URL 5: Celiakia nie je len ochorenie detí a môže sa vyskytovať v ktorom koľvek veku, 2014. [online] s.a. [cit.2015-12-21] Available at: http://www.bezlepku.sk/?titlee=celiakia-nie-je-len-ochorenie-deti-a-moze-sa-vyskytovat-v-ktoromkolvek-veku&id=89&kat1=2&kat2=2&kat3=&shopid=2

URL 6: Celiac Disease, 2015. [online] s.a. [cit.2015-12-22] Available at: https://www.nlm.nih.gov/medlineplus/celiacdisease.html

URL 7: Diagnosis, 2015. [online] s.a. [cit.2015-12-22] Available at: https://celiac.org/celiac-disease/diagnosing-celiac-disease/diagnosis/

URL 8: Celiac dinase facts and figures, 2014. [online] s.a. [cit.2015-12-21] Available at: http://www.cureceliacdisease.org/ wpcontent/uploads/2014/06/CeliacDiseaseFactsAndFigures0614.pdf

URL 9: Výskyt celiakie sa v Európe zvyšuje, 2015. [online] s.a. [cit.2015-12-23] Available at: http://www.eufic.org/article/ sk/artid/Coeliac-disease-on-the-rise-in-Europe

URL 10: Celiac Disease Treatment, 2015. [online] s.a. [cit.2015-12-23] Available at: http://www.webmd.com/digestive-disorders/ celiac-disease/celiac-disease-treatment

URL 11: Výskytceliakie v Európesazvyšuje, 2012. Food today [online] 2016-06-09 [cit.2015-12-20] Available at: http://www.eufic.org/article/sk/artid/Coeliac-disease-on-the-rise-in-Europe

URL 12: Celiakia - život bez lepku, 2014. [online] s.a. [cit.2015-12-22] Available at: http://www.celiakia.sk/files/subory-na-stiahnutie/ostatne-subory/celiakia-uvod.pdf

Villanaci, V., Ceppa P., Tavani, E., Vindigni, C., Volta, U. 2011. Coeliac disease: The histology report. *Digestive and Lver Disease*, vol. 43, no. 4, p. 385-395. http://dx.doi.org/10.1016/S1590-8658(11)60594-X

Walker, M. M., Murray, J. A. 2010. An update in the diagnosis of coeliac disease. *Histopathology*, vol. 59, 2010, no. 2, p. 166-169.

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INFLUENCE OF THE XANTHAN GUM ADDITION ON THE TECHNOLOGICAL AND SENSORY QUALITY OF BAKING PRODUCTS DURING THE FREEZING STORAGE

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ABSTRACT

The influence of the 0.16% xanthan gum addition in the recipe of the bread production and its influence on the baking and sensory quality of products was monitored during the process of our research. Prepared dough was inserted in the freezing box directly (-18°C) and it was stored for one, two, three, four, five and six months. When the baking process was finished, the products with xanthan gum and the products without it were compared and evaluated by both objective and subjective methods. It was found that freezing, cooling and storage of the products without xanthan gum addition influenced the volume, vaulting and general appearance of the products in a negative way and loaves of bread were evaluated as unacceptable after four months of freezing. The quality of experimental loaves of bread with xanthan gum was, even after six months of freezing storage, comparable with freshly baked products. Despite the freezing, the volume of the products had an increased value. After first month of freezing the volume increased by 28.6% and after two months of freezing it increased by 23.8% both compared to the control. The vaulting in products processed by freezing was in the required optimal level during the whole period of freezing. Sensory evaluation results of loaves of bread with xanthan gum were the best after three, four and five months of storage in a freezer, when 98 points were achieved. During the monitored period of freezing, the addition of 0.16% of xanthan gum markedly contributed to the preservation of sensory and baking quality of the frozen wheat dough.

Keywords: xanthan gum; freezing storage; baking quality; sensory quality

INTRODUCTION

The producer's aim is to preserve the product fresh as long as possible, therefore it is feasible to influence the forms of slowing down the bread staling by regulating the room temperature where the bread is stored and by the modification of production process, or recipes **(Cauvain, 1998; Hampl et al., 1981)**. Hydrocolloids are perspective additives with the ability of effective linking of water molecules which become fixed, thus with this mechanism it is possible to eliminate the presence of unfavourable crystals that influence product texture in a negative way **(Gimeno et al., 2004; Khan et al., 2007)**.

Hydrocolloids are high-molecular hydrophilic biopolymers which has many functions in food industry. The most important function could be the ability to control the rheologic attributes and food texture. In baking industry they are mostly added for the purposes of emulsions, suspensions and foams stabilization, to improve the processing attributes, because of their inhibition ability of starch retrogradation, their efficient humidity retaining, improvement of the whole structure, staling inhibition of the products, but also as the replacement of fat and eggs **(Collar et al., 1999; García-Ochoa et al., 2000; Arozarena et al., 2001;Kohajdová et al., 2008; Kohajdová et al., 2009; Magala et al., 2011)**. The most popular hydrocolloid of the microbial origin is a xanthan gum, which is exocellular polysaccharide produced by aerobical sugar fermentation by the *Xanthomonas campestris* bacterium **(Hojerová et al., 2005; Mikuš et al., 2011; Tao et al., 2012)**; the main bond of xanthan consists of β-D-(1.4) glucosic elements and lateral bonds are formed by the D-glucoronic acid leftover and two leftovers of D-mannose **(Velíšek, 2002)**. According to **Hojerová et al. (2005)**, it was discovered that xanthan gum reaches the fastest hydration, the smallest temperature sensibility and its stability in soft acid to neutral pH only.

The xanthan gum is characterized by the ability of creation the reversible gels in conjunction with galactomannans, e.g. carob gum **(Milani et al., 2012)**. It is possible to use xanthan gum as a partial replacement of egg white in cakes **(Miller and Setser, 1983)**. Moreover it was discovered that the xanthan gum addition during the dough kneading, prevented the shrinking of products and markedly improved the volume and height of a bread in comparison with the specimen **(Miller and Hoseney, 1993)**, provided the highest viscosity of dough **(Ashwini et al., 2009)**, firmed the structure of dough **(Ashwini et al., 2009)** and strengthened the bonds between flour

proteins **(Collar et al., 1999)**. There are various possibilities of xanthan gum usage: applications in dressings, syrups, or diet, frozen and baking products, but also in other branches of industry and agriculture **(García-Ochoa et al., 2000)**. The xanthan gum has a very important function as an additive in gluten-free bread recipes where, due to the absence of gluten, it is improving technological and sensorial quality of the products for celiatics **(Gambuś et al., 2007)**.

MATERIAL AND METHODOLOGY

The first experimental group of loaves of bread were prepared from wheat extra fine flour T 650 (Mlyn Pohronský Ruskov a.s.) in amount of 500 g, sugar (1%), salt (1.6%), yeast (4%) (Trenčianske droždie, OLD HEROLD HEFE, s.r.o.) and water which was added according to farinograph **(ICC - Standard 115/1, 1992, AACC Method 54-21, 1995)** farinograph water absorption of the flour (150 cm^3). The second experimental group of bread loaves was created with the same ingredients in same proportions, but in addition it contained 0.16% of xanthan gum (f. Natural Jihlava) in regard to the flour weight.

The dough was kneaded in a laboratory kneader Diosna SP 12. Then it was designed into loaves of bread, which rose in rise rooms for 20 minutes at 30°C and after that were baked at 240°C for 20 minutes with a steaming in Miwe Condo oven. The control specimen of wheat bread loaves was prepared this way. The rest of the loaves was put without yeasting into the freezer with temperature of -18°C (AFG 070 AP, company: Whirlpool Slovakia spol. s.r.o.,) and stored at this temperature for one, two, three, four, five and six months. The defrosting of the loaves before the yeasting and baking lasted 2 hours at room temperature of 22°C ±2°C.

The flour was quantitatively evaluated and the following attributes were set: *determination of the moisture content, %* **(ICC Standards No. 110/1 (1976))**, *ash on products, %* **(ICC Standard No. 104/1, (1990))**, *determination of the "Falling number", s* **(Falling Number, measurer FN 1800, Perten, according ICC Standard No. 107/1, (1995))**, *the wet gluten rate, %* Glutomatic 22000, Perten, (**ICC Standard No. 155, (1994))**, *determination of the sedimentation value by Zeleny, cm^3* **(ICC Standard No. 116/1, (1994))**, *determination of crude protein* **(ICC Standard No. 105/2 (1994))**.

Baked loaves of bread were evaluated by objective methods for baking quality examination during the storage at -18°C. Within them the following attributes were set by standard procedures and calculations used at the working place: *volume of the products* (cm^3), *specific volume of the products* ($cm^3.100g^{-1}$), *volume recovery* ($cm^3.100g^{-1}$ flour), *yield of products* (%), *baking losses* (%) and *vaulting of the products* (the ratio of the height and width of the loaves). Also, the loaves were evaluated sensorically by using the one hundred points test applied by the retrained evaluators at the Department of Plant Products Storing and Processing. These attributes were evaluated: *general appearance and shape* (the coefficient of the importance 1), *surface and characters of the crust* (the coefficient of the importance 2), *rising and the appearance of the crumb* (the coefficient of the importance 4), *structure and elasticity of the crumb* (the coefficient of the importance 4), *smell and taste* (the coefficient of the importance 9) with the maximum possible reached points of 100. In terms of sensory evaluation, the general profile of experimental controlled loaves (which were stored for six months in a freezer) with xanthan gum, was created and compared. The experiment results are pictured graphically and they were evaluated by the application of non-parametric statistic methods: Wilcoxon test and Kruskal-Wallis test and evaluated in **R Core Team (2014)**.

RESULTS AND DISCUSSION

The year consumption of pastry for one inhabitant of the Slovak Republic is approximately 40 kg of bread and 30 kg of wheat pastry **(ŠÚ SR)**. The quality of bread and pastry as the essential food is a very current topic. It is markedly influenced by the quality of used ingredients, especially flour and additives. In the flour we used (T650) it was detected: moisture content 13.9%, ash on products 0.49%, the Falling Number test 324 s, the wet gluten rate 32.6%, sedimentation value according to Zeleny 40.0 cm^3, crude protein (Nx5.7) 11.5%. Based on the analysis of **Muchová et al. (2001)** the used flour can be characterized as mid-strong with proportional amylases activity and sufficient amount of good quality gluten; so it can be used for risen products.

The prepared dough in the bowl mixed directly after the rising and the qualitatively evaluated specimen was used for the control. After the process of dough freezing, storage (from one to six months) and defrosting, the next specimens were risen again and baked according to the

Table 1 Results of the experiment with wheat bread loaves without the xanthan gum addition

period of deep-freeze storage	volume of products cm^3	specific volume $cm^3.100g$	volume recovery $cm^3.100$ g flour	yield of products %
immediately baked/ control [a]	262.5	297.1	420.0	141.3
one month [a]	212.5	231.9	340.0	146.5
two months [a]	225.0	258.3	360.0	139.3
three months [a]	240.0	264.6	384.0	145.1
four months [a]	235.0	283.7	380.0	133.9
five months [a]	215.0	239.1	344.0	143.8
six months [a]	200.0	230.9	320.0	138.5

methodology described above. The chosen results of the baking experiment without the xanthan gum are pictured in Table 1. From the Table 1, it is seen that the volume of the baked loaves without the addition of xanthan gum has been gradually decreasing till the third month, then the decrease was stopped and the slight increase of volume was observed compared to the first and the second month of freezing. Based on the results of Kruskal – Wallis test it was discovered that there is no statistic difference between the samples (every month of freezing was compared with the control), because the rate of $p = 0.45$. After five and six months of freezing, the loaves of bread achieved the lowest volume and insufficient vaulting (Figure 1) in comparison to the control and thus these products can be evaluated as unsatisfactory.

Results of the work, which confirm the similar decreasing baking quality during the freezing storage, were also noticed and described after six months of freezing storage by the authors **Berglund et al.** (**1991**).

The disrupted and cracked gluten structure, which was separated from the starch granules, was discovered by them. More authors mention that the most significant changes of frozen dough were related to yeast, because dead ice damaged yeast cells release glutathione, which disables the gluten structure. This consequently leads to the worse retention of gases and extension of the rise time of dough **(Kline et al., 1968; Hsu et al., 1979; Autio et al., 1992; Gelinas et al., 1995; Pepe et al., 2005)**. A decreased ability of the dough to retain emergent gases during the rising process is practically expressed by insufficient and low volume of experimental loaves of bread. So it is possible to state, that freezing storage gradually degrades the baking quality of loaves of bread, which was confirmed also by our experiments.

Table 2 shows experimental loaves of bread staling, which in contrast to products pictured in Table 1 contained

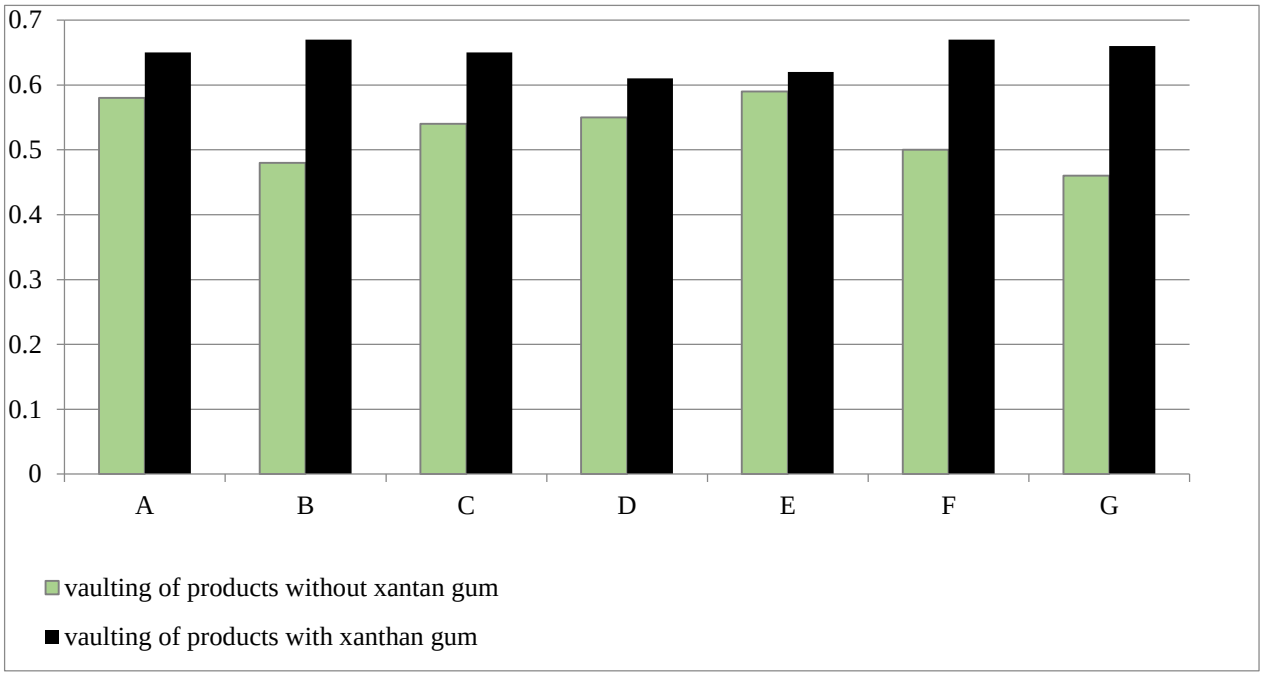

Figure 1 Comparison of the vaulting of the products with and without xanthan gum.

Note: A – products baked at the beginning - the control, B – products stored in freezer for one month, C – two months, D – three months, E – four months, F – five months, G – six months. The 0.65 value is optimal for vaulting of the loaves of bread. The values under 0.6 and over 0.7 are insufficient for vaulting of the products.

Table 2 Baking experiment results with the wheat loaves of bread containing 0.16% of xanthan gum.

period of deep-freeze storage	volume of products cm^3	specific volume $cm^3.100g$	volume recovery $cm^3.100$ g flour	yield of products %
immediately baked/ control [a]	262.5	286.4	420.0	146.6
one month [a]	337.5	362.5	540.0	148.9
two months [a]	325.0	351.5	520.0	147.9
three months [a]	262.5	241.8	420.0	153.4
four months [a]	312.5	338.2	500.0	147.8
five months [a]	275.0	301.2	440.0	146.0
six months [a]	262.5	280.9	420.0	149.5

0.16% of xanthan gum. The dough prepared in kneader was risen and baked. The qualitatively evaluated sample was used as the control. The other samples (experimental loaves of dough) were put in a freezing storage (from one to six months) and then defrosted, risen, baked and evaluated. Consequently, their baking quality was compared to the products without the addition of xanthan gum.

The Table 2, where results of baking experiment with frozen and baked loaves of bread with 0.16% of xanthan gum are introduced, shows that freezing storage does not affect the quality of products negatively. After the first month the volume of later baked loaves of bread increased by 28.6% compared to the control, after two months of freezing storage it increased by 23.8% also compared to the control and the vaulting rates (Figure 1) were on the required optimal level constantly. After three and six months of freezing storage the same volume as in the control was discovered and after four and five months of storage the increase of volume was observed, while the vaulting was constantly good (0.65). Statistic results reached by Kruskal – Wallis test (every month of freezing storage is compared to the control again) indicated the value of $p = 0.7$ which means that no statistical difference was found between compared samples and the control. By the Wilcoxon test, the results of particular months of freezing storage without xanthan gum were statistically compared to the results of products, which were frozen for the same time span, but they contained xanthan gum. It is possible to claim again that there was no statistically significant difference between compared samples.

In the past, many scientific groups were engaged in removing and eliminating negative impacts on baking products by the addition of xanthan gum, e.g. **Collar et al. (1999)**, who published that xanthan gum in recipes improves the retention of gases which is visible by bigger volume, **Rosell et al. (2001)** discovered the positive effect of increased water activity in a crumb, **Dodić et al. (2007)** measured out the higher specific volume in comparison with frozen products without the addition of hydrocolloids and **Mandala (2005)** discovered that the xanthan gum addition higher than 0.16% causes a decrease of specific volume of breads, so it recommended not to exceed this level. The results of works of the authors who were engaged in this issue were also confirmed by our experiments. It is possible to claim that the baking quality of the products with 0.16% xanthan gum addition was perfect even after six months of freezing storage and it was comparable to freshly baked products. After the evaluation of baking experiment, the loaves of bread were subjectively rated by 100 points questionnaires for the comparison of the xanthan gum influence on sensory quality of the products. Figure 2 shows the results of sensory evaluation.

As it results from Figure 2, the sensory quality of experimental loaves of bread without the addition of xanthan gum has been proportionally declining as the time of freezing storage has been prolonging. The most expressive decline of sensory quality was observed after four months of storage (Letter E). The experimental loaves of bread which contained xanthan gum reached almost the same sensory quality during the whole period of freezing. That proves the positive influence of the additive.

For better comparison of xanthan gum influence on a general appearance, an appearance of crust and crumb, elasticity, smell and taste; the sensory profile of bread loaves without xanthan gum (Figure 3) and sensory profile of measured index of bread loaves with xanthan gum (Figure 4) was created. By comparison of the Figure 3 and the Figure 4, it is possible to claim that the addition of 0.16% of xanthan gum had a positive effect on surface of loaves of bread, quality of crust and crumb and elasticity

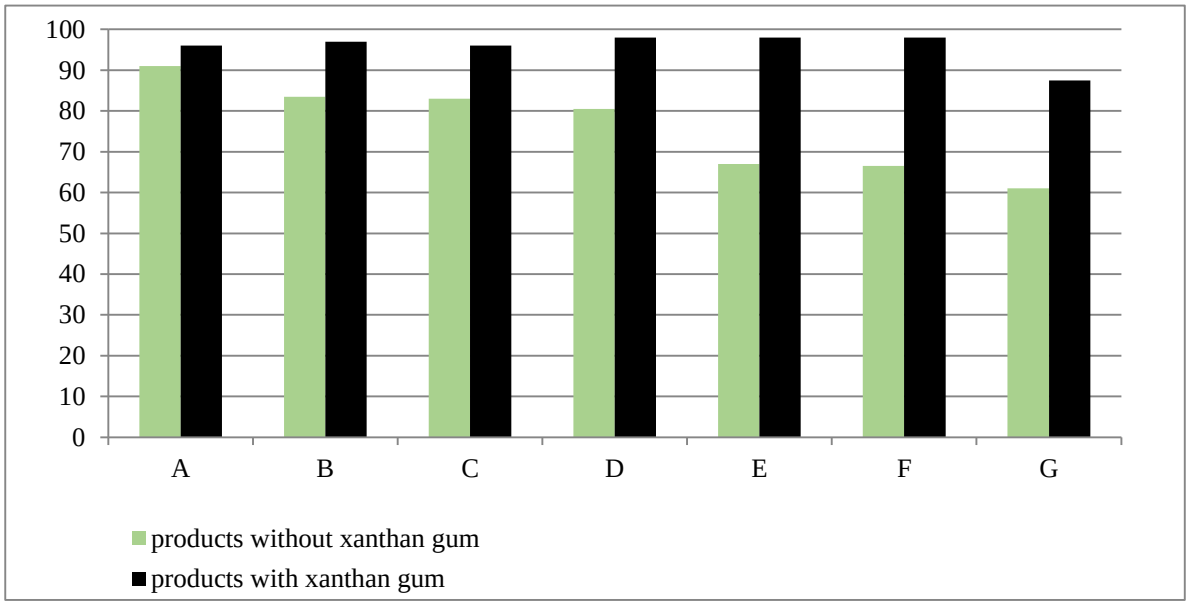

Figure 2 The comparison of sensorial qualities process during the freezing storage.

Note: A – products baked at the beginning - the control, B – products stored in freezer for one month, C – two months, D – three months, E – four months, F – five months, G – six months.

of crumb. The evaluators claimed that even after six months of freezing storage, the crust was softer, crispier and easier to chew in the products with xanthan gum, compared to the products without it.

Moreover, the positive influence of the xanthan gum addition was discovered in the quality of crumb, especially its structure, elasticity and uniform porosity of the products.

The sensory and technological baking quality of baked bread loaves without the addition of xanthan gum were after six months of freezing storage evaluated by **Bojňanská et al.** (**2013**), as decreased in comparison to the control was. Our experiments confirm the results for example of **Gimeno et al.** (**2004**). The sensory quality of experimental loaves of bread with xanthan gum was in balance for almost the whole period of the storage. A mild decline was observed after six months of freezing (85.5 points), but the point score can still be considered as convenient. The addition of xanthan gum had clearly positive effect on the sensory quality of the products during the whole period of freezing storage.

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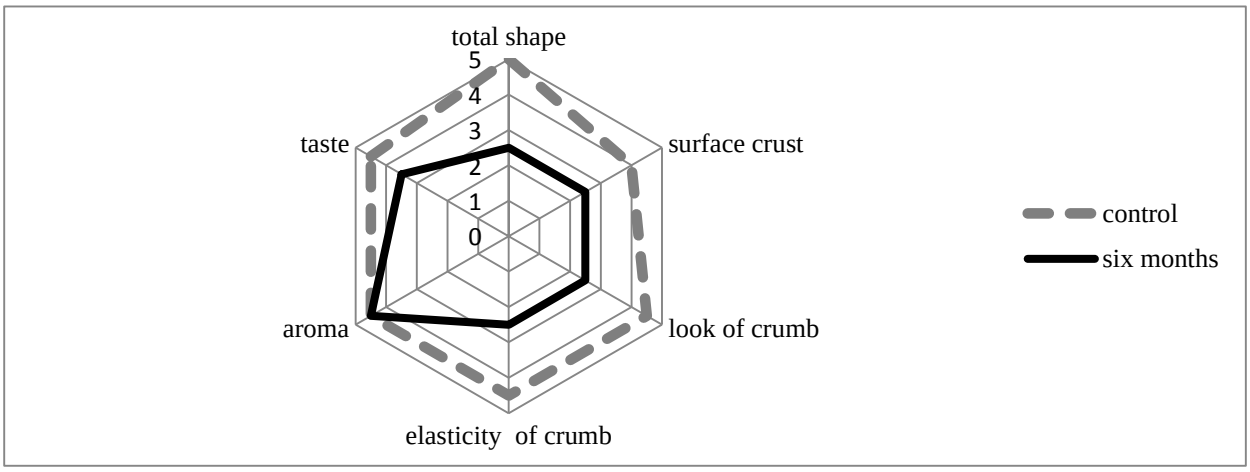

Figure 3 Sensory profile of the products without xanthan gum.

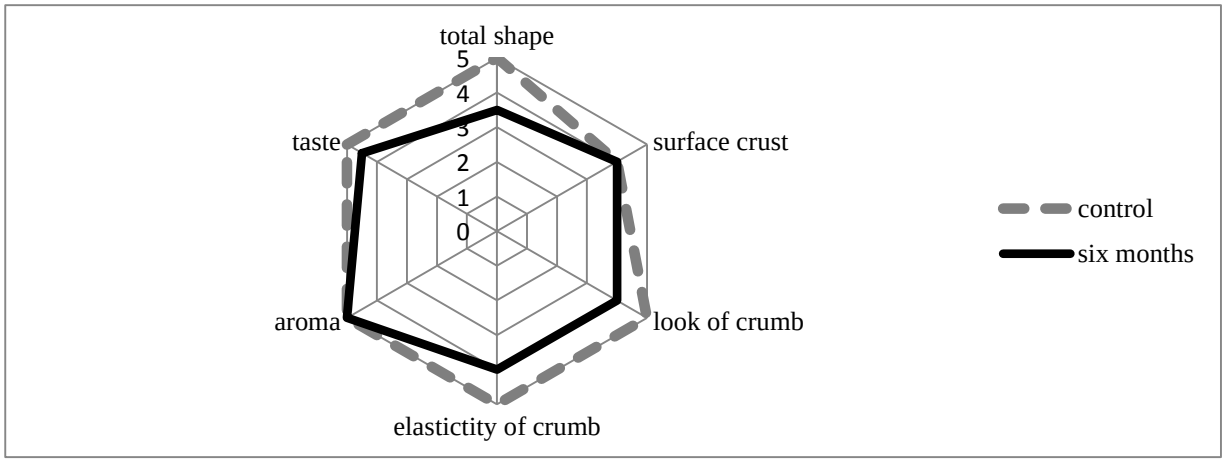

Figure 4 Sensory profile of the products with xanthan gum.

CONCLUSION

Based on the comparison of the baking experiment results and the sensory evaluation of baked wheat bread loaves and bread loaves with xanthan gum addition, it is possible to claim that this additive in recipe has markedly contributed to the elimination of undesirable effects of freezing storage and the products achieved perfect quality also after six months of storage. Technological and sensory quality of the products without xanthan gum can be, after four months of the storage in a freezing box, evaluated as unsatisfactory. This was in contrast to the loaves of bread with 0.16% xanthan gum addition which volume, specific volume, vaulting and sensorial indicators were, even after six months of freezing storage, still excellent and comparable with freshly baked products (the control).

REFERENCES

Arozarena, I., Bertholo, H., Empis, J., Bunger, A., De Sousa, I. 2001. Study of the total replacement of eff by white lupine protein, emulsifiers and xanthan gum in yellow cakes. *European Food Research and Technology*, vol. 213, no. 4-5, p. 312-316. http://dx.doi.org/10.1007/S002170100391

Ashwini, A., Jyotsna, R., Indrani, D. 2009. Effect of hydrocolloids and emulsifiers on the rheological, microstructural and quality characteristics of eggless cake. *Food Hydrocolloids*, vol. 23, no. 3, p. 700-707. http://dx.doi.org/10.1016/j.foodhyd.2008.06.002

Autio, K., Sinda, E., 1992. Frozen doughs: Rheological changes and yeast viability. *Cereal Chemistry*, vol. 69, no. 4, p. 409-413.

Berglund, P., Shelton, D., Freeman, T., 1991. Frozen bread dough ultra strucutre as affected by duration of frozen storage and freeze-thaw cycles. *Cereal Chemistry*, vol. 68, no. 1, p. 105-107.

Bojňanská, T., Muchová, Z., Frančáková, H., Urminská, D., Mendelová, A., Mareček, J., Solgajová, M., Vietoris, V., Czako, P. 2013. *Hodnotenie surovín a potravín rastlinného pôvodu* (Evaluation of plant raw materials and foodstuffs). Nitra : SPU, 335 p. ISBN 978-80-552-1086-5

Cauvain, S. P.1998. Improving the control of stalling in frozen bakery products. *Trends in Food Science and Technology*, vol. 9, p. 56-61. ISSN 0924-2244.

Collar, C., Andreu, P., Martínez, J. C., Armero, E. 1999. Optimization of hydrocolloid addition to improve wheat bread dough functionality: a response surface methodology study. *Food Hydrocolloids*, vol. 13, no. 6. p. 467-475. http://dx.doi.org/10.1016/S0268-005X(99)00030-2

Dodić, J., Pejin, D., Dodić, S., Popov, S., Mastilović, J., Popov-Raljić, J., Zivanovic, S., 2007. Effects of hydrophilic hydrocolloids on dough and bread performance of samples made from frozen doughs. *Journal of Food Science*, vol. 72, no. 4, p. 35-41. http://dx.doi.10.111/j.1750-3841.2007.00337.x

Gambuś, H., Sikora, M., Ziobro, R. 2007. The effect of composition of hydrocolloids on properties of gluten–free bread. *Acta Scientiarum Polonorum*. vol. 6, no. 3, p. 61-74.

Garciá-Ochoa, F., Santosa, V. E., Casasb, J. A., Gómez, E. 2000. Xanthan gum:production, recovery, and properties. *Biotechnology Advances*. vol. 18, no. 7, p. 549-579. http://dx.doi.org/10.1016/S0734-9750(00)00050-1

Gelinas, P., Deaudelin, I., Grenier, M. 1995. Frozen dough: effects of dough shape, water content, and sheeting-molding conditions. *Cereal Foods World*. vol. 40, no. 3, p. 124-126.

Gimeno, E., Morau, C. I., Kokini, J. L. 2004. Effect of xanthan gum and CMC on the structure and texture of corn flour pellets expanded by microwave heating. *Cereal Chemistry*. vol. 81, no. 1, p. 100-107. http://dx.doi.org/10.1094/CCHEM.2004.81.1.100

Hampl, J., Havel, F., Kadlec, F., Příhodová, J. 1981. *Jakost pekárenských a cukrárenských výrobků* (The quality bakery and confectionery product). Praha : SNTL, 232 p.

Hojerová, J., Štern, P., Zsemlye, R. 2005. Reológia potravinárskych hydrokoloidov (The rheology of food hydrocolloids). *Bulletin of Food Research*, vol. 44, no. 1-2, p. 83-99.

Hsu, K. H., Hoseney, R. C., Seib, P. A. 1979. Frozen dough II. Effects of freezing and storing conditions on the stability of yeasted doughs. *Cereal Chemistry*, vol. 56, no. 5, p. 424-426.

ICC Standard No. 104/1:1990 *Determination of ash in cereals and cereal products.*

ICC Standard No. 105/2:1994 *Determination of crude protein.*

ICC Standard No. 107/1:1995 *Determination of the "Falling number" according to Hagberg* – as a measure of the degree of alpha-amylase activity in grain and flour.

ICC Standard No. 110/1:1976 *Determination of the moisture content of cereals and cereal products* (Practical method).

ICC Standard 115/1:1992 AACC Method 54-21:1995 *Method for using the Brabender Farinograph*.

ICC Standard No. 116/1:1994 *Determination of the sedimentation value* (according to Zeleny) as an approximate measure of baking quality.

ICC Standard 155/1994 *Determination of wet gluten quantity and quality* (Gluten index ac. to Perten) of whole wheat meal and wheat flour (*Triticum aestivum* L.).

Khan, T., Park, J. K., Kwon, J. 2007. Functional biopolymers produced by biochemical technology considering applications in food engineering. *Korean Journal of Chemical Engineering*, vol. 24, no. 5, p. 816-826.

Kohajdová, Z., Karovičová, J., Gajdošová, Ž. 2008. Importance of hydrocolloids in bakery industry. *Potravinarstvo*. vol. 2, p. 9-18.

Kohajdová, Z., Karovičová, J., Schmidt, Š. 2009. Significance of emulsifiers and hydrocolloids in bakery industry. *Acta Chimica Slovaca*. vol. 2, no. 1, p. 46-61. ISSN 1337-978X.

Mandala, I. G. 2005. Physical properties of fresh and frozen stored microwave-reheated breads, containing hydrocolloids. *Journal of Food Engineering*. vol. 66, no. 3, p. 291-300. http://dx.doi:org/10.1016/j.jfoodeng.2004.03.020

Mikuš, Ľ., Valík, Ľ., Dodok, L. 2001. Usage of hydrocolloids in cereal technology. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 59, no. 5, p. 325-333.

Milani, J., Maleki, G. 2012. *Hydrocolloids in Food Industry*. Food Industrial Processes–Methods and Equipment, Iran : Tech, p. 17-38. ISBN 978-953-307-905-9.

Miller, R. A., Hoseney, R. C. 1993. The role of xanthan gum in white layer cakes. *Cereal Chemistry*, vol. 70, no. 5, p. 585-588. ISSN 0009-0352.

Miller, L. L., Setser, C. 1982. Xanthan gum in a reduced egg white angel food cake. *Cereal Chemistry*, vol. 60, no. 1, p. 62-64.

Muchová, Z., Frančáková, H. 2001. *Hodnotenie a využitie rastlinných produktov*. (Evaluation and using of plant products). Nitra : SPU. 168 p. ISBN 80-7137-887-9.

Pepe, O., Anastasio, M., Villani, F. 2005. Improvement of frozen dough stability using a cryoresistant yeast strain and refreshment. *Cereal Chemistry*. vol. 82, no. 3, p. 239-241. ISSN 0009-0352.

Ribotta, P. D., Leon, A. E., Anon, M. C. 2003. Effects of yeast freezing in frozen dough. *Cereal Chemistry*, vol. 80, no. 4, p. 454-458.

Rosell, C. M., Rojas, J. A., Benedito de Barnber, C. 2001. Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocolloids*. vol. 15, no. 1, p. 75-81. http://dx.doi:10.1016/S0268-005X(00)00054-0

Statistical Office of the Slovac republic: *Spotreba chleba a pečiva za roky 2008-2012* (Consumption of bread and pastries for years 2008-2012): http://www.statistics.sk/pls/elisw/casovy_Rad.procDlg

Tao, F., Wang, X., Ma, C., Yang, Ch., Tang, H. 2012. Genome sequence of *Xanthomonas campestris* JX, an industrially productive strin for xanthan gum. *Journal of Bacteriology*. vol. 194, no. 17, p. 4755-4756. http://dx.doi.org/10.1128/JB.00965-12

Velíšek, J. 2002. *Chemie potravin 1* (Food chemistry 1), Tábor, 2nd ed, Tábor : Ossis, 2002, 344 p. ISBN 80-86659-00-3.

R Core Team, 2014. *R: A language and environment for statistical computing. R.* Foundation for Statistical Computing. Vienna, Austria.

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Intersection of mycotoxins from grains to finished baking

Viera Šottníková, Luděk Hřivna, Iva Burešová, Jan Nedělník

ABSTRACT

This work is focused on the evaluation of the content of deoxynivalenol and zearalenone in samples of winter wheat of the following varieties: Sultan, Cubus, Akteur, Seladon, Mulan, Chevalier, Evina, Hewitt, Bohemia, Baletka. The total amount of 10 samples harvested in 2011 and 2012 was evaluated and included variants both treated and untreated against fungal diseases. The samples were adjusted for mycotoxicological determination and subsequently measured by the ELISA method. The content of deoxynivalenol (DON) and zearalenone (ZEA) was measured in grain, flour and breadrolls in all samples. Out of all samples 43% were found to have positive content of DON and 75% of ZEA. In the treated variants, the average DON content was found to be 115 $\mu g.kg^{-1}$ in grain, 77 $\mu g.kg^{-1}$ in flour and 97 $\mu g.kg^{-1}$ in pastries. In the untreated variants, the average DON content was found to be 208 $\mu g.kg^{-1}$ in grain, 103 $\mu g.kg^{-1}$ in flour and 128 $\mu g.kg^{-1}$ in pastries. Moreover, the average ZEA content in the treated variant was 4.95 $\mu g.kg^{-1}$ in grain, 3.38 $\mu g.kg^{-1}$ in flour and 4.51 $\mu g.kg^{-1}$ in pastries, in the non-treated variant average ZEA content in grain was 3.07 $\mu g.kg^{-1}$, 4.97 $\mu g.kg^{-1}$ in flour and 2.81 $\mu g.kg^{-1}$ in pastries. The maximal acceptable limits given by the valid legislation were not exceeded in any analysed sample. It can be concluded wheat grain grown in the Czech Republic, whether it is treated or untreated by fungicides, is not dangerous for consumers. The content of both mycotoxins is not dependent on each other, and the untreated variant has a slightly higher dependency between DON and ZEA.

Keywords: mycotoxins; deoxynivalenol; zearalenone; grain; flour; finished product

INTRODUCTION

Microscopic fibrous micromycetes (moulds) create an important part of all organisms, especially in relation to humans and animals. They can cause skin, mucosal and internal organ diseases, collectively they are called mycoses **(Cempírková et al., 1997)**. Mycotoxicosis occurs after consumption, inhalation or after contact with toxic secondary metabolites of microscopic fungi. In favourable conditions, microscopis fungi may contaminate and destroy huge quantity of stock, food and feed by its adverse actions **(Tančinova et al., 2012)**. Every type of micromycet has different requirements for environment conditions, in which it grows, and this is also valid for types of the same species **(Diekman and Green, 1992)**. We can divide them into external factors (temperature, relative humidity, oxygen, period of stock storage), and internal factors (water activity, pH, texture, composition of stock, antimicrobial substances in stock) **(Tančinová et al., 2001, Mašková et al., 2012)**.

Danger of majority of mycotoxins lies rather in chronical effect of slight amounts than in actual toxicity **(Patočka et al., 2004)**. Deoxynivalenol (DON) is probably the most known and the most common mycotoxin contaminating feed and food made of grain crops. DON was for the first time isolated from corn infested by mould *Fusarium graminearum* in 1973. Further it is produced by toxicogenic species of *Fusarium culmorum, F. graminearum, F. sporotrichioides*a*F. poae*. It occurs anywhere in the world where grains are grown. It has been found in a set of other food, e.g. in baby food made of grains, in corn, rice, millet, bran, ginger, garlic, beer, muesli, spaghetti and soybeans **(Malíř and Ostrý, 2003)**.

Zearalenone (ZEA, also F-2 toxin) was for the first time isolated in 1964 from the culture *Gibberellazeae* (anamorph of *F. graminearum*) of corn. It is produced by the genus *Fusarium*, especially *Fusarium graminearum, F. culmorum, F. equiseti, F. moniliformea* and *F. semitectum* belong to the most significant producers. The ideal temperatures for production of zearalenone are in the range of 12 – 14 °C, but also lower than 10 °C. *F. graminearum* is able to produce zearalenone even in the concentration 1900 µg/kg **(Magan and Olsen, 2004)**. First indirect evidence about masked mycotoxins appeared already in the first half of 80s in the 20th century. In animals, there were largely observed symptoms typical for mycotoxicosis in spite of the quantity of mycotoxins set in feed did not correspond with that. High toxicity of feed was apparently caused by conjugated forms of mycotoxins, which leaked from analytical determination **(Berthiller et al., 2013)**. While observing dynamics of mycotoxins in intentionally infected wheat, the increase of deoxynivalenol and subsequently its decrease was proved, which was probably caused by transformation of initial deoxynivalenol into its metabolites **(Hajšlová et al., 2009)**. Studies dealing with transformations of mycotoxins proved that conjugated forms of mycotoxins apparently occur during detoxification processes of grain crops. Until today, metabolites of deoxynivalenol, zearalenone, ochratoxin A and T-2 toxin have been identified **(Berthiller et al., 2013)**. Currently, only deoxynivalenol and zearalenone are

observed as markers of grain contamination caused by fusarium mycotoxins. However, many studies proved that in a majority of cases, trichotecenenivalenol, T-2 toxin or HT-2 toxin are dominant. However, if deoxynivalenol is the only one analytically observed representative, the severity of contamination can be underestimated **(Nedělník et al., 2005)**. Commission Regulation (ES) No. 1126/2007 from 28th September 2007 determines maximum limits for deoxynivalenol, zearalenone (cereals - 100, flour - 75 and breadrolls - 50 µg per 1 kg) and tolerable daily intakes of these substances to 1 kg of body weight.

The objective of work was to compare and evaluate changes in the content of mycotoxins DON and ZEA in wheat grain, flour and breadrolls.

MATERIAL AND METHODOLOGY

Samples of winter wheat Sultan, Cubus, Akteur, Seladon, Mulan, Chevalier, Evina, Hewitt, Bohemia, Baletka were used for measuring, while they were obtained from experiments of the Central Controlling and Testing Institute for Agriculture, from its research station Hradec nad Svitavou. Grain was harvested from standardly treated crops (seed disinfected against bunt, dwarf bunt, morph regulator was used during vegetation, fungicide was applied against illnesses of haulm heels and against leaf and spikelet illnesses: T1 – treatment end of bracketing, T2 – beginning of heading until flowering). The test stations were located in the altitude 450 m, where long-term average temperature is 7.4 °C, long-term average rainfall is 616 mm. The soil type was typical brown soil and the soil class clay-loam (heavy soil). Content of DON and ZEA was observed in grain samples (especially whole grain groats, flour and breadrolls made of it were analysed).

Milled flour was let to ripen for a month. Breadrolls were made from the flour by the RMT method adjusted to laboratory conditions of MENDELU Brno. For quantitative evaluation of content of both mycotoxins, ELISA kits Veratox from the company Neogen were used. Kits contain plates with microtiter wells with bound antibody.

Results were evaluated through Statistika 8, the variable projection method into a factorial plane and pair test.

RESULTS AND DISCUSSION

All tested samples of wheat were positive for content of DON in case of the treated as well as non-treated variant. Measured values of DON were quite low in spite of the year 2013 was characteristic by considerable rainfall in the period of fungicide application and its efficiency did not have to be absolute. None of measured values exceeded the legislative limit. Concerning cereal supplementary food for infants and little children, the limit (200 µg.kg^{-1}) was exceeded in 5 samples of the non-treated variant. In the treated variant, DON in flour decreased on average by 33.04% in comparison to grain, in pastries it increased by 25.97% in comparison to flour, and in pastries it decreased by 15.65% in comparison to grain. In case of the non-treated variant, content of DON in flour in comparison to grain on average decreased by 50.48%, in pastries in comparison to grain it decreased by 38.46%. Decrease of values in both variants of flour in comparison to grain can be explained by low penetration of mycotoxins into endosperm, thus higher content was concentrated in outer covering layers, which were removed during milling. Increase of DON values in breadrolls in both variants can be explained by release of masked forms of mycotoxins (derivatives and conjugates of DON) and thus increase of their free forms, what was confirmed also by **Malachová et al., (2010)**. In addition to this, DON is relatively thermally stable, and therefore there are minimal losses during baking. Thus, passing of DON into finished products was confirmed. Moreover, yeast is added into recipe, enzymes of which are able to transform some of precursors of mycotoxins contained in flour into mycotoxins and thus to increase their quantity in pastries, what was also proved by **Young et al., (1984)**, that during fermentation processing of flour, the level of DON increased almost by 100%.

According to **Horáková (2013)** Cubus, Hewitt and Seladon belong to varieties very prone to content of DON, which can be also confirmed by measured results. The lowest content of DON was in the variety Akteur. Resistant varieties have not been bred yet.

In the Figure 1 and 2 we can see excessive quantity of DON and ZEA in both observed years.

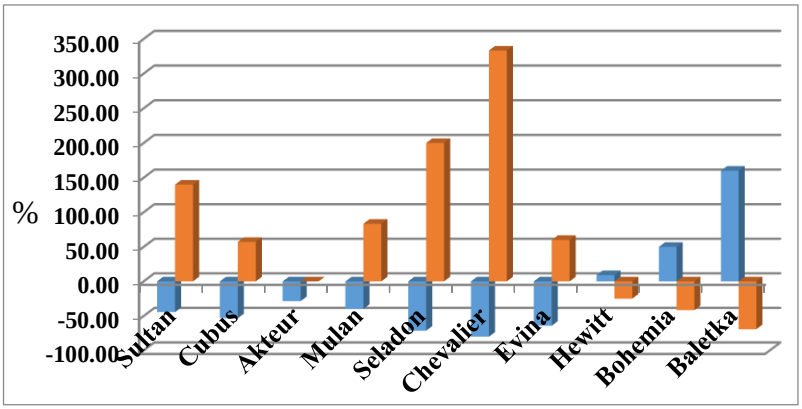

Figure 1 Change of DONcontent after milling and baking in the treated variant in %.

Note: blue-changing content DON grain / flour, red- changing content DON flour/pastries.

By comparison of the treated and non-treated variant, measured values of DON were clearly higher in the non-treated variant, on average by 80.87 % for grain, 33.77 % for flour and 31.96 % for pastries. Thus, we can say that positive effect of fungicide treatment was proved.

Statistical evaluation of measured values of DON is stated in the Table 1, where the two sided T-test for files of the same size was used. Decrease of DON in flour in comparison to grain seems to be statistically significant in case of the treated as well as the non-treated variant and further difference of DON quantity in grain in the treated in comparison to the non-treated variant. Many studies state that DON is thermally stable even during baking. **Scott et al., (1983)** and also **Lancová et al., (2007)** state that baking did not have any influence on DON content.

Zhang and Wang (2015) found**,** that concentrations of DON were verifiably higher in baked bread than in flour. On the contrary, according to **Young et al (1984)** baking has verifiable influence on decrease of DON concentration in bread, on average by 17 to 33% in comparison to the dough. **Boyacioglu et al., (1993)** state that content of DON decreased by 7% after baking bread, however the content of DON in bread with L-cystein as an additive in flour before baking decreased by 38 to 46 %. **Lešnik et al., (2008)** in his study baked 6 types of bread from 3 types of differently milled flour and in two different ovens (industrially used and classic ceramic). Average decrease of DON concentration in bread was 47. 2% in industrial ovens and 48.7% in ceramic ovens. The flour used was strongly contaminated by DON (average concentrations were 850–950 $\mu g.kg^{-1}$), however, breads after baking reached under 500 $\mu g.kg^{-1}$, and thus they met legislative limits. We can summarize that during technological processing, there occurs reduction of DON, but it is not completely removed from a finished product. **Hajšlová et al., (2008)** confirm that the most of waste fractions and bran tend to be the most contaminated and the lowest levels are in scouring flour. Further they stated that with increased time of dough ripening, there occurs increase of free DON and at the same time decrease of the conjugated form of D3G and that with increasing baking time, DON content decreases on average by 32% in comparison to shorter baking time.

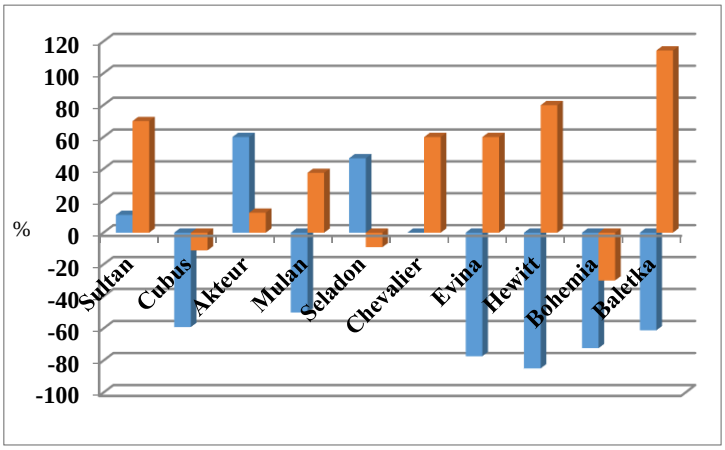

Figure 2 Change of DON content after milling and baking in the non-treated variant in %.

Note: blue-changing content DON grain / flour, red- changing content DON flour/pastries.

Table 1 Statistical significance (P <0.05) decrease/increase of DON values and comparison of the treated and the non-treated variant.

		p	Statistically significant difference
DON treated	grain / flour	0,028	+
	flour / pastries	0,188	-
	grain/ pastries	0,213	-
DON untreated	grain / flour	0,029	+
	flour / pastries	0,282	-
	grain/ pastries	0,079	-
DON treated/ untreated	grain T/ flour U	0,04	+
	flourT/ pastries U	0,232	-
	grain T/ pastries U	0,08	-

Note: *T-treated, U-untreated.

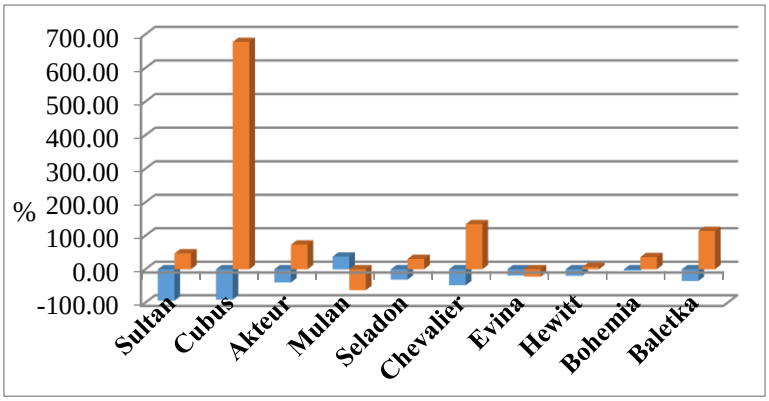

Figure 3 Change of ZEA content after milling and baking in the treated variant in %.

Note: blue-changing content ZEA grain / flour, red- changing content ZEA flour/pastries.

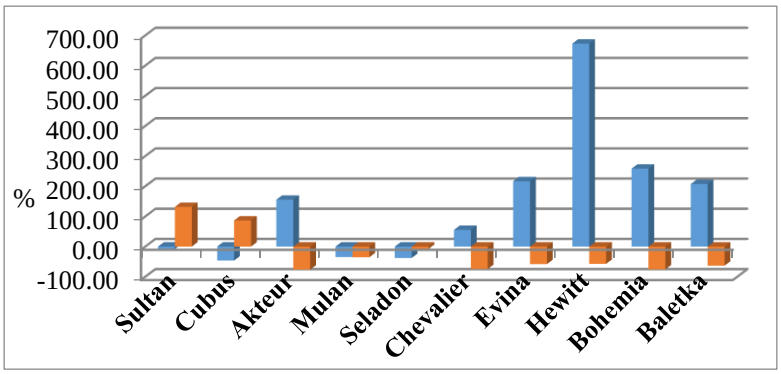

Figure 4 Change of ZEA content after milling and baking in the non-treated variant.

Note: blue-changing content ZEA grain / flour, red-changing content ZEA flour/pastries.

In the Figure 3 and 4 there are illustrated differences in the treated and non-treated variants and changes in ZEA content after milling and baking and in a finished product in comparison to grain.

All measured samples of wheat were positive for content of ZEA. None of the measured values of the treated and non-treated variant exceeded legislative limit. In total, values were very low and at so low level of contamination it is not possible to make clear conclusions.

When comparing the treated and the non-treated variant, higher ZEA values were measured in the treated variant, on average by 62.00% for grain and 62.23% for pastries. In flour, higher values were in the non-treated variant, on average by 46.85 %. Thus, the ZEA fungicide treatment did not have positive effect. This fact can also be caused by high rainfall at the time of fungicide application and its

Table 2 Evaluation of statistical significance (p <0.05) decrease/increase of ZEA values and comparison of the treated and non-treated variant

		p-value	Statistically significant difference
ZEA treated	grain / flour	0,07	-
	flour / pastries	0,273	-
	grain/ pastries	0,64	-
ZEA untreated	grain / flour	0,032	+
	flour / pastries	0,012	+
	grain/ pastries	0,713	-
ZEA treated/untreated	grain T/ flour U	0,019	+
	flour T/ pastries U	0,09	-

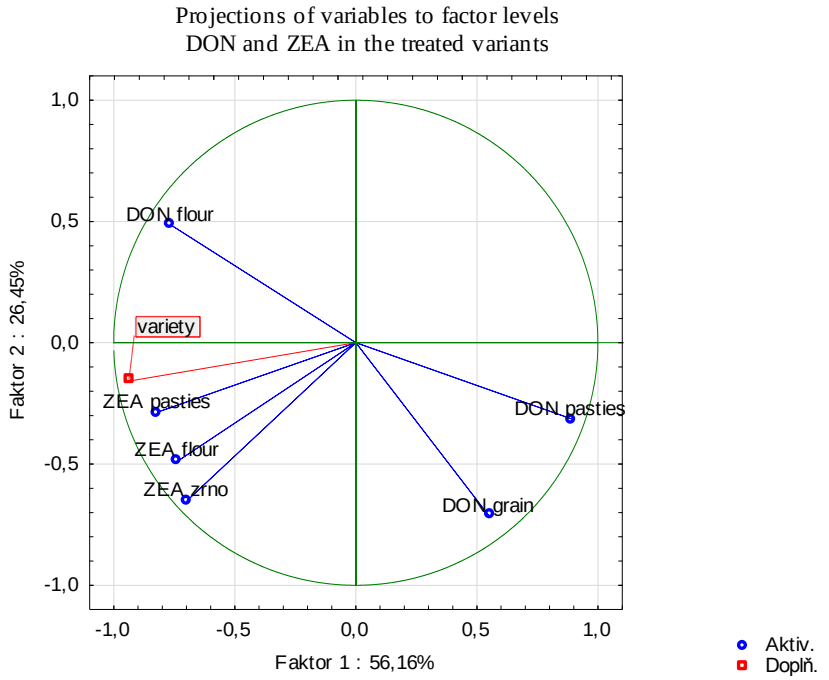

Figure 5 Projection of variables into factor plane – DON and ZEA in the treated variant.

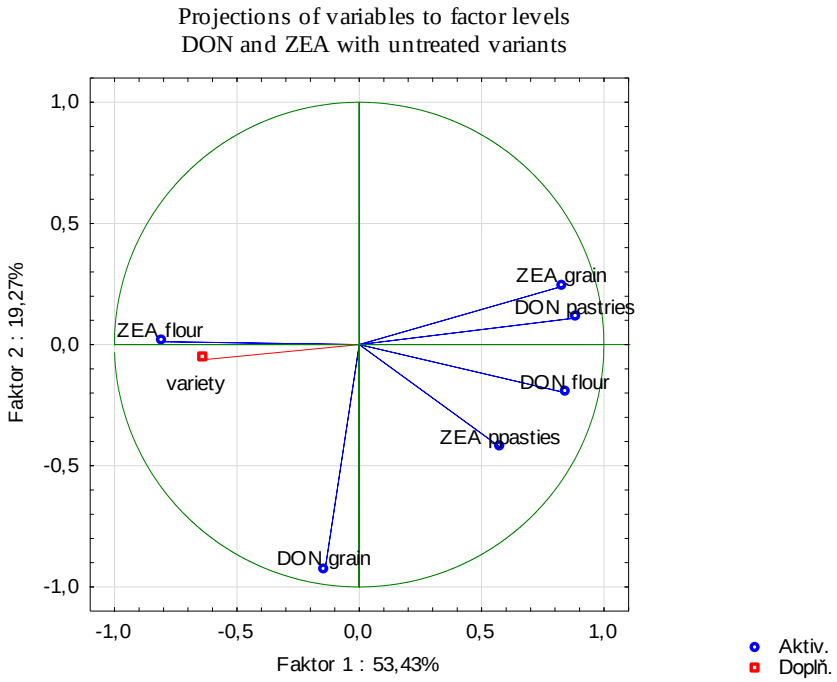

Figure 6 Projection of variables into factor plane – DON and ZEA in the non-treated variant.

efficiency thus did not have to be absolute. According to **Nedělník et al., (2005)**, it commonly happens that after fungicide treatment (especially of strobilurins) the increase of mycotoxins occurs because the stress factor affects fungus and it tries to save itself before extinction and thus it produces even higher quantity of mycotoxins, which is also confirmed by **Šafránková et al., (2010)** and **Malachová et al., (2010)**.

In the treated variant, ZEA in flour in comparison to grain decreased on average by 31.63%, in pastries in comparison to flour increased by 33.34% and in pastries in comparison to grain it decreased by 8.83%. In the non-treated variant ZEA content in flour in comparison to grain increased on average by 61.93% and in pastries in comparison to flour it decreased by 43.50% and in pastries in comparison to grain it decreased by 8.51%.

Decrease of values in flour in comparison to grain in the treated version can be explained by low penetration of mycotoxin into endosperm, and thus higher content was concentrated in outer covering layers, which were removed during milling. On contrary, in the non-treated variant, there is clear increase in flour in comparison to grain,

which can be explained by opposite situation that penetration was high thus mycotoxins were concentrated in grain endosperm. Increase of ZEA values in pastries in the treated variant can be, similarly as for DON, explained by release of masked forms of mycotoxins. ZEA is also relatively thermally stable and losses in baking are small.

Statistical evaluation of measured ZEA values is stated in the Table 2. The two sided T-test was used for files of the same size. Increase of ZEA in flour in comparison to grain and decrease of ZEA in pastries in comparison to flour in the non-treated variant appears to be statistically significant. Furthermore, difference between ZEA quantity in grain of the treated version and non-treated version is statistically significant

Figure 5 and 6 are outcomes of processing of measured data in the program Statistica 8 and they illustrate projection of variables into the factor plane. There is the level of dependence of individual variables evaluated according to the angle size that they enclose. The smaller the angle, the stronger the dependence. At the same time, the angle cosine determines approximate value of correlation coefficient. If arrows are horizontally in the same direction, it means that variables are positively dependent on each other. If arrows are horizontal but in opposite direction, it means that variables are negatively dependent on each other. If arrows are perpendicular on each other, variables can be considered as independent. Length of an arrow indicates variability of measured data.

At the Figure 5 we can see that in the treated variant, species influence ZEA content in all products and at the same time, there is strong correlation dependency. DON content in individual products is independent on each other. DON and ZEA content are not dependent on each other. The highest variability of measured values was recorded in ZEA content in grain (the longest arrow).

In the Figure 6 in the non-treated variant, we can see strong dependency of species on ZEA content in flour. DON content (breadrolls, flour) and ZEA (grain, breadrolls) area closely related, especially ZEA content in grain and in breadrolls and DON in breadrolls and flour. The highest variability of measured values was also recorded in DON content in grain.

CONCLUSION

Average DON content in the treated variant was 115 $\mu g.kg^{-1}$ in grain, 77 $\mu g.kg^{-1}$ in flour and 97 $\mu g.kg^{-1}$ in pastries, in the non-treated variant average DON content in grain was 208 $\mu g.kg^{-1}$, 103 $\mu g.kg^{-1}$ in flour and 128 $\mu g.kg^{-1}$ in pastries. Average ZEA content in the treated version was 4.95 $\mu g.kg^{-1}$ in grain, 3.38 $\mu g.kg^{-1}$ in flour and 4.51 $\mu g.kg^{-1}$ in pastries, in the non-treated version average ZEA content in grain was 3.07 $\mu g.kg^{-1}$, 4.97 $\mu g.kg^{-1}$ in flour and 2.81 $\mu g.kg^{-1}$ in pastries. Measured ZEA contents are on a very low level of contamination. The maximal acceptable limits given by the valid legislation were not exceeded in any analysed sample. It implies that grain grown in the Czech Republic, whether it is treated or non-treated by fungicides, is not dangerous for consumers.

Content of both mycotoxins is not dependent on each other, in the non-treated variant there is slightly higher dependency of DON and ZEA. Species that have the highest influence on ZEA content, did not have any influence on DON content. In the treated variant ZEA contents in grain, flour and breadrolls contents closely related.

In the treated variant, DON content was decreased by milling on average by 33.04% in comparison to grain and on the other hand, its content in breadrolls was increased by 25.97%. In the non-treated variant, DON content decreased by 50.48% in comparison to grain and by baking, it again increased in comparison to flour by 24.27%. ZEA content in the treated version in flour decreased on average by 31.63% in comparison to grain, in pastries in comparison to flour, it increased by 33.34%. On the other hand, in the non-treated version, ZEA content was increased through milling by 61.93% in comparison to grain and during baking, its content decreased by 43.50% in comparison to flour. Decrease of mycotoxin content after milling means that mycotoxins were concentrated more in outer covering layers and less in endosperm. Covering layers are removed during milling and thus mycotoxin levels are decreased in flour. On the contrary, in the non-treated variant ZEA was more concentrated in endosperm and thus there was its increase in flour. Subsequently, increase of mycotoxins after baking in pastries can be explained by release of masked forms of mycotoxins or by activity of yeast during fermentation, what was confirmed by number of studies stated above. Both mycotoxins are quite thermally stable and thus they are not degraded during baking too much.

REFERENCES

Berthiller, F., Crews, C., Dall'asta, Ch., Saeger, S., Haesaert, G., Karlovsky, P., Oswald, I. P., Seefelder, W., Speijers, G., Stroka, J. 2013. Masked mycotoxins: A review. *Molecular Nutritionand Food Research*. vol. 57, p. 165-186. http://dx.doi.org/10.1002/mnfr.201100764 PMid:23047235

Boyacioglu, D., Hettiarachchy, N., S., D'appolonia, B, L. 1993. Additives affect deoxynivalenol (vomitoxin) flour during bread baking. *Journal of Food Science*, vol. 58, p.416-418. http://dx.doi.org/10.1111/j.13652621.1993.tb04288.x

Cempírková, R., Lukášová, J., Hejlová, Š. 1997. *Mikrobiologie potravin (Microbiology of food).* Jihočeská univerzita, Zemědělská fakulta České Budějovice, České Budějovice, 165 p.

Diekman, M., A., Green, M., L. 1992. Mycotoxins and reproduction in domestic nlivestock. *Journal of animal science*, vol. 70, p. 1615-1627.

Hajšlová, J., Malachová, A., Zachariášová, M., Kostelanská, M., Kocourek, V., Poustka, J. 2008. *Mykotoxiny a jejich konjugáty v potravinářských surovinách a krmivech: trendy, rizika dietární expozice, možnosti prognózy osudu při zpracování (Mycotoxins and their conjugates in food and feed materials: trends, risks of dietary exposure, possibility of forecasting fate processing).* Výzkumný ústav rostlinné výroby [cit. 2015-03-22]. http://www.phytosanitary.org/?link=cs/projekty/2008/.

Hajšlová, J., Malachová, A., Zachariášová, M., Kostelanská, M., Kocourek, V. 2009. *Mykotoxiny (The mycotoxins).* Výzkumný ústav rostlinné výroby [cit. 2015-03-22]. http://www.phytosanitary.org/?link=cs/projekty/2009/.

Horáková, V. 2013. *Fuzariózové mykotoxiny v odrůdách ozimé pšenice (Fusarium mycotoxins in varieties of winter wheat).* Ústřední kontrolní a zkušební ústav zemědělský. Sekce rostlinné výroby Brno. Národní odrůdový úřad [cit. 2015-02-20].

http://eagri.cz/public/web/file/232495/Horakova_Mykotoxiny_2013.pdf.

Lancová, K., Hajšlová, J., Kostelanská, M., Kohoutková, J., Nedělník, J., Moravcová, H., Váňová, M. 2008. *Fate of trichothecene mycotoxins during the processing: milling and baking. Food Additived and Contaminants*, vol. 25, p. 650-659. http://dx.doi.org/10.1080/02652030701660536 PMid:18473219

Lešnik, M., Cencič, A., Vajs, S., Simončič, A. 2008. Milling and bread baking techniques significantly affect themycotoxin (deoxynivalenon and nivalenon) level in bread. *Acta Alimentaria*, vol. 37, no 4, p. 471-483. http://dx.doi.org/10.1556/AAlim.2008.0015

Magan, N., Olsen, M. 2004. *Mycotoxins in food: detection and control.* Woodhead Publishing, Cambridge, 471 p. ISBN: 978-1-85573-733-4 http://dx.doi.org/10.1533/9781855739086

Malachová, A., Hajšlová, J., Ehrenbergerová, J., Kostelanská, M., Zachariášová, M., Urbanová, J., Cerkal, R., Šafránková, I., Marková, J., Vaculová, K., Hrstková, P. 2010. *Fusarium mycotoxins in spring barley and their transfer into malt. Fermentation industry*, vol. 3, p. 131-137.

Mašková, Z., Tančinová, D., Barboráková, Z., Felšöciová, S., Císarová, M. 2012. Comparison of occurrence and toxinogenity of Alternaria spp. isolated from samples of conventional and new crossbred wheat of Slovak origin. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 1, p. 552-562.

Malíř, F., Ostrý, V. 2003. *Vláknité mikromycety (plísně), mykotoxiny a zdraví člověka*. (Fibrous micromycets (mold), mycotoxins and human health). Národní centrum ošetřovatelství a nelékařských zdravotnických oborů, Brno, 349 p.

Nedělník, J., Hajšlová, J., Sýkorová, S. 2005. *Validační studie Mykotoxiny – detekce, dynamika a podmínky kontaminace potravin a krmiv (Validation studies The mycotoxins - detection, dynamics and conditions of contamination of food and feed).* Výzkumný ústav rostlinné výroby [cit. 2015-02-20]. http://www.phytosanitary.org/?link=cs/projekty/2005/.

Patočka, J., Bajgar, J., Cabal, J., Fusek, J., Herink, J., Kassa, J., Štětina, R. 2004. *Vojenská toxikologie (Military of toxicology).* Grada Publishing, Praha, 178 p.

Scott, P. M., Kanhere, S. R., Lau, P. Y., Dexter, J. E., Greenhalgh, R. 1983. Effects of Experimental flourmilling and breadbaking on retention of deoxynivalenol (vomitoxin) in hard red spring wheat. *Cereal Chemistry*, vol. 60, p. 421-424.

Šafránková, I., Marková, J.,Kmoch M. 2010. Mykoflóra zrn sladovnických odrůd a linií ječmene jarního na lokalitách Kroměříž a Žabčice *(The microflora of grain malting varieties and lines of spring barley in localities Žabčice and Kroměříž). Fermentation industry* (*Kvasný průmysl*), vol. 56, p. 138-144.

Vidal, A., Morales, H., Sanchis, V., Ramos, A. J., Marín, S. 2014. Stability of DON and OTA during the breadmaking proces and determinativ of process and performance kriteria. *Food Control*, vol. 40, p. 234-242.

Tančinová, D., Kačániová, M., Javoreková, S. 2001. Natural occurrence of fungi in feeding beat after harvest and during storage in the agricultural farmfacilities. *Biologia*, vol. 56, no. 3, p. 247-250.

Tančinová, D., Mašková, Z., Dovičičová, M., Barboráková, Z., Felšöciová, S. 2012. *Mykocenóza pšenice (Representation of wheat fungus).* Slovenská poľnohospodárská univerzita v Nitre, Nitra, 110 p.

Young, J. C., Fulcher, R. G., Hayhoe, J. H., Scott, P. M., Dexter, J. E. 1984. Effect of milling and baking on deoxynivalenol (vomitoxin) content of eastern Canadian wheats. *Journal of agricultural and food chemistry*, vol. 32, no. 3, p. 659-664. http://dx.doi.org/10.1021/jf00123a058

Zhang, H., Wang, B. 2015. Fates of deoxynivalenol and deoxynivalenol-3-glukoside during bread and noodle processing. *Food control*, vol. 50, p. 754-757.

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OXIDATIVE STABILITY OF CHICKEN'S BREAST AFTER VACUUM PACKAGING, EDTA, SAGE AND ROSEMARY ESSENTIAL OILS TREATMENT

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ABSTRACT

In the present work, the effect of the sage and rosemary essential oils on oxidative stability of chicken breast muscles during chilled storage was investigated. In the experiment were chickens of hybrid combination Cobb 500 after 42 days of the fattening period slaughtered. All the broiler chickens were fed with the same feed mixtures and were kept under the same conditions. The feed mixtures were produced without any antibiotic preparations and coccidiostats. After slaughtering was dissection obtained fresh chicken breast with skin from left half-carcass, which were divided into five groups (n = 5): C - control air-packaged group; A1 - vacuum-packaged experimental group; A2 - vacuum-packaged experimental group with EDTA solution 1.50% w/w; A3 - vacuum-packaged experimental group with *Salvia officinalis* L. oil 2.0% v/w and A4 - vacuum-packaged experimental group with *Rosmarinus officinalis* L. essential oil 2.0% v/w. The sage and rosemary essential oils were applicate on surface chicken breasts and immediately after dipping, each sample was packaged using a vacuum packaging machine and storage in refrigerate at 4 ±0.5 °C. The value of thiobarbituric acid (TBA) expressed as amount of malondialdehyde (MDA) in 1 kg sample was measured during storage in 1st, 4th, 8th, 12th and 16th day. The treatments of chicken breasts with sage and rosemary essential oils show statistically significant differences between all testing groups and control group, where higher average value of MDA measured in breast muscle of broiler chickens was in samples of control group (0.396 $mg.kg^{-1}$) compared to experimental groups A1 (0.060 $mg.kg^{-1}$), A2 (0.052 $mg.kg^{-1}$), A3 (0.042 $mg.kg^{-1}$) and A4 (0.041 $mg.kg^{-1}$) after 16-day of chilled storage. The results of experiment showed that the treatment of chicken breast with sage and rosemary essential oils had positive effect on the decrease of oxidative processes in breast muscles during chilling storage and use of plant essential oils is one of the possibilities increase shelf life of fresh chicken meat.

Keywords: oxidative stability; chicken breast; essential oil; sage, rosemary

INTRODUCTION

Meat and meat products are essential components in the human diets and their consumption is affected by various factors, e.g. product characteristics, consumer and environment related **(Jiménez-Colmenero et al., 2001)**. Chicken meat has many desirable nutritional characteristics such as a low lipid content and relatively high concentration of polyunsaturated fatty acids (PUFAs) which can be further increased by specific dietary strategies **(Bourre, 2005)**. However, a high degree of polyunsaturation accelerates oxidative processes leading to deterioration in meat flavour, colour, texture and nutritional value **(Mielnick et al., 2006)**.

Lipid oxidation causes degradation of polyunsaturated fatty acids (PUFA) and generation of residual products, such as malondialdehyde (MDA) and lipid-derived volatiles leading to sensory and nutritional deterioration of meat **(Kanner et al., 1991)**. Oxidative reactions in foodstuffs are enhanced after cooking and refrigerated storage through the increase of their oxidative instability due to the degradation of natural antioxidants and the release of free fatty acids and iron from the haem molecule **(Estévez and Cava, 2004; Kingston et al., 1998; Kristensen and Purslow, 2001)**.

The higher level of PUFAs in muscle membranes increases the susceptibility of oxidative deterioration of lipid **(Engberg et al., 1996)**, which impairs the organoleptic characteristics and shortens the shelf-life of meat and meat products.

The major strategies for preventing lipid oxidation are the use of antioxidants and restricting the access to oxygen during storage vacuum-packaging **(Tang et al., 2001)**. The antioxidant additives are added to fresh and further processed meats to prevent oxidative rancidity, retard development of off-flavours, and improve colour stability **(Nam and Ahn, 2003)**.

For chicken meat products, freshness, as one of the most important quality attributes, has attracted attention from producers and consumers and has a strong relationship with product sales and consumption **(Rzepka et al., 2013)**. One option for reducing lipid oxidation is the use of various natural plant antioxidants presented in essential oils.

The use of natural preservatives to increase the shelf life of meat products is a promising technology since many

vegetal substances have antioxidant and antimicrobial properties. Functional ingredients in meat products may improve the nutritional and health qualities and prolonging their self-life **(Fernández-Ginés et al., 2005)**. Plants' extracts rich in polyphenols are good candidates, since they are easily obtained from natural sources and they efficiently prevent lipid oxidation in food products.

Studies have shown wide effective in spices to retard lipid oxidation in meat products **(Juntachote et al., 2006, 2007; Chouliara et al., 2007; Mariutti et al., 2008; Sasse et al., 2009; Lee et al., 2010; Marcinčák et al., 2010; Viuda-Martos et al., 2011; Tkáčová et al., 2015)**.

Essential oils (EOs) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, enfleurage or extraction but the method of steam distillation is most commonly used for commercial production of EOs **(Van de Braak and Leijten, 1999)**. EOs obtained from various herbs are widely used in cosmetics and food manufacturing and can be used for prolonging the shelf-life of food for their antimicrobial **(Skandamis et al., 2002; Mihajilov-Krstev et al., 2009)**, and antioxidant activities **(Burt, 2004; Bobko et al., 2015a, b)**.

In the last years, many researchers have evaluated the antioxidant properties of extracts from different plants and vegetables **(Chen et al., 2002; Ibanez et al., 2003; Ichikawa et al., 2003)**.

Essential oils represent a small fraction of the plant composition; the main compounds are terpenes and sesquiterpenes, and several oxygenated derivatives compounds (alcohols, aldehydes, ketones, acids, phenols, ethers, esters, etc.) all of them responsible for the characteristic plant odour and flavour **(Yanishlieva et al., 2006)**. These compounds include natural flavourings such as sage, oregano, rosemary and others **(Mariutti et al., 2008)**.

Sage (*Salvia officinalis*) and rosemary (*Rosmarinus officianalis*) are popular *Labiatae* herbs with a verified potent antioxidant activity **(Dorman et al., 2003)**. The antioxidant activity of sage and rosemary essential oils is mainly related to two phenolic diterpenes: carnosic acid and carnosol which are considered two effective free-radical scavengers **(Dorman et al., 2003; Ibanez et al., 2003)**.

Sage (*Salvia officinalis*) is a variety of aromatic herb which has been planted widely throughout much of the world. It is not only used as raw material in the pharmaceutical and cosmetic industries but also used to improve flavours of foods **(Tepe et al., 2006)**. Sage has been reported to have excellent activities in scavenging radicals, reducing metal ions and inhibiting lipid peroxidation **(Dorman et al., 2003; Grzegorczyk et al., 2007)**. The phenolic compounds, such as carnosol, carnosic acid and rosmarinic acid, in the plant may account for the antioxidant activity of sage. Some researchers have reported that sage, or sage extracts, can effectively retard lipid oxidation of muscle foods **(Fasseas et al., 2007; McCarthy et al. 2001a; Tanabe et al., 2002)**.

Among natural antioxidant sources, rosemary (*Rosmarinus officinalis* L.), a woody aromatic herb that is native to the Mediterranean countries, has recently been authorized by the European Union under Directive 95/2/EC and assigned E-392 as its E number (European Union Directives 2010/67/EU and 2010/69/EU) for use in meat product preservation. The addition of rosemary extract to poultry products has been shown to be effective in retarding lipid oxidation, and previous studies in chicken sausages **(Liu et al., 2009)** and patties **(Naveena et al., 2013)** have pointed to the protective effect of rosemary extract (500–1500 ppm) and leaves (22.5–130 ppm) in inhibiting lipid oxidation.

Rosemary antioxidant activity is related to components such as phenolic diterpenes, carnosol (CAS No. 5957-80-2) and carnosic acid (CAS No. 3650-09-7) **(Rodriguez-Rojo et al., 2012)**. The antioxidant capacity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms and chelate metal cations **(Shan et al., 2005)**. Previous studies **(Azmir et al., 2013; Wang et al., 2013)** have reported that the yield of bioactive compounds can be changed or modified by using different extraction procedures, solvents, temperatures, pressures and times.

In this study we aimed to investigate the combined effect of ethylenediaminetetraacetate (EDTA) and plant essential oils (*Salvia officinalis* L. and *Rosmarinus officinalis* L.) on the oxidative stability of fresh chicken breasts stored under vacuum packaging (VP), at 4 ±0.5 °C for a period of 16 days.

MATERIAL AND METHODOLOGY

The experiment was implemented in the local poultry station (Hydinaren a.s., Zamostie). The tested were broiler chickens of hybrid combination Cobb 500 both sexes. All the broiler chickens were fed with the same feed mixtures and were kept under the same conditions. The feed mixtures were produced without any antibiotic preparations and coccidiostats. At the end of the fattening period (42. day) were chickens slaughtered for analysis in laboratory of Slovak University of Agriculture in Nitra. After slaughtering was dissection obtained fresh chicken breast with skin from left half-carcass, which were divided into five groups (n = 5):

- Air-packaged (C, control group): chicken breast fresh meat was packaging to polyethylene backs and stored aerobically in refrigerator;
- Vacuum-packaged (A1, experimental group): chicken breast fresh meat was packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator;
- VP with EDTA solution 1.50% w/w (A2, experimental group): chicken breast fresh meat was treated with EDTA for 1 min and packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator;
- VP with *Salvia officinalis* L. 2.0% v/w (A3, experimental group): chicken breast fresh meat was treated with *Salvia officinalis* L. oil for 1 min and packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator;
- VP with *Rosmarinus officinalis* L. 2.0% v/w, (A4, experimental group): chicken breast fresh meat was treated with *Rosmarinus officinalis* L. oil for 1 min and packaging to polyethylene backs and stored anaerobically in vacuum and in refrigerator.

Immediately after dipping, each sample was packaged using a vacuum packaging machine type VB-6 (RM Gastro, Czech Republic).

Ethylenediaminetetraacetic acid (EDTA) (C10H14N2O8.Na2.2H2O) was 99.5% purity, analytical grade, (Invitrogen, USA). A stock solution of 500 mM concentration was prepared by diluting 186.15 $g.L^{-1}$ distilled water. A final concentration of 50 mM EDTA solution was prepared from the stock solution. The pH of the solution was adjusted to 8.0 with the addition of the appropriate quantity of NaOH solution. The amount of EDTA added to the treat chicken breasts was 0.28 $g.kg^{-1}$. Essential oil (Calendula, Nova Lubovna, Slovakia) were added to the coated chicken beast surface (both sides) of each sample using a micropipette so as to achieve a 0.2% v/w final concentration of essential oils.

TBA value expressed in number of malondialdehyde (MDA) was measured in the process of first storage day of 1^{st}, 4^{th}, 8^{th}, 12^{th} and 16^{th} day. TBA number was determined by **Marcinčák et al. (2004)**. Absorbance of samples was measured on UV-VIS spectrophotometer T80 (PG Limeted Instruments, UK) at a wavelength of 532 nm, the translation results on the amount of MDA in 1 kg samples.

Results of the experiment were evaluated by statistical program SAS 9.3 with using application Enterprise Guide 4.2. The variation-statistical values (mean, standard deviation) were calculated and to determine the significant difference between groups was used variance analyse.

RESULTS AND DISCUSSION

Jo et al. (2006) stated that oxidation of lipids can have significant impact to meat industry. Meat containing unsaturated fatty acids is very sensitive to lipid oxidation especially during storage, because polyunsaturated fatty acid esters are easily oxidized by molecular oxygen. This kind of oxidation is called autoxidation and proceeds by a free radical chain mechanism **(Brewer, 2011)**.

The results of the oxidation stability of fresh chicken breast muscles of chicken Cobb 500 after application EDTA and plant essential oils (*Salvia officinalis* L. and *Rosmarinus officinalis* L.) during 16 days storage at 4 °C are shown in Table 1 and Figure 1.

The higher average value of MDA measured in breast muscle in 0 day of experiment was in samples of vacuum-packaged chicken breasts group with *Rosmarinus officinalis* L. oil 2.0% v/w group A4 (0.026 $mg.kg^{-1}$) compared to experimental groups A1 (0.022 $mg.kg^{-1}$), A2 (0.023 $mg.kg^{-1}$), A3 (0.024 $mg.kg^{-1}$) and air-packaged control group (0.024 $mg.kg^{-1}$). We have not found statistically significant differences between testing groups chicken breasts. During chilled storage of the breast muscles were noticed increased content of malondialdehyde in comparison to the first day of storage.

On the fourth day of storage were measured below the values of malondialdehyde in all experimental groups (0.028 $mg.kg^{-1}$ in group A2, 0.030 $mg.kg^{-1}$ in group A3, 0.034 $mg.kg^{-1}$ in group A4, and 0.036 $mg.kg^{-1}$ – group A1) opposite control group C (0.182 $mg.kg^{-1}$). We have found statistically significant differences ($p \leq 0.05$) between control group C and all tested groups.

A similar tendency of improving the oxidation stability after eight days of refrigerate storage in the breast muscle of hybrid combination Cobb 500 we found in the experimental groups (0.031 $mg.kg^{-1}$ – A3, A4 to 0.048 $mg.kg^{-1}$ - A1) compared with control group C (0.191 $mg.kg^{-1}$).

After 12 days of breast muscle storage was statistic significantly ($p \leq 0.05$) improved the oxidative stability of all test groups chicken breasts (0.033 $mg.kg^{-1}$ – A4 to 0.055 $mg.kg^{-1}$ – A1) compared to the control group C (0.229 $mg.kg^{-1}$). We have found statistically significant differences ($p \leq 0.05$) between control group C and tested groups, between group A1 and A2, A4 and between tested group A2 and groups A3, A4.

During testing period of chilled storage were higher values of malondialdehyde measured in control group C

Table 1 Effect of sage and rosemary essential oils on the concentration of MDA ($mg.kg^{-1}$) in breast muscle (mean ±*SD*) (n = 5).

Day	C	A1	A2	A3	A4
0	0.024 ±0.006	0.022 ±0.007	0.023 ±0.006	0.024 ±0.005	0.026 ±0.007
4	0.182 ±0.007^{a}	0.036 ±0.004^{b}	0.028 ±0.007^{b}	0.030 ±0.004^{b}	0.034 ±0.004^{b}
8	0.191 ±0.006^{a}	0.048 ±0.005^{b}	0.044 ±0.007^{b}	0.031 ±0.008^{c}	0.031 ±0.007^{c}
12	0.229 ±0.019^{a}	0.055±0.006^{b}	0.043 ±0.005^{c}	0.037 ±0.009^{cd}	0.033 ±0.005^{d}
16	0.396 ±0.027^{a}	0.060 ±0.005^{b}	0.052 ±0.004^{c}	0.042 ±0.004^{d}	0.041 ±0.005^{d}

Note: C - air-packaged control group; A1 - vacuum-packaged control group; A2 - vacuum-packaged control samples with EDTA solution 1.50% w/w; A3 - vacuum-packaged experimental group with *Salvia officinalis* L. oil 2.0% v/w; A4 - vacuum-packaged experimental group with *Rosmarinus officinalis* L. oil 2.0% v/w. Mean values in the same lines with different superscripts (a, b, c) are significantly different at $p \leq 0.05$ level.

compare to experimental groups. The higher average value of MDA measured in breast muscle of broiler chickens Cobb 500 was in samples of control group C (0.396 mg.kg^{-1}) compared to experimental groups A1 (0.060 mg.kg^{-1}), A2 (0.052 mg.kg^{-1}), A3 (0.042 mg.kg^{-1}) and A4 (0.041 mg.kg^{-1}) after 16-day of chilled storage. At the end of the test period we have found statistically significant differences ($p \leq 0.05$) between all testing groups and control group of chicken breasts.

Botsoglou et al. (2007) reported that a higher concentration of antioxidants in poultry meat has the effect of reducing lipid oxidation, i.e. there is a reduction in malondialdehyde values during chilling storage. **Gong et al. (2010)** used TBARs values as an indicator of secondary lipid oxidation products, which were determined in minced breast and thigh muscles from chicken, turkey and duck during -4 °C storage. TBARs formation was slowest in minced chicken thigh, intermediate in duck thigh and fastest in turkey thigh ($p < 0.01$).

The plant essential oils such as oregano, thyme, sage etc. **(Economou et al., 1991; Yanishlieva and Marinova, 1995; Man and Jaswir, 2000)**, show positive effect on oxidation stability of lipids in meat.

In contrast to synthetic antioxidants, the use of natural antioxidants from spices is increasing since their application is less stringently regulated in most countries around the world. Active essential oil compounds in rosemary, oregano, borage and sage are for example phenolic diterpenes, derivates of hydroxycinnamic acid, flavonoides and triterpenes **(Oberdieck, 2004; Ryan et al., 2009; Sanchez-Escalante et al., 2003).** For rosemary, sage and oregano, the most active substances with a high antioxidant potential are carnosic acid, carnosol, and rosmarinic acid **(Oberdieck, 2004).**

Estévez et al. (2007) evaluated the antioxidant effect of two plant essential oils (sage and rosemary essential oils) and one synthetic antioxidant (BHT) on refrigerated stored liver pâté (4 °C/90 days). The addition of antioxidants significantly ($p \leq 0.05$) reduced the total amount of lipid-derived volatiles isolated from liver pâtés HS. Plant essential oils inhibited oxidative deterioration of liver pâtés to a higher extent than BHT did.

Fasseas et al. (2007) showed that porcine and bovine ground meat treated with the essential oils of oregano and sage (3%w/w) had increased oxidative stability and the antioxidant capacity of the raw and cooked meat (85 °C for 30 min) was high during storage at 4 °C for 12 days. They also suggested that addition of antioxidants is much more important for cooked meat products than the raw products.

Mohamed et al. (2011) reported that addition of herbal extracts of marjoram, rosemary and sage at concentration of 0.04% (v/w) to ground beef prior to irradiation (2 and 4.5 kGy) significantly lowered the TBARS values, off odour scores and increased colour and acceptability scores.

Sampaio et al. (2012) examined the effect of combinations of sage, oregano and honey on lipid oxidation in cooked chicken meat (thigh and breast) during refrigeration at 4 ±0.5 °C for 96 h as measured by TBARs numbers. The analysis of variance on the TBARs data indicated that the TBARs values were significantly affected by natural antioxidants throughout refrigeration ($p < 0.05$). Analysis their data showed that all of the three combinations of natural antioxidants tested would be beneficial for reducing the velocity of lipid oxidation in

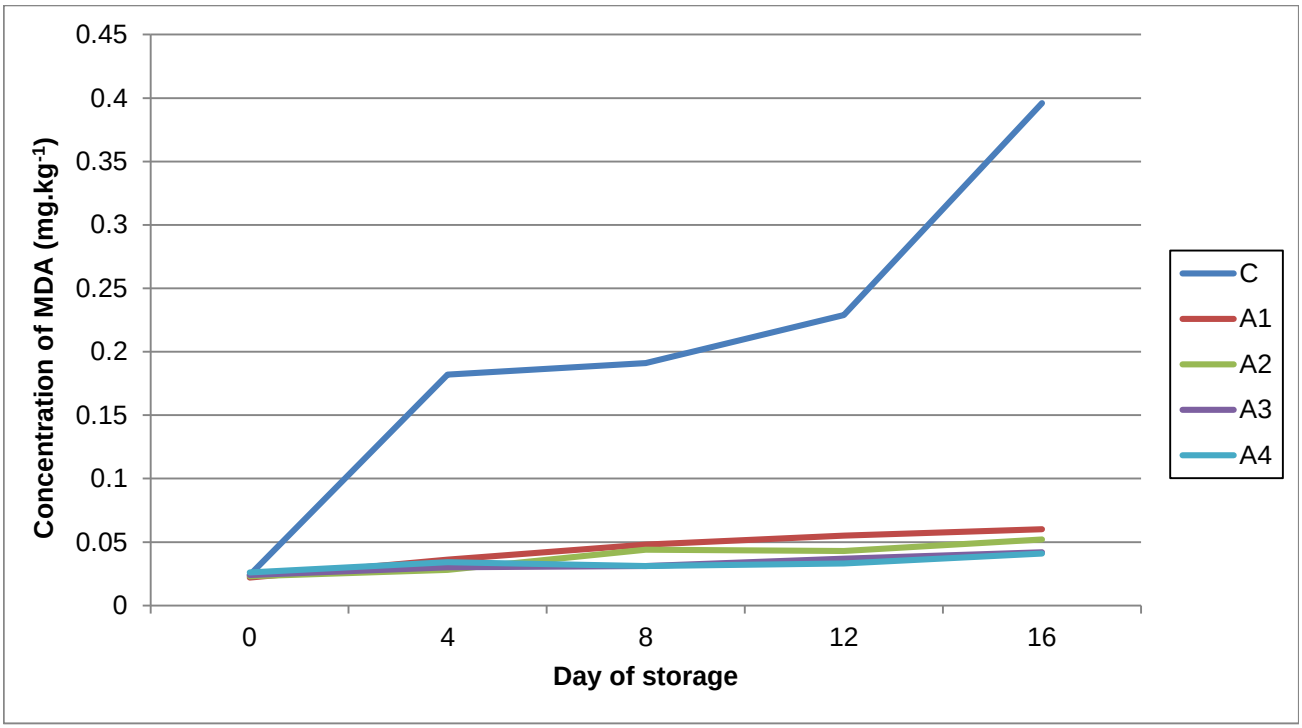

Figure 1 Concentration of MDA (mg.kg^{-1}) in breast muscle.

Note: C - air-packaged control group; A1 - vacuum-packaged control group; A2 - vacuum-packaged control samples with EDTA solution 1.50% w/w; A3 - vacuum-packaged experimental group with *Salvia officinalis* L. oil 2.0% v/w; A4 - vacuum-packaged experimental group with *Rosmarinus officinalis* L. oil 2.0% v/w.

both chicken meats during storage, what are corroborated by other authors who have added honey and herbs and thereby inhibited the development of lipid oxidation in cooked meats during refrigeration time **(McKibben and Engeseth, 2002; Juntachote et al., 2007)**.

Mohamed et al. (2011) reported that addition of herbal extracts of marjoram, rosemary and sage at concentration of 0.04% (v/w) to ground beef prior to irradiation (2 and 4.5 kGy) significantly lowered the TBARS values, off odour scores and increased colour and acceptability scores.

The effectiveness of rosemary essential oil as an inhibitor of lipid oxidation in meat products has been documented **(Esteévez and Cava, 2006; McCarthy et al., 2001; Sebranek et al., 2005)**.

Plant essential oils have been successfully introduced to inhibit oxidative deterioration of meat and fat products, this deterioration being generally referred to the accumulation of lipid-oxidation-derived products and to the generation of lipid-derived volatiles in meat products **(Ahn et al., 2002; Yu et al., 2002)**. **Formanek et al. (2001)** and **McCarthy et al. (2001)** reported the high effectiveness of antioxidants from natural resources against oxidative reactions that showed similar activity to those from synthetic origin such as BHT. **Sebranek et al. (2005)** reported similar antioxidant activities of rosemary essential oils and synthetic ones (BHT/BHA) regarding MDA generation in refrigerated sausages.

Ramos Avila et al. (2013) stated that the degradation pathways of fatty substances play one of the main causes of foods deterioration and unpleasant odours. This factor is also responsible for the loss of sensory properties.

Rhee et al. (1996) observed that raw poultry meat is less prone to lipid oxidation than beef or pork meat because of its lower iron content.

CONCLUSION

The essential oil as well essential oils from *Labiatae* herbs can be used as substitutes to chemical food additives which could prolong of shelf life of the meat and meat products. Results achieved in the experiment show that the treatment of chicken breast muscles with *Salvia officinalis* L. and *Rosmarinus officinalis* L. essential oils in concentration 0.20% v/w with combination vacuum packaging had positive effect on the decrease of oxidative processes in chicken breast muscles during chilling storage at 4 ±0.5 °C in comparative with tested groups - control air-packaged group, vacuum-packaged experimental group and vacuum-packaged experimental group with EDTA solution 1.50% w/w.

REFERENCES

Ahn, J., Grün, I. U., Fernando, L. N. 2002. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *Journal of Food Science*, vol. 67, no. 4, p. 1364-1369. http://dx.doi.org/10.1111/j.1365-2621.2002.tb10290.x

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N., Omar, A.K.M. 2013. Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, vol. 117, no. 4, p. 426-436. http://dx.doi.org/10.1016/j.jfoodeng.2013.01.014

Bobko, M., Haščík, P., Bobková, A., Pavelková, A., Tkáčová, J., Trembecká, L. 2015b. Lipid oxidation in chicken meat after application of bee pollen extract, propolis extract and probiotic in their diets. *Potravinarstvo*, vol. 9, no. 1, p. 342-346. http://dx.doi.org/10.5219/495

Bobko, M., Kročko, M., Haščík, P., Bobková, A. 2015a. Oxidative stability of chicken meat after propolis extract application in their diets. *Potravinarstvo*, vol. 9, no. 1, p. 48-52. http://dx.doi.org/10.5219/427

Botsoglou, N. A., Govaris, A., Giannenas, I., Botsoglou, E., Papapageorgiou, G. 2007. The incorporation of dehydrated rosemary leaves in therations of turkeys and their impact on the oxidative stability of the produced raw and cooked meat. *International Journal of Food Science and Technology*, vol. 58, no. 4, p. 312-320. http://dx.doi.org/10.1080/09637480701228583

Bourre, J. M. 2005. Where to find omega-3-fatty acids and how feeding animals with diet enriched in omega-3-fatty acids to increase nutritional value derived products for human: what is actually useful? *Journal of Nutrition, Health and Aging*, vol. 9, no. 4, p. 232-242.

Brewer, M. S. 2011. Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety*, vol. 10, no. 4, p. 221-247. http://dx.doi.org/10.1111/j.1541-4337.2011.00156.x

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. *International Journal of Food Microbiology*, vol. 94, no. 3, 223-253. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022

Dorman, H. J. D., Peltoketo, A., Hiltunen, R., Tikkanen, M. J. 2003. Characterisation of the antioxidant properties of deodourised aqueous extracts from selected *Lamiaceae* herbs. *Food Chemistry*, vol. 83, no. 2, p. 255-262. http://dx.doi.org/10.1016/s0308-8146(03)00088-8

Economou, K. D., Oreopoulou, V., Thomopouls, C. D. 1991. Antioxidant properties of some plant extract of the labiatae family. *JOACS*, vol. 68, no. 2, p. 109-113.

Engberg, R. M., Lauridsen, C., Jensen, S. K., Jakobsen, K. 1996. Inclusion of oxidised vegetable oil in broiler diets. Its influence on nutrient balance and on antioxidative status of broilers. *Poultry Science*, vol. 75, no. 8, p. 1003-1011. http://dx.doi.org/10.3382/ps.0751003

Estévez, M., Cava, R. 2004. Lipid and protein oxidation, release of iron from heme molecule and colour deterioration during refrigerated storage of liver pâté. *Meat Science*, vol. 68, no. 4, p. 551-558. http://dx.doi.org/10.1016/j.meatsci.2004.05.007

Estévez, M., Cava, R. 2006. Effectiveness of rosemary essential oil as an inhibitor of lipid and protein oxidation: Contradictory effects in different types of frankfurters. *Meat Science*, vol. 72, no. 2, p. 348-355. http://dx.doi.org/10.1016/j.meatsci.2005.08.005

Estévez, M., Ramírez, R., Ventanas, S., Cava, R. 2007. Sage and rosemary essential oils versus BHT for the inhibition of lipid oxidative reactions in liver pâté. *LWT*, vol. 40, no. 1, p. 58-65. http://dx.doi.org/10.1016/j.lwt.2005.07.010

Fasseas, M. K., Mountzouris, K. C., Tarantilis, P. A., Polissiou, M., Zervas, G. 2007. Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chemistry*, vol. 106, no. 3, p. 1188-1194. http://dx.doi.org/10.1016/j.foodchem.2007.11.068

Fernández-Ginés, J. M., Fernández-López, J., Sayas-Barberá, E., Pérez-Alvarez, J. A. 2005. Meat products as

functional foods: a review. *Journal of Food Science*, vol. 70, no. 2, p. R37-R43. http://dx.doi.org/10.1111/j.1365-2621.2005.tb07110.x

Formanek, Z., Kerry, J. P., Higgins, F. M., Buckley, D. J., Morrissey, P. A., Farkas, J. 2001. Addition of synthetic and natural antioxidants to a-tocopheryl acetate supplemented beef patties: Effects of antioxidants and packaging on lipid oxidation. *Meat Science*, vol. 58, no. 4, p. 337-341. http://dx.doi.org/10.1016/s0309-1740(00)00149-2

Gong, Y., Parker, R. S., Richards, M. P. 2010. Factors affecting lipid oxidation in breast and thigh muscle from chicken, turkey and duck. *Journal of Food Biochemistry*, vol. 34, no. 4, p. 869-885. http://dx.doi.org/10.1111/j.1745-4514.2010.00341.x

Grzegorczyk, I., Matkowski, A., Wysokińska, H. 2007. Antioxidant activity of extracts from in vitro cultures of Salvia officinalis L. *Food Chemistry*, vol. 104, no. 2, p. 536-541. http://dx.doi.org/10.1016/j.foodchem.2006.12.003

Chen, Z.-Y., Wong, I. Y. F., Leung, M. W. S., He, Z.-D., & Huang, Y. 2002. Characterization of antioxidants present in bitter tea (Ligustrum pedunculare). *Journal of Agricultural and Food Chemistry*, vol. 50, no. 26, p. 7530-7535. http://dx.doi.org/10.1021/jf0206421

Chouliara, E., Karatapanis, A., Savvaidis, I. N., Kontominas, M. G. 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 °C. *Food Microbiology*, vol. 24, no. 6, p. 607-617. http://dx.doi.org/10.1016/j.fm.2006.12.005

Ibanez, E., Kubatova, A., Senorans, F. J., Cavero, S., Reglero, G., Hawthorne, S. B. 2003. Subcritical water extraction of antioxidant compounds from rosemary plants. *Journal of Agricultural and Food Chemistry*, vol. 51, no. 2, p. 375-382. http://dx.doi.org/10.1021/jf025878j

Ichikawa, M., Ryu, K., Yoshida, J., Ide, N., Kodera, Y., Sasaoka, T., Rosen, R.T. 2003. Identification of six phenylpropanoids from garlic skin as major antioxidants. *Journal of Agricultural and Food Chemistry*, vol. 51, no. 25, p. 7313-7317. http://dx.doi.org/10.1021/jf034791a

Jiménez-Colmenero, F., Carballo, J., Cofrades, S. 2001. Review. Healthier meat and meat products: their role as functional foods. *Meat Science*, vol. 59, no. 1, p. 5-13. http://dx.doi.org/10.1016/s0309-1740(01)00053-5

Jo, S., Nam, K., Min, B., Ahn, D., Cho, S., Park, W. 2006. Antioxidant activity of prunus mume extract in cooked chicken breast meat. *International Journal of Food Science and Technology*, vol. 41, no. s1, p. 15-19. http://dx.doi.org/10.1111/j.1365-2621.2006.01234.x

Juntachote, T., Berghofer, E., Siebenhandl, S., Bauer, F. 2006. The antioxidative properties of Holy basil and Galangal in cooked ground pork. *Meat Science*, vol. 72, no. 3, p. 446-456. http://dx.doi.org/10.1016/j.meatsci.2005.08.009

Juntachote, T., Berghofer, E., Siebenhandl, S., Bauer, F. 2007a. Antioxidative effect of added dried Holy basil and its ethanolic extracts on susceptibility of cooked ground pork to lipid oxidation. *Food Chemistry*, vol. 100, no. 1, p. 129-135. http://dx.doi.org/10.1016/j.foodchem.2005.09.033

Juntachote, T., Berghofer, E., Siebenhandl, S., Bauer, F. 2007a. Antioxidative effect of added dried Holy basil and its ethanolic extracts on susceptibility of cooked ground pork to lipid oxidation. *Food Chemistry*, vol. 100, no. 1, p. 129-135. http://dx.doi.org/10.1016/j.foodchem.2005.09.033

Kanner, J., Hazan, B., Doll, L. 1991. Catalytic 'free' iron ions in muscle foods. *Journal of Agricultural and Food Chemistry*, vol. 36, no. 3, p. 412-415. http://dx.doi.org/10.1021/jf00081a002

Kingston, E. R., Monahan, F. J., Buckley, D. J., Lynch, P. B. 1998. Lipid oxidation in cooked pork as affected by vitamin E, cooking and storage conditions. *Journal of Food Science*, vol. 63, no. 3, p. 386-389. http://dx.doi.org/10.1111/j.1365-2621.1998.tb15748.x

Kristensen, L., Purslow, P. P. 2001. The effect of processing temperature and addition of mono- and di- valent salts on the heme–nonheme-iron ratio in meat. *Food Chemistry*, vol. 73, no. 4, p. 433-439. http://dx.doi.org/10.1016/s0308-8146(00)00319-8

Lee, M.-A., Choi, J.-H., Choi, Y.-S., Han, D.-J., Kim, H.-Y., Shim, S.-Y., Chung, H.-K., Kim, Ch.-J. 2010. The antioxidative properties of mustard leaf (*Brassica juncea*) kimchi extracts on refrigerated raw ground pork meat against lipid oxidation. *Meat Science*, vol. 84, no. 3, p. 498-504. http://dx.doi.org/10.1016/j.meatsci.2009.10.004

Liu, D. C., Tsau, R. T., Lin, Y. C., Jan, S. S., Tan, F. J. 2009. Effect of various levels of rosemary or Chinese mahogany on the quality of fresh chicken sausage during refrigerated storage. *Food Chemistry*, vol. 117, no. 1, p. 106-113. http://dx.doi.org/10.1016/j.foodchem.2009.03.083

Man, Y., Jaswir, I. 2000. Effect of rosemary and sage extracts on frying performance of refind, bleached and deodorized plam olein during deep-fat frying. *Food Chemistry*, vol. 69, no. 3, p. 301-307. http://dx.doi.org/10.1016/s0308-8146(99)00270-8

Marcinčák, S., Popelka, P., Šimková, J., Marcinčáková, D., Martonová, M. 2010. Oxidative stability of chilled chicken meat after feeding of selected plants. *Potravinarstvo*, vol. 4, no. 3, p. 46-49. http://dx.doi.org/10.5219/38

Marcinčák, S., Sokol, J., Bystrický, P., Popelka, P., Turek, P., Máté, D. 2004. Determination of lipid oxidation level in broiler meat by liquid chromatography. *Journal of AOAC International*, vol. 87, p. 1148-1152.

Mariutti, L. R. B., Orlien, V., Bragagnolo, N., Skibsted, L. H. 2008. Effect of sage and garlic on lipid oxidation in high-pressure processed chicken meat. *European Food Research and Technology*, vol. 227, no. 2, p. 337-344. http://dx.doi.org/10.1007/s00217-007-0726-5

McCarthy, T. L., Kerry, J. P., Kerry, J. F., Lynch, P. B., Buckley, D. J. 2001. Assessment of the antioxidant potential of natural food and plant extracts in fresh and previously frozen pork patties. *Meat Science*, vol. 57, no. 2, p. 177-184. http://dx.doi.org/10.1016/s0309-1740(00)00090-5

McCarthy, T. L., Kerry, J. P., Kerry, J. F., Lynch, P. B., Buckley, D. J. 2001. Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. *Meat Science*, vol. 58, no. 1, p. 45-52. http://dx.doi.org/10.1016/s0309-1740(00)00129-7

McKibben, J., Engeseth, N. J. 2002. Honey as a protective agent against lipid oxidation in ground turkey. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 3, p. 592-595. http://dx.doi.org/10.1021/jf010820a

Mielnick, M. B., Olsen, E., Vogt, G., Adeline, D., Skrede, G. 2006. Grape seed extract as antioxidant in cooked, cold stored turkey meat. *LWT Food Science and Technology*, vol. 39, no. 3, p. 191-198. http://dx.doi.org/10.1016/j.lwt.2005.02.003

Mihajilov-Krstev, T., Radnović, D., Kitić, D., Zlatković, B., Ristić, M., Branković, S. 2009. Chemical composition and antimicrobial activity of *Satureja hortensis* L. essential oil.

Open Life Sciences, vol. 4, no. 3, p. 411-416. http://dx.doi.org/10.2478/s11535-009-0027-z

Mohamed, H. M., Mansour, H. A., Farag, M. D. 2011. The use of natural herbal extracts for improving the lipid stability and sensory characteristics of irradiated ground beef. *Meat Science*, vol. 87, no. 1, p. 33-39. http://dx.doi.org/10.1016/j.meatsci.2010.06.026

Nam, K. C., Ahn, D. U. 2003. Use of antioxidants to reduce lipid oxidationand off-odor volatiles of irradiated pork homogenates and patties. *Meat Science*, vol. 63, no. 1, p. 1-8. http://dx.doi.org/10.1016/s0309-1740(02)00043-8

Naveena, B. M., Sen, A. R., Vaithiyanathan, S., Babji, Y., Kondaiah, N. 2008. Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Meat Science*, vol. 80, no. 4, p. 304-308. http://dx.doi.org/10.1016/j.meatsci.2008.06.005

Oberdieck, R. 2004. Fat protection for foods, especially meat products. *Fleischwirtschaft*, vol. 84, no. 10, p. 91−95.

Ramos Avila, F., Pro-Martínez, A., Sosa-Montes. E., Cuca-García,. J. M., Becerril-Pérez, C., Figueroa-Velasco, J. L., Ruiz-Feria, C. A., Hernández-Cázares, A. S., Narciso-Gaytán, C. 2013. Dietary supplemented and meat-added antioxidants effect on the lipid oxidative stability of refrigerated and frozen cooked chicken meat. *Poultry Science*, vol. 92, no. 1, p. 243-249. http://dx.doi.org/10.3382/ps.2012-02409

Rhee K. S., Anderson L. M., Sams A. R. 1996. Lipid oxidation potential of beef chicken, and pork. *Journal of Food Scince*, vol. 61, no. 1, p. 8-12. http://dx.doi.org/10.1111/j.1365-2621.1996.tb14714.x

Rodriguez-Rojo, S., Visentin, A., Maestri, D., Cocero, M. J. 2012. Assisted extraction of rosemary antioxidants with green solvents. *Journal of Food Engineering*, vol. 109, no. 1, p. 98-103. http://dx.doi.org/10.1016/j.jfoodeng.2011.09.029

Ryan, E., Aherne, S. A., O'Grady, M. N., McGovern, L., Kerry, J. P., O'Brien, N. M. 2009. Bioactivity of herb-enriched beef patties. *Journal of Medicinal Food*, vol. 12, no. 4, p. 893−901. http://dx.doi.org/10.1089/jmf.2008.0069

Rzepka, M., Ozogul, F., Surowka, K., Michalczyk, M. 2013. Freshness and quality attributes of cold stored Atlantic bonito (*Sarda sarda*) gravad. *International Journal of Food Science and Technology*, vol. 48, no. 6, p. 1318-1326. http://dx.doi.org/10.1111/ijfs.12094

Sampaio, G. R., Saldanha, T., Soares, R. A. M., Torres, E. A. F. S. 2012. Effect of natural antioxidant combinations on lipid oxidation in cooked chicken meat during refrigerated storage. *Food Chemistry*, vol. 135, no. 3, p. 1383-1390. http://dx.doi.org/10.1016/j.foodchem.2012.05.103

Sanchez-Escalante, A., Djenane, D., Torrescano, G., Beltran, J. A., Roncales, P. 2003. Antioxidant action of borage, rosemary, oregano, and ascorbic acid in beef patties packaged in modified atmosphere. *Journal of Food Science*, vol. 68, no. 1, p. 339-344. http://dx.doi.org/10.1111/j.1365-2621.2003.tb14162.x

Sasse, A., Colindres, P., Brewer, M. S. 2009. Effect of natural and synthetic antioxidants on the oxidative stability of cooked, frozen pork patties. *Journal of Food Science*, vol. 74, no. 1, S30-S35. http://dx.doi.org/10.1111/j.1750-3841.2008.00979.x

Sebranek, J. G., Sewalt, V. J. H., Robbins, K. L., Houser, T. A. 2005. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. *Meat Science*, vol. 69, no. 2, p. 289-296. http://dx.doi.org/10.1016/j.meatsci.2004.07.010

Shan, B., Cai, Y. Z., Sun, M., Corke, H. 2005. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, vol. 53, no. 20, p. 7749-7759. http://dx.doi.org/10.1021/jf051513y

Skandamis, P., Tsigarida, E., Nychas, G.-J. E. 2002. The effect of oregano essential oil on survival/death of Salmonella typhimuriumin meat stored at 5 °C under aerobic, VP/MAP conditions. *Food Microbiology*, vol. 19, no. 1, p. 97-103. http://dx.doi.org/10.1006/fmic.2001.0447

Tanabe, H., Yoshida, M., Tomita, N. 2002. Comparison of the antioxidant activities of 22 commonly used culinary herbs and spices on the lipid oxidation of pork meat. *Animal Science Journal*, vol. 73, no. 5, p. 389-393. http://dx.doi.org/10.1046/j.1344-3941.2002.00054.x

Tang, S., Kerry, J. P., Sheehan, D., Buckley, D. J., Morrissey, P. A. 2001. Antioxidative effect of added tea catechins on susceptibility of cooked red meat, poultry and fish patties to lipid oxidation. *Food Research International*, vol. 34, no. 8, p. 651-657. http://dx.doi.org/10.1016/s0963-9969(00)00190-3

Tepe, B., Sokmen, M., Akpulat, H. A., Sokmen, A. 2006. Screening of the antioxidant potentials of six Salvia species from Turkey. *Food Chemistry*, vol. 95, no. 2, p. 200-204. http://dx.doi.org/10.1016/j.foodchem.2004.12.031

Tkáčová, J., Angelovičová, M., Haščík, P., Bobko, M. 2015. Oxidative stability of chicken meat during storage influenced by the feeding of alfalfa meal. *Potravinarstvo*, vol. 9, no. 1, p. 106-111. http://dx.doi.org/10.5219/444

Van de Braak, S. A. A. J, Leijten, G. C. J. J. 1999. Essential Oils and Oleoresins: A survey in the Netherlands and other major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam. p. 116.

Viuda-Martos, M., Mohamady, M. A., Fernández-López, J., Abd ElRazik, K. A., Omer, E. A., Pérez-Alvarez, J. A., Sendra, E. 2011. *In vitro* antioxidant and antibacterial activities of essentials oils obtained from Egyptian aromatic plants. *Food Control*, vol. 22, no. 11, p. 1715-1722. http://dx.doi.org/10.1016/j.foodcont.2011.04.003

Wang, L., Wang, Z., Li, X. 2013. Preliminary phytochemical and biological activities study of solvent extracts from a cold-field fruit (Malus baccata Linn) Borkh. *Industrial Crops and Products*, vol. 47, p. 20-28. http://dx.doi.org/10.1016/j.indcrop.2013.02.029

Yanishhlieva, V., Marinova, M. 1995. Antioxidant activity of selected species of the family Lamiaceae grown in Bulgaria. *Food / Nahrung*, vol. 39, no. 5-6, p. 458-463. http://dx.doi.org/10.1002/food.19950390510

Yanishlieva, N. V., Marinova, E., Pokorny, J. 2006. Natural antioxidants from herbs and spices. *European Journal of Lipid Science and Technology*, vol. 108, no. 9, p. 776-793. http://dx.doi.org/10.1002/ejlt.200600127

Yu, L., Scanlin, L., Wilson, J., Schmidt, G. 2002. Rosemary extracts as inhibitors of lipid oxidation and color change in cooked turkey products during refrigerated storage. *Journal of Food Science*, vol. 67, no. 2, p. 582-585. http://dx.doi.org/10.1111/j.1365-2621.2002.tb10642.x

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THE ROLE OF COLOR SORTING MACHINE IN REDUCING FOOD SAFETY RISKS

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ABSTRACT

It is the very difficult problem how we can decrease food safety risks in the product, which was polluted in process of cropping. According to professional literature almost the prevention is considered as an exclusive method to keep below safe level the content of DON toxin. The source of food safety in food chain is that the primary products suit the food safety requirements. It is a very difficult or sometimes it is not possible to correct food safety risk factors - which got into the products during cultivation - in the course of processing. Such factor is fusariotoxin in fodder and bread wheat. DON toxin is the most frequent toxin in cereals. The objective of the searching was to investigate, if it is possible to decrease DON toxin content of durum wheat and to minimize the food safety risk by application milling technology with good production practice and technological conditions. The samples were taken in the first phase of milling technology just before and after color sorting. According to measuring results Sortex Z+ optical sorting decreased DON toxin content of wheat. This mean that the food safety risks can be reduced by Sortex Z+ optical sorting machine. Our experiments proved if there is color sorting in the cleaning process preceding the milling of wheat then a part of the grain of wheat infected by Fusarium sp. can be selected. This improves the food safety parameters of given lot of wheat and decrease the toxin content. The flour made from contaminated grains of wheat can be a serious food safety risk. We would like to support scientifically the technical development of milling technology with our experimental data.

Keywords: food safety; DON toxin; fusarium; wheat; *Triticum durum*

INTRODUCTION

During the course of wheat process, we have to make efforts to minimize toxin content of wheat before milling, but at the least to keep below allowable limits.

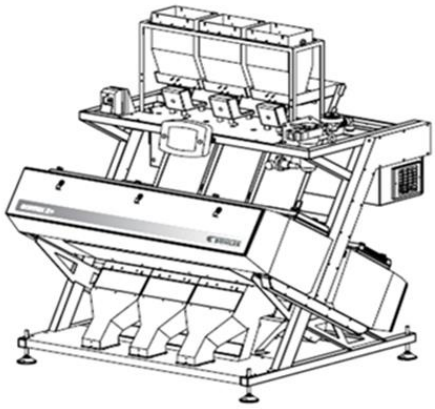

Figure 1 Sketch of Sortex Z+ optical sorter.
(Source: Sortex Z operator's manual).

Basic requirement is that these products don't contain microbiological, chemical and other contaminations, or at least not more than the maximum allowable limits **(Commission regulation (EC) No 1881, 2006)**. Products made of cereals, such as flour, bread and bakery products are classifiable as basic foods, but we consume lot of pasta and cake also. Large-scale consumption of basic foods is a feature of adult and children population equally. At these products, the fundamental importance to use primary materials which are free from biological and chemical pollutants is unambiguous.

The mycotoxins in food are secondary metabolic products of moulds, which have strong toxic effect. These can cause heavy complications in human and animal organizations, can result illness shorter or longer time, and can cause lasting damage. The species belong to Fusarium family produce significant quantity of toxin, which contain several fuzariotoxin. The Fusarium species are parasites on several cultivated plants. In most cases they infect the cereals. So they might cause significant damage both in plant cultivation and animal husbandry as well as there might be considerable human-health consequences. **(Mesterházy, 2015; Summerell at al., 2010; Stanic at al., 2015; Leslie and Logrieco, 2014; Remža at al., 2011; Adam at al., 2002)**. Deoxinivalenol, otherwise vomitoxin became known as DON toxin, is frequent representative of fusariotoxins. This toxin can be found in wheat very often according to **Mesterházy (2007)**. This toxin might be present both in cereals and in processed cereal products, so it is important from the point of food safety **(Hrubošová, 2015)**.

Contrary to data of literature we started from the hypothesis, that nowadays toxin content can be decreased

after harvesting and storing also by application of modern milling industry equipments and technical conditions.

Our first investigations were focused on toxin decreasing effect of Sortex Z+ color sorting machine (Figure 1). This is a new, more precise, quicker technology with less loss in milling processing. It's possible to select the components which size are similar to unbroken, health grain, but they are optically different. The sorting machines applied previously were not satisfactorily efficient to select components which brought down the quality. The manufacturer offers the application of color sorting machine Sortex Z+ as the alternative of the mechanical cleaning equipments. But it possible to gain more efficient sorting than at the mechanical cleaners, and the result is cleaner, contaminants-free product. Color sorting is not yet generally applied in the milling technology. As it was mentioned the application of Sortex Z+ makes possible not just to remove the physical dirt but the grains with different color can be selected also. It is known the color of infected grains can change depending on characteristic and time of infection.

Meteorological factors have significant influence on development of fusarium infection and on degree of toxin content also. The weather conditions are risk factors which can't be influenced during the wheat growing **(Commission recommendation, 2006)**. The prevention against these factors maybe the usage of resistant species. But it is known the species-structure has not changed in the last years in this regard. This means we have to calculate with fusarium infection in the following periods also, especially afterward rainy early summer weather. The degree and the characteristic of fusarium infection depends in which phenology phases was the wheat contaminated. This determines whether just the seed-coat is infected, or the endosperm also. If the weather is favourable for fusarium infection after the fertilization and at the beginning of development of cereal grains, then the rate of cereal grains with fungi in the endosperm is higher. This case the color of grains changes. It becomes bright, primarily greyish-white, but lilac or pinky color might occur also. The color change is very important in the respect of our investigation. In case of early infection a part of wheat grains are smaller and their texture are softer, in parallel with the above mentioned characteristics. As far as the weather becomes rainy in more advanced status of wheat that is at the beginning of the full ripening, then the infection of fungi represents lower rate in the inner part of wheat grain, and the center of infection develops in the layers of seed-coat. In cases of that kind the change of grain color is less typical and the size of grain is not considerably smaller. But the texture of infected grains are softer than the healthy ones. At the end of full ripening the infected seeds barely differ from healthy ones and the inner part of grains remains intact. Accidently mycelium on surface or slight discoloration indicates the infection.

The experimental results of **Hrubošová (2015)** clearly confirm the above mentioned process. The analysis of infected lots of wheat proved there is no correlation between internal and external infection that is they develop independently from each other. Based on the above mentioned results it is difficult to remove the infected seeds from wheat lots after harvest period. It is not possible to decrease the toxin content reliably and efficiently by simple cleaning, selection process. Therefore, scientific literature assumes that the opportunity of decreasing mycotoxin content and thereby the food safety risk is very restricted and uncertain during processing.

Font et al., (2013) implemented model research to decrease DON toxin content, when they created and built machineries in laboratory which were operated similarly to surface cleaning treatment applied in the milling process. The starting hypothesis was that majority of toxin concentrates in the coat and the germ of the wheat. They evaluated the test of a small number of samples which was taken from a given lot of wheat. Their results are important because they proved it is possible to decrease the toxin content of wheat by application certain surface cleaning methods. However, we shouldn't ignore that the degree and characteristic of fusarium infection depends in which phenology phases was the wheat contaminated. This determines whether just the coat and the germ infected or the endosperm affected also. But it shouldn't be ignored that the cleaning treatment and its efficiency is different in mill industrial and in laboratory circumstances due to blending of raw material lots and different processes. Conversely it is very important from food safety aspect to get information not only about theoretical possibilities, but about efficiency of process which actually takes place.

Presumable beyond removing physical dirt the quantity of mycotoxin being the cause of chemical danger can be decreased also. We try to prove the rightness of our hypothesis by our experiments and data.

Toxin-test of wheat harvested in 2013 indicated that the color sorting of grain resulted decrease of toxin content. But the other hand the investigation of the degree of decrease didn't resulted correlation between toxin content before and after color sorting **(Kecskés-Nagy et al., 2015)**. The reason for this is that toxin concentration can be different in the internal layers of the grain depending on the characteristic of the Fusarium infection **(Veha et al., 2015)**. This is why we continued our experiment. We tested wheat samples harvested in 2014 to prove it is possible to decrease toxin level in the mill technology under different ecological circumstances by operation of modern equipment like Sortex Z+ color sorter.

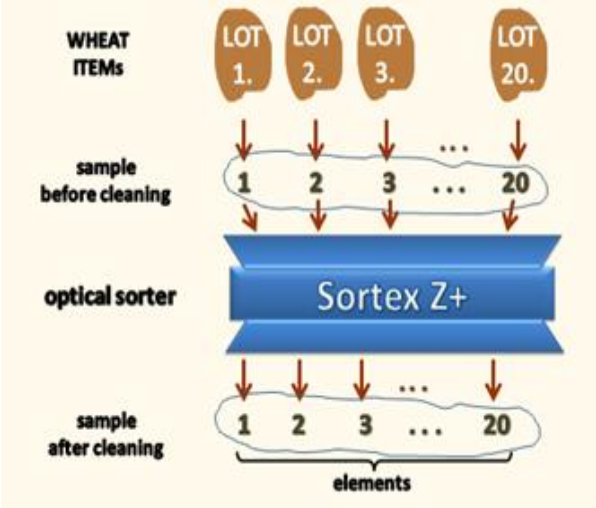

Figure 2 The method of sampling.

MATERIAL AND METHODOLOGY

We carried out the experiment at Júlia Malom Ltd. and investigated the DON toxin content of durum wheat (*Triticum durum*) before and after the cleaning process in the course of milling.

Durum wheat which was the subject of our investigation is cultivated among the wheat species on the second largest territory in the world. It is particularly popular in the mediterranean region. The flour made from hard, glassy wheat grains is used primarily by pasta industry but Sicilians bake bread from that also. Its nutritional value is better than that of *T. aestivum*, which is widely grown in our area. Its consumption is advantageous because of its beta-carotene content, amino acid and protein composition, slowly resorbable carbohydrate content. Owing to its beta-carotene and protein content, pasta can be produced from durum wheat without eggs. But growing experiences indicate that durum wheat is more sensitive to Fusarium infection than other wheat species.

The samples were taken in the first phase of milling technology just before and after color sorting. The time of sampling was settled in accordance with performance of Sortex Z+ machine. This method ensured the test same samples before and after sorting.

We investigated DON toxin content of 20 samples during the experiment. The samples taken before sorting by Sortex Z indicate the initial toxin content of investigated wheat (Figure 2). After sorting the mycotoxin decreasing efficiency of the process can be evaluated by means of analytical results of relevant samples.

Toxin analysis was carried out in own laboratory of Júlia Malom Ltd with AgraQuant Deoxinyvalenol test kit.

The evaluation was made by hypothesis analysis. The elements of two samples came in pairs (before and after sorting element in a pair) from the same lot of wheat, and DON toxin content was tested in each element. Thus the lot and the elements of samples are not independent from each other from mathematical respect. We applied „one-sample T-test" to the statistical analysis, in which the difference of two values that is difference (d_i - t) was ordered to the element.

We used „null-hypothesis" at 5% significance level to answer whether the difference between DON values before and after color sorting under same condition is negligible.

From mathematical aspects we can investigate in four logical steps whether the hypothesis is correct or it should be rejected. Values of t-probe function were calculated with MS Excel software, thus we investigated the rightness of null-hypothesis in two steps. (The last three steps were drawn together.)

First step:

We analyzed fulfillment of precondition to carrying out pared t-probe According to our assumption the distribution of population is normal. The results of measuring was completed with equipment in practice and employed chemical and physical rules. Thus they fulfilled the precondition of normality.

Mathematically we should verify normality with so-called $\chi 2$-probe, but owing to number of data (n = 20 <50) this wouldn't be exact. Presumably if we would make quite a number measurement, we would experience that DON values and their differences have normal distribution.

Second (drawn together) step:

We defined the value of „t-probe function" with Excel program. The mathematical basis of calculation can be described by the following equations:

$$t = \frac{\bar{d}}{s_{\bar{d}}}, \text{ where:}$$

$$\bar{d} = \frac{\sum_{i=1}^{n} d_i}{n} \quad \text{and} \quad s_{\bar{d}} = \sqrt{\frac{\sum_{i=1}^{n}(d_i - \bar{d})^2}{n(n-1)}}$$

Thereafter we compared the values of t-probe belonging to the relevant degree of freedom (which can be seen in the Excel table) with t values of probe function. That was the basis for acception or rejection the null-hypothesis.

RESULTS AND DISCUSSION

Data of initial DON toxin content of wheat can be seen in the Figure 3, where the elements of sample arranged by size. The figure indicates well that in the case of tested elements Sortex Z color sorting decreased DON toxin content of wheat. But there is big difference between degree of decrease if we examine the individual elements.

The hypothesis analysis is necessary because the effectiveness of sorting must be proved undoubtedly. We have to clearly express, the decrease is not owing to chance.

Drawn up the starting point: there are tandem samples with „n" elements and it is supposed those come from population with normal distribution. The arithmetic mean and standard deviation isn't known. Toxin data of samples before cleaning is indicated by „x", and data of cleaned wheat samples by „y". Namely:

Elements of wheat samples before cleaning (X): x_i

Elements of cleaned wheat samples (Y): y_i

where i= 1,…, n

As it was above-mentioned according to arrangement of research samples data that belonging together were analyzed by paired t-probe. The average of data before cleaning indicated μ_1, and standard deviation σ_1. Accordingly with this logic the average of cleaned wheat samples is μ_2, and standard deviation is σ_2.

The hypothesis are following:

H0 = null-hypothesis when there is no significant difference between theoretical mean of two samples. That is

$$\mu_1 = \mu_2$$

H1= according to alternative hypothesis theoretical average of samples before mean is significantly higher than the average of sample after cleaning

$$\mu_1 > \mu_2$$

In our case the unilateral alternative hypothesis has sense. Figure 3 clearly presents the color sorting have an influence on decreasing of DON toxin content of wheat.

The Table 1 demonstrates the critical value of Student's

t- distribution at 5% significance level less than calculated value. That is null-hypothesis should be rejected, because theoretical means of two samples present significant difference. That is

$$\mu_1 \neq \mu_2$$

So it can be stated the selection by color proved to be effective in certain circumstances, at 95% probability level. Results were not induced by chance.

In the matter of food safety questions it is worth to examine null-hypothesis at lower significant level also. Data of the table displays, that

$$\alpha=0.0005 \rightarrow t=3.883$$

That is the effect of treatment is justifiable at higher probability level also, and the null-hypothesis can be rejected.

Similarly to examination of wheat samples harvested in 2013, we proved the efficiency of color sorting on decreasing of DON toxin content of wheat in samples cultivated in 2014. The plan of the experiment was started from the results of **Veres and Borbély (2007)** and **Kótai et al., (2012)**. They didn't find correlation between external and internal infection of grains, respectively toxin content. Depending on the characteristic of the infection the toxin concentration might be different in the grain **(Mesterházy, 1995)**. Thus we got to the color sorting method. Using this process principally those grains can be selected, which were infected in early stage of grain development. We come to the conclusion from the examination of results, that color sorting of wheat has statistically verifiable effect on the reduction of toxin content. That is the effect of cleaning is provable on 95% probability level. In this way our experiments proved that the toxin content of wheat can be efficiently decreased by application of modern machinery during the processing. Since this is a food safety question, it is important to clarify the results to what extent can be considered stable and repeatable depending on the different weather conditions of the years. We didn't get unambiguous answer to this. Further investigations are required to determine the extent of correlation between different initial mycotoxin content of wheat before sorting and efficiency of cleaning. Although the efficiency of a Sortex Z+ color sorting machine was justifiable in two very different years with different weather and infection circumstances, but the degree of decreasing of risk is can not be predetermined, because it depends on characteristic of fusarium infection. The grains infected in the full ripening period can not be selected by color sorting, because they are not discolored. In this case DON toxin accumulates in the wheat coat, which becomes bran during the processing. **Sándor et al., (2010)** and **Frank (2010)** investigated the efficiency of traditional surface cleaning in model experiments. Although they experienced different results, these methods had influence on decreasing of DON toxin content. For that very reason we plan to examine a modern surface cleaning method further on. The surface of grain can be cleaned by intense scrubbing machine with good

Table 1 Two sample paired t-probe for probable value.

	DON value before cleaning	DON value after cleaning
Experted value	0.6005	0.5320
Variance	0.0824366	0.084154
Observations	20	20
df	19	
t value	8.2097227	
P(T <=t) unpaired	5.694E-08	
t critical unpaired	1.7291328	
P(T <=t) paired	1.139E-07	
t critical paired	2.093024	

Note: Table style: Top and bottom border lines 1.5 point, other lines 1 point.

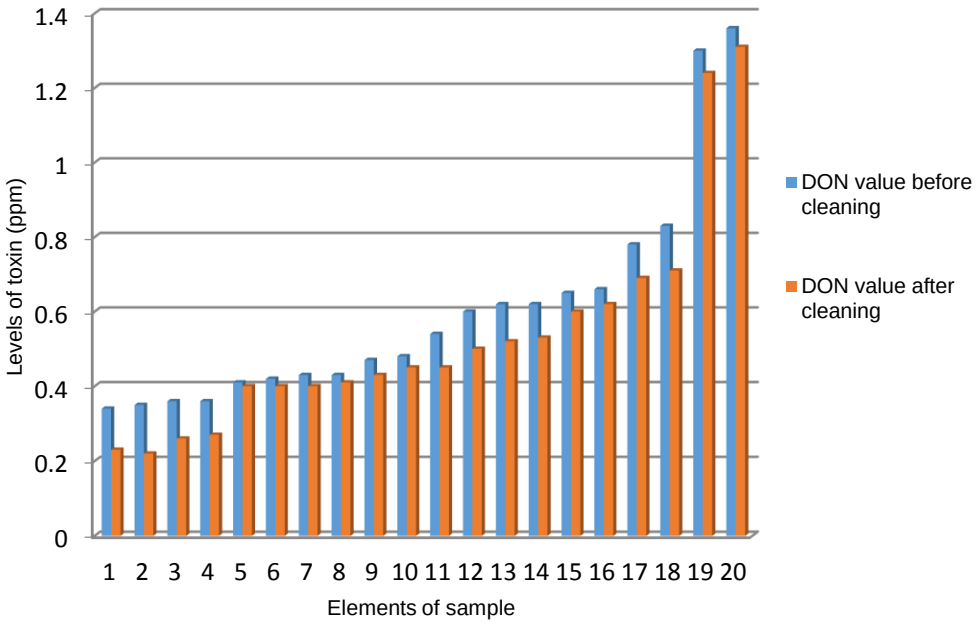

Figure 3 DON-toxin content of wheat before and after cleaning.

efficiency. Júlia Malom Ltd. applies Schule Verticone VPC 480 intense surface cleaner, which is a modern machine. In the next phase of experiment we will examine the efficiency of DON toxin content by application this machine.

CONCLUSION

Requirements of good manufacturing practice (GMP) and good hygiene practice (GHP) must be followed with attention and must be kept in mill industrial production also. On the one hand this means just as instruments and machinery can be applied in production which comply with these requirements. On the other hand it must be kept in mind that by the application of proper machinery in technological process enable to keep the regulation and to decrease risks. For the latter it is good example the opportunity of decreasing of DON toxin during processing. During our research work we prove by experiments, that by applying adequate instruments and machinery the degree of food safety risk can be decreased and the requirements of good manufacturing practice can be fulfilled. It can be summarized, that application of adequate technical equipment contributes to fulfil food safety requirements on a higher level in the course of food processing. According to the results of the experiment the application of Sortex Z+ color sorting can be suggested in the milling industry.

REFERENCES

Adam, A., Lopez, W. L., Tressol, J., Leuillet, M., Demigné, Ch., Rémésy, Ch. 2002. Impact of Whole Wheat Flour and Its Milling Fractions on the Cecal Fermentations and the Plasma and Liver Lipids in Rats, *Journal of Agricurtural snd Food Chemistry*, vol. 50, no. 22, p. 6557-6562. http://dx.doi.org/10.1021/jf020418b

Commission recommendation (EC) of 17 August 2006 on the prevention and reduction of Fusarium toxins in cereals and cereal products. Oj L 234, 29.8.2006, p 35-40.

Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.20060, p. 5-24.

Font, G., Rodríguez-Carrasco, Y., Moltó, J. C., Berrada, H., 2013. Determination of deoxynivalenol in wheat-based snacks by gas chromatography-triple quadrupole tandem mass spectrometry. *Revista de Toxicología*, vol. 30, p. 198-202.

Frank, P. 2010. Technológiai kísérletek a búza fuzárium toxin szennyezettségének csökkentésére. *Élelmiszer Tudomány Technológia*, vol. 64, no. 2, p. 16-20.

Hrubošová, D., Vytřasová, J., Brožková, I. 2015. Production of T-2 Toxin and Deoxynivalenol in the Presence of Different Disinfectants, *Potravinarstvo*, vol. 9, no. 1, p. 18-23. http://dx.doi.org/10.5219/417

Kecskésné, N. E., Korzenszky, P., Sembery, P. 2015. Reduction of toxin content of *Triticum durum* in the milling process. *Review on agriculture and rural development*, vol. 4, no. 1, p. 11-16.

Kótai, Cs., Lehoczki-Krsjak, Sz., Mesterházy Á., Varga M. 2012. Kalászfuzárium és más betegségek elleni védekezés búzában, egy új megközelítés. *Agro Napló*, vol. 16. p. 49-54.

Leslie, F. L., Logrieco, F. A. 2014. *Mycotoxin Reduction in Grain Chains*. John Wiley & Sons, ISBN 978-0-8138-2083-5, p. 376. http://dx.doi.org/10.1002/9781118832790

Mesterházy, Á. 1995. Types and components of resistance to Fusarium head blight of wheat. *Plant Breeding*, vol. 114, no. 5, p. 377-386. http://dx.doi.org/10.1111/j.1439-0523.1995.tb00816.x

Mesterházy, A., Lehoczki-Krsjak, S., Varga, M., Szabó-Hevér, Á., Tóth, B., Lemmens, M. 2015. Breeding for FHB Resistance via Fusarium Damaged Kernels and Deoxynivalenol Accumulation as Well as Inoculation Methods in Winter Wheat. *Agricultural Sciences*, vol. 6, p. 970-1002. http://dx.doi.org/10.4236/as.2015.69094

Remža, J., Bíreš, J., Matúšová, M., Lacko-Bartošová, M., 2011. Official Controls of Foodstuffs – Contamination of Cereals by Mycotoxins of the Genus Fusarium and Ochratoxin A. *Potravinarstvo*, vol. 5, no. 2, p. 63-66. http://dx.doi.org/10.5219/139

Sándor, M., Győri, Z., Sípos, P. 2010. Malomipari lehetőségek értékelése az őszi búza mikotoxin szennyezettségének csökkentésében. *Élelmiszer Tudomány Technológia*, vol. 64, no. 2. p. 4-9.

Stanic, A., Uhlig, S., Solhaug, A., Rise, F., Wilkins L. A., Miles O. Ch. 2015. Nucleophilic Addition of Thiols to Deoxynivalenol. *Journal of Agricultural and Food Chemistry*, vol. 63, no. 34, p. 7556-7566. http://dx.doi.org/10.1021/acs.jafc.5b02864 PMid:26242781

Summerell, B., Laurence, H. M., Liew, C. Y. E., Leslie F. J. 2010. Biogeography and phylogeography of Fusarium: a review. *Fungal Diversity*, vol. 44, no. 1, p. 3-13. http://dx.doi.org/10.1007/s13225-010-0060-2

Véha, A., Szabó, P., Bartók, T., Gyimes, E. 2015. Milling technological experiments to reduce Fusarium toxin contamination in wheat, *Acta Universitatis Sapientiae Alimentaria*, vol. 8, no 1, p. 55-62.

Veres, E., Borbély, M. 2007. Az őszi búza felhasználhatósága a vizuális és mikrobiológiai Fusarium fertőzöttség-, valamint a toxin vizsgálatok alapján. *Agrártudományi Közlemények*, vol. 12, p. 26-34.

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Péter Semberyy, Szent István University, Faculty of Mechanical Engineering, Institut of Process Engineering, Department of Measurement Technic, 2100 Gödöllő Páter K. út 1. Hungary, E-mail: Sembery.Peter@gek.szie.hu.

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INDUSTRIALLY PROCESSED OILSEED RAPE IN THE PRODUCTION OF TABLE EGGS

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ABSTRACT

The purpose of this study was to investigate the effectiveness of feed mixtures with varying proportions of rape cakes to the weight of table eggs, its components, thickness and strength of egg shell. The eggs were from the final laying hybrid ISA Brown reared in the enriched cage system under experimental conditions. An age of laying hens was from 48 to 54 weeks. Egg weight and its components were measured on scales type KERN 440-35N, with an accuracy of 0.01 g and a maximum weight of 400 g. Egg white weight was calculated. The thickness and strength of the egg shell were measured from the dried samples at 55 °C. From each egg shell were cut 3 pcs of samples in the equatorial plane, one sample from the blunt end and one sample from the sharp end. Egg shell thickness was measured by test instrument SOME, type 60/0.01mm with a range of 0 – 10 mm. Egg shell strength was measured according to test instrument Instron with the small body, having a diameter 4.48 mm to exert pressure on the egg shell. The obtained data were assessed in the program system SAS, version 8.2. Based on the results observed in egg weight of our experiment we can conclude that in the group with share 5% of rape cakes was non-statistically significant (p >0.05) decreased egg weight compared to the control group. Egg weight was reduced in the group with share 10% of rape cakes, which confirmed a statistically significant difference compared to egg weight of control group (p <0.05). The differences among experimental groups with share 5% and 10% of rape cakes in feed mixture and as well as to control group were not statistically significant (p >0.05) in weight of egg yolk, egg white, egg shell and egg shell strength. **E**gg shell thickness was no statistically significant (p >0.05) increased in experimental group with share 5% of rape cakes and decreased in experimental group with share 10% of rape cakes versus control group. Increase of egg shell thickness in experimental group with share 5% of rape cakes versus decrease in experimental group with share 10% of rape cakes was statistically significant (p <0.05).

Keywords: table egg; white; yolk; shell; quality; oilseed rape

INTRODUCTION

Poultry breeding recorded a boom in technology of nutrition but also breeding in the twentieth century. The average body weight and the number of laid the eggs were increased. It changes the methods used in poultry breeding, the programs of hybridization and breeding, as well as the principles of nutrition and feeding. The production of hybrid chickens a laid type of hens is in the developed world by breeding provided through specialized reproductive breeding **(Capcarová et al., 2009)**.

Modern chickens were domesticated from the Red Jungle Fowl (*Gallus gallus*) over the last four or five thousand years for eggs and meat, for game and for exhibition **(Klasing, 2005)** and they are scientifically classified as the same species **(Wong et al., 2004)**.

Consumers of agricultural commodities are increasingly sensitive to animal welfare, and new systems aiming at improving this have recently been introduced to EU livestock production. For example, the conventional battery cage system used for chicken egg production was banned in the European Union in 2012 **(Council Directive 1999/74/EC)**, and fully housed production was mainly replaced by new enriched colony cages (or free range production) **(Leinonen et al., 2014)**.

According to a study conducted in the U.S.A., the quality of eggs from different production systems does not substantially differ. United States department of agriculture-agricultural research service developed a study in which examined various quality criteria for eggs. One of the many findings was that the organically produced eggs and eggs of usual production, there is no significant difference in quality **(Kvasničková, 2010)**.

Nutrition of laying hens is an important factor for the quality and safety of the table egg production. A feeding of laying hens with share 20% of secondary industrial products of oilseed rape has no negative effects on egg weight. These results indicate that the utilization of nutrients from the feed mixture containing secondary industrial products were similar as in the control group without secondary rapeseed products. Based on these results it can be assumed that laying hens good use the nutrients from rapeseed meal **(Gheisari et al., 2011)**.

The selection of appropriate feedstuffs for nutrition of the laying hens is limited to concentrated kinds with a high concentration of nutrients and energy. Corn together with

wheat and soybean meal presents the basis of feed mixture. Currently, the corn and wheat constitute a proportion 70% and soybean meal 16 – 20% of feed mixture **(Angelovičová, 1999)**.

The oil crops are considered a strategic material. The seeds contain economically significant quantity of oil. The fat is irreplaceable in human nutrition, in animal feed rations but also for an increasing proportion of oil in biodiesel. **Angelovič et al., (2013)** state that rapeseed oil constitutes a main raw material in the EU and presents two thirds of total input in biodiesel production.

Rapeseeds are a rich source of oil and their industrial remnants also of proteins. Oilseed rape is one of the most important and most fertile oilseeds in Slovakia **(Božík, 2007)**. Oilseed rape ranks the second among oilseeds **(USDA, 2011)**.

The chemical composition of oilseed rape was significantly changed by breeding. The term "rape" is currently used in Canada. It is incorporated in Great Britain, Australia and the United States to the characteristics of the species *Brassica napus* L. Oilseed rape provides edible oil with an erucic acid content of less than 2% and less than 30 mmol per gram of aliphatic glucosinolates. The use of rapeseed meal or cakes is restricted to full replacement of soybean meal due to the low level of available energy and the occurrence of anti-nutritional factors **(Khajali and Slominski, 2012)**.

Anti-nutrients of secondary rapeseed products include glucosinolates, sinapines, tannins and phytates, indigestible oligosaccharides and non-starchpolysacharides **(Kocher et al., 2000)**.

So far it has been identified more than 120 different species of glucosinolates **(Chen and Andreasson, 2001)**.

It is generally assumed that the glucosinolates by themselves are non-toxic. However, they are always accompanied by the enzyme myrosinase (thioglucoside glucohydrolase) in seed. Glucosinolates are subject to hydrolysis in moist and cracked seed. The results of glucosinolate hydrolysis are degradation products, such as isothiocyanates, goitrin, nitriles and isothiocyanates, which interfere with the function of the thyroid gland **(Tripathi and Mishra, 2007)**.

As a result of these effects on thyroid function, it is affected the metabolism of almost all tissues, including the reproductive system. In addition, various hydrolysis products of glucosinolates are irritating to the mucous membranes of the gastrointestinal tract and consequently are result local necrosis and hepatotoxicity **(Mawson et al., 1994b; Mithen et al., 2000; Burel et al., 2001; Conaway et al., 2002)**.

The negative consequences are described in farm animals as growth retardation, decreased production, impaired reproductive activity, and hepatic and renal function **(Mawson et al., 1994a)**.

The earlier published work indicating that the effect of glucosinolates is associated with reduced production of eggs, and the mortality syndrome related to bleeding of the liver **(Ibrahim and Hill, 1980)**.

In another study warn **Smith and Campbell (1976)** on the harmful effects of high levels of glucosinolates in rapeseed meal and rapeseed cake for laying hens. According to them, these adverse effects may be caused by the degradation products of glucosinolates, such as nitrile. They found that these decomposition products of glucosinolates are in the digestive tract of laying hens.

In the conclusions of the published work by **Mawson et al., (1994a)** is indicated the recommended share of low glucosinolate oilseed rape in feed mixture for laying hens.

The recommended proportion of low glucosinolate oilseed rape ranges up to 10%, in which there were no adverse effects on egg production **Khajali and Slominski (2012)**. They recommend share 20% of low oilseed rapes. Over the past few years, glucosinolate content of was decreased by breeding, particularly as regards double zero oilseed rapes compared to indigenous species.

According to the current annual report of the Canadian International Grains Institute, current oilseed rapes contain 10 μmol per gram of seed (in non-fat dry matter) **(Newkirk, 2009)**. Earlier report indicates reduced glucosinolate content, an average 11 μmol per gram of rapeseed meal **(Mailer and Cornish, 1987)** or 18 μmol per gram (in non-fat dry matter) **(Brand et al., 2007)**.

Oilseed rape cultivated in conditions of Europe, concretely in France, reached glucosinolate content 10 μmol per gram **(Labalette et al., 2011)**.

The level of glucosinolates 4.3 μmol per gram of grown oilseed rapes was also achieved in conditions of Poland **(Mikulski et al., 2012)**.

Glucosinolate content 1.5 μmol per gram could correspond to a share of rape cakes or rapeseed meal from 15 to 20% of current types '00' oilseed rape **(Khajali and Slominski, 2012)**.

The genetic potential of laying hens can be fully utilized. The condition is that laying hens must efficiently convert feed nutrients to eggs as food for human consumption. Laying hens must be healthy and their breeding must be well managed. Laying hens must consume highly digestible, concentrated and well balanced a feed mixture **(Jeroch et al., 2013b)**.

Industrial secondary products of oilseed rape can constitute a substantial proportion of laying hens feed mixture. The valuable components of feed mixture are modern genotypes (F1, F2 and F3 generation, i.e. 0-, 00- and 000-oilseed rape) **(Jahreis, 2003; Jahreisa Schöne, 2006; Jeroch et al., 2013a)**.

Rapeseed secondary products can replace 35 – 40% of soybean meal from the aspect of physiologically acceptable combination of amino acids **(Roth-Maier et al., 2004)**.

A representation of different share of soybean meal by rapeseed industrial secondary products were recommended based on the results of experiments **(Janjecic et al., 2002; Mushtaq et al., 2007; Tripathi a Mishra, 2007)**.

The purpose of this study was to investigate the effectiveness of feed mixtures with varying proportions of rape cakes to the weight of table eggs, its components, thickness and strength of egg shell.

MATERIAL AND METHODOLOGY

Characteristics of the object for research

The objects of the research were the table eggs and quality their components. The eggs were from the final laying hybrid ISA Brown reared in the enriched cage system under experimental conditions. An age of laying hens was from 48 to 54 weeks.

Experimental conditions of laying hens ISA Brown

Hens of laying type ISA Brown were included in the experiment. They lay eggs with brown shell. ISA Brown hens are a hybrid combination of color sexing type of lower body weight. Body weight to end of young hen rearing is 1450 g and to end of egg laying 2100 g. They reach sexual maturity at 145 days of age, when they begin laying. They reached high laying eggs 295 pcs to 500 days of age. Average egg weight is 63.3 grams. It is currently one of the most common hen hybrid combinations in the European Union, about 60% of large-scale breeding. Feed consumption, is around 115.0 g to 118.0 g per day, and 2.2 kg of feed per one kg of egg mass.

The experiment was conducted at the experimental facilities of Slovak University of Agriculture, Faculty of Biotechnology and Food, Department of Food Hygiene and Safety no. SK P 10011. Hens were housed individually in two-storey enriched cages. The cage space is in accordance with the recommendation for the implementation of the natural activity of laying hens, i.e. 750 cm^2. Laying hens had unrestricted access to feed in the feeder and water in the watering place. Feeder and watering place were completed daily. Laying hens was fed by feed mixture of soybean-cereal type, which is usually used in practical conditions. A share of corn and wheat constitute about 66% (33% corn and 33% wheat) and soybean meal 20% of feed mixture.

This feed mixture was used in control group. In the first experimental group was fed a feed mixture with a 5% rape cakes at the expense of soybean meal and other experimental group with share 10% of rape cakes at the expense of soybean meal. Rape cakes were obtained as remnant after pressing of seeds of oilseed rape. Double-zero rape seeds generally contain less than 30 μmol of glucosinolates per gram **(Widharna, 2012)**.

Replenishment of feed and water, as well as egg collection was carried out by hand, and each day at 9:00 am.

Sampling and investigated indicators

Sampling of eggs was carried out three times for 10 eggs in each group of laying hens. An age of laying hens was 50, 52 and 54 weeks when sampling.

Investigated indicators were: egg weight, yolk weight, white weight, shell weight, shell thickness and shell strength.

The methods of investigation of indicators

An egg weight was measured on scales type KERN 440-35N, with an accuracy of 0.01 g and a maximum weight of 400 g.

Sample preparation: The egg was broken, separated the yolk and the white. The yolk placed in pre-weighed watch glass and the egg shell with membranes were washed with tap water and dried in a drying cabinet preheated to 55 °C.

Yolk and shell were weighed on scales of type KERN 440-35N, with an accuracy of 0.01 g and a maximum weight of 400 g.

White weight was calculated using the formula:

$$x = \text{egg weight, g} - (\text{weight of egg yolk, g} + \text{weight of egg shell, g})$$

The thickness and strength of the egg shell were measured from the dried samples at 55 °C. From each egg shell were cut 3 pcs of samples in the equatorial plane, one sample from the blunt end and one sample from the sharp end.

Egg shell thickness was measured in the laboratory of the *Department of Machines* and Production Systems, Slovak University of Agriculture in Nitra; by test instrument SOME, type 60/0.01mm with a range of 0 – 10 mm.

Egg shell strength was measured in the laboratory of the Department of Machines and Production Systems, Slovak University of Agriculture in Nitra; according to test instrument Instron with the small body, having a diameter 4.48 mm to exert pressure on the egg shell by the method of **Angelovičová et al. (1994)** and **Rataj (1994)**.

Statistical methods

The obtained data were assessed according to basic statistical characteristics ($\overline{x}$ = mean, *SD* = standard deviation and c_v = coefficient of variation). Scheffe´s test at the significance level of $\alpha = 0.05$ was used to compare a difference between indicator values in the program system SAS, version 8.2.

RESULTS AND DISCUSSION

Table eggs are among the valuable foodstuffs **(Sparks, 2006)**. Type of laying hens is used to produce table eggs. In our experiment, it was used type of ISA Brown hens. Composition of eggs is influenced by genetic factors, age and diet. Nutrition is a very important factor for the production of quality and safe table eggs. The feed mixture of soybean-type cereal was used in our experiment.

According to **Angelovičová (1999)**, excluding maize and wheat is the basis of feed mixture the soybean meal too. We do not know soybeans to grow in our country, so we are forced to import it. We are focused on solving partial substitution of soybean meal with our domestic feed-rape cake in our experiment. It is a product that has a relatively high nutritional value and can be a useful component in feed mixture for laying hens in the production of the table eggs.

Mawson et al., (1994a) recommend incorporating into the feed mixture for laying hens with share of low glucosinolated oilseed rape. This amount is up to 10%. These authors conclude that in such proportion were no adverse effects on the production of table eggs. Some authors state that the appropriate proportion may be up to 20% **(Khajali and Slomiński, 2012)**.

The study by **Ibrahim and Hill (1980)** stated that the feed mixture of 20% rapeseed high in glucosinolates suppressed egg production in laying hens. However, the feed mixture with 20% of rapeseed meal produced from low glucosinolated rape did not cause a reduction in egg production.

Weight of eggs, yolk and white

Egg weight

Najib and Al-Kateeb (2004) noted that the results of their experiment confirmed that 10% of rapeseed in the feed mixture of laying hens not adversely affects the egg mass and egg weight. These authors further stated that the

daily egg production, egg mass and egg weight was lower if the in the feed mixture of laying hens was an addition of 30% rapeseed. They also stressed that the highest egg production was found if the laying hens fed feed mixture with a share of 5% and 10% of rape seed.

Based on the results observed in egg weight of our experiment we can conclude that in the group with share 5% of rape cakes was non-statistically significant (p >0.05) decreased egg weight compared to the control group. Egg weight was reduced in the group with share 10% of rape cakes, which confirmed a statistically significant difference compared to egg weight of control group (p <0.05). In the group with share 10% of rape cakes were observed the greatest variation in egg weight values expressed by standard deviation and coefficient of variation.

Table 1 Average egg weight.

Group	n	$\bar{x}$, g	*SD*	c_v, %
Control	30	61.8[a]	1.72	2.78
Experimental 5	30	60.3[ab]	2.10	3.48
Experimental 10	30	59.7[b]	2.36	3.95

n – number of samples, $\bar{x}$ – mean, *SD* – standard deviation, c_v – coefficient of variation,
a, b – value within a column compared between groups with different superscript letter is significantly different (p <0.05).

In contrast to our results, **Gheisar et al., (2011)** reported that feeding of the laying hens with share 20% of rapeseed products in feed mixture did not cause a reduction in egg weight. However **Ciurescu (2009)** takes the opposite view. The author notes based on the results his experiments, if the proportion of the rapeseed meal is greater than 15%, egg weight decreases.

Egg yolk weight

With this statement we can agree with the difference that in our experiment, there was a decrease in egg weight at share 10% of rape cakes. Average egg weight was in our experiment, by the proportion of rape cakes in the feed mixture, from 59.70 g in experimental group 10 and 60.30 g in experimental group 5. Higher egg weight (65.00, 71.25 g, respectively) in laying hens of the same age reported **Angelovičová and Polačková (2015)**. The laying hens were different type, Moravia SSL.

Based on our results of yolk weight we can conclude that differences among experimental groups with share 5% and 10% of rape cakes in feed mixture and as well as to control group were not statistically significant (p >0.05). Our results are in agreement with the results of yolk weight recorded by **Ciurescu (2009)**. However, our results indicate that the greatest variation of yolk weight values were in the group with share 10% of rape cakes.

Table 2 Average yolk weight.

Group	n	$\bar{x}$, g	*SD*	c_v, %
Control	30	15.7	0.83	5.29
Experimental 5	30	15.9	1.14	7.17
Experimental 10	30	15.2	1.19	7.83

n – number of samples, $\bar{x}$ – mean, *SD* – standard deviation, c_v – coefficient of variation,
No significant differences between groups *in* yolk weight between groups (p >0.05).

Egg white weight

Table 3 Average white weight.

Group	n	$\bar{x}$, g	*SD*	c_v, %
Control	30	40.2	2.17	5.4
Experimental 5	30	38.5	2.09	5.43
Experimental 10	30	38.8	3.14	8.09

n – number of samples, $\bar{x}$ – mean, *SD* – standard deviation, c_v – coefficient of variation,
No significant differences between groups in white weight between groups (p >0.05).

We did not met s literary knowledge about the impact of rape products on an egg white quality. The results of our experiment showed that between groups (control, with share 5% of rape cakes and 10% of rape cakes) no statistically significant difference (p >0.05). Even at egg white weight was observed the biggest variation of values in the experimental group with share 10% of rape cakes.

Egg shell quality

Egg shell weight

The egg shell constitutes the skeletal or external support of the egg **(Ar et al., 1979)** and, as such, egg shell quality is very important to the poultry industry **(Takahashi et al., 2009)**.

Table 4 Average egg shell weight.

Group	n	$\bar{x}$, g	*SD*	c_v, %
Control	30	5.9	0.12	2.03
Experimental 5	30	5.9	0.16	2.71
Experimental 10	30	5.7	0.19	3.33

n – number of samples, $\bar{x}$ – mean, *SD* – standard deviation, c_v – coefficient of variation,
No significant differences between groups *in* shell weight between groups (p >0.05).

We did not met s literary knowledge about the impact of rape cakes on an egg white quality. Literary knowledge is known about the impact of rapeseed meal on this indicator. **Riyazi et al., (2009)** indicate that the proportion 10% of rapeseed meal in feed mixture caused an increase of the egg shell weight. Our results disagree with this conclusion. We have found that, among the groups no statistically significant difference (p <0.05) in weight of the egg shell being compared according to the share 5 and 10% of rape cakes in feed mixture or compared to control group.

Egg shell thickness

Likewise at a thickness of the egg shell **Riyaz et al. (2009)** state that was observed no significant difference in the thickness, as well as in strength of egg shells, if laying hens fed rapeseed meal. Authors further state that the values of these indicators were higher in group of laying hens that were fed share 10% of rapeseed meal in feed mixture compared with the control group. Our results are consistent with ones of these authors.

Table 5 Average egg shell thickness.

Group	n	$\bar{x}$, mm	*SD*	c_v, %
Control	30	0.38	0.012	3.16
Experimental 5	30	0.4[a]	0.021	5.25
Experimental 10	30	0.361[b]	0.019	5.26

n – number of samples, $\bar{x}$ – mean, *SD* – standard deviation, c_v – coefficient of variation,
a, b – value within a column compared between groups with different superscript letter is significantly different (*p <0.05).*

Egg shell strength

Our results on the strength of egg shell are different compared to ones by **Khajali and Słomiński (2012)**. They argue that the quality of the egg shell was statistically significant in the control group compared with groups of laying hens, which fed feed with a share of industrial rapeseed products.

Table 6 Average egg shell strength.

Group	n	$\bar{x}$, N	*SD*	c_v, %
Control	30	33.1	1.36	4.11
Experimental 5	30	33.5	1.59	4.75
Experimental 10	30	33.8	1.72	5.09

n – number of samples, $\bar{x}$ – mean, *SD* – standard deviation, c_v – coefficient of variation,
No significant differences between groups in shell strength between groups (*p >0.05).*

Khajali and Słomiński (2012) reported on the base of experimental results that the values of quality indicators of egg shell were significantly higher (p <0.05) in the control group compared with the indicators of quality of the egg shell hens that fed feed mixture with a share of industrial by-products of oilseed rape. It is believed that the presence of phytic acid in the by industrial oilseed rape forms an insoluble complex with the protein and some minerals, such as e.g. calcium, iron, zinc, manganese and magnesium in a biologically unavailable for laying hens. This complex in turn leads to difficulties in the use of these minerals, protein and other nutrients for the organism of laying hens **(Šašytė et al., 2006)**. In addition, a high level of sulfur in rapeseed meal causes indigestion and absorption of calcium **(Summers et al., 1992)**. Increasing the pH of the small intestine chyme is another reason that might have impact on the quality characteristics the egg shell, if rapeseed meal is fed, in comparison with the pH of the small intestine chime, if soybean meal is fed **(Zdunczyk et al., 2013)**.

Oilseed rape is a farm significant crop suitable for cultivation in our country. Its products after industrial processing of seeds for oil are an important feed material for animals intended for food production. However, definite conclusions about their use as feed material require further investigation. Conclusions of existing experiments are not unanimous as to the extent of their inclusion in feed mixture in relation to the quality production of table eggs, or their safety.

CONCLUSION

Seeds of oilseed rape are an important raw material for obtaining oil. By-product after industrial processing has a relatively high nutritional value. By-product may be classified as feed material for laying hens. More research is needed for single-valued recommendation of by-product share of oilseed rape in the feed mixture for laying hens. Oilseed rape and industrial by-products contain anti-nutrients according to literary knowledge.

In our work we focus on the verification of rape cake, for their use as feed for laying hens. Rape cakes were obtained as a by-product after pressing of seeds. By pressing of seeds was obtained oil to produce of biodiesel.

On the basis of evaluated results, we can state the following:

- share 5% of rapeseed cakes in the feed mixture had no statistically significant effect on egg weight, its component parts, egg shell thickness and strength,
- share 10% of rapeseed cakes in feed mixture statistically significant negative influenced on egg weight and measured values showed the greatest fluctuations in investigated indicators expressed as the standard deviation and coefficient of variation,
- the issue of the use of rapeseed cakes as feed is highly topical in terms of their production in obtaining of oil for human consumption and biodiesel,
- the conditions for inclusion of rapeseed cakes in the feed mixture for laying hens remain open for further research.

REFERENCES

Angelovič, M., Tkáč, Z., Angelovič, M. 2013. Oilseed rape as feedstock for biodiesel production in relation to the environment and human health. *Potravinarstvo*, vol. 7, no. 1, p. 101-106. http://dx.doi.org/10.5219/278

Angelovičová, M., Mihálik, V., Rataj, V. 1994. Stability of egg shell depends on nutrition of laying type of hens. *International Agrophysics*, vol. 8, no. 4, p. 607-610.

Angelovičová, M. 1999. *Nutrition and feeding of high-productive poultry*. Nitra: SPU, 1999. 85 p. ISBN 80-7137-608-6.

Angelovičová, M., Polačková, D. 2015. Assessment of welfare and egg production of laying hens Moravia SSL in small-scale breeding. *Potravinarstvo*, vol. 9, no. 1, p. 365-374. http://dx.doi.org/10.5219/514

Ar, A., Rahn, H., Paganelli, C. V. 1979. The avian egg: mass and strength. *The Condor*, vol. 81, p. 331-337. http://dx.doi.org/10.2307/1366955

Božík, M. 2007. Economy and prospects of growing oilseed rape in Slovakia and the EU. *Intensive cultivation of oilseed rape during periods of high demand*, no. 12, p. 2.

Brand, T. S., Smith, N., Hoffman, L. C. 2007. Anti-nutritional factors in canola produced in the Western and Southern Cape areas of South Africa. *South African Journal of Animal Science*, vol. 37, no. 1, p. 45-50.

Burel, C., Boujard, T., Kaushik, S. J., Boeuf, G., Mol, K. A., Van Der, G. S., Darras, V. M., Kuhn, E. R., Pradet-Balade, B., Querat, B., Quinsac, A., Krouti, M., Ribaillier, D. 2001. Effects of rapeseed meal-glucosinolates on thyroid metabolism and feed utilization in rainbow trout. *General and Comparative Endocrinology*, vol. 124, no. 3, p. 343-358. http://dx.doi.org/10.1006/gcen.2001.7723 PMid:11742518

Capcarová, M., Kováčik, J., Mellen, M. 2009. *Changes of biochemical indicators in poultry blood after application of probiotics*. 1st ed. Nitra: SUA, 61 p. ISBN: 978-80-552-0206-8.

Chen, S., Andreasson, E. 2001. Update of glucosinolate metabolism and transport. *Plant Physiology and*

Biochemistry, vol. 39, no. 9, p. 743-758. http://dx.doi.org/10.1016/S0981-9428(01)01301-8

Ciurescu, G. 2009. Efficiency of soybean meal replacement by rapeseed meal and/or canola seeds in commercial layer diets. *Archiva Zootechnica*, vol. 12, no. 1, p. 27-33.

Conaway, C. C., Yang, Y. M., Chung, F. L. 2002. Isothiocyanates as cancer chemopreventive agents: their biological activities and metabolism in rodents and humans. *Current Drug Metabolism*, vol. 3, no. 3, p. 233-255. http://dx.doi.org/10.2174/1389200023337496 PMid:12083319

Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. OJ L 203, 3.8.1999, p. 53-57.

Gheisari, A. A., Ghayor, P., Egbal-Saeid, S., Toghyani, M., Najafi, A. A. 2011. Effect of different dietary levels of rapeseed meal on reproductive performance of iranian indigenous breeder hens. *Asian Journal of Animal and Veterinary Advance*, vol. 6, no. 1, p. 62-70.

Ibrahim, I. K., Hill, R. 1980. The effects of rapeseed meals from brassica napus varieties and the variety tower on the production and health of laying fowl. *British Poultry Science*, vol. 21, no. 6, p. 423-430.

Jahreis, G. 2003. Physiological effects of plant oils in human nutrition (in German). *UFOP-Schriften,Öl-und Faserpflanzen*, vol. 20, p. 91-99.

Jahreis, G., Schöne, F. 2006. Rapeseed oil – physiologically particularly valuable chosen from oils (in German). In *Oil and proteinplants - The conference proceedings Oil 2005*, UFOP-Schriften, vol. 29, p. 7-17.

Janjecic, Z., Grbeša, D., Muzic, S., Curic, S., Rupic, V., Liker, B., Dikic, M., Antunovic, B., Zupanic, D. 2002. Influence of rapeseed meal on productivity and health of broiler chicks. *Acta Veterinaria Hungarica*, vol. 50, no. 1, p. 37-50. http://dx.doi.org/10.1556/AVet.50.2002.1.6 PMid:12061234

Jeroch, H., Jankowski, J., Lipiec, A., Kozłoeski, K., Matusevičius, P., Mikołajczak, J., Schöne, F. 2013a. *Rapeseed feedstuffs in animal nutrition* (in Polish). Olsztyn : UWM.

Jeroch, H., Simon, A., Zentek, J. 2013b. *Poultry nutrition* (in German). Stuttgart : Eugen Ulmer Verlag.

Khajali, F., Slominski, B. A. 2012. Factors that affect the nutritive value of canola meal for poultry. *Poultry Science*, vol. 91, no. 10, p. 2564-2575. http://dx.doi.org/10.3382/ps.2012-02332 PMid:22991543

Klasing, K. C. 2005. Poultry nutrition: a comparative approach. *The Journal of Applied Poultry Research*, vol. 14, no. 2, p. 426-436. http://dx.doi.org/10.1093/japr/14.2.426

Kocher, A., Choct, M., Porter, M. D., Broz, J. 2000. The effects of enzyme addition to broiler diets containing high concentration of canola or sunflower meal. *Poultry Science*, vol. 79, no. 12, p. 1767-1774. http://dx.doi.org/10.1093/ps/79.12.1767 PMid:11194039

Kvasničková, A. 2010. The quality of eggs from conventional and organic production [online] Praha : Informative Centre of the Ministry of Agriculture [cit. 2015-09-08]. Available at: http://www.bezpecnostpotravin.cz/kvalita-vajec-z-konvencni-a-bio-produkce.aspx.

Labalette, F. R., Dauguet, S., Merrien, A., Peyronnet, C., Quinsac, A. 2011. Glucosinolate content, an important quality parameter monitored at each stage of the French rapeseed production chain. In *Proceeding 16th Rapeseed Congress*. Prague, Czech Republic, p. 438-442.

Leinonen, I., Williams, A. G., Kyriazakis, I. 2013. The effects of welfare-enhancing system changes on the environmental impacts of broiler and egg production. *Poultry Science*, vol. 93, no. 2, p. 256-266. http://dx.doi.org/10.3382/ps.2013-03252 PMid:24570446

Mailer, R. J., Cornish, P. S. 1987. Effects of water stress on glucosinolates and oil concentrations in the seeds of rape (*Brassica napus* L.). *Australian Journal of Experimental Agriculture*, vol. 27, no. 5, p. 707-711. http://dx.doi.org/10.1071/EA9870707

Mawson, R., Heaney, R. K., Zdunczyk, Z., Kozlowska, H. 1994a. Rapeseed mealglucosinolates and their antinutritional effects. Part 3. Animal growth and performance. *Nährung*, vol. 38, no. 2, p. 167-177. http://dx.doi.org/10.1002/food.19940380209 PMid:8196745

Mawson, R., Heaney, R. K., Zdunczyk, Z., Kozlowska, H. 1994b. Rapeseed meal-glucosinolates and their antinutritional effects. Part 4. Goitrogenicity and internal organs abnormalities in animals. *Nährung*, vol. 38, no. 2, p. 178-191. http://dx.doi.org/10.1002/food.19940380210 PMid:8196746

Mikulski, D., Jankowski, J., Zdunczyk, Z., Juskiewicz, J., Slominski, B. A. 2012. The effect of different dietary levels of rapeseed meal on growth performance, carcass traits and meat quality in turkeys. *Poultry Science*, vol. 91, no. 1, p. 215-223. http://dx.doi.org/10.3382/ps.2011-01587 PMid:22184447

Mithen, R. F., Dekker, M., Verkerk, R., Rabot, S., Johnson, I. T. 2000. The nutritional significance, biosynthesis and bioavailability of glucosinolates in human food. *Journal of the Science of Food and Agriculture*, vol. 80, no. 7, p. 967-984. http://dx.doi.org/10.1002/(SICI)1097-0010(20000515)80:7<967::AID-JSFA597>3.0.CO;2-V

Mushtaq, T., Sarwar, M., Ahmad, G., Mirza, M. A., Nawaz, H., Haroonmushtaq, M. M., Noreen, U. 2007. Influence of canola meal-based diets supplemented with exogenous enzyme and digestible lysine on performance, digestibility, carcass, and immunity responses of broiler chickens. *Poultry Science*, vol. 86, no. 10, p. 2144-2151. http://dx.doi.org/10.1093/ps/86.10.2144 PMid:17878444

Najib, H., Al-Kateeb, S. A. 2004. The effect of incorporating different levels of locally produced canola seeds (*Brassica napus*, L.) in the diet of laying hen. *International Journal of Poultry Science*, vol. 3, no. 7, p. 490-496. http://dx.doi.org/10.3923/ijps.2004.490.496

Newkirk, R. W. 2009. *Canola Meal Feed Industry Guide*. 4th ed. Canadian International. Grains Institute, Winnipeg, Manitoba.

Rataj, V. 1994. Determination of strength of agricultural materials by loading. *Zemědělská technika*, vol. 40, no. 2, p. 87-93.

Roth-Maier, D. A., Böhmer, B. M., Roth, F. X. 2004. Effects of feeding canola meal and sweet lupin (*L. luteus*, *L. angustifolius*) in aminoacid balance diets on growth performance and carcass characteristics of growing-finishing pigs. *Animal Research*, vol. 53, p. 21-34. http://dx.doi.org/10.1051/animres:2003048

Riyazi, S. R., Ebrahimnezhad, Y., Nazeradl, K. Maheri-Sis, N., Salamatdust, R., Vahdatpour, T. 2009. The effects of replacing soybean meal with different levels of rapeseed meal on egg quality characteristics of commercial laying hens. *Asian Journal of Animal and Veterinary Advance*, vol. 4, no. 6, p. 337-341. http://dx.doi.org/10.3923/ajava.2009.337.341

Šašytė, V. A., Gruzauskas, R., Mosenthin, R. 2006. Effect of phytase on P and Ca utilization at different age periods of laying hens fed higher amount of rapeseed meal. *Biologia*, vol. 1, p. 69-72.

Smith, T. K., Campbell, L. D. 1976. Rapeseed meal glucosinolates: Metabolism and effect on performance in laying hens. *Poultry Science*, vol. 55, no. 3, p. 861-867. http://dx.doi.org/10.3382/ps.0550861 PMid:935053

Sparks, H. C. N. 2006. The hens egg – its role in human nutrition changing. *World's Poultry Science Journal* [online], vol. 62, no. 2, p. 308-315 [cit. 2015-09-12]. Available at: http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=624024&fileId=S0043933906000201.

Summers, J. D., Sprat, D., Bedford, M. 1992. Sulfur and calcium supplementation of soybean and canola meal diets. *Canadian Journal of Animal Science*, vol. 72, no. 1, p. 127-133. http://dx.doi.org/10.4141/cjas92-014

Takahashi, H., Yang, D., Sasaki, O., Furukawa, T., Nirasawa, K. 2009. Mapping of quantitative trait loci affecting eggshell quality on chromosome 9 in an F2 intercross between two chicken lines divergently selected for eggshell strength. *Animal Genetics*, vol. 40, no. 5, p. 779-782. http://dx.doi.org/10.1111/j.1365-2052.2009.01914.x PMid:19780721

Tripathi, M. K., Mishra, A. S. 2007. Glucosinolates in animal nutrition: A review. *Animal Feed Science and Technology*, vol. 132, no. 1-2, p. 1-27. http://dx.doi.org/10.1016/j.anifeedsci.2006.03.003

USDA (United States Department of Agriculture), 2011. Oilseads: World market and trade. *Circular Series*, FOP 11.-12. 12. 2011.

Widharna, R. M. 2012. Evaluation of means of reducing glucosinolate in canola meal. *Jurnal Medika Planta*, vol. 1, no. 5, p. 24-35.

Wong, G. K., Liu, B., Wang, J., Zhang, Y., Yang, X., Zhang, Z., Meng, Q., Zhou, J., Li, D., Zhang, J., Ni, P., Li, S., Ran, L., Li, H., Zhang, J., Li, R., Li, S., Zheng, H., Lin, W., Li, G., Wang, X., Zhao, W., Li, J., Ye, C., Dai, M., Ruan, J., Zhou, Y., Li, Y., He, X., Zhang, Y., Wang, J., Huang, X., Tong, W., Chen, J., Ye, J., Chen, C., Wei, N., Li, G., Dong, L., Lan, F., Sun, Y., Zhang, Z., Yang, Z., Yu, Y., Huang, Y., He, D., Xi, Y., Wei, D., Qi, Q., Li, W., Shi, J., Wang, M., Xie, F., Wang, J., Zhang, X., Wang, P., Zhao, Y., Li ,N., Yang, N., Dong, W., Hu, S., Zeng, C., Zheng, W., Hao, B., Hillier, L.W., Yang, S.P., Warren, W. C., Wilson, R. K., Brandström, M., Ellegren, H., Crooijmans, R. P., van der Poel, J. J., Bovenhuis, H., Groenen, M. A., Ovcharenko, I., Gordon, L., Stubbs, L., Lucas, S., Glavina, T., Aerts, A., Kaiser, P., Rothwell, L., Young, J. R., Rogers, S., Walker, B. A., van Hateren, A., Kaufman, J., Bumstead, N., Lamont, S. J., Zhou, H., Hocking, P. M., Morrice, D., de Kning, D. J., Law, A., Bartley, N., Burt, D. W., Hunt, H., Cheng, H. H., Gunnarsson, U., Wahlberg, P., Andersson, L., Kindlund, E., Tammi, M. T., Andersson, B., Webber, C., Ponting, C. P., Overton, I. M., Boardman, P. E., Tang, H., Hubbard, S. J., Wilson, S. A., Yu, J., Wang, J., Yang, H. 2004. A genetic variation map for chicken with 2.8 million single – nucleotide polymorphisms. International Chicken Polymorphism Map Consortium. *Nature*, vol. 432, no. 7018, p. 717-722. http://dx.doi.org/10.1038/nature03156 PMid:15592405

Zduńczyk, Z., Jankowski, J., Juśkiewicz, J., Mikulski, D., Slominski, B. A. 2013. Effect of different dietary levels of low-glucosinolate rapeseed (canola) meal and non-starch polysaccharide-degrading enzymes on growth performance and gut physiology of growing turkeys. *Canadian Journal of Animal Science*, vol. 93, p. 353-362.

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A COMPARISON OF THE DETERMINATION OF THE RENNET COAGULATION PROPERTIES OF BOVINE MILK

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ABSTRACT

The aim of the work was compared of two different methods (the visual method and the nephelo turbidimetry method) for determination of rennet coagulation time. It was observed the effect of heat treatment of milk; types of rennet and addition of different amount of $CaCl_2$ into the pasteurized milk. It was used two different chymosin rennet. For the visual method was milk sample (100 mL) equilibrated at 35 °C, 1 mL of rennet was added into milk and was measured the time required for the first visible flakes (visual method). For the determination rennet coagulation time by nephelo-turbidimetry was removed part of milk with rennet and placed into nephelo-turbidimetry. Milk had a titratable acidity in the range from 6.5 to 7.0 °SH, average pH of milk was 6.68. Dry matter content was in range from 12.351 to 13.142%. The average content of protein by Kjeldahl was 3.14%, fat by Gerber 4.34%, lactose by polarimetry 4.68% and calcium content 1.1%. The pasteurized milk had the worst rennet coagulation time about 32 s compared to the raw milk. The difference coagulation time between milk with addition of 20 µL $CaCl_2$ and 40 µL $CaCl_2$ was in range 21 s to 26 s by visual method. The difference coagulation time between milk with addition of 20 µL $CaCl_2$ and 40 µL $CaCl_2$ was 15 s by nephelo-turbidimetry method. There occurred statistically non-significant differences in most of the measurements, comparing the visual and the nephelo-turbidimetric method. The heat treatment, addition of $CaCl_2$ and using of different rennet had an influence on the curd category. It was obtained, that using nephelo-turbidimetry shown objective results for measuring the rennet coagulation time contrary the subjective visual method. Further, the results obtained by nephelo-turbidimetry are accurate and determined with the lower variation.

Keywords: rennet coagulation time; nephelo-turbidimetry; bovine milk; calcium chloride

INTRODUCTION

Milk coagulation properties are one of the most important technological properties of milk which have influence on the cheese production. The addition of rennet into the sample of milk will be cause of physical and biochemical changes in milk, which include modification of casein micelles. The result of renneting is the change of viscosity and elasticity. Milk coagulation properties is collection of property which are traditionally expressed as the rennet coagulation time, time to curd firmness of 20 mm and curd firmness 30 min after enzyme addition **(Bittante, 2011)**.

The principle of cheese production is the conversion of a viscous liquid (milk) into solid material (curd) which retain casein protein and fat (which was included in milk). If needs a solid material, the whey from curd have to be removed. In the whey is left a major part of water, whey protein and majority of lactose. For this step is important to precipitate the casein from the milk and release the whey from the curds **(Law and Tamime, 2010)**. After the addition of rennet para casein micelles starts to aggregate, thereby increasing the viscosity and elasticity. This transition may also change other physical properties of milk e.g. a light reflectance or a thermal conductivity. Several methods are principally based on the detection of these changes. These methods were developed for measuring and determination of rennet coagulation properties **(Fuquay et al., 2011)**.

The milk coagulation property is influenced by many factors, which have an effect on primary or secondary phase of precipitate of milk or both. The milk composition has direct effect on precipitate of milk and total yield of cheese. Especially important is the content of calcium, particularly the ionic form, which has influence on formation of curd gels consistency **(Roginski et al., 2003)**. Further factors which had an effect on rennet coagulation time are coagulation temperature, pH and concentration of $CaCl_2$. The coagulation temperature had highly significant effect on rennet coagulation time, coagulum firmness, gel firming rate and curd firmness. The effect of pH was highly significant for all parameters too, especially on curd firmness. Concentration of $CaCl_2$ was significant only for rennet coagulation time and coagulum firmness **(Nájera et al., 2003)**. The milk salts have an influence on rennet coagulation of milk and structure of cheese **(Lucey and Fox, 1993)**. The addition of $CaCl_2$ had an effect on the yield of cheese **(Wolfschoon-Pombo, 1997)**. The main salts are Ca and PO_4. Addition of Ca decrease the rennet coagulation time of milk that is due to neutralization of negatively charged residues of casein, which increased the aggregation
of renneted micelles **(Lucey and Fox, 1993)**. **Yuksel**

(2013) confirmed that rennet flocculation and clotting times are decreased with increasing concentration $CaCl_2$.

In this study was showed that $CaCl_2$ has effects on both – the primary and secondary phase of renneting. $CaCl_2$ reduced the time of coagulation. On the other hand addition of disodium phosphate into homogenized milk increases the time of coagulation **(Maxcy et al., 1955**).

High heat treatment or a low calcium concentration had an effect on prolonged flocculation (slow aggregation rate) with slow firming rate in the samples of reconstituted milk. The coagulation time was negatively correlated with the firming parameters **(Klandar et al., 2007)**.

Several methods were used for measuring and determining the rennet coagulation property of milk. The visual determination of rennet coagulation time is very often used. A nephelo-turbidimetry and the measurement on the formagraph are also used.

The visual method – the coagulation of proteins is often assessed using a visual method that consists of the observation of precipitating milk with naked eye. After the addition of rennet, it was measured the time until the first visible flakes of aggregation casein could be spotted with naked eye. The sample of coagulation milk is observed under continuous gentle mixing, mostly against the light **(Sbodio and Revelli, 2012)**. The result of visual method affects subjective assessment and the experience of the person monitoring the rennetability. For these reasons, it is not regarded as an objective **(Čejna and Přibyla, 2006)**.

The characteristic of rennet was based on the determination of rennet strength. To determine the strength of the rennet, there can be often used these methods: The rennet strength by Soxhlet is defined as the quantity of raw milk, which can be precipitated by 1 mL rennet at 35 °C for 40 min **(Law and Tamime, 2010)**. The method by Berridge – this method is used for determination of the defined strength of rennet to precipitate 10 mL of reconstituted standardized milk (standardized sample of powdered milk reconstituted 0.01 mol/L $CaCl_2$ at pH 6.3 at 30 °C for 100 s **(Fuquay et al., 2011)**. The method of Rolling bottle which is based on giving milk samples in special tubes or bottles. These bottles are heated up to 30 °C, adding 1 mL of diluted chymosin and placed in water bath at an angle of approximately 20 °. Bottles are spinned in this bath at speed of 8 rpm. This test is finished when the film of small casein flocks is formed on the inner wall of the bottle. The coagulation time is determined from number of revolutions from start to the end of this test **(Sommer and Matsen, 1935)**. This method is among nondynamic method. Nondynamic methods are mostly empirical and based on the measurement of rheological or optical properties **(Castillo, 2006)**.

The nephelo-turbidimetry works in the principle of the nephelometry and the turbidimetry. The nephelometry is an optical method, engaged in measuring the intensity of diffusely scattered light to the dispersed particles **(Sojková et al., 2011)**. The optical detector device converts light intensity to an electrical signal. The strain at the output is a function of the intensity of light incident on the optical detector. The milk coagulate reduces the optical signal (turbidimetry) and thereby also a reduction of the measured strain. The resulting recording signal is immediately derived. This result corresponds to the precipitate para-casein and the maximum value of derivate curve **(Chládek and Čejna, 2005)**.

The detection of rennet coagulation properties by formagraph is based on the principle of the movement of small pendulum which is linearly moved in the milk coagulation sample. This movement is recorded on a photographic paper and this curve gives us the dependence of strength to the time. When milk is in liquid form and has a low viscosity, this milk doesn´t have a sufficient strength to move the pendulum, whereas the coagulation milk has a higher viscosity and causes synchronous movement of the pendulum **(McMahon and Brown, 1982)**. Other methods for determination rennet coagulation time are: refractometry, where was measured change in the refractive index during milk coagulation **(Korolczuk et al., 1988)**, Near Infrared spectroscopy (NIR) used a light transmission or reflectance measured in the NIR range for coagulation milk **(O´Callaghan et al., 2000)**, vibration viscometry **(Marshall et al., 1982; O´Callaghan et al., 2000)**, fluorescence spectroscopy where is monitored emission fluorescence spectra of tryptophan residues during coagulation **(Herbert et al., 1999)**, electroacoustic **(Wade and Beattie, 1998)**, ultrasound low frequency used a frequencies in range 50 to 100 kHz **(Nassar et al., 2001)** and reflection photometry where are monitoring coordinates L* and b* which rise during coagulation of milk **(Hardy and Fanni, 1981)**.

MATERIAL AND METHODOLOGY

The bovine milk used for this work was collected in summer 2015. The samples of milk were heated up prior to analyses to 40 °C and then cooled down to 20 °C for better dispersion of the fat globules. After heating and cooling, the samples were immediately analysed. Before measuring the milk samples, there were done some basic laboratory analyses that have an effect on rennet coagulation time. The titratable acidity, pH, the calcium content and the lactose by polarimetry were determined according to Czech state standard No 57 0530. The content of fat was determined by Gerber's method **(ISO 2446:2008)**, the protein content was determined by Kjeldahl's method **(EN ISO 8968-1:2002)**, dry matter content was determined by gravimetry **(ISO 6731:2010)**.

After these analyses, the part of milk for rennet coagulation time was removed. This part of milk was analysed as raw milk. The remaining part of milk was pasteurized at 72 °C for 20 s. This combination is very often used at cheese production. It was premised that heat treatment has an influence on the rennet coagulation time (that the pasteurized milk needs more time for coagulation then raw milk).

Subsequently, the pasteurized milk has been divided into four groups. The first group was only pasteurized. The solution of 36% $CaCl_2$ was added into the next three groups at the quantities of 20 µL, 30 µL and 40 µL 100 $mL^{-1}$of pasteurized milk.

In our work was used a proteolytic enzyme which causes precipitation of proteins. It was used two different chymosin RENNET A (CHY-MAX M200, CHR. HANSEN, Denmark, 190 IMCU/1 mL) and RENNET B (Laktochym CZ 1068 ES, MILCOM a.s.,

Czech Republic, 59.5 IMCU/1 mL). These rennet were diluted so, that the precipitation was in range from 120 s to 240 s.

Each group (raw milk, pasteurized milk, pasteurized milk with 20 μL 100 mL^{-1}, pasteurized milk with 30 μL 100 mL^{-1}, pasteurized milk with 40 μL 100 mL^{-1}) had 6 samples for RENNET A and 6 samples for RENNET B. The measurements were repeated three times.

The measurement of rennet coagulation time of milk was carried out by visual methods and method using the nephelo-turbidimetric sensor for milk coagulation property.

The milk sample (100 mL) was equilibrated at 35 °C, 1 mL of rennet was added into milk and was measured the time required for the first visible flakes (visual method). For the determination rennet coagulation time of coagulation, which was measured by nephelo-turbidimetry.

The milk with adding rennet was placed in a thermostat at 35 °C. Samples of coagulation milk were evaluated according five grade scale (in the Table 1) after one hour in a thermostat.

Statistically, the difference between the two methods using the t-test was evaluated. The results were statistically processed by program STATISTICA 12.

The aim of the work was compare of two difference methods (the visual method and nephelo-turbidimetry method) for determination of rennet coagulation time. It was observed the effect of heat treatment of milk; types of rennet and addition of difference concentration of $CaCl_2$ into the pasteurized milk.

Table 1 Evaluation of rennet curd quality (Kuchtík et al., 2008).

Category	Appearance and firmness of curd and appearance of whey
1	very good and hard curd, keeping its shape after its removal from the container; whey is clear, of yellowgreenish colour
2	good but a little softer curd, not keeping its shape quite perfectly; excretion of whey not perfect; whey is greenish
3	not good, soft curd, partly not keeping its shape; milky white whey
4	very bad curd, not keeping its shape; milky white whey
5	very weak or invisible flocculation of casein

Table 2 The composition of milk and milk properties.

	Average	Standard deviation	Minimum	Maximum	Coefficient of variation (%)
Titratable acidity (°SH)	6.7	0.3	6.5	7.0	3.7
pH	6.68	0.04	6.63	6.71	0.62
Dry matter content (%)	12.740	0.396	12.351	13.142	3.106
Protein content (%)	3.14	0.11	3.04	3.26	3.58
Fat content (%)	4.34	0.31	4.01	4.62	7.11
Lactose content (%)	4.68	0.08	4.61	4.77	1.75

Table 3 The comparison of rennet coagulation time of raw and pasteurized milk by the visual and the nephelo-turbidimetry method (the average value with the standard deviation).

Groups of milk	Rennet	Visual method (s)	Nephelo-turbidimetry (s)	Significance
Raw	A	165 ±4	148 ±6	*
	B	135 ±15	130 ±16	NS
Pasteurized	A	184 ±23	162 ±23	*
	B	159 ±27	142 ±15	NS

Note: * – significant difference at $p < 0.05$; NS – nonsignificant difference at $p > 0.05$.

by nephelo-turbidimetry was removed part of milk with rennet and placed into nephelo-turbidimetry. The result of nephelo-turbidimetry measurement of coagulation of milk is a curve with an inflection point representing the milk coagulation time. From each group, there was calculated an average compared to the time

RESULTS AND DISCUSSION

Milk, which has been used for comparing of rennet coagulation time, had a titratable acidity in the range from 6.5 to 7.0 °SH, average pH of milk was 6.68. Dry matter content was in range from 12.351 to 13.142%. The average content of protein by Kjeldahl was 3.14%, fat by Gerber

Groups of milk	Rennet	Visual method (s)	Nephelo-turbidimetry (s)	Significance
Raw	A	165 ±4	148 ±6	*
	B	135 ±15	130 ±16	NS
Pasteurized	A	184 ±23	162 ±23	*
	B	159 ±27	142 ±15	NS

4.34%, lactose by polarimetry 4.68% and calcium content 1.1%. The detail results of milk composition and properties are shown in Table 2.

It was compared visual and nephelo-turbidimetry method for determination of rennet coagulation time in raw and pasteurized milk. The rennet coagulation time determined by two different methods is shown in the Table 3.

It was used two different rennet for determination of coagulation time in this work. The RENNET A was added in the raw milk. The coagulation time determinated by visual method was 165 s and the coagulation time determined by nephelo-turbidimetry was 148 s. According **Bujko et al., (2011)** this rennet coagulation time is evaluated as "less good" because coagulation time is in range 140 – 200 s. The difference between the visual and the nephelo-turbidimetry method was 17 s. For raw milk, there is statistically significant difference between these methods.

The same samples of milk were obscured by RENNET B. The coagulation time determinated by visual method was 135 s and the coagulation time determined by nephelo-turbidimetry was 130 s. According **Bujko et al., (2011)** this rennet coagulation time is evaluated as "good" because coagulation time is in range 110 – 140 s. The difference between the visual and the nephelo-turbidimetry method was 5 s. This difference is not statistically significant.

The pasteurized milk had the worst rennet coagulation properties because heat treatment caused changes in solubility of calcium ions. The rennet coagulation time was increased by about 32 s compared to the raw milk. The pasteurized milk with RENNET A gave a statistically significant difference between both methods. However comparing these methods with RENNET B, it was found that there is no significant difference.

It was observed an influence of different amount $CaCl_2$ to rennet coagulation time. The results of rennet coagulation time are shown in the Table 4.

The rennet clotting time for pasteurized milk with 20 µL by visual method (RENNET A) was 150 s. The rennet clotting time by nephelo-turbidimetry was 134 s. Difference between these methods was 16 s with a statistically significant difference.

The rennet clotting time for pasteurized milk with 20 µL by visual method (RENNET B) was 129 s. The rennet clotting time by nephelo-turbidimetry was 124 s. Difference between these methods was 5 s. This difference between these methods is a statistically not significant.

This statistically not significant difference is due to the fact that RENNET B with $CaCl_2$ forms larger flakes which are better visibly detected by naked eye.

The higher addition than 20 µL of calcium chloride into the milk caused that the difference between the visual and the nephelo-turbidimetry methods is not statistically significant. With the increasing levels of calcium chloride begin to form the larger flakes without influence to type of rennet. The rennet coagulation time are decreasing with increasing the addition of $CaCl_2$. The difference

Table 4 The comparison of rennet coagulation time of pasteurized milk with the addition of different amount of $CaCl_2$ by the visual and the nephelo-turbidimetry methods (average value with standard deviation).

Groups of milk	Rennet	Visual method (s)	Nephelo-turbidimetry (s)	Significance
Pasteurized with 20 µL	A	150 ±20	134 ±11	*
	B	129 ±22	124 ±18	NS
Pasteurized with 30 µL	A	136 ±20	124 ±13	NS
	B	121 ±17	111 ± 9	NS
Pasteurized with 40 µL	A	124 ±19	119 ±15	NS
	B	108 ±17	109 ±15	NS

Note: * – significant difference at p <0.05; NS – nonsignificant difference at p >0.05.

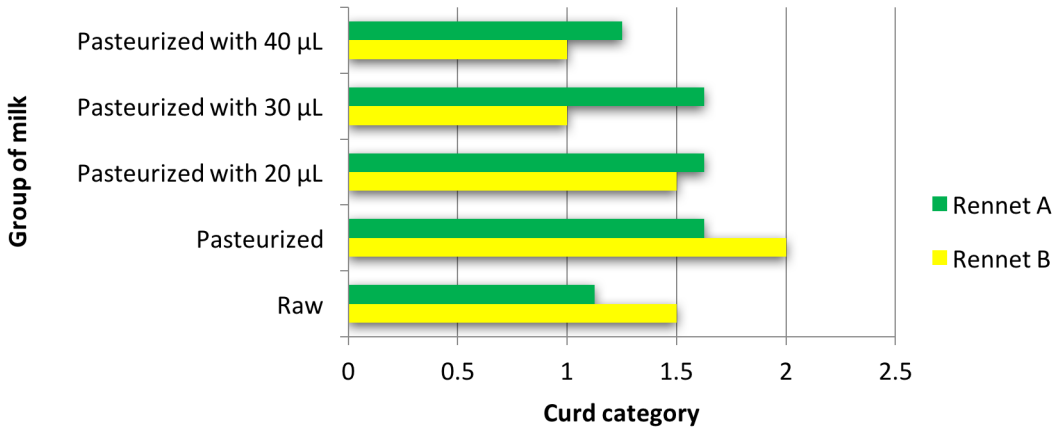

Figure 1 Curd category evaluation of milk group.

coagulation time between milk with addition of 20 µL $CaCl_2$ and 40 µL $CaCl_2$ was in range 21 s to 26 s by visual method. The identical results present **Erdem (1997)**, which studied the effect of $CaCl_2$ concentration on the clotting time. **Fox et al. (2004)** report, that the best rennet coagulation time had pasteurized milk with added calcium chloride. This finding was confirmed by our results – rennet coagulation time for milk with addition of 40 µL was 124 s while the rennet coagulation time for pasteurized milk was 184 s (RENNET A).

According to the study by **Čejna and Přibyla (2006)** the good milk coagulation property means that milk needs a short time to precipitate the milk by adding rennet. The results of this study confirmed possibility to reduce rennet coagulation time by addition of $CaCl_2$.

When was compared the curd quality, it was confirmed the premise that the heat treatment had an effect to curd quality. These curd were soft but the curd harder released a whey. The released whey was a slightly turbid. The curd quality was also affect by rennet which was used (Figure 1). At the higher amount $CaCl_2$ is better used a RENNET B. This curd was solid, keep the shape and whey was yellowgreen clear colour. At amount of 30 or 40 µL $CaCl_2$ was formed a first category of curd. At amount of 20 µL $CaCl_2$ was formed a worse curd category. The curd was a less solid and releasing of whey was more difficult. RENNET A formed a better curd category without addition of $CaCl_2$ or with an addition of 20 µL $CaCl_2$.

CONCLUSION

The visual method for determining rennet coagulation time is a subjective method, which is influenced by the experience of person monitoring rennet coagulation time, by the type of rennet and its concentration. It was obtained, that using nephelo-turbidimetry shown objective results for measuring the rennet coagulation time contrary the subjective visual method. There were found statistically non-significant differences in most of the measurements, comparing these methods. Using nephelo-turbidimetry was obtained the objective results for measuring the rennet coagulation time. Further, the results obtained by nephelo-turbidimetry are accurate and there are determined with the lower deviation. The determination rennet coagulation time by nephelo-turbidimetry is more expensive, but is unaffected by the experience of person monitoring these properties. Thus, this method can replace the subjective visual method.

The heat treatment, addition of $CaCl_2$ and using of different rennet had an influence on the curd category. For milk where is used a less amount of $CaCl_2$ or without $CaCl_2$ is better used RENNET A, which formed a better curd category. The combination of higher amount $CaCl_2$ with RENNET A is not suitable for formed good curd category. RENNET B is better used with higher amount of $CaCl_2$ for formed good curd category.

REFERENCES

Bittante, G. 2011. Modeling rennet coagulation time and curd firmness of milk. *Journal of Dairy Science*, vol. 94, no. 12, p. 5821-5832. http://dx.doi.org/10.3168/jds.2011-4514

Bujko, J., Kocman, R., Žitný, J., Trakovická, A., Hrnčár, C. 2011. Analyse of traits of milk production in dairy cows. *Potravinarstvo*, vol. 5, no. 1, p. 5-9. http://dx.doi.org/10.5219/93

Castillo, M. 2006. Cutting time prediction methods in cheese making. In Heldman, D. R., Moraru, C. I. *Encyclopedia of Agricultural, Food, and Biological Engineering*. 2nd ed. Boca Raton: CRC Press. p. 1-7. ISBN 13: 9781439811115

Čejna, V., Přibyla, L. 2006. Porovnání vizuální a nefelo-turbidimetrické metody pro měření syřitelnosti mléka (Comparison visual and nephelo-turbidimetry methods for rennet coagulation time of milk). *Den mléka 2006*. Praha: Česká zemědělská univerzita v Praze, p. 110-111. ISBN: 80-213-14898-2.

Chládek, G., Čejna, V. 2005. Měření syřitelnosti mléka pomocí nefeloturbidimetrického snímače (A measurement of rennet coagulation time by nephelo-turbidimetry). *Mléko a sýry 2005*. Praha: Česká společnost chemická, p. 127-130. *ISBN: 80-86238-48-2.*

Czech state standart No 57 0530. 1974. *Metody zkoušení mléka a tekutých mléčných výrobků (Methods for testing of milk and milk products)*, Praha: Vydavatelství Úřadu pro normalizaci a měření, 108 p.

EN ISO 8968-1:2002. *Mléko - Stanovení obsahu dusíku. Část 1, Metoda dle Kjeldahla (Milk and milk products – Determination of nitrogen content. Part 1, Kjeldahl method and crude protein calculation)*, Praha: Český normalizační institut, 16 p.

Erdem, Y. K. 1997. The affect of calcium chloride concentration and pH on the clotting time during the renneting of milk. *Gida*, vol. 22, no. 6, p. 449–455.

Fox, F., McSweeney, P. L. H., Cohan, T. M., Guinee, T. P. 2004. *Cheese Chemistry, Physics and Microbiology. Volume 1*. 3rd ed. Elsevier Ltd., Oxford. 617 p. ISBN-13: 978-0122636523

Fuquay, J. W., Fox, P. F., McSweeney, P. L. H. 2011. *Encyclopedia of Dairy Sciences*. 2nd ed. Amsterdam: Academic Press. 923 p. ISBN-13: 9780123744029.

Hardy, J., Fanni, J. 1981. Application of reflection photometry to the measurement of milk coagulation. *Journal of Food Science*, vol. 46, no. 6, p. 1956-1957. http://dx.doi.org/10.1111/j.1365-2621.1981.tb04531.x

Herbert, S., Riaublanc, A., Bouchet, B., Gallant, D. J., Dufour, E. 1999. Fluorescence spectroscopy investigation of acid- or rennetinduced coagulation of milk. *Journal of Dairy Science,* vol. 82, no. 10, p. 2056-2062. http://dx.doi.org/10.3168/jds.S0022-0302(99)75446-9

ISO 2446:2008. *Mléko - Stanovení obsahu tuku (Milk - Determination of fat content)*, Praha: Úřad pro technickou normalizaci, metrologii a státní zkušebnictví, 16 p.

ISO 6731:2010. *Mléko, smetana a zahuštěné neslazené mléko - Stanovení obsahu celkové sušiny (Milk, cream and evaporated milk - Determination of total solids content),* Praha: Úřad pro technickou normalizaci, metrologii a státní zkušebnictví, 12 p.

Klandar, A. H., Lagaude, A., Chevalier-Lucia, D. 2007. Assessment of the rennet coagulation of skim milk: A comparison of methods. *International Dairy Journal*, vol. 17, no. 10, p. 1151-1160. http://dx.doi.org/10.1016/j.idairyj.2007.03.005

Korolczuk, J., Maubois, J. L. 1988. Effect of pH, calcium concentration and temperature on the evolution of the refractometric signal produced during rennet coagulation of milk. *Journal of Dairy Research,* vol. 55, p. 81-88.

Kuchtík, J., Šustová, K., Urban, T., Zapletal, D. 2008. Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian

ewes. *Czech Journal of Animal Science,* vol. 53, no. 2, p. 55-63. ISSN: 12121819

Law, B. A., Tamime, A. 2010. *Technology of cheesemaking.* 2nd ed. Malden, MA: Blackwell, 482 p. ISBN-13: 978-140-5182-980.

Lucey, J. A., Fox, P. F. 1993. Importance of Calcium and Phosphate in Cheese Manufacture: A Review. *Journal of Dairy Science*, vol. 76, no. 6, p. 1714-1724. http://dx.doi.org/10.3168/jds.S0022-0302(93)77504-9

Marshall, R. J., Hatfield, D. S., Green, M. L. 1982. Assessment of two instruments for continuous measurement of the curd-firming of renneted milk. *Journal of Dairy Research,* vol. 49, p. 127-135. http://dx.doi.org/10.1017/S0022029900022202

Maxcy, R. B., Price, W. V., Irvine, D. M. 1955. Improving curd-forming properties of homogenized milk. *Journal of Dairy Science*, vol. 38, p. 80-86.

McMahon, D. J., Brown, R. J. 1982. Evaluation of formagraph for comparing rennet solutions. *Journal of Dairy Science*, vol. 65, no. 8, p. 1639-1642. http://dx.doi.org/10.3168/jds.S0022-0302(82)82390-4

Nájera, A. I., De Renobales, M., Barron, L. J. R. 2003. Effects of pH, temperature, $CaCl_2$ and enzyme concentrations on the rennet-clotting properties of milk: a multifactorial study. *Food Chemistry,* vol. 80, no. 3, p. 345-352. http://dx.doi.org/10.1016/S0308-8146(02)00270-4

Nassar, G., Nongaillard, B., Noel, Y. 2001. Monitoring of milk gelation using a low-frequency ultrasonic technique. *Journal of Food Engineering,* vol. 48, no. 4, p. 351-359. http://dx.doi.org/10.1016/S0260-8774(00)00178-3

O'Callaghan, D. J., O'Donnell, C. P., Payne, F. A. 2000. On-line sensing techniques for coagulum setting in renneted milks. *Journal of Food Engineering,* vol. 43, no. 3, p. 155-165. http://dx.doi.org/10.1016/S0260-8774(99)00145-4

Roginski, H., Fuquay, J. W., Fox, P. 2003. *Encyclopedia of Dairy Sciences*: *Volume 1*. New York: Academic Press, 557 p. ISBN-13: 0122272358.

Sbodio, O. A., Revelli, G. R. 2012. Milk's coagulation. Development of a device for online "monitoring" of the process. Progress made in Argentina. *Revista de Investigaciones Agropecuarias*, vol. 38.

Sojková, K., Hanuš, O., Dufek, A., Kopecký, J., Jedelská, R. 2011. Srovnání nefelometricky a tradičně stanovené koagulace proteinů syrového kravského mléka jako technologické vlastnosti (Compared nephelometry and traditionally determined protein coagulation of raw cow's milk as the technological properties). *Výzkum v chovu skotu / Cattle Research,* vol. 1, p. 52-59.

Sommer, H. H., Matsen, H. 1935. The relation of mastitis to rennet coagulability and curd strength of milk. *Journal of Dairy Science*, vol. 18, no. 11, p. 741-749. http://dx.doi.org/10.3168/jds.S0022-0302(35)93196-4

Wade, T., Beattie, J. K. 1998. situ observation of rennetting by electroacoustics. *Milchwissenschaft*, vol. 53, no. 9, p. 490-493.

Wolfschoon-Pombo, A. F. 1997. Influence of calcium chloride addition to milk on the cheese yield. *International Dairy Journal,* vol. 7, no. 4, p. 249-254 http://dx.doi.org/10.1016/S0958-6946(97)00005-8

Yuksel, Z. 2013. Determination of rennet clotting time by texture analysis method. *Food and Nutrition Sciences*, vol. 4, no. 11, p. 1108-1113. http://dx.doi.org/10.4236/fns.2013.411144

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STEREOLOGICAL ANALYSIS OF PEA PROTEIN IN MODEL SAMPLES

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ABSTRACT

With the growing popularity of various plant proteins used as raw materials for meat production, interest of manufacturers to extend the range of such raw materials is increasing as well. Manufacturers are trying to minimize the cost of manufacturing their products with simultaneous preserving the nutritional value of their products to the maximum extent possible. Such cheaper raw materials, which are also nutritionally rich, include pea protein. Another advantage for manufacturers is the fact that legislation does not order them to indicate pea protein presence in case of its addition, as it does for other allergenic ingredients, although this legume contains storage proteins which can cause a variety of allergic reactions, just like other legumes. Currently no method used for its qualitative determination has been described in literature, let alone its quantitative determination. Our work describes a possible method that can be applied for its quantification. It is a stereological method applied to microscopic sections stained by immunohistochemical staining based on the avidin-biotin complex using monoclonal legumin (1H9) as the primary antibody. The stereological method is based on geometry, it applies knowledge of geometry to analyze a sample of diverse origin, size and internal structure. Despite potential shortcomings in staining microscopic preparations, stereology allows us to perform quantification based on knowledge of morphology of the observed structures. This work describes a procedure of a known pea protein addition quantification in model meat products by means of Ellipse software. Pea protein quantification was performed in two ways. In the first case ten microimages of all sections prepared were examined, while in the second case one scan of the entire section was analyzed. Based on the results, Spearman's correlation coefficient was calculated, which confirmed our assumption of correlation between the protein added into the product and the measured area in microimages. In both ways Spearman's correlation coefficient was rSp = 1000. We obtained regression equations in MS Excel, which can be used for calculation of pea protein addition based on measured area of this protein in microscopic section.

Keywords: Vegetable proteins; microscopy; immunohistochemistry; allergens; meat products

INTRODUCTION

In the meat industry, raw materials in the form of vegetable proteins, which are used as a meat substitute, are very common **(Modi et al., 2004)**. During meat production is frequently used various vegetable and animal proteins. The most commonly employed plant - origin proteins are wheat and soy proteins. Meat product also contain from animal - origin proteins as plasma, collagen or milk protein **(Petrášová, 2015)**. Some of these vegetable proteins are classified as allergens in the legislation **(Regulation (EU) No. 1169/2011)**. Besides other reasons, this motivates the producers' efforts to replace them with other vegetable proteins that are not ranked among the allergens that must be indicated in harmony with the aforementioned legislation. Pea protein belongs among the most common ones **(Baticz, 2001)**. Like other legumes, however, pea proteins also include storage proteins which can cause allergic reactions. The literature identifies analysis of polyphenols characteristic of certain legumes and HPLC method which can detect up to 0.1% addition of soy protein in a meat product, as potential detection methods. Detection of lupine can be performed similarly, nevertheless, reliable detection of pea has not been achieved yet **(Mession et al., 2012; Mellenthin and Galensa, 1999)**. Other possible methods that can be used to detect vegetable protein are microscopic methods.

Microscopic methods belong among the oldest analytical methods and can be applied to demonstrate food components. These methods are simple, able to differentiate and identify individual basic components in the foodstuffs. The most commonly used methods in practice are histochemical methods, but now there is a wide range of options for processing and preparation of samples and also investigative techniques from classic to those that apply the most innovative technical equipment. Imaging techniques belong among the most suitable techniques to examine the structure of food **(Kaláb et al., 1995)**. As argued by **Tremlová et al. (2013)**, addition of vegetable protein can be detected using microscopic methods if they are present in the product in a sufficient size for light microscopy.

Javůrková et al. (2015) mentioned the use of modern microscopy methods for a qualitative as well as quantitative examination of the products. These methods provide information about location all components of the sample examined. One of the methods is image analysis.

Image analysis is often using as qualitative methods for meat products. The image obtained by microscopic methods can undergo quantitative analysis while preserving all the advantages of microscopy. In such a case, the input is image data and the output is a description of the image. Quantitative microscopic examination may be indicative or accurate **(Pospiech, 2008)**. Quantitative image analysis allows us to describe and specify all information obtained by microscopic (as well as macroscopic) scanning. It allows a detailed comparison of samples, accurate processing of information obtained and different ways of expressing the results achieved. The procedure for image analysis consists of creating photographs and their subsequent analysis using a program. To scan microscopic slides, a set composed of a light microscope and a digital camera or camcorder can be utilized. The very analysis involves creating a template (colour and brightness are usually selected from among image parameters) to identify the selected components and subsequently to measure their surface area and the entire section area. This results in numerical data obtained from the image, thereby permitting a detailed comparison of different samples, accurate processing of information obtained and different ways of expressing the results. Recorded data can be evaluated using different statistical methods. Another great advantage is the ability to compare objects scanned currently with objects stored previously. Integration of image analysis into the manufacturing process allows on-line measuring which is very useful, even necessary, in the inspection process in food production. The main advantage is the possibility to obtain a result without direct contact with the sample. This completely minimizes the risk of e.g. cross-contamination **(Javůrková, 2014)**. Image analysis based on computer technology is developing rapidly and allows to obtain objective results, because it uses a large number of images in statistical processing. This means that one of the biggest pitfalls of microscopy can be avoided, namely selecting and publishing only the best images as sample „representatives“ for demonstration of results and publishing **(Tremlová et al., 2013)**. Therefore, the literature considers the results obtained by image analysis in the examination of meat and meat products objective, accurate and comparable with data produced by chemical methods.

Development of image analysis in the field of microscopy largely coincides with the development of stereology. Stereology is based on geometry, it applies its knowledge to analyze samples of diverse origin, size and internal structure. It deals with statistical derivation of geometric properties of the examined structures and object from test probes applied to oriented sample sections **(Glaser and Glaser, 2000)**. Stereology is used by **Flintová and Meech (1978)** in their work. They used a method based on counting the points in a grid in quantifying textured soy protein, where estimated surface area of the object being measured was based on counting the area belonging to one point and the number of grid intersections with the object being measured. The advantage of this measurement includes its ease and affordability and the possibility to examine the image not only based on colour contrast, but also on the basis of morphological criteria. The disadvantage of stereology is manual processing that is time consuming and not always more accurate than automatic examination. Image analysis used as quantification method requires optimum contrast between the monitored component and other components in the product **(Aguilera and Stanley, 1990)**, while stereology does not have this requirement **(Lukášková Řezáčová, 2011)**.

An integral part of quantitative studies is statistical evaluation of results. Correctness of the analysis may be affected by so-called deflections of the measuring system itself, processing (various thickness of sections, uneven stainability, creation of artifacts, change of protocols etc.), examiner (whether in manual measuring or error rate in mathematical processing of results) or improper calibration of the digital recording collection equipment. In current practice, variability of sample processing can be reduced by standardizing and automating the examination workflow **(Tonar, 2008)**.

Currently, there is no commercially available method for demonstrating the addition of pea protein, let alone its quantification in a meat product. Therefore, the aim of our work was to create a method and protocols for its quantitative determination in meat products.

MATERIAL AND METHODOLOGY

Model meat products (MMP) containing pea protein additions in concentrations of 0.1, 1.0, 2.0, 3.0, 4.0 and 5.0% were produced for the examination. The products were made of ground chicken breast meat with the addition of pea protein. These model products were cooked at 70 °C for 10 minutes. Four blocks (A, B, C, and D) of 1 mL were collected from each product and frozen. These blocks were then sliced into sections 10 µm thick using cryostat HM 550 (Germany, Microm).

Subsequently, these cryosections were stained with immunohistochemical (IHC) staining method of ABC complex. The primary antibody used was monoclonal legumin (1H9). With respect to previous testing of immunohistochemical staining and consideration of costs, antibody concentration of 1:1000 was selected.

Quantification of the immunohistochemical examination results was performed in two different ways. First, quantification was performed in digital images of MMP, which had been taken in Eclipse E200 microscope (Nikon, JPN) using EOS 1100D camera (Canon, JPN) and processed by DSLR REMOTE Ver. 2.2.2.1 (UK) at a magnification of 100x (Ellipse 1). The entire sections were scanned in this way and a random selection of 10 images from all blocks of the sample was performed. As reported by **Řezáčová Lukášková (2011)**, who used stereology to quantify the addition of wheat protein in her work, in order to achieve the coefficient of error (CE) <0.2, at least 8 images of the sample with added proteins should be investigated.

Also, these samples were scanned using Eclipse Ci-L microscope (Nikon, JPN), DFK 23U274 camera (Imaging Source, GER) and motorized stage of Prosca III (Prior, USA) in NIS Elements Basic Research 04.13.04 software (Laboratory Imaging, Czech Republic) at magnification of 40x (Ellipse 1). Thanks to the motorized stage and NIS software, the entire sections could be scanned and

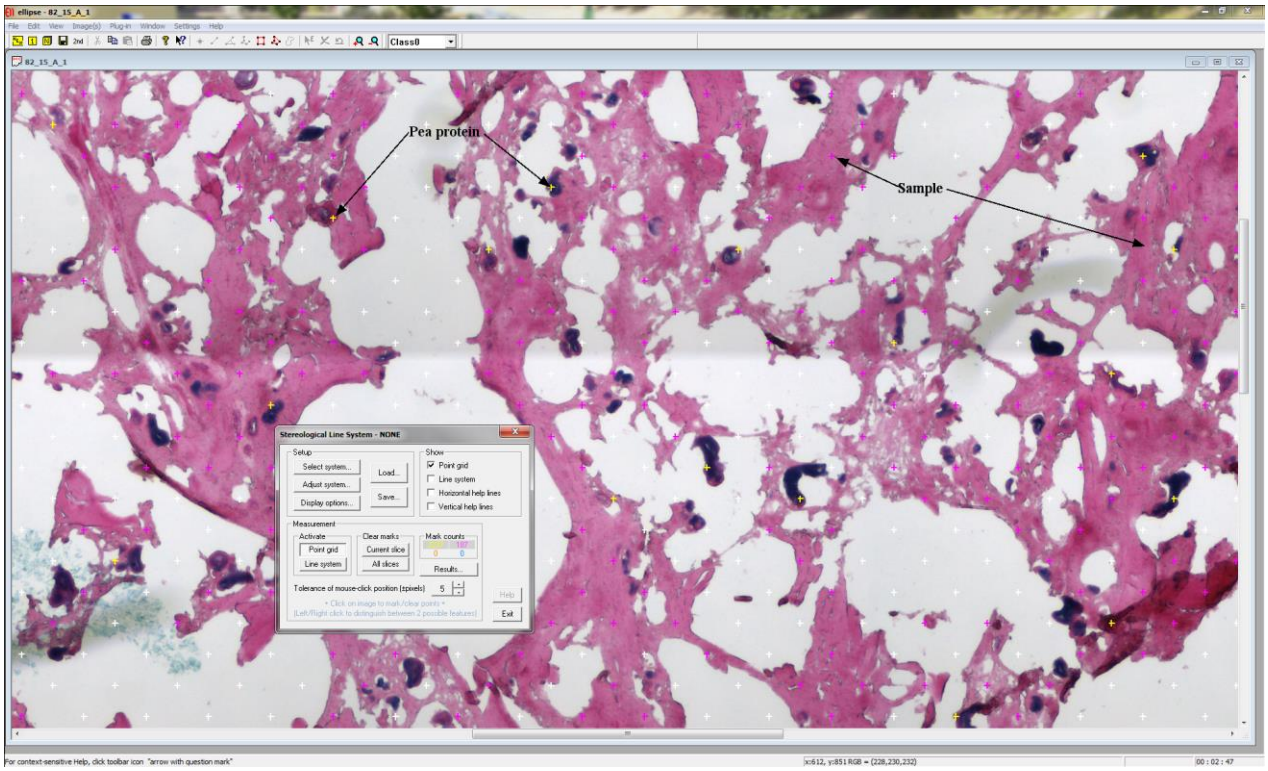

Figure 1 Example of pea protein quantification in the Ellipse software, sample no. 82_15 with 4% pea protein addition, IHC staining method, 40x magnification.

subsequently merged into a single image by the program and thus the Ellipse 1 software was able to examine 1 image (the entire section) from each sample.

Subsequently, the actual quantification of the pea protein addition was performed using Stereological Line System program by Ellipse version 2.0.7.1. (ViDiTo, Slovakia) (Figure 1) with adjusting the size of the grid point for the quantification of individual images and for the entire sections to 20745.5 μm^2 (a total of 157 points in the image) and 20764.8 μm^2 (a total of 7616 points in the image), respectively.

Results obtained by the stereological method of microimages of model meat product sections were contrasted to the contained values in the prepared concentrations of protein additions by means of the Spearman's correlation coefficient rSp (a nonparametric method that uses the order of values of the monitored variables in the calculation, and which can be used to describe any relation (linear and nonlinear). Relation of variables may have a generally upward or downward character **(Bedáňová and Večerek, 2007)**. The coefficients were calculated in the UNISTAT ver. 6.0 software. Moreover, a regression analysis (studying what relationship exists between the variables – linear, quadratic, logarithmic, etc. – and how a dependent variable Y changes depending on changing its predictor (independent variable) X. It is thus a one-sided dependence, unlike the correlation analysis studying bilateral reciprocal relation between two random variables was performed in MS Excel **(Bedáňová and Večerek, 2007)**.

RESULTS AND DISCUSSION

The measured areas of pea protein added for each concentration for both methods of scanning are listed in Table 1 and Figure 2. Table 1 and Figure 2 compare addition of proteins in the weight percentage and section areas in area percentages measured. The results indicate that with increasing addition of the proteins increases the measured area in section by lowest concentration (0.1 percentage).

Figure 3 illustrates a comparison of the examined areas of microscopic slides of model meat product samples when the examined area was 2.9x to 4.7x greater in the event of Ellipse 2 than the examined area Ellipse 1.

Using the first method of capturing images (Ellipse 1) by means of the Ellipse SW, a total of 60 images (10 images of a sample for each concentration) were quantified. Protein surface areas of 1.71%, 3.40%, 6.18%, 8.47%, 9.42%, and 11.26% were detected for the meat product samples with pea protein additions of 0.1%, 1.0%, 2.0%, 3.0%, 4.0%, and 5.0%, respectively. Based on the calculated Spearman's correlation coefficient, statistical dependence (rSp = 1000) was demonstrated for each concentration of pea proteins addition in model meat products.

In the latter method of capturing images (Ellipse 2), where sections were scanned whole, six images (one image of the entire section for each concentration) were examined. Protein surface areas of 0.66%, 2.82%, 4.46%, 6.09%, 7.71%, and 9.52% were measured for pea protein additions of 0.1%, 1.0%, 2.0%, 3.0%, 4.0%, and 5.0%, respectively. Statistical relation was also confirmed by the calculated Spearman's correlation coefficient (rSp = 1000), which confirm high dependence between pea protein addition and measured area of this protein in microscopic sections.

Table 1: Measured areas of pea protein for each MMP concentration.

Prepared MMP concentrations	Measured area of proteins [%] by Ellipse SW	
	Ellipse 1	Ellipse 2
0.1	1.71	0.66
1.0	3.40	2.82
2.0	6.18	4.46
3.0	8.47	6.09
4.0	9.42	7.71
5.0	11.26	9.52

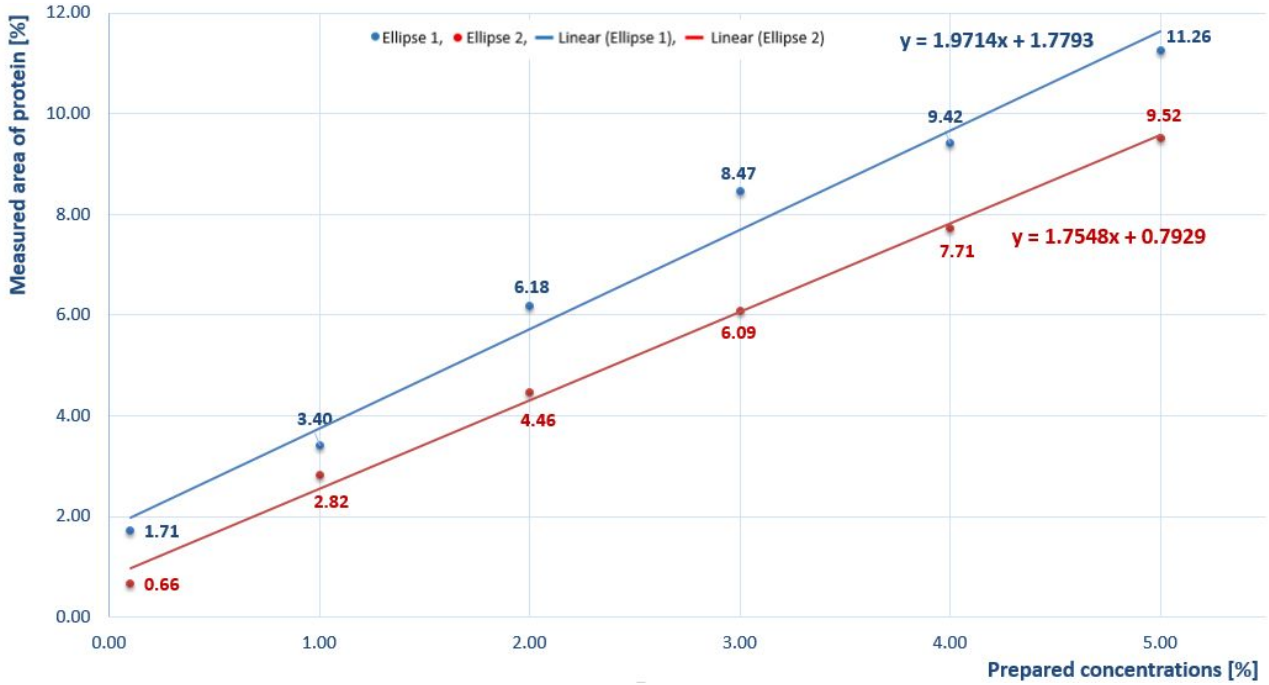

Figure 2 Dependence of protein area measured by Ellipse on the prepared concentrations.

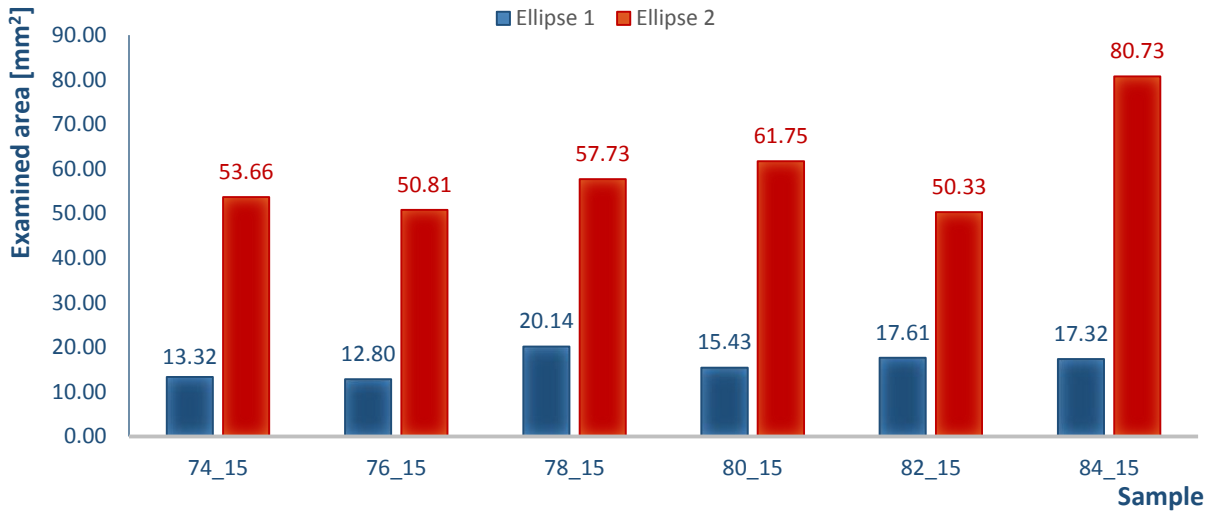

Figure 3 Comparison of areas of the examined sections.

Note: Sample number 74_15 contains 0.1% of pea protein, sample number 76_15 contains 1.0% of pea protein, sample number 78_15 contains 2.0% of pea protein, sample number 80_15 contains 3.0% of pea protein, sample number 82_15 contains 4.0% of pea protein and sample number 84_15 contains 5.0% of pea protein.

In addition to evaluating both procedures of capturing images and their results, regression analysis of the results obtained, which evaluates the dependence of quantitative statistical features, was also conducted. Obtained regression equations are shown in Figure 2. Regression equations can be used for calculation of pea protein addition based on measured area of this protein in microscopic section.

CONCLUSION

Based on the results obtained and calculated Spearman's correlation coefficients, hypothesis regarding the suitability of stereology for the quantitative determination of pea protein additions in model meat products was confirmed. As reported by **Aguilera and Stanley (1990)**, stereological quantification is more time consuming than image analysis. However, in view of incompletely 100% results of immunohistochemical staining, where the image analysis software would fail to mark the protein automatically leading to an incorrect result, this method appears to be appropriate. Also, reduction of stereological points in the grid and thus shortening the time for the quantification itself is worth considering. In case of using scans of entire section, one section would be enough for the quantification, which would also shortened the examination.

REFERENCES

Aguilera, J. M, Stanley, D. W. 1999. *Microstructural principles of food processing & engineering*. New York: Elsevier Science Publishers LTD, ISBN 978-0-8342-1256-5.

Baticz, O. 2001. Isolation and study of the functional properties of pea proteins. *Nahrung/Food*, vol. 45, no. 6, p. 399-401.

Bedáňová, I., Večerek, V. 2007. *Basics of statistics for studying veterinary medicine and pharmacy*. 1st Ed. Brno: University of Veterinary and Pharmaceutical Sciences Brno, 130 p.

Flint, O. F., Meech, M. V. 1978. Quantitative determination of texturised soya protein by a stereological technique. *The Analyst*, vol. 103, no. 1224, p. 252-258. http://dx.doi.org/10.1039/an9780300252

Glaser, J. R., Glaser, E. M. 2000. Stereology, morphometry, and mapping: the whole is greater than the sum of its parts. *Journal of Chemical Neuroanatomy*, vol. 20, no. 1, p. 115-126. http://dx.doi.org/10.1016/S0891-0618(00)00073-9

Javůrková, Z. 2014. *Food quality evaluation by image analysis*. Brno, 133 p. Doctoral thesis on Faculty of Veterinary Hygiene and Ecology on University of Veterinary and Pharmaceutical Sciences Brno. Leader dissertation doc. MVDr. Bohuslava Tremlová, Ph.D.

Javůrková, Z., Pospiech, M., Zelenková, M., Kameník, J., Petrášová, M., Tremlová, B. 2015. Microscopic determination of bamboo fiber in meat products. *Potravinarstvo*, vol. 9, no. 1, p. 190-194. http://dx.doi.org/10.5219/452

Kaláb, M., Wojtas, P. A., Miller, S. S. 1995. Microscopy and other imaging techniques in food structure analysis. *Trends in Food Sciences and Technology*, vol. 6, no. 6, p. 177-186. http://dx.doi.org/10.1016/S0924-2244(00)89052-4

Lukáškova, Z. Řezáčová, Tremlová, B., Pospiech, M., Renčová, E., Randulová, Z., Steinhauser, L., Reichová, A., Bednář, J. 2011. Comparison of immunohistochemical, histochemical and immunochemical methods for the detection of wheat protein allergens in meat samples and cooked, dry, raw and fermented sausage samples, *Food Additives and Contaminants Part A-Chemistry Analysis Control Exposure & Risk Assessment*, vol. 28, no. 7, p. 817-825. http://dx.doi.org/10.1080/19440049.2011.572292

Mellenthin, O., Galensa, R. 1999. Analysis of polyphenols using capillary zone electrophoresis and HPLC: detection of soy, lupin, and pea protein in meat products. *Journal of agricultural and food chemistry*. vol. 47, no. 2, p. 594-602. http://dx.doi.org/10.1021/jf980749h

Messiona, J. L., Assifaouia, A., Cayota, P., Saurela, R. 2012. Effect of pea proteins extraction and vicilin/legumin fractionation on the phase behavior in admixture with alginate. *Food hydrocolloids*. vol. 29, no. 2, p. 335-346. http://dx.doi.org/10.1016/j.foodhyd.2012.03.003

Modi, V. K., Mahendrakar, N. S., Narasimha Rao, D. Sachindra, N. M. 2004. Quality of buffalo meat burger containing legume flours as binders. *Meat Science*, vol. 66, no. 1, p. 143-149. http://dx.doi.org/10.1016/S0309-1740(03)00078-0

Petrášová, M., Pospiech, M., Tremlová, B., Randulová, Z. 2015. Immunofluorescence detection of milk protein in meat products. *Potravinarstvo*, vol. 9, no. 1, p. 101-105, http://dx.doi.org/10.5219/431

Pospiech M. 2008. *Histochemical and immunohistochemical methods in food analysis with focus on soya protein detection*. Brno, 133 p. Doctoral thesis on Faculty of Veterinary Hygiene and Ecology on University of Veterinary and Pharmaceutical Sciences Brno. Leader dissertation doc. MVDr. Bohuslava Tremlová, Ph.D.

Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October. Official Journal of the European Union. 2011, L 04, p. 18-63.

Řezáčová Lukášková, Z., Pospiech, M., Tremlová, B., Randulová, Z. 2011. Quantification of immunohistochemical detection of wheat protein in model samples. *Proteins 2011*, Zlín, p. 104-107.

Tremlová, B., Pospiech, M., Randulová, Z. 2013. *Microscopic methods in the analysis of meat and meat products*. 1st ed. Brno: knihovnicka.cz, 133 p.

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PHTHALATES IN MEAT PRODUCTS IN DEPENDENCE ON THE FAT CONTENT

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ABSTRACT

The content of dibutylphthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in samples of packages intended for thermally processed meat products and release of phthalates from packages into meat products in dependence on the fat content were observed. 80 samples of packages were analyzed, 5 of them wereselected due to exceeding the specific migration limit. The raw meat was prepared, one type with the fat content of 10% and second one with the fat content of 50%. The both types of raw meat were analyzed for the content of DBP and DEHP and packed into chosen packages.The samples of meat products were thermally processed (70 ℃, 10 min in the core), stored until the expiration date at 4 °C and gradually analyzed after 1st, 7th, 14th, 21st and 28th day of storage. Determination of phthalates was carried out by high performance liquid chromatography (HPLC) in the Zorbax Eclipse C8column and by UV detection at a wavelength of 224 mm. The phthalate content in the raw meat was under the limit of detection. According to the EU Commission Regulation no. 10/2011 the specific migration limit of products intended for the contact with food for DEHP (max. 1.5 mg.kg^{-1}of food stimulant and DBP max. 0.3 mg.kg^{-1} of food stimulant), wasexceeded already after first day of storage, in case of DBP in two samples with 10% of fat and after 7th day of storage in one sample. In the samples with 50% of fat, SML was exceeded after first day of storage in four samples and in one sample after 14th day of storage. Regarding DEHP in the samples with 10% of fat SML was exceeded after 1st day of storage in one sample and after 7th day of storage also in one sample and after 21st day of storage similarly in one sample. Four samples with 50% of fat had SML exceeded in case of DEHP already after 1st day of storage. By comparison of PAE migration depending on the fat content we concluded that leaching of PAE from a package into food was 2 – 21 times higher in samples with 50% of fat than in samples with 10% of fat.

Keywords: phthalates; DBP; DEHP; package; migration; fat content

INTRODUCTION

Phthalates are synthetic substances used mainly as plasticizers of polyvinyl chloride (PVC). As additives, they provide plastics with softness and flexibility. Their wide spectrum of use results in the contamination of the environment since pthalates are not firmLy bound by a covalent bond in the plastic, and can leach out, migrate or evaporate into the surrounding air, atmosphere, food or other materials. Phthalates enter the human body via ingestion, inhalation or dermal transfer throughout life, and even during intrauterine development. Due to the potential risks posed to human health and the environment, some phthalates have been added to the list of priority pollutants of the European Union. Although phthalates are not persistent substances, due to the predominance of ingestion when compared to metabolic conversion, the parent compounds and metabolites cumulate in the bodies of both animals and humans. These substances do not remain in the body for long. However, throughout their stay, they are responsible for serious health issues **(Heudorf et al. 2007)**.

Regarding the fact that phthalates (PAE) are not tightly bound in polymer matrices, they can easily migrate from products to the hydrosphere, atmosphere and biosphere during production, usage and liquidation. Regarding the wide use and resistance to microorganism they became omnipresent in the environment and they were found almost in all elements of the environment in the whole world such as soil, precipitation, surface water, sea water, underground water, sediment, biota, air, waste water and sewage sludge **(Schiedek et al., 1995, Zhang et al., 2014, Selvaraj et al., 2015, Net et al., 2015, Wang et al., 2014,Wang et al., 2002)**.

Humans are exposed to phthalates through several ways of exposition including breathing, nutrition and dermal absorption **(Das et al., 2014, Meng et al., 2014)**. The main source of exposition for a human is food, therefore various types of food were researched in various countries of the world from the perspective of phthalate contamination **(Das et al., 2014)**. Phthalates may enter into the food chain also through migration from contact materials during growing, production, storage or even during food preparation in a household.

Evaluation of the dietary intake of phthalates in the Belgian adult population revealed that there is the highest intake of DEHP, followed by di-n-butyl phthalate (DNBP), butyl benzyl phthalate (BBP) and diethyl phthalate (DEP). The groups of food, which contribute to the dietary

exposition to the stated group of phthalates include grain and grain products, milk and dairy products, meat and meat products. According to the executed research the intake of DEHP through food was found ($mg.kg^{-1}.d^{-1}$) in Great Britain, Denmark, Germany, France in the values of 3.40 - 4.00; 2.70 - 4.30; 14.0 and 1.46 respectively **(Yang et al., 2015, Fierens et al., 2014a, Ji et al., 2014, Fierens et al., 2014b)**.

Another studies for example dealt with the phthalate content in caps of beer bottles, in olive oil, in infant feeding bottles, yogurt packages and packages intended for microwave ovens. It was also found that with an increased temperature during preparation there is increasing danger of migration of phthalates from packages into food **(Gonzales-Castro et al., 2011, Li et al., 2012, Nanni et al., 2011)**.

The objective of the work was to carry out the analysis of packages used for production of thermally processed meat products. Another objective was to create products with various fat content, fill them into packages and observe if there occurs release of phthalates into a meat product after thermal processing.

MATERIAL AND METHODOLOGY

Packages (n = 80) used for meat products were supplied by a German company. They included plastic and cellulose packages with printing intended for production of thermally processed meat products. 5 packages were used for filling of the products, in which the specific migration limit was exceeded. The samples were analyzed twice. In total, 80 packages were analyzed and 160 analyses were carried out.

Meat products intended for thermal processing were produced in pilot production conditions at the Department of Food Technology at Mendel University in Brno. Two types of raw meat were prepared (the fat content 10% and 50%), which were filled into the packages (n = 5). For every fat content there were 30 samples produced. The samples were stored at 4 °C and they were taken for analysis after 1st, 7th, 14th, 21st and 28th day of storage. The samples were analyzed twice.

In total, there were 12 samples of raw meat analyzed, 300 samples of meat product and there were 624 analyses carried out.

The package samples were leached in the mixture of dissolvent *n*-hexan:dichlormethan (1:1) for 72 hours and subsequently three times extracted (60, 30, 30 minutes). The combined extracts were filtered, evaporated on a rotary vacuum evaporator and dried by nitrogen. Afterwards, the extract was transferred into vials and centrifuged by hexane (5 mL). The top part of the extract (1.5 mL) was isolated into a vial for determination by HPLC (high-performance liquid chromatography) and dried by nitrogen. Vials were again centrifuged, the top part of the extract was isolated (1.5 mL), dried by nitrogen and subsequently the vials were filled up to the volume of 1 mL. If the extracts were colored or bleary, they were cleared by sulfuric acid.

The verified methods for determination of DBP and DEHP in food were used for the analysis of PAE in the samples of raw meat and meat products **(Jarošová et al., 1998, 1999).**

The samples of raw meat and meat products were homogenized, weighed on metal bowls and frozen. Gradually, the frozen samples were lyophilized and subsequently residues of PAE were extracted by n-hexane. PAE was separated from the co-extracts by gel permeation chromatography on the gel Bio beads S-X3. The cleaning procedure with concentrated sulfuric acid was used for completion of cleaning of eluates. Phthalates were determined by the HPLC method with column of Zorbax Eclipse C8 and UV detection at a wavelength of 224 mm. The injection of samples on the column was 10 µl. The final concentrations were calculated based on the calibration curve by the software Agilent Chemstationfor LC and LC/MS systems. The scope of the calibration curves for DBP was from 1.06 $\mu g.mL^{-1}$to106.00 $\mu g.mL^{-1}$ and for DEHP from 1.01 $\mu g.mL^{-1}$ to 100.50 $\mu g.mL^{-1}$. The correlation coefficient for DBP was 0.9999 and for DEHP also 0.9999. The detection limit for DBP was 0.05 $\mu g.mL^{-1}$ and for DEHP 0.11 $\mu g.mL^{-1}$. The results were statistically processed by the program Statistica 12.

The majority of lab glass was flushed by hexane during preparation of samples. At the same time the dry matter and fat content were determined. Concentrations of DEHP and DBP are related to an initial sample.

RESULTS AND DISCUSSION

The concentration of phthalates in the analyzed packages are expressed in$\mu g.dm^2$ and they are stated in Table 1. Every value represents an average from two parallel determinations.

According to the EU Commission Regulation No. 10/2011 for products intended for the contact with food and dishes, a package cannot release its own components into food in the quantity exceeding10 $mg.dm^2$ or 60 $mg.kg^{-1}$ of food or food stimulant. The stated regulation includes also the specific migration limit which is max. 1.5 $mg.kg^{-1}$ of food stimulant for DEHP and max. 0.3 $mg.kg^{-1}$ of food stimulant for DBP.

Tab. 1 Average concentration of DBP and DEHP ($\mu g.dm^{-2}$) in samples of packages used of packing of meat products

Sample	DBP	DEHP
	$\mu g.dm^{-2}$	
1	21.55	95.45
2	14.12	64.75
3	18.35	88.12
4	39.13	134.97
5	27.43	108.61

From all the analyzed packages (n = 80) there were five packages selected, in which the highest concentration (Table 2) of the both phthalates occurred and there was the assumption that they may contaminate final products after thermal processing and storage. The values of DBP in packages moved from 14.12 to 39.13 $\mu g.dm^{-2}$ and in case of DEHP from 64.75 to 134.97 $\mu g.dm^{-2}$.

The phthalate concentrations (DBP and DEHP) in the raw meat and meat products are expressed in $mg.kg^{-1}$ of the initial sample and they are stated in Table 2. Every value represents an average of 12 values (6 parallel samples and every sample analyzed twice).

In the samples separated immediately after mixingthe raw meat, the concentration of phthalates moved under the limit of detection (≤ 0.2 $mg.kg^{-1}$).

In the first sample of product with 10% of fat, DBP gradually increased from the value 0.21 (after first day of storage) up to 0.68 $mg.kg^{-1}$of the initial sample. In the sample with 50% of fat it reached the values from 3.64 up to 4.7 $mg.kg^{-1}$ of the initial sample. Based on the measured values we can state that at the end of expiration period (28^{th} day) the DBP content was approximately 7x higher in products with 50% of fat than in products with 10% of fat. DEHP reached in the sample with 10% of fat values from 2.19 to 3.57 $mg.kg^{-1}$ of the initial sample and in the sample with 50% of fat from 4.96 to 6.48 $mg.kg^{-1}$ of the initial sample. At the end of the expiration period, there was 2x higher content of DEHP in the product with 50% of fat than in the product with 10% of fat.

In the second sample of product with 10% of fat there was DBP detected only at the end of the expiration period (0.22 $mg.kg^{-1}$ of the initial sample), but it did not exceed SML (0.3 $mg.kg^{-1}$). In the sample with 50% of fat, DBP reached values from 1.18 to 3.61 $mg.kg^{-1}$ of the initial sample. At the end of the expiration period there was 16x higher content of DBP in the product with 50% of fat than in the product with 10% of fat. DEHP in samples with 10% of fat was, similarly as in case of DBP, detected only at the end of the expiration period (0.40 $mg.kg^{-1}$ of the initial sample) but it did not exceed SML (1.5 $mg.kg^{-1}$). In the sample with 50% of fat its content ranged from 4.81 to 8.34 $mg.kg^{-1}$ of the initial sample. At the end of the expiration period, there was 21x higher content of DEHP in the product with 50% of fat than in the product with 10% of fat.

In the third sample of product, DBP was not detected in any sample of both fat contents (10% and 50%) during the whole time of expiration period. DEHP was detected in the sample with 10% of fat only at the end of the expiration period (0.20 $mg.kg^{-1}$ of the initial sample) but it did not exceed SML (1.5 $mg.kg^{-1}$). In the sample with 50% of fat its content ranged from 1.26 to 2.5 $mg.kg^{-1}$ of the initial sample, but it exceeded SML only after 7^{th} day of storage. At the end of expiration period, there was 13x higher content of DEHP in the product with 50% of fat than in the product with 10% of fat.

In the fourth sample of product with 10% of fat DBP gradually increased from the value 0.72 (after first day of storage) up to 1.30 $mg.kg^{-1}$ of the initial sample. In the sample with 50% of fat it reached values from 0.85 up to 1.63 $mg.kg^{-1}$ of the initial sample. At the end of the expiration period (28^{th} day), the content of DBP in both products (10% and 50%) did not differ too much. DEHP in the sample with 10% of fat reached values from 0.77 to 2.01 $mg.kg^{-1}$ of the initial sample and in the sample with 50% of fat from 3.87 to 11.67 $mg.kg^{-1}$ of the initial sample. At the end of the expiration period (28^{th} day) there was almost 6x higher content of DEHP in the product with 50% of fat than in the product with 10% of fat.

In the fifth sample of product with 10% of fat, DBP gradually increased from the value of 0.68 (after first day of storage) up to 1.19 $mg.kg^{-1}$of the initial sample. In the sample with 50% of fat it reached values from 3.60 up to 7.95 $mg.kg^{-1}$of the initial sample. Based on the measured values we can state that at the end of expiration period (28^{th} day) there was 7x higher content of DBP in the product with 50% of fat than in the product with 10% of fat. DEHP in the sample with 10% of fat reached values from 1.44 to 2.89 $mg.kg^{-1}$of the initial sample and in the sample with 50% of fat from 7.12 to 8.54 $mg.kg^{-1}$of the initial sample. At the end of expiration period there was 3x higher content of DEHP in the product with 50% of fat than in the product with 10% of fat. There was confirmed highly statistically significant evidence of migration of DBP and DEHP depending on the fat content (p=0,000**) and depending on the period of storage (p=0,000**).

According to the EU Commission Regulation No. 10/2011 there was exceeded the specific migration limit already after 1^{st} day of storage in case of DBP in two samples with 10% of fat (4 and 5) and after 7^{th} day of storage in one sample (1). In samples with 50% of fat there was SML exceed already after first day of storage in four samples (1, 2, 4, 5) and in one sample (3) after 14^{th} day of storage.

In case of DEHP in the sample with 10% of fat there was SML exceeded after 1^{st} day of storage in one sample (1), after 7^{th} day of storage also in one sample (5) and after 21^{st} day of storage in one sample (4). The samples (1, 2, 4, 5) with 50% of fat in case of DEHP exceeded SML already after 1^{st} day of storage.

Based on the results (Table 1) we can state that DBP content in packages contributed by 20 % and in case of DEHP by 80% to the overall content of PAE. This finding corresponds with results of cumulation in meat products, higher concentration in meat products were found in case of DEHP (Table 2). By the comparison of PAE depending on the fat content we concluded that leaching of PAE was 2 – 21 times higher in samples with 50% of fat than in samples with 10% of fat.

Our experiment pointed at the fact that the content of plasticizers leached from packages increases with temperature, period of storage and fat content and this finding corresponds also with results of another authors. There was found increasing average concentration of DEHP in pasteurized skimmed milk (20 $\mu g.kg^{-1}$), in comparison to full fat milk (35 $\mu g.kg^{-1}$) and cream (1400 $\mu g.kg^{-1}$) **(Castle et al., 1990)**. Values of DEHP correlating with the fat content in milk were also confirmed in the study of Sharman et al. (1994). In milk with up to 1% of fat there was detected DEHP in the range of 0.02 – 0.04 $mg.kg^{-1}$, 0.05 $mg.kg^{-1}$of DEHP in milk with 1% of fat, 0.10 – 0.38 $mg.kg^{-1}$ in milk with 3% of fat and 1.06 – 1.67 $mg.kg^{-1}$ in cream with 35% of fat **(Sharman et al., 1994)**. **Shuangling and Kangquan (2009)** found that migration of DEHP from a PVC foil into meat increased with rising temperature and time.

The maximum migration was recorded at 90 ˚C and 30 minutes of effecting (75.12 mg.dm^{-2}).
The overall migration limit (10 mg.dm^{-2}) was exceeded in all observed combinations of time and temperature, except for the combination of 10 ˚C and <41 hours when migration was not observed.

Barros et al. (2011) in his study executed the analysis of food that might be contaminated by DEHP and di-etylhexyladipátem (DEHA). There were observed 18 different types of food with less than 3% of fat with possibility to be packed in plastic foils. The study proved that all food was contaminated by DEHP and DEHA while the content of observed phthalates was increasing with the period of storage.

Guo et al. (2010) proved a decreasing tendency in DEHP content with increasing distance from the surface. The authors monitored the migration of DEHP from the packaging film into ham sausages with relatively low fat content. The DEHP content in the sausages dropped significantly as the distance from the surface increased. The DEHP concentration was 8.7 mg.g^{-1} in the packaging film and 206.5 ng.g^{-1} in the first outer layer of the sausage. The first and second layer contained approximately 90 % of the total DEHP amount which migrated from the packaging. Significant levels of DEHP in the inner layers of the sausages were detected only after six months of storage.

A study by **Wang et al. (2015)** investigated the presence of phthalates in greenhouse soils and vegetables. Wang et al. monitored dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP) and di-n-octyl phthalate (DnOP) content which was analysed in 44 vegetables grown in greenhouses made of plastic film and in the corresponding soil. The total phthalate content ranged from 0.51 to 7.16 mg.kg^{-1} in vegetables and from 0.4 to 6.20 mg.kg^{-1} in soils with an average concentration of 2.56 and 2.23 mg.kg^{-1}. DnBP, DEHP and DnOP contributed to the overall phthalate content in vegetables and soils in more than 90%, but the ratios of DnOP and DnBP in vegetables were significantly (p <0.05) higher than in soils. The average concentration of phthalates in mustard, celery and lettuce was >3.00 mg.kg^{-1} but <2.50 mg.kg^{-1} in the corresponding soil. Stems and leaves of the vegetables accumulated larger amounts of phthalates. No mutual relationship was detected between the phthalate content in vegetables and in the soils.

Tsumara et al. (2001) observed the content of phthalates in ten samples of lunch half-products packed in plastic packages. The quantity of DEHP in the samples moved from 45 to 517 ng.g^{-1}, with the average value of 198 ng.g^{-1}. DBP was not detected in any sample.

The main source of phthalates in food, especially in those with a high fat content, is their direct contact with surfaces of production equipment and package materials. **Tsumara et al. (2001)** demonstrated increase of DEHP in chicken. From the initial value 0.080 mg.kg^{-1} before thermal processing the content of DEHP increased to 13.10 mg.kg^{-1}after frying at a nonstick pan and further to 16.90 mg.kg^{-1} after packaging.

In a study by **Moreira et al. (2015)**, the content of 8 plasticisers in spices and in roast chicken meat stored in plastic bags was monitored. The values detected ranged between 0.01 and 0.18 g.kg^{-1}. The samples showed presence of diisobutyl phthalate and dibutyl phthalate. The highest concentration of plasticisers was detected in spice used for roasting chicken meat.

A study by **Wang et al. (2013)** discussed the migration behaviour of 9 phthalate plasticizers in food with higher fat content, and the influence of temperature on the migration amount of these substances. The studied substances were: dimethyl phthalate (DMP), diethyl phthalate (DEP), diallyl phthalate (DAP), diisobutyl phthalate (DIBP), dibutyl phthalate (DBP), benzylbutyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), diisononyl ortho-phthalate (DINP) and diisodecyl ortho-phthalate (DIDP). The results have shown that the thickness of the plastic film is an essential factor in the process of phthalate migration. Another important condition in the study of the migration behaviour was

Table 2 Average concentrations of DBP and DEHP (mg.kg^{-1}) in the samples of raw meat and meat products (n = 5) with the fat content of 10 % and 50 % after 1st, 7th, 14th, 21st and 28th days of storage at 4 °C.

Sample	FAT (%)	Product		1st day		7th day		14th day		21st day		28th day	
		DBP	DEHP	DBP	DEHP	DBP	DEHP	DBP	DEHP	DBP	DEHP	DBP	DEHP
1	10	ND		0.21	2.19	0.45	3.27	0.45	3.39	0.47	3.42	0.68	3.57
	50			3.64	4.96	3.86	5.02	4.64	6.33	4.66	6.4	4.7	6.48
2	10			ND	ND	ND	ND	ND	ND	ND	ND	0.22	0.4
	50			1.18	4.81	2.37	6.45	2.42	6.65	3.08	8.21	3.61	8.34
3	10			ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	50			ND	1.26	ND	1.66	ND	1.8	ND	1.84	ND	2.5
4	10			0.72	0.77	0.8	0.98	0.83	1	1.22	1.75	1.3	2.01
	50			0.85	3.87	1.08	8.53	1.17	9.55	1.56	11.3	1.63	11.67
5	10			0.68	1.44	0.69	1.47	0.72	1.5	0.91	2.34	1.19	2.89
	50			3.6	7.12	4.92	7.94	6.31	8.04	6.68	8.5	7.95	8.54

Note: ND – the limit of detection of DBP and DEHP in fat matrices – 0.2 mg.kg^{-1}.

temperature. Measurements have proven that higher temperature accelerates the transfer and the migration of phthalate plasticisers increases. Each of the studied substances was affected differently by the increasing temperature. For instance, DINP and DIDP were affected minimally, since equilibrium was established and increasing the temperature did not change the migration amount. The migration mount measured in the temperature range of 5°C to 70°C ranged between 80 and 350 $mg.kg^{-1}$ for DMP, 75 to 375 $mg.kg^{-1}$ for DEP, 75 to 350 $mg.kg^{-1}$ for DAP, 50 to 350 $mg.kg^{-1}$ for DIBP, 75 to 325 for DBP $mg.kg^{-1}$, 100 to 275 $mg.kg^{-1}$ for BBP and 110 to 170 $mg.kg^{-1}$ for DEHP. The migration amount for DINP and DIDP reached equilibrium. This equilibrium migration amount for DINP was 140 $mg.kg^{-1}$ and for DIDP 160 $mg.kg^{-1}$. The migration values of phthalate plasticisers differ.

In dairy products, more than 80% of the total concentration of phthalates ranging from 50 to 200 $\mu g.kg^{-1}$ in ordinary milk came from suction machines. Further processing and packaging may lead to increase of the DEHP concentration in cream and cheese products **(Casajuan a Lacorte, 2004).**

CONCLUSION

The objective of the work was to observe the content of phthalates (DBP and DEHP) in packages intended for packing of meat products and observation of potential migration of phthalates from packages into products after thermal processing and storage until the expiration time depending on the fat content.

According to the EU Commission Regulation no. 10/2011 in comparison to the specific migration limits for DBP (0.3 $mg.kg^{-1}$) and DEHP (1.5 $mg.kg^{-1}$), 3 samples of packages stated in Table 1 (1, 4, 5) would not meet the regulation with respect to the specific migration limit (Table 2) after 1st day of storage. The samples of packages 2 and 3 would be suitable during the whole time of storage if it would be filled with theraw meat with the fat content of 10% (Table 2).

REFERENCES

Barros, H. D., Zamith, H. P. S., Bazílio, F. S., Carvalho, L. J., Abrantes, S. M. P. 2011. Identification of fatty foods with contamination possibilities by plasticizers when stored in PVC film packaging. *Ciencia Tecnologia de Alimentos*, vol. 31, no. 2, p. 547-552. http://dx.doi.org/10.1590/S0101-20612011000200041

Casajuana, N., Lacorte, S., 2004. New methodology for the determination of phthalate esters, bisphenol A, bisphenol A diglycidyl ether, and nonylphenol in commercial whole milk samples. *Journal of Agricultural and Food Chemistry*, vol. 52, p. 2702-2707. http://dx.doi.org/10.1021/jf040027s PMid:15186085

Castle, L., Gilbert, J., Eklund, T., 1990. Migration of plasticizer from poly(vinyl chloride) milk tubing. *Food Additives and Contaminants*, vol. 7, p. 591-596. http://dx.doi.org/10.1080/02652039009373924 PMid:2253803

Commission Regulation No. 2011/10/EC of theCommisssion of the European Communities of 14 January 2011 relating to plastic materials and articles intended to come into contact with foodstuffs 2011; OJ L 12, p. 4-12.

Das, M. T., Ghosh, P., Thakur, I. S., 2014. Intake estimates of phthalate esters for South Delhi population based on exposure media assessment. *Environ. Pollut.*, vol. 189, p. 118-125. http://dx.doi.org/10.1016/j.envpol.2014.02.021 PMid:24657605

Fierens, T., Cornelis, C., Standaert, A., Sioen, I., Henauw, S., Holderbeke, M., 2014a. Modelling the environmental transfer of phthalates and polychlorinated dibenzo-p-dioxins and dibenzofurans into agricultural products: The EN-forc model. *Environ. Res.*, vol. 133, p. 282-293. http://dx.doi.org/10.1016/j.envres.2014.06.005 PMid:24981827

Fierens, T., Standaert, A., Cornelis, C., Sioen, I., Henauw, S., Willems, H., Bellemans, M., Maeyer, M., Holderbeke, M., 2014b. A semi-probabilistic modelling approach for the estimation of dietary exposure to phthalates in the Belgian adult population. *Environ. Int.*, vol. 73, p. 117-127. ISSN 01604120. http://dx.doi.org/10.1016/j.envint.2014.07.017 PMid:25113625

Heudorf, U., Mersch-Sundermann, V., Angerer, E. 2007. Phthalates: toxicology and exposure. *International Journal of Hygiene and Environmental Health*, vol. 210, no. 5, p. 623-634. http://dx.doi.org/10.1016/j.ijheh.2007.07.011 PMid:17889607

Gonzáles-Castro, M. I., Olea-Serrano, M. F., Rivas-Velasco, A. M., Medina-Rivero, E., Ordoñez-Acevedo, L. G., León-Rodríguez, A., 2011. Phthalates and Bisphenols Migration in Mexican Food Cans and Plastic Food Containers. *Bull. Environ. Contam. Toxicol.*, vol. 86, no. 6, p. 627-631. http://dx.doi.org/10.1007/s00128-011-0266-3 PMid:21509467

Guo, Z., Wang, S., Wei, D., Wang, M., Zhang, H., Gai, P., Duan, J. 2010. Development and application of a method for analysis of phthalates in ham sausages by solid-phase extraction and gas chromatography-mass spectrometry. *Meat Science*, vol. 84, no. 3, p. 484-490. http://dx.doi.org/10.1016/j.meatsci.2009.10.002 PMid:20374814

Jarošová, A., Gajdůšková, V., Raszyk, J., Ševela, K. 1998. Determination of phthalic acid esters (PAEs) in biological materials by HPLC. *Czech Journal of Food Sciences*, vol. 16, p. 122-130.

Jarošová, A., Gajdůšková, V., Raszyk, J., Ševela, K. 1999. Di-2-ethylhexyl phthalate and di-n-butyl phthalate in the tissues of pigs and broiler chicks after their oral administration. *Veterinární medicína*, vol. 44, p. 61-70.

Ji, Y., Wang, F., Zhang, L., Shan, CH., Bai, Z., Sun, Z., Liu, L., Shen, B., 2014. A comprehensive assessment of human exposure to phthalates from environmental media and food in Tianjin, China. *J. Hazard. Mater.*, vol. 279, p. 133-140. http://dx.doi.org/10.1016/j.jhazmat.2014.06.055 PMid:25051237

Li, Y., Fei, F., Zhang, K., Chen, Q., Li, Y., 2012. Migration analysis of DEHP from Inner Liner of Beer Bottle Caps by HPLC. *Procedia Environ. Sci.*, vol. 12, p. 17-21. ISSN 1878-0296. http://dx.doi.org/10.1016/j.proenv.2012.01.241

Meng, X., Wang, Y., Xiang, N., Chen, L., Liu, Z., Wu, B., Dai, X., Zhan, Y., Xie, Z., Ebinghaus, R., 2014. Flow of sewage sludge-borne phthalate esters (PAEs) from human release to human intake: Implication for risk assessment of sludge applied to soil. *Sci. Total Environ.*, vol. 476-477, p. 242-249. http://dx.doi.org/10.1016/j.scitotenv.2014.01.007 PMid:24468498

Moreira, M. A., Andre, L. C., Cardeal, Z. D. 2015. Analysis of plasticiser migration to meat roasted in plastic bags by SPME-GC/MS. *Food Chemistry*, vol. 1, no. 178, p. 195-200.

http://dx.doi.org/10.1016/j.foodchem.2015.01.078
PMid:25704701

Nanni, N., Fiselier, K., Grob, K., DI Pasquale, M., Fabrizi, L., Aureli, P., Coni, E., 2011. Contamination of vegetable oils marketed in Italy by phthalic acid esters. Food Control, vol. 22, no. 2, p. 209-214. http://dx.doi.org/10.1016/j.foodcont.2010.05.022

Net, S., Delmont, A., Sempéré, R., Paluselli, A., Ouddane, B., 2015. Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): A review. *Sci. Total Environ.*, vol. 515-516, p. 162-180. http://dx.doi.org/10.1016/j.scitotenv.2015.02.013
PMid:25723871

Selvaraj, K. K, Sundaramoorthy, G., Ravichandran, P. K., Girijan, G. K., Sampath, S., Ramaswamy, B. R., 2015. Phthalate esters in water and sediments of the Kaveri River, India: environmental levels and ecotoxicological evaluations. Environ. *Geochem. Health*, vol. 37, no. 1, p. 83-96. http://dx.doi.org/10.1007/s10653-014-9632-5
PMid:25056812

Sharman, M., Read, W. A., Castle, L., Gilbert, J., 1994. Levels of di-(2-ethylhexyl) phthalate and total phthalate esters in milk, cream, butter and cheese. *Food Additives and Contaminants*, vol. 11, p. 375-385. http://dx.doi.org/10.1080/02652039409374236
PMid:7926171

Shuangling, Z., Kangquan, G., 2009. Migration amount of Di-2-ethylhexyl phthalate from food-grade PVC film into meat at three temperatures. *Transactions of the Chinese Society of Agricultural Engineering*, vol. 25, p. 291-293.

Schiedek, T., 1995. Impact of plasticizers (phthalic acid esters) on soil and groundwater quality, p. 149-156. In: Groundwater Quality: Remediation and Protection (Proceedings of the Prague Conference, May 1995). IAHS Publ. no. 225, 1995.

Tsumara, Y., Ishimitsu, S., Kaihara, A., Yoshii, K., Nakamura, Y., Tonogai, Y., 2001. Di(2-ethylhexyl) phthalate contamination of retail packed lunches caused by PVC gloves used in the preparation of foods. *Food additives and contaminants*, vol. 18, p- 569-579. PMid:11407756

Wang, X. K., Guo, W. L., Meng, P. R., Gan, J. A., 2002. Analysis of Phthalate Esters in Air, Soil and Plants in Plastic Film Greenhouse. *Chin. Chem. Lett.*, vol. 13, no. 6, p. 557-560.

Wang, S., Yang, W., Shi, M., Sun, X., Pang, W., Wang, G. 2013. GC-MS Assisted with Chemometric Methods Applied for Investigation of Migration Behavior of Phthalate Plasticizers in Fatty Foods Simulant. *Chromatographia*, vol. 76, no. 9-10, p. 529-534. http://dx.doi.org/10.1007/s10337-013-2410-1

Wang, J., Bo, L., Li, L., Wang, D., Chen, G., Christie, P., Teng, Y., 2014. Occurrence of phthalate esters in river sediments in areas with different land use patterns. *Sci. Total Environ.*, vol. 500-501, p. 113-119. http://dx.doi.org/10.1016/j.scitotenv.2014.08.092
PMid:25217750

Wang, J., Chen, G. C., Christie, P., Zhang, M. Y., Luo, Y. M., Teng, Y. 2015. Occurrence and risk assessment of phthalate esters (PAEs) in vegetables and soils of suburban plastic film greenhouses. *Science of the Total Environment*, vol. 1, no. 523, p. 129-137. http://dx.doi.org/10.1016/j.scitotenv.2015.02.101
PMid:25863503

Yang, J., Li, Y., Wang, Y., Ruan, J., Zhang, J., Sun, CH., 2015. Recent advances in analysis of phthalate esters in foods. *Trends Anal. Chem.*, vol. 72, p. 10-26. http://dx.doi.org/10.1016/j.trac.2015.03.018

Zhang, Z., He, G., Peng, X., Lu, L., 2014. Distribution and sources of phthalate esters in the topsoils of Beijing, China. *Environ. Geochem. Health*, vol. 36, no. 3, p. 505-515. ISSN 0269-4042. http://dx.doi.org/10.1007/s10653-013-9577-0
PMid:24203261

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SWEET POTATO (*IPOMOEA BATATAS* L.) GROWING IN CONDITIONS OF SOUTHERN SLOVAK REPUBLIC

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ABSTRACT

The sweet potato (*Ipomoea batatas* L.) belongs to very important crops from aspect of its world production. It is grown in large areas in Asia, on the contrary, sweet potato production in Europe presents minimal part of its total world rate. The sweet potato is less-known crop, grown only on small area in home gardens in Slovak Republic. Tubers of sweet potato are characterized by anti-diabetic, anti-oxidant and anti-proliferative properties due to the presence of valuable health-promoting components, such as carotenoids or vitamin C. The main objective of study was testing of sweet potato growing in conditions of southern Slovak Republic with focus on quantity and quality of its yield. The field trial was realised on land of the Slovak University of Agriculture in Nitra in 2015. Within trial, effect of cultivar and mulching on the selected quantitative (average tuber weight; yield per plant; yield in $t.ha^{-1}$) and qualitative (total carotenoids; vitamin C) parameters were tested. One certified cultivar of sweet potato 'Beauregard' was used as a comparative cultivar. Other two cultivars were marked according to the market place at which were purchased and sequentially used for seedling preparation. Tubers of first un-known cultivar were purchased in the Serbian market (marked as 'Serbian'). Tubers of next sweet potato cultivar were purchased on the market in Zagreb (marked as 'Zagrebian'). Outplating of sweet potato seedlings were realised on the 19th May 2015. The sweet potato was grown by hillock system. Each cultivar was planted in two variants (rows): non-mulching (bare soil) and mulching by black non-woven textile. All variants were divided to three replications with 6 plants. Difference between rows was 1.20 m and seedlings were planted in distance of 0.30 m in row. The harvested tubers were classified in two size classes: >150 g (marketable yield) and <150 g (non-marketable yield). Total carotenoid content was determined spectrophotometrically. The vitamin C content was measured chromatographically (HPLC). The highest values of average tuber weight, yield per plant and total yield ($t.ha^{-1}$) were found in cultivar 'Serbian'. Statistical analysis showed statistically significant difference in all yield quantitative parameters of cultivar 'Serbian' against cultivars 'Beauregard' and 'Zagrebian'. The highest content of total carotenoids was determined in cultivar 'Serbian' (99.52 $mg.kg^{-1}$ fresh weight) with orange-creme flesh color, followed by cultivar 'Beauregard' (94.78 $mg.kg^{-1}$) with orange flesh color and cultivar 'Zagrebian' (28.79 $mg.kg^{-1}$) with yellow-creme flesh color. Differences among all cultivars were showed as statistically significant. The highest vitamin C content was detected in tubers of cultivar 'Serbian' (155.70 $mg.kg^{-1}$), followed by cultivar 'Beauregard' (154.37 $mg.kg^{-1}$) and cultivar 'Zagrebian' (146.33 $mg.kg^{-1}$). Statistical analysis confirmed differences among cultivars as statistically non-significant. The mulching of sweet potato plants had statistically significant impact to all quantitative and qualitative characteristics of sweet potato. The application of black non-woven textile resulted in increase of average tuber weight, tuber yield and vitamin C content in sweet potato tubers. On the contrary, higher total carotenoid content was found in non-mulching variant compared to the variant with mulching.

Keywords: Slovak Republic; sweet potato; yield; carotenoids; vitamin C

INTRODUCTION

The sweet potato (*Ipomea batatas* L.) belongs to the *Convolvulaceae* family and it is original from South America. Due to Christopher Columbus, it was imported to the Europe about century earlier than classical potatoes - *Solanum tuberosum* L. **(Valíček et al., 2002)**. According to **FAOSTAT (2016)**, total world production of sweet potato tubers was more than 100 milions tones in 2014. The main production area was Asia (75.3 %), followed by Africa (20.2 %), American continents (3.7 %) and Oceania (0.8 %). Sweet potato production in Europe presented the least part of its total world value (0.1 %) and the European production was only 56 113 tones in 2014. The main European producers of sweet potatoes was Spain and Italy. From world-wide aspect, China is the main producer of sweet potatoes within recent period. The production of this commodity was more than 70 milions tones in 2014. The other important producers was Nigeria, Tanzania, Ethiopia or Indonesia. **Šlosár (2016)** state that sweet potatoes are less-known crop, grown only on small area in home local gardens in Slovak republic.

The sweet potato, known as batatas, is well known long-term species in a warm and hot climate zone and an annual plant (spring) in temperate zone. It produces moist and

delicate tubers with a sweetish taste, pleasant and aromatic smell. In addition, young leaves can also be used for consumption **(Antonio et al., 2011)**. Tubers are characterized by diverse size, shape (round, ovate, elliptic etc.), skin and flesh color (white, cream, yellow, orange, red or purple), depending on a cultivar **(Moulin et al., 2012)**.

The main nutritional compounds in tubers of sweet potato are carbohydrates (simple sugars and starches), proteins, fats and fat-soluble vitamins **(Allen et al., 2012)**. The glycemic index of sweet potatoes is quite high, thus, it is unsuitable for diabetics and overweight persons. Total carbohydrate content of this crop is 201 g. kg^{-1} of fresh weight (f. w.); starch content is 160 $g.kg^{-1}$ and soluble sugar content is 42 $g.kg^{-1}$ f. w. The proteins and fats are contained in sweet potatoes in small quantities **(Maria and Rodica, 2015)**. According to **USDA (2015)**, energetical value of fresh sweet potato is 359 kJ per 100 g. From mineral complex, potassium (337 $mg.100\ g^{-1}$), sodium (55 mg) and phosphorus (47 mg) are the most abundant in sweet potato tubers.

Tubers of sweet potato are characterized by anti-diabetic, anti-oxidant and anti-proliferative properties due to the presence of valuable nutritional and mineral components **(Abubakar et al., 2010)**. Sweet potato cultivars with an orange or yellow flesh contain significant amounts of carotenoids which are known as provitamins A **(Allen et al., 2012)**. Carotenoids show strong antioxidant capacity to scavenge free radicals because of their conjugated double bonds **(Fu et al., 2011)**. **Lichtenstein (2009)** indicates that carotenoids or their metabolites are associated with cardiovascular diseases. According to **Rao and Rao (2007)**, higher carotenoid intake in the food form helps to decrease of several cancer type risk (stomach, colon or larynx) and prevent to bone calcification, eye degeneration and neurotic diseases.

The vitamin C, also known as ascorbic acid, is another important substances within vitamin complex in sweet potato tubers **(USDA, 2015)**. Due its properties, vitamin C is characterized as very effective antioxidant. The human organism is not able to synthesize vitamin C, thus, it must be ingested in the food form, mainly vegetables and fruits **(Keresteš et al., 2011)**. The vitamin C plays an important role in immune system, stimulation of leucocytes to the increased bacteria degradation, secretion of antibodies and body resistance increase to the coldness **(Hacişevki 2009)**. According to **Feiz and Mobarhan (2002)**, sufficient vitamin C intake helps to eliminate *Helicobacter pylori* bacteria considered as important risk factor in stomach cancer formation. **Iqbal, Khan and Khan Khattak (2004)** state that vitamin C contributes to prevent human organism by elimination of nitrosamine formation which descend from nitrates contained in many food sources.

The main objective of present study was testing of sweet potato growing in conditions of southern Slovak Republic with focus on selected quantitative and qualitative parameters of its yield.

MATERIAL AND METHODOLOGY

The field trial with sweet potato was realised on the land of the Slovak University of Agriculture in Nitra in 2015. The experimental area is situated at an absolute altitude of 144 m above sea level. The climate of experimental area is characterized by warm and dry summer and slightly warm, dry or very dry winter. According to the climatic normal 1951-2000 for Nitra, annual mean temperature is 9.9 °C and mean rainfall total is 548 mm **(Šlosár and Uher, 2013)**. Within trial year 2015, the average month air temperature was 11.5 °C. The rainfall total was 418.2 mm in 2015.

Plant material

Sweet potato seedlings were purchased from Croatian producer (Ing. Darko Ďurica, Ilok). According to him, the situation with cultivar sortiment of sweet potato in Europe is often unclear and confusing. A lot of producers, including him, produces seedlings according to the tuber availability on the market. Thus, the origin of sweet potato seedlings on market is often un-known.

Within trial, one certified cultivar of sweet potato 'Beauregard' was used as a comparative cultivar. Other two cultivars were marked according to the market place at which were purchased and sequentially used for seedling preparation. Tubers of first un-known cultivar were purchased in the Serbian market. In this study, cultivar is marked as 'Serbian'. Tubers of next cultivar of sweet potato were purchased on the market in Zagreb (Croatia). Within study, the cultivar is marked as 'Zagrebian'.

Experiment organisation

The sweet potato is warm-requiring crop. It needs warm season lasting at least four month with an average temperature more than 20°C and without freeze **(Antonio et al., 2011)**. From this reason, outplanting of sweet potato seedlings was realised on the 19th May 2015 when the risk of later spring freeze is reduced.

Within soil preparation for sweet potato growing, nitrogen was only applied on the soil supply level of 60 $kg.ha^{-1}$ according to results of agrochemical soil analysis (tab. 1). The sweet potato plants were grown by hillock system, similar to the carrot growing (height of 0.30 m). The distance between hillock rows was 1.20 m. In each row, 18 sweet potato seedlings were planted in distance of 0.30 m. Rows for all tested cultivars and variant were divided to three replications with 6 sweet potato plants.

Within experiment, two variants for each cultivar were tested:

- non-mulching - bare soil (one row),

Table 1 Agrochemical soil characteristics before trial realisation.

pH_{KCl}	Humus (%)	Nutrient content ($mg.kg^{-1}$ of soil)					
		N_{min}*	P	K	Ca	Mg	S
7.16	3.25	19.1	245	149.5	6340	643.5	7.5

Note: *N_{min} - N mineral (N inorganic).

- mulching by black non-woven textile (one row).

The harvest of sweet potato tubers was realised on the 6th October 2015.

Morphological characteristics of cultivar

Selected morphological parameters of tubers for each cultivars were evaluated in order to its more accurate characteristics (table 2). It was realised by using of relevant international descriptor for sweet potato - *Ipomoea batatas* L. **(UPOV, 2010)**. The evaluation of morphological characteristics was done in 20 tubers of each cultivar. Following parameters of tubers were evaluated:

- shape,
- main color of skin,
- secondary color of skin,
- main color of flesh,
- intensity of main color flesh,
- secondary color of flesh,
- depth of eyes.

Quantitative parameters of sweet potato

Harvested tubers of sweet potato were classified according to average weight of tubers in two size classes:

- >150 g - marketable yield of tubers,
- <150 g - non-marketable yield of tubers.

Within experiment, average weight of tubers (g) and average yield quantity per plant (g) were evaluated. The sweet potato yield in $t.ha^{-1}$ was calculated on the basis of average plant yield. The density of plants, used for calculation, was 27 000 seedlings per hectare with using the same plant spacing as it was in realised experiment.

Total carotenoid content estimation

The estimation of total carotenoid content was realised in the laboratory of Department of Vegetable Production SUA in Nitra. The content of total carotenoids was estimated by spectrophotometric measurement of substances absorbance in petroleum ether extract on spectrophotometer PHARO 100 at 445 nm wavelengths. As an extraction reagent, acetone was used acetone **(Hegedüsová et al., 2007)**.

Vitamin C content estimation

The estimation of total carotenoid content was realised in the certified laboratory of Regional Public Health Authority in Nitra. HPLC method of vitamin C content estimation **(Stan, Soran and Marutoiu, 2014)** was used by the help of liquid chromatograph with UV detector, for separation was used RP C18 column, mobile phase was methanol : water (5:95, v/v), UV detection was adjusted to 258 nm (HPLC fy. VARIAN).

Statistical analysis

A statistical analysis was performed using Statgraphic Centurion XVII (StatPoint Inc. USA). Obtained results were evaluated by analysis of variance (ANOVA) and average values were tested by Tukey HSD test performed at the significance level of 95%.

RESULTS AND DISCUSSION

Average weight of sweet potato tubers

From aspect of marketable yield, the statistical analysis showed statistically significant differences of average tuber weight (AW) among cultivar 'Serbian' and cultivars 'Beauregard' and 'Zagrebian' (tab. 3). Difference between cultivars 'Beauregard' and 'Zagrebian' was evaluated as statistically non-significant. From aspect of marketable yield part (tuber > 150 g), values of AW were varied from 332.73 g ('Zagrebian') to 428.15 g ('Serbian'). Values in this range were similar to results in study of **Maria and Rodica (2015)** who found variability of AW from 210 g to 400 g in experiment in Romania. Similarly, **Ellong, Billard and Adenet (2014)** also found higher AW (308.91-647.75 g) in Martinique compared to our results. Thus, it is evident that locality for sweet potato growing expressively affects the awerage tuber weight, one of the

Figure 1 Sweet potato cultivars in realised treatment.

most important parameters of sweet potato from aspect of its total production.

Regarding to non-marketable part of sweet potato yield (<150 g), AW was ranged from 36.00 g ('Serbian') to 58.94 g ('Beauregard'). Statistical analysis of results showed statistically significant differences of its values among all tested cultivars.

Yield of sweet potato tubers per plant

Values of yield/plant (marketable yield) were increasing from 1185.62 g 'Zagrebian' to 1455.54 g 'Serbian'. Difference between mentioned values was evaluated as statistically significant. On the contrary, statistical analysis showed statistically non-significant difference between cultivars 'Beauregard' and 'Zagrebian'. **Yildirim, Tokuşoğlu and Öztürk (2011)** tested the impact of genotype on the yield of sweet potato per plant (13 genotypes) in Turkey. Its values, found by authors, were ranged from 210.5 g 621.8 g. It means markedly lower range of values compared to our trial results. The lower yield of sweet potato per plant (380-460 g) was also presented in study of **Uwah et al. (2013)**. On the contrary, **Maria and Rodica (2015)** reached comparable tuber yield per plant (1071-1600 g) to obtained trial results.

The yield of non-marketable tubers per plant was varied from 106.07 g ('Zagrebian') to 241.96 g ('Beauregard'). According to the statistical analysis, statistically significant differences between cultivars 'Beauregard' and 'Serbian'/'Zagrebian' were found.

Yield of marketable sweet potato tubers per hectare

The total marketable yield of sweet potato, in dependency on cultivar, was ranged from 32.01 $t.ha^{-1}$ ('Zagrebian') to 39.30 $t.ha^{-1}$ ('Serbian'). Statistical analysis confirmed statistically significant difference of yield between mentioned cultivars, similarly as between 'Beauregard' and 'Serbian'. Yield difference between cultivars 'Beauregard' and 'Zagrebian' was evaluated as statistically significant. **Maria and Rodica (2015)** found expressively higher yield in sweet potato cultivar 'Pumpkin' (53.3 $t.ha^{-1}$) compared to our results. On the contrary, total yield of cultivar 'Chestnut' (35.6 $t.ha^{-1}$) was comparable to results in our study. Comparable values of sweet potato yield were presented by **Jian-wei et al.**

Table 2 Evaluated morphological characteristics of sweet potato tubers.

Tuber parameters	Beauregard	Serbian	Zagrebian
Shape	oblong	ovate	ovate
Main color of skin	brownish orange	purple red	medium purple
Secondary color of skin	pink	orange	orange
Main color of flesh	orange	orange	yellow-creme
Main flesh color intensity	medium	medium	light
Secondary color of flesh	absent	beige	orange
Depth of eyes	shallow	medium	shallow

Table 3 Effect of cultivar on quantitative parameters of sweet potato yield.

Cultivar	Marketable tubers (>150 g)			Non-marketable tubers (<150 g)		Ratio of marketable tubers
	AW* (g)	Yield/plant (g)	Yield ($t.ha^{-1}$)	AW* (g)	Yield/plant (g)	(%)
Beauregard	348.54^{a}	1213.34^{a}	32.76^{a}	58.94^{c}	241.96^{b}	81.58^{a}
Serbian	428.15^{b}	1455.54^{b}	39.30^{b}	36.00^{a}	116.63^{a}	92.82^{b}
Zagrebian	332.73^{a}	1185.62^{a}	32.01^{a}	48.36^{b}	106.07^{a}	91.31^{b}

Note: * AW - average weight of sweet potato tubers.
Different letters (a; b; c) within the same column means statistically significant difference (at 95.0 % confidence level).

Table 4 Effect of mulching variant on quantitative parameters of sweet potato yield.

Cultivar	Marketable tubers (>150 g)			Non-marketable tubers (<150 g)		Marketable share of tubers
	AW* (g)	Yield/plant (g)	Yield ($t.ha^{-1}$)	AW* (g)	Yield/plant (g)	(%)
Non-mulching	232.24^{a}	981.05^{a}	26.49^{a}	52.03^{b}	164.42^{a}	85.46^{a}
Mulching	351.76^{b}	1588.62^{b}	42.89^{b}	43.51^{a}	145.53^{b}	91.68^{b}

Note: * AW - average weight of sweet potato tubers.
Different letters (a; b; c) within the same column means statistically significant difference (at 95.0 % confidence level).

(2001) who grown this crop in nine localities in China. The average yield of sweet potatoes, found in Chinese study, was 36.7 $t.ha^{-1}$. The marketable yield of sweet potato cultivar 'Beauregard' (22.5 – 36.8 $t.ha^{-1}$ in depdendency on vegetation period) presented by **Bonte and Wilson (2008)** was also comparable to the yield of this cultivar found in our trial. Within study of **Uwah et al. (2013)**, total yield of sweet potato was varied from 20.8 $t.ha^{-1}$ to 25.5 $t.ha^{-1}$ in dependence on the cultivar and experimental year. The markedly lower yield of sweet potato tubers (3.4 –14.4 $t.ha^{-1}$), compared to obtained results, was reached within study of **Yildirim, Tokuşoğlu and Öztürk (2011)**. The lower yield of marketable sweet potato tubers, compared to our trial, was also found in studies of other authors **(Hartemink, 2003**; **Oliveira et al., 2010**; **Sowley, Neindow and Abubakari, 2015)**.

Ratio of marketable yield

The highest ratio of marketable sweet potato tubers from total yield was found in cultivars 'Serbian' (92.82%), followed by cultivars 'Zagrebian' (91.31%) and 'Beauregard' (81.58%). Statistically non-significant difference was between cultivars 'Serbian' and 'Zagrebian'. Other differences between cultivars were evaluated as statistically significant. **Sokoto, Magaji and Singh (2007)** examined the effect of various intra-row spacing on the ratio of marketable yield in trial with sweet potato. Within variant, in which the same spacing was used as in our trial (0.30 m), marketable ratio of sweet potato was 48.99 %. It was expressively under value found in our trial. **Hartemink (2003)** found that marketable ratio of sweet potato tubers was varied from 83.53% to 89.56%, dependent on the growing year. It is comparable to results obtained in our trial.

Total carotenoid content

According to **Sebuliba, Nsubuga and Muyonga (2001)**, orange-fleshed sweet potato tubers are rich sources of carotenoids. Compared to cultivars with yellow and white flesh color, orange-fleshed sweet potato has expressively higher content of total carotenoids.

The content of total carotenoids was increasing in following cultivar order: 28.79 $mg.kg^{-1}$ fresh weight ('Zagrebian') <94.78 $mg.kg^{-1}$ f. w. ('Beauregard') <99.52 $mg.kg^{-1}$ f. w. ('Serbian'). The statistical analysis of results showed statistically significant differences among cultivars with orange flesh color ('Beauregard' and 'Serbian') and cultivar 'Zagrebian' with yellow-creme color of tuber flesh. Difference between cultivars with orange flesh color was not statistically significant.

Kammona et al. (2015) found the significant variability of carotenoid content in dependency on the color of sweet potato flesh. Total carotenoid content in orange-fleshed tubers was more than three-fold higher compared to sweet potatoes with yellow, purple and white flesh color. The strong interaction between flesh color and total carotenoid content in sweet potato was also presented in study of **Ellong, Billard and Adenet (2014)**, **Grace et al. (2014)** and **Hussein et al (2014)**. Within trial in China**, Tang, Cai and Xu (2015)** found higer total carotenoid in sweet potato tubers compared to results showed in our trial. The total carotenoid content in cultivar with orange flesh color was 157.9 $mg.kg^{-1}$ f. w. Cultivars with yellow-creme (75.4 $mg.kg^{-1}$), light-purple (5.19 $mg.kg^{-1}$), white (4.46 $mg.kg^{-1}$) and deep-purple color of tuber flesh had several-fold lower content of total carotenoid compared to orange sweet potato cultivar.

The most important and predominant carotenoid substance in sweet potatoes is β-carotene (**USDA, 2015**). According to study of **Kammona et al. (2015)**, β-carotene ratio from total carotenoid content is variable in relation with flesh color of sweet potato. The highest β-carotene ratio was found in purple-fleshed tubers (97.9%), followed by tubers with orange (93.8%), yellow (84.1%) and white (79.0%) flesh color.

Within study with ten cultivars of sweet potato, **Yildirim, Tokuşoğlu and Öztürk (2011)** found marked variability of β-carotene content in cultivars with yellow-creme color of tuber flesh (50.1 – 70.3 $mg.kg^{-1}$ f. w.). The β-carotene content in orange tubers was 70.3 $mg.kg^{-1}$. This results were in contrast to our study where expressive difference between orange and yellow-creme sweet potato was found. **Suparno, Prabawardani and Pattikawa (2016)** found similar β-carotene content values in yellow-creme sweet potato (62.98 – 64.69 $mg.kg^{-1}$ f. w.) compared

Table 5 Effect of cultivar on qualitative parameters of sweet potato tubers (marketable yield).

Cultivar	Total carotenoids ($mg.kg^{-1}$ fresh weight)	Vitamin C ($mg.kg^{-1}$ fresh weight)
Beauregard	94.78^{b}	154.37^{a}
Serbian	99.52^{b}	155.70^{a}
Zagrebian	28.79^{a}	146.43^{a}

Note: Different letters (a; b; c) within the same column means statistically significant difference (at 95.0 % confidence level).

Table 6 Effect of mulching variant on qualitative parameters of sweet potato tubers (marketable yield).

Cultivar	Total carotenoids ($mg.kg^{-1}$ fresh weight)	Vitamin C ($mg.kg^{-1}$ fresh weight)
Non-mulching	77.81^{b}	143.23^{a}
Mulching	70.92^{a}	161.10^{b}

Note: Different letters (a; b; c) within the same column means statistically significant difference (at 95.0 % confidence level).

to study of previous authors. Results in study of **Aywa, Nawiri and Nyambaka (2013)** also confirmed fact that orange flesh cultivars of sweet potato have markedly higher content of β-carotene (46.19 – 48.89 mg.kg^{-1} f. w.) compared to the tubers with yellow flesh color (20.17 – 26.28 mg.kg^{-1} f. w.).

Obtained results confirmed that cultivar is important factor influencing on the content of carotenoids in sweet potatoes. The expressive impact of cultivar to the total carotenoid content was found in the experiments with tomato **(Mendelová et al., 2012)**, bell pepper **(Ignat et al., 2013)** or sea buckthorn **(Mendelová et al., 2016)**.

Vitamin C content

Compared to total carotenoid content, variability of vitamin C content among cultivars was not marked. The vitamin C content was from 146.43 mg.kg^{-1} ('Zagrebian') to 155.70 mg.kg^{-1} f. w. ('Serbian'). According to statistical analysis, differences of vitamin C among cultivars were evaluated as statistically non-significant. Comparable values of vitamin C content (129-142 mg.kg^{-1} f. w.) in sweet potato were presented by **Maria and Rodica (2015)** in field trial in Romania.

Within trial in Poland, **Krochmal-Marczak et al. (2013)** found higher vitamin C in tubers of sweet potato compared to our trial results. Its values were varied, dependent on cultivars, from 202.6 mg.kg^{-1} to 242.0 mg.kg^{-1} f. w. The higher content of vitamin C in sweet potato tubers, compared to our study, was also presented in study of **Suparno, Prabawardani and Pattikawa (2016)**. Authors found variable content of vitamin C in dependency on the flesh color of sweet potato tubers. The highest vitamin C content was determined in purple cultivars (727.1 mg.kg^{-1}), followed by cultivars with white (672.2 mg.kg^{-1}) and yellow flesh color (204.7-254.4 mg.kg^{-1}). According to study of **Ellong, Billard and Adenet (2014)**, determined vitamin C content in yellow or creme sweet potatoes varied from 177.5 mg.kg^{-1} to 290.5 mg.kg^{-1} f. w. It meant higher values of vitamin C content in comparison with our trial. **Yildirim, Tokuşoğlu and Öztürk (2011)** also stated that cultivar and flesh color had an significant impact on the vitamin C content in sweet potato tubers. Within yellow-creme flesh cultivars, vitamin C content was ranged from 237 mg.kg^{-1} to 386 mg.kg^{-1} f. w. The cultivar with orange flesh color ('Regal') showed lower vitamin C content than most of yellow-creme cultivars.

On the contrary, markedly lower content, compared to trial results, was presented in study of **Gichuhi, Kokoasse Kpomblekou and Bowel-Benjamin (2014)**. In cultivar 'Beauregard' (the same cultivar as in our study), value of vitamin C content was 64.3 mg.kg^{-1} f. w. Similarly, lower values of vitamin C content were found (48.5 – 57.3 mg.kg^{-1} f. w.) in the field trial realised in different localities of Western Kenya **(Aywa, Nawiri and Nyambaka, 2013)**.

Compared to obtained results, more expressive and statistically significant impact of cultivar to the vitamin C content was found in studies with vegetable pepper and tomatoes **(Valšíková et al., 2010)**, broccoli **(Koh et al., 2009)** potatoes **(Mareček et al., 2016)**.

Effect of mulching on sweet potato yield

According to **Novak et al. (2007)**, sweet potato [*Ipomoea batatas* (L.) Lam] needs a yearly minimum of three month with air temperatures above 15 ºC for its growth and development. For the purpose of achieving the highest possible sweet potato yield during a relatively short vegetation period in middle Europe, using of mulching material (PE foil, non-woven textile or organic materials) is necessary for succesfull growing. **Wees, Seguin and Boisclair (2016)** similarly emphasize that use of black mulch to heat the soil can markedly improve and optimize yields of sweet potato and attain its market quality standards in cooler climate.

The statistical analysis showed statistically significant increase of particular quantitative parameters of sweet potato marketable yield in mulching variant (black non-woven textile) compared to variant without mulching (tab. 6). Between mentioned variants, increase of average tuber weight and total sweet potato yield (t.ha^{-1}) was presented by values of 51.5% and 61.8%. In mulching variant, the marketable ratio of tubers was higher about 7.3% compared to the non-mulching variant (bare soil). According to study of **Novak, Zutić and Toth (2007)**, mulching with black PE-film had a significant effect on the yield and average weight of sweet potato tubers. Within study, higher yield about 5.3 kg.m^{2} was found compared to non-mulching variant.**Novak et al. (2007)** found a significantly higher yield of marketable tubers of sweet potato in mulching variant by black PE film mulch compared to uncovered soil. In mentioned study, realised in Croatia, marketable yield was increased from 1.16 to 2.53 kg.m^{-2} (118 %). **Aregheore and Tofinga (2004)** tested effect of mulching by using of organic materials (guinea grass, dadap leaves) on the yield of sweet potato tubers. In all tested mulching treatments, sweet potato yield icncrease was reached compared to the treatment without mulch, varying from 4.6% to 12.2%. According to **Laurie et al. (2015)**, using of organic (grass straw) and inorganic (black plastic foil) mulching materials resulted in higher total and marketable yield of sweet potato tubers compared to the un-control treatment. The application of grass straw was showed by increase of total yield about 33.3% and marketable yield about 63.5%. In the treatment with black plastic foil, total and marketable yield of sweet potato were higher about 77.7% and 69.4% subsequently. Positive impact of mulching on the sweet potato yield was also presented in study of **Ossom et al. (2001)**.

Compared to experimental results, **McKinley Sullen (2010)** found minimal and non-significant impact of mulching by variously colored plastic material on the total carotenoid content in sweet potato (cultivar Beauregard). Its value was varying in this treatment order: black PE foil (159.4 mg.kg^{-1} f. w.) < red PE foil (159.6 mg.kg^{-1} f. w.) < control - bare soil (159.9 mg.kg^{-1} f. w.) < silver PE foil (160.1 mg.kg^{-1} f. w.) < white PE foil (160.4 mg.kg^{-1} f. w.) < blue PE foil (160.6 mg.kg^{-1} f. w.). The effect of mulching on the total carotenoid content (TCC) in sweet potato is not well-documented. On the other side, TCC in dependency on mulching was examined in studies with other crops. **Siwek, Libik and Zawiska (2012)** tested various biodegradable mulching materials and their impact on the TCC in butterhead lettuce. Authors found non-statistically significant increase of TCC in treatments with applied mulching materials compared to the control

treatment. Differences to control treatment in TCC were varying from 1.5% to 6.7%. **Szafirowska and Elkner (2009)** found variable effect of mulching on the content of β-carotene, main carotenoid substance, in sweet pepper by using of various materials. The application of cloves (organic mulching) resulted in statistically significantly higher TCC in pepper fruits compared to the control treatment. On the other hand, TCC decrease was detected in treatment with black polypropylene foil used for mulching in comparison with control. According to **Moreno et al. (2014)**, lycopene is the main carotenoid susbtance in tomato fruits. The using of different mulching materials (papers; biodegradable foil; black PE foil; straw) was showed by increase of lycopene content in tomato fruits in the range from 1.6% to 15.1% compared to control treatment.

In realised trial, positive impact of mulching on the vitamin C content in sweet potato was found. It is consistent with study results presented by **McKinley Sullen (2010)** who tested variously colored plastic foil on the sweet potato yield. Author found higher vitamin C content in sweet potato tubers compared to the control treatment (bare soil), presenting increase of its value in the range from 4.6% (blue foil) to 22.3% (black foil). Positive impact of mulching on vitamin C content was also found in trial with chilli pepper (*Capsicum annuum* L.) realised by **Ashrafuzzaman et al. (2011)**. Authors found higher vitamin C content in chilli pepper fruits in all treatments with variously-colored plastic mulching foil (transparent, black and blue) compared to the control treatment. The most significant increase of its value was reached by using of black plastic foil. **Franczuk et al. (2009)** tested effect of different types of organic mulching (phacelia, vetch, serradella, oat) on the yield quality of tomato and onion. Authors also presented increase of vitamin C content in both vegetables as the results of all used mulching materials during growing period. According to **Dvořák et al. (2012)**, using of black textile mulch resulted in statistically non-significant increase of vitamin C content (9,8%) in potato tubers compared to the treatment with bare soil.

CONCLUSION

The sweet potato is marked warm-requiring crop, grown mainly in Asia. The mulching is important intesification factor of production influencing yield of sweet potato. From aspect of quantitative parameters of sweet potato yield, all tested cultivars were showed significant increase of selected quantitative parameters (yield per plant; average tuber weight; yield in $t.ha^{-1}$; marketable tuber ratio) in mulching treatment compared to the control treatment. The highest yield of tubers (39.30 $t.ha^{-1}$) was showed in sweet potato cultivar 'Serbian' . The cultivar and mulching had also expressive impact on the quality of sweet potato tubers (total carotenoids; vitamin C). The highest content of total carotenoids (99.52 $mg.kg^{-1}$ fresh weight) and vitamin C (155.70 $mg.kg^{-1}$ f. w.) was also found in tubers of cultivar 'Serbian'. Results gained in presented study indicate that sweet potato can be succesfully grown in conditions of Southern Slovak Republic.

REFERENCES

Abubakar, H. N, Olayiwola, I. O., Sanni, S. A., Idowu, M. A. 2010. Chemical composition of sweet potato (*Ipomea batatas* Lam) dishes as consumed in Kwara state, Nigeria. *International Food Research Journal*, vol. 17, no. 2, p. 411-416. Available at: http://www.ifrj.upm.edu.my/17%20(02)%202010/IFRJ-2010-411-416_nigeria[1].pdf

Allen, J. C., Corbitt, A. D., Maloney, K. P., Butt, M. S., Truong, V. D. 2012. Glycemic index of sweet potato as affected by cooking methods. *Open Nutrition Journal*, vol. 6, p. 1-11. http://dx.doi.org/10.2174/1874288201206010001

Antonio, G. C., Takeiti, C. Y., de Oliveira, R. A., Park, K. J. 2011. Sweet Potato: Production, Morphological and Physiochemical Characteristics, and Technological Process. *Fruit, Vegetable and Cereal Science and Biotechnology*, vol. 5, special no. 2, p. 1-18. Available at: http://www.globalsciencebooks.info/Online/GSBOnline/images/2011/FVCSB_5(SI2)/FVCSB_5(SI2)1-18o.pdf

Ashrafuzzaman, M., Halim, M. A., Ismail, M. R., Shahidullah, S. M., Hossain, M. A. 2011. Effect of plastic mulch on growth and yield of chilli (*Capsicum annuum* L.). *Brazilian Archives of Biology and Technology*, vol. 54, no. 2, p. 321-330. http://dx.doi.org/10.1590/S1516-89132011000200014

Aywa, A. K., Nawiri, M. P., Nyambaka, H. N. 2013. Nutrient variation in colored varieties of *Ipomea batatas* grown in Vihiga County, Western Kenya. *International Food Research Journal*, vol. 20, no. 2, p. 819-825. Available at: http://www.ccsenet.org/journal/index.php/jas/article/view/55458/30650

Bonte, D. R. L., Wilson, P. W. 2008. 'Evangeline' Sweetpotato. *HortiScience*, vol. 43, no. 1, p. 258-259. Available at: http://hortsci.ashspublications.org/content/43/1/258.full.pdf+html

Dvořák, P., Tomášek, J., Kuchtová, P., Hamouz, K., Hajšlová, J., Schulzová, V. 2012. Effect of mulching materials on potato production in different soil-climatic conditions. *Romanian Agricultural Research*, vol. 29, p. 201-209. Available at: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.462.6215&rep=rep1&type=pdf

Ellong, E. N., Billard, C., Adenet, S. 2014. Comparison of Physicochemical, Organoleptic and Nutritional Abilities of Eight Sweet Potato (*Ipomoea batatas*) Varieties. *Food and Nutrition Sciences*, vol. 5, no. 2, p. 196-211. http://dx.doi.org/10.4236/fns.2014.52025

Feiz, H. R., Mobarhan, S. 2002. Does vitamin C intake slow the progression of gastric cancer in *Helicobacter pylori*-infected populations? *Nutrition Reviews*, vol. 60, no. 1, p. 34-36. http://dx.doi.org/10.1301/002966402760240345

Food and Agriculture Organization of the United Nations (FAOSTAT) 2016. Available at: http://faostat3.fao.org/browse/Q/*/E

Franczuk, J., Jabłońska-Ceglarek, R., Zaniewicz-Bajkowska, A., Kosterna, E., Rosa, R. 2009. The effect of plant mulches on the nutritive value of red cabbage and onion. *Vegetable Crops Research Bulletin*, vol. 70, no. 1, p. 125-134. http://dx.doi.org/10.2478/v10032-009-0012-0

Fu, H., Xie, B., Ma, S., Zhu, X., Fan, G., Pan, S. 2011. Evaluation of antioxidant activities of principal carotenoids available in water spinach (*Ipomoea aquatica*). *Journal of Food Composition and Analysis*, vol. 24, no. 2, p. 288-297. http://dx.doi.org/10.1016/j.jfca.2010.08.007

Gichuhi, P. N., Kokoasse Kpomblekou, A., Bowel-Benjamin, A. C. 2014. Nutritional and physical properties of organic Beauregard sweet potato [*Ipomoea batatas* (L.)] as influenced by broiler litter application rate. *Food Science & Nutrition*, vol. 2, no. 4, p. 332-340. http://dx.doi.org/10.1002/fsn3.108

Grace, M. H., Yousef, G. G., Gustafson, S. J., Truong, V. D., Yencho G. C., Lila, M. A. 2014. Phytochemical changes in phenolics, anthocyanins, ascorbic acid, and carotenoids associated with sweetpotato storage and impacts on bioactive properties. *Food Chemistry*, vol. 145, p. 717-724. http://dx.doi.org/10.1016/j.foodchem.2013.08.107

Hacişevki, A. 2009. An overview of ascorbic acid biochemistry. *Journal of Faculty of Pharmacy of Ankara*, vol. 38, no. 3, p. 233-255. Available at: http://dergiler.ankara.edu.tr/dergiler/24/1716/18327.pdf

Hartemink, A. E. 2003. Sweet potato yields and nutrient dynamics after short-term fallows in the humid lowlands of Papua New Guinea. *NJAS - Wageningen Journal of Life Sciences*, vol. 50, no. 3-4, p. 297-319. http://dx.doi.org/10.1016/S1573-5214(03)80014-3

Hegedüsová, A., Musilová, J., Jomová, K., Hegedűs, O., Bystrická, J. 2007. *Laboratórne experiment z organickej chémie a biochémie pre špecializáciu Chémia životného prostredia* (*Laboratory experiments from organic chemistry and biochemistry for specialization Chemistry of environment*). NITRA, SK : UKF. 103 p. ISBN 978-80-8094-211-3.

Hussein, S. M., Jaswir, I., Jamal, P., Othman, R. 2014. Carotenoid Stability and Quantity of Different Sweet Potato Flesh Colour over Postharvest Storage Time. *Advances in Environmental Biology*, vol. 8, no. 3, special no. 2014, p. 667-671. Available at: http://www.aensiweb.com/old/aeb/2014/667-671.pdf

Iqbal, K., Khan, A., Khan Khattak, M. A. 2004. Biological Significance of Acid Ascorbic Acid (Vitamin C) in Human Health – A Review. *Pakistan Journal of Nutrition*, vol. 3, no. 1, p. 5-13. http://dx.doi.org/10.3923/pjn.2004.5.13

Ignat, T., Schmilovitch, Z., Feföldi, J., Bernstein, N., Steiner, B., Egozi, H., Hoffman, A. 2013. Nonlinear methods for estimation of maturity stage, total chlorophyll, and carotenoid content in intact bell peppers. *Biosystems engineering*, vol. 114, no. 4, p. 414-425. http://dx.doi.org/10.1016/j.biosystemseng.2012.10.001

Jian-wei, L., Fang, C., You-sheng, X., Yun-fan, W., Dong-bi, L. 2001. Sweet Potato Response to Potassium. *Better Crops International*, vol. 15, no. 1, p. 10-12. Available at: http://www.ipni.net/publication/bci.nsf/0/E1E140F6CD1365B585257BBA0068AC0F/$FILE/Better%20Crops%20International%202001-1%20p10.pdf

Kammona, S., Othman, R., Jaswir, I., Jamal, P. 2015. Characterisation of carotenoid content in diverse local sweet potato (*Ipomoea batatas*) flesh tubers. *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 7, no. 2, p. 347-351. Available at: http://innovareacademics.in/journals/index.php/ijpps/article/view/3643

Keresteš, J. et al. 2011. *Zdravie a výživa ľudí* (*Human health and nutrition*). BRATISLAVA, SK : CAD Press. 1037 p. ISBN 978-80-88969-57-0.

Koh, E., Wimalasim, K. M. S., Chassy, A. W., Mitchell, A. E. 2009. Content of ascorbic acid, quercetin, kaempferol and total phenolics in commercial broccoli. *Journal of Food Composition and Analysis*, vol. 22, no. 7-8, p. 637-643. http://dx.doi.org/10.1016/j.jfca.2009.01.019

Krochmal-Marczak, B., Sawicka, B., Supski, J., Cebulak, T., Paradowska, K. 2013. Nutrition value of the sweet potato (*Ipomoea batatas* (L.) Lam) cultivated in south – eastern Polish conditions. *International Journal of Agronomy and Agricultural Research (IJAAR)*, vol. 4, no. 4, p. 169-178. Available at: http://www.innspub.net/wp-content/uploads/2014/04/IJAAR-V4No4-p169-178.pdf

Laurie, S. M., Maja, M. N., Ngobeni, H. M., Du Plooy, C. P. 2015. Effect of Different Types of Mulching and Plant Spacing on Weed Control, Canopy Cover and Yield of Sweet Potato (*Ipomoea batatas* (L.) Lam). *American Journal of Experimental Agriculture*, vol. 5, no. 5, p. 450-458. http://dx.doi.org/10.9734/AJEA/2015/12404

Lichtenstein, A. H. 2009. Nutrient supplements and cardiovascular disease: a heartbreaking story. *Journal of Lipid Research*, vol. 50, supplement, p. 429-433. http://dx.doi.org/10.1194/jlr.R800027-JLR200

Mareček, J., Ivanišová, E., Frančáková, H., Musilová, J., Krajčovič, T., Mendelová, A. 2016. Evaluation of primary and secondary metabolites in selected varieties of potatoes. *Potravinarstvo*, vol. 10, no. 1, p. 145-151. http://dx.doi.org/10.5219/562

Maria, D., Rodica, S. 2015. Researches on the sweet potato (*Ipomea batatas* L.) behaviour under the soil and climatic conditions of the South-West of Romania. *Journal of Horticulture, Forestry and Biotechnology*, vol. 19, no. 1, p. 79-84. Available at: http://www.journal-hfb.usab-tm.ro/romana/2015/Lucrari%20PDF/Lucrari%20PDF%2019(1)/15Dinu%20Maria.pdf

McKinley Sullen, D. 2010. *Effects of Color Plastic Mulches and Row Cover on the Yield and Quality of Sweet Potato [Ipomea batatas cv. 'Beauregard']: thesis for Degree of Master of Science.* AUBURN, USA: Auburn University. 74 p. Available at: https://etd.auburn.edu/handle/10415/2344?show=full

Mendelová, A., Andrejiová, A., Líšková, M., Kozelová, D., Mareček, J. 2012. Analysis of carotenoids and lycopene in tomato (*Lycopersicon esculentum* Mill.) and their retention in tomato juice. *Potravinarstvo*, vol. 6, no. 2, p. 36-38. http://dx.doi.org/10.5219/195

Mendelová, A., Mendel, Ľ., Czako, P., Mareček, J. 2016. Evaluation of carotenoids, polyphenols content and antioxidant activity in the sea buckthorn fruit juice. *Potravinarstvo*, vol. 10, no. 1, p. 59-64. http://dx.doi.org/10.5219/551

Moreno, C., Mancebo, I., Tarquis, A. M., Moreno, M. M. 2014. Univariate and multivariate analysis on processing tomato quality under different mulches. *Scientia Agricola*, vol. 71, no. 2, p. 114-119. http://dx.doi.org/10.1590/S0103-90162014000200004

Moulin, M. M., Rodrigues, R., Gonçalves, L. S. A., Sudré, C. P., dos Santos, M. H., da Silva, J. S. P. 2012. Collection and morphological characterization of sweet potato landraces in north of Rio de Janeiro state. *Horticultura Brasileira*, vol. 30, no. 2, p. 286-292. http://dx.doi.org/10.1590/S0102-05362012000200017

Novak, B., Zutić, I., Toth, N. 2007. Effects of mycorrhizal and colored mulch in sweet potato production. *Acta Horticulturae*, vol. 729, p. 245-248. http://dx.doi.org/10.17660/ActaHortic.2007.729.39

Novak, B., Žutić, I., Toth, N., Dobričević, N. 2007. Sweet Potato [*Ipomoea batatas* (L.) Lam] Yield Influenced by Seedlings and Mulching. *Agriculturae Conspectus Scientificus*, vol. 72, no. 4, p. 357-359. Available at: http://www.agr.unizg.hr/smotra/pdf_72/acs72_58.pdf

Oliveira, A. P., Santos, J. F., Cavalcante, L. F., Pereira, W. E., do Carmo, M., Santos, C. A., Oliveira, A. N. P., Silva, N. V. 2010. Yield of sweet potato fertilized with cattle manure and biofertilizer. *Horticultura Brasileira*, vol. 28, no. 3, p. 277-281. http://dx.doi.org/10.1590/S0102-05362010000300006

Ossom, E. M., Pace, P. F., Rhykero, R. L., Rhykero, C. L. 2001. Effect of mulch on weed infestation, soil temperature, nutrient concentration, and tuber yield in *Ipomoea batatas* (L.) lam. in Papua New Guinea. *Tropical Agriculture*, vol. 78, no. 3, p. 144-151. Available at: http://www.ajol.info/index.php/uniswa-jast/article/view/4737

Rao, A. V., Rao, L. G. 2007. Carotenoids and human health. *Pharmacological Research*, vol. 55, no. 3, p. 207-216. http://dx.doi.org/10.1016/j.phrs.2007.01.012

Sebuliba, J. M., Nsubuga, E. N., Muyonga, J. H. 2001. Potential of Orange and Yellow Fleshed Sweetpotato Cultivars for Improving Vitamin A Nutrition in Central Uganda. *African Crop Science Journal*, vol. 9, no. 10, p. 309-316. http://dx.doi.org/10.4314/acsj.v9i1.27653

Siwek, P., Libik, A., Zawiska, I. 2012. The effect of biodegradable nonwovens in butterhead lettuce cultivation for early harvest. *Folia Horticulturae*, vol. 24, no. 2, p. 161-166. http://dx.doi.org/10.2478/v10245-012-0020-2

Šlosár, M. 2016. Batáty - poznáte ich (Batatas - do you know them)? *Záhradnictví*, vol. 15, no. 3, p. 24-25.

Šlosár, M., Uher, A. 2013. *Fertilization and phytochemicals in broccoli and cauliflower: impact of nitrogen and sulphur fertilization to the content of phytochemicals in broccoli and cauliflower*. Saarbrücken, DE : LAP Lambert Academic Publishing. 128 p. ISBN-13 978-3659435911.

Sokoto, M. B., Magaji, M. D., Singh, A. 2007. Growth and Yield of Irrigated Sweet Potato (*Ipomoea batatas* (L.) Lam.) As Influenced by Intra-Row Spacing and Potassium. *Journal of Plant Sciences*, vol. 2, no. 1, p. 54-60. http://dx.doi.org/10.3923/jps.2007.54.60

Sowley, E. N. K., Neindow, M., Abubakari, A. H. 2015. Effect of poultry manure and NPK on yield and storability of orange- and white-fleshed sweet potaot [*Ipomoea batatas* (L.) Lam]. *ISABB - Journal of Food and Agriculture Sciences*, vol. 5, no. 1, p. 1-6. http://dx.doi.org/10.5897/ISABB-JFAS2014.0005

Stan, M., Soran, M. L., Marutoiu, C. 2014. Extraction and HPLC Determination of the Ascorbic Acid Content of Three Indigenous Spice Plants. *Journal of Analytical Chemistry*, vol. 69, no. 10, p. 998-1002. http://dx.doi.org/10.1134/S106193481410013X

Suparno, A., Prabawardani, S., Pattikawa, A. B. 2016. The Nutritional Value of Sweet Potato Tubers [*Ipomoea batatas* (L.) Lamb.] Consumed by Infants and Children of Dani Tribe in Kurulu District, Baliem-Jayawijaya. *Journal of Agricultural Sciences*, vol. 8, no. 3, p. 64-69. http://dx.doi.org/10.5539/jas.v8n3p64

Szafirowska, A., Elkner, K. 2009. The comparison of yielding and nutritive value of organic and conventional pepper fruits. *Vegetable Crops Research Bulletin*, vol. 71, no. 1, p. 111-121. http://dx.doi.org/10.2478/v10032-009-0032-9

Tang, Y., Cai, W., Xu, B. 2015. Profiles of phenolics, carotenoids and antioxidative capacities of thermal processed white, yellow, orange and purple sweet potatoes grown in Guilin, China. *Food Science and Human Wellness*, vol. 4, no. 3, p. 123-132. http://dx.doi.org/10.1016/j.fshw.2015.07.003

UPOV 2010. *Sweet potato*. Geneva : International Union for the Protection of new varieties of plants [online]. 27 p. [cit. 2016-03-15]. Retrieved from the web: http://www.upov.int/edocs/tgdocs/en/tg258.pdf

USDA 2015. USDA National Nutrient Database for Standard Reference, release 28, U.S. Department of Agriculture, Agricultural Research Service, Retrieved from the web: http://ndb.nal.usda.gov/ndb/search

Uwah, D. F., Undie, U. L., John, N. M., Ukoha, G. O. (2013). Growth and Yield Response of Improved Sweet Potato (*Ipomoea batatas* (L.) Lam) Varieties to Different Rates of Potassium Fertilizer in Calabar, Nigeria. *Journal of Agricultural Sciences*, vol. 5, no. 7, p. 61-69. http://dx.doi.org/10.5539/jas.v5n7p61

Valíček, P. et al. 2002. *Užitkové rostliny tropů a subtropů* (*Useful plants of tropical and substropical areas*). PRAHA, CZ : Academia. 486 p. ISBN 80-200-0939-6.

Valšíková, M., Červenka, J., Barkoci, Š., Sudzina, M. 2010. The evaluation of vitamin C content in fruits of vegetable pepper and tomato. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, vol. 58, no. 2, p. 281-286. http://dx.doi.org/10.11118/actaun201058020281

Wees, D., Seguin, P., Boisclair, J. 2016. Sweet potato production in a short-season area utilizing black plastic mulch: Effects of cultivar, in-row plant spacing, and harvest date on yield parameters. *Canadian Journal of Plant Science*, vol. 96, no. 1, p. 139-147. http://dx.doi.org/10.1139/cjps-2015-0150

Yield of sweet potato fertilized with cattle manure and biofertilizer. *Horticultura Brasileira*, vol. 28, no. 3, p. 277-281. http://dx.doi.org/10.1590/S0102-05362010000300006

Yildirim, Z., Tokuşoğlu, Ö., Öztürk, G. 2011. Determination of Sweet Potato [*Ipomoea batatas* (L.) Lam] Genotypes Suitable to the Aegean Region of Turkey. *Turkish Journal of Field Crops*, vol. 16, no. 1, p. 48-53. [cit. 2016-03-14] Available at: http://www.field-crops.org/assets/pdf/product512fa99bd3c05.pdf

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HISTOLOGICAL ANALYSIS OF FEMORAL BONES IN RABBITS ADMINISTERED BY AMYGDALIN

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ABSTRACT

Cyanogenic glycosides are present in several economically important plant foods. Amygdalin, one of the most common cyanoglucoside, can be found abundantly in the seeds of apples, bitter almonds, apricots, peaches, various beans, cereals, cassava and sorghum. Amygdalin has been used for the treatment of cancer, it shows killing effects on cancer cells by release of cyanide. However, its effect on bone structure has not been investigated to date. Therefore, the objective of this study was to determine a possible effect of amygdalin application on femoral bone microstructure in adult rabbits. Four month old rabbits were randomly divided into two groups of three animals each. Rabbits from E group received amygdalin intramuscularly at a dose 0.6 mg.kg^{-1} body weight (bw) (group E, n = 3) one time per day during 28 days. The second group of rabbits without amygdalin supplementation served as a control (group C, n = 3). After 28 days, histological structure of femoral bones in both groups of rabbits was analysed and compared. Rabbits from E group displayed different microstructure in middle part of the compact bone and near endosteal bone surface. For endosteal border, an absence of the primary vascular longitudinal bone tissue was typical. This part of the bone was formed by irregular Haversian and/or by dense Haversian bone tissues. In the middle part of *substantia compacta*, primary vascular longitudinal bone tissue was observed. Cortical bone thickness did not change between rabbits from E and C groups. However, rabbits from E group had a significantly lower values of primary osteons' vascular canals and secondary osteons as compared to the C group. On the other hand, all measured parameters of Haversian canals did not differ between rabbits from both groups. Our results demonstrate that intramuscular application of amygdalin at the dose used in our study affects femoral bone microstructure in rabbits.

Keywords: amygdalin; femoral bone; rabbit; histomorphometry

INTRODUCTION

Cyanogenic glycosides are natural plant toxicants **(Bolarinwa et al., 2015)**. All cyanogenic glycosides are potentially dangerous due to production of hydrogen cyanide (HCN) by their hydrolysis **(Vetter, 2000)**, known as prussic acid **(Francisco and Pinotti, 2000)**. Consumption of cyanogenic plants may cause acute and chronic toxicity in both animals and humans **(Yildirim and Askin, 2010)**.

Amygdalin, D-mandelonitrile-β-D-gentiobioside, $C_{20}H_{27}NO_{11}$, is one of the most frequently occurring cyanogenic glycosides **(Bolarinwa et al., 2014)**. It is especially presented in fruit kernels of peaches, apricots, bitter almonds **(Blaheta et al., 2016)**, pears, plums and apples **(Kolesár et al., 2015)**. Amygdalin is composed of two molecules of glucose, one of benzaldehyde and one of hydrocyanic acid **(Chang et al., 2006; Abdel-Rahman, 2011).**

Amygdalin itself is non-toxic, but it is able to generate toxic hydrogen cyanide (HCN) **(Bolarinwa et al., 2014)** which is decomposed by some endogenous plant enzymes **(Song and Xu, 2014; Kolesár et al., 2015)**. Amygdalin has been used as a one of the most popular alternative treatments of cancer, asthma, atherosclerosis, hypertension, migraine and chronic inflammation **(Cheng et al., 2015)**. However, the Food and Drug Administration (FDA) has not approved amygdalin as a therapeutic agent owing to insufficient clinical evidence of its positive efficacy and potential toxicity **(Zhou et al., 2012; Halenár et al., 2015)**. The acute lethal dose of peroral application of HCN for human ranges between 0.5 and 3.5 mg.kg^{-1} bw **(Speijers, 1993; Bolarinwa et al., 2014)**. The maximum tolerance dose of intramuscular injection of amygdalin is 3 g.kg^{-1} bw in rabbits **(Song and Xu, 2014)**.

Amygdalin has a stimulating effect on the growth of skeletal muscle cells (line C2C12) which is dose-dependent **(Yang et al., 2014)**. However, the impact of amygdalin on bone microstructure is still unknown.

Therefore, the aim of our study was to investigate the effect of intramuscular application of amygdalin on femoral bones microstructure in adult male rabbits.

MATERIAL AND METHODOLOGY

Adult male rabbits (n = 6) of outbred line P91 (Californian broiler line) were used in the experiment. The animals (at the age of 4 months, weighing 4 ±0.2 kg) were obtained from an experimental farm of the Animal Production Research Centre in Nitra, Slovak Republic.

Males were housed in individual flat-deck wire cages (area 0.3 m^2) under standard conditions (temperature 20 – 22 °C, humidity 55 ±10%, 12/12 h cycle of light and darkness) with access to food (feed mixture) and drinking water *ad libitum*.

Clinically healthy animals were randomly divided into two groups of three individuals each. In the experimental group (E), adult rabbits were intramuscularly injected with amygdalin (99% purity, Sigma-Aldrich, St. Louis, MO, USA) at the dose 0.6 $mg.kg^{-1}$ bw one time per day during 28 days. The second group (C; n = 3) without amygdalin addition served as a control. All experimental procedures were approved by the State Veterinary and Food Institute of Slovak Republic, no. 3398/11-221/3 and Ethic Committee.

After 28 days, all the rabbits were euthanized and their femurs were collected for microscopical analysis. Right femurs were sectioned at the midshaft of the diaphysis and the segments were fixed in HistoChoice fixative (Amresco, USA). The segments were then dehydrated in increasing grades (40 to 100%) of ethanol and embedded in Biodur epoxy resin (Günter von Hagens, Heidelberg, Germany) according to the method described by **Martiniaková et al., (2008)**. Transverse thin sections (70 – 80 μm) were prepared with a sawing microtome (Leitz 1600, Leica, Wetzlar, Germany) and fixed onto glass slides by Eukitt (Merck, Darmstadt, Germany) as previously described **(Martiniaková et al., 2010)**. The qualitative histological characteristics of the compact bone tissue were determined according to the internationally accepted classification systems of **Enlow and Brown (1956)** and **Ricqlés et al. (1991)**. The quantitative (histomorphometrical) variables were assessed using the software Motic Images Plus 2.0 ML (Motic China Group Co., Ltd.). We measured area, perimeter and the minimum and maximum diameters of primary osteons' vascular canals, Haversian canals and secondary osteons in all views (i.e., anterior, posterior, medial and lateral) of the thin sections in order to minimize inter-animal differences. Diaphyseal cortical bone thickness was also measured by Motic Images Plus 2.0 ML software. Twenty random areas were selected, and average thickness was calculated for each femur.

Statistical analysis was performed using SPSS 8.0 software. All data were expressed as mean ±standard deviation (SD). The unpaired Student's T-test was used for establishing statistical significance (p <0.05) between both groups of rabbits.

RESULTS AND DISCUSSION

Femoral diaphysis of rabbits from the C group had the following microstructure in common. Primary vascular longitudinal bone tissue, as a basic structural pattern of rabbit's bones, formed the endosteal and periosteal borders. This tissue contained vascular canals which ran in a direction essentially parallel to the long axis of the bone. Near endosteal and periosteal borders, primary vascular radial bone tissue occurred (mainly in anterolateral and anteromedial views). It was created by branching or non-branching vascular canals radiating from the marrow cavity or *periosteum*. The middle part of the compact bone was composed by dense Haversian bone tissue with a high concentration of secondary osteons. Several secondary osteons were also observed near the endosteal surface (especially in the anterior and posterior views) (Figure 1).

Rabbits intramuscularly administered by amygdalin (E group) had different bone microstructure in the middle part of the *substantia compacta* and near endosteal bone surface. For endosteal border, an absence of the primary vascular longitudinal bone tissue was typical (mainly in anterolateral and anteromedial views). Endosteal surface was formed by irregular Haversian tissue (characterized by occurrence of scattered secondary osteons) and/or by dense Haversian bone tissue. In the middle part of *substantia compacta*, primary vascular longitudinal bone tissue was observed (it extended there from *periosteum*). Also, no secondary osteons were presented there. The periosteal surface was composed of primary vascular longitudinal bone tissue (Figure 2).

The results of qualitative histological analysis in rabbits from the C group corresponded with previous studies **(Enlow and Brown, 1956; Martiniaková et al., 2003; Martiniaková et al., 2009)**. However, intramuscular application of amygdalin (in rabbits from E group) induced changes near endosteal bone surface and also in the middle part of the compact bone. Central area of the compact bone was composed of primary vascular longitudinal bone tissue. Secondary osteons were found only near endosteal border. This phenomenon can be associated with adaptation of bone tissue to amygdalin exposure. This mechanism might prevent towards bone cells apoptosis and bone tissue necrosis. The experiment by **Shou et al., (2000)** has shown that cyanide treatment induced apoptosis by inducing oxidative stress in cortical neurons. It is known that bone is richly innervated **(Marenzana and Chenu, 2008)**. Nerve endings are in direct contact with bone cells **(He et al., 2013)**, indicating that nerve fibers may regulate growth and remodeling of the bone. It was found that sympathetic denervation induced abnormal formation and resorption of bone **(Chenu and Marenzana, 2005)**. Also, increased bone resorption was observed in rats after removal of sympathetic nerve supply **(He et al., 2011)**.

Therefore, the absence of primary vascular longitudinal bone tissue near endosteal surface in rabbits from E group can be connected with intensive endosteal resorption due to amygdalin administration. Generally, skeletal system is a dynamic organ and is constantly undergoing remodeling, which is enabled by osteoblasts and osteoclasts **(Guntur and Rosen, 2012; Arakaki et al., 2013)**. The formation and activation of osteoclasts to enhance bone resorption have shown to be associated with the higher generation of oxygen-derived free radicals **(Garrett et al., 1990; Baek et al., 2010)**. **Gunasekar et al., (1998)** reported that excessive production of reactive oxygen species (ROS) might be cyanide-induced. With accordance with this finding, **Daya et al., (2000)** reported that cyanide led to generation of oxidative stress and also lipid peroxidation which are associated with hypoxia **(Gunasekar et al., 1998)**. **Chang et al., (2014)** found that hypoxia reduced proliferative activity of osteoblasts and inhibited mineralization and bone formation. On the other hand, hypoxia increased formation of osteoclasts **(Patntirapong and Hauschka, 2007; Arnett, 2010)** and activity of these cells **(Knowles and Athanasou, 2009)**. The authors **(Patntirapong and Hauschka, 2007)** further stated that

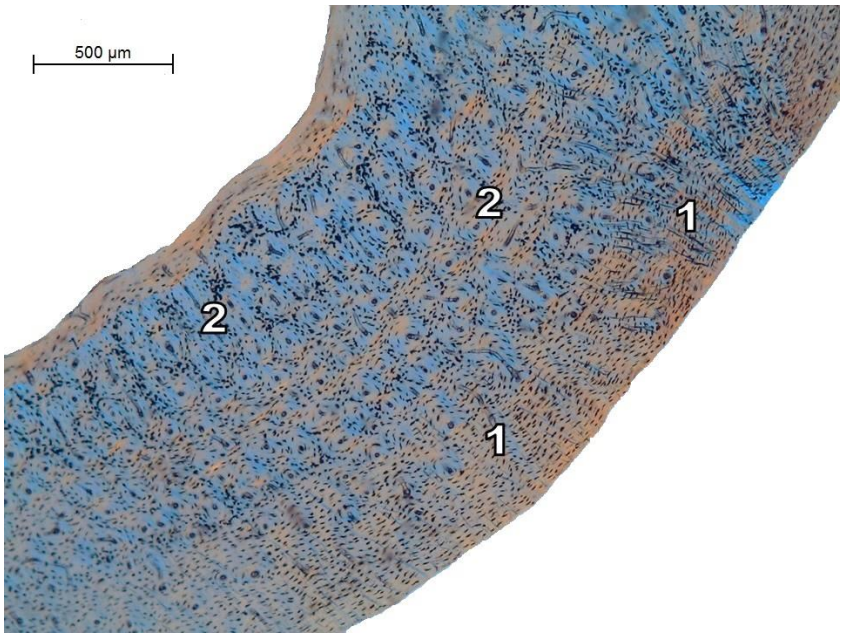

Figure 1 Microscopical structure of femoral bone in rabbits from the C group:
1 – primary vascular longitudinal bone tissue,
2 – dense Haversian bone tissue.

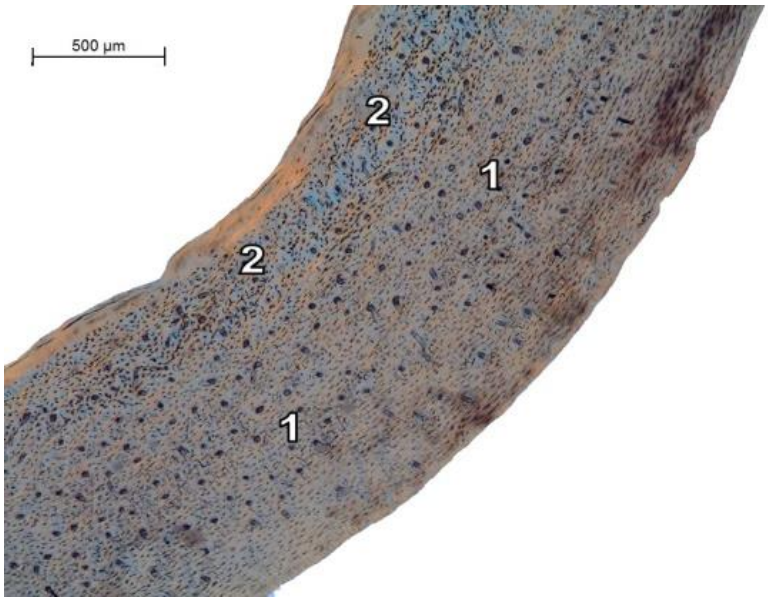

Figure 2 Microscopical structure of femoral bone in abbits from the E group:
1 – primary vascular longitudinal bone tissue,
2 – dense Haversian bone tissue.

low concentration of oxygen may change homeostasis of bone, leading to osteolysis, osteonecrosis **(Martiniaková et al., 2013b)** and osteoporosis **(Alagiakrishnan et al., 2003)**. Our results also showed increased periosteal bone apposition due to amygdalin administration which can serve as a compensatory mechanism to enormous endosteal bone reduction **(Szulc et al., 2006)**.

Our results showed an – non-significant effect of amygdalin application on cortical bone thickness in male rabbits (1085.15 ±145.54 mm and 1053.90 ±153.17 mm in rabbits from E and C groups, respectively).

For the quantitative histological analysis, 196 vascular canals of primary osteons, 98 Haversian canals and 98 secondary osteons were measured in both groups of rabbits. The results are summarized in Tables 1, 2 and 3. We have found that intramuscular application of amygdalin significantly affected sizes of the primary osteons' vascular canals and secondary osteons. Primary osteons' vascular canals and secondary osteons were significantly lower (p <0.05) in males from the E group. On the other hand, the size of Haversian canals did not differ between rabbits from both groups.

Rabbits injected with amygdalin had significantly lower values of primary osteons' vascular canals. This finding could be connected with a negative impact of amygdalin on blood vessels which are situated in vascular canals of primary osteons **(Currey, 2002; Greenlee and Dunnell, 2010)**.

Table 1 Data on primary osteons' vascular canals in rabbits from E and C groups.

Rabbit´s group	N	Area (μm^2)	Perimeter (μm)	Max. diameter (μm)	Min. diameter (μm)
E	96	290.67 ±48.08	61.40 ±5.40	10.76 ±1.31	8.66 ±0.99
C	100	314.33 ±63.56	63.73 ±6.67	11.14 ±1.51	9.01 ±1.18
T-test		p <0.05	p <0.05	NS	NS

Note: N: number of measured structures; NS: non-significant changes.

Table 2 Data on Haversian canals in rabbits from E and C groups.

Rabbit´s group	N	Area (μm^2)	Perimeter (μm)	Max. diameter (μm)	Min. diameter (μm)
E	49	338.25 ±73.78	66.05 ±6.83	11.64 ±1.30	9.23 ±1.29
C	49	343.88 ±58.93	67.18 ±5.63	11.93 ±1.40	9.24 ±1.22
T-test		NS	NS	NS	NS

Note: N: number of measured structures; NS: non-significant changes.

Table 3 Data on secondary osteons in rabbits from E and C groups.

Rabbit´s group	N	Area (μm^2)	Perimeter (μm)	Max. diameter (μm)	Min. diameter (μm)
E	49	4887.19 ±2408.89	245.70 ±62.40	43.42 ±11.55	33.95 ±9.16
C	49	6849.68 ±2795.79	291.70 ±57.75	50.66 ±9.85	41.54 ±9.50
T-test		p <0.05	p <0.05	p <0.05	p <0.05

Note: N: number of measured structures**.**

Waypa et al., (2001) reported that cyanide caused vasoconstriction. According to **Hamel (2011)** hypoxia has a negative effect on cardiovascular system and induced constriction of pulmonary arteries and dilatation in systemic arteries **(Weir and Archer, 1995)**. Higher levels of ROS production are associated with endothelial dysfunction like atherosclerosis and hypertension **(Guzik et al., 2011)**. **Cheng et al., (2015)** found that amygdalin increased the intracellular calcium level which can be associated with atherosclerosis **(Henry, 1985; Orimo and Ouchi, 1990)**. According to many authors **(Baum and Moe, 2008; Yarema and Yost, 2011)** glucocorticoid hormones have essential roles in homeostatic regulation and stress adaptation. These types of hormones cause vasoconstriction of blood vessels and mediate hypertension **(Saruta, 1996; Ponticelli and Glassock, 2009)**. According to **Ullian (1999)** application of hydrocortisone or corticosterone into rabbit aortic strips leads to greatly potentiated contractile responses to norepinephrine. Also **Berecek and Bohr (1978)** mentioned that deoxycorticosterone application stimulated vasoconstrictive effect of norepinephrine and angiotensin II in pigs. Based on these findings, we assume that the significant reduction of the size of primary osteons' vascular canals could be associated with these aspects.

On the other hand, non-significant changes in the size of Haversian canals were observed in rabbits from E group. It is generally known that the structure of primary and secondary osteons is different. Secondary osteons and also Haversian canals are surrounded by a cement line which is not found in primary osteons **(Currey, 2002; Martiniaková et al., 2013b)**. We suppose that the cement line is a main reason for different results in histomorphometry of both canals.

We have also found significantly lower secondary osteons in rabbits from E group. It is known that collagen type I (the major organic component of mineralized bone matrix, **Buchwald et al., 2012**) is produced by osteoblasts **(Bosetti et al., 2003; Wang et al., 2012)**. Secondary osteons are formed by lamellae in which collagen fibers run parallel to each other **(Martiniaková et al., 2013b)**. The arrangement of collagen fibers in lamellaes of secondary osteons ensures strength **(Dylevský, 2009)** and biochemical properties of the compact bone **(Martiniaková et al., 2013a)**. According to **Wang et al., (2012)** osteoblasts also synthesize alkaline phosphatase (ALP) which promotes mineralization of bone matrix. **Mody et al., (2001)** observed increase of intracellular oxidative stress and inhibition of differentiation markers in osteoblasts. The same findings have also been documented in the study of **Bai et al., (2004)**. In addition, these authors observed a reduction in the expression of collagen type I and ALP in rabbits calvarial osteoblasts. The experiment by **Arai et al., (2007)** has shown that bone mineralization significantly decreased by hydrogen peroxide (the most important ROS) exposure. Therefore, changes in the size of secondary osteon's may be associated with the inhibitory effect of oxidative stress (caused by amygdalin) on the osteoblastic activity which is connected with a reduction of collagen **(Patntirapong and Hauschka, 2007)**.

CONCLUSION

Natural plant substances like amygdalin are still a major part of traditional medicine. However, its effect on animal and human organisms is still not clear. Our results demonstrate that administration of amygdalin at the dose 0.6 $mg.kg^{-1}$ bw one time per day during 28 days induced changes in femoral bone microstructure of rabbits. Primary vascular longitudinal bone tissue was not found near endosteal surface. On the other hand, it was observed near *periosteum* and also in the middle part of *substantia*

compacta. Moreover, an absence of secondary osteons in the central area of the bone was identified in amygdalin-injected rabbits. Also, rabbits from E group had significantly lower (p <0.05) values of primary osteons' vascular canals and secondary osteons as compared to the C group.

REFERENCES

Abdel-Rahman, M. K. 2011. Can apricot kernels fatty acids delay the atrophied hepatocytes from progression to fibrosis in dimethylnitrosamine (DMN)-induced liver injury in rats? *Lipids in Health and Disease*, vol. 10, no. 114, p. 1-10. http://dx.doi.org/10.1186/1476-511X-10-114

Alagiakrishnan, K., Juby, A., Hanley, D., Tymchak, W., Sclater, A. 2003. Role of Vascular Factors in Osteoporosis. *The Journal of Gerontology. Series A, Biological Sciences and Medical Sciences*, vol. 58, no. 4, p. 362-366. http://dx.doi.org/10.1093/gerona/58.4.M362 PMid: 12663699

Arai, M., Shibata, Y., Pugdee, K., Abiko, Y., Ogata, Y. 2007. Effects of reactive oxygen species (ROS) on antioxidant system and osteoblastic differentiation in MC3T3-E1 cells. *UIBMB Life*, vol. 59, no. 1. p. 27-33. doi:10.1080/15216540601156188 PMid: 17365177

Arakaki, N., Yamashita, A., Niimi, S., Yamazaki, T. 2013. Involvement of reactive oxygen species in osteoblastic differentiation of MC3T3-E1 cells accompanied by mitochondrial morphological dynamics. *Biomedical Research*, vol. 34, no. 3, p. 161-166. http://dx.doi.org/10.2220/biomedres.34.161 PMid: 23782750

Arnett, T. R. 2010. Acidosis, hypoxia and bone. *Archives of Biochemistry and Biophysics*, vol. 503, no. 1, p. 103-109. http://dx.doi.org/10.1016/j.abb.2010.07.021

Baek, K. H., Oh, K. W., Lee W. Y., Lee, S. S., Kim, M. K., Kwon, H. S., Rhee, E. J., Han, J. H., Song, K. H., Cha, B. Y., Lee, K. W., Kang, M. I. 2010. Association of Oxidative Stress with Postmenopausal Osteoporosis and the Effects of Hydrogen Peroxide on Osteoclast Formation in Human Bone Marrow Cell Culture. *Calcified Tissue International*, vol. 87, no. 3, p. 226-235. http://dx.doi.org/10.1007/s00223-010-9393-9 PMid: 20614110

Bai, X., Lu, D., Bai, J., Zheng, H., Ke, Z., Li, X., Luo, S. 2004. Oxidative stress inhibits osteoblastic differentiation of bone cells by ERK and NF-κB. *Biochemical and Biophysical Research Communications*, vol. 314, no. 1, p. 197-207. http://dx.doi.org/10.1016/j.bbrc.2003.12.073 PMid: 14715266

Baum, M., Moe, O. W. 2008. Glucocorticoid-Mediated Hypertension: Does the Vascular Smooth Muscle Hold All the Answers? *Journal of the American Society of Nephrology*, vol. 19, no. 7, p. 1251-1253. http://dx.doi.org/10.1681/ASN.2008040410 PMid: 18508960

Berecek, K. H., Bohr, D. F. 1978. Whole Body Vascular Reactivity during the Development of Deoxycorticosterone Acetate Hypertension in the Pig. *Circulation Research*, vol. 42, no. 6, p. 764-771. http://dx.doi.org/10.1161/01.RES.42.6.764 PMid: 657435

Blaheta, R. A., Nelson, K., Haferkamp, A., Juengel, E. 2016. Amygdalin, quackery or cure? *Phytomedicine*, vol. 23, no. 4, p. 367-376. http://dx.doi.org/10.1016/j.phymed.2016.02.004

Bolarinwa, I. F., Orfila, C., Morgan, M. R. A. 2014. Amygdalin content of seeds, kernels and food products commercially-available in the UK. *Food Chemistry*, vol. 152, p. 133-139. http://dx.doi.org/10.1016/j.foodchem.2013.11.002 PMid: 24444917

Bolarinwa, I. F., Orfila, C., Morgan, M. R. A. 2015. Determination of amygdalin in apple seeds, fresh apples and processed apple juices. *Food Chemistry*, vol. 170, p. 437-442. http://dx.doi.org/10.1016/j.foodchem.2014.08.083 PMid: 25306368

Bosetti, M., Zanardi, L., Hench, L., Cannas, M. 2003. Type I collagen production by osteoblast-like cells cultured in contact with different bioactive glasses. *Journal of Biomedical Materials Research part A*, vol. 64, no. 1, p. 189-195. http://dx.doi.org/10.1002/jbm.a.10415 PMid: 12483713

Buchwald, T., Kozielski, M., Szybowicz, M. 2012. Determination of Collagen Fibers Arrangement in Bone Tissue by Using Transformations of Raman Spectra Maps. *Spectroscopy: An International Journal*, vol. 27, no. 2, p. 107-117. http://dx.doi.org/10.1155/2012/261487

Chang, H. K., Shin, M. S., Yang, H. Y., Lee, J. W., Kim, Y. S., Lee, M. H., Kim, J., Kim, K. H., Kim, C. J. 2006. Amygdalin Induces Apoptosis through Regulation of Bax and Bcl-2 Expressions in Human DU145 and LNCaP Prostate Cancer Cells. *Biological and Pharmaceutical Bulletin*, vol. 29, no. 8, p. 1597-1602. http://dx.doi.org/10.1248/bpb.29.1597 PMid: 16880611

Chang, J., Jackson, S. G., Wardale, J., Jones, S. W. 2014. Hypoxia Modulates the Phenotype of Osteoblasts Isolated From Knee Osteoarthritis Patients, Leading to Undermineralized Bone Nodule Formation. *Arthritis and Rheumathology*, vol. 66, no. 7, p. 1789-1799. http://dx.doi.org/10.1002/art.38403 PMid: 24574272

Cheng Y., Yang, C., Zhao, J., Tse, H. F., Ring, J. 2015. Proteomic identification of calcium-binding chaperone calreticulin as a potential mediator for the neuroprotective and neuritogenic activities of fruit-derived glycoside amygdalin. *The Journal of Nutritional Biochemistry*, vol. 26, no. 2, p. 146-154. http://dx.doi.org/10.1016/j.jnutbio.2014.09.012 PMid:25465157

Chenu, C., Marenzana, M. 2005. Sympathetic nervous system and bone remodeling. *Joint Bone Spine*, vol. 72, no. 6, p. 481-483. http://dx.doi.org/10.1016/j.jbspin.2005.10.007

Currey, J. D. 2002. *Bones - Structure and Mechanics* United States: Princeton University Press, p. 436. ISBN 9780691128047.

Daya, S., Walker, R. B., Anoopkumar-Dukie, S. 2000. Cyanide-Induced Free Radical Production and Lipid Peroxidation in Rat Homogenate is Reduced by Aspirin. *Metabolic Brain Disease*, vol. 15, no. 3, p. 203-210. http://dx.doi.org/10.1023/A:1011163725740 PMid: 11206589

Dylevský, I. 2009. Funkční anatomie. *The Functional Anatomy*. Praha: Grada Publishing, a. s., p. 544. ISBN 978-80-247-3240-4.

Enlow, D. H., Brown, S. O. 1956. A comparative histological study of fossil and recent bone tissues. Part I. *Texas Journal of Science*, vol. 8, p. 405-412.

Francisco, I. A., Pinotti, M. H. P. 2000. Cyanogenic Glycosides in Plants. *Brazilian Archives of Biology and Technology*, vol. 43, no. 5, p. 487-492. http://dx.doi.org/10.1590/S1516-89132000000500007

Garrett, I. R., Boyce, B. F., Oreffo, R. O. C., Bonewald, L., Poser, J., Mundy, G. R. 1990. Oxygen-derived Free Radicals Stimulate Osteoclastic Bone Resorption in Rodent Bone In Vitro and In Vivo. *The Journal of Clinical Investigation*, vol. 85, no. 3, p. 632-639. http://dx.doi.org/10.1172/JCI114485 PMid: 2312718

Greenlee, D. M., Dunnell, R. C. 2010. Identification of fragmentary bone from the Pacific. *Journal of Archaeological Science*, vol. 37, no. 5, p. 957-970. http://dx.doi.org/10.1016/j.jas.2009.11.029

Gunasekar, P. G., Borowitz, J. L., Isom, G. E. 1998. Cyanide-Induced Generation of Oxidative Species: Involvement of Nitric Oxide Synthase and Cyclooxygenase-2. *Journal of Pharmacological and Experimental Therapeutics*, vol. 285, no. 1, p. 236-241. PMid: 9536016

Guntur, A. R., Rosen, C. J. 2012. Bone as an endocrine organ. *Endocrine Practice*, vol. 18, no. 5, p. 758-762. http://dx.doi.org/10.4158/EP12141.RA PMid: 22784851

Guzik, B., Chwala, M., Matusik, P., Ludew, D., Skiba, D., Wilk, G., Mrowiecki, W., Batko, B., Cencora, A., Kapelak, B., Sadowski, J., Korbut, R., Guzik, T. J. 2011. Mechanisms of increased vascular superoxide production in human varicose veins. *Polskie Archiwum Medycyny Wewnnetrznej*, vol. 121, no. 9, p. 279-286. PMid: 21860369

Halenár, M., Medveďová, M., Maruniaková, N., Kolesárová, A. 2015. Ovarian hormone production affected by amygdalin addition *in vitro*. *Journal of Microbilogy, Biotechnology and Food Sciences*, vol. 4, p. 19-22. http://dx.doi.org/10.15414/jmbfs.2015.4.special2.19-22

Hamel, J. 2011. A Review of Acute Cyanide Poisoning. *Critical Care Nurse*, vol. 31, no. 1, p. 72-81. http://dx.doi.org/10.4037/ccn2011799

He, J. Y., Jiang, L. S., Dai, L. Y. 2011. The roles of the sympathetic nervous system in osteoporotic diseases: A review of experimental and clinical studies. *Ageing Research Reviews*, vol. 10, no. 2, p. 253-263. http://dx.doi.org/10.1016/j.arr.2011.01.002 PMid: 21262391

He, J. Y., Zheng, X. F., Jiang, S. D., Chen, X. D., Jiang, L. S. 2013. Sympathetic neuron can promote osteoblast differentiation through BMP signaling pathway. *Cellular Signalling*, vol. 25, no. 6, p. 1372-1378. http://dx.doi.org/10.1016/j.cellsig.2013.02.016 PMid: 23454096

Henry, P. D. 1985. Atherosclerosis, calcium, and calcium antagonists. *Circulation*, vol. 72, no. 3, p. 456-459. http://dx.doi.org/10.1161/01.CIR.72.3.456 PMid: 3893790

Knowles, H. J., Athanasou, N. A. 2009. Acute hypoxia and osteoclast activity: a balance between enhanced resorption and increased apoptosis. *Journal of Pathology*, vol. 218, p. 256-264. http://dx.doi.org/10.1002/path.2534 PMid: 19291710

Kolesár, E., Halenár, M., Kolesárová, A., Massányi, P. 2015. Natural plant toxicant - cyanogenic glycoside amygdalin: characteristic, metabolism and the effect on animal reproduction. *Journal of Microbilogy, Biotechnology and Food Sciences*, vol. 4, p. 49-50. http://dx.doi.org/10.15414/jmbfs.2015.4.special2.49-50

Marenzana, M., Chenu, C. 2008. Sympathetic nervous system and bone adaptive response to its mechanical environment. *Journal of Musculoskeletal and Neuronal Interactions*, vol. 8, no. 2, p. 111-120. PMid: 18622080

Martiniaková, M., Vondráková, M., Fabiš, M. 2003. Investigation of the microscopic structure of rabbit compact bone tissue. *Scripta medica (Brno)*, vol. 76, no. 4, p. 215-220.

Martiniaková, M., Omelka, R., Grosskopf, B., Sirotkin, A. V., Chrenek, P. 2008. Sex-related variation in compact bone microstructure of the femoral diaphysis in juvenile rabbits. *Acta Veterinaria Scandinavica*, vol. 50, p. 15. http://dx.doi.org/10.1186/1751-0147-50-15 PMid:18522730

Martiniaková, M., Omelka, R., Grosskopf, B., Chovancová, H., Massányi, P., Chrenek, P. 2009. Effects of dietary supplementation of nickel and nickel-zinc on femoral bone structure in rabbits. *Acta Veterinaria Scandinavica*, vol. 50, p. 15. http://dx.doi.org/10.1186/1751-0147-51-52 PMid: 20003522

Martiniaková, M., Omelka, R., Jančová, A., Stawarz, R., Formicki, G. 2010. Heavy metal content in the femora of yellow-necked mouse (*Apodemus flavicollis*) and wood mouse (*Apodemus sylvaticus*) from different types of polluted environment in Slovakia. *Environmental Monitoring Assessment*, vol. 171, no. 1-4, p. 651-660. http://dx.doi.org/10.1007/s10661-010-1310-1 PMid: 20135219

Martiniaková, M., Chovancová, H., Boboňová, I., Omelka, R. 2013a. *Účinky rizikových látok na štruktúru kostného tkaniva potkanov*. Nitra: Fakulta prírodných vied UKF, p. 187. ISBN 978-80-558-0295-4.

Martiniaková, M., Boboňová, I., Omelka, R., Grosskopf, B., Chovancová, H., Španková, J., Toman, R. 2013b. Simultaneous subchronic exposure to selenium and diazinon as possible risk factor for osteoporosis in adult male rats. *Acta Veterinaria Scandinavica*, vol. 55, p. 8. http://dx.doi.org/10.1186/1751-0147-55-81 PMid: 24237628

Mody, N., Parhami, F., Sarafian, T. A., Demer, L. L. 2001. Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. *Free Radical Biology and Medicine*, vol. 31, no. 4, p. 509-519. http://dx.doi.org/10.1016/S0891-5849(01)00610-4 PMid: 11498284

Orimo, H., Ouchi, Y. 1990. The role of calcium and magnesium in the development of atherosclerosis. Experimental and clinical evidence. *Annals of the New York Academy of Sciences*, vol. 598, p. 444-457. http://dx.doi.org/10.1111/j.1749-6632.1990.tb42315.x PMid: 2248457

Patntirapong, S., Hauschka, P. V. 2007. Molecular Regulation of Bone Resorption by Hypoxia. *Orthopaedic Journal, Harvard Medical School*, p. 72-75.

Ponticelli, C., Glassock, R. J. 2009. In: *Treatment of Primary Glomerulonephritis*. New York: Oxfort University Press, p. 481. ISBN 978-0-19-955288-7. http://dx.doi.org/10.1093/med/9780199552887.001.0001

Ricqlès, A. J., Meunier, F. J., Castanet, J., Francillon-Vieillot, H. 1991. *Comparative microstructure of bone. Bone 3, Bone Matrix and Bone Specific Products*. Hall BK. Boca Raton: CRC Press; p. 1-78. ISBN 0-8493-8823-6.

Saruta, T. 1996. Mechanism of Glucocorticoid-Induced Hypertension. *Hypertension Researcg*, vol. 19, no. 1, p. 1-8. http://dx.doi.org/10.1291/hypres.19.1 PMid: 8829818

Shou, Y., Gunasekar, P. G., Borowitz, J. L., Isom, G. E. 2000. Cyanide-induced apoptosis involves oxidative-stress-activated NF-kappaB in cortical neurons. *Toxicology and Applied Pharmacology*, vol. 164, no. 2, p. 196-205.

http://dx.doi.org/10.1006/taap.2000.8900
PMid: 10764633

Song, Z., Xu, X. 2014. Advanced research on anti-tumor effects of amygdalin. *Journal of Cancer Research and Therapeutics*, vol. 10, suppl. no. 1, p. 3-7. http://dx.doi.org/10.4103/0973-1482.139743
PMid: 25207888

Speijers, G. 1993. Cyanogenic glycosides. WHO Food Additives Series 30. Geneva. JECFA.

Szulc, P., Seeman, E., Duboeuf, F., Sornay-Rendu, E., Delmas, P. D. 2006. Bone fragility: failure of periosteal apposition to compensate for increased endocortical resorption in postmenopausal women. *Journal of Bone and Mineral Research*, vol. 21, no. 12, p. 1856-1863. http://dx.doi.org/10.1359/jbmr.060904

Tewe, O. O., Maner, J. H. 1980. Cyanide, protein and iodine interactions in the performance, metabolism and pathology of pigs. *Research in Veterinary Science*, vol. 29, no. 3, p. 271-276. PMid: 7255888

Ullian, M. E. 1999. The role of corticosteroids in the regulation of vascular tone. *Cardiovascular Research*, vol. 41, no. 1, p. 55-64. http://dx.doi.org/10.1016/S0008-6363(98)00230-2

Vetter, J. 2000. Plant cyanogenic glycosides. *Toxicon*, vol. 38, no. 1, p. 11-36. http://dx.doi.org/10.1016/S0041-0101(99)00128-2
PMid: 10669009

Wang, Y., Zhao, L., Wang, Y., Xu, J., Nie, Y., Guo, Y., Tong, Y., Qin, L., Zhang, Q. 2012. Curculigoside isolated from *Curculigo orchioides* prevents hydrogen peroxide-induced dysfunction and oxidative damage in calvarial osteoblasts. *Acta Biochimica et Biophysica Sinica (Shanghai)*, vol. 44, no. 5, p. 431-441. http://dx.doi.org/10.1093/abbs/gms014
PMid: 22427460

Waypa, G. B., Chandel, N. S., Schumacker, P. T. 2001. Model for Hypoxic Pulmonary Vasoconstriction Involving Mitochondrial Oxygen Sensing. *Circulation Research*, vol. 88, no. 12, p. 1259-1266. http://dx.doi.org/10.1161/hh1201.091960
PMid: 11420302

Weir, E. K., Archer, S. L. 1995. The mechanism of acute hypoxic pulmonary vasoconstriction: the tale of two channels. *Federation of American Societies for Experimental Biology Journal*, vol. 9, no. 2, p. 183-189. PMid: 7781921

Yang, C., Li, X., Rong, J. 2014. Amygdalin isolated from *Semen Persicae* (*Tao Ren*) extracts induces the expression of follistatin in HepG2 and C2C12 cell lines. *Chinese Medicine*, vol. 9, p. 1-8. http://dx.doi.org/10.1186/1749-8546-9-23
PMid: 25237385

Yarema, T. C., Yost, S. 2011. Low-Dose Corticosteroids to Treat Septic Shock: A critical Literature Review. *Critical Care Nurse*, vol. 31, no. 6, p. 16-26. http://dx.doi.org/10.4037/ccn2011551
PMid: 22135328

Yildirim, F. A., Askin, M. A. 2010. Variability of amygdalin content in seeds of sweet and bitter apricot cultivars in Turkey. *Africal Journal of Biotechnology*, vol. 9, no. 39, p. 6522-6524.

Zhou, C., Qian, L., Ma, H., Yu, X., Zhang, Y., Qu, W., Zhang, X., Xia, W. 2012. Enhancement of amygdalin activated with β-D-glucosidase on HepG2 cells proliferation and apoptosis. *Carbohydrate Polymers*, vol. 90, no. 1, p. 516-523. http://dx.doi.org/10.1016/j.carbpol.2012.05.073
PMid: 24751072

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MINOR LIPOPHILIC COMPOUNDS IN EDIBLE INSECTS

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ABSTRACT

Contemporary society is faced with the question how to ensure suffiecient nutrition (quantity and quality) for rapidly growing population. One solution can be consumption of edible insect, which can have very good nutritional value (dietary energy, protein, fatty acids, fibers, dietary minerals and vitamins composition). Some edible insects species, which contains a relatively large amount of fat, can have a potential to be a „good" (interesting, new) source of minor lipophilic compounds such as sterols (cholesterol and phytosterols) and tocopherols in our diet. For this reason, the objective of this work was to characterize the sterols and tocopherols composition of fat from larvae of edible insect *Zophobas morio* L. and *Tenebrio mollitor* L. Cholesterol and three phytosterols (campesterol, stigmasterol and β-sitosterol) were reliably identified and quantified after hot saponification and derivatization by GC-MS. Other steroid compounds, including 5,6-trans-cholecalciferol were identified only according to the NIST library. Cholesterol was the predominant sterol in all analysed samples. Both types of larvae also contained high amount of phytosterols. Different region of origin had a no significant impact on sterols composition, while the effect of beetle genus was crucial. Tocopherols were analysed by reverse phase HPLC coupled with amperometric detection. Tocopherols content in mealworm larvae was lower than content in edible oils, but important from the nutritional point of view. Change of tocopherols composition was not observed during the storage under different conditions. Larvae of edible insect can be a potential good dietary source of cholesterol, but also vitamin D_3 isomers, phytosterols and tocopherols.

Keywords: sterol; tocopherol; *Tenebrio mollitor*; *Zophobas morio*

INTRODUCTION

The large group of minor lipophylic compounds include higher hydrocarbons, alcohols, ketones and diketones, steroids, lipophilic vitamins, pigments and other compounds. Steroids and lipophilic vitamins are most important compounds from this group.

Several steroid compounds are usually present in plants and organisms, but the major steroids are phytosterols and cholesterol. Steroids are synthesised in organisms via complex mechanisms from isoprene units, isopentenyl diphosphate and dimethylallyl diphosphate. Reaction gives an important intermediate, farnesyl diphosphate. Two molecules of farnesyl phosphate give rise to triterpenic hydrocarbon squalene, which in the body of animals yields triterpenic alcohol lanosterol and the triterpenic alcohol cycloartenol in plants. Lanosterol in animals is a precursor for the biosynthesis of the most important zoosterol cholesterol. An intermediate in the biosynthesis of cholesterol is 7-dehydrocholesterol, which is a precursor of vitamin D_3. Cholesterol in the body is used for the biosynthesis of steroid hormones and bile acids **(Velíšek, 2014)**.

Insects also need cholesterol for the synthesis of vitamin D_3 and also for the synthesis of insect steroid hormones called as ecdysteroids (e.g. ecdysone, 20-hydroxy-ecdysone, makisterone A etc.). But, insects are not able to synthesize cholesterol *de novo* and they have to use plant phytosterols (β-sitosterol, campesterol, stigmasterol) for cholesterol synthesis by the side dealkylation on the C-24 alkyl group of dietary phytosterols. Cholesterol and 7-dehydrocholesterol are formed during synthetic pathway. For example, the beetle *Tenebrio molitor* produces about 17% of 7-dehydrocholesterol and about 67% of cholesterol from sitosterol **(Leclerq, 1948; Svoboda and Feldlaufer, 1991; Ikekawa et al., 1993; Svoboda and Lusby, 1994; Ikekawa et al., 2013)**.

Cholesterol is mainly found in animal fats and in human tissues. In lower animals other sterols, known collectively as zoosterols may also be present. Cholesterol and its esters are present in all membranes and in blood lipids, but particularly rich sources are nervous tissues, especially the brain. Egg yolk is other very important source of dietary cholesterol. Other sources include meat, milk and cheeses, but also animal fats, lard and butter to a greater extent **(Velíšek, 2014)**. Sterols are essential components of lipoproteins and lipid membranes in animals. They are particularly important in nerve tissues and in the transport of lipids, which are bound in lipoproteins. In humans, dietary cholesterol intake is lower than the daily requirement, therefore the body synthesises (in the liver) the majority of cholesterol that is needed. Cholesterol in the diet is easily absorbed, but problems may occur during transport of cholesterol from the intestinal wall during lymph and blood circulation. Excessive cholesterol transport in low-density lipoproteins may cause cardiovascular diseases. It is therefore recommended that the intake of dietary cholesterol should not exceed 300 mg per day **(Dinh et al.,**

2011; Golebiowski et al., 2014; Velíšek, 2014).

Vitamin D_3 is formed from 7-dehydrocholesterol after UV irradiation (wavelength 280 – 320 nm) through the intermediate precholecalciferol. Calcitriol (1α,25-dihydroxycholecalciferol) is the active form of vitamin D_3 that is created in oxidation in the liver and subsequently in the kidney. Along with two other hormones, calcitonin (from thyroid gland) and parathormone (parathyroid gland) act in the resorption, metabolism and excretion of calcium and phosphorus **(Lawson, 1971; Velíšek, 2014; Finke, 2015)**. Two geometrical isomers of the vitamin D_3 and its 25-hydroxyderivative can be formed during synthesis from 7-dehydrocholesterol and consecutive oxidation in the liver. 5,6-*cis* isomer, after oxidation in the kidney, stimulates intestinal calcium transport. Vitamin activity of 5,6-*trans* isomer is significantly lower, but this compound shows another biological activity – mainly antiproliferative activity **(Holick et al., 1972; Chen et al., 2000; Filip et al., 2010)**.

Plants synthesise a number of steroid substances from cycloartenol, mainly demethylsterols (campesterol and other phytosterols) and 4,4-dimethylsterols (mainly as saponins – betulinic acid etc.) or 4-methylsterols as minor compounds **(Kuksis, 2001; Velíšek, 2014)**.

Vitamin E, formerly also known as antisterile vitamin, has eight basic structurally-related derivatives of chroman-6-ol (2H-1-benzopyran-6-ol). Structural bases common to all compounds with the reported activity of vitamin E are tocol and tocotrienol, which contain a hydrophobic chromane ring with a saturated or unsaturated isoprenoid side chain of 16 carbon atoms **(Velíšek, 2014)**. Vitamin E, especially α-tocopherol is the most important lipophilic antioxidant that acts in eucaryotic cells to protect (poly)unsaturated lipids against free radical damage. Tocopherols show the antioxidant activity *in vivo* and also *in vitro*. It protects the structure and integrity of biomembranes, such as the cytoplasmic cell membrane (or plasmolema) and intracellular membranes of organelles (nucleus, mitochondria, lysosome and endoplasmic reticulum). It is also employed in the protection of lipoproteins present in plasma. It is transported in the bloodstream by association with the lipid phase of low density lipoprotein (LDL) particles. Each LDL particle contains six molecules of vitamin E **(Li et al., 1996; Munné-Bosch and Alegre, 2002; Hofius and Sonnewald, 2003; Velíšek, 2014)**.

The aim of this study was to characterize the profile of minor lipophilic (unsaponifiable) compounds (mainly steroids and vitamin E) in three types of the edible insect.

MATERIAL AND METHODOLOGY

Analyzed samples

Insect species used for analysis were larvae of mealworm (*Zophobas morio* L.) and larvae of superworm (*Tenebrio mollitor* L.). The stage of development of analyzed insect was suitable for culinary preparation. *Zophobas morio* and *Tenebrio mollitor* were purchased lyophilized in local market in Sumatra. *Zophobas morio* sample were also purchased in Radek Frýžela Company, Brno, Czech Republic. Insects were starved for 48 hours, killed with boiling water (100 °C) and dried at 105 °C to constant weight. These samples were homogenized and stored at 4 – 7 °C until analysis. Sampels of *Zophobas morio* (from Czech Republic) used for tocopherols analysis were stored at two different conditions: at 5 – 6 °C and at 25 °C (room temperature).

Fat extraction

Fat from analysed samples was extracted by Soxtherm® apparatus (Gerhardt, Königswinter, Germany). Approximately 5 g of homogenized sample was weight into extraction cartridge and extracted by petroleum ether 120 min at 70 °C. Fat content was measured gravimetrically after drying to the constant weight at 103 °C. Extracted fat was used for sterols and tocopherols analysis.

Sterols analysis

Sterols content was determined according to AOCS Official Method Ch 6-91, American Oil Chemists´ Society, USA, 1997. Approximately 0.5 g of fat was boiled by 50 ml ethanolic KOH (2 $mol.L^{-1}$) for 1 hour. The unsaponifiable fraction was extracted with diethylether. The solvent was evaporated using rotary vacuum evaporator. Dried samples were silylated by pyridine and BSTFA (Bis(trimethylsilyl)-trifluor-acetamide; Merck, Czech Republic). Sterols were determined by GC Agilent 7820A coupled with mass detector Agilent 5975 Series MSD (Agilent Technologies, Palo Alto, CA, USA). Analytes were separated by capillary column Supelco (SACTM5, 22 m x 025 mm I.D. x 0.25 µm film). High purity helium was used as carrier gas at a flow rate of 20 $mL.min^{-1}$. Column temperature program started at

245 °C for 1 min, than heated at a rate 10 °C/min to 290 °C for 33 min, than increased by 5 °C/min to 310 °C for 15 min. The injector temperature was set to 280 °C. Samples were injected (1µL) in a split mode (20:1). 5α-cholestane was used as internal standard for quantification of cholesterol, campesterol, stigmasterol and ß-sitosterol in SCAN mode. Peaks were identified by their retention time compared with pure standard, comparison of their mass spectra with the NIST library spectra and also by comparison with literature.

Tocopherols analysis

Tocopherols were determined by reverse phase HPLC with amperometric detection The analysis was performed under the following conditions: a mobile phase - mixture of acetonitrile/methanol (1:1, v/v) with $LiClO_4$ (0.02 $mol.L^{-1}$) and NaCl (0.005 $mol.L^{-1}$); a flow rate 1 ml/min (LCP 4020.31 nonmetallic pump Ecom, Prague, Czech Republic); injected volume 20 µL. Separation was performed by steel column (4 x 250 mm, a particle size 5 µm, Tessek, Prague, Czech Republic); column temperature 28 °C (LCO 101 column heater, Ecom, Prague, Czech Republic); detection potential - +0.7 V (HP 1049A amperometric detector with a glassy – carbon working and solid state Ag/AgCl reference electrode (Agilent Technologies, St. Clara, USA). The quantification of tocopherols was provide by external calibration. For the determination of tocopherols insect fat samples were prepared as follows. Approximately 0.25 g of fat extracted from insect was weighed into 25 mL volumetric flask and filled to the mark with acetone.

RESULTS AND DISCUSSION

Amounts of sterols in samples of edible insects are shown in Table 1.

Table 1 Sterol composition of edible insect species.

Insect	Sterol			
	Cholesterol (mg.kg^{-1}±SD)	**Campesterol (mg.kg^{-1}±SD)**	**Stigmasterol (mg.kg^{-1}±SD)**	**ß–sitosterol (mg.kg^{-1}±SD)**
Zophobas morio	1784.1 ±30.4	227.6 ±19.9	79.3 ±9.4	344.1 ±35.8
Zophobas morio*	1594.9 ±164.1	169.2 ±8.45	unquantified	260.2 ±12.3
Tenebrio mollitor	669.4 ±34.7	350.5 ±56.0	71.9 ±2.5	244.7 ±12.0

*Czech Republic

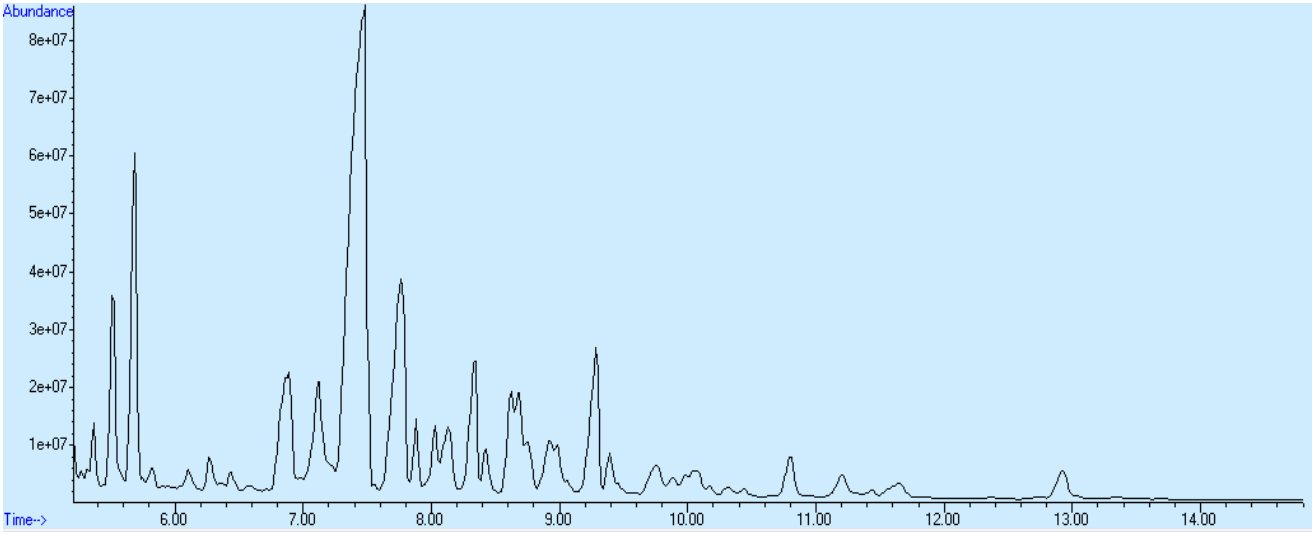
Table 2 Tocopherols composition of *Zophobas morio* (Czech Republic) stored at different conditions.

Conditions (°C)	α – tocopherol mg.kg^{-1} ±SD	ß + γ – tocopherol mg.kg^{-1} ±SD	δ – tocopherol mg.kg^{-1} ±SD
5 – 6	75.7 ±3.2	5.3 ±0.1	LOQ
25	77.2 ±0.1	5.3 ±0.3	LOQ

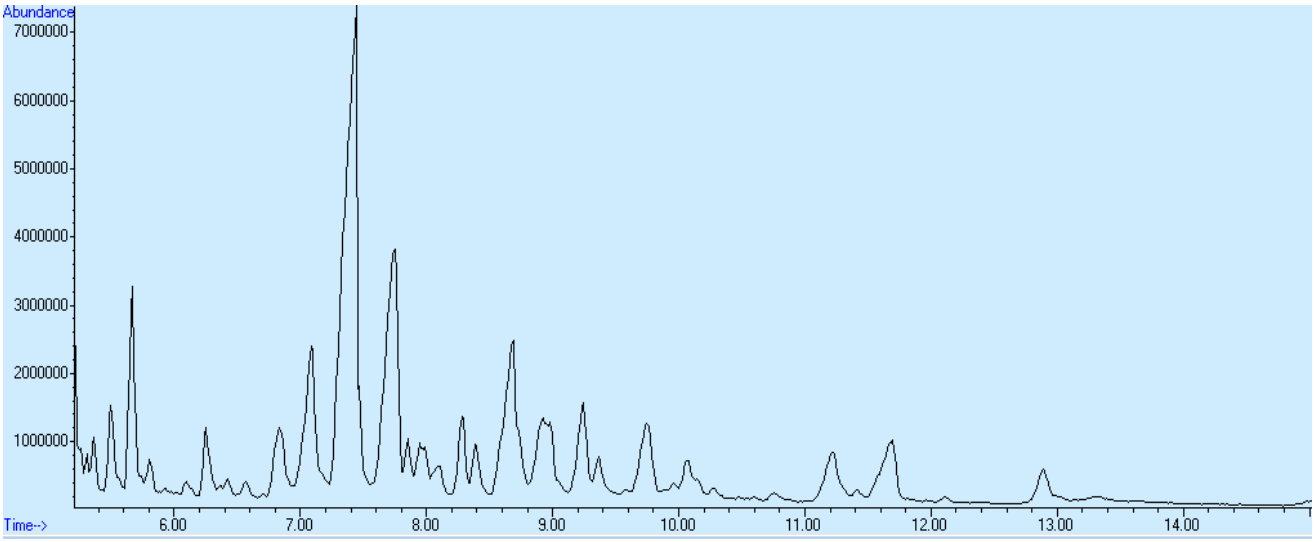
Cholesterol, typical animal sterol, was the most abundant sterol found in analysed samples. There are only minimum amount of cholesterol in food source of plant origin. Negative health impact (metabolic syndrome) of cholesterol is well known for a long time **(WHO, 2004).** On the other hand, cholesterol lowering effect of phytosterols is well known as well **(Peterson, 1951)**. Phytosterols reduce intestinal cholesterol absorbtion **(Normén et al., 2000)** and plasma LDL-cholesterol **(Piironen et al., 2000)**. Phytosterols (namely ß-sitosterol, campesterol and stigmasterol), typical plant origin sterols, were also determined in insects' samples. Identity of these compounds was confirmed by comparison with standards.

Cholesterol content was quite similar for the same insect species which came from different regions *Z. morio* from the Czech Republic (1594.9 mg.kg^{-1}) and *Z. morio* from Sumatra (1784.1 mg.kg^{-1}). The results indicate, that the extent of cholesterol synthesis is probably determined genetically and the influence of climate or type of feed is negligible. But larger set of samples would be needed to confirm these conclusions. However, sterols composition differences were much bigger between various insect species. *Z. morio* contained more cholesterol (1594.9 – 1784.1 mg.kg^{-1}) than *T. mollitor* (669.4 mg.kg^{-1}). An interesting trend can be seen in the content of phytosterols, which is the opposite content ratios of campesterol and β-sitosterol of mealworm larvae (228 and 344 mg.kg^{-1}) and superworm larvae (350 and 246 mg.kg^{-1}). This could indicate a different mechanism of cholesterol biosynthesis. Mealworm probably favors a demethylation of β-sitosterol (C_{29}-C_{28}), superworm favors easier demethylation of campesterol (C_{28}). The cholesterol content of the *T. mollitor* was comparable with content in meat of common livestock. Larvae of *Z. morio* synthesized much larger amount of cholesterol than there is in eggs – cholesterol content in the whole eggs is 2000 – 3500 mg per kg **(Velíšek, 2014)**. High cholesterol level could mean a nutritional problem.

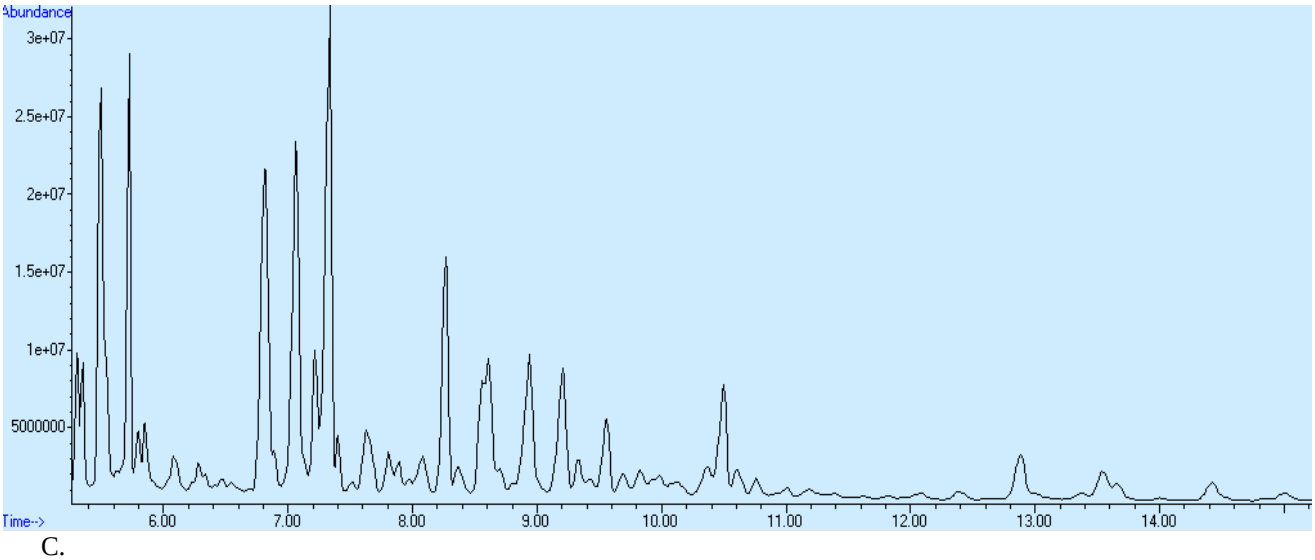
Chromatograms from sterols analysis in fat from different edible insect are shown in Figure 1. According to mass spectra, the following sterols and stanol (saturated sterol) were identified by the NIST library: lanosterol (RT 7.9), ergosterol (RT 8.1) and stigmastanol (RT 9.4). Ergosterol was found as pro-vitamin D_2 in food from plant and microbial origin **(Gorman et al., 1987; Tsiaras et al., 2011)**. All analyzed samples contained significant amount of the compound, that was identified as cholecalciferol (vitamin D_3). The main food sources of cholecalciferol are fish (mainly fatty fish such as herring, mackerel and salmon), meat, eggs, milk and dairy products **(Schmid and Walther, 2013; Velíšek, 2014)**. The compound with retention time 7.7 was identified as cholecalciferol according to the NIST library. However, retention time of cholecalciferol standard was different (6.1 to 7.1). Comparison of standard, real sample and the NIST library mass spectra of cholecalciferol is shown in Figure 2. As shown in this figure, the spectra are practically identical. Similarity of mass spectra and differences in retention times of these compounds suggests that these compounds are probably different cholecalciferol isomers. This compound is probably 5,6-*trans*-cholecalciferol, which is described in literature. This isomer was identified with high probability only at *Z. morio* samples. This isomer was identified with high probability only at *Z. morio* samples. 5,6-t*rans* vitamin D_3 does not have the same biological activity as 5,6-*cis* vitamin D_3, but it has other biological activities **(Borsje et al., 1977; Chen et al., 2000; Filip et al., 2010)**. 5,6-*cis* vitamin D_3 plays the crucial role in calcium and phosphorus metabolism (bone development and maintenance) and is also important for cell differentiation process and immune system **(Ovesen et al., 2003; Velíšek, 2014)**. Concerning other peaks of analytes, they were not identified with sufficient reliability.



A.

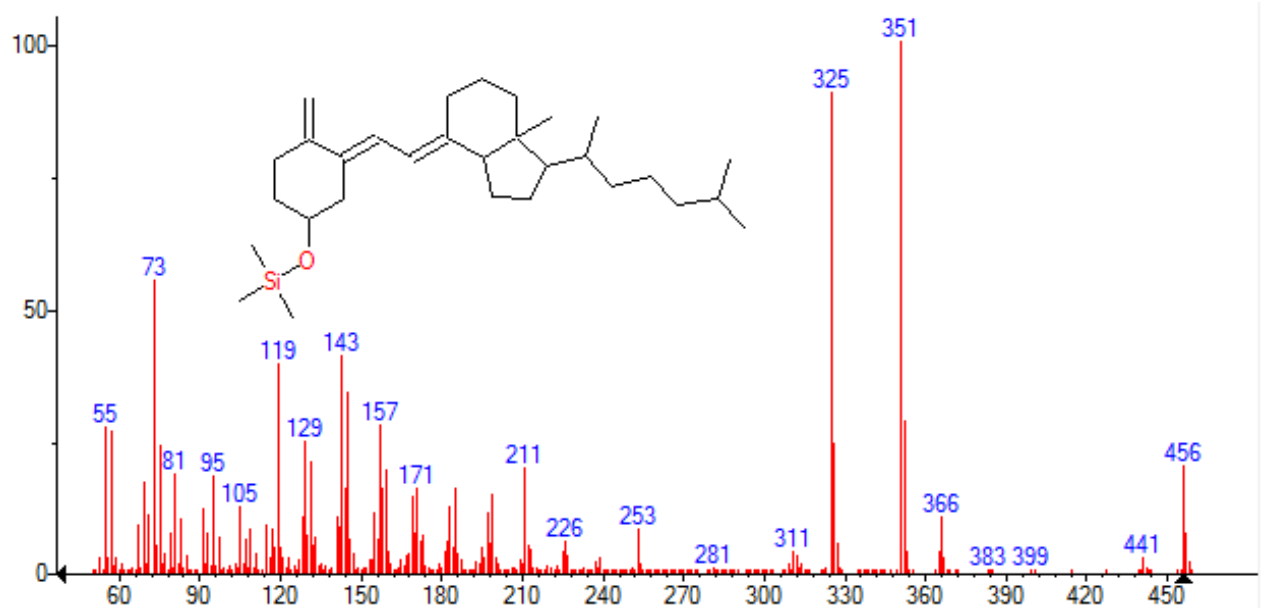


B.

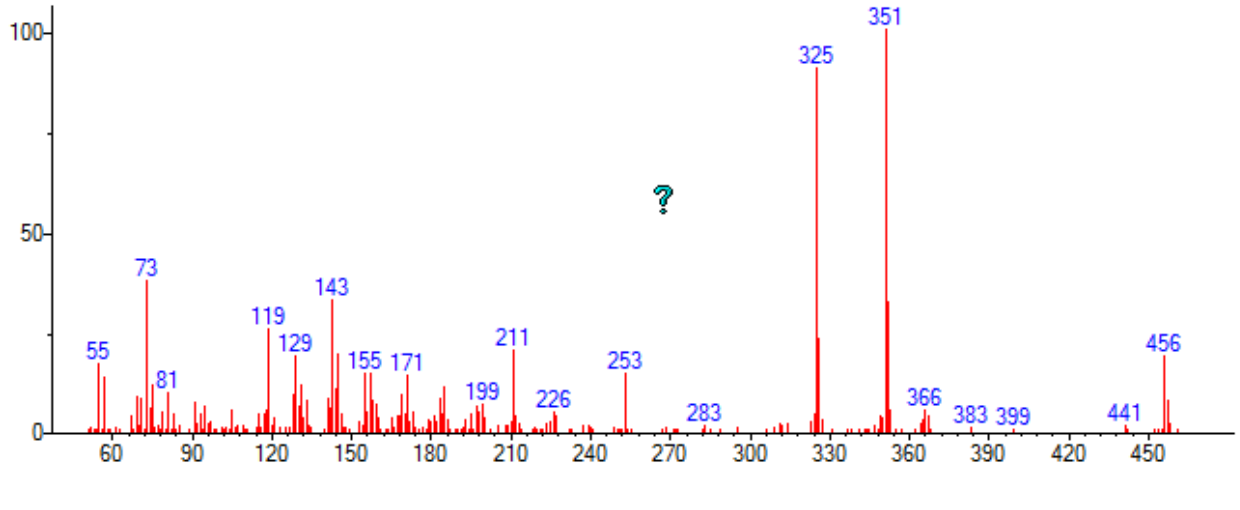


C.

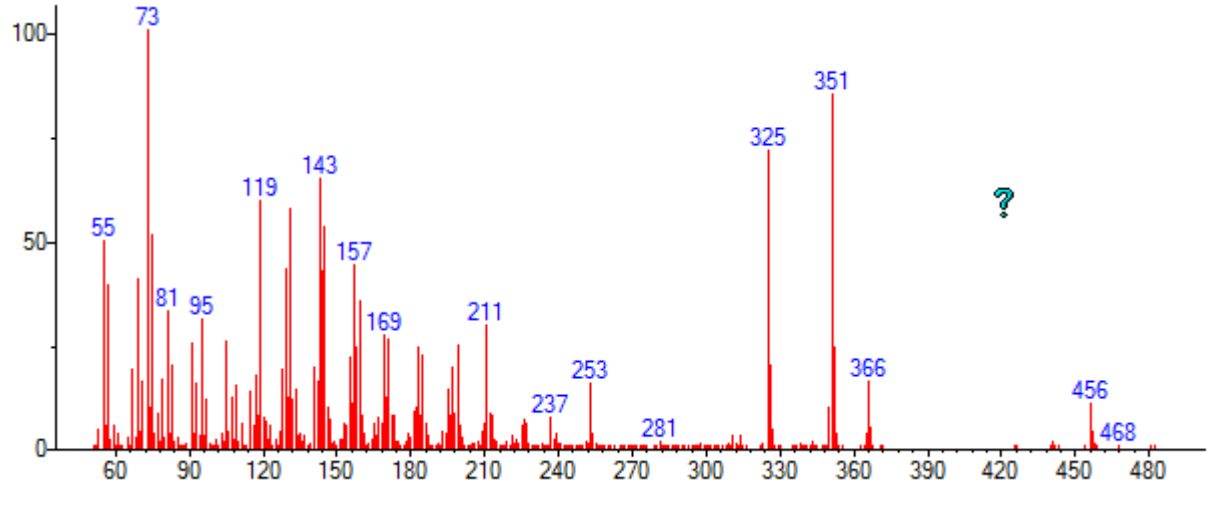
Figure 1 Chromatograms – sterols composition of A - *Zophobas morio* (Sumatra), B - *Zophobas morio* (Czech Republic), C - *Tenebrio mollitor* (Sumatra); retention times: 5α-cholestane 5.4, cholesterol 7.4, 5,6-*trans*-cholecalciferol 7.7, lanosterol 7.9, ergosterol 8.1, campesterol 8.3, stigmasterol 8.6, ß-sitosterol 9.2, stigmastanol 9.4.



A.



B.



C.

Figure 2 Mass spectra of cholecalciferol: A – NIST libary, B – standard, C – analyzed sample.

Tocopherols content in edible insect samples is shown in Table 2. The α-tocopherol was the most abundant tocopherol analyzed in larvae of mealworm *Zophobas morio*, followed by β- and γ-tocopherols. Δ-tocopherol content was under the limit of quantification (LOQ). The content of α-tocopherol can be interesting from the nutritional point of view. The requirement of α-tocopherol for adults is about 15 mg of tocopherol equivalent per day. In this case, the consumption of about 50 g of edible insect brings approximately 25% of recommended daily intake. The storage temperature had no impact to the tocopherols content in analyzed insect samples.

The content of α-tocopherol was 75.7 mg per kg and 77.2 mg per kg in sample stored at 5 – 6 °C and 25 °C, respectively.

CONCLUSION

Larvae of beetle *Zophobas morio* L. and *Tenebrio mollitor* L. had a relatively high content of minor lipophilic compounds, which can be interesting from a nutritional point of view. The cholesterol content at superworm larvae was comparable with its content in meat, mealworm larvae had its content significantly higher. Both types of larvae contain approximately the same amount of phytosterols, their total content was comparable with cholesterol in superworm larvae, and in case of mealworm larvae. Mealworm larvae contained significant amounts of α-tocopherol, other tocopherols content was insignificant.

Relatively high level of phytosterols and α-tocopherol may be one of the major benefits of this new food commodity.

REFERENCES

AOCS Official Method Ch 6-91. 1997. Determination of the composition of the sterol fraction of animal and vegetable oils and fats by TLC and capillary GLC. *Official Methods and Recommended Practices of the American Oil Chemists´ Society*. Champaigne, USA: AOCS Press.

Borsje, B., Heyting, A., Roborgh, J. R., Ross, D. B., Shillam, K. W. G. 1977. Analysis of Fat-Soluble Vitamins. XVI. Antirachitic Activity of 5,6-*trans*-Vitamin D_3 Alone and in the Presence of 5,6-*cis*-Vitamin D_3 Resin, Using Chick Bioassay. *Journal – Association of Official Analytical Chemists*, vol. 60, no. 5 , p. 1003-1009. PMid:197056

Chen, T. C., Persons, K. S., Lu, Z., Mathieu, J. S., Holick, M. F. 2000. An evaluation of the biologic activity and vitamin D receptor binding affinity of the photoisomers of vitamin D_3 and previtamin D_3. *Journal of Nutritional Biochemistry*, vol. 11, no. 5, p. 267-272. http://dx.doi.org/10.1016/S0955-2863(00)00077-2

Dinh, T. T. N., Thompson, L. D., Galyean, M. L., Brooks, J. C., Patterson, K. Y., Boylan, L. M. 2011. Cholesterol content and methods for cholesterol determination in meat and poultry. *Comprehensive Reviews in Food Science and Food Safety*, vol. 10, p. 269-289. http://dx.doi.org/10.1111/j.1541-4337.2011.00158.x

Filip, B., Milczarek, M., Wietrzyk, J., Chodynski, M., Kutner, A. 2010. Antitumor properties of (5E,7E) analogs of vitamin D_3. *Journal of Steroid Biochemistry and Molecular Biology*, vol. 121, no. 1-2, p. 399-402. http://dx.doi.org/10.1016/j.jsbmb.2010.03.017

Finke, M. D. 2015. Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *ZOO Biology*, vol. 34, no. 6, p. 554-564. http://dx.doi.org/10.1002/zoo.21246

Golebiowski, M., Cerkowniak, M., Urbanek, A., Slocinska, M., Rosinski, G., Stepnowski, P. 2014. Adipokinetic hormone induces changes in the fat body lipid composition of the beetle *Zophobas atratus*. *Peptides*, vol. 58, p. 65-73. http://dx.doi.org/10.1016/j.peptides.2014.05.013

Gorman, A. A., Hamblett, I., Rodgers, M. A. J. 1987. Ergosterol (Provitamin D2) Triplet State: An Efficient Sensitier of Singlet O_2 Formation. *Photochemistry and Photobiology*, vol. 45, no. 2, p. 215-221. http://dx.doi.org/10.1111/j.1751-1097.1987.tb05366.x PMid:3562585

Hofius, D., Sonnewald, U. 2003. Vitamin E biosynthesis: Biochemistry meets cell biology. *Trends in Plant Sciences*, vol. 8, p. 6-8. http://dx.doi.org/10.1016/S1360-1385(02)00002-X

Holick, M. F., Garabedian, M., DeLuca, H. F. 1972. 5,6-Trans isomers of cholecalciferol and 25-hydroxycholecalciferol substitutes for 1,25-dihydroxycholecalciferol in anephric animals. *Biochemistry*, vol. 11, no. 14, p. 2715-2719. http://dx.doi.org/10.1021/bi00764a026

Ikekawa, N., Fujimoto, Y., Ishiguro, M. 2013. Reminiscences of research on the chemistry and biology of natural sterols in insects, plants and humans. *Proceedings of the Japan Academy, Series B – Physical and Biological Sciences*, vol. 89, no. 8, p. 349-369. http://dx.doi.org/10.2183/pjab.89.349

Ikekawa, N., Morisaki, M., Fujimoto, Y. 1993. Sterol metabolism in insects: Dealkylation of phytosterol to cholesterol. *Accounts of Chemical Research*, vol. 26, no. 4, p. 139-146. http://dx.doi.org/10.1021/ar00028a002

Kuksis, A. 2001. Plasma non-cholesterol sterols. *Journal of Chromatography*, vol. 935, p. 203-236. http://dx.doi.org/10.1016/S0021-9673(01)01226-2

Lawson, D. E. M. 1971. Vitamin D: new findings on its metabolism and its role in calcium nutrition. *Proceedings of the Nutrition Society*, vol. 30, no. 1, p. 47-58. PMid:4326653

Leclercq, J. 1948. Sur les besoins en stérols des larves de *Tenebrio molitor* L. *Biochimica et Biophysica Acta*, vol. 2, p. 614-617. http://dx.doi.org/10.1016/0006-3002(48)90079-1

Li, S. C. X., Cherian, G., Ahn, D. U., Hardin, R. T, Sim, J. S. 1996. Storage, Heating, and Tocopherols Affect Cholesterol Oxide Formation in Food Oils. *Journal of Agricultural and Food Chemistry*, vol. 44, no. 12, p. 3830-3834. http://dx.doi.org/10.1021/jf950732o

Munne-Bosch, S., Alegre, L. 2002. The function of tocopherols and tocotrienols in plants. *Critical Review in Plant Sciences*, vol. 21, no. 1, p. 31-57. http://dx.doi.org/10.1016/S0735-2689(02)80037-5

Normén, L., Dutta, P., Lia, A., Andersson, H. 2000. Soy Sterol Esters and Beta-sitostanol Ester as Inhibitors of Cholesterol Absorbtion in Human Small Bowel. *The American Journal of Clinical Nutrition*, vol. 71, no. 4, p. 908-913. PMid:10731496

Ovesen, L., Brot, Ch., Jakobsen, J. 2003. Food Contents and Biological Activity of 25-Hydroxyvitamin D: A Vitamin D Metabolite to be Reckoned With? *Annals of Nutrition & Metabolism*, vol. 47, no. 3-4, p. 107-113. http://dx.doi.org/10.1159/000070031

Peterson, D. W. 1951. Effect of Soybean Sterols in the Diet on Plasma and Liver Cholesterol in Chicks. *Proceeding of the Society for Experimental Biology and Medicine*, vol. 78, no. 1, p. 143-147. http://dx.doi.org/10.3181/00379727-78-19002 PMid:14891948

Piironen, V., Lindsay, D. G., Miettinen, T. A., Toivo, J., Lampi, A. M. 2000. Plant Sterols: Biosynthesis, Biological Function and their Importance to Human Nutrition. *Journal of the Science of Food and Agriculture*, vol. 80, no. 7, p. 939-966. http://dx.doi.org/10.1002/(SICI)1097-0010(20000515)80:7<939::AID-JSFA644>3.0.CO;2-C

Schmid, A., Walther, B. 2013. Natural Vitamin D Content in Animal Products. *Advances in Nutrition*, vol. 4, p. 453-462. http://dx.doi.org/10.3945/an.113.003780

Svoboda, J. A., Feldlaufer, M. F. 1991. Neutral sterol-metabolism in insects. *Lipids*, vol. 26, no. 8, p. 614-618. http://dx.doi.org/10.1007/BF02536425

Svoboda, J. A., Lusby, W. R. 1994. Variability of sterol utilization in stored-products insects. *Experientia*, vol. 50, no. 1, p. 72-74. http://dx.doi.org/10.1007/BF01992053

Tsiaras, W. G., Weinstock, M. A. 2011. Factors Influencing Vitamin D Status. *Acta Dermato-venereologica*, vol. 91, no. 2 , p. 115-124. http://dx.doi.org/10.2340/00015555-0980

Velíšek, J. 2014. *The Chemistry of Food*. 1st ed. CHICHESTER, UK: John Wiley & Sons, Ltd. 348 p. ISBN 978-1-118-38381-0.

WHO. 2004. A strategy to prevent chronic disease in Europe. A focus on public health action. The CINDI vision. Available at: http://www.euro.who.int/__data/assets/pdf_file/0010/134848/E83057.pdf

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MICROBIOLOGICAL EVALUATION OF FISH

Olga Cwiková

ABSTRACT

Fish meat has a specific composition that positively influences human health. Thanks to this composition, it is an excellent nutritional medium for growth and reproduction of undesirable microorganisms, which may cause spoilage and they can also lead to alimentary illnesses. Microbiota of fish is dominated by Gram negative and psychrophilic bacteria**.** Microbial contamination causes fish deterioration and leads to the end of its shelf-life when reaches levels between 10^7 and 10^9 CFU.g^{-1}. The most appropriate temperature for storage of fish is between -1 °C and 4 °C and the ideal relative air humidity is 80 to 85%. The objective of the work was to evaluate microbiological quality of fresh fish (Rainbow Trout, Atlantic Salmon, Atlantic Cod) bought in various types of stores in the Czech Republic and to evaluate if different storage temperatures have influence on the quantity of microorganisms. The following microorganisms were monitored: the total aerobic count (TAC), coliform bacteria, *E. coli*, *Salmonella* spp. and *Vibrio parahaemolyticus.* Based on the obtained results it is possible to state that difference between individual stores (p >0.05) in the total aerobic count and the quantity of *E. coli* (except for cod) was not proven. After 2 days of storage there was increase (p <0.05) of the total aerobic count in case of all monitored fish species from all stores. In case of coliform bacteria and *E. coli* there was increase (p <0.05) of their quantity in a majority of the analysed samples. Different storage temperature (4 °C and 8 °C) did not have influence (p <0.05) on the TAC, the quantity of coliform bacteria (except for cod) and the quantity of *E. coli* (except for trout).

Keywords: fish; TAC; *E. coli*; *Salmonella* spp.; *Vibrio parahaemolyticus*; storage temperature

INTRODUCTION

Fish plays an important role in the human diet and there is an observed increase in the consumption of fish per capita in Europe **(Novoslavskij et al., 2016)**. Consumption of fish meat in the Czech Republic is low in comparison to another EU countries **(Buchtová 2001)**. Consumption of two portions of fish per week is recommended as prevention of cardiovascular and oncological diseases **(Clonan et al., 2012)**. Fish is a source of many beneficial substances such as vitamins, polyunsaturated fatty acids, mineral substances and in some countries of the world they are practically the only source of animal protein **(Matyáš et al., 2002)**. Generally, fish meat is sterile while it is alive. However, a large number of bacteria are found on the outer surface, scales, gills and intestine **(Hempel et al., 2011)**. Microbiota of fish is dominated by Gram negative and psychrophilic bacteria **(Görner, Valík, 2004)**. During the capture and handling of fish, the muscle is colonized by these microorganisms. Microbial contamination causes fish deterioration and leads to the end of its shelf-life when reaches levels between 10^7 and 10^9 CFU.g^{-1} **(Scheleguedaa et al., 2016)**. Fish meat is a very suitable substrate for growth and reproduction of microorganisms due to high water content **(Pipová et al., 2006)**. Most fish contain only very little carbohydrate (<0.5%) in the muscle tissue and only small amounts of lactic acid are produced post mortem. This has important consequences for the microbiology of fish **(Gram, Huss, 1996)**.

Safety of fish products and their quality assurance is one of the main problems of food industry today. The presence or absence of foodborn pathogens in a fish product is a function of the harvest environment, sanitary conditions, and practices associated with equipment and personnel in the processing environment **(Grigoryan, Badalyan Andriasyan, 2010)**.

The most suitable storage temperature for fish is between -1 °C and 4 °C. The ideal relative air humidity is 80 to 85%. Chilled fish stays "fresh" approximately for 4 days (in crushed ice), if fish is sliced, the shelf-time decreases to two days at 4 °C **(Buchtová, 2001)**.

The objective of the work was to evaluate microbiological quality of meat of Rainbow Trout, Atlantic Salmon and Atlantic Cod, and to find out if storage temperature and the type of store, where fish was bought, have impact on the quantity of monitored microorganisms.

MATERIAL AND METHODOLOGY

Samples of fresh fish bought in the regular sales network in the Czech Republic were observed. 3 representative samples of every fish species were analysed. They included samples of Rainbow Trout (*Oncorhynchus mykiss*), Atlantic Salmon (*Salmo salar*) and Atlantic Cod *(Gadus morhua*). Samples were bought in the store focusing on sale of fresh fish and fish products, it has several branches in the Czech Republic and it guarantees fast transport of fish from a place of catching and thus also

freshness of freshwater as well as saltwater fish (denoted as the store 1). The second set of samples was bought in one of the store chains that offers food as well as non-food products (denoted as the store 2). The third set of samples was bought in a small store that is specialized on sales of fish and it is owned by private persons (denoted as the store 3).

Samples were transported into the microbiological laboratory of the Institute of Food Technology at MENDELU in Brno in a cooling box, stored at the temperature of melting ice and the microbiological analysis was carried out on the day of purchasing. The subsequent microbiological analysis was carried out after 2 days of storage at different temperature conditions at 4 °C and 8 °C.

For every sample of fish the following microbiologic indicators were determined:

The total aerobic count (TAC). Culture on the growth medium Plate Count Agar (PCA, NOACK, France) according to ISO 4833 at 30 °C for 72 hours.

Quantity of Coliform bacteria. Culture on the growth medium Violet Red Agar (VRBL, NOACK, France) according to ISO 4832 at 37 °C for 24 – 48 hours.

Quantity of bacteria *E. coli*. Cultivation on Chromocult Coliform agar (Merck, Francie) for 24 to 48 hours at 37 C.

Vibrio parahaemolyticus. Pre-reproduction in peptone water with higher salt content at 41.5 °C for 18 hrs. Cultivation on Thiosulfate-citrate-bile salts-sucrose agar (TCBS, Biokar Diagnostics, Francie) for 24 hrs at 37 °C. Biochemical confirmation, oxidase test.

***Salmonella* Species.** Cultivation after pre-reproduction on Salmonella Enrichment with addition of IRIS Salmonella selective supplement (18 hrs at 41.5 °C) in IRIS Salmonella® Agar (Biokar Diagnostics, Francie) for 24 hrs at 37 °C. Biochemical confirmation.

Sampling and processing was carried out based on ČSN ISO 7218 and ČSN EN ISO 6887-1.

The following methods were used for statistical evaluation: the calculation of basic statistical parameters (mean, standard deviation, standard deviation of the mean) and the simple sorting method of analysis of variance (ANOVA, Turkey test). Evaluation was performed using the programme STATISTICA CZ, version 10.

RESULTS AND DISCUSSION

The total aerobic count

The total aerobic count (Figure 1) found in the samples of Rainbow Trout bought in the individual stores was comparable (p >0.05). In the store 1, the total aerobic count was 6.1 log CFU.g^{-1} (1.3 x 10^6 CFU.g^{-1}), in the store (2) 6.4 log CFU.g^{-1} (2.2 x 10^6 CFU.g^{-1}) and in the store (3) 6.6 log CFU.g^{-1} (3.7 x 10^6 CFU.g^{-1}).

Also in the samples of Atlantic Salmon the total aerobic count was comparable (p >0.05). The highest values of the total aerobic count were found in the sample from the store (3), specifically 6.6 log CFU.g^{-1} (3.6 x 10^6 CFU.g^{-1}), the lowest in the store (1) 6.1 log CFU.g^{-1} (1.2 x 10^6 CFU.g^{-1}).

Regarding cod, the highest (p >0.05) total aerobic count was recorded in the sample bought in the store (1) specifically 7.0 log CFU.g^{-1} (10.7 x 10^6 CFU.g^{-1}), the lowest quantity (p <0.05) was found in the sample from the store (2), specifically 6.3 log CFU.g^{-1} (1.9 x 10^6 CFU.g^{-1}). As it is stated by **Ingr (2010)**, according to currently invalid Notice no. 132/2004 Coll., fish and its parts intended for heat treatment may contain the total aerobic count 6 log CFU.g^{-1} (10^6 CFU.g^{-1}), according to **ČSN 56 9609 (2008**) the quantity of 6.7 log CFU.g^{-1} (5.10^6 CFU.g^{-1}) is permissible. The total aerobic count in the observed samples was lower in all fish samples bought in all stores, except for cod bought in the store (1). If we compare the found values of TAC and results of **Kordiovská et al. (2004)**, then the quantities found by our research are higher. In the above mentioned experiment of **Kordiovská et al. (2004)**, the total aerobic count fresh fish bought in the sales network was 6.2 x 10^4 CFU.g^{-1} (4.8 log CFU.g^{-1}). On the contrary **Nespolo et al. (2012)** present in salmon TAC comparable with our study (1.1 x 10^3 to 3.9 x 10^6 CFU.g^{-1}).

During capture and storage fish are almost invariably come into contact with nets, decks, ropes, boxes, human hands and clothing. These contacts are introducing microorganisms from other sources such as humans, birds and soil **(Fernandes, 2009).**

During storage (Figure 2) after 2 day there was increase (p <0.05) of the total aerobic count in the all observed species of fish from all stores. Considering storage of fish at different temperatures, there was no difference in TAC recorded (p <0.05). TAC was comparable after two days of storage at the temperature 4 °C as well as 8 °C. The highest TAC was found in the sample of trout, specifically 9 log CFU.g^{-1}. With this high total aerobic count there already occurs spoilage and sensory changes **(Miks-Krajnik et al., 2016)**. **Parlapani and Boziaris (2016)** also carried out monitoring spoilage microbiota, where the total aerobic count detected at the beginning of storage was 3.5 log CFU.g^{-1}, and after storage at 5 °C then 8.1 log CFU.g^{-1}, which is lower than we found during the analyses.

The quantity of coliform bacteria

The quantity of coliform bacteria (Figure 3) in the samples of Rainbow Trout bought in the individual stores was comparable (p >0.05), only in the store (1) there was higher quantity of coliforms recorded (p >0.05) in comparison to the store (2), specifically 4.7 log CFU.g^{-1} (4.8 x 10^4 CFU.g^{-1}); respectively 4.4 CFU.g^{-1} (2.3 x 10^4 CFU.g^{-1}) in the store (2).

In case of Atlantic Salmon higher (p <0.05) quantity of coliform bacteria was recorded in the store (2) in comparison to the store (1), 4.5 log CFU.g^{-1} (3.2 x 10^4 CFU.g^{-1}) and 3.5 log CFU.g^{-1} (3.2 x 10^3 CFU.g^{-1}) respectively.

Regarding the store (3), 3.7 log CFU.g^{-1} (5.10^3 CFU.g^{-1}) was found in salmon, which is comparable quantity of coliforms as in the store (1). In comparison to **Kordiovská et al. (2004)**, higher quantities of coliform bacteria were recorded in our experiment. In the above mentioned experiment there was found the quantity 2.9 log CFU.g^{-1} (7.6 x 10^2 CFU.g^{-1}) of coliform bacteria. Lower quantities were also recorded in fresh fish before storage by **Miks-Krajnik (2016)**, specifically up to 4 log CFU.g^{-1}.

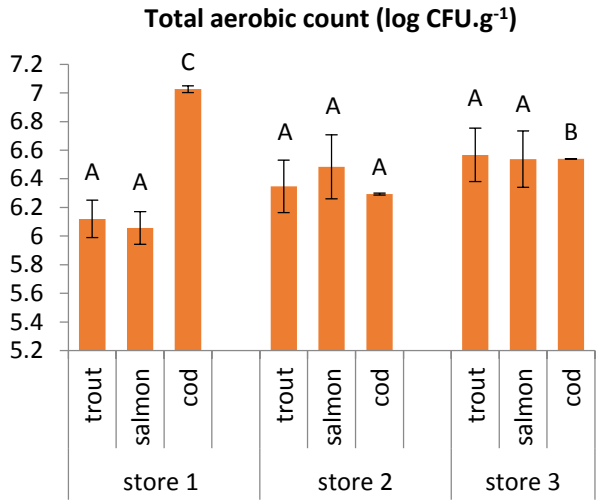

Figure 1 Comparison of the total aerobic count (log CFU g^{-1}) in the samples of fresh fish from three different stores (1 = specialized wholesale of fish, 2 = chain store, 3 = fishmonger´s). The averages marked with different letters are statistically different (p <0.05); n = 3 within the observed factor (store).

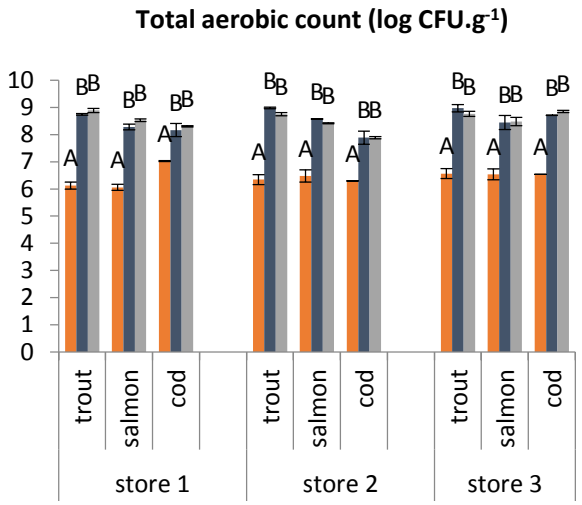

Figure 2 Comparison of the total aerobic count (log CFU g^{-1}) in the samples of fresh fish (marked red) and fish stored 2 days at the temperature 4°C (marked blue) and at the temperature 8°C (marked greed) from three different stores (1=specialized wholesale of fish, 2=chain store, 3=fishmonger´s). The averages denoted by different letters are statistically different (p < 0.05); n = 3 within the observed factor (storage temperature).

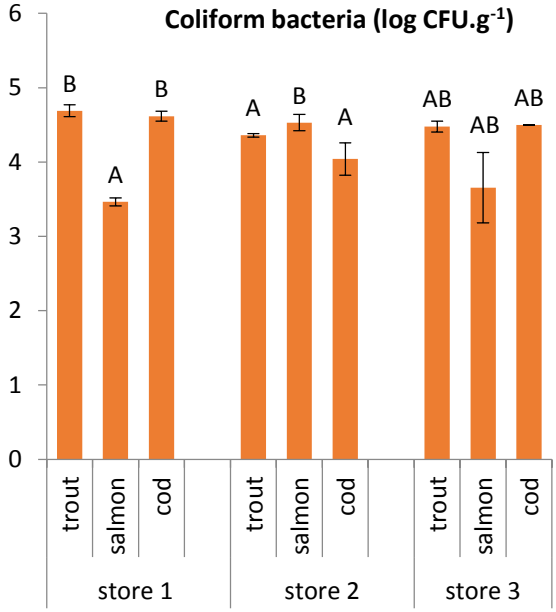

Figure 3 Comparison of the quantity of coliform bacteria (log CFU.g^{-1}) in the samples of fresh fish from three different stores (1 = specialized wholesale of fish, 2 = chain store, 3 = fishmonger´s). The averages denoted by different letters are statistically different (p <0.05); n = 3 within the observed factor (store).

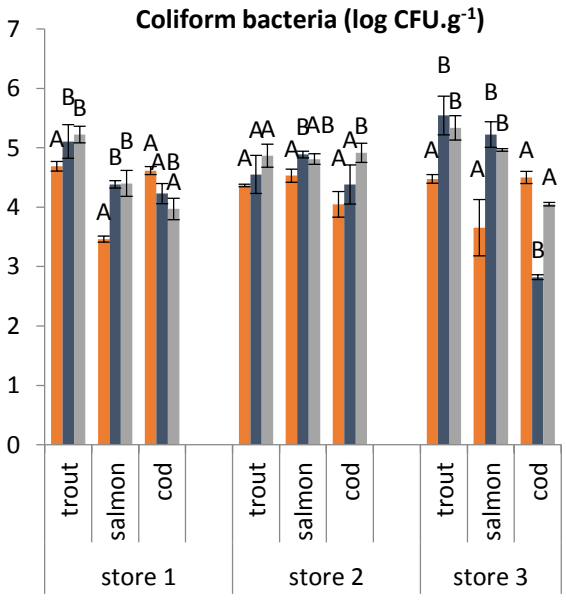

Figure 4 Comparison of the quantity of coliform bacteria (log CFU.g^{-1}) in the samples of fresh fish (marked red) and fish stored for 2 day at the temperature 4 °C (marked blue) and at the temperature 8 °C (marked greed) from three different stores (1 = specialized wholesale of fish, 2 = chain store, 3 = fishmonger´s). The averages denoted by different letters are statistically different (p <0.05); n = 3 within the observed factor (storage temperature).

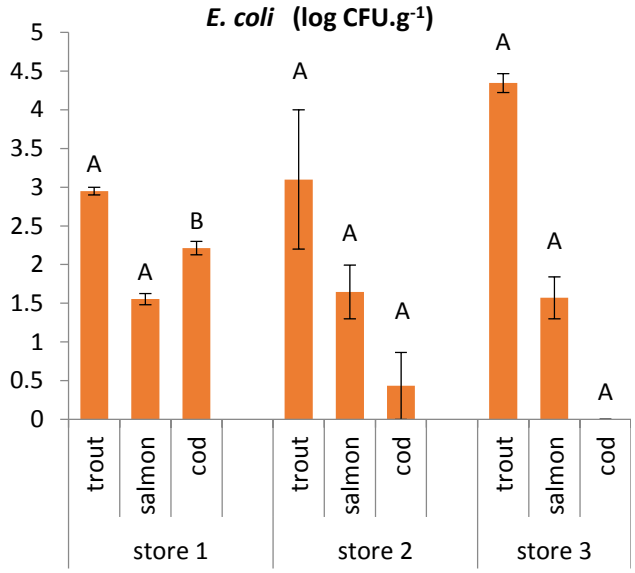

Figure 5 Comparison of the quantity of *E. coli* (log CFU.g^{-1}) in the sample of fresh fish from three different stores (1 = specialized wholesale of fish, 2 = chain store, 3 = fishmonger´s). The averages denoted by different letters are statistically different (p <0.05); n = 3 within the observed factor (store).

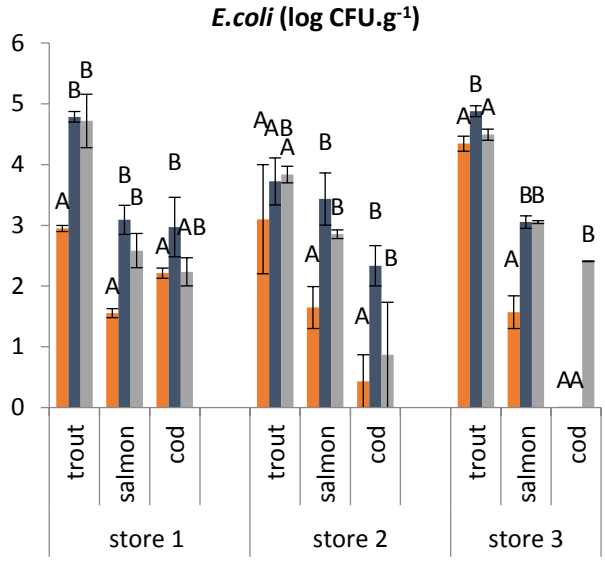

Figure 6 Comparison of the quantity of *E. coli* (log CFU.g^{-1}) in samples of fresh fish (marked red) and fish stored for 2 days at the temperature 4 °C (marked blue) and at the temperature 8 °C (marked greed) from three different stores (1 = specialized wholesale of fish, 2 = chain store, 3 = fishmonger´s). The averages denoted by different letters are statistically different (p <0.05); n = 3 within the observed factor (storage temperature).

Coliform bacteria belongs to the family of *Enterobacteriaceae*, which can play a key role in food spoilage due to their ability to metabolize amino acids to malodorous volatile compounds **(Remenanta et al., 2015)**. Higher quantity of coliforms in food is attributed especially to incorrect treatment of food and to not keeping of storage temperatures (**Görner, Valík, 2004)**.

During storage (Figure 4) there was increase (p <0.05) of the quantity of coliform bacteria in a majority of the observed samples. The highest value was recorded in trout bought in the store (3), specifically 5.5 log CFU.g^{-1}. Different storage temperature did not have (except for the cod samples) impact on the quantity of coliform bacteria (p >0.05).

The* quantity of bacteria *E. coli

In case of Rainbow Trout and Atlantic Salmon (Figure 5) the individual stores were not different in the quantity of *E. coli* (p >0.05). The highest values were recorded in the sample of trout bough in the store (3), specifically 4.4 log CFU.g^{-1} ($2.2 \cdot 10^4$ CFU·g^{-1}). Considering salmon, the quantities of *E. coli* were comparable and they were from 1.6 log CFU.g^{-1} (3.5×10^1 CFU.g^{-1}) to 1.7 log CFU.g^{-1} (4.5×10^4 CFU.g^{-1}).

In the samples of Atlantic cod, higher quantity (p <005) of *E. coli* in the sample from the store (1) was recorded: 2.2 log CFU.g^{-1} (1.6×10^2 CFU.g^{-1}), in the samples from the store (3) bacteria *E. coli* were not detected.

According to **ČSN 56 9609 (2008)** the highest permitted quantity for *E. coli* in fresh fish and their parts intended for heat processing is 2 log CFU.g^{-1}, respectively 2.7 log CFU.g^{-1} (5×10^2 CFU.g^{-1}) in two samples from five. In our experiment the maximal legislative limit was not kept only in case of the trout samples. Despite norms do not have a binding character, it is necessary to take these values as limiting in case of missing legislative requirements. **The Commission Regulation (ES) 2073 (2005)** states the highest permitted values of *E. coli* only for products of boiled crustaceans and molluscs.

During storage (Figure 6) there was increase (p <0.05) in the quantity of *E. coli* a majority of the observed fish samples, expect for trout (the store 2). The highest quantity of *E. coli* was recorded in trout bought in the store (3), specifically 4.9 log CFU.g^{-1}. Different storage temperature did not have (except for the samples of trout from the store 3) impact on the quantity of *E. coli* (p >0.05). The highest permitted quantity (2.7 log CFU.g^{-1}) was exceeded by all fish samples at the storage temperature 4 °C, except for two cod samples. At the storage temperature 8 °C it was 4 samples. If we would take 2 log CFU.g^{-1} as the highest threshold, then only two analysed samples would meet this requirement.

Vibrio parahaemolyticus

Bacteria *Vibrio parahaemolyticus* were detected in 4 samples of fresh fish, especially in the samples of trout from the store (2) 0.9 log CFU.g^{-1}, in the samples of salmon from the stores (2) or (3) 1.8 log CFU.g^{-1}, and 0.5 log CFU.g^{-1} respectively, and in the sample of cod from the store (1) 0.3 log CFU.g^{-1}.

Vibrio parahaemolyticus is widely distributed in the marine environments and considered as the leading cause of human gastroenteritis **(Alaboudi et al., 2016)**. This pathogen is connected especially with fish products and sea products **(Komprda, 2004)**. Higher quantities of the species *Vibrio* were proved in fish by **Aagesen and Häse (2014)**, especially in case of violation of cooling chain or repeated temperature fluctuations. Major outbreaks are associated with the warmer month. Control of *Vibrio parahaemolyticus* growth in shellfish meats is temperature

depended in the first place **(Fernandes, 2009)**. The negative criteria in 25 grams is stated in fish in case of *V. parahaemolyticus* **(ČSN 56 9609, 2008)**.

***Salmonella* species**

Bacteria of the Salmonella species were detected in 4 samples. It included two samples of trout from the store (1), one sample of salmon from the store (1) and one sample of salmon from the store (2). **The Commission regulation (ES) 2073 (2005)** on microbiologic criteria for food requires no presence of salmonella in 25 g of a sample. The above stated samples thus do not correspond with the legislative limit. On contrary **Patil et al. (2013)** during observing microbiological quality of fish stored at 4 °C did not detect salmonella in samples.

The presence of *Salmonella* in seafood may derive from contamination occurring in the natural aquatic environment, in aquaculture or cross-contamination during store, transportation and processing **(Amagliani et al., 2012)**. Salmonella was not isolated also from the freshwater fish in the study of Terenjeva et al**.** According this study salmonella-negative results are in agreement with those of previous reports for France, Great Britain, Portugal, Czech Republic, Slovakia, and the United States for marine and freshwater fish. But, the presence of *Salmonella* in fish was detected in several countries of Asia and Africa **(Terenjeva et al., 2015)**. **Yang et al. (2015)** study implied that pollution by human or animal feces and sewage may be a major reason for the high prevalence of *Salmonella* in freshwater fish samples.

CONCLUSION

Ensuring health safety of food is a basic responsibility of all producers. Controlling bacterial contamination is important all the way from catching and handling to processing. In processing and producing operation there are therefore implemented HACCP systems, which are responsible not only for analysing danger, but especially for eliminating this danger at an acceptable level and to choose effective preventive measures.

Microbial criteria and monitoring of microbial levels are important part of food inspection and determine acceptability or unacceptability of fish and seafood for the consumers.

In conclusion, it is possible to state that quality of fish bought by us was in all types of stores comparable and that a consumer does not have to be afraid to buy fish also in stores that are not exclusively specialized on sale of these products. Despite our results of the quantity of observed microorganisms in comparison to results of another studies were higher, they met legislative requirements (except for five analysed samples). Obviously, pathogens (*Salmonella* spp., *V. parahaemolyticus*) should not occur in products offered to consumers. Considering fish storage, it is not possible to recommend storage at fridge temperatures because this temperature is often higher than 8 °C and fish stored like this would reach limits of spoilage after 2 days (8 log CFU.g^{-1}). Based on the obtained results it is possible to recommend consumption of fish on the day of purchase or storage at the temperature of melting ice, specifically from -1 °C to 2 °C.

REFERENCES

Aagesen, A., M., Häse, C. C. 2014. Seasonal effects of heat shock on bacterial populations, including artificial *Vibrio parahaemolyticus* exposure, in the Pacific oyster, *Crassostrea gigas*. *Food Microbiology*, vol. 38, p. 93-103. http://dx.doi.org/10.1016/j.fm.2013.08.008 PMid:24290632

Alaboudi, A. R., Ababneh, M., Osaili, T. M., Al Shloul, K. 2016. Detection, Identification, and Prevalence of Pathogenic *Vibrio parahaemolyticus* in Fish and Coastal Environment in Jordan. *Journal of Food Science*, vol. 81, no. 1, p. 130-134. http://dx.doi.org/10.1111/1750-3841.13151 PMid:26554333

Amagliani, G., Brandi, G., Schiavano, G. F. 2012. Incidence and role of *Salmonella* in seafood safety (Review). *Food Research International*, vol. 45, no. 2, p. 780-788. http://dx.doi.org/10.1016/j.foodres.2011.06.022

Buchtová, H. 2001. *Hygiene and Technology of Fish and seafood Treatment: Alimentary illnes of fish; Freezing Industry.* (*Hygiena a technologie zpracování ryb a ostatních vodních živočichů: Alimentární onemocnění z ryb; Mrazírenství).* 1st ed. Brno: University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Hygiene and Ecology, 164 p. ISBN 80-7305-401-9.

Clonan, A., Holdsworth, M., Swift, J., Leibovici, D., Wilson, P. 2012. The dilemma of healthy eating and environmental sustainability: The case of fish. *Public Health Nutrition*, vol. 15, p. 277-284. http://dx.doi.org/10.1017/S1368980011000930 PMid:21619717

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs. OJ L 338, 22/12/2005, p. 1-6.

ČSN 569609:2008. *Guides to good hygiene and manufacturing practice-Microbiological criteria for foods. Principles for the establishment and application.*

Fernandes, R. 2009. *Fish and Seafood.* UK, Leather Food International Ltd. 258 p. ISBN: 978-1-905224-76-0.

Görner, F. and Valík, L. 2004. *Applied food microbiology*. (*Aplikovaná mikrobiológia poživatín).* Bratislava, Malé Centrum, PPA. 528 p. ISBN 80-967064-9-7.

Gram, L., Huss, H. H. 1996. Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*, vol. 33, p. 121-137. http://dx.doi.org/10.1016/0168-1605(96)01134-8

Grigoryan, K., Badalyan, G., Andriasyan, D. 2010. Prevalence of *Staphylococcus aureus* in Fish Processing Factory. *Potravinarstvo*, vol. 4, no. 2, p. 25-28. http://dx.doi.org/10.5219/47

Hempel, A., Borchert, N., Walsh, H., Choudhury, K. R., Kerry, J. P., Papkovsky, D. B. 2011. Analysis of Total Aerobic Viable Counts in Raw Fish by High-Throughput Optical Oxygen Respirometry. *Journal of Food Protection*, vol. 74, no. 5, p. 776-82. http://dx.doi.org/10.4315/0362-028X.JFP-10-352 PMid:21549048

Ingr, I. 2010. *Fish Quality and Processing*. (*Jakost a zpracování ryb).* 2nd ed. Brno: Mendelova univerzita v Brně, 102 p. ISBN 978-80-7375-382-5.

ISO 4832:2010. *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coliforms, Colony-count technique*.

ISO 4833:2003. *Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of micro-organisms, Colony-counttechnique at 30 °C*.

ISO 6887-2:2003. *Microbiology of food and animal feeding stuffs-Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 2: Specific rules for the preparation of meat and meat products.*

ISO 7218:2007. *Microbiology of food and animal feeding stuffs-General requirements and guidance for microbiological examinations*.

Komprda, T. 2004. *General food hygiene*. *(Obecná hygiena potravin)*. 2nd ed. Brno: MZLU, 112 p. ISBN 80-7157-757-X.

Kordiovská, P., Vorlová, L., Karpíšková, R., Lukášová, J. 2004. Potential risk of biogenic amine formation in carp muscle (*Cyprinus carpio*). In Proceeding of the IV. international conference Rizikové faktory potravového reťazca (Risk factors of food chain) SPU : Nitra, Slovakia, 7th of October 2004. Available at: https://www.researchgate.net/publication/267197428.

Matyáš, Z., Pavlíček, J., Sovjak, R., Kopřiva, V., Pažout, V., Hejlová, Š., Vojtěch, J., Horký J., Hlaváček, J. 2002. *Resources for implementation of HACCP plan to branch raw materials treatment and food of animal origin: fish, mollusc, crustacean, game, poultry, eggs, honey, delicatessen*. *Podklady pro zavedení HACCP do oboru zpracování surovin a potravin živočišného původu: ryby, měkkýši, korýši, zvěřina, drůbež, vejce, med, lahůdky*. 1st ed. Brno, Czech Republic : Veterinary and Pharmacy University, Faculty of Veterinary hygiene and ecology, 141 p. ISBN 80-7305-428-0.

Miks-Krajnik, M., Yoonc, Y-J., Ukukud, D. O., Yuka H-G. 2016. Volatile chemical spoilage indexes of raw Atlantic salmon (*Salmo salar*) stored under aerobic condition in relation to microbiological and sensory shelf lives. *Food Microbiology*, vol. 53, Part B, p. 182-191. http://dx.doi.org/10.1016/j.fm.2015.10.001 PMid:26678146

Nespolo, N. M., Martineli, T. M., Rossi, O. D. 2012. Microbiological Quality of Salmon (*Salmo salar*) Sold in Cities of the State of Sao Paulo, Brazil. *Brazilian Journal of Microbiology*, vol. 43, no. 4, p. 1393-1400. http://dx.doi.org/10.1590/S1517-83822012000400021 PMid:24031968

Novoslavskij, A., Terentjeva, M., Eizenberga, I., Valciņa, O., Bartkevičs, V., Bērziņš, A. 2016. Major foodborne pathogens in fish and fish products: a review. *Annals of Microbiology*, vol. 66, no. 1, p. 1-15. http://dx.doi.org/10.1007/s13213-015-1102-5

Parlapani, F. F., Boziaris, I. S. 2016. Monitoring of spoilage and determination of microbial communities based on 16S rRNA gene sequence analysis of whole sea bream stored at various temperatures. *LWT-Food Science and Technology*, vol. 66, p. 553-559. http://dx.doi.org/10.1016/j.lwt.2015.11.007

Patil, S. S., Koli, J. M., Sharangdher, S. T., Sharangdher, M. T. 2013. Storage charakteristice of „fish pakoda“ from pink perch (*Nemipterus japonicus*) meat at 4 °C. *Ecology, Environment and Conservation*, vol. 19, no. 3, p. 877-880.

Pipová, M., Buchtová, H., Cabadaj, R., Gima, J., Hanzel, S., Iglovská, N., Kantíková, M., Kohút, J., Košuth, P., Kozák, A., Nagy, J., Pliešovský, J., Rajský, D., Sokol, J., Steinhauserová, I., Večerek, V. 2006. *Hygiene and technology of freshwater and sea fish treatment.* (*Hygiena a technológia spracovania sladkovodných a morských rýb).* Košice, Slovakia: University of Veterinary Medicine, 417 p. ISBN 80-8077-048-4.

Remenanta, B., Jaffrèsa, E., Dousseta, X., Pileta, M-F., Zagoreca, M. 2015. Spoilers, wonder spores and diehard microorganisms: New insights to integrate these super foes in food spoilage risk management. *Food Microbiology*, vol. 45, Part A, p 45-53.

Scheleguedaa, L. I., Zalazara, A. L., Gliemmoa, M. F., Camposa, C. A. 2016. Inhibitory effect and cell damage on bacterial flora of fish caused by chitosan, nisin and sodium lactate. *Int. J. Biol. Macromol.*, vol. 83, p. 396-402. http://dx.doi.org/10.1016/j.ijbiomac.2015.11.033 PMid:26597566

Terentjeva, M., Eizenberga, I., Valcina, O., Novoslavskij, A., Strazdina, V., Berzins, A. 2015. Prevalence of Foodborne Pathogens in Freshwater Fish in Latvia. *Journal of Food Protection*, vol 78, no. 11, p. 2093-2098. http://dx.doi.org/10.4315/0362-028X.JFP-15-121 PMid:26555535

Yang, X., Wu, Q., Zhang, J., Huang, J., Chen, L., Liu, S., Yu, S., Cai, S. 2015. Prevalence, enumeration, and characterization of Salmonella isolated from aquatic food products from retail markets in China. *Food Control*, vol. 57, p. 308-313. http://dx.doi.org/10.1016/j.foodcont.2015.03.046

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EFFECT OF FARMING SYSTEM ON COLOUR COMPONENTS OF WHEAT NOODLES

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ABSTRACT

Colour of noodles is definitely a key element of a consumer's buying decisions. It can be influenced by many factors. Conditions, under which is winter wheat grown, can be considered as one of these factors. The aim of this work was to evaluate colour of noodles that were prepared from winter wheat grown in ecological and integrated arable farming systems, after different forecrops with two levels of fertilization (fertilized and unfertilized) during the years 2009, 2010 and 2011. Winter wheat noodles were prepared from white flour and wholegrain flour and its colour was evaluated using the spectro-colorimeter. Colour was measured by three coordinates: lightness L*, red/ green value a* and yellow/ blue value b*. Wholegrain noodles had lower L* value, so they were darker than white flour noodles, with higher redness and higher yellowness. Colour of white flour noodles and wholegrain noodles was significantly influenced by crop nutrition (fertilized and unfertilized variants), farming system and meteorological conditions during experimental years. Wholegrain noodles from ecological system were darker, with lower lightness and higher redness compared to noodles from integrated system. White flour noodles from ecological system were also darker compared to noodles from integrated system. Fertilization decreased lightness of white flour noodles, on the contrary, fertilization increased the lightness and decreased the redness of wholegrain noodles. In non-fertilized treatment, ecological wheat noodles were darker, with higher redness and yellowness than noodles prepared from winter wheat grown in integrated arable farming system.

Keywords: ecological arable system; integrated arable system; wheat noodle colour

INTRODUCTION

The key quality attributes in the evaluation of noodles include texture and colour, which are important quality factors since they are associated with flour **(Chang and Wu, 2008)**. Flour colour influences the quality of end-use products of common wheat. It is determined by the content of yellow pigment in the endosperm. A high yellow pigment content is preferred for yellow alkaline noodles. A minimal addition of alkaline salts leads to an improved texture and yellowness, as well as the antioxidant functions, derived from its main component carotenoids during the aging process **(Zhang et al., 2009)**. Flour colour is mainly controlled by genetic factors, several studies mentioned the effects of the 1B.1R translocation on flour colour and yellow pigment content in common and durum wheat **(Liu et al., 2005)**.

Flour extraction rate has an important influence on noodle attributes, especially colour. Studies on dried noodles showed a decline in brightness and increase in yellowness with increased extraction rate **(Kruger et al., 1994; Lee et al., 1987)**. Noodle darkening increases with the increase of flour extraction rate. This is due to the action of polyphenol oxidase enzymes which are largely located in the bran layer **(Fuerst et al., 2006)**. Low flour extraction and ash levels are preferred for the manufacture of noodles with a clean and bright appearance. Low ash content (1.4% and less) in flour is always an advantage for noodles since flour ash is traditionally viewed as causing noodle discoloration. For white salted noodles, a white or creamy white colour is desirable with firm texture. The level of natural yellow pigment levels in flour is highly correlated with noodle colour, which is wheat variety dependent. For yellow alkaline noodles, a bright yellow colour is required. The primary component of yellow colour development in alkaline noodles is due to a pH dependent, chemically induced colour shift in water-soluble flour flavonoids, with a secondary effect due to flour xanthophylls **(Fu, 2008; Asentorfer et al., 2006)**. It is well known that the amber-yellow colour of semolina is due to the presence of natural pigments from carotenoid and xanthophyll families in wheat. As these pigments increase, the yellow colour detected by the human eye becomes brighter and more vivid. It is also known that semolina with a high pigment content do not always produce very yellow pasta. This is because carotenoids and xanthophylls have components which are affected by several oxidizing enzymes. It is important to have a durum wheat with a low content of the enzymes, exercising this negative action **(Landi, 1995)**. Since colour is a key element of a consumer's buying decisions, it is important to ensure noodle colour options availability on the market. The influence of farming system and plant nutrition pattern on colour components of wheat noodles on the basis of CIE L*a*b* readings is not broadly documented. In this study, the relationship between winter wheat noodle colour components, obtained from different farming systems,

nutrition treatment and flour extraction rate was investigated.

MATERIAL AND METHODOLOGY

Materials

Field experiments of ecological and integrated arable farming systems were conducted at the Research experimental station of the Faculty of Agrobiology and Food Resources in Nitra during 2009, 2010 and 2011 growing periods. Experiments were established on a Haplic Luvisol developed at proluvial sediments mixed with loess. The altitude of the experimental field is 178 m. The location has a continental climate with an average temperature 19.7 °C in July and -1.7 °C in January, an average annual precipitations are 561mm. Both arable farming systems were composed of six-course crop rotations. The ecological system was composed of the crop rotation: beans + alfalfa – alfalfa – winter wheat – peas – maize – spring barley. The integrated system consisted of the crop rotation: winter wheat – peas – winter wheat — maize – spring barley – alfalfa (3 years at the same plot). Subplots were fertilized (F) and unfertilized (NF). The fertilized variant in ecological system was based on 40 t of manure while the integrated system also received 40 t of manure plus synthetic fertilizers (Table 1), treatments were replicated four times. Sowing and harvesting dates, rainfall and average temperature calculated for vegetative period of the crop, synthetic fertilizer inputs ($kg.ha^{-1}$) applied in the integrated system are shown also in the Table 1. Nitrogen fertilizers were applied in three split applications. Winter wheat was grown within both farming systems, after different forecrops, fertilized and unfertilized variants. Winter wheat noodles were prepared from white flour and from wholegrain flour. Egg noodles with moisture of 30.5% were produced on the apparatus for pasta producing P3 (La Monferina) and were dried at 50 °C for 12 hours.

Milling

Winter wheat samples were milled without further conditioning on a Quadrumat Senior Mill (Brabender, Germany). Combined fractions I. + II. are referred to here as „white flour". Wholegrain flour was obtained by grinding on the special mill PSY MP (Mezos, s.r.o, Czech Republic.

Colour Analysis

Noodle colour was evaluated using the spectrophotometer SP60 (X-Rite, Inc., Germany) which allows very accurate measurement of the basic optical properties of the surface. The International Commission on Illumination (CIE, Commission Internationale l'Eclairage) has standardized colour order system to derive values for describing colour. The CIE Colour System utilize three coordinates to locate a colour in a three-dimensional colour space and is used to compare the colours of two objects. Colour of an object is defined by three coordinates (L*a*b*). CIE L* values represent lightness, a* denotes the red/green value and b* the yellow/blue value. CIE L*a*b* uses Cartesian coordinates to calculate a colour in a colour space. The a* axis runs from left to right, from -60 (pure green) to +60 (pure red), + value indicates a shift toward red. The b* axis is at an angle of 90 degrees to a* axis. Values on the b* axis range from -60 (pure blue) to +60 (pure yellow), so + value represents a shift toward yellow, - value means a shift toward blue. The L* axis range from 0 (pure black) at the bottom to 100, which represents pure white.

Colour evaluation of cooked noodles was carried out in triplicate; analysis of variance was used for statistical evaluation. The statistical tests were performed with the software STATISTICA version 10.0.

RESULTS AND DISCUSSION

L*values

CIE L* values of wheat white flour noodles and wholegrain noodles were significantly ($p \leq 0,05$) influenced by farming system (Table 2). Average lightness of white flour noodles was 75.92 and wholegrain noodles 56.46. There was significant variation between ecological and integrated system in both, white flour and wholegrain noodles. Noodles prepared from w. wheat grown in ecological system were darker with significantly lower CIE L* values. Crop nutrition also significantly influenced the lightness of noodles.

a*values

The mean CIE a* value for the white flour noodles was 0.92, for wholegrain noodles 9.8, what means that the redness (a*) of wholegrain noodles was almost ten times higher compared to white flour noodles. Significant differences were observed for a* value in wholegrain noodles between farming systems, the redness of ecological wholegrain noodles was higher than integrated noodles. However, there was no significant difference in the a* values in white flour noodles. Crop nutrition significantly influenced the redness of wholegrain noodles, with higher CIE a* value for non-fertilized variant. Variation between a* values for white flour noodles and two crop nutrition treatments was not significant.

b* values

The mean CIE b* value for wholegrain noodles was 20.52, for white flour noodles 18.83, the yellowness (b*) of wholegrain noodles was higher than white flour

Table 1 Crop management data for winter wheat 2009-2011.

Growing season	Sowing date	Harvest date	Rainfall (mm)	Average temperature (°C)	Nitrogen ($kg.ha^{-1}$)	Phosphorus ($kg.ha^{-1}$)	Potassium ($kg.ha^{-1}$)
2008 – 2009	13/10/08	15/07/09	426	9.6	82.5	37.5	20.0
2009 – 2010	07/10/09	28/07/10	610	8.8	62.5	7.5	40.0
2010 – 2011	12/10/10	13/07/11	326	8.6	76.0	30.0	120.0

Table 2 Effect of farming system, crop nutrition and year on colour evaluation of wheat noodles.

white flour noodles				
		lightness L*	**colour a***	**colour b***
Crop nutrition	F	74.49 a	0.95 a	19.14 a
	NF	77.34 b	0.88 a	18.51 a
Farming system	ES	75.58 a	0.92 a	19.05 a
	IS	76.26 b	0.92 a	18.60 a
Year	2009	79.92 c	1.75 c	22.54 c
	2010	74.95 b	0.88 b	20.03 b
	2011	72.89 a	0.12 a	13.91 a
Standard error		±3.479	±0.717	±3.869
wholegrain noodles				
		L*	**a***	**b***
Crop nutrition	F	57.45 b	8.82 a	20.35 a
	NF	55.47 a	9.35 b	20.69 a
Farming system	ES	55.61 a	9.24 b	20.65 a
	IS	57.32 b	8.93 a	20.39 a
Year	2009	57.06 a	10.15 c	21.01 b
	2010	55.42 b	8.44 a	19.58 a
	2011	56.91 a	8.67 b	20.97 b
Standard error		±2.594	±0.974	±0.897

Legend: F = fertilized; NF = non-fertilized; ES = ecological system; IS = integrated system.

noodles. The difference was significant (Table 3). Variations between CIE b* values for farming systems and crop nutrition, both white flour and wholegrain flour noodles, were not significant (Table 2).

There was significant variation for all colour components L*a*b* caused by variable meteorological conditions during three growing seasons (years 2009 – 2011).

Effect of fertilisation on colour components was significant (Table 3). CIE L*a*b* values were significantly different for farming system in non-fertilized treatment. Ecological wheat noodles were darker (lower L*), with higher redness (a*) and yellowness value (b*). No significant effect of farming system on CIE L*a*b* values was recorded under fertilized conditions. The effect of wholegrain flour and white flour on noodle colour components showed the same tendency under both, fertilized and non-fertilized treatment. Wholegrain noodles were darker, with higher redness and yellowness.

The correlation between the L* value and a* value, but also b* value, was positive, significant (r^2 = 0.79;

Table 3 Winter wheat noodle colour evaluation, effect of fertilisation.

non-fertilized treatment				
		lightness L*	**colour a***	**colour b***
Farming system	ES	65.82 a	5.32 b	20.08 b
	IS	66.99 b	4.92 a	19.11 a
Noodles	W	55.47 a	9.35 b	20.68 b
	Fl	77.34 b	0.88 a	18.51 a
Standard error		±11.426	±4.344	±2.876
fertilized treatment				
		L*	**a***	**b***
Farming system	ES	66.04	4.84	19.61
	IS	65.91 n.s.	4.93 n.s.	19.88 n.s.
Noodles	W	57.45 a	8.82 b	20.35 b
	Fl	74.49 b	0.95 a	19.14 a
Standard error		±3.479	±0.716	±3.869

Legend: ES = ecological system; IS = integrated system; W = wholegrain flour noodles; Fl = white flour noodles.

Table 4 Correlation analysis of CIE L*a*b* values.

white flour noodles			wholegrain noodles		
	a*	**b***		**a***	**b***
L*	0.79 **	0.71 **	**L***	-0.39 *	0.02
a*		0.89 **	**a***		0.58 *

Marked values are significant at ** p <0.01, * p <0.05.

$r^2 = 0.71$) for white flour noodles. Low CIE a* values in white flour noodles are strongly correlated with lightness (L*). For wholegrain noodles the correlation between L* and a* value was negative, but not strong ($r^2 = -0.39$). The correlation between L* and b* value was not significant (Table 4).

Miskelly (1984) studied the influence of components contributing to the colour and brightness of flour, flour paste, and Chinese and Japanese style noodles. Differences in brightness and yellowness were attributable to a multitude of factors including wheat cultivar, milling extraction rate, protein content, starch damage, and brown and yellow pigments. Most of the variation was attributed to genetic factors, but growing environment and milling procedures were also important. Noodle brightness is related inversely to protein content and to flour-grade colour. Lutein is a yellow plant pigment that belongs to the carotenoid family, namely to Xanthophylls. It acts as an effective antioxidant, it protects the organism against heart diseases and cancer **(Šivel et al., 2014)**.

Humphries et al., (2004) analysed whole-meal wheat, including common, durum varieties, and triticale samples for their carotenoid content and colour. A positive correlation between CIE b* (yellowness) and lutein concentration was shown, there was little correlation between CIE L* or CIE a* (redness) and lutein, α or β carotene. In contrast, the b* value correlated well with the concentration of α and β carotene, but those wheat groups with the lowest CIE b* values did not have a strong correlation. Study has identified CIE b* as a useful diagnostic for rapid screening of wheat varieties for lutein content and was also indicative of the provitamin A carotenoid content. In our experiment, variation in CIE b* values caused by farming system, crop nutrition treatment and flour extraction rate was lower than variation in CIE a* value. We assumed, that differences in CIE L* and CIE a* values can't be attributable to one factor – the concentration of carotenoids.

Ma et al., (2007) showed that N fertilizer increased the redness and yellowness, while brightness decreased. **Wang et al., (2004)** reported that wheat grain protein content strongly correlated with noodle colour.

In small quantities, flavonoids are also present in cereals, located in the pericarp **(Dykes and Rooney, 2007)**. In our previous study, higher concentration of total flavonoid and free flavonoid content was found in wholegrain flour compared to white flour. Free flavonoids represented 77.9% of the total content for wholegrain flour and 68.7% for white flour. Significant effect of farming system and fertilization treatment on free and total flavonoid contents was recorded for wholegrain flour. Concentrations of phenolic compounds were significantly higher in wholegrain flour in all, free, bound and total content. In contrary, farming system showed significant differences in white flour, when total, free and bound phenolic contents were higher in ecological system. Fertilization treatment was significant also for white flour **(Kosík et al., 2014)**. It is estimated that flavonoids account for approximately two thirds of the phenolics in our diet and the remaining one third are from phenolic acids **(Liu, 2004)**.

Large controlled studies with a more factorial approach to the effect of the different components involved in cultivation systems have shown that the ecological production is more likely to favour the synthesis of secondary compounds in food plants. Both environmental factors and production methods have been shown to affect plant growth and composition including the content of secondary bioactive metabolites that may be important to health **(Holmboe-Ottesen, 2010)**.

Bran is a key factor in determining wholegrain products health benefits. Bran has higher vitamin and mineral contents than endosperm, high antioxidant activity and higher secondary metabolites content. These characteristics give wheat bran and wholegrain food very interesting nutritional properties, by reducing the risks of developing chronic diseases **(Liu, 2007; Li et al., 2007; Kosík et al., 2014)**.

Bednářová et al., (2015) concluded that breads produced of blue coloured wheat wholemeal flour were not below the average in sensory properties and its market position could be very high in the future, due to the content of health promoting substances.

Variation in concentration of phytochemicals caused by growing environment and milling extraction rate may affect the colour of end product. A brown or red hue in pasta is detrimental to consumer acceptance in many countries where a bright amber colour is preferred **(Owens, 2011)**.

CONCLUSION

Colour of noodles is an important quality factor influencing the decision of a consumer. Farming system, fertilization, flour extraction rate, forecrop and weather conditions during growing period of winter wheat may have significant effect on the colour of wheat noodles. Colour was measured by three coordinates: lightness L*, red/ green value a* and yellow/ blue value b*. Wholegrain noodles had lower L* value, so they were darker than white flour noodles, with higher redness and higher yellowness. Wholegrain noodles from ecological system were darker, with lower lightness and higher redness compared to noodles from integrated system. White flour noodles from ecological system were also darker compared to noodles from integrated system. Fertilization decreased lightness of white flour noodles, on the contrary, fertilization increased the lightness and decreased the redness of wholegrain noodles. In non-fertilized treatment, ecological wheat noodles were darker, with higher redness

and yellowness than noodles prepared from winter wheat in integrated arable farming system. Since colour is a key element of buying preferences, increased interest of consumers for more healthy, wholegrain food may shift their acceptance of darker colour of pasta.

REFERENCES

Asentorfer, R. E., Wang, Y., Mares, D. J. 2006. Chemical structure of flavonoid compounds in wheat that contribute to the yellow colour of Asian alkaline noodles. *Journal of Cereal Science*, vol. 43, p. 108-119. http://dx.doi.org/10.1016/j.jcs.2005.09.001

Bednářová, M., Ošťádalová, M., Král, M., Tremlová, B. 2015. Comparison of selected sensory properties of wholemeal breads. *Potravinarstvo*, vol. 9, no. 1, p. 211-216. http://dx.doi.org/10.5219/454

Dykes, L., Rooney, L. W. 2007. Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World*, vol. 52, no. 3, p. 105-111. http://dx.doi.org/10.1094/CFW-52-3-0105

Fu, B. X. 2008. Asian noodles. History, classification, raw materials and processing. *Food Research International*, vol. 41, p. 888-902. http://dx.doi.org/10.1016/j.foodres.2007.11.007

Fuerst, E. P., Anderson, J. V., Morris, G. F. 2006. Delineating the role of polyphenol oxidase in the darkening of alkaline wheat noodles. *Journal of Agricultural and Food Chemistry*, vol. 54, p. 2378-2384. http://dx.doi.org/10.1021/jf0526386

Holmboe-Ottesen, G. 2010. Increased levels of bioactive compounds in organically grown food plants. Possible health effects? In Aksel Bernhoft (ed.) *Bioactive compounds in plants - benefits and risks for man and animals. Proceedings from a symposium held at the Norwegian Academy of Science and Letters, Oslo, 13-14 November 2008*. Novus Forlag. p. 236-252. ISBN 978-82-7099-583-7.

Humphries, J. M., Graham, R. D., Mares D. J. 2004. Application of reflectance colour measurement to the estimation of carotene and lutein content in wheat and triticale. *Journal of Cereal Science*, vol. 40, p. 151-159. http://dx.doi.org/10.1016/j.jcs.2004.07.005

Chang, H. C., Wu, L. C. 2008. Texture and quality properties of Chinese fresh egg noodle formulated with green seaweed (*Monostroma nitidum*). *Journal of Food Science*, vol. 73, no. 8, p. 398-404. http://dx.doi.org/10.1111/j.1750-3841.2008.00912.x

Kosík, T., Lacko-Bartošová, M., Kobida, Ľ. 2014. Influence of agricultural practices on phenolics and flavonoids of winter wheat. In Inovácie technológií špeciálnych výrobkov biopotravín pre zdravú výživu ľudí. Nitra, p. 145-151, ISBN 978-80-552-1272-2.

Kruger, J. E., Anderson, M. A., Dexter, J. E. 1994. Effect of flour refinement on raw Cantonese noodle color and texture. *Cereal Chemistry*, vol. 71, no. 2, p. 177-182.

Landi, A. 1995. Durum wheat, semolina and pasta quality characteristics for an Italian food company. In Durum wheat quality in the Mediterranean region. Di Fonzo N. (ed.), Kann F. (ed.), Nachit M. (ed.). Zaragoza: CIHEAM, no. 22, p. 33-42.

Lee, C. H., Gore, P. J., Lee, H. D., Yoo, B. S., Hong, S. H. 1987. Utilization of Australian wheat for Korea style dried noodle making. *Journal of Cereal Science*, vol. 6, no. 3, p. 283-287. http://dx.doi.org/10.1016/S0733-5210(87)80065-6

Li, W. D., Pickard, M. D., Beta, T. 2007. Effect of thermal processing on antioxidant properties of purple wheat bran. *Food Chemistry*, vol. 104, no. 3, p. 1080-1086. http://dx.doi.org/10.1016/j.foodchem.2007.01.024

Liu, L., He, Z., Yan, J., Zhang, Y., Xia, X., Peña, R. J. 2005. Allelic variation at the Glu-1 and Glu-3 loci, presence of the 1B.1R translocation, and their effects on mixographic properties in Chinese bread wheats. *Euphytica*, vol. 142, no. 3, p. 197-204. http://dx.doi.org/10.1007/s10681-005-1682-4

Liu, R. H. 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of Nutrition*, vol. 134, no. 12, p. 3479S-3485S. PMid:15570057

Liu, R. H. 2007. Whole grain phytochemicals and health. *Journal of Cereal Science*, vol. 46, no. 3, p. 207-219. http://dx.doi.org/10.1016/j.jcs.2007.06.010

Ma, W., Sutherland, M. W., Kammholza, S., Banks, P., Brennan, P., Bovill, W., Daggard, G. 2007. Wheat flour protein content and water absorption analysis in a doubled haploid population. *Journal of Cereal Science*, vol. 45, no. 3, p. 302-308. http://dx.doi.org/10.1016/j.jcs.2006.10.005

Miskelly, D. H. 1984. Flour components affecting paste and noodle colour. *Journal of the Science of Food and Agriculture*, vol. 35, no. 4, p. 463- 471. http://dx.doi.org/10.1002/jsfa.2740350417

Owens, G. 2011. *Cereals processing technology*, CRC Press, 238p., ISBN 978-1-85573-561-3.

Šivel, M., Fišera, M., Klejdus, B., Kráčmar, S., Kubáň, V., Golian, J., Svobodová, B. 2014. Lutein in food supplements available on the markets of the Viszegrad countries. *Potravinarstvo*, vol. 8, no. 1, p. 261-266, http://dx.doi.org/10.5219/387

Wang, Ch., Kovacs, M. I. P., Fowler, D. B., Holley, R. 2004. Effects of Protein Content and Composition on White Noodle Making Quality: Color. *Cereal Chemistry*, vol. 81, no. 6, p. 777-784. http://dx.doi.org/10.1094/CCHEM.2004.81.6.777

Zhang, Y., Wu, Y., Xiao, Y., He, Z., Zhang, Y., Yan, J., Zhang, Y., Xia, X., Ma, C. 2009. QTL mapping for flour and noodle colour components and yellow pigment content in common wheat. *Euphytica*, vol. 165, p. 435-444. http://dx.doi.org/10.1007/s10681-008-9744-z

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CHEMICAL AND PHYSICAL PARAMETERS OF DRIED SALTED PORK MEAT

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ABSTRACT

The aim of the present study was analysed and evaluated chemical and physical parameters of dried salted pork neck and ham. Dried salted meat is one of the main meat products typically produced with a variety of flavors and textures. Neck (14 samples) and ham (14 samples) was salted by nitrite salt mixture during 1week. The nitrite salt mixture for salting process (dry salting) was used. This salt mixture contains: salt, dextrose, maltodextrin, flavourings, stabilizer E316, taste enhancer E621, nitrite mixture. The meat samples were dried at 4 °C and relative humudity 85% after 1 week salting. The weight of each sample was approximately 1 kg. After salting were vacuum-packed and analysed after 1 week. The traditional dry-cured meat such as dry-cured ham and neck obtained after 12 – 24 months of ripening under controlled conditions. The average protein content was significantly (p <0.001) lower in dried pork neck in comparison with dried salted pork ham. The average intramuscular fat was significantly (p <0.001) lower in dried pork ham in comparison with dried salted pork neck. The average moisture was significantly lower (p ≤0.05) in dried salted ham in comparison with dried pork neck. The average pH value was 5.50 in dried salted pork ham and 5.75 in dried salted pork neck. The content of arginine, phenylalanine, isoleucine, leucine and threonine in dried salted ham was significantly lower (p <0.001) in comparison with dried salted pork neck. The proportion of analysed amino acids from total proteins was 56.31% in pork salted dried ham and 56.50% in pork salted dried neck.

Keywords: 5 pork; neck; ham; chemical quality; intramuscular fat; moisture; amino acids; proteins; pH value

INTRODUCTION

Meat quality has always been important to the consumer, and it is an especially critical issue for the meat industry in the 21st century **(Joo et al., 2013)**. In the course of production, the nutritional composition of commercial meat products undergoes changes due to variations in the meat and non-meat ingredients and the processing conditions. Animal production practices (genetic and dietary strategies) play an important role in the nutritional quality of meat raw materials **(Jiménez-Colmenero, 2011)**. Consumers require meat products with high quality, health benefits and high safety. Nevertheless, processing of the muscle into ready-to-eat products with acceptable and high nutritional value, convenience and palatability to use is indispensable to add value to this muscle much higher it´s conventional profitability. The pork dry-cured quality is defined as a combination of different characteristics of raw and processed meat **(Joo et al., 2013)**.

The characteristics like water-holding capacity (WHC) of muscle and pH value are important because it affects both qualitative and quantitative aspects of meat and meat products **(Van der Wal et al., 1997)**. These important factors have the biggest influence on biochemical changes in the muscle *post mortem*. The most popular meat products processed mostly from pork muscle is dry-cured meat according consumers expressions due its typical flavour and palatability **(Ventanas et. al., 2007)**.

Dry-cured ham is one of the main meat products typically produced with a variety of flavors and textures. The processing of dry-cured ham is based on traditional manufacturing practices consisting primarily of salting and drying steps, followed by a more or less extensive ripening period, which is dependent on the desired final product quality **(Toldrá, 2015)**. The dry – cured meat is produced salting, drying and ripening. The ripening process of dry-cured meat involves complex of biochemical and chemical changes **(Ruitz et. al., 2002)**.The ripening, is the processing step that develops the unique and characteristic aroma and flavor. The intensity of the dry- cured aroma and flavor is a result of extensive lipolysis and proteolysis that are proportional to the length of the aging time. The quality characteristics of dry-cured meat products are related to the raw material and processing technology **(Jurado et al., 2007)**.

Dry-aging is typically the aging of premium meat under critically controlled ambient conditions of temperature, relative humidity and airflow. These parameters need to be carefully balanced and monitored to inhibit microbial growth and minimise weight loss while producing excellent eating quality resulting from tenderisation and enhanced flavour **(Yuan et al., 2016)**. The acceptance of dry-cured hams by consumers is mainly determined by their sensory quality. The aroma is perhaps the most important quality parameter and it is markedly affected by the raw material and the processing of the dry- cured meat.

In the case of dry-cured hams, the aroma is due to the presence of many volatile compounds, most of them produced by chemical and enzymatic mechanisms during the post-mortem process **(Sánchez-Peña et al., 2005)**. Flavor and aroma are key attributes that impact the overall acceptance of dry-cured hams and are markedly affected

by raw material processing techniques and aging time. The flavor and aroma of dry-cured ham can be determined by sensory descriptive analysis and the composition of aroma impact compounds, most of which are produced *post-mortem* by chemical and enzymatic mechanisms **(Pham et al., 2008)**. Flavour is a very important attribute contributing to the sensory quality of meat and meat products. Although the sensory quality of meat includes orthonasal and retronasal aroma, taste as well as appearance, juiciness and other textural attributes **(Neethling et al., 2016)**. Flavour affects on the customer acceptance **(Ruiz et al., 2002)**. In general, important reductions in both moisture content and water activity take place during the production process of dry-cured meat products. This reduction depends on the drying conditions and the decreasing water activity may affect enzyme activity which influences the sensory characteristics of the final product **(Jiménez-Colmenero et al., 2010)**.

Sodium chloride is the very important ingredient, its manufacturing process begins with a salting step during which salt and other curing ingredients (nitrate or nitrite) and additives (ascorbic acid) slowly diffuse into the meat followed by brushing or washing of hams to remove the excess of salt, a post-salting step and a ripening or drying stage **(Martínez-Onandi et al., 2016)**. Salt, nitrate and nitrite are the major ingredients in the cure mix. Salt inhibits the growth of spoilage microorganisms by reducing the water activity and solubilizing some of the myofibrillar proteins. Nitrate is reduced to nitrite and then nitric oxide by nitrate reductase a natural enzyme in the ham. The typical red/pinkish color of ham is due to the reaction of nitric oxide and myoglobin which forms nitrosyl myoglobin **(Zhao et al., 2016)**.

Lipid oxidation is a very important biochemical reaction in dry-cured meat products. Many studies have investigated the relationship between the muscle lipid oxidation and flavor formation in dry-cured meat products and proved that lipid oxidation plays an important role in the formation of the final flavor of dry-cured meat products **(Guofeng et al., 2015)**.

The effect of environmental, nutritional and production factors on intramuscular fat have formerly been quantified as well as their consubsequent influence on meat quality. Genetic factors also influence intramuscular fat deposition **(Pannier et al., 2014)**. Intramuscular fat of dry-cured meat contributes to odour and flavour impression during mechanisms such as lipid oxidation. Fat content is believed to be one of the most crucial quality traits of cured hams (the higher the fat content, the greater the acceptability of cured hams) but what most affects the appearance, texture (juiciness) and intensity and persistence of flavour of dry-cured hams is the intramuscular fat content **(Jiménez-Colmenero et al., 2010)**. Intramuscular fat plays one of important role in the impression of the texture of dry-cured meat, especially in juiciness **(Ventanas et al., 2005)**. Maillard reactions concerned creation of volatile compounds formation **(Ruiz et al., 2002)**.

Proteolysis is one of the most important biochemical processes during the ripening of ham and neck. This biochemical process influences texture and flavour due to the formation of free amino acids and other low-molecular weight compounds. Free amino acids influence directly in taste. The major ways for generation of volatile compounds from amino acids in ham and neck are Maillard and Strecker reactions **(Jurado et al., 2007)**. The sensory quality depends not only of the curing process but also on factors such as the age, breed and feeding of pigs. The chemical changes occurring in different muscles during the ripening of hams and necks influence the ham and neck aroma and flavour **(Diego et al., 2008)**. The flavour of high quality is the result of enzymatic reactions (proteolysis and lipolysis) and chemical processes (lipid autooxidation, Strecker degradation and Maillard reactions) **(González et al., 2008)**.

According to the nutrition importance for human the amino acids are divided into essential: valine (Val), leucine (Leu), isoleucine (Ile), threonine (Thr), methionine (Met), lysine (Lys), phenylalanine (Phe) and tryptophan (Trp); semi-essential: arginine (Arg) and histidine (His); and nonessential ones: glycine (Gly), alanine (Ala), serine (Ser), cysteine (Cys), aspartic acid (Asp), asparagine (Asn), glutamic acid (Glu), glutamine (Gln), tyrosine (Tyr) and proline (Pro) **(Belitz et al., 2001)**.

Passi and de Luca (1998) stated that in the human nutrition it is possible to consider only 10 amino acids as principal, i.e. essential nutrients, which the humans must obtain from various diets. The remaining amino acids may be synthesized from the products of metabolism and of essential amino acids.

The aim of the study was analysed basic chemical and aminoacids composition of dry-cured pork ham and neck salted and matured during 1week. After salting were vacuum-packed and analysed after 1 week.

MATERIAL AND METHODOLOGY

The aim of the present study was to determine and evaluate chemical parameters of dried, salted pork neck and ham. Neck (14 samples) and ham (14 samples) was matured and salted by nitrite salt mixture during 1week. The nitrite salt mixture was used for salting process (dry salting). This salt mixture contains: salt, dextrose, maltodextrin, flavourings, stabilizer E316, taste enhancer E621, nitrite mixture. The meat samples were matured at 4 °C and relative humudity 85% after 1 week of salting. The samples were vacuum-packed and storage 1 week after salting. The weight of each sample was approximately 1 kg.

Determination of chemical composition analysis and amino-acids analysis

The chemical composition and amino-acids composition of the ham and the neck (50 g) was measured by the device Nicolet 6700 (Thermo Scientific, USA). The intramuscular fat content in $g.100g^{-1}$, total proteins in $g.100g^{-1}$, total water in $g.100g^{-1}$, amino-acids in $g.100g^{-1}$ were analysed by the FTIR method. FTIR spectroscopy provides information about the secondary structure content of proteins. This spectroscopy works by shining infrared radiation on a sample and seeing which wavelengths of radiation in the infrared region of the spectrum are absorbed by the sample. Each compound has a characteristic set of absorption bands in its infrared spectrum. The infrared spectrum of the muscular

homogenate analysis was transferred out by molecular spectroskopy method.

Determination of NaCl (salt content)

Samples (approximately 2 g) with 2 mL of indicator were titrated by solution of silver nitrate by using the indicator potassium chromate. This suspension was titrated by solution of silver nitrate until a light orange colour. The amount of chloride ions was evaluated. The titration amount of silver nitrate was divided by weight of sample.

Determination of pH value

The pH value was measured using the pH meter Gryf 209L (Sigma-Aldrich, Czech Republic). The pH value of dried neck and ham at different ripening periods were measured.

Determination of water activity a_w

Water activity of salted and dried neck and ham was determined at 25 °C by using the device FA-st lab (GBX advanced technology, Switzerland).

Statistical analyse

The data were subjected to statistical analysis using the SAS (Statistical Analysis System) package SAS 9.3 using of application Enterprise Guide 4.2. Differences between groups were analysed by t-test.

RESULTS AND DISCUSSION

The traditional dry-cured meat such as dry-cured ham and neck obtained after 12 – 24 months of ripening under controlled conditions **(Dall´asta et al., 2010)**. Nowadays is tendency to make aging time shorter. Chemical parameters of dried salted pork neck and ham were analysed in this article.

Table 1 shows basic chemical parameters of dried salted pork neck and ham. The moisture content ranged from 61.11% to 67.13% in dried salted pork ham and from 52.98% to 64.18% in dried salted pork neck. The average moisture content was 63.52% in dried salted pork ham and 58.88% in dried salted pork neck. There was found significantly lower ($p \leq 0.05$) moisture content in dried salted pork neck in comparison with dried salted pork ham. **Benedini et al., (2012)** found out similar results of moisture in ham (61.2%) in dried salted *biceps femoris* muscle.

The protein content ranged from 22.91% to 26.34% in dried salted pork ham and from 18.41% to 22.22% in dried salted pork neck. The average protein content was 23.37% in dried salted pork ham and 19.98% in dried salted pork neck. There was found significantly lower ($p < 0.001$) protein content in dried salted pork neck in comparison with dried salted pork ham.

Kunová et al., (2015) found out similar our results protein content 24.87% in dried salted pork ham and 20.51% in dried salted pork neck. **Lorido et al., (2015)** found out higher protein content (39.26%) in *musculus semimembranosus.* **Benedini et al., (2012)** found out protein content 27.00% in *biceps femoris* muscle.

The intramuscular fat ranged from 3.38% to 8.99% in dried salted pork ham and from 7.26% to 20.80% in dried salted pork neck. The average intramuscular fat was 4.05% in dried salted pork ham and 14.11% in dried salted pork neck. There was found significantly higher ($p < 0.001$) intramusculat fat in dried salted pork neck in comparison with dried salted pork ham. **Lorido et al., (2015)** found out higher content of intramuscular fat (10.62%) in *semimembranosus.* **Jiménez-Colmenero et al., (2010)** fond out in Iberian ham content of intramuscular fat in range 2.6 – 9.5%.

The salt content ranged from 3.01% to 6.68% in dried salted pork ham and from 4.35% to 6.05% in dried salted pork neck. The average salt content was 4.85% in dried salted pork ham and 4.41% in dried salted pork neck. There wasn't found statistical difference between salt content in dried salted pork neck in comparison with dried salted pork ham.

Matínez – Onandi et al., (2016) found out salt content in average 5.49% and ranged from 2.87% to 7.91% in

Table 1 The basic chemical composition of dried salted pork ham and neck.

	Moisture (%)	Proteins (%)	Intramuscular fat (%)	Salt (%)
		Ham		
x	63.52	23.37	4.05	4.85
s	2.09	0.68	2.30	1.19
s_x	0.75	0.21	0.65	0.36
min.	61.11	22.91	3.38	3.01
max.	67.13	26.34	8.99	6.68
v%	3.09	2.79	40.10	21.90
		Neck		
x	58.88	19.98	14.11	4.41
s	4.42	0.88	4.55	0.55
s_x	1.64	0.22	1.64	0.19
min.	52.98	18.41	7.26	4.35
max.	64.18	22.22	20.80	6.05
v%	7.55	4.33	32.90	14.00
t-test	+	+++	+++	-

Note: -$p > 0.05$; +$p \leq 0.05$, ++$p < 0.01$, +++$p < 0.001$.

Table 2 Physical parameters of dried salted pork ham and neck.

Parameters	pH value	Water activity (a_w)
Ham		
x	5.50	0.899
s	0.06	0.019
s_x	0.03	0.003
min.	5.75	0.822
max.	6.05	0.945
v%	0.75	1.235
Neck		
x	5.75	0.935
s	0.25	0.035
s_x	0.03	0.005
min.	5.42	0.888
max.	6.10	0.955
v%	1.62	2.236
t-test	-	-

Note: -p >0.05; +p ≤0.05, ++p <0.01, +++p <0.001.

Table 3 Content of amino acids of dried salted pork neck and ham (g.100g^{-1}).

Prarameters	x	s	s_x	v%	x	s	s_x	v%	t-test
	neck				ham				
arginine	1.72	0.13	0.05	7.69	1.44	0.10	0.04	7.09	+++
cysteine	0.44	0.03	0.01	6.71	0.47	0.06	0.02	11.9	-
phenylalanine	1.09	0.08	0.03	7.48	0.92	0.07	0.03	7.07	+++
histidine	1.17	0.12	0.05	10.2	0.99	0.13	0.05	13.4	+
isoleucine	1.04	0.09	0.03	8.44	0.84	0.08	0.03	9.23	+++
leucine	2.09	0.17	0.06	7.96	1.72	0.15	0.06	8.44	+++
lysine	2.35	0.18	0.07	7.51	2.01	0.12	0.05	6.06	++
methionine	0.94	0.06	0.02	6.17	0.91	0.04	0.02	4.60	-
threonine	1.19	0.09	0.03	7.42	1.02	0.05	0.02	4.97	+++
valine	1.12	0.11	0.04	9.83	0.97	0.09	0.04	10.2	+

Note: -p >0.05; +p ≤0.05, ++p <0.01, +++p <0.001.

Serrano hams. **Lorido et al., (2015)** found out similar salt content (4.38%) in *musculus semimembranosus.*

Table 2 shows the change in pH value and water activity (a_w) of dried salted pork neck and ham. The pH value ranged from 5.75 to 6.05 in dried salted pork ham and from 5.42 to 6.10 in dried salted pork neck. The average pH value was 5.50 in dried salted pork ham and 5.75 in dried salted pork neck. The pH value of both products showed that meat has not been ripened. **Bednářová et al., (2014)** found out in *musculus semimembranosus* pH values in range from 5.56 to 5.63.

The water activity ranged from 0.822 to 0.945 (a_w) in dried salted pork ham and from 0.888 to 0.955 (a_w) in dried salted pork neck. The average water activity was 0.899 in dried salted pork ham and 0.935 (a_w) in dried salted pork neck. **Bjarnadottit et al., (2015)** found out similar value of water activity in dried ham.

Table 3 shows contein of amino acids composition of dried salted pork neck and ham. The average content of arginine in dried salted pork ham was 1.44 ±0.10 g.100g^{-1} and 1.72 ±0.13 g.100g^{-1}. There was found significantly higher (p <0.001) content of arginine in dried salted pork neck in comparison with dried salted pork ham. The average content of lysine was 2.01 ±0.12 g.100g^{-1} in dried salted pork ham and 2.35 ±0.18 g.100g^{-1} in dried salted pork neck. There was found significantly higher (p <0.01) content of lysine in dried salted pork neck in comparison with dried salted pork ham. The average content of leucine was 1.71 ±0.15 g.100g^{-1} in dried salted pork ham and 2.09 ±0.17 g.100g^{-1} in dried salted pork neck. There was found significantly higher (p <0.001) content of leucine in dried salted pork neck in comparison with dried salted pork ham. The average content of methionine was 0.91 ±0.04 g.100g^{-1} in dried salted pork ham and 0.94 ±0.06 g.100g^{-1} in dried salted pork neck. There wasn't found statistical difference between content of methionine in dried salted pork neck in comparison with dried salted pork ham.

Bučko et al., (2015) found out similar content of aminoacids in the pork *musculus longissimus dorsi* but with lower intramuscular fat content (1.19 g.100g^{-1}). Contents of arginine found out 1.50 g.100g^{-1}, cysteine 0.36 g.100g^{-1}, lysine 2.01 g.100g^{-1} and histidine 1.09 g.100g^{-1}. **Wilkinson et al., (2014)** and **Okrouhlá et al., (2006)** found out the proportion of amino acid from total amount of amino acids. **Wilkinson et al., (2014)** found out content

of arginine 7.16 g.100g^{-1}, lysine 8.64 g.100g^{-1}, leucine 8.68 g.100g^{-1} and methionine 2.97 g.100g^{-1}. **Okrouhlá et al., (2006)** found out content of arginine 7.3 g.100g^{-1}, lysine 9.71 g.100g^{-1} and leucine 8.38 g.100g^{-1}. The proportion of analysed amino acids from total proteins was 56.31% in pork salted dried ham and 56.50% in pork salted dried neck. **Wilkinson et al., (2014)** found out proportion of amino acids from total proteins 66.42%, but they analysed more amino acids in comparison with our experiment.

CONCLUSION

The aim of this article was to determine physical and chemical parameters of dried salted pork neck and ham. The protein content in dried salted pork ham was significantly higher in comparison with dried salted pork neck. The value of intramuscular fat in dried salted pork neck was significantly higher in comparison with dried salted pork ham. The moisture was significantly lower in neck in comparison with dried salted pork ham. The salt content was comparable in neck with ham. The pH value was similar in dried salted pork neck as in dried salted pork ham. The value of water activity (a_w) was similar in ham as in neck. The content of arginine, phenylalanine, isoleucine, leucine and threonine in dried salted ham was significantly lower (p <0.001) in comparison with dried salted pork neck. The proportion of analysed amino acids from total proteins was 56.31% in pork salted dried ham and 56.50% in pork salted dried neck.

REFERENCES

Bednářová, M., Kameník, J., Saláková, A., Pavlík, Z., Tremlov, B. 2014. Monitoring of color and ph in muscles of pork leg (*m. adductor* and *m. semimembranosus*). *Potravinarstvo*, vol. 8, no. 1, p. 48-53. http://dx.doi.org/10.5219/337

Belitz, H. D., Grosch, W., Scieberle, P. 2001. *Food Chemistry*. 5. Auflage. Berlin : Springer Verlag. 572 p.

Benedini, R., Parolari, G., Toscani, T., Virgili, R. 2012. Sensory and texture properties of Italian typical dry-cured hams as related to maturation time and salt content. *Meat Science,* vol. 90, no. 2, p. 431-437. http://dx.doi.org/10.1016/j.meatsci.2011.09.001

Bjarnadottir, S. G., Lunde, K., Alvseike, O., Mason, A., Al-Shamma'a, A. I. 2015. Assessing quality parameters in dry-cured ham using microwave spectroscopy. *Meat Science*, vol. 108, p. 109-114. http://dx.doi.org/10.1016/j.meatsci.2015.06.004

Kim, Y. H. B., Kemp, R., Samuelsson, L. M. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. *Meat Science*, vol. 111, no. 1, p. 168-176. http://dx.doi.org/10.1016/j.meatsci.2015.09.008

Bučko, O., Bahelka, I., Petrák, J., Hellová, D., Debrecéni, O. 2015. The difference in the nutritional composition of pork and fat of barrows, boars and gilts of finishing pigs. *Research in pig breeding*, vol. 9, no. 1, p. 1-5.

Dall'Asta, C., Galaverna, G., Bertuzzi, T., Noseriti, A., Pietri, A., Dossena, A. Marchelli, R. 2010. Occurrence of chratoxin A in raw ham muscle, salami and dry-cured ham from pigs fed with contaminated diet. *Food Chemistry*, vol. 120, no. 4, p. 978-983. http://dx.doi.org/10.1016/j.foodchem.2009.11.036

Garcı´a-Gonza´lez, D. L., Tena, N., Aparicio-Ruiz, R., Morales, M. T. 2008. Relationship between sensory attributes and volatile compounds qualifying dry-cured hams, *Meat Science*, vol. 80, no. 2, p. 315-325. http://dx.doi.org/10.1016/j.meatsci.2007.12.015 PMid:22063336

Guofeng, J., Lichao, H., Chengliang, L., Yuhua, Z., Cheng, Ch., Yuehan, Z., Jianhao, Z., Meihu, M. 2015. Effect of pulsed pressure-assisted brining on lipid oxidation and volatiles development in pork bacon during salting and dryingripening. *Food Science and Technology*, vol. 64, no. 2, p. 1099-1106.

Jiménez-Colmenero, F., Ventanas, J., Toldrá, F. 2010. Nutritional composition of dry-cured ham and its role in a healthy diet. *Meat Science,* vol. 84, no. 4, p. 585-593. http://dx.doi.org/10.1016/j.meatsci.2009.10.029 PMid:20374828

Jiménez-Colmenero, F., Pintado, T., Cofrades, S., Ruiz-Capillas, C., Bastida, S. 2011. Production variations of nutritional composition of commercial meat products. *Food Research International*, vol. 43, no. 10, p. 2378-2384. http://dx.doi.org/10.1016/j.foodres.2010.09.009

Joo, S. T., Kim, G. D, Hwang, Y. H., Ryu, Y. C. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. *Meat Science*, vol. 95, no. 3, p. 828-836. http://dx.doi.org/10.1016/j.meatsci.2013.04.044

Jurado, A., Garcıa, C., Timon, M. L., Carrapiso, A. I. 2007. Effect of ripening time and rearing system on amino acid-related flavour compounds of Iberian ham. *Meat Science*, vol. 75, no. 4, p. 585-594. http://dx.doi.org/10.1016/j.meatsci.2006.09.006

Lorido, L., Estévez, M., Ventanas, J., Ventanas, S. 2015. Salt and intramuscular fat modulate dynamic perception of flavour and texture in dry-cured hams. *Meat Science*, vol. 107, p. 39-48. http://dx.doi.org/10.1016/j.meatsci.2015.03.025

Martínez-Onandi, N., Rivas-Cañedo, A., Nuñez, M., Picon, A. 2016. Effect of chemical composition and high pressure processing on the volatile fraction of Serrano dry-cured ham. *Meat Science*, vol. 111, no. 1, p. 130-138. http://dx.doi.org/10.1016/j.meatsci.2015.09.004 PMid:26398007

Neethling, J., Hoffman, L. C., Muller, M. 2016. Factors influencing the flavour of game meat: A review. *Meat Science*, vol. 113, no. 3, p. 139-153. http://dx.doi.org/10.1016/j.meatsci.2015.11.022 PMid:26658009

Okrouhlá, M., Stupka, R., Čitek, J., Šprysl, M., Kluzakova, E., Trnka, M., Štolc, L. 2006. Amino acid composition of pig meat in relation to live weight and sex. *Czech Journal of Animal Science,* vol. 51, no. 12, p. 529-534.

Pannier, L., Pethick, D. W., Geesink, G. H., Ball, A. J., Jacob, R. H., Gardner, G. E. 2014. Intramuscular fat in the longissimus muscle is reduced in lambs from sires selected for leanness. *Meat Science*, vol. 96, no. 2, p. 1068-1075. http://dx.doi.org/10.1016/j.meatsci.2013.06.014

Passi S., de Luca C. 1998. Dietetic advice for immunodeficiency. Proceedings of Conference Cell Aging Center, Instituto Dermopatico delÍImmacolata (IDI), Rome, Italy, p. 268-272.

Pham, A. J., Schilling, M. W., Mikel, W. B., Williams, J. B., Martin, J. M., Coggins, P. C. 2008. Relationships between sensory descriptors, consumer acceptability and volatile flavor compounds of American dry-cured ham. *Meat Science*, vol. 80, no. 3, p. 728-737. http://dx.doi.org/10.1016/j.meatsci.2008.03.015

Ruiz, J., Muriel, E., Ventanas, J. 2002. The flavour of Iberian ham. *Research advances in the quality of meat and meat products*, p. 255-271.

Ruiz, J., García, C., Muriel, E., Andrés, A. I., Ventanas, J. 2002. Influence of sensory characteristics on the acceptability of dry-cured ham. *Meat Science*, vol. 61, no. 4, p. 347-354. http://dx.doi.org/10.1016/s0309-1740(01)00204-2

Sánchez-Peña, C. M., Luna, G., García-Gomez, D. L., Aparicio, R. 2005. Characterization of French and Spanish dry-cured hams: Influence of the volatiles from the muscles and the subcutaneous fat quantified by SPME-GC. *Meat Science*, vol. 69, no. 4, p. 635-645. http://dx.doi.org/10.1016/j.meatsci.2004.10.015

Toldrá, F. 2016. Ham: Dry-cured Ham. In. Caballero et al. *Encyclopedia of food and health,* Kidlington, Oxford : Academic Press, p. 307-310. http://dx.doi.org/10.1016/B978-0-12-384947-2.00368-8

Van der Wal, P. G., Engel, B., Hulsegge, B. 1997. Causes for variation in pork quality. *Meat Science*, vol. 46, no. 4, p. 319-327. http://dx.doi.org/10.1016/S0309-1740(97)00026-0

Ventanas S., Ventanas J., Ruiz J. 2007. Sensory characteristics of Iberian dry-cured loins: Influence of crossbreeding and rearing system. *Meat Science*, vol. 75, no. 2, p. 211-219. http://dx.doi.org/10.1016/j.meatsci.2006.07.003

Ventanas, S., Ventanas, J., Ruiz, J., Estévez, M. 2005. Iberian pigs for the development of high-quality cured products. *Recent Research in Development in Agricultural and Food Chemistry*, vol. 6, p. 27-53.

Wilkinson, B. H. P., Lee, E., Purchas, R. W., Morel, P. C. H. 2014. The retention and recovery of amino acids frompork longissimus muscle following cooking to either 60 °C or 75 °C. *Meat Science*, vol. 96, no. 1, p. 361-365. http://dx.doi.org/10.1016/j.meatsci.2013.07.019 PMid:23954276

Zhao, Y., Abbar, S., Amoah, B., Phillips, T. W., Schilling, M. W. 2016. Controlling pests in dry-cured ham: A review. *Meat Science*, vol. 111, no. 1, p. 183-191. http://dx.doi.org/10.1016/j.meatsci.2015.09.009

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COMPARISON OF PHYSICOCHEMICAL PROPERTIES OF SELECTED LOCALLY AVAILABLE LEGUMES VARIETIES (MUNG BEAN, COWPEA AND SOYBEAN)

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ABSTRACT

Grain legumes are widely used as high-protein contained crops that play a secondary role to cereal or root crops. In Sri Lanka various legume species are cultivated and often utilised in the whole grain boiled form. The objective of present study was to analyse and compare locally grown legumes varieties; Mung bean (MI 5, MI 6), Cowpea (Bombay, Waruni, Dhawal, MICP1, ANKCP1) and soybean (pb1, MISB1) for their morphological characteristics, proximate and mineral composition (Fe, Ca, Zn, K, P). Seed shape, seed coat texture and colour, seed size and 100 seed weight (g) were observed morphological characteristics in present study. Most of the characteristics of mung bean and soybean were similar within their species whereas characteristics of cowpea varieties largely differed. Values of 100 seed weight among the varieties of mung bean, soybean and cowpea were ranged from 5.8 – 6.5 g, 13.5 – 14.1 g and 13.4 – 17.2 g, respectively. The moisture content of all legume seeds ranged from 6.81% to 11.99%. Results were shown that the protein content significantly higher in soybean (36.56 – 39.70%) followed by mung bean (26.56 – 25.99%) and cowpea (25.22 – 22.84%) respectively. Range of total carbohydrate, crude fat, crude fibre and total ash contents of nine legume varieties varied from 15.29 – 62.97%, 1.25 – 22.02%, 3.04 – 7.93% and 3.43 – 6.35 respectively. potassium (K), phosphorus (P), calcium (Ca), iron (Fe) and zinc (Zn) ranged from 1000 – 1900, 360 – 669, 15.0 – 192.3, 2.26 – 11.6 and 1.67 – 4.26 mg.100g^{-1} respectively in all the species of studied legume varieties. The wide variation in the chemical and physical properties of observed nine legume varieties, suggesting possible applications for various end-use products.

Keywords: legume; morphological characteristics; proximate; minerals composition

INTRODUCTION

In Sri Lanka, various legume species are cultivated. Being a cheap source of protein for the low-income group of the population, legumes are commonly used as a substitute for meat and they play a significant role in alleviating the protein-energy malnutrition. Most undernourished people live on a mono carbohydrate diet (i.e. maize or rice) which are in lacking of the required protein, fat, vitamin A, iodine, zinc and iron. Therefore incorporation of legume and pulses with other locally grown grains has a potential to reduce some extend of the protein malnutrition problems. Usually legumes are consume as whole or split form and it is cooked by follwing precooking process such as soaking **(Timoracká et al., 2010)**. Legume contain about 17 – 40% of protein which is comparable to cereals, 7 – 13% and to meat, 18 – 25% **(Genovese and Lajolo, 2001)**. The vitamin and mineral content of pulses also significance. They are rich in both major mineral elements (Mg, Ca, K, P) as well as trace elements (Fe, Cu, Zn, Mn) but very little amount of sodium **(Timoracká et al., 2011; Uebersax and Occena, 1991)**. Mung bean (*Vigna radiate wilczek*), Cowpea (*Vigna unguiculate*), soybean (*Glycine max L.*), black gram (*Vigna mungo L.*), groundnut (*Arachis hypogaea L.*) and Dhal (*Lens culinaris*) are mostly consumed legumes among Sri Lankan people and find different applications.

In the present study, some locally grown selected legumes have been recognised as economically important (Mung bean-*Vigna radiate L,* Cowpea-*Vigna unguiculata L* and Soybean-*Glycine max L*) were evaluated for their morphological characteristics, proximate and mineral composition with an intention to screen better variation for processing in future use.

MATERIAL AND METHODOLOGY

Two varieties of mung bean (MI5 and MI6), two varieties of soybean (pb1and MISB1) and five varieties of cowpea (ANKCP1, MICP1, Bombay, Wauni and Dhawala) recommended by the Department of Agriculture, Sri Lanka were selected for this study (Figure 1, 2 and 3) and they were obtained from Grain Legumes and Oil Seed Crops Research and Development Centre (GLOSCRDC), Angunakolapelessa, the main agriculture research centre located in Southern Dry Zone in Sri Lanka.

Sampling method

For the selection of legume seeds, random sampling method was performed and all varieties were collected from the same field with same environmental conditions and agricultural practices.


MI 5


(b) MI 6


(a) pb1


(b) MISB

Figure 1 Mungbean varieties. **Figure 2** Soybean varieties.


(a) Bombay


(b) Waruni


(c) Dhawala


(d) MICP 1


(e) ANKCP

Figure 3 Cowpea varieties.

Assessment of morphological characteristics

To identify and define the specific morphological characteristics, Seed shape, seed coat texture (wrinkled/ smooth), seed coat colour were described after visual examination. Seed size and seed weight (on their 100 seed weight) were determined following the procedure described by **Henshaw (2008)**. Weight less than 15.0 g were described as small; 15.1 – 20 g were as medium size while large seeds have 20.1 – 25 g and seeds over 25 g of weight defined as very large seeds.

Sample preparation for proximate and mineral analysis

Clean and dry whole legume seeds were ground to pass a 0.5 mm sieve using a laboratory type mill (Model-RETSCH S/S CROSS BEATER Hammer Mill Sk1). Then the powdered samples were homogenised and stored in polyethene bags at 10 ºC until use for analysis.

Proximate analysis

Proximate composition of legume seeds were carried out according to the methods described in **AOAC (2012)**. Every determination of composition values were performed in triplicates. Moisture contents of the legume seed flours were determined according to the oven drying method as described in **AOAC (2012)** 925.09B, applying gravimetric principal. Crude protein content of the legume seed flour was determined by micro-kjeldahl method as specified in **AOAC (2012)** 920.87 using Kjeldahl heating digestion unit (VELP Scientifica DK 20) and Kjeldahl semi distillation unit (VELP Scientifica DK 139). Crude fat content was determined by soxhlet extraction method according to **AOAC (2012)** 920.39C using Automatic extraction systems Soxtherm (C. GERHARDT GMBH & CO. KG Analytical Systems). Crude fibre content was determined according to the method described in **AOAC (2012)** 962.09E using Fibertec™ M6 Fibre Analysis System (FOSS-1020 HOT EXTRACTOR). Ash content was determined as specified in AOAC (2012) 923.03 by dry ashing method with gravimetric principal. Total carbohydrate content was determined according to the method described by **Sompong (2011)**.

Mineral analysis

Varian SpectrAA 220 Fast Sequential Atomic Absorption Spectrophotometer was used for the analysis of calcium, potassium, iron and zinc by following the method of 975.03 as specified in **AOAC (2012)**. Phosphorous contents of seeds were determined colorimetrically sodium molybdate according to the method 995.11 as specified in **AOAC (2000)**.

Statistical analysis

The data were statistically evaluated by one-way analysis of variance (ANOVA) by using Minitab 17 software (Minitab, Ltd. Brandon Court Unit E1-E2, Progress Way, Coventry CV3 2TE, UNITED KINGDOM). General linear model was used for comparison between legume varieties. All test procedures were made at 5% significant level. Also Microsoft Office Excel 2010 was used to graphical representation of data.

RESULTS AND DISCUSSION

Determination of morphological characteristics of selected legume variety

Studying of morphological characteristics helps to the selection of suitable variety for the purpose of cultivation as well as distinguishes between particular species and varieties within a species. Morphological characteristics of studied legume varieties are mentioned in Table 1. Most of the characteristics of mung bean and soybean are similar within their species whereas characteristics are largely different within cowpea. Mung beans are usually oblong in shaped and cowpea seeds varied from the typical kidney shape (Bombay, MICP 1) to rhomboid (Waruni, Dhawala, ANKCP 1) shape. The common shape of soybean varieties observed in this study was spherical. Shape of legume seed is mainly applicable for consumer preference for consuming and processing like snacks, canning, autoclaving, etc. Cooking and moisture absorption properties are accordance with the nature of seed coat texture, either smooth or wrinkled **(Sefa-Dedeh et al., 1978)**. Seeds with wrinkled seed coat texture have ability to absorb more water than seeds having smooth seed coat. Method of dehulling and soaking determine the color of final seed flour. Hence seed coat texture can be considered as an important criterion when processing seeds into flour **Henshaw (2008)**. Only two cowpea varieties (Bombay and Dhawala) were showed wrinkled texture among observed seeds. When considering seed coat colors, typically mung bean is in green color and soybean is in cream color. Color of cowpea varieties were largely varied and highly influenced consumer acceptance.

Here, it was observed that colors of cowpea varieties have been given particular diversity which is directly helped to distinguish each variety within the species. The cream colored seed (Dhawala and ANKCP 1) are preferred

Table 1 Morphological characteristics of selected mung bean, cowpea and soybean varieties.

Name of Variety	Seed shape	Seed coat texture	Seed coat colour	Seed Size	Seed weight of 100 seeds(g)
Mung Bean					
1. MI 5	Oblong	Smooth	Green	Small	5.8
2. MI 6	Oblong	Smooth	Green	Small	6.5
Cowpea					
3.Bombay	Kidney	Wrinkled	Speckled grey brown	Medium	15.3
4. Waruni	Rhomboid	Smooth	Reddish brown	Small	14.5
5.Dhawala	Rhomboid	Wrinkled	Cream colour with black eyed	Medium	17.2
6. MICP1	Kidney	Smooth	Cream color	Small	13.8
7.ANKCP 1	Rhomboid	Smooth	Pale brown colour	Small	13.4
Soybean					
8. pb 1	Spherical	Smooth	Cream color with a buff colour hilum	Small	13.5
9. MISB 1	Spherical	Smooth	Cream colour with a buff colour hilum	Small	14.1

than brown, red colored (Bombay and Waruni) seeds because they provide a sensory appeal by their color. Seed weight is mostly contributed from the kernel (Cotyledons and embryo) which make up about 88.8% and seed coat takes about 11.1% of the seed weight **(Singh et al., 1995; Kurien, 1977)**. Mung bean is the smallest seed among cowpea and soybean varieties and had less in weight, but both MI 5-1982 and MI 6-2004 are comparatively larger than other mung varieties recommended in Sri Lanka such as Harsha-1990 (4.8g in 100 seed wt) and Ari-1999 (5.8 g in 100 seed wt) **(Wasala et al., 2011)**. Smaller seeds of the mung bean variety Harsha fetched a lower price whereas MI5 always fetched a higher price even though Harsha possessed same physical characteristics with less mature time of seeds **(Hettiarachchi et al., 1998)**. Therefore seed weight of legume variety could be a useful criterion for determining suitability for a particular end-use application. Most of local cowpea varieties were small in size and Dhawala and Bombay were medium in size. There are 28 cowpea varieties have been studied by **Henshaw (2008)** and 100 seed weight varied between 10.1 g to 25.8 g. **Amiruzzaman (2003)** indicated that the average seed weight of soybean seeds are ranged between 15 – 40 g in 100 seeds. In this study, pb 1 and MISB 1 varieties were classified with small in size and the corresponding weights were 13.5 g and 14.1 g (in 100 seed weight) respectively.

Quantitative determination of proximate composition of legume seeds (Mung bean, Cowpea and Soybean)

In generally, cotyledons provide majority of the nutritional components, which makes 93% seed proteins, 95% fat, 87% ash and 88% nitrogen free extract-NFE in whole seed **(Singh et al., 1968)**. In present study moisture content of observed legume species were expressed in Table 2 and results ranged from 6.81 ±0.05% to 11.99 ±0.48%. The highest value was obtained from mung bean, MI 5 (11.99 ±0.48%) and the lowest from cowpea, MICP 1 (6.81 ±0.05%). In the case of mung bean, similar findings were observed by other scientists but with slight variations. **Akaerue and Onwuka (2010)** reported that the moisture content of the raw undehulled mung bean flour (*Vigna radiate*) was 10.25%. A study from, **Butt and Batool (2010)** showed comparatively lower value for moisture content of mung bean (8.81% – 7.79%). However, other researchers had earlier reported that *Phaseolus aureus* variety had 9.75% of moisture content which were in agreement of our results **(Mubarak, 2005)**. Moisture content of Bombay, Waruni and ANKCP 1 were significantly ($p \leq 0.05$) higher than those for Dhawala and MICP 1. Similar observations on the moisture content of different cowpea varieties have been reported by several investigations. **Butt and Batool (2010)** had reported that moisture content of *Vigna unguiculata L* is 9.66% – 11.12% and 13.22% is the results of **Mwasaru et al., (1999)**.

When consider the mean values of soybean, no significant difference ($p > 0.05$) was found between pb 1 and MISB 1 in their moisture content. It is in agreement with those reported by **Joshi et al., (2015),** the moisture content for full fat seed flour ranged between from 8.54% to 10.20%. However, slight variations may be due to genotype and environmental conditions **(Qayyum et al., 2012)**.

According to the results mentioned in Table 3 the crude protein content of the whole ground legume (undehulled) ranged between 22.84 ±0.09% (Dhawala) to 39.70 ±0.43% (MISB 1). The findings of **Adam et al., (1989)** were in conformity with these values and which amplified that crude protein content of the selected legumes ranged from 15% to 45%. In this context, no significant difference ($p > 0.05$) was observed between the protein content within mung bean varieties. Current results are resemblance with other research, which was reported that protein content of *P. aureus* and *Vigna radiate* remained as 27.5% **(Mubarak, 2005)** and 24.08% **(Blessing and Gregory, 2010)** respectively. In cowpea varieties, the protein content of Dhawala was significantly ($p \leq 0.05$) lower than observed other four cowpea varieties. In this regards,

Table 2 Moisture content of selected legume varieties of mung bean, cowpea and soybean.

Name of Variety	Moisture content (g.100g^{-1} of sample ±SD)
Mung bean	
1. MI 5	11.99 ±0.48 [a]
2. MI 6	11.48 ±0.22 [ab]
Cowpea	
3. Bombay	11.05 ±0.39 [ab]
4. Waruni	11.05 ±0.06 [ab]
5. Dhawala	9.50 ± 0.05 [c]
6. MICP1	6.81 ±0.05 [d]
7. ANKCP 1	10.99 ±0.10 [b]
Soybean	
8. pb 1	9.24 ±0.62 [c]
9. MISB 1	9.57 ±0.37 [c]

Note: Results were expressed in Mean ±Standard deviation of triplicates and means with same superscript in column are not significantly different (p >0.05).

Elharadallou (2013) explicated that protein content of *Vigna unguiculata L.* was 22.30% while value obtained by **Elias et al., (1964)** for *Vigna sinensis* was 27.5%. The array of investigations, variations in protein content have been observed owing to analytical methods, genotype, different environments and agricultural practices. Generally speaking, soybean are rich in protein is collaborate with present findings. According to that protein content of soybean varieties were notably higher than both mung bean and cowpea. But protein content of MISB1 was significantly (p ≤0.05) higher than pb 1. Protein concentration is highest in the embryo, followed by cotyledons and least in the seed coats. Because of the size, cotyledons contribute for the maximum protein amount. Protein concentration of grains also varies with the cultivar and the same cultivar grown at different areas **(Gottschalk and Mũller, 1983)**.

The fat content of soybean is prominent than both mung bean and cowpea varieties. By the reason, soybean generally speaks as oil seed. The low-fat content in mung bean and cowpea is an advantage during processing it into flour, since there is no need for a defatting step in seed flour production **(Henshaw, 2008)**. In values reported in this study, fat content of all three legume species ranged from 1.25 ±0.03% (MICP 1) to 22.02 ±0.05% (pb1). Fat content of mung bean varieties were not significantly differ (p >0.05) from each other while similar findings have been reported previously by **Mubarak (2005)** and **Blessing and Gregory (2010)**. Most of cowpea varieties exhibited slightly high-fat content rather than mung bean varieties and the values show no significant difference (p >0.05) between each other. Studies conducted by **Elharadallou (2013)** and **Elias et al., (1964)** found same value (2.1%) for fat content of *Vigna unguiculata L* and *Vigna sinensis* which is collaborated with present findings. In the case of soybean, fat content of pb 1 was significantly (p ≤0.05) higher than the value of MISB 1. Results are also in agreement with the findings of **Namiki (1995)** 21.88% for *Glycine max*.

Legumes contained more fibre than any major food group. Some fibre are soluble and others insoluble. In most legumes consumed by humans, the fibre content ranges from 8% to nearly 28% **(McGreevy, 2008)**. As the values presented in Table 3 there is no significant difference (p >0.05) between crude fibre content of two mung bean varieties and these findings are supported by **Mubarak, (2005)** 4.63% for *P. aureus* and **Blessing and Gregory,**

Table 3 Proximate composition of different legume varieties of mung bean, cowpea and soybean (on dry weight basis).

	Composition (g.100g^{-1} of sample ±SD)				
	Protein	**Fat**	**Fibre**	**Ash**	**Carbohydrate***
Mung Bean					
MI 5	25.99 ±0.24 [cd]	1.54 ±0.01 [cd]	5.55 ±0.05 [cd]	3.96 ±0.04 [e]	62.97
MI 6	26.56 ±0.10 [c]	1.25 ±0.03 [d]	5.01 ±0.13 [d]	3.95 ±0.04 [e]	51.75
Cowpea					
Bombay	24.98 ±0.24 [e]	1.81 ±0.06 [cd]	4.36 ±0.16 [e]	3.43 ±0.01 [h]	52.22
Waruni	25.03 ±0.25 [e]	1.51 ±0.04 [cd]	6.84 ±0.15 [b]	3.78 ±0.01 [f]	58.76
Dhawala	22.84 ±0.09 [f]	1.72 ±0.08 [cd]	5.06 ±0.21 [d]	3.62 ±0.03 [g]	54.37
MICP1	25.22 ±0.27 [de]	1.86 ±0.04 [cd]	3.04 ±0.10 [f]	4.3 ±0.03 [c]	51.79
ANKCP1	24.90 ±0.23 [e]	2.03 ±0.57 [c]	5.75 ±0.37 [c]	4.10 ±0.05 [d]	57.24
Soybean					
pb 1	36.56 ±0.22 [b]	22.02 ±0.05 [a]	7.93 ±0.13 [a]	6.14 ±0.00 [b]	18.11
MISB 1	39.70 ±0.43 [f]	21.17 ±0.18 [b]	7.93 ±0.25 [a]	6.35 ±0.01 [a]	15.29

Note: Results were expressed in Mean ±Standard deviation of triplicates and means with same superscript in column are not significantly different (p >0.05).

* Standard deviations are not applicable for figures of carbohydrate since they are obtained by subtracting sum of average values of other nutrients from 100%.

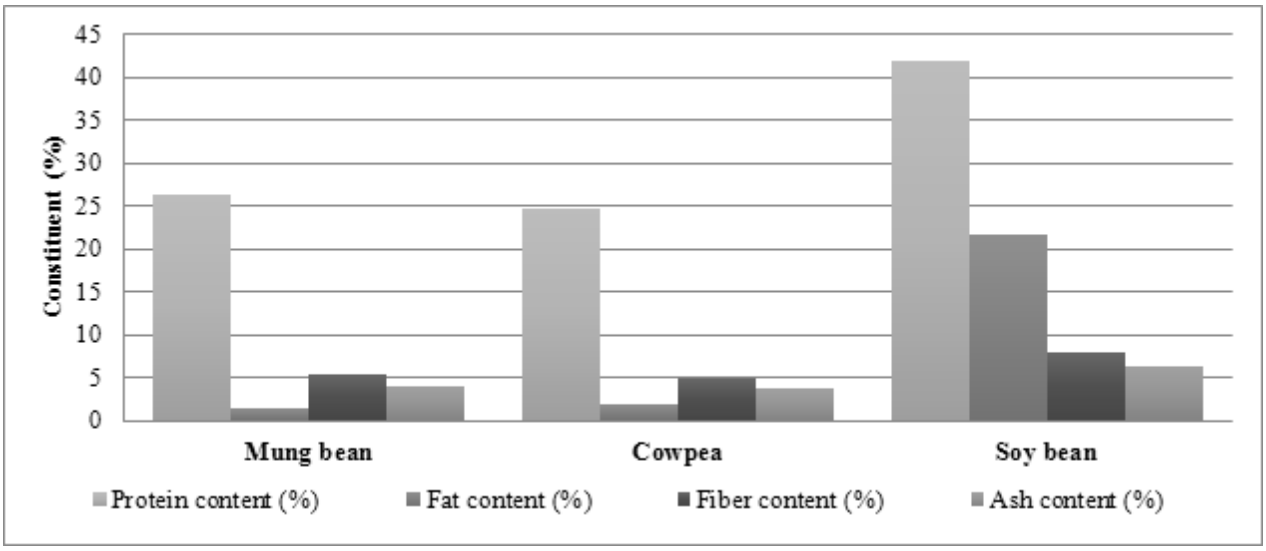

Figure 4 Average proximate composition (on dry basis) of mung bean, cowpea and soy bean. Mean (n = 3).

(2010) 5.00% for *Vigna radiate* in fibre content. Soybean also did not exhibits significant difference (p >0.05) between fibre content while these represent higher values among selected nine varieties in this study. However, present results slightly vary from previous literature, as the value reported by **Namiki (1995)**, fibre content of *Glycine max* was 9.0%**.** Although mung bean and soybean show no significant difference within their species, significant variations (p ≤0.05) were existed in fibre content in cowpea varieties. However, lowest value was observed in MICP 1 and the highest value was in Waruni.

Present results are comparable to the earlier findings **(Elharadallou, 2013; Elias et al., 1964)**. They reported that 4.10% and 7.0% of fibre contents for *Vigna unguiculata L.* and *Vigna sinensis* respectively. The causes for observed variations in cowpea varieties are depend on the type of legume species, the variety within same species, and the processing of the legume (Milling conditions, particle size, etc.) **(McGreevy, 2008)**.

The mean values for total ash content of selected nine legume varieties ranged from highest 3.43 ±0.01% (Bombay) to lowest 6.35 ±0.01% (MISB 1). There is not significant difference reported (p >0.05) in the ash contents of mung bean varieties. Previous studies have been found that 3.76% ash content for *P. aureus* and 3.00% for *Vigna radiate* **(Mubarak, 2005; Blessing and Gregory, 2010)**, which are in agreement with the ash content of MI 5 and MI 6. Total ash content of cowpea varieties show significant difference (p ≤0.05) from each other. It was reported that ash content of *Vigna unguiculata L*. was 3.77% and the value for *Vigna sinensis* was 4.9% **(Elharadallou, 2013; Elias et al., 1964)**, showing that present results are in accordance with previous research. When considering the results of ash content in soybean varieties, the value for pb 1 was significantly *(p ≤0.05)* lower than the value for MISB1. Also, ash contents in present study are very much deviate from the studies of **Cheftel et al., (1985)** (i.e. 4.9%) and **Namiki (1995)** (i.e. 2.59%). The significance variations of the result would be better interpretation to that variety cultivated under different cultural conditions such as soil composition, climatic and agronomic practices **(Henshaw, 2008)**.

Carbohydrate content of legume seed ranged from 15.29% (MISB 1) to 62.97% (MI5). For most of legumes, the largest part of the carbohydrate fraction is starch, accounting for about 35% – 45% of the seed weight depending on the legume species **(Hedley, 2001)**. Carbohydrate values of MI 5 and MI 6 in present study are in agreement with the results of **Mubarak (2005**) and **Blessing and Gregory (2010)**. As they reported, values of carbohydrate contents are 62.3% for *p. Aureus* and 55.74% for *vigna radiate* respectively.

Among cowpea varieties, carbohydrate content ranged from 51.79% to 58.76%. Similar values were followed by **Elharadallou (2013)** for *Vigna unguiculata L.* (60.07%) and **Elias et al., (1964)** for *Vigna sinensis* (58.5%). In case of soybean the highest carbohydrate content was reported from pb 1 (18.11%) while lowest was MISB 1 (15.29%). But both values are severely deviated from the value (44.06%) reported by **Namiki (1995)**. Total carbohydrate content analysis which is not determined analytically but is calculated by difference. Since the result is obtained by subtracting the total percentages calculated for each macro nutrient from 100, any errors in evaluation will be reflected in the final calculation. Hence lower value for carbohydrate in soybean seed could be observed in present study due to higher number of other compositional components (i.e. mainly protein) than the findings of others.

Quantitative determination of mineral composition of legume seeds (mung bean, cowpea and soybean)

Results for the mineral analysis are presented in Table 4. Iqbal et al., (2006) indicated that potassium is the most abundant mineral among legume seeds. It has been observed from the current study and values ranged from 1000 to 1900 mg.100g^{-1} of sample. Phosphorous, copper, iron, calcium and magnesium are some of other important minerals found in legumes in significant amount **(Eskin and Shahidi, 2012)**. Whereas concentrations will vary in response to both genetic and environmental factors. Both

Table 4 Mineral composition of different legume varieties of mung bean, cowpea and soybean (on dry weight basis).

Name of Variety	Composition (mg.100g^{-1} of sample ±SD)				
	Iron (Fe)	Calcium(Ca)	Zinc(Zn)	Potassium(K)	Phosphorus (P)
Mung Bean					
MI 5	2.69 ±1.07 ^{c}	29.0 ±4.33 ^{c}	1.67 ±0.12 ^{c}	1000 ±5.51 ^{f}	394 ±1.53 ^{e}
MI 6	2.83 ±0.18 ^{c}	27.4 ±3.13 ^{c}	1.71 ±0.2 ^{c}	1200 ±6.24 ^{d}	438 ±4.04 ^{c}
Cowpea					
Bombay	3.54 ±0.32 ^{c}	27.8 ±3.03 ^{c}	2.82 ±0.39 ^{bc}	1300 ±14.18 ^{c}	360 ±2.08 ^{g}
Waruni	3.49 ±0. 06 ^{c}	29.9 ±5.11 ^{c}	2.63 ±0.19 ^{c}	1200 ±9.07 ^{d}	424 ±2.65 ^{d}
Dhawala	2.42 ±0.45 ^{c}	23.3 ±2.39 ^{cd}	2.20 ±0.17 ^{c}	1100 ±11.93 ^{e}	372 ±1.53 ^{f}
MICP1	2.26 ±0.03 ^{c}	29.4 ±3.58 ^{c}	2.04 ±0.72 ^{c}	1000 ±6.03 ^{f}	441 ±5.03 ^{c}
ANKCP1	2.83 ±0.33 ^{c}	15.0 ±2.81 ^{d}	2.30 ±0.47 ^{c}	1200 ±7.64 ^{d}	396 ±4.04 ^{e}
Soybean					
pb 1	11.6 ±0.42 ^{a}	153.3 ±1.47 ^{b}	4.26 ±0.91 ^{a}	1700 ±8.00 ^{b}	587 ±2.52 ^{b}
MISB 1	7.91 ±0.41 ^{b}	192.3 ±2.18 ^{a}	4.07 ±0.28 ^{ab}	1900 ±9.07 ^{a}	669 ±2.08 ^{a}

Note: Results were expressed in Mean ±Standard deviation of triplicates. Means with same superscript in column are not significantly different (p >0.05).

soybean varieties (pb 1 and MISB 1) contained remarkable quantities of iron, calcium, zinc, potassium and phosphorus when to compare mung bean and cowpea varieties and might thus be of nutritional interest. Iron and zinc contents are remarkably higher in legumes than the cereals. Therefore it is very beneficial to go for composite feeding and supplementary food formulations for under nourished groups using legumes because in biological system, trace minerals (Mn, Zn and Fe) play a vital role **(Timoracká et al., 2011)**.

CONCLUSION

Based on visual and instrumental evaluations seed assessments discovered that more variations could be seen between varieties within cowpea, but mung bean and soybean showed minor variation by only in the seed weight. As general speaking, soybean recorded markedly higher protein content and fat content while observed values show next higher protein content and fat content in mung bean varieties. Legumes have more fibre than any major food group, among them soy bean reported highest. Ash contents of soybean were significantly higher than mung bean and cowpea varieties and it is explicated by relatively higher amount of potassium, phosphorus, calcium, iron and zinc in mineral analysis. In nutritional point of view, tested legumes; mung bean, cowpea and soybean are good sources of protein, zinc and iron compare to cereal and it is better for composite mix formulations for malnourished population.

REFERENCES

Akaerue, B., Onwuka, G. 2010. Evaluation of the Yeild, Protein Content and Functional Properties of Mungbean [Vigna radiata (L.) Wilczek] Protein Isolates as Affected by Processing. *Pakistan Journal of Nutrition*, vol. 9, no. 8, p. 728-735. http://dx.doi.org/10.3923/pjn.2010.728.735

Amiruzzaman, M. 2003. Report of the APO Seminar on Processing and utilisation of legumes. [online] s.a. [cit.2016-04-22] Available at: http://www.apo-tokyo.org/00e-books/AG.

Blessing, I. A., Gregory, I. O. 2010. Effect of Processing on the Proximate Composition of the Dehulled and Undehulled Mungbean [Vigna radiata (L.) Wilczek] Flours. *Pakistan Journal of Nutrition*, vol. 9, no. 10, p.1006-1016. http://dx.doi.org/10.3923/pjn.2010.1006.1016

Butt, M., Batool, R. 2010. Nutritional and Functional Properties of Some Promising Legumes Protein Isolates. *Pakistan Journal of Nutrition*, vol. 9, no. 4, p. 373-379. http://dx.doi.org/10.3923/pjn.2010.373.379

Cheftel, J., Cuq, J., Lorient, D. 1985. Protéines alimentaires : biochimie, propriétés fonctionnelles, valeur nutritionnelle, modifications chimiques. Paris : Tec & Doc Lavoisier. ISBN: 2-85206-870-2.

Elias, L. G., Colindres, R., Bressani, R. 1964. Nutritive value of eight varieties of cowpea (*Vigna sinensis*). *Journal of Food Science*, vol. 29, no. 1, p. 118-122.

Eskin, N. A. M., Shahidi, F. 2012. Biochemistry of Foods. Academic Press, p. 584. ISBN: 978-0122423529.

Genovese, M. I., Lajolo, F. M. 2001. Trypsin inhibitory activity of the bean (*Phaseolus vulgaris* L.): evaluation of critical method of the determinacy. *Archivos Latinoamericanos de Nutrición*, vol. 51, no. 4, p. 386-394. PMID: 12012566

Gottschalk, W., Mǔller, H. 1983. Seed proteins. Netherlands : Springer, p. 531. ISBN:978-90-247-2789-6. http://www.springer.com/in/book/9789024727896

Hedley, C. 2000. Carbohydrates in grain legume seeds. UK : John Innes Centre, p. 11-13. ISBN: 0851994679. http://dx.doi.org/10.1079/9780851994673.0000

Henshaw, F. 2008. Varietal Differences in Physical Characteristics and Proximate Composition of Cowpea (Vigna unguiculata). *World Journal of Agricultural Sciences*, vol. 4, no. 3, p. 302-306.

Hettiarachchi, M. P., De Costa, W. A. J. M., Jayasekara, S. J. B. A. 1998. Factors responsible for productivity of food

legumes: Finding of a farmer survey in the Kurunegala district, Sri lanka. *Tropical Agricultural Reasearch and Extension*, vol. 1, no. 2, p. 165-168.

Iqbal, A., Khalil, I., Ateeq, N., Sayyar Khan, M. 2006. Nutritional quality of important food legumes. *Food Chemistry*, vol. 97, no. 2, p. 331-335. http://dx.doi.org/10.1016/j.foodchem.2005.05.011

Joshi, A., Liu, C., Sathe, S. 2015. Functional properties of select seed flours. *LWT - Food Science and Technology*, vol. 60, no. 1, p. 325-331. http://dx.doi.org/10.1016/j.lwt.2014.08.038

Kadam, S. S., Deshpande, S., Jambhale, N. 1989. Handbook of world food legumes: Nutritional Chemistry, Processsing Technology, and Utilization, Boca Raton: CRC press, vol. 1, p. 23-50.

Khalid, I. I., Elharadallou, S. B. I. 2013. Functional properties of Cowpea (*Vigna Ungiculata* L.Walp), and lupin (*Lupinus termis*) flour and protein isolates. *Journal of Nutrition and Food Science*, vol. 03, no. 06, p. 1-6. http://dx.doi.org/10.4172/2155-9600.1000234

Kurien, P. P. 1977. Grain Legume Milling Technology. In *FAO Expert Consultation on Grain Legume Processing*, Mysore, India : CFTRI, pp. 31.

McGreevy, T. 2008. General Properties of Dry Peas, Lentils and Chickpeas. [online] s.a. [cit.2016-04-22] Available at: http://www.pealentil.com/core/files/pealentil/uploads/files/Chapter2.pdf.

Mubarak, A. E., 2005. Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. *Food Chemistry*, vol. 9, no. 4, p. 489-495. http://dx.doi.org/10.1016/j.foodchem.2004.01.007

Mwasaru, M., Muhammad, K., Bakar, J., Man, Y. 1999. Effects of isolation technique and conditions on the extractability, physicochemical and functional properties of pigeonpea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. I. Physicochemical properties. *Food Chemistry*, vol. 67, no. 4, p. 435-443. http://dx.doi.org/10.1016/S0308-8146(99)00150-8

Namiki, M. 1995. The chemistry and physiological functions of sesame. *Food Reviews International*, vol. 11, no. 2, p. 281-329. http://dx.doi.org/10.1080/87559129509541043

Official Methods of Analysis of AOAC INTERNATIONAL. 2012. 18th ed. USA: Gaithersburg, Md.

Qayyum, M. M. N., Butt, M. S., Anjum, F. M., Nawaz, H. 2012. Composition analysis of some selected legumes for protein isolates recovery. *The Journal of Animal and Plant Sciences*, vol. 22, no. 4, p. 1156-1162. http://www.thejaps.org.pk/docs/V-22-4/55.pdf

Sefa-Dedeh, S., Stanley, D., Voisey, P. 1978. Effects of soaking time and cooking conditions on texture and microstructure of cowpeas (*Vigna unguiculata*). *Journal of Food Science*, vol. 43, no. 6, p. 1832-1838. http://dx.doi.org/10.1111/j.1365-2621.1978.tb07426.x

Singh, Jai, Verma, P. 1995. *Status of Pulse Milling in India*. Ludhiana : Central Institute of Post Harvest Engineering and Technology (CIPHET). 22 p.

Singh, S., Singh, H. O., Sikka, K. C. 1968. Distribution of nutrients in anatomical parts of common Indian pulses. *Cereal Chemistry*, vol. 45, p. 13-18. http://www.aaccnet.org/publications/cc/backissues/1968/Documents/chem45_13.pdf

Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G., Berghofer, E. 2011. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chemistry*, vol. 124, no. 1, p. 132-140. http://dx.doi.org/10.1016/j.foodchem.2010.05.115

Tharanathan, R., Mahadevamma, S. 2003. Grain legumes a boon to human nutrition. *Trends in Food Science and Technology*, vol. 14, no. 12, p. 507-518. http://dx.doi.org/10.1016/j.tifs.2003.07.002

Timoracká, M., Vollmannová, A., Bystrická, J. 2010. Polyphenols in chosen species of legume - A Review. *Potravinarstvo*, vol. 4, no. 4, p. 65-72. http://dx.doi.org/10.5219/81

Timoracká, M., Vollmannová, A., Ismael, D. 2011. Minerals, trace elements and flavonoids content in white and coloured kidney bean. *Potravinarstvo*, vol. 5, no. 1, p. 56-60. http://dx.doi.org/10.5219/116

Uebersax, M. A., Occena, L. G. 1991. Composition and nutritive value of dry edible beans: commercial and the world food relief applications. *Michigan Dry Bean Digest*, vol. 15, no. 5, p. 3-12.

Wasala, S., Fernando, W. and Narasinghe, I. 2011. Hardseededness of Local Mungbean (Vigna radiata) Varieties. Tropical Agricultural Research and Extension, vol. 14, no. 1, p. 5-6. http://dx.doi.org/10.4038/tare.v14i1.4833

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MICROBIOLOGICAL AND CHEMICAL QUALITY OF FRESH AND FROZEN WHOLE TROUT AND TROUT FILLETS

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ABSTRACT

The rainbow trout (*Oncorhynchus mykiss*) is considered as an important fish in the freshwater aquaculture and play a significant role in the human diet. The final quality of fish depends on the chemical and microbiological quality of fish at the time of freezing as well as on other factors including storage temperature and processing. The purpose of the study was to determine the microbiological status of 30 samples cooled and frozen trouts collected from approved farm in the Turiec region, territory of middle Slovakia. Total viable counts (TVCs), psychrotrophic bacteria, *Pseudomonas* spp. and also total volatile base-nitrogen (TVB-N) and pH were measured in samples of fresh (1. and 7. day of storage at 0 – 2 °C) and frozen whole trout and trout fillets. Frozen samples were stored at -18 °C during 1, 3, and 6 months. Samples were collected from the skin, muscles (sterile) and muscles after filleting. The microbiological quality of samples varied between fresh and frozen (6th month of storage) regarding TVCs and also between samples taken from the skin and muscles after filleting compared to muscle samples collected sterile regarding all tested bacteria. A large number of bacteria (pathogens and spoilage bacteria) enter with the raw material and in particular the skin contamination had a negative impact on the increase of microbial load in fillets. All processing techniques and procedures including filleting therefore must be designed and aimed to minimise contamination and growth of microorganisms in fish. However, based on the results of TVB-N analysis, differences between fresh and frozen samples were found, but all the samples were suitable for human consumption.

Keywords: total viable counts; psychrotrophic bacteria; *Pseudomonas*; total volatile base-nitrogen, rainbow trout

INTRODUCTION

The subsurface flesh of live, healthy fish is considered sterile and should not present any bacteria or other microorganisms. On the contrary, as with other vertebrates, microorganisms colonise the skin, gills and the gastrointestinal tract of fish. The number and diversity of microbes associated with fish depend on the geographical location, the season and the method of harvest. In general, the natural fish microflora tends to reflect the microbial communities of the surrounding waters **(Fernandes, 2009)**. The fish could be also contaminated after being caught or during transportation to retail markets. Fish quality is influenced by many factors as the source, cooling methods, processing, packaging, storage conditions. Especially, during the filleting of fish as practised in filleting plants or shops, it is impossible to avoid contamination of the initially virtually sterile fish flesh **(Shewan, 1961)**. After contamination and replication of microorganisms, decay occurs and the consumption becomes dangerous **(Alparslan et al., 2014)**.

The quality and freshness of fish are rapidly deteriorated through microbial and biochemical mechanisms **(Al-Jasser and Al-Jasass, 2014)**. The autochthonous bacterial flora of fish is dominated by Gram-negative genera including: *Acinetobacter*, *Flavobacterium*, *Moraxella*, *Shewanella* and *Pseudomonas*. Members of the families *Vibrionaceae* (*Vibrio* and *Photobacterium*) and the *Aeromonadaceae* (*Aeromonas* spp.) are also common aquatic bacteria **(Huss, 1995)**. Fish quality is mainly assessed through the total aerobic plate counts and counts of bacteria with public health relevance. A monitoring of these microorganisms has been suggested as a measure of fish quality. Psychrotrophs are these bacteria that grow well at or below 7 °C and have their optimum temperature for growth between 20 – 30 °C. Some psychrotrophic pathogens can grow in the refrigerated food with little or no obvious change of sensory characteristics. *Pseudomonas* species are important spoilage microorganisms in many chilled food products especially fish in which they become the dominant microflora during chill storage **(Gram, 1993)**. Thus, their presence in food creates a great risk as they lead to food poisoning and spoilage of food **(Jay, 2000)**. *Pseudomonas* spp. mediated spoilage is characterised by unpleasant odours from the production of ketones, aldehydes, esters and sulphur-containing compounds such as methyl sulphide **(Vogel et al., 2005)**.

Food spoilage can be considered as any change that renders the product unacceptable for human consumption **(Sivertsvik et al., 2002)**. Spoilage of fish starts upon death due to autoxidation (oxidation of unsaturated lipids), reactions caused by activities of the fish's own enzymes, and metabolic activities of microorganisms present in the fish. Over time, loss of the fresh characteristics may be simply measured by comparative visual and smell analysis. Bacterial activity results in unpleasant odour due to

conversion of amino acids into biogenic amines, sulfides, organic acids etc. **(Velu et al., 2013)**. However, volatile bases are the best-characterised chemical indicators of fresh fish spoilage. Evaluation of Total volatile base-nitrogen (TVB-N), or a specific fraction of the volatile bases, for example the TMA fraction, using Conway diffusion chambers allows determination of changes of mg-N/100 g fish.

The activity of bacterial enzymes could be minimised by observing hygiene rules, proper processing, conservation and low temperatures. Cooling and freezing are widely used methods for preservation of fish. However, cooling could not prevent spoilage but shelf-life could be prolonged **(Shamsuzzaman et al., 2011)**. Maintenance of product temperature at -18ºC will not permit bacterial growth or enzymic spoilage. The conditions and freezing process most favourable for good-quality products will, however, also be those that have the least effect on the viability of microorganisms.

The purpose of this survey was to evaluate the microbiological status of fresh and frozen rainbow trout and to assess the degree of bacterial colonisation, amount of TVB-N and pH of fresh and consequently frozen whole fish and fillets.

MATERIAL AND METHODOLOGY

Sample collection

The study was conducted with 30 samples cooled and frozen fish products. Fresh fish were collected from approved farm Rybarstvo Požehy Ltd. located in the Turiec region, territory of middle Slovakia. After catching the fish were slaughtered, vacuum packed and cooled and then transported to the laboratory for analysis. The fresh samples have been stored at 0 – 2 °C (12 pcs) and 18 fish have been frozen immediately after receiving in the laboratory and stored 1, 3 and 6 month at -18 °C. The samples were taken from the fresh fish on 1. (fresh 1) and 7.day (fresh 7) of storage for microbiological examination from the skin (first step), muscle sterile (second step) and from the muscle after filleting (third step). The same procedure was repeated also for frozen fish on 1. (frozen 1), 3. (frozen 3), and 6. (frozen 6) month of storage. At the same time samples were taken for estimation of pH and determination TVB-N. All the analysis were performed on 6 samples in each group.

Sample analysis

In the beginning, swabs were taken from the skin and then 10 grams of muscle tissue were collected aseptically and placed into a Stomacher bag. Ninety ml sample diluent were added, and samples were homogenised at 256 rpm for 1 min. Tenfold dilutions were performed in tubes with 9 ml sample diluent. The total viable counts (TVCs) was determined using the pour plate method according to **ISO 4833 (2003)** and plates were incubated at 30 °C for 48 – 72 h. Horizontal method was used for enumeration of psychrotrophic bacteria **(ISO 6730, 2005)**, and colonies were counted in a solid medium after incubation at 6.5 °C for 10 days. **ISO 13720 (2010)** specifies a method for the enumeration of presumptive Pseudomonas spp. present in meat and meat products, including poultry. The results are presented as log cfu/g.

The pH of the meat was measured by a digital pH meter with glass electrode (AMA digit AD 140, Germany). TVB-N values were determined using micro diffusion Conway method and expressed as mg TVB-N/100 g rainbow trout flesh **(Conway and Byrne, 1933)**.

Statistical analysis

Group means and standard deviations were calculated using column statistics, followed by one-way ANOVA analysis of variance, Tukey's multiple comparison test **(GraphPad Prism 5, 2007)**; and treatments were considered significantly different at $p < 0.05$.

RESULTS

Total viable counts (TVCs) in fresh and frozen fish taken from skin, muscle and fillet are presented in Table 1. The highest microbial loads were established in frozen samples after six month of storage. Contamination of fish samples analyse during first three months of frozen storage was comparable to bacterial load in fresh samples. Slight increase in TVCs was found in fresh samples after 7 days of storage under chilling conditions. The amount of TVCs in samples collected sterile from muscles have been significantly lower ($p < 0.05$) compared to bacteria number on the skin and in the muscle samples collected after filleting.

Most contaminated with psychrotrophic bacteria were samples taken from skin of fresh trouts within 7 days of storage under chilling conditions and high load of bacteria

Table 1 Total viable counts (log CFU.g^{-1}) in fresh and frozen fish and fillets.

Sample	Fresh 1	Fresh 7	Frozen 1	Frozen 3	Frozen 6
Skin	4.75 ±0.15	5.4 ±1.0	4.75 ±0.25	4.8 ±0.2	7.05 ±0.45
Muscle	2.7 ±0.2	2.75 ±0.25	2.1 ±0.4	2.2 ±0.2	5.05 ±0.36
Fillet	4.55 ±0.35	4.85 ±0.45	3.7 ±0.3	4.1 ±0.3	6.05 ±0.55

Table 2 Psychrotrophic bacteria (log CFU.g^{-1}) in fresh and frozen fish and fillets.

Sample	Fresh 1	Fresh 7	Frozen 1	Frozen 3	Frozen 6
Skin	4.8 ±0.1	5.2 ±0.8	4.8 ±0.1	4.5 ±0.6	4.9 ±0.5
Muscle	2.75 ±0.05	2.75 ±0.25	2.05 ±0.05	1.9 ±1.14	1.75 ±0.45
Fillet	4.55 ±0.45	4.95 ±0.55	3.8 ±0.2	4.1 ±0.4	3.9 ±0.1

Table 3 *Pseudomonas* spp. (log CFU.g^{-1}) in fresh and frozen fish and fillets.

Sample	Fresh 1	Fresh 7	Frozen 1	Frozen 3	Frozen 6
Skin	1.5 ±1.06	1.63 ±0.96	2.63 ±0.08	2.65 ±0.26	3.33 ±0.37
Muscle	1.13 ±1.41	1.18 ±0.7	0	0	0
Fillet	1.28 ±1.68	1.83 ±1.45	2.53 ±0.13	2.73 ±0.38	2.85 ±0.57

Table 4 presents the results for pH and TVB-N (mg.100g^{-1}) in fresh and frozen fish.

Sample	Fresh 1	Fresh 7	Frozen 1	Frozen 3	Frozen 6
pH	6.51 ±0.03	6.64 ±0.05	6.79 ±0.01	6.78 ±0.05	6.75 ±0.02
TVB-N	12.75 ±0.52	13.96 ±0.34	17.03 ±0.1	17.03 ±0.21	18.74 ±1.71

was also found in the fillets examined at the same time. The amount of psychrophilic bacteria in samples collected sterile from muscles have been significantly lower (p <0.05) compared to bacteria number on the skin and in the muscle samples collected after filleting. Freezing did not cause the increase in the number of bacteria in all samples (Table 2).

Non-significant increase in microbial counts was observed in samples of fresh trout after seven days of storage (0 – 2 °C). Frozen fish samples taken from the skin and muscles after filleting exhibited higher number of *Pseudomonas* spp. compared to cooled fish samples. The amount of *Pseudomonas* spp. in samples collected sterile from muscles have been significantly lower (p <0.05), in frozen samples has been absented, compared to bacteria number on the skin and in the muscle samples collected after filleting (Table 3).

Results of pH value and TVB-N values in samples of rainbow trout are shown in Table 4. There was no significant difference (p >0.05) in pH values, however slightly higher pH values were found in frozen samples. Significantly higher TVB-N concentrations (p <0.05) were recorded in frozen fish compared to fresh ones, but all the values were below the 30 mg.100g^{-1} considered to be value when spoilage become obvious.

DISCUSSION

Quality of meat products is affected by the quality of raw meat, storage temperature and handling conditions. Current challenges and concerns related to consumption of meat products may be divided into those associated with microbial pathogens and into other meat safety issues. Major challenges related to microbial pathogens include foodborne illness outbreaks, associated product recalls, regulatory compliance, and issues related to microbiological control **(Kunová et al., 2014)**. The complex concept of fish quality consists of safety, nutritional value, availability, integrity, freshness, eating quality, product size and type **(Abbas et al., 2008)**. Cooling and freezing are the usual methods for conservation of fish. Freezing of fish has the advantage of providing the consumer with unprocessed fish that retain to a much greater extent the flavour, odour, appearance and texture of the freshly caught fish **(Jeon et al., 2002)**. The most widely accepted microbiological criteria for chilled and frozen raw fish are those set for aerobic plate counts (APC) at 25 °C proposed by the International Commission on Microbiological Specifications for Foods **(ICMSF, 1986)**. An increase of APC to levels in excess of 10^6 cfu/g is usually indicative of inadequate refrigeration, long storage under refrigeration or one of the former prior to freezing. According to **Broekaert et al., (2011)** loads of 10^7 – 10^8 CFU.g^{-1} make spoilage organoleptically detectable.

The effect of chilling (0 – 2 ºC) on the quality deterioration of whole ungutted aquacultured rainbow trout was studied by integrated evaluations of microbiological, biochemical, and sensory attributes. The counts of aerobic mesophilic, psychrotrophic bacteria and *Pseudomonas* increased exponentially. Aerobic mesophilic and psychrotrophic bacteria grew exponentially from an initial load of 3 – 5 $\log_{10}$ CFU.g^{-1} reaching 7.6 $\log_{10}$ CFU.g^{-1} on day 15 **(Ninan et al., 2011)**. The initial values are comparable with our results in samples taken from skin and fillets. However on day 7 increase of bacteria count has not been significant and fish were evaluated as a fit for human consumption. It is widely accepted that the initial microbial load of fresh water varies depending on water conditions and temperature. In our study, mesophilic and psychrotrophic counts in fresh fish tissues were close to or lower than 5.7 log CFU.g^{-1} recommended by **ICMSF (1986)** for whole fresh water fish. In their experiments **Diler et al. (2000)** demonstrated that mesophilic bacterial counts on trout skin varied between 2 and 7 log CFU.g^{-1}. Our studies showed average total viable microbial counts was 4.75 – 5.4 log CFU.g^{-1} on the skin and in the fillet and only in frozen trout after six months of storage was higher (7.05 log cfu/g). Lower results have been achieved by **Gonzales et al. (1999)** established TVCs of 2.90 log CFU.g^{-1} in rainbow trout. However most of the authors reported the similar TVCs in rainbow trout to be increased from a baseline of 4.0 log CFU.g^{-1} up to 7.04 log CFU.g^{-1} during storage **(Rezaei and Hosseini, 2008)**. According to **Özogul et al. (2013)** a TVC load of 3.59 log CFU.g^{-1} is a parameter for high quality of trouts.

The high psychrotrophic count of frozen fish may be attributed to the contamination of raw materials which come in contact with fish unsatisfactory sanitation during handling, processing and distribution as well as inadequate

chilling and/or freezing which increase the existing microorganisms. *Pseudomonas* species are widely distributed in nature, unsanitized equipment's, pouted water and fishermen hands especially during harvesting, transportation and storage are considered as the source of fish contamination. The contamination of *Pseudomonas* organisms may be attributed to the heavily contamination boats and boxes which transfer the organisms to fish during cleaning. Accordingly, the consumption of such frozen fish contaminated with different members of psychrotrophic bacteria particularly and *Pseudomonas* species may constitute, at times public health hazards **(Hassan et al., 2014)**. *Pseudomonas* spp. may only represent the minority of the total microflora at the beginning, but become the dominant at the end of the shelf life. The quantity of present SSOs can be used to predict the residual shelf life of products causing identification of this organism as a main concern. The shelf life of any food product can be detected from beginning of storage period and ending when the SSOs reach the least spoilage level. *Pseudomonas* spp. decrease the shelf life of food products and so their quality by producing lipolytic and proteolytic enzymes which is the main cause of food spoilage during the storage **(Franzetti and Scarpellini, 2007)**. **Khidhir et al. (2014)** found that *Pseudomonas* spp. was 3.77 log CFU.g^{-1} for silver carp sold in Sulaimania markets, Iraq. Higher count was recorded by **Begum et al. (2010)** who collected fish samples from the super shop in Bangladesh, and reported that the highest count of *Pseudomonas* spp. was found in Tilapia (5.94 log CFU.g^{-1}). The counts of psychrotrophic bacteria and *Pseudomonas* were even lower in frozen samples compared to fresh samples. The similar results were found in our previous study, when thawing and repeated freezing in double frozen fish samples led to an increase in TVCs, but the counts of psychrotrophic bacteria in frozen was lower over the period of storage compared to fresh fish **(Popelka et al., 2014)**.

The amount of TVCs, psychrotrophic bacteria and *Pseudomonas* in samples collected sterile from muscles have been significantly lower (p <0.05) compared to bacteria number on the skin and in the muscle samples collected after filleting. Fish product safety is influenced by many factors as origin of the fish, product characteristics, processing mode and cooking. During cold storage of fish in a chill room or in melting ice these bacteria will grow, and together with those acquired by contact with contaminated surfaces on board ships or ashore they form the natural spoilage flora in fish. During filleting more spoilage bacteria will contaminate the fish flesh from debris and slime on filleting boards and knives and from the filleters' hands **(van den Broek et al., 1984)**. To attain the best possible bacteriological condition of fish fillets it is essential to start with freshly caught fish, consistently cooled in ice of good bacteriological quality **(Shewan, 1961)**. Before filleting, the fish must be washed thoroughly in clean drinking water to keep contamination of the fish flesh during filleting as low as possible. Filleting itself must be done on cutting boards made of synthetic material under running water. The cutting boards and other utensils must be cleaned and disinfected daily and the fillets must be cooled immediately after cutting. These measures in combination represent good manufacturing practice (GMP). The numbers of TVCs did not exceed 10^6 CFU.g^{-1} **(van den Broek et al., 1984)**.

There was no significant difference in the pH values, however slightly higher pH values were found in frozen samples (6.75 – 6.79) compared to fresh fish on day 7 (6.64). Significantly higher TVB-N concentrations were recorded in frozen fish compared to fresh ones, but all the values were below the 30 mg.100g^{-1}. In the study performed by **Ninan et al. (2011)** the pH values increased from an initial value of 6.74 to 7.13 on day 15 of chilled storage. The pH values indicating bacterial growth and production of volatile basic compounds such as ammonia by fish spoilage bacteria. Increase in pH due to accumulation of alkaline compounds through autolytic activities and microbial metabolism has been reported. Many microbes including *Pseudomonas* produce ammonia during amino acid metabolism. TVB-N values exceeded 27.87 mg.100g^{-1} on day 14 when the psychrotrophic counts exceeded 10^7 CFU.g^{-1}. *Pseudomonas* also displayed the typical growth pattern of psychrotrophic bacteria without a lag phase increasing from initial counts of 3.0 to 5.02 log CFU.g^{-1} on day 15. Based on the TVB-N and microbiological limits, the shelf life of trout at 0 – 2 ˚C was 9 – 12 days. In this study, the values exceeded the limit of acceptability of 25 mg.100g^{-1} proposed by **Stansby and Olcott (1963)**. However, higher concentration of 30–35 mg TVB-N.100g^{-1} flesh is considered the limit of acceptability for ice-stored cold water fish by **Connell (1995)**. Critical limits of 25, 30 and 35 mg.100g^{-1} of TVB-N were established for different groups of fishes **(Commission Regulation 2074/2005)**, but no limit for acceptability has been established for rainbow trout. However, comparing results for different fish species, does not show correlation between muscle pH and the amount of volatile bases contained within the fish at rejection. Neither does the change in pH during storage correlate well with the production of TVB-N **(Fernandes, 2009)**. During spoilage, the majority of volatile bases are produced from the soluble non-protein nitrogen of the fish (free amino acids and other low-molecular-weight nitrogenous compounds), as significant proteolysis is observed only during the latest stages of spoilage and after rejection. For some fish species, a correlation can be made between the spoilage of the fresh fish and the production of TVB-N.

Post mortem spoilage of food products can be caused by chemical, enzymatic or microbial activities and is accompanied by the formation of compounds responsible for changes in odour, flavour and texture of fish meat. One of the chemical markers of spoilage in fish is the total volatile basic nitrogen, including ammonia, trimethylamine (TMA) and dimethylamine (DMA), the concentrations of which increase with spoilage by either bacterial or enzymatic degradation. The total volatile basic nitrogen is produced during degradation of proteins and non-protein nitrogenous compounds, mainly as a result of microbial activity **(Özogul and Özogul 2000)**. **Commission Regulation (EC) 2074/2005** set the limits for TVB-N in sea fish, however, no limits are available for fresh-water fish. Since TVB-N is produced mainly during bacterial decomposition of fish meat, the higher content of TVC of samples throughout the period of frozen storage could account for higher TVB-N values of rainbow trout.

CONCLUSION

Taking into consideration the obtained results and the recommendations of the International Commission on Microbiological Specifications for Foods, it could be concluded that all the samples were fit for human consumption in respect to method and time of storage and processing. The highest microbial contamination was observed in samples taken from the skin of cooled and frozen trout, followed by fillets, and lowest microbial load was in samples of muscles collected sterile. The higher number of contaminated processed fish products, both cooled and frozen, and the *Pseudomas* spp. counts could pose a risk for human health after consumption of undercooked fish.

Contaminated fish could be dangerous and the efficient bacteriological control of hygiene is important to ensure acceptable levels of contamination and prevention of food intoxications. In the fish processing chain managing risks should be based on scientific knowledge of the microbiological hazards and the understanding of the primary production, processing and manufacturing technologies and handling during fish storage and transport, retail and catering.

REFERENCES

Abbas, K. A., Mohamed, A., Jamilah, B. Ebrahimian, M. 2008. A review on correlations between fish freshness and pH during cold storage. *American Journal of Biochemistry and Biotechnology*, vol. 4, no. 4, p. 416-421. http://dx.doi.org/10.3844/ajbbsp.2008.416.421

Al-Jasser, M. S., Al-Jasass, F. M. 2014. Study the Chemical, Physical Changes and Microbial Growth as Quality Measurement of Fish. *Annual Research and Review in Biology*, vol. 4 no. 9, p. 1406-1420. http://dx.doi.org/10.9734/ARRB/2014/7131

Alparslan, Y., Hasanhocaoglu, H., Metin, C., Baygar, T. 2014. Determination of meat quality of sea bass (Dicentrarchus labrax) sold at different selling areas. *Emirates Journal of Food and Agriculture*, vol. 26, no. 3, p. 293-301.

Begum, M., Abu Ahmed, A., Das, M., Parveen, S. 2010. A comparative microbiological assessment of five types of selected fishes collected from two different markets. *Advances in Biological Research*, vol. 4, no. 5, p. 259-265.

Broekaert, K., Heyndrickx, M., Herman, L., Devlieghere, F., Vlaemynck, G. 2011. Seafood quality analysis: Molecular identification of dominant microbiota after ice storage on several general growth media. *Food Microbiology*, vol. 28, no. 6, p. 1162-1169. http://dx.doi.org/10.1016/j.fm.2011.03.009

Commission Regulation (EC) No 2074/2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 and for the organisation of official controls under Regulation (EC) No 854/2004 and Regulation (EC) No 882/2004. OJ L 338, 22.12.2005, 15-18.

Connell, J. J. 1995. *Control of fish quality*. 4th ed. LONDON, UK : Wiley-Blackwell, Fishing News Books Limited. 256 p. ISBN-13: 978-0-85238-226-4.

Conway, E. J., Byrne, A. 1933. An absorption apparatus for the micro-determination of certain volatile substances. 1. The micro-determination of ammonia. *Biochemical Journal*, vol. 27, 419-29. PMid:16745115

Diler, O., Altun, S., Calikusu, F., Diler, A. 2000. A study on qualitative and quantitative bacterial flora of the rainbow trout (*Oncorhynchus mykiss*) living in different fish farms. *Turkish Journal of Veterinary and Animal Sciences*, vol. 24, p. 251-259.

Fernandes, R. 2009. *Microbiology handbook fish and seafood*. 1st ed. Cambridge, UK: Rsc Publishing. ISBN: 978-1-905224-76-0. http://dx.doi.org/10.1039/9781847559432

Franzetti, L., Scarpellini, M. 2007. Characterization of *Pseudomonas* spp. isolated from foods. *Annals of Microbiology*, vol. 57, no. 1, p. 39-47. http://dx.doi.org/10.1007/BF03175048

Gonzalez, C. J., Lopez-Diaz, T. M., Garciia-Lopez, M. L., Prieto, M., Otero, A. 1999. Bacterial microflora of wild brown trout (*Salmo trutta*), wild pike (*Esox lucius*), and aquacultured rainbow trout (*Oncorhynchus mykiss*). Journal of Food Protection, vol. 62, no. 11, p. 1270-1277. PMid:10571316

Gram, L. 1993. Inhibitory effect against pathogenic and spoilage bacteria of *Pseudomonas* strains isolated from spoiled and fresh fish. *Journal of Applied Environmental Microbiology*, vol. 59, p. 2197-2203. PMid:8357253

Hassan, M. A., Shaltout, F. A., Maarouf, A. A., El-Shafey, W. S. 2014. Psychrotrophic bacteria in frozen fish with special reference to pseudomonas species. *Benha Veterinary Medical Journal*, vol. 27, no. 1, p. 78-83.

Huss, H. H. 1995. *Quality and quality changes in fresh fish*. FAO Fisheries Technical Paper No. 348. Food and Agriculture Organisation of the United Nations, Rome. ISBN 92-5-103507-5.

ICMSF (International Commission on Microbiological Specifications for Foods). 1986. *Sampling plans for fish and shellfish*. In ICMSF (Eds.). Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Scientific Applications. 2nd edition, TORONTO, CANADA : University of Toronto Press. p. 181-196.

ISO 4833: 2003. *Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony-count technique at 30 °C*.

ISO 6730: 2005. *Milk - Enumeration of colony-forming units of psychrotrophic microorganisms - Colony-count technique at 6.5 °C*.

ISO 13720: 2010. *Meat and meat products - Enumeration of Pseudomonas spp*.

Jay, J. M. 2000. Food preservation with modified atmospheres. In: Jay, J. M. *Modern Food Microbiology*. GAITHERSBURG, MARYLAND : Aspen publishers, Inc., p. 283-300. ISBN 978-0-8342-1671-6,

Jeon, Y. J., Kamil, J. Y., Shahidi, F. 2002. Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 18, p. 5167-5178. http://dx.doi.org/10.1021/jf011693l

Khidhir, Z.K., Jaff, B. M. A., Saleh, H. H. 2014. Assessment of the microbial quality of five types of Iraqi fresh fish in Sulaimania markets. *Journal of Zankoy Sulaimani- Part A*, vol. 16, p. 251- 260.

Kunová, S., Bobková, A., Lopašovský, L., Kačániová, M. 2014. Microbiological evaluation of poultry sausages stored at different temperatures. *Potravinarstvo*, vol. 8, no. 1, p. 141-145. http://dx.doi.org/10.5219/338

Ninan, G., Zynudheen, A.A, Joseph, J. 2011. Effect of Chilling on Microbiological, Biochemical and Sensory Attributes of Whole Aquacultured Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792). *Journal of Aquaculture Research and Development*, S5 http://dx.doi.org/10.4172/2155-9546.S5-001

Özogul, F., Özogul, Y. 2000. Comparison of methods used for determination of total volatile basic nitrogen (TVB-N) in rainbow trout (*Oncorhynchus mykiss*). *Turkish Journal of Zoology*, vol. 24, p. 113-120.

Özogul, F., Yavuzer, E., Özogul, Y., Kuley, E. 2013. Comparative quality loss in wild and cultured rainbow trout (*Oncorhynchus mykiss*) during chilling storage. *Food Sciences and Technology Research*, vol. 19, no. 3, p. 450-454. http://dx.doi.org/10.3136/fstr.19.445

Popelka, P., Nagy, J., Pipová, M., Marcinčák, S., Lenhardt, L. 2014. Comparison of chemical, microbiological and histological changes in fresh, frozen and double frozen rainbow trout (*Oncorhynchus mykiss*). *Acta Veterinaria Brno*, vol. 83, p. 157-161. http://dx.doi.org/10.2754/avb201483020157

Rezaei, M., Hosseini, S. F. 2008. Quality assessment of farmed rainbow trout (*Oncorhynchus mykiss*) during chilled storage. *Journal of Food Sciences*, vol. 73, no. 6, p. 93-96. http://dx.doi.org/10.1111/j.1750-3841.2008.00792.x

Shamsuzzaman, M. M., Mazumder, S. K., Siddique, M. A., Miah, M. N. U. 2011. Microbial quality of hilsa shad (*Tenualosa ilisha*) at different stages of Processing. *Journal of the Bangladesh Agricultural University*, vol. 9, no. 2, p. 339-344.

Shewan, J. M. 1961. The microbiology of seawater fish. In: Borgstrom, G. *Fish as Food*. LONDON, UK : Academic Press, p. 487-560. http://dx.doi.org/10.1016/B978-0-12-395569-2.50020-0

Sivertsvik, M., Jeksrud, W. K., Rosnes, J. T. 2002. A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. *International Journal of Food Sciences and Techonology*, vol. 37, p. 107-27. http://dx.doi.org/10.1046/j.1365-2621.2002.00548.x

Stansby, M. E., Olcott, H. S. 1963. Industrial Fishery Technology Composition of Fish. Reinhold Publishing Corporation. p. 339-349.

van den Broek, M. J. M., Mossel D. A. A., Mol, H. 1984. Microbiological quality of retail fresh fish fillets in The Netherlands. *International Journal of Food Microbiology*, vol. 1, p. 53-61. http://dx.doi.org/10.1016/0168-1605(84)90008-4

Velu, S., Bakar, A. F., Mahyudin, N. A., Saari, N., Zaman, M. Z. 2013. Effect of modified atmosphere packaging on microbial flora changes in fishery products. *International Food Research Journal*, vol. 20, no. 1, p. 17-26.

Vogel, B. F., Venkateswaran, K., Satomi, M., Gram, L. 2005. Identification of *Shewanella baltica* as the most important H2S-producing species during iced storage of Danish marine fish. *Applied and Environmental Microbiology*, vol. 71, p. 6689-97. http://dx.doi.org/10.1128/AEM.71.11.6689-6697.2005

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THE CHANGES IN BIOCHEMICAL PARAMETERS DUE TO WINE CONSUMPTION DEPENDING ON GENDER

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ABSTRACT

The aim of this study was to investigate the effect of red wine consumption on the lipid profile and glucose in the group of male (13 men aged 34 – 64 years) and the group of female (11 women aged 28 – 57 years). Research consisted of moderate red wine consumption for 6 weeks. The dose of alcohol ranged from 200 to 300 mL per day of red wine Lemberger (Winery Masaryk, Slovakia). The blood samples were obtained after overnight fasting and were collected at baseline and after three days, three weeks and six weeks of wine consumption. Differences between male and female subjects were reflected in the results of different biochemical parameters in the dynamics of wine consumption. We found out that while in females the total cholesterol level did not change significantly and had a predominantly downward trend, for male subjects we observed at the beginning the slight increase of the levels, which, however, after 6 weeks of consumption significantly decreased from an initial value of 5.75 ±1.32 mmol.L^{-1} to 5.35 ±1.25 mmol.L^{-1} (p <0.05). The blood concentration of triglycerides in the dynamics of the experiment did not change significantly in either one gender, although small differences were observed, because while the female subjects had triglyceride development over consumption upward trend in male subjects it was vice versa. LDL-cholesterol changed significantly only in the group of female. Level of this lipid parameter decreased significantly during the six weeks of consumption of Lemberger from an initial value 3.37 ±0.68 mmol.L^{-1} to the lowest 2.99 ±0.61 mmol.L^{-1}, which was recorded in the third week of consumption (p <0.0001), but statistically significant differences versus baseline we monitored after three days and six weeks of consumption (p <0.01). In the group of male, we did not observe such significant changes, but it should be noted, that the men had changes in LDL-cholesterol downward direction and all the values were in the range of benchmarks. In the group of female, HDL-cholesterol increased to 2.05 ±0.6 mmol.L^{-1} after six weeks of consumption from baseline of 1.7 ±0.69 mmol.L^{-1}, and the difference was statistically significant (p <0.05), in the group of male, its level changed first significantly after three days of consumption of steep increase (1.46 ±0.61 mmol.L^{-1}, p <0.05), and for the next six weeks, it was the significantly increase (1.59 ±0.5 mmol.L^{-1}, p <0.01). The glucose concentration did not change significantly in the dynamics of wine consumption among men and women, and all the values were between the limits of the standard.

Keywords: red wine; alcohol; health; gender

INTRODUCTION

Among the wine experts as the best wines in the world they consider French, Italian and German white wines. Slovak vineyards also offer a wide range of wine grape varieties from which it is possible to produce high-quality and fine wines. Even the results of our measurements confirm **(Gažarová et al., 2008)**, the Slovak dry red and white wines contain health-promoting substances which are not only comparable with quality foreign wines, but in some cases, greater than. It is proved by the content of the total polyphenols, especially specific substances such as trans-resveratrol, quercetin and anthocyanins in red wines. In addition, Slovak wines have significant anti-radical capabilities, which allow them to compete with high-quality foreign wines.

Various mechanisms are involved in the development of fatal and non-fatal coronary heart diseases and other vascular diseases, including inflammatory and oxidative stress, that leading to hypertension, diabetes and other risk factors that accelerate atherosclerosis. The creation of conditions preventing the overproduction of a toxic accumulation of free radicals becomes the foundation for both the prophylaxis and also in enhancing the effectiveness of treatment of lifestyle and civilization diseases including cardiovascular diseases. Red wine provides alcohol and polyphenolic compounds. The beneficial effects of moderate red wine consumption may be attributed to the overall mix of its components and not to one in particular – the alcoholic fraction increases HDL-cholesterol, non-alcoholic fraction (polyphenolic compounds) inhibits the oxidation of LDL particles, improvements of endothelial function, blood lipid profile and decreases blood pressure **(Tsang et al., 2005; Karatzi et al., 2009; Xin et al., 2010; Costanzo et al., 2011)**.

In the last decades, wine is the subject of various studies, while it is monitored not only the short-term but also the long-term effects of wine consumption in the relation to the various diseases. Many of these studies not only

suggest but even confirm the beneficial effects of wine on human health compared to other alcoholic beverages. Also other authors **(Renaud and de Lorgeril, 1992; Mosinger, 1994; Marques-Vidal et al., 1995; Carbonneau et al., 1998; Serafini et al., 1998; Paganga et al., 1999; Gorinstein et al., 2000; Gronbaek et al., 2000; Klatsky et al., 2003; Renaud et al., 2004)** share the same opinion based on their research, that among the alcoholic beverages the red wine is the most effective against atherosclerosis.

Numerous epidemiological studies observed a J-shaped relationship between increasing wine consumption and cardiovascular risk **(Fuller, 2011; Rostron, 2012)**, low HDL-cholesterol, high triglycerides **(Park and Kim, 2012)**. Meta-analyses of cohort studies confirmed a U-shaped relationship between alcohol intake and type-2 diabetes **(Koppes et al., 2005; Baliunas et al., 2009)**. Moderate alcohol consumption is up to 30 g alcohol.day^{-1} for men and 10 – 15 g alcohol.day^{-1} for women. Different amounts of alcohol have different health benefits according to gender. Some studies suggest that the beneficial effects of alcohol consumption are higher in men than in women **(Xin et al., 2001)**, but many studies show similar effects in both genders **(Mukamal et al., 2005)**. **Lorková et al., (2015)** found that the most preferred alcoholic beverages among men were beer and wine and among women wine and liqueurs. The regular use of alcohol was reported by 55.35% of men and 33.4% of women consume 0.2 – 0.5 liters of alcoholic beverage per week. **Bellavia et al., (2014)** found that women with alcohol intake of 5 – 10 g.day^{-1} had lower risk of cardiovascular diseases and cancer mortality and men with alcohol consumption of 15 – 20 g.day^{-1} had lower only risk of cardiovascular diseases mortality.

At some stage of life, male´s and female´s organism works under the influence of several different factors. This can be mostly reflected the different health based on gender. In the case of civilization diseases, one gender is more prone to one type of disease, while the opposite gender turns into a different type of diseases. Morphologically and physiologically there are some differences between the genders that can play an important role in the development and predisposition to certain diseases. Framingham study data showed a lower incidence of cardiovascular disease events among premenopausal women compared with postmenopausal women **(Kannel, Hjortland and McNamara 1976)**. Premenopausal women represent a specific part of the population due to the effect of female sex hormone estrogen, which protect them from the cardiovascular disease by reducing this risk, while in the case of male sex hormones androgens opposite is true. It means that premenopausal women are on average at substantially lower risk of premature cardiovascular disease than men of the same age. An adverse effect of menopause on cardiovascular diseases risk is explained by the deterioration of the lipid profile towards postmenopause **(Matthews et al., 2009)**. This is reflected in increasing levels of total cholesterol, LDL-cholesterol and triglycerides and the reduction of HDL-cholesterol **(Anagnostis et al., 2015)**.

Since the consumption of alcoholic beverages is associated with the risk of both cardiovascular and other civilization diseases, it seems interesting to observe the effect of the consumption of the Slovak dry red wine Lemberger to the individual biochemical, anthropometric and clinical indicators depending on gender. Based on this fact, we investigated the effect on female subjects, and especially in male subjects.

MATERIAL AND METHODOLOGY

The aim of this study was to investigate the effect of red wine consumption on the lipid profile and glucose in the group of male and the group of female. Research consisted of moderate red wine consumption. 1 – 3 glasses of red wine at dinner after day of abstinence were administered to 24 healthy volunteers (13 men aged 34 – 64 years - mean age 51 years and 11 women aged 28 – 57 years - mean age 45 years) for 6 weeks. The dose of alcohol ranged from 200 to 300 mL per day of red wine Lemberger (Winery Masaryk, Slovakia). Most of the subjects were categorized in the group at high risk for cardiovascular complications. The participants were carefully instructed to abstain from any pharmacological treatment and all of the volunteers were asked to maintain their habitual diets and lifestyles during the 6 weeks of the study. The blood samples were obtained after overnight fasting and were collected at baseline (1st blood sampling) and after three days (2nd blood sampling), three weeks (3rd blood sampling) and six weeks (4th blood sampling) of wine consumption. Serum and EDTA-plasma were collected and stored at the temperature -80 °C. A total fasting blood sample was collected to assess a total cholesterol, low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), triglyceride and glucose. A written informed consent to participate in the study was provided to all subjects involved in the study after they were informed of all risks, discomforts and benefits. The Ethic Committee at the Specialized Hospital St. Zoerardus Zobor, n. o. approved the study protocol. All the biochemical parameters were analyzed at the Department of Human Nutrition at Slovak University of Agricultural using the selective multiparameter analyzer LISA 200. The results were evaluated with standard mathematical-statistical methods (STATISTICA Cz, MS Excel 2007). Differences were tested with the *t*-test, Tukey and Fisher test. The results were listed in the Table 1 and Table 2.

RESULTS AND DISCUSSION

Differences between male and female subjects were also reflected in the results of different biochemical parameters in the dynamics of wine consumption. Cholesterol is one of the basic compounds essential for cells life. Its blood concentration is relatively stable through dietary intake restrictions, a change in its concentration evident only after a long time. The reference values for the optimum cholesterol levels range from 3 to 5.2 mmol.L^{-1}. In the lipid profile, we found out that while in females the total cholesterol level did not change significantly and had a predominantly downward trend, for male subjects we observed at the beginning the slight increase of the levels, which, however, after 6 weeks of consumption statistically significant decreased from an initial value of 5.75 ±1.32 mmol.L^{-1} to 5.35 ±1.25 mmol.L^{-1} (p <0.05). For both, male and female group, the total cholesterol levels remained

above the upper limit of normal reference values. Similar results reached the authors **Basu et al., (2006).**

Triglycerides do not interfere in the process of atherogenesis, but the elevated levels point at the increased levels of fatty substances and certain lipoproteins in the blood. The optimum range of blood triglyceride values is from 0.2 to1.92 mmol.L^{-1}. The blood concentration of triglycerides in the dynamics of the experiment did not change significantly in either one gender, although small differences were observed, because while the female subjects had triglyceride development over consumption upward trend in male subjects it was vice versa. It is also important to stress that while triglyceride levels in women ranged within the normal range, men had all values above the upper critical threshold. The negative effect of alcohol consumption is the increase in triglycerides. **Cesena et al., (2011)** reported that red wine increased plasma levels of triglycerides and the triglycerides/HDL-C ratio and those individuals with higher BMI were at higher risk for elevation in plasma triglycerides. Although fasting levels of triglyceride have been shown to increase in some experimental observations, the change is minor compared with increases in HDL **(Leighton and Urquiaga, 2007)**.

To determine the risk of atherosclerosis it is not as important total cholesterol, but in particular the binding to the various types of lipoproteins and their concentrations in blood. It is proved the direct dependence between serum levels of LDL-cholesterol and mortality from cardiovascular diseases. The optimal level of LDL-cholesterol is in the range from 0 to 3.9 mmol.L^{-1}. In the cardiovascular prevention the reduction of LDL-C by 1% results in a reduction in the risk of coronary heart disease by 1%. Every reduction in LDL-C by 1 mmol.L^{-1} (~40 mg.dL^{-1}) leads to the corresponding 22% reduction in cardiovascular mortality and morbidity in patients with risk. In general, the US national recommendations advise the following reference values for serum lipids: (total cholesterol <200 mg.dL^{-1}, triglycerides <150 mg.dL^{-1}, LDL-C <130 mg.dL^{-1} and HDL-C >40 mg.dL^{-1}). Extrapolated from the clinical studies overall reduction in LDL-cholesterol below <1.8 mmol.L^{-1} (less than ~70 mg.dL^{-1}), or at least 50% has the largest benefit in terms of the decrease of cardiovascular diseases. In patients with a very high cardiovascular risk is the therapeutic target of LDL-C value <1.8 mmol.L^{-1} or more than 50% decrease from baseline. In patients with high cardiovascular risk a target LDL blood value is <2.5 mmol.L^{-1} and in patients with moderate risk <3 mmol.L^{-1} **(ESC Pocket Guidelines)**. In our study, LDL-cholesterol changed significantly only in the group of female. Level of this lipid parameter significantly decreased during the six weeks of consumption of Lemberger from an initial value 3.37 ±0.68 mmol.L^{-1} to the lowest 2.99 ±0.61 mmol.L^{-1}, which was recorded in the third week of consumption (p <0.0001), but statistically significant differences versus baseline we monitored after three days and six weeks of consumption (p <0.01). In the group of male, we did not observe such significant changes, but it should be noted, that the men had changes in LDL-cholesterol downward direction and all the values were in the range of benchmarks. **Kanner et al., (2012)** found that LDL-C was slightly reduced after red wine consumption. The same decline also observed **Shai et al., (2004)**; **Micallef, Lexis and Lewandowski (2007)** and **Joosten et al., (2008)**.

HDL-cholesterol is considered to be a long-term protective factor for the development of cardiovascular diseases. The total level of HDL-C is inversely associated with the risk of the developing coronary heart disease and an increase in HDL-C by 0.39 mmol.L^{-1} results in a risk reduction by 22%, and every increase in HDL-C by 1% results in a reduction in the risk of coronary heart disease by 2 – 3% **(Sabaka et al., 2012)**. When we assessed changes in levels of "good" HDL-C in the dynamics of consumption of Lemberger we found out that values were changed very beneficially in both genders in the direction of a positive increase, but in group of male reflected these changes significantly. In the group of female, HDL-C increased to 2.05 ±0.6 mmol.L^{-1} after six weeks of consumption from baseline of 1.7 ±0.69 mmol.L^{-1}, and the difference was statistically significant (p <0.05), in the group of male, its level changed first significantly after three days of consumption of steep increase (1.46 ±0.61 mmol.L^{-1}, p <0.05), and for the next six weeks, it was the significantly increase (1.59 ±0.5 mmol.L^{-1}, p <0.01). This positive upturn described most authors dealing with this topic **(Gaag et al., 1999; Estruch, 2000; Devaraj et al., 2002; Shai et al., 2004; Sierksma et al., 2004; Joosten et al., 2008)**. **Brien et al., (2011)** reported that moderate red wine consumption increased HDL-C. **Leighton et al., (2000)** showed that 30 g of alcohol a day would cause an average increase of 8.3% in HDL levels. **Leighton and Urquiaga (2007)** observed increases in HDL-C ranging between 4% and 18% among wine consumers and red wine appeared to be slightly better than white wine.

Although women should be more protected against cardiovascular risk, but there are also other factors to eliminate this benefit of women, for example diabetes mellitus. In this regard, monitoring of the impact of dry red wine intake on blood glucose changes has been very interesting. After evaluating the results we observed that the level of glucose in the dynamics of consumption did not change significantly in either men or women and that all the values were between the limits of the standards. After 3 days of wine consumption we observed a slight increase in its levels in both genders, which in the next weeks in women returned back to the level of initial value (4.93 ±0.45 mmol.L^{-1} vs baseline 4.91 ±0.47 mmol.L^{-1}) and in men even lower (5.54 ±0.91 mmol.L^{-1} of 5.72 ±0.87 mmol.L^{-1}). A U-shaped relationship between alcohol consumption and risk of type-2 diabetes has been confirmed **(Koppes et al., 2005; Baliunas et al., 2009)**. Several studies have suggested that moderate alcohol intake reduces the risk of type-2 diabetes by 20 – 40% **(Joosten et al., 2010; Sato et al., 2012; Shi et al., 2013)**. **Rasouli et al., (2013)** reported that high consumers had similar risk to abstainers. **Heianza et al., (2013)** reported a higher risk of type-2 diabetes among binge drinkers and **Cullmann, Hilding and Ostenson (2012)** found a higher type-2 diabetes risk among heavy consumers. **Chiva-Blanch et al., (2013)** found that red wine with or without alcohol improved metabolism of glucose. **Rasouli et al., (2013)** found that consumption of wine has been linked to reduced diabetes risk.

Table 1 Changes of biochemical parameters in group of male.

Parameters	Reference standard	Baseline	3 day-consumption	p-value	Significance	3 week-consumption	p-value	Significance	6 week-consumption	p-value	Significance
Total cholesterol (mmol.L^{-1})	**3 – 5.2**	**5.75 ±1.32**	5.98 ±1.34	0.247	-	5.94 ±1.29	0.320	-	5.35 ±1.25	0.046	+
Triglycerides (mmol.L^{-1})	**0.2 – 1.92**	**2.31 ±1.36**	2.55 ±1.73	0.502	-	2.17 ±1.24	0.699	-	1.78 ±0.66	0.141	-
HDL-C (mmol.L^{-1})	**1.16 – 1.6**	**1.19 ±0.49**	1.46 ±0.61	0.014	+	1.58 ±0.71	0.001	++	1.59 ±0.50	0.001	++
LDL-C (mmol.L^{-1})	**0 – 3.9**	**3.53 ±0.92**	3.34 ±0.91	0.128	-	3.36 ±0.81	0.168	-	3.37 ±0.89	0.183	-
Glucose (mmol.L^{-1})	**3.9 – 6.1**	**5.72 ±0.87**	5.99 ±0.70	0.079	-	5.76 ±0.71	0.787	-	5.54 ±0.91	0.235	-

Table 2 Changes of the biochemical parameters in a group of female.

Parameters	Reference standard	Baseline	3 day-consumption	p-value	Significance	3 week-consumption	p-value	Significance	6 week-consumption	p-value	Significance
Total cholesterol (mmol.L^{-1})	**3 – 5.2**	**5.55 ±0.88**	5.5 ±1.09	0.797	-	5.3 ±0.83	0.232	-	5.37 ±0.79	0.377	-
Triglycerides (mmol.L^{-1})	**0.2 – 1.92**	**0.94 ±0.34**	1.08 ±0.48	0.380	-	1.2 ±0.55	0.102	-	1.11 ±0.44	0.263	-
HDL-C (mmol.L^{-1})	**1.16 – 1.6**	**1.7 ±0.69**	1.9 ±0.58	0.197	-	2.01 ±0.81	0.047	+	2.05 ±0.6	0.027	+
LDL-C (mmol.L^{-1})	**0 – 3.9**	**3.37 ±0.68**	3.08 ±0.76	0.001	++	2.99 ±0.61	<0.0001	+++	3.14 ±0.58	0.009	++
Glucose (mmol.L^{-1})	**3.9 – 6.1**	**4.91 ±0.47**	5.08 ±0.45	0.296	-	4.93 ±0.52	0.916	-	4.93 ±0.45	0.880	-

CONCLUSION

As mentioned above and in many other researches it is therefore clear that wine is much richer in content of nutrients and natural products beneficial for the human body than expected. Its consumption, if it is drunk sensibly and in moderation, is not harmful to the human body and forms an appropriate part of the diet and is beneficial for health. It should be taken into consideration that the moderate consumption of alcohol is recommended to the healthy people. It is necessary to fight against excessive and inappropriate consumption of alcoholic beverages and therefore the wine and it is needed to drink it in moderation. Although everyone has different opinions on what is moderate drinking, the fact is that wine is fine, and therefore deserves the noble usage and use only to the extent that it has a pleasant effect and benefit to our body and strengthened the health because drinking too much wine is harmful to the human body!

We found out that during consumption of red wine the serum of total cholesterol did not change significantly and had a predominantly downward trend in the group of female. In the group of male, we initially observed a slight increase in the levels, which after 6 weeks of consumption statistically significantly decreased. Total cholesterol levels in both genders, we found above the upper range of reference values. The level of triglycerides in the dynamics of the experiment did not change significantly in either one gender, although there were observed small differences. In female subjects had changed in triglycerides upward trend, in male subjects it was just the opposite. In contrast to women, in men all values of triglycerides were above the upper limit of normal. LDL-cholesterol was significantly changed only in women whose levels decreased significantly. Changes in LDL-cholesterol in group of male had downward direction, although statistically not significant, and all the values were in the range of benchmarks. HDL-cholesterol in both genders in the dynamic of consumption of Lemberger was changed very favorably in the direction of a positive increase, but in male subjects reflected to these changes significantly. Women should be more protected against cardiovascular disease, but there are impacts that eliminate this benefit of women, such as diabetes mellitus. Diabetes increases the risks of heart attack, stroke and other complications of the atherosclerosis in women much more than in men. It is therefore favorable fact that the glucose concentration did not change significantly in the dynamics of wine consumption among men and women, and all the values were between the limits of the standard.

REFERENCES

Anagnostis, P., Stevenson, J. C., Crook, D., Johnston, D. G., Godsland, I. F. 2015. Effects of menopause, gender and age on lipids and high-density lipoprotein cholesterol subfractions. *Maturitas*, 2015, vol. 81, no. 1, p. 62-68. http://dx.doi.org/10.1016/j.maturitas.2015.02.262 PMid:25804951

Baliunas, D. O., Taylor, B. J., Irving, H., Roerecke, M., Patra, J., Mohapatra, S., Rehm, J. 2009. Alcohol as a risk factor for type 2 diabetes: a systematic review andmeta-

analysis. *Diabetes Care*, vol. 32, no. 11, p. 2123-2132. http://dx.doi.org/10.2337/dc09-0227
PMid:19875607

Basu, H., Pernecky, S., Sengupta, A., Liepa, G. U. 2006. Coronary Heart Disease: How Do the Benefits of -3 Fatty Acids Compare with Those of Aspirin, Alcohol/Red Wine, and Statin Drugs? *Journal of American Oil Chemists´ Society*, vol. 83, no. 12, p. 985-997. http://dx.doi.org/10.1007/s11746-006-5153-4

Bellavia, A., Bottai, M., Wolk, A., Orsini, N. 2014. Alcohol consumption and mortality: a dose-response analysis in terms of time. *Annals of Epidemiology*, vol. 24, no. 4, p. 291-296. http://dx.doi.org/10.1016/j.annepidem.2013.12.012
PMid:24486142

Brien, S. E., Ronksley, P. E., Turner, B. J., Mukamal, K. J., Ghali, W. A. 2011. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. *BMJ*, vol. 342, p. 1-15. http://dx.doi.org/10.1136/bmj.d636
PMid:21343206

Carbonneau, M. A., Leger, C. L., Descomps, B., Michel, F., Monnier, L. 1998. Improvement in the antioxidant status of plasma and low-density lipoprotein in subjects receiving a red wine phenolics mixture. *Journal of the American Oil Chemists´ Society*, vol. 75, no. 2, p. 235-240.

Cesena, F. H. Y., Coimbra, S. R., Andrade, A. C. M., da Luz, P. L. 2011. The relationship between body mass index and the variation in plasma levels of triglycerides after short-term red wine consumption. *Journal of Clinical Lipidology*, vol. 5, no. 4, 4-8. http://dx.doi.org/10.1016/j.jacl.2011.04.007
PMid:21784375

Chiva-Blanch, G., Urpi-Sarda, M., Ros, E., Valderas-Martinez, P., Casas, R., Arranz, S., Guillén, M., Lamuela-Raventós, R. M., Llorach, R., Andres-Lacueva, C., Estruch, R. 2013. Effects of red wine polyphenols and alcohol on glucose metabolism and the lipid profile: A randomized clinical trial. *Clinical Nutrition*, vol. 32, no. 2, p. 200-206. http://dx.doi.org/10.1016/j.clnu.2012.08.022
PMid:22999066

Costanzo, S., Di Castelnuovo, A., Donati, M. B., Iacoviello, L., de Gaetano, G. 2011. Wine, beeror spirit drinking in relation to fatal and non-fatal cardiovascular events: a meta-analysis. *European Journal of Epidemiology*, vol. 26, no. 11, p. 833-850. http://dx.doi.org/10.1007/s10654-011-9631-0
PMid:22076059

Cullmann, M., Hilding, A., Ostenson, C. G. 2012. Alcohol consumption andrisk of pre-diabetes and type 2 diabetes development in a Swedish population. *Diabetic Medicine*, vol. 29, no. 4, p. 441-452. http://dx.doi.org/10.1111/j.1464-5491.2011.03450.x
PMid:21916972

Devaraj, S., Vega-Lopéz, S., Kaul, N., Schönlau, F., Rohdewald, P., Jialal, I. 2002. Supplementation with a pine bark extract rich in polyphenols increases plasma antioxidant capacity and alters the plasma lipoprotein profile. Lipids, vol. 37, no. 10, p. 931-934. http://dx.doi.org/10.1007/s11745-006-0982-3
PMid:12530550

ESC Pocket Guidelines. 2016. Committee for Practice Guidelines to improve the quality of clinical practice and patient care in Europe [online] s.a. [cit.2016-01-09-8] Available at: www.escardio.org/guidelines.

Estruch, R. 2000. Wine and cardiovascular diseases. *Food Research International*, vol. 33, no. 3, p. 219-226. http://dx.doi.org/10.1016/S0963-9969(00)00037-5

Fuller, T. D. 2011. Moderate alcohol consumption and the risk of mortality. *Demography*, vol. 48, no. 3, p. 1105-1125. http://dx.doi.org/10.1007/s13524-011-0035-2
PMid:21594734

Gaag, M. S., Tol, A., Scheek, L. M., James, R. W., Urgert, R., Schaafsma, G., Hendriks, H. F. J. 1999. Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomised intervention study in middle-aged men. *Atherosclerosis*, vol. 147, no. 2, p. 405-410. http://dx.doi.org/10.1016/S0021-9150(99)00243-9

Gažarová, M., Chlebo, P., Turianica, I., Slezák, F., Rostoka, L. 2008. Analysis of selected wine types focused on their antioxidant capacity and activity. *Visnik Stomatologii*, vol. 64, no. 4, p. 45-46.

Gorinstein, S., Caspi, A., Zemser, M., Trakhtenberg, S. 2000. Comparative contents of some phenolics in beer, red and white wines. *Nutrition Research*, vol. 20, no. 1, p. 131-139. http://dx.doi.org/10.1016/S0271-5317(99)00145-1

Grønbaek, M., Becker, U., Johansen, D., Gottschau, A., Schnohr, P., Hein, H. O., Jensen, G., Sørensen, T. I. 2000. Type of alcohol consumed and mortality from all causes, coronary heart disease, and cancer. *Annals of Internal Medicine*, vol. 133, no. 6, p. 411-419. http://dx.doi.org/10.7326/0003-4819-133-6-200009190-00008
PMid:10975958

Heianza, Y., Arase, Y., Saito, K., Tsuji, H., Fujihara, K., Hsieh, S. D., Kodama, S., Shimano, H., Yamada, N., Hara, S., Sone, H. 2013. Role of alcohol drinking pattern in type 2 diabetes in Japanese men: the Toranomon Hospital Health Management Center study11 (TOPICS 11). *American Journal of Clinical Nutrition*, vol. 97, no. 3, p. 561-568. http://dx.doi.org/10.3945/ajcn.112.043364
PMid:23343972

Joosten, M. M., Beulens, J. W. J., Kersten, S., Hendriks, H. F. J. 2008. Moderate alcohol consumption increases insulin sensitivity and ADIPOQ expression in postmenopausal women: a randomised, crossover trial. *Diabetologia*, vol. 51, no. 8, p. 1375-1381. http://dx.doi.org/10.1007/s00125-008-1031-y
PMid:18504547

Joosten, M. M., Grobbee, D. E., van der A, D. L., Verschuren, W. M., Hendriks, H. F., Beulens, J. W. 2010. Combined effect of alcohol consumption and lifestyle behaviors on risk of type 2 diabetes. *American Journal of Clinical Nutrition*, vol. 91, p. 1777-1783. http://dx.doi.org/10.3945/ajcn.2010.29170
PMid:20410096

Kannel, W., Hjortland, M., McNamara, P. 1976. Menopause and risk of cardiovascular disease: the Framingham study. *Annals of Internal Medicine*, vol. 85, no. 4, p. 447-452. http://dx.doi.org/10.7326/0003-4819-85-4-447
PMid:970770

Kanner, J., Gorelik, S., Roman, S., Kohen, R. 2012. Protection by polyphenols of post-prandial human plasma and low-density lipoprotein modification: the stomachas a bioreactor. *Journal of Agricultural and Food Chemistry*, vol. 60, no. 63, p. 8790-8796. http://dx.doi.org/10.1021/jf300193g
PMid:22530973

Karatzi, K., Karatzis, E., Papamichael, C., Lekakis, J., Zampelas, A. 2009. Effects of red wine onendothelial function: postprandial studies vs clinical trials. *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 19, no. 10, p. 744-750. http://dx.doi.org/10.1016/j.numecd.2009.04.006
PMid:19570663

Klatsky, A.L., Friedman, G. D., Armstrong, M. A., Kipp, H. 2003. Wine, liquor, beer, and mortality. *American Journal of*

Epidemiology, vol. 158, no. 6, p. 585-595. http://dx.doi.org/10.1093/aje/kwg184 PMid:12965884

Koloverou, E., Panagiotakos, D. B., Pitsavos, C., Chrysohoou, C., Georgousopoulou, E. N., Metaxa, V., Stefanadis, C., ATTICA Study group. 2014. Effects of alcohol consumption and the metabolic syndrome on 10-year incidence of diabetes: The ATTICA study. *Diabetes and Metabolism*, vol. 41, no. 2, p. 152-159. http://dx.doi.org/10.1016/j.diabet.2014.06.003 PMid:25190450

Koppes, L. L., Dekker, J. M., Hendriks, H. F., Bouter, L. M., Heine, R. J. 2005. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. *Diabetes Care*, vol. 28, no. 3, p. 719-725. http://dx.doi.org/10.2337/diacare.28.3.719 PMid:15735217

Leighton, F., San Martin, A., Castillo, O., Pollak, F., Perez, D. D., Strobel, P., et al. 2000. Red wine, white wine and diet, intervention study. Effect on cardiovascular risk factors. Proc XXV Congrès Mondial de la Vigne et du Vin, Parìs, pp. 63-70.

Leighton, F., Urquiaga, I. 2007. Changes in Cardiovascular Risk Factors Associated with WineConsumption in Intervention Studies in Humans. *Annals of Epidemiology*, vol. 17, p. 32-36. http://dx.doi.org/10.1016/j.annepidem.2007.01.007

Lorková, M., Kopčeková, J., Gažarová, M., Habánová, M., Chlebo, P., Magula, D., Mrázová, J. 2015. Dietary Patterns And Lifestyle Of PatientsAfter Myocardial Infarction. *Potravinarstvo*, vol. 9, no. 1, p. 315-323. http://dx.doi.org/10.5219/490

Marques-Vidal, P., Cambou, J. P., Nicaud, V., Luc, G., Evans, A., Arveiler, D., Bingham, A., Cambien, F. 1995. Cardiovascular risk factors and alcohol consumption in France and northern Ireland. *Atherosclerosis*, vol. 115, no. 5, p. 225-232. http://dx.doi.org/10.1016/0021-9150(94)05517-M

Matthews, K. A., Crawford, S. L., Chae, C. U., Everson-Rose, S. A., Sowers, M. F., Sternfeld, B., Sutton-Tyrrell, K. 2009. Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition? *Journal of the American College of Cardiology*, vol. 5, no. 25, p. 2366-2373. http://dx.doi.org/10.1016/j.jacc.2009.10.009 PMid:20082925

Micallef, M., Lexis, L., Lewandowski, P. 2007. Red wine consumption increases antioxidant status and decreases oxidative stress in the circulation of both young and old humans. *Nutrition Journal*, vol. 27, no. 6, p. 113-119. http://dx.doi.org/10.1186/1475-2891-6-27

Mosinger, B. 1994. Polyphenolics but not alcohol in beer and wine protect serum low-density lipoprotein against atherogenic modification. *Cor Vasa*, vol. 4, p. 171-174.

Mukamal, K. J., Jensen, M. K., Gronbaek, M., Stampfer M. J., Manson, J. E., Pischon, T., Rimm, E. B.. 2005. Drinking frequency, mediating biomarkers,and risk of myocardial infarction in women and men. *Circulation*, vol. 112, no. 10, p. 1406-1413. http://dx.doi.org/10.1161/CIRCULATIONAHA.105.537704 PMid:16129796

Paganga, G., Miller, N., Rice-Evans, C. A. 1999. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? *Free Radical Research*, vol. 30, no. 2, p. 153-162. http://dx.doi.org/10.1080/10715769900300161 PMid:10193583

Park, H., Kim, K. 2012. Association of alcohol consumption with lipidprofile in hypertensive men. *Alcohol and Alcoholism*, vol. 47, no. 3, p. 282-287. http://dx.doi.org/10.1093/alcalc/ags019 PMid:22371847

Rasouli, B., Ahlbom, A., Andersson, T., Grill, V., Midthjell, K., Olsson, L., Carlsson, S. 2013. Alcohol consumption is associated with reduced risk of type 2 diabetesand autoimmune diabetes in adults: results from the Nord-Trondelag healthstudy. *Diabetic Medicine*, vol. 30, no. 1, p. 56-64. http://dx.doi.org/10.1111/j.1464-5491.2012.03713.x PMid:22612671

Renaud, S. C., Guéguen, R., Conard, P., Lanzmann-Petithory, D., Orgogozo, J. M., Henry, O. 2004. Moderate wine drinkers have lower hypertension-related mortality: a prospective cohort study in French men. *American Journal of Clinical Nutrition*, vol. 80, no. 3, p. 621-625. PMid:15321801

Renaud, S., de Lorgeril, M. 1992. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*, vol. 339, no. 8808, p. 1523-1526. http://dx.doi.org/10.1016/0140-6736(92)91277-F

Rostron, B. 2012. Alcohol consumption and mortality risks in the USA. *Alcohol and Alcoholism*, vol. 4, no. 3, p. 334-339. http://dx.doi.org/10.1093/alcalc/agr171 PMid:22278318

Sabaka, P., Mistríková, L., Wawruch, M., Baláž, D., Oravec, S., Dukát, A. 2012. HDL-cholesterol – relevance in clinical practice. Súčasná Klinická Prax, vol. 1, p. 31-35.

Sato, K. K., Hayashi, T., Harita, N., Koh, H., Maeda, I., Endo. G., Nakamura, Y., Kambe, H., Kiyotaki, C. 2012. Relationship between drinking patterns and the risk of type 2diabetes: the Kansai Healthcare Study. *Journal of Epidemiology and Community Health*, vol. 66, p. 507-511. http://dx.doi.org/10.1136/jech.2010.109777 PMid:21131305

Serafini, M., Maiani, G., Ferro-Luzzi, A. F. 1998. Alcohol free red wine enhances plasma antioxidant capacity in humans. *Journal of Nutrition*, 1998, vol. 128, no. 6, p. 1003-1007. PMid:9614160

Shai, I., Rimm, E. B., Schulze, M. B., Rifai, N., Stampfer, M. J., Hu, F. B. 2004. Moderate alcohol intake and markers of inflammation and endothelial dysfunction among diabetic men. *Diabetologia*, vol. 47, no. 10, p. 1760-1767. http://dx.doi.org/10.1007/s00125-004-1526-0 PMid:15502925

Shi, L., Shu, X. O., Li, H., Cai, H., Liu Q., Zheng W. Xiang, Y. B., Villegas, R. 2013. Physical activity,smoking, and alcohol consumption in association with incidenceof type 2 diabetes among middle-aged and elderly Chinese men. *PLoS One*, vol. 8, no. 11, p. e77919. http://dx.doi.org/10.1371/journal.pone.0077919 PMid:24223743

Sierksma, A., Vermunt, S. H. F., Lankhuizen, I. M., van der Gaag, M. S. Scheek, L. M., Grobbee, D. E., van Tol, A., Hendriks, H. F. J. 2004. Effect of Moderate Alcohol Consumption on Parameters of Reverse Cholesterol Transport in Postmenopausal Women. Alcoholism: Clinical and Experimental Research, vol. 28, no. 4, p. http://dx.doi.org/10.1097/01.ALC.0000122763.30770.F5

Tsang, C., Higgins, S., Duthie, G. G., Duthie, S. J., Howie, M., Mullen, W., Lean, E. J., Crozier, A. 2005. The influence of moderate red wine consumption on antioxidant status and indices of oxidative stress associated with CHD in healthy volunteers. *British Journal of Nutrition*, vol. 93, p. 233-240. http://dx.doi.org/10.1079/BJN20041311 PMid:15788107

Xin, P., Pan, Y., Zhu, W., Huang, S., Wel, M., Chen, C. 2010. Favorable effects of resferatrol on sympathetic neural remodeling in rats following myocardial infarction. *European Journal of Pharmacology*, vol.649, no. 1, p. 293-300. http://dx.doi.org/10.1016/j.ejphar.2010.09.036 PMid:20869962

Xin, X., He, J., Frontini, M. G., Ogden, L. G., Motsamai, O. I., Whelton, P. K. 2001. Effects of alcohol reduction on blood pressure: a meta-analysisof randomized controlled trials. *Hypertension*, vol. 38, no. 5, p. 1112-1117. http://dx.doi.org/10.1161/hy1101.093424 PMid:11711507

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CONTENT OF TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN VARIETIES OF ONION AND GARLIC

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ABSTRACT

Garlic as onion family and the most important Allium species consumed all over the world. Garlic possesses potential health promoting effects due to its high phenolic content and a good source of vitamins, minerals and major component like polyphenols, flavonoids, thiosulfinates and other sulfur compounds. Red onion and garlic are among the important parts of diet in many world populations, and there is also a long-held belief in their health enhancing properties. In this work evaluated content of total polyphenols and antioxidant activity in onion and garlic. Samples of plant material (onion, garlic) were collected at the stage of full maturity in the village (Harmota) in Koysinjac town in Erbil city. The content of the total polyphenols was determined by using the Folin-Ciocalteu reagent (FCR). Antioxidant activity was measured by using a compound DPPH (2.2-diphenyl-1-picrylhydrazyl). Phenolic content and antioxidant activities of one variety of garlic and four varieties of onions have been studied. In the present experiment it was detected, that total polyphenols content in samples onion ranges from 626.61 – 322.83 mg.kg^{-1} (onion) and 506.7 mg.kg^{-1} for (garlic). Statistically significant highest value of total polyphenols was recorded in red onion the lowest content of total polyphenols was recorded in white onion 322.83.mg.kg^{-1}, and statistically significant highest value of antioxidant activity was recorded in red onion (33.42%) the lowest value was in white onion (22.68%). The value of antioxidant activity of garlic was (28.47%). Data analysis showed that the red variety onion presents highest value of total polyphenolic compounds. Consequently, antioxidant capacity was highest for red variety compared to other variety of onion and garlic. The order value of TPC was follow: Red Onion (626.61) >Garlic (506.70) >yellow onion (423.94) >pink onion (345.36) >white onion (322.83). Based on the measured values of AOA in onion and garlic samples can be classified as follows: Red Onion (33.42) >Garlic (28.47 >yellow onion (25.95) >pink onion (23.74) >white onion (22.68).

Keywords: onion; garlic; total polyphenols; antioxidant activity; ethanolic extracts

INTRODUCTION

Plants and plant-derived produce are among the prime utilities of mankind for food, shelter and cure since the dawn of civilization and it wouldn't be inappropriate to state that the use of medicinal plants predates written human history **(Harrison et al., 2015)**. Among these medicinal plants, garlic (*Allium sativum* L.) has secured its repute of a therapeutic panacea. Onion and garlic are the most important Alliums species consumed all over the world. Onion (*Allium cepa* L.) is one of the most consumed vegetable planted widely across the world. Huge quantities of onions are consumed all over the world, as it is very popular flavoring agent. However, available information on their free radical scavenging activities is scanty. In Europe Onion is one of the main vegetables consumed either raw or processed in different ways **Benitez et al. (2011a)**. Medicinal plants are important in pharmacological research and drug de- elopement **(Li and Vederas, 2009)** Polyphenols are natural substances in plants that are antioxidants with the potential to protect the body from some diseases. Interest in natural antioxidants and particularly in dietary antioxidants which, are present in vegetables and contribute to protection against oxidative stress in humans, is increasing. Antioxidant is defined as any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate **Li et al. (2007)**. The essential oil reveals interesting properties, such as antimicrobial agent and moderate reducing power feasible to implement in food **Ye et al. (2013)**. Also, phenolic extracts obtained from wastes of onion were used to evaluate the capacity inhibiting of processes inflammatory and oxidation of low-density lipoprotein (LDL) **Albishi et al. (2013)**. Onions (*Allium cepa* var. cepa) are an important source of bioactive compounds including phenolic compounds, flavonoids, fructooligosaccharides (FOS), thiosulfinates and other sulfur compounds, and many of these compounds have potential beneficial properties for human health **Soinen et al. (2012)**. Onion is one of the highly rich sources of main flavonols, quercetin in human diet **(Sellappan, Akoh, 2002)**. Onion is a source of minerals such as iron, selenium, iodine, potassium, calcium, sulfur, and many others. Onion is characterized by not only rich in vitamins and minerals, but is characterized by a strong content of biologically active substances, especially polyphenolic compounds. Many groups of polyphenolic compounds contained in onion e.g. phenolcarboxylic acids, such as

protocatechuic, caffeic, ferulic, p-coumaric, p-hydroxybenzoic and vanillic acid. Many reports have indicated that onions have a wide range of beneficial properties for human health, such as anti-mutagenic **Singh et al. (2009)**, and antioxidant capacity **(Lu et al., 2011; Pérez-Gregorio et al., 2011)**. Epidemiological studies suggest that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases **(Pandey and Rizvi, 2009)**.

Garlic (*Allium sativum* L.) is one of the most commonly produced vegetables worldwide. Fruits and vegetables are the major functional foods because they are the main sources of nutraceuticals such as vitamins, minerals and phenolic compounds **(Tomás-Barberán and Espin, 2001; Rupasinghe and Clegg, 2007)**. Garlic possesses potential health-promoting effects due to its high phenolic phytochemical content and is a source of natural antioxidants **Nuutila et al. (2003)**. The health properties of garlic depend on its bioactive compounds and especially on phenolic compounds **(Lanzotti, 2006; Corzo, et al., 2007)**, which have interesting pharmacological properties, are present in relatively high amounts **Beato, et al. (2011)**. Although researchers have paid considerable attention to isolate and identify bioactive compounds of garlic which account for its marvelous therapeutic repute, less reports are available regarding the diversity for abundance of the active allelochemicals within different garlic ecotypes and very few literature documents the considerable differences accounted for total phenolic compounds **Chen et al. (2013)**. The wealth of scientific literature supports the proposal that garlic consumption have significant effects on lowering blood pressure, prevention of atherosclerosis, reduction of serum cholesterol and triglyceride, inhibition of platelet aggregation, and increasing fibrinolytic activity **(Chan et al., 2013)**. Antioxidants are absorbed and metabolized in the body in a variety of ways and some antioxidants are more bioavailability than others **(Kalt, 2005)**.The natural antioxidants in foods, fruits, vegetables, beverages, spices and supplements have received much attention for their nutritive value in recent years and various synthetic antioxidants have also been in commercial use. There is an increasing demand for natural antioxidants to replace synthetic additives in the food industry. Natural antioxidant substances are presumed to be safe since they occur in plant foods. Antioxidants can scavenge radicals by three major mechanisms: hydrogen atom transfer, electron transfer, and combination of both these transfers **Prior et al. (2005)**. Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical induced oxidative stress. A variety of free radical scavenging antioxidants is found in a number of dietary sources **Qusti et al. (2010)**. The main characteristic of antioxidant is its ability to trap free radicals. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidants scavenge free radicals that reduce risk of cancer and cardiovascular diseases. According to **Benkeblia (2005)**, Allium species are revered to possess anti-bacterial and anti-fungal activities, and they contain the powerful antioxidants, sulphur and other numerous phenolic compounds which have aroused great interests for food industries. A mong the species of onion, the red onion is abundant in polyphenols, flavonol and tannin **(Gorinstein et al., 2010)**. The essential oil from Allium cepa may be anew agents applied in food system **(Ye et al., 2013)**. The aim of this study was to determine levels of total polyphenolic and antioxidant activities among local commonly consumed vegetables (Onion and garlic) in Kurdistan region Iraq.

MATERIAL AND METHODOLOGY

Chemicals and reagents

Folin–Ciocalteu reagent, 2, 2- diphenyl-1- picrylhydrazyl (DPPH), Sodium carbonate (Na_2CO_3), Methanol (CH_3OH).

Sample preparation

Four onion varieties, namely red onion, white onion, pink onion, yellow onion and garlic. Samples of plant material for this experiment were harvested at full maturity stage from area in the village (Harmota) in Koysinjac town in Erbil city in Iraq Republic.

Extraction of polyphenols

Samples of fresh onion and garlic were homogenized and were prepared an extract: 25 g cut onion, garlic extracted by 50 mL 80% ethanol according 24 hours. These extracts were use for analyze. Extractions were performed in light exposure was avoided during the extraction process.

Determination of TPC

Total phenolic content of each extract was determined by the Folin-Ciocalteu procedures according compounds in to the method of **Lachmann et al. (2003)** and expressed as mg of gallic acid equivalent per kg fresh mater(GAE) which corresponds to the mean response of all the major phenolic fruits and vegetables **(Georgé et al., 2005)**. And Gallic acid is usually used as a standard unit for phenolics content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteau assay. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 μL of extract. The content was mixed and 5 mL of a sodium carbonate solution (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min. and the absorbance was measured at 765 nm of wavelength against blank (Shimadzu UV/VIS-1240, Japan). The concentration of polyphenols was calculated from a standard curve plotted with known concentration of garlic acid (Sigma Aldrich). The average content of polyphenol compounds in the samples was obtained from six replicates.

Determination of antioxidant activity (TAC)

The antioxidant capacity (TAC) of the onion and garlic extracts was measured using a DPPH method described by **Brand and Williams et al. (1995)** method-using a compound $DPPH^{\cdot}$ (2.2-diphenyl-1-pikrylhydrazyl)).

2.2-diphenyl-1-pikrylhydrazyl (DPPH) was pipetted to cuvette (3.9 cm^3) then the value of absorbance, which corresponded to the initial concentration of $DPPH^{\cdot}$

solution in time Ao, was written. Then 0.1 cm³ of the followed solution was added and then the dependence A = f (t) was immediately started to measure. The absorbance of 0 and 10 minutes at 515.6 nm in the Spectropho- tometer Shimadzu UV/VIS-1240 was mixed and measured. The percentage of inhibition reflects how antioxidant compound are able to remove DPPH˙ radical at the given time.

Inhibition (%) = (A0 - At / A0) x 100

Statistical analysis

Results were statistically evaluated by the Analysis of Variance. All the assays were carried out in triplicates and results are expressed as mean ±SD. The data were subjected to the F-test in the one-way analysis of variance (ANOVA) If the *p*-value of the F-test is less than 0.05, there is a statistically significant difference between the means at the 95% confidence level; the Multiple Range Tests will tell which means are significantly different from which others. The method currently being used to discriminate among the means of Fisher's least significant difference (LSD) procedure. Analysis was conducted using SAS software 9.4.

RESULTS AND DISCUSSION

On the base of reached results there were estimated changes in the total polyphenols content and also changes in total antioxidant capacity values in dependence on garlic and onion.

Evaluation of total polyphenol content and values of antioxidant capacity in onion and garlic

The distribution of polyphenolic content and antioxidant properties among different varieties onion and one variety garlic. The frequency distributions of TPC and TAC are shown in Figure 1, 2 and Table 1, 2. In the present experiment it was detected, that total polyphenols content in samples ranged from 322.83 mg.kg^{-1} GAE (in white variety of onion) to 626.61mg.kg^{-1} GAE (in red variety of onion) (Table. 1). According to the obtained results, the polyphenols content (TPC) in the tested was significantly different and was influenced by variety and color. Statistically the highest value of total polyphenols (626.61 mg.kg^{-1} GAE) was recorded in variety Red onion. The lowest content of total polyphenols was recorded in white variety (322.83 mg.kg^{-1} GAE). According to the average contents of total polyphenols in fresh matter of onion and garlic there is the following line in present work: red onion (626.61 mg.kg^{-1} GAE) >garlic (506.70 mg.kg^{-1} GAE) >yellow onion (423.94 mg.kg^{-1} GAE) >pink onion (345.36 mg.kg^{-1} GAE) >white onion (322.83 mg.kg^{-1} GAE). **Andrejiová et al. (2011)** found that the content of total polyphenols in onion was in the interval from 105 to 134 mg.kg^{-1}. **Amin et al. (2013)** reffered that the content of polyphenols was 132.2 mg.kg^{-1}. **Brat et al. (2006)** published that the content of total polyphenols in onion was761 mg.kg^{-1}. comparision to our determined values of polyphenols in onion with their results our results are higher than the values of **(Andrejiová et al., 2011: Amin et al., 2013)** but lower than 761 mg.kg^{-1} for **Brat et al. (2006)**. **Bystrická et al. (2014)** found the values of total polyphenols in onion were in the range from 389.64 ±20.31 mg.kg^{-1} to 429.58 ±17.44 mg.kg^{-1}. **Karadeniz et al. (2005)** reported that the polyphenols in onion was in the amount 536 mg.kg^{-1}. **Benkeblia et al. (2005)** reported that the highest content of polyphenols was in red onion (473 mg.kg^{-1} GAE), followed by yellow variety (347 mg.kg^{-1} GAE). In comparision to our determined values of red and yellow onion, our result was higher content polyphenols in red onion (626.61 mg.kg^{-1} GAE) followed by yellow variety (423.94 mg.kg^{-1} GAE). **Kavalcová et al. (2015)** published that the content of polyphenols in the case of yellow varieties of onion Sherpa (455.22 mg.kg^{-1} GAE), Bingo (451.71 mg.kg^{-1} GAE) and Boston (441.32 mg.kg^{-1} GAE). In comparision to our determined values of polyphenols in yellow onion, their results were in similar interval. **Armand et al. (2012)** reported that the content of polyphenols in onion was 620 mg.kg^{-1}. This report is in agreement with the values of polyphenols content in red variety of our result. **Kavalcová et al. (2015)** published that the content of polyphenols in the case of white dry variety of onion (142.01 mg.kg^{-1} GAE). In comparison to our determined values (322.83 mg.kg^{-1} GAE) of polyphenols content in white variety, their results were lower. Our results correspond to the results of **Armand et al. (2012)**, which reported the highest values of total polyphenols in variety of red onion followed by yellow variety and white variety. **Andrejiová et al. (2011)** also reported the content of total polyphenols in red onion 1088.51 mg.kg^{-1}, followed by yellow onion 652.15 mg.kg^{-1} and in white onion 105.19 mg.kg^{-1}.

In the present work antioxidant activity in samples ranges from 22.68% to 33.42%. The DPPH method is frequently used to determine the antioxidant activity. DPPH assay is a primary antioxidant activity test that determines the free radical scavenging activity of the respective samples. When comparing onions and garlic from the antioxidant capacity (TAC%) point of view (Figure 1 and 3; Table1, 2). *p*-value <0.05 there is a statistically significant difference between the means of the 4 varieties onion and one variety garlic at the 95.0% confidence level. The highest value was reached in case of red onion variety with the value 33.42%, the lowest value was found in case of white variety with the value 22.68%, in the case of garlic(28.47%) was recorded. Based on the measured values of AOA in garlic and onion varieties samples can be classified as follows: red onion (33.4242%) garlic (28.47%) >yellow onion (25.95%) >pink onion (23.74%) >white onion (22.68%). **Prakash et al. (2007)** published that the value of antioxidant activity in red onion was 50.6% and in white onion 13.6%. In comparison to our measured values of antioxidant activity in red onion was higher but in white onion was lower than our result. **Kavalcová et al. (2014)** published that the value of antioxidant activity in yellow onion was (25.7%). In comparison to our measured values (25.95) of antioxidant activity in yellow onion their results were in similar interval. **Škerget et al. (2009)** published that the value of antioxidant activity in yellow onion was (35%) there result were higher than our value (25.95) measured in yellow variety of onion. **Cheng et al. (2013)** determined that red onion extracts showed good antioxidant activity varying from 53.36% to 85.53% and better than in the yellow

Table 1. Total phenolic content and antioxidant capacity (mean and standard deviation values). In chosen variety Onion and Garlic (mg.kg^{-1}).

Variety	TAC%	TPC
Garlic	28.47 ±1.59 b	506.70 ±17.67 b
Pink onion	23.74 ±0.86 d	345.36 ±38.79 d
Red onion	33.42 ±0.87 a	626.61 ±11.22 a
White onion	22.68 ±1.14 d	322.83 ±11.37d
Yelow onion	25.95 ±0.51 c	423.94 ±9.21c

Note: Data expressed as means of six replications ± standard deviation. Values in the same column with the different letters present significant differences p <0.05 using F-test for independent samples.

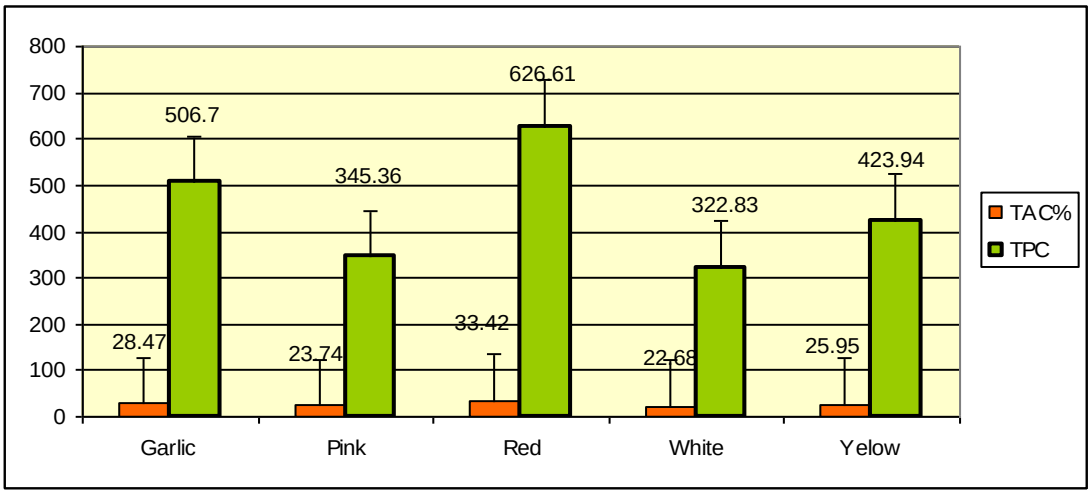

Figure 1 Average content of total polyphenols TPC (mg.kg^{-1}) and Average content of total antioxidant capacity TAC (%) in chosen varieties of onion and garlic.

Table 2 Total phenolic content and antioxidant capacity (mean and standard deviation values with the group). In chosen variety Onion and Garlic (mg.kg^{-1}).

	TAC (%)			TPC		
Variety	Mean	Std Dev	Group	Mean	Std Dev	Group
Garlic	28.47	1.59	b	506.70	17.67	b
Pink onion	23.74	0.86	d	345.36	38.79	d
Red onion	33.42	0.87	a	626.61	11.22	a
White onion	22.68	1.14	d	322.83	11.37	d
Yellow onion	25.95	0.51	c	423.94	9.21	c

Note: All the assays were carried out in triplicates and results are expressed as mean ±SD. The data were subjected to one-way analysis of variance (ANOVA) and the differences between various concentrations were determined Fisher LSD test using SAS software.

variety ranging from 52.32% to 72.25%. In comparison to our measured values their results were higher.

In case of garlic, **Priecina et al. (2013)** reported that the polyphenols in garlic was in the amounts from (272.28 to 1818.81 mg.kg^{-1}). In comparison to our determined values (506.70 mg.kg^{-1}), their results were higher. **Bystrická et al. (2014)** published that the content of polyphenols in the case of garlic (260.62 to 279.74 mg.kg^{-1}). In comparison to our determined values, their results were lower. **Manach et al. (2004)** noted that environmental and genetic factors have a major effect on polyphenols content. The content of polyphenolic compounds in onion also can be affected by the type of variety and color of bulb of onion. Several factors may affect the polyphenol content of plants, such as ripeness at the time of harvest, genotype, environmental factors, processing and storage **Picchi et al. (2012)**. Various factors, such as genotype of cultivar, growing season, post harvest treatment, and cultivation place are responsible for the variation in contents of such photochemical in garlic **Beato et al. (2011)**.

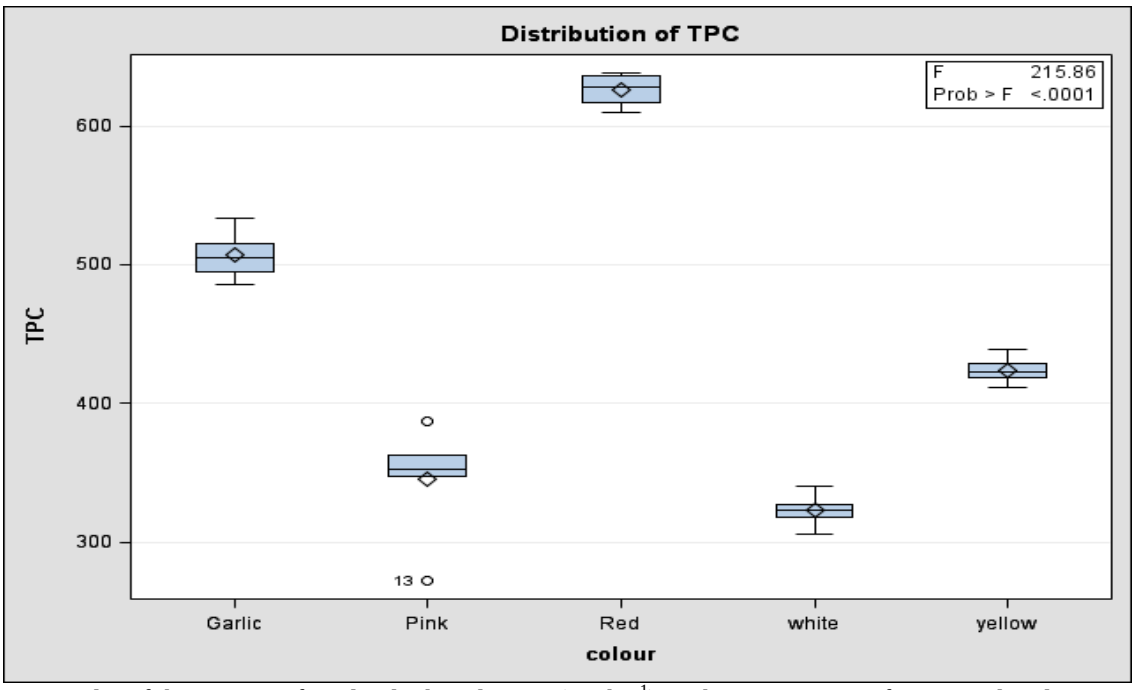

Figure 2 Box-plot of the content of total polyphenols TPC (mg.kg^{-1}) in chosen varieties of onion and garlic.

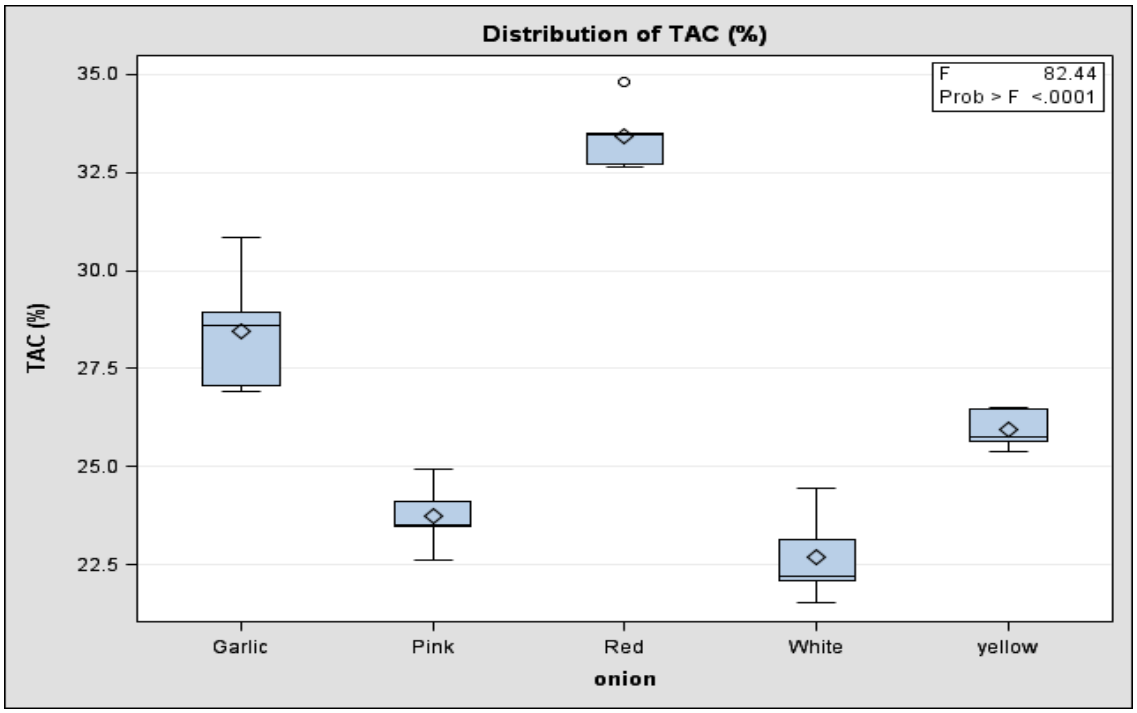

Figure 3 Box-plot of the content of total antioxidant capacity TAC (%) in chosen varieties of onion and garlic.

Statistical evaluation of total polyphenol (TPC) content differences significance within of chosen varieties

When comparing average content of total polyphenols TPC (mg.kg^{-1}) in four onion varieties and garlic in Table 2 and Figure 2 there were significant differences according to used statistical methods on the all types. Four types onion (red, yellow, pink, white) and garlic, observed confidence levels between almost the all observed varieties. Effect of varieties analysis (Table 2, Figure 2) for the phenolic compounds contents (TPC) of varieties onion and garlic of tested showed the presence of significant variety differences (p <0.05).

As shown in Figure 2. Garlic has higher content of total polyphenols TPC than yellow, pink and white but lower content of TPC than red variety onion.

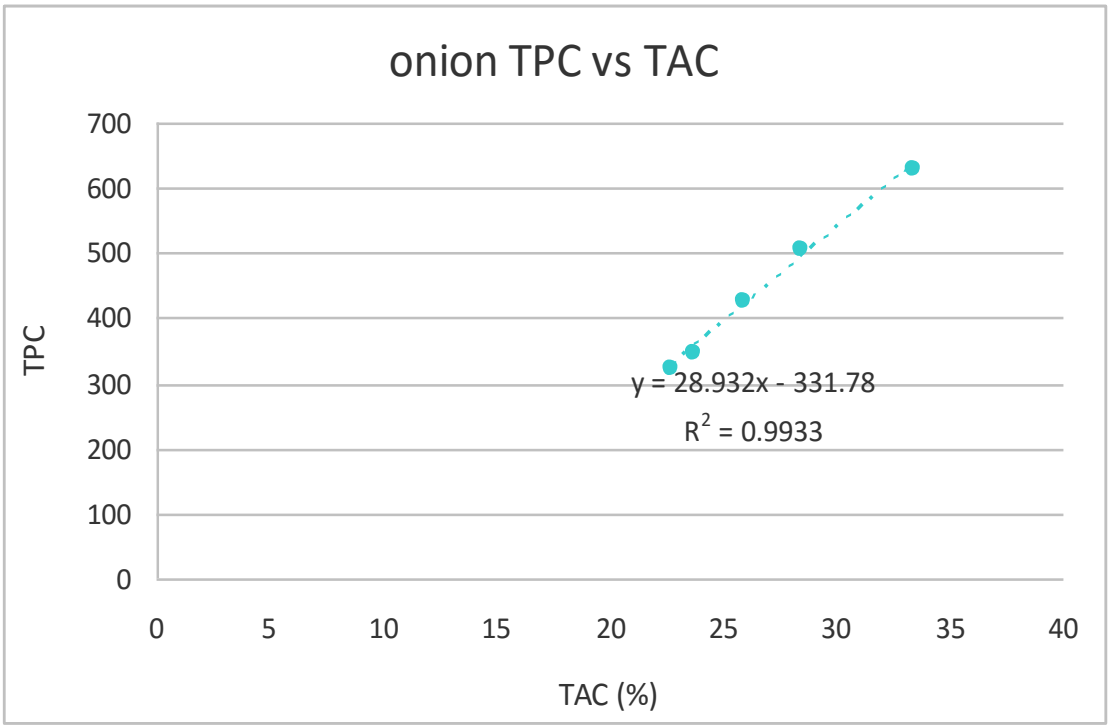

Figure 4 Correlation between TP and TAC of varieties of onion and garlic.

In the present work it was detected, that antioxidant activity in samples ranges from (22.68% to 33.42%). Statistically significant highest value of antioxidant activity was recorded in red onion the lowest value in white onion. **Kavalcova et al. (2014)**, published the interval of statistically significant highest value of antioxidant activity was recorded in onion from 20.22 ±0.53% to 25.76 ±0.53% and statistically significant the lowest value of antioxidant activity was recorded in garlic from 4.05 ±0.20% to 5.07 ±0.47%, our measured values of AOA in onion and garlic samples can be classified as follows: Red Onion (33.42%) >Garlic (28.47% >yellow onion (25.95%) >pink onion (23.74%) >white onion (22.68%). In comparison to our measured values incase of garlic and Onion our value of AOA was higher.

Correlation between the total antioxidant activity values and total phenolics contents

ANOVA linear correlation coefficients were used to assess the relationships between TPC and TAC, Correlation: Our result confirmed a strong statically correlations between total polyphenol content and total antioxidant capacity values. A statistically strongly significant correlation (R = 0.9966; p <0.05) was found (Figure 5). **Amarowicz et al. (2005)** analyzed the extracts of fababean, broad bean, adzuki bean, red bean, pea, red lentil and green lentil seeds using 80% (v/v) acetone and confirmed a statistically significant correlation between the total antioxidant activity values and total phenolics (p = 0.01). A strong correlation between total polyphenol content and antioxidant activity (R = 0.86; p <0.05) was observed also by **Akond et al. (2011)** in common bean and a statistically strongly significant correlation (p-value 2.391E-06; R = 0.802) was found between total polyphenol content and total antioxidant capacity values by **Dalaram et al. (2013)** in lentil. According these authors this finding suggests that total polyphenol content is a good predictor of in vitro antioxidant activity.

CONCLUSION

The phenolic content, antioxidant activity, and the correlation between phenolic content and antioxidant capacity were studied in varieties onion and garlic, Cultivar significantly affected phenolic accumulation and antioxidant capacity. Current results of total phenolic content (TPC) assay indicate that TPC is higher in red onion, also (TAC); the positive interrelationship between these two parameters demonstrates that the antioxidant activity depends mainly on polyphenols contents. Onions were rich sources of polyphenols and flavonoids, and showed the promising antioxidant and free radical scavenging activities. The results suggest that statistically the highest value of total polyphenols and antioxidant activity was in red onion. In the case of white varieties of onion, determined significantly the lower content of total polyphenols and antioxidant activity was recorded. The value of total polyphenols content and antioxidant activity of garlic were lower than red variety onion but higher value of total polyphenols and antioxidant activity than other varieties onion(yellow, pink, white).The content of total polyphenols and antioxidant activity may be affected by many factors for example postharvest (storage) and climatic conditions (altitude, rainfall, mean annual temperature) and the agrochemical composition of the soil (humus of content, nutrients) and type varieties.

REFERENCES

Akond, G. M. A. S. M., Khandaker, L., Berthold, J., Gates, L., Peters, K., Delong, H., Hossain, K. 2011. Anthocyanin, total polyphenols and antioxidant activity of common bean. *American Journal of Food Technology*, vol. 6, no. 5, p. 385-394. http://dx.doi.org/10.3923/ajft.2011.385.394

Albishi, T., John, J. A., Al-Khalifa, A. S., Shahidi, F. 2013 Antioxidant, Anti-Inflammatory and DNA Scission Inhibitory Activities of Phenolic Compounds in Selected Onion and Potato Varieties. *Journal of Functional Foods*, vol. 5, no. 2, p. 930-939. http://dx.doi.org/10.1016/j.jff.2013.02.005

Amin, F., Wani, S. M., Gani, A., Masoodi, F. A. 2013. Polyphenolic Estimation and Antioxidant Activity of Some Vegetables of J&K India-A Correlation Study. *International Journal of Engineering Research and Applications*, vol. 3, no. 2, p. 595-603.

Andrejiová, A., Kóňa, J., Barátová, S. 2011. Effect of fertilization and cultivar on total polyphenol content in onion (*Allium cepa* L.). In *Nutrition and Health*, Proceeding from the Conference, SPU : Nitra. p. 12-17.

Armand, B. A., Toua, V., Bernard, G. N., Nicolas, Y. N., Dimitry, Y. M., Montet, D., Joel, S., Omf, M. 2012. Effect of Solar and Electric Dryingon the Content of the Phenolic Compounds and Antioxidant activity of three varietes of onion (*Allium cepa* L.). *International Journal of Biology, Pharmacy and Allied Sciences*, vol. 1, no. 3, p. 204-220.

Beato, V. M., Orgaz, F. Mansilla, F. Montaño, A. 2011. Changes in phenolic compounds in garlic (*Allium sativum* L.) owing to the cultivar and location of growth. *Plant Food for Human Nutrition*, vol. 66, no. 3, p. 218-223. http://dx.doi.org/10.1007/s11130-011- 0236-2

Benítez, V. Molla, E. Martin-Cabrejas, M. A, Aguilera, Y, Lopez-Andreu, F. J, Cools, K. Terry, L. A. Esteban, R. M. 2011a. Characterization of Industrial Onion Wastes (*Allium cepa* L.): Ditary fiber and Bioactive compounds. *Plant Food for Human Nutrition*, vol. 66, no. 1, p. 48-57. http://dx.doi.org/10.1016/j.chroma.2011.02.059

Benkeblia, N. 2005. Free-radical scavenging capacity and antioxidant properties of some selected onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts. *Brazilian Archives of Biology and Technology*, vol. 48, no. 5, vol. 753-759. http://dx.doi.org/10.1590/S1516-89132005000600011

Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft and Technologie*, vol. 28, no. 1, p. 25-30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5

Bystrická, J., Kavalcová, P., Vollmannová, A., Tomáš, J., Orsák, M. 2014. The role of sulphur on the content of total polyphenols and antioxidant activity in onion (*Allium cepa* L.). *Potravinarstvo*, vol. 8, no. 1, p. 284-289. http://dx.doi.org/10.5219/401

Chan, J. Y, Yuen, A. C, Chan, R. Y., Chan, S. W. 2013. A review of the cardiovascular benefits and antioxidant properties of allicin. *Phytotherapy Research*, vol. 27, no. 5, p. 637-646. http://dx.doi.org/10.1002/ptr.4796 PMid:22888009

Chen S., Shen X., Cheng S., Li P., Du J., Chang Y., Meng, H. 2013. Evaluation of garlic cultivars for polyphenolic content and antioxidant properties. *Plos Medicine*, vol. 8, no. 11, p. e79730. http://dx.doi.org/10.1371/journal.pone.0079730

Corzo-Martínez, M. Corzo, N. Villamiel, M. 2007. Biological properties of onions and garlic. *Trends in Food Science and Technology*, vol. 18, no. 12, p. 609-625. http://dx.doi.org/10.1021/jf960339y

Georgé, S., Brat, P., Alter, P., Amiot, M. J. 2005. Rapid determination of polyphenols and vitamin C in plant-derived products. *Journal of Agricultural and Food Chemistry*, vol. 53, no. 5, p. 1370-1373. http://dx.doi.org/10.1021/jf048396b

Gorinsteine, S., Leontowicz, M., Jastrzebisky, Z., Najman, K., Tashma, Z., Katrich, E., Heo, B. J. G., Cho, J. Y., Trakhtenberg, S. 2010. The influnce of raw and processed garlic and onions on plasma classical atherosclerosis indices investigation in vitro and in vivo. *Phytotherapy Reserach*, vol. 24, no. 5, p. 706-714.

Harrison, F., Roberts, A. E. L., Gabrilska, R., Rumbaugh, C. L., Diggle, S. P. 2015. A 1000-Year-Old antimicrobial remedy with antistaphylococcal activity. *American Society for Microbiology*, vol. 6, no. 4, p. 1-7.

Kalt, W. 2005. Effects of Production and Processing Factors on Major Fruit and Vegetable Antioxidants. *Journal of Food Science*, vol. 70, no. 1, 2005, p. 11-19. http://dx.doi.org/10.1111/j.1365-2621.2005.tb09053.x

Karadenyz, F., Burdurlu, H. S., Koca, N., Soyer, Y. 2005. Antioxidant activity of selected fruits and vegetables grown in Turkey. *Turkish Jurnal of Agriculture & Forestry*. vol. 29, p. 297-303.

Kavalcová, P., Bystrická, J., Tomáš, J., Karovičová, J., Kuchtová, V. 2014. Evaluation and comparing of the content of total polyphenols and antioxidant activity in onion, garlic and leek. *Potravinarstvo*, vol. 8, no. 1, p. 272-276. http://dx.doi.org/10.5219/394

Kavalcová, P., Bystrická, J., Tóth, T., Trebichalský, P., Hrstková, M., Lenková, M., Šiatkovský, O. 2015. Content of total polyphenols and antioxidant activity in selected varieties of onion (*Allium cepa* L.). *Potravinarstvo*, vol. 9, no. 1, p. 494-500. http://dx.doi.org/10.5219/524

Lachman, J., Proněk, D., Hejtmánková, A., Pivec, V., Faitová, K. 2003. Total polyphenol and main flavonoid antioxidants in different onion (*Allium cepa* L.) varieties. *Scientia Horticulturae*, vol. 30, p. 142-147.

Lanzotti V. 2006. The analysis of onion and garlic. *Journal of Chromatography A*, vol. 1112, no. 1, p. 3-22. http://dx.doi.org/10.1016/j.chroma.2005.12.016

Li, H. B., Cheng, K. W., Wong, C-C., Fan, K. W., Chen, F., Jiang, Y. 2007. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*, vol. 102, no. 3, p. 771-776. http://dx.doi.org/10.1016/g.foodchem.2006.06.022

Li, J. W. and Vederas, J. C. 2009. Drug discovery and natural products: End of an era or an endless frontier? *Science*, vol. 325, no. 5937, p. 161-165. http://dx.doi.org/10.1126/science.1168243

Lu, X., Wang, J., Hamzaf, M., Ross, C. F., Powers, J. R., Tang, J., Rasco, B. A. 2011. Determination of total phenolic content and antioxidant capacity of onion and shallots using infrared spectroscopy. *Food Chemistry*, vol. 129, no. 2, p. 637-644. http://dx.doi.org/10.1016/j.foodchem.2011.04.105

Manach, C., Scalbert, H., Morand, C., Remesy, C., Jimenez, L. 2004. Polyphenols in foods and bioavailability. *The American Journal Clinical Nutrition*, vol. 79, no. 5, p. 727-747. PMid:15113710

Nuutila, A. M., Puupponen-Pimia, R., Aarni, M., Oksman-Caldentey, K. 2003. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem.*, vol. 81, no. 4, p. 485-493. http://dx.doi.org/10.1016/S0308-8146(02)00476-4

Pandey, K. B., Rizvi, S. I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, vol. 2, no. 5, p. 270-278. http://dx.doi.org/10.4161/oxim.2.5.9498

Pérez-Gregorio, R. M., González-Barreiro, C., Rial- Otero, R., Simal-Gándara, J. 2011b. Comparison of sanitizing technologies on the quality appearance and antioxidant levels in onion slices. *Food Control*, vol. 22, no. 12, p. 2052-2058. http://dx.doi.org/10.1016/j.foodchem.2012.10.134

Picchi, R., V., Migliori, C., Lo Scalzo, R., Campanelli, G., Ferrari, V., Di Cesare, L. F. 2012. Phytochemical content in

organic and conventionally grown Italian cauliflower. *Food Chemistry*, vol. 130, no. 3, p. 501-509. http://dx.doi.org/10.1016/j.foodchem.2011.07.036

Prakash, D., Singh, B. N., Upadhyah, G. 2007. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa* L.) *Food chemistry*, vol. 102, no. 4, p. 1389-1393. http://dx.doi.org/10.1016/j.foodchem.2006.06.063

Prior, L. R., Wu, X., Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, vol. 53, no. 10, p. 4290-4302. http://dx.doi.org/10.102/jf0502698

Qusti, S. Y., Ahmed, N., Abo-khatwa, Bin Lahwa, M. A. 2010. Screening of antioxidant activity and phenolic content of selected food items cited in the holly quran. *Journal Biological Science*, vol. 2, no. 1, p. 40-51.

Rupasinghe, V. H., Clegg, P. 2007. Total antioxidant capacity, total phenolic content, mineral elements, and histamine concentrations in wine of different fruit sources. *Journal of Food Composition and Analysis*, vol. 20, no. 2, p. 133-137. http://dx.doi.org/10.5897/AJB10.2010

Sellappan, S., Akoh, C. C., 2002. Flavonoids and antioxidant capacity of Georgia-grown Vidalia onions. *Journal of Agricurtural and Food Chemistry*, vol. 50, no. 19, p. 5338-5342. http://dx.doi.org/10.1021/jf 020333a

Škerget, M., Majheniè, L., Bezjak, M. 2009. Antioxidant, Radical Scavenging and Antimicrobial Activities of Red Onion (*Allium cepa* L.) Skin and Edible Part Extracts. *Chemical and Biochemical Engineering Quarterly*, vol. 23, no. 4, p. 435-444.

Soinen, T. H., Jukarainen, N., Julkunen-Titto, R., Karjalainen, R., Vepsalainen, J. 2012. The combined use of constrained total-line-shape 1H NMR and LC-MS/MS for quantitative analysis of bioactive components in yellow onion. *Journal of Food Composition*, vol. 25, no. 2, p. 208-214. http://dx.doi.org/10.1016/j.jfca.2011.09.001

Stajner, D., Varga, I. S. 2003. An Evaluation of the Antioxi dant Abilities of Allium Species," *Acta Biologica Szegediensis*, vol. 47, no. 1-4, p. 103-106. http://dx.doi.org/10.3923/pjn.2016.347.351

Tomás-Barberán, Espín., C. J. 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture*, vol. 81, p. 853-876. http://dx.doi.org/10.5897/AJB10.2010 PMid:12570662

Ye C. L., Dai, D. H., Hu, W. L. 2013. Antimicrobial and antioxidant activities of the essestial oil from onion (*Allium Cepa* L.). *Food control*, vol. 30, no. 1, p. 48-53 http://dx.doi.org/10.1016/j.food cont.2014.11.009

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A QUALITY ENHANCEMENT GREEN STRATEGY FOR BROILER MEAT BY APPLICATION OF TURMERIC (*Curcuma Longa*) POWDER AS LITTER AMENDMENT TO AFFECT MICROBES, AMMONIA EMISSION, PH AND MOISTURE

Katukurunda Gamage Shanaka Chaminda Katukurunda, Hewa Anthonige Amal Yasas Buddhika, Manoja Krishanthi Wijethunge Gamage, Prabhashini Dissanayake and Dulcy Senaratna

ABSTRACT

In multi-cultural Sri Lankan conditions, poultry meat is paramount importance in ensuring food security and improving nutrition. Issues as contact dermatitis and ammonia emission in broiler industry which caused by diminished litter parameters cause reduction of meat quality, profits and environmental conditions. Therefore use of Turmeric (Curcuma longa) (TM) powder as an antiseptic litter amendment at several application levels to enhance litter parameters with microbial demolition was attempted. Three months old broiler litter (2 kg) sample was taken and initial pH and moisture was determined. Turmeric was used to mix at levels of 0%, 1%, 3%, 5% and 8% (w/w). After mixing, 150 g of mixed litter was placed in container for each level of the 4 replicates, incubated for 5h and analyzed for Total Plate Count (TPC), Yeast and Mold Count (YMC), total Nematode Count (NC), ammonia emission, pH and moisture. Significant reduction (p <0.05) of total bacteria was seen (20%, 46%, 95% and 96%) when 1%, 3%, 5% and 8% applications of TM. The YMC reduction was also significant (p <0.05) (34%, 41%, 55% and 65%). Total nematode reduction (p <0.05) was 22%, 45%, 62.5% and 70%. A significant (p <0.05) pH reduction with increment of TM also seen (0.1, 2, 3 and 3%). Moisture (%) was increased (p <0.05) (6, 0.78, 19 and 1%). Ammonia emission was significantly decreased (p *<0.05*) by increased TM (64, 68, 73 and 84%) against control. It was concluded that the bacterial, fungal, nematode counts, pH and Ammonia emission of broiler litter can be significantly reduced with the application of 8% (w/w) of turmeric powder.

Keywords: Turmeric powder; Total Plate Count; Mold Count; Nematode Count; NH_3

INTRODUCTION

Chicken meat is the most popular meat type of Sri Lanka which had a production of 14.45 million MT, exports of 1524.46 MT and per capita availability of 7.09 kg **(Department of Animal Production and Health, 2014)**. Under multi-cultural conditions, poultry meat is paramount importance in ensuring food security and improving nutritional status, because the consumption of the same and the production of poultry for meat are not severely regulated by ethno-religious taboos. As an industry which relies on quality, production and highly perishable nature, the profit maximization is bit challenging. Diseases to live animals and other management costs affect the industry in general. Basically chickens spend their entire life on a litter which has a higher microbial population may lead to contaminated processed carcasses by increasing the microbial load of skin and feathers and providing a source for upper gastrointestinal contamination during pre-harvest feed withdrawal. Pine shavings, peanut hulls, rice hulls, sand or other materials were commonly used as litter materials. Addition of birds onto litter adds large amount of excreta, feathers, feed and water. The quality of the in-house environment is highly dependent upon litter quality because of thousands of birds on thousand tons of litter. Moreover, the microbes are responsible for production of ammonia in poultry houses which has a global issue of green house gases and also affect badly to performance, health, behavior and welfare of animals. Health and welfare problems accompanying with high NH_3 concentrations include damage to the respiratory tract increased vulnerability to Newcastle disease, incidence of airsacculitis, increased Mycoplasma gallisepticum and incidence of keratoconjunctivitis **(Katukurunda et al., 2015)**. High NH_3 concentrations in poultry houses decrease growth rate of birds which reduces meat yield, feed efficiency **(Katukurunda et al., 2015)**, feed conversion and weight gains **(Carlile, 1984)** which lead to smaller animals and egg production **(Katukurunda et al., 2015)**. Uses of artificial litter amendments to address these problems were seen in the industry which reduces the fertilizer value of litter and increase of operational costs.

The intestinal flora and litter microbes of poultry play a vital role for their growth performance and health. On the other hand, knowledge about these intestinal flora and litter microbial ecology is still limited. Previous explorations which chiefly used culture dependent approaches proved that the common cultural bacteria in small intestine of birds belong to *lactobacilli*, *enterococci*

and *enterobacteria* and in the caecum, principally *lactobacilli, enterococci, bacteroides*, and *clostridia* **(Mead, 1989; Barnes, 1979; Engberg et al., 2000; Salanitro et al., 1978)**. Some modern molecular studies have dedicated more comprehensive insight into the constitution of the microbial community of poultry ecosystem **(Amit-Romach et al., 2004; Lan et al., 2002; Gong et al., 2002; Lu et al., 2003; Zhu et al., 2002)**. The constitution and composition of litter microbial community is inclined by factors such as temperature, humidity, day length, light color and intensity etc. **(Zhu et al., 2002)**, though **Yardimci and Kenar (2008)** reported that the effect of stocking density had no significant effect on microbial load and stocking density changing from 10 to 17 birds.m^{-2} did not affect microbial count of broiler litters. Therefore, microbial communities which found under different environmental factors in poultry management can have significant differences and deviations among them. Especially in poultry industry, the dietary and environmental factors affect the microbial status of gastrointestinal tracts of birds. Wet or caked litters and other animal management practices affect microbial composition of poultry gastrointestinal tract directly by giving a continuous bacteria source and indirectly by improving the physical condition and immunity **(Apajalahti, 2004)**.

Carr et al. (1990) and **Schefferle (1965)** reported that the total litter bacteria concentrations fall within the range of 10^{10} to 10^{11} colony forming units per gram (CFU.g^{-1}) of litter. As per the findings of **Lu et al. (2003)** and **Macklin et al. (2005)** the total aerobic bacteria counts are lower at 10^8 to 10^{10}. These amounts can vary with age of litter and age of birds **(Macklin et. al., 2005)** moreover fresh litter were found to have 10^5 CFU.g^{-1}, as soon as birds were placed on it, numbers of bacteria amplified by several levels of magnitude to 10^8.

NH_3 pollution is a major problem cause acid rains and overriding source of NH_3 in Europe is livestock waste **(Apsimon et. al., 1987)**. Ammonia is generated during the microbial breakdown of undigested proteins and excretory uric acids which in feces of poultry flock. Conditions that favor microbes will result in boosted NH_3 production. These conditions include warm temperature, pH in the neutral range or slightly higher (around 7.0 – 8.5) moisture and the presence of organic matter **(Miles et. al., 2004)**. Rarely the NH_3 concentrations in poultry houses can reach high levels, causing poor poultry performance **(Carlisle, 1984)**. **Anderson et al., (1964)** concluded that the NH_3 levels low as 20 ppm compromised the immune system of birds, making more vulnerable to respiratory diseases and damaged respiratory systems of animals. By considering all the observations of these negative effects on performance, **Carlile (1984)** recommended the NH_3 concentrations in poultry sheds should be kept less than 25 ppm. Higher levels of NH_3 may also affect the health of associated workers in these sheds **(Donham, 1977)** causing a health hazard.

Use of Turmeric (*Curcuma longa*) [TM] as a disinfectant was seen in many cultures especially throughout Asia. **Klimešová et al. (2015)** reports about the beneficial influence of turmeric on lipid metabolism, anti-hypercholesterolemic effect, anti-lithogenic effect and antioxidant effects in their study. In relation to poultry studies, turmeric improves feed intake in poultry when used at 0.25% level in feed. Several research studies suggested that turmeric can assist to fight microbial actions. Aqueous extracts of turmeric showed good antimicrobial activity against common bacteria, fungi, viruses, yeast, and round worms. **Katukurunda et al., (2015)** concluded that the application of turmeric higher than 3% (w/w) basis of poultry litter as an amendment was effective in lowering pH and the NH_3 emission of the litter material. **Hossain et al. (2015)** concluded that the fresh leaves juice (78.9%) and leaf dust (73.3%) of Turmeric plant inhibited the development of *Ascaridia galli* eggs, one of the most common parasitic roundworms of poultry at 20% w/w concentration. Moreover, **Rahman (2002)** reported that the highest efficacy against gastrointestinal nematodes in goats by turmeric leaves in alcoholic extracts (100%) than aqueous extract (92%). The overall objective of the study was to investigate the applicability of Turmeric powder as a natural antiseptic litter amendment to diminish microbial populations, ammonia emission rates by antimicrobial effects and wiping out conditions that favor microbes such as pH and moisture to increase the quality of broiler production with comprehensive green alternative.

MATERIAL AND METHODOLOGY

A representative broiler litter sample (±2 kg) was obtained from the broiler unit of the Faculty of Agriculture, University of Ruhuna, Kamburupitiya Sri Lanka, in black poly bag, sealed and well mixed. Turmeric powder available in local market was used to mix with litter (w/w) at 5 different levels; 0%, 1%, 3%, 5% and 8% (Figure 1). After mixing, 150 g of mixed litter was placed in container for each level as 4 replicated samples (5 X 4) and incubated at 30 °C for 5h and analyzed for Total Plate Count (TPC) **(Scott et al., 1998)** (ISO Standard procedure), Yeast and Mold count (YMC) **(Obire et. al., 2008)** (ISO Standard procedure), total Nematode Count (NC), pH, moisture % and litter ammonia emission rate **(Moore et al., 1996)** (ISO Standard procedure). The TPC and YMC were determined by using microbial cultures on PCA and PDA media which prepared to ISO standard procedures respectively. Colonies were counted using the colony counter to determine TPC and YMC.

McMaster Worm Egg Counting Slide (Vet Lab Supplies, United Kingdom) and the ISO Standard procedure which described by **Vadlejch et al. (2011)** was used to determine NC. Ammonia emissions were determined as described by **Moore et al. (1996)** (ISO Standard procedure), with modifications and determined the emission as milligrams of NH_3 emitted /kilogram of fresh litter/hour. Complete randomize design (ANOVA) was used with 4 replicates. Data were analyzed using Minitab 17 (2013).

RESULTS AND DISCUSSION

Jingrang et al., (2003) reported that total aerobic bacteria in poultry litter were detected by culture at 10^9 CFU.g^{-1} (colony forming units per gram). Majority are gram-positive, and often represented by the *coryneform* bacteria or *Staphylococcus* spp. Fungi and moulds are commonly present **(Gaiero, 2014)**. Dominant genera are *Aspergillus, Fusarium, Penicillium, Mucor, Scopulariopsis*, and *Onychocola*. Pathogenic strains like *E.*

coli O157:H7 and *Salmonella*, mostly absent. *E. coli* and *Enterococci* are both found in relatively high in litter **(Gaiero, 2014)**.

Brooks et al. (2010) concluded that around 90% of cultured aerobic bacteria in broiler houses were *staphylococci*.

In this experiment a very significant p <0.05 removal of total bacteria and YMC were found after treatment with different level of application of TM powder (Table 1). The reduction of total bacteria was 20%, 46%, 95% and 96% for 1%, 3%, 5% and 8% applications of TM powder respectively (Figure 1d). The significant reduction of YMC was 34, 41, 55 and 65% from control (Figure 1e). Nematode count reduction p <0.05 was 22, 45, 62 and 70% (Figure 1f). Considering large scale poultry houses, this kind of microbial reduction may help to increase quality of produce which can declined by contact dermatitis problems like foot pad dermatitis, hock burn and breast blisters. Besides, the quality of chicken meat is now of major importance, since meat is usually consumed as cuts or as processed goods moderately than as whole carcasses **(Haščík et.al., 2015)**, these problems largely affect meat weight, meat quality and also animal welfare. Unlike synthetic litter amendments with acidifiers such as alum, sodium bisulfate, calcium sulfate, magnesium chloride **(Pokharel, 2010)**, turmeric powder is a 100% natural produce and this green strategy may result fruitful outcomes to the industry as an antiseptic.

Effect of turmeric at different level of applications with poultry litter for NH_3 emission, moisture % and pH values are shown in Table 1. The pH of all application levels were significantly different with the control. Increasing the level of application of TM shows a significant reduction of pH values from 0.1, 2, 3 and 3% than control (Figure 1a). All TM levels have significant p <0.05 increase of moisture and the 3% level has the lowest increase (Figure 1b).

Table 1 Effect of turmeric at different levels of application with broiler litter for NH_3 emission, moisture%, pH values microbial properties. Values with different letters are significantly different (p <0.05), (↑: Percentage increase and ↓: % decrease than control).

Parameter	**Level of Turmeric application**				
	0%	**1%**	**3%**	**5%**	**8%**
(a) pH	9.06a ±0.01	8.97b ±0.01 (↓1%)	8.81c ±0.01 (↓2.75%)	8.73d ±0.01 (↓3.6%)	8.75c ±0 (↓3.4%)
(b) Moisture	51.34e ±0	54.54b ±0.05 (↑6%)	51.74d ±0.02 (↑0.7%)	61.26a ±0.01 (↑19%)	51.94c ±0.02 (↑1.2%)
(c) NH_3	0.68a ±0.27	0.24b ±0 (↓64%)	0.21b ±0.01 (↓68%)	0.18bc ±0.02 (↓73%)	0.1c ±0.05 (↓84%)
(d) TPC	5.75×10^4a ±0	4.58×10^4b ±0 (↓20%)	3.11×10^4c ±0 (↓46%)	2.45×10^3d ±0 (↓95%)	2.25×10^3e ±0 (↓96%)
(e) YMC	4.53×10^4a ±0	2.99×10^4b ±0 (↓34%)	2.64×10^4c ±0 (↓41%)	2.04×10^4d ±0 (↓55%)	1.57×10^4e ±0 (↓65%)
(f) NEM	400a ±36.9	333.5b ±21.3 (↓22%)	216.75c ±21.3 (↓45%)	150d ±0 (↓62%)	116.5e ±21.3 (↓70%)

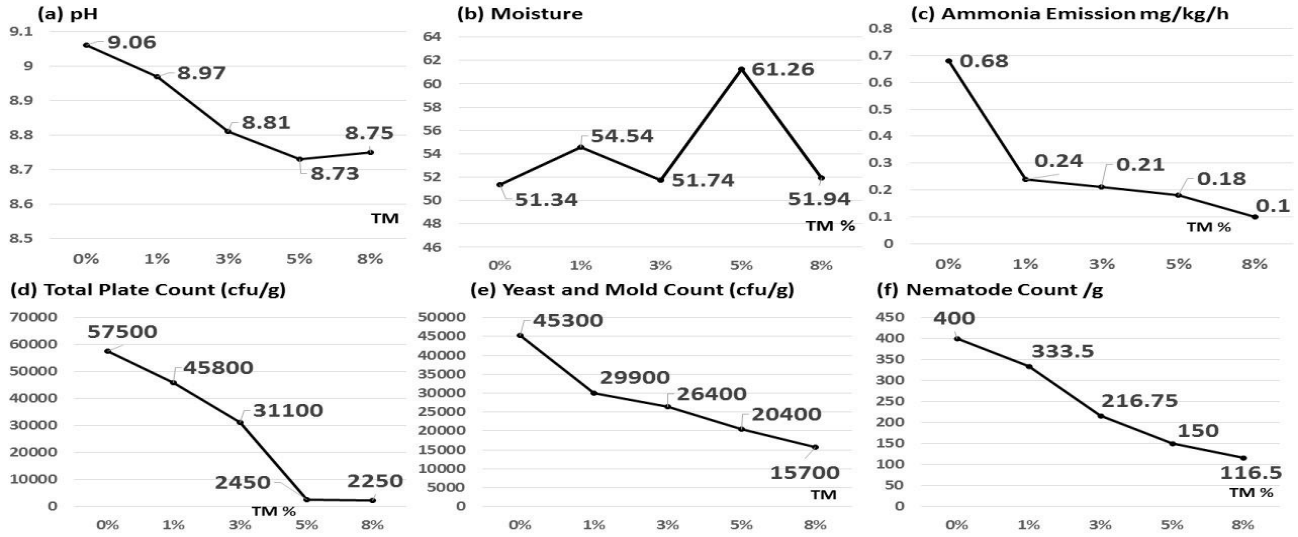

Figure 1 Effect of turmeric at different levels of application with broiler litter (a) pH (b) moisture% (c) NH_3 emission, (d)TPC (e)YMC (f) NC.

Practically lowering of moisture in a litter may cause lowering of NH_3 production **(Pokharel, 2010)**. The emission of NH_3 is lowered with the increase of turmeric levels significantly p <0.05 by 64, 68, 73 and 84% respectively (Figure 1c) and seems the increase of moisture did not affect the microbial count reduction.

Bad litter management conditions such as high moisture and pH levels increase the incidence of footpad dermatitis, hock burning damage and breast blisters which have become serious welfare problems in the industry. These problems primarily affect the surface of the footpad, hock joint and in severe cases extends to breast area and reduces meat quality. Birds accompanied with harsh lesions show slower weight gain and reluctance to move as they are obviously lame and experience pain-induced decline in desire for food **(Nagaraj, 2006)**. **Nagaraj (2006)** concluded that the presence of ammonia or other chemical substances in litter may play a role in the further development of lesions but does not appear to cause it straightly and lesions enhance in severity as litter moisture increases. Moreover, dietary factors have direct influence on litter qualities. Increased salt diets may increase the water intake of birds and increase the litter moisture content via increased excreta. Some animal owners use higher dietary crude protein levels in diets to get higher growth performance to maximize the profits and eventually increase the nutrient content of litter which favors microbial growth. Hence ultimately these conditions may lead to bad litter quality and problems assorted with birds from increased NH_3 and moisture. Moreover, maintaining litter quality especially in broiler industry is a must to overcome these problems by using litter amendments.

Rothrock et al. (2008) evaluated the effect of alum on the microbial population in litter and concluded that the application of alum shifts the microbial population from bacterially to fungal dominated populations by making litter more acidic. Ammonia emissions depend on how much of ammonia nitrogen in solution counters to generate Ammonia against ionized ammonium (NH_4^+), which nonvolatile and dropping pH in litter can decrease the NH_3 emission by leading the equilibrium among NH_3 and NH_4^+ on the way to the NH_4^+ ions. **Pope and Cherry (2000)** concluded that sodium bisulfate is highly effective in reducing the ammonia, pH and *E. coli* in commercial facilities. However, **Gholap (2012)** concluded that the effects of acidified clay and sodium bisulfate on litter characteristics were ineffective in maintaining low ammonia levels after second week of application. Sodium bisulfate was more effective in reducing litter pH compared to acidified clay. Neither of the litter treatments was ineffective in reducing the bacterial counts of litter. Since all these amendments are synthetic, it is better to switch on to environmental friendly low cost alternatives.

Witkowska and Sowińska (2013) concluded that fogging of poultry houses with aqueous solutions of peppermint and thyme oils effectively reduced bacterial contamination in broiler houses. During their experiment, total counts of mesophilic bacteria in barns fogged with peppermint and thyme oils were lower than control. Both oils were effective in reducing bacterial counts, though thyme oil was more effective in lowering coliform bacteria, whereas peppermint oil had a higher inhibitory effect on *staphylococci* proliferation. Moreover, **Inouye et al. (2001, 2003)** observed the inhibited growth of selected bacteria and fungi under laboratory conditions when treated with essential oils in vapor and liquid form. Therefore, use of natural antiseptics to reduce litter bacterial counts seems to be possible in various ways. Effect of turmeric at different levels of application with broiler litter was further shown graphically in Figure 1.

Application of turmeric powder to broiler litters is more effective to reduce microbial counts and NH_3 emission with a low operational cost compared to other synthetic litter amendments. This strategy practically suits for small or large scale broiler meat producers to improve the hygiene and quality of their produce. As per demand of poultry meat for its paramount importance in ensuring food security and improving nutrition this strategy will reduce the post-harvest losses of industry. Use of turmeric as a litter amendment may provide a green alternative towards global warming and environmental pollution via reducing ammonia emission in poultry farms. Further researches in this topic is much needed to apply these techniques and strategies in large scale operations thus to maximize profits in the industry and reduce pollution.

CONCLUSION

The bacterial, fungal and nematode counts of broiler litter can be significantly reduced with application of turmeric powder 8% (w/w) as an amendment up to 96, 65 and 70% respectively. The pH values of treatments were significantly reduced but maximum reduction of 3% was seen in 5% (w/w) level. Moisture % was significantly increased than the control along treatments but it did not affect the microbial reduction. Ammonia emission within the treatments were significantly lowered by application of turmeric 8% (w/w) from 64 to 84% along treatments

REFERENCES

Amit-Romach, E., Sklan, D., Uni, Z. 2004. Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. *Poultry Science*, vol. 83, no. 7, p. 1093-1098. http://dx.doi.org/10.1093/ps/83.7.1093 PMid:15285498

Anderson, D. P., Beard, C. W., Hanson, R. P. 1964. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. *Avian Diseases*, vol. 8, no. 3, p. 369-379. http://dx.doi.org/10.2307/1587967

Apajalahti, J. H. A., Kettunen, A., Graham, H. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *World's Poultry Science Journal*, vol. 60, no. 2, p. 223-232. http://dx.doi.org/10.1079/WPS20040017

Apsimon, H., Kruse, M., Bell, J. N. P. 1987. Ammonia emissions and their role in deposition. Atmospheric Environment, vol. 21, no. 9, p. 1939-1946. http://dx.doi.org/10.1016/0004-6981(87)90154-5

Barnes, E. M., 1979. The intestinal microflora of poultry and game birds during life and after storage. *Journal of Applied Bacteriology*, vol. 46, no. 3, p. 407-419. http://dx.doi.org/10.1111/j.1365-2672.1979.tb00838.x PMid:383674

Brooks, J. P., McLaughlin, M. R., Scheffler, B., Miles, D. M. 2010. Microbial and antibiotic resistant constituents associated with biological aerosols and poultry litter within a commercial poultry house. *Science of the Total Environment*,

vol. 408, no. 20, p. 4770-4777. http://dx.doi.org/10.1016/j.scitotenv.2010.06.038 PMid:20655094

Carlile, F. S. 1984. Ammonia in poultry houses: A literature review, *World's Poultry Science Journal*, vol. 40, no. 2, p. 99-113. http://dx.doi.org/10.1079/WPS19840008

Carr, L. E., Wheaton, F. W., Douglass, L. W. 1990. Empirical models to determine ammonia concentrations from broiler chicken litter. *Transaction of the ASAE*, vol. 33, no. 1, p. 260-265. http://dx.doi.org/10.13031/2013.31325

Caveny, D. D., Quarles, C. L., Greathouse, G. A. 1981. Atmospheric ammonia and broiler cockerel performance. Poultry Science, vol. 60, no. 3, p. 513-516. http://dx.doi.org/10.3382/ps.0600513

Department of Animal production and Health. 2014. Annual Report of the Department of Animal production and Health, Sri Lanka, 2014. Livestock statistics [online] s.a. [cit. 2015-11-09] Available at: http://www.daph.gov.lk/web/index.php?lang=en.

Donham, K. J., Rubino, M. J., Thedell, T. D. Kammermeyer, J. 1977. Potential health hazards to agricultural workers in swine confinement buildings. *Journal of Occupational and Environmental Medicine*, vol. 19, no. 6, p. 383-387. http://dx.doi.org/10.1097/00043764-197706000-00004

Engberg, R. M., Hedemann, M. S., Leser, T. D., Jensen, B. B. 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. *Poultry Science*, vol. 79, no. 9, p. 1311-1319. http://dx.doi.org/10.1093/ps/79.9.1311 PMid:11020077

Gaiero, J. R. 2014. *Microbiology and Nitrogen Mineralization in Composted Poultry Litter Amended with Biodiesel Wash Water* : dissertation thesis. Guelph, Ontario, Canada : The University of Guelph. 58 p.

Gholap, D. B. 2012. *Evaluation of Air and Litter Quality with Microbiological Fluctuations in Commercial Broiler Facilities Using a Biological or Chemical Litter Treatment* : dissertation thesis. Auburn, Alabama : Auburn University. p. 79-80.

Gong, J., Forster, R. J., Yu, H., Chambers, J. R., Sabour, P. M., Wheatcroft, R., Chen, S. 2002. Diversity and phylogenetic analysis of bacteria in the mucosa of chicken caeca and comparison with bacteria in the cecal lumen. *FEMS Microbiology Letters*, vol. 208, no. 1, p. 1-7. http://dx.doi.org/10.1111/j.1574-6968.2002.tb11051.x PMid:11934485

Gruzauskas, R., Semaskaite, A., Jarule, V., Stupeliene, A.R., Sasyte, V., Angelovicova, M., Hagarova, M., Kliment M. 2011. The impact of phytobiotics origami aetheroleum and probiotics pediococcus acidilactici ma 18/5 m on biochemical blood indices and preceacal availability of calcium and phosphorus in broiler chickens. *Potravinárstvo*, vol. 5, no. 1, p. 355-358.

Haščík, P., Trembecká, L., Bobko, M., Čuboň, J., Bučko, O., Tkáčová, J. 2015. Evaluation of meat quality after application of different feed additives in diet of broiler chickens. *Potravinarstvo*, vol. 9, no. 1, p. 174-182. http://dx.doi.org/10.5219/429

Hossain, M. S., Dey, A. R., Alim, M. A., Begum, N. 2015. In vitro efficacy of medicinal plant material on the inhibition of development of egg of Ascaridia galli. *The Journal of Advances in Parasitology*, vol. 2, no. 1, p. 5-10. http://dx.doi.org/10.14737/journal.jap/2015/2.1.5.10

Inouye, S., Abe S., Yamaguchi H., Asakura M. 2003. Comparative study of antimicrobial and cytotoxic effects of selected essential oils by gaseous and solution contacts. *International Journal of Aromatherapy*, vol. 13, no. 1, p. 33-41. http://dx.doi.org/10.1016/S0962-4562(03)00057-2

Inouye, S., Takizawa T. and Yamaguchi H. 2001. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *Journal of Antimicrobial Chemotherapy*, vol. 47, no. 5, p. 565-573. http://dx.doi.org/10.1093/jac/47.5.565 PMid:11328766

Jingrang, L., Sanchez, S., Hofacre, C., Maurer, J. J., Harmon, B. G., Lee, M. D. 2003. Evaluation of Broiler Litter with Reference to the Microbial Composition as Assessed by Using 16S rRNA and Functional Gene Markers. *Applied and Environmental Microbiology*, vol. 69, p. 901-908. http://dx.doi.org/10.1128/AEM.69.2.901-908.2003 PMCid:PMC143620

Katukurunda, K. G. S. C., Gamage, M. K. W., Buddhika, H. A. A. Y., Prabhashini, S. D., Senaratna, D. 2015. Comparison of Microbial Aspects, Ammonia Emission Rates and Properties of Broiler and Layer Litters after Application of Turmeric (Curcuma longa) Powder. *International Journal of Innovative Research in Technology*, vol. 2, no. 4, 19-24.

Klimešová, M., Horáček, J., Ondřej, M., Manga, I., Koláčková, I., Nejeschlebová, L., Ponížil, A. 2015. Microbial contamination of spices used in production of meat products. *Potravinarstvo*, vol. 9, no. 1, p. 154-159. http://dx.doi.org/10.5219/440

Lan, P. T., Hayashi, H., Sakamoto, M. Benno, Y. 2002. Phylogenetic analysis of cecal microbiota in chicken by the use of 16S rDNA clone libraries. *Microbiology and Immunology*, vol. 46, no. 6, p. 371-382. http://dx.doi.org/10.1111/j.1348-0421.2002.tb02709.x PMid:12153114

Lu, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J. J., Lee, M. D. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Applied and Environmental Microbiology*, vol. 69, no. 11, p. 6816-6824. http://dx.doi.org/10.1128/AEM.69.11.6816-6824.2003 PMid:14602645

Lu, J., Shanchez, S., Hofacre, C., Maurer, J. J., Harmon, B. G., Lee, M. D. 2003. Evaluation of broiler litter with reference to the microbial composition as assessed by using 16s rRNA and functional gene markers. *Applied and Environmental Microbiology*, vol. 69, no. 2, p. 901-908. http://dx.doi.org/10.1128/AEM.69.2.901-908.2003 PMid:12571010

Macklin, K. S., Hess, J. B., Bilgili, S. F., Norton, R. A. 2005. Bacterial levels of pine shavings and sand used as poultry litter. *The Journal of Applied Poultry Research*, vol. 14, p. 238-245. http://dx.doi.org/10.1093/japr/14.2.238

Mead, G. C. 1989. Microbes of the avian cecum: Types present and substrates utilized. *The Journal of Experimental Zoology Supplement*, vol. 3, p. 48-54. http://dx.doi.org/10.1002/jez.1402520508

Miles, D., Branton, S. L., Lott, B. D. 2004. Atmospheric ammonia is detrimental to the performance of modern commercial broilers. *Poultry Science*, vol. 83, no. 10, p. 1650-1654. http://dx.doi.org/10.1093/ps/83.10.1650 PMid:15510548

Moore, P. A. Jr., Daniel, T. C., Edwards, D. R. 1999. Reducing phosphorus runoff and improving poultry production with alum. *Poultry Science*, vol. 78, no. 5, p. 692-698. http://dx.doi.org/10.1093/ps/78.5.692 PMid:10228965

Nagaraj, M. 2006. *Evaluation of nutrition and management factors in the etiology of pododermatitis in broiler chickens* :

dissertation thesis. Auburn, Alabama : Auburn University. 121 p.

Obire, O., Anyanwu, E. C., Okigbo, R. N. 2008. Saprophytic and crude oil degrading fungi from cow dung and poultry droppings as bioremediating agents, *Journal of Agricultural Technology*, vol. 4, no. 2, p. 81-89.

Pokharel, B. B. 2010. Ammonia emission from poultry industry, its effect and mitigation mechanism [online] s.a. [cit. 2016-07-12] Available at: http://www.academia.edu/1799968/AMMONIA_EMISSION_FROM_POULTRY_INDUSTRY_ITS_EFFECTS_AND_MITIGATION_MECHANISM.

Pope, M. J. and Cherry, T. E. 2000. An evaluation of the presence of pathogens on broilers raised on Poultry Litter Treatment (PLT) treated litter. *Poultry Science*, vol. 79, no. 9, p. 1351-1355. http://dx.doi.org/10.1093/ps/79.9.1351 PMid:11020084

Rahman, M. 2002. In vitro and in vivo anthelmintic effects of some plants against gastro intestinal nematodes of goats : dissertation thesis. Mymensingh, Bangladesh : Agricultural University, 121 p.

Rothrock, M. J., Cook, K. L., Warren, J. G., Sistani, K. 2008. The effect of alum addition on microbial communities in poultry litter. *Poultry Science*, vol. 87, no. 8, p. 1493-1503. http://dx.doi.org/10.3382/ps.2007-00491 PMid:18648040

Salanitro, J. P., Blake, I. G., Muirehead, P. A., Maglio, M., Goodman, J. R. 1978. Bacteria isolated from the duodenum, ileum and cecum of young chicks. *Applied and Environmental Microbiology*, vol. 35, no. 4, p. 782-790. PMid:646359

Schefferle, H. E. 1965. The microbiology of built up poultry litter. *Journal of Applied Microbiology*, vol. 28, no. 3, p. 403-411. http://dx.doi.org/10.1111/j.1365-2672.1965.tb02170.x

Scott, A. M., McCann, M. A., Waltman, W. D. 1998. Microbiological Survey of Georgia Poultry Litter. *Journal of Applied Poultry Research*, vol. 7, no. 1, p. 90-98. http://dx.doi.org/10.1093/japr/7.1.90

Vadlejch, J., Petrtýl, M., Zaichenko, I., Čadková, Z., Jankovská, I., Langrová, I., Moravec, M. 2011. Which McMaster egg counting technique is the most reliable? *Parasitology Research*, vol. 109, no. 5, p. 1387-1394. http://dx.doi.org/10.1007/s00436-011-2385-5 PMid:21526406

Witkowska, D., Sowińska, J. 2013. The effectiveness of peppermint and thyme essential oil mist in reducing bacterial contamination in broiler houses. Poultry Science, vol. 92, no. 11, p. 2834-2843. http://dx.doi.org/10.3382/ps.2013-03147 PMid:24135585

Yardimci, M. and Kenar, B. 2008. Effect of stocking density on litter microbial load in broiler chickens. *Archiva Zootechnica*, vol. 11, no. 3, p. 75-81.

Zhu, X. Y., Zhong T., Pandya, Y., Joerger, R. D. 2002. 16S rRNA based analysis of microbiota from the caecum of broiler chickens. Applied and Environmental Microbiology, vol. 68, no. 1, p. 124-137.

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BIOCHEMICAL COMPOSITION OF TANGERINE FRUITS UNDER MICROFERTILIZERS

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ABSTRACT

The paper presents the long-term research and its results in the field of biochemical composition and mechanical analysis of dwarf tangerine fruits ('*Miagava-Vase*') growing in the subtropical zone of the Black Sea coast, Krasnodar region. The given results have been obtained after treatments with the following micro fertilizers: H_3BO_3 (at a concentration of 0.06%), $MnSO_4$ H_2O (0.4%), $ZnSO_4$ H_2O (0.3%) and $CuSO_4$ H_2O (0.06%), an option with foliar water spraying served as a control. It is shown that treatments with copper, zinc and boron significantly (at 2.37 – 3.66 $mg.100g^{-1}$) increase the amount of vitamin C in fruits compared to the control option. The total amount of sugars in fruits was slightly increased under foliar application of manganese salts. The results show an effect of manganese and zinc to the ratio of total sugars and titratable acids and consequently, on the sugar-acid index value. We found that the content of ascorbic acid in tangerine depends on harvesting term, fruit location on the crown, light level, etc. Equally important is the fact that if storage makes up 3 months the loss of ascorbic acid is not more than 12%. Micro fertilizers had a positive effect on market quality: heavier fruits with thin peel were recorded on options with boric acid and manganese treatments. Treatments with boron and copper sulphate increased juice output from the pulp (74 and 76%, respectively, which is 3 – 7% higher than the control option). A higher dry substance accumulation was observed in the option with foliar application of zinc (13.93%). The despite differences in climatic characteristics of research years and annual adverse periods, foliar feeding with micro fertilizers had a positive effect not only on the accumulation of assimilates in the fruit, but also on the preservation of their presentation. The studies have shown that foliar treatments with micronutrients are promising for tangerine crop, which allowed us to develop guidelines for their use as an agro technical method on dwarf tangerine plantations.

Keywords: tangerines; foliar fertilizing; microelements; sugars; acidity; ascorbic acid; sugar-acid index; market quality.

INTRODUCTION

The Black Sea coast of Russia, where Sochi is located, refers to the humid subtropics, where it is possible to grow a large number of tropical and subtropical crops, including citrus fruits – the most frost-resistant and very popular among local citizens and visitors. Citrus crops are more popular and include a large group of evergreen woody plants belonging to the botanical genus *Citrus*, *Aurantioideae* subfamily, *Rutaceae* family **(Vorontsov, 1979)**. Tangerines are playing a leading role on the territory of Russian humid subtropics. Due to their rich chemical composition tangerine fruits are special for their excellent taste, as well as great nutritional, dietary and medicinal values **(Metlitskiy, 1955)**.

Russian humid subtropics are northern and their natural and climatic characteristics are slightly different from the classic subtropical regions with a bit lower winter temperatures. Dwarf tangerines of '*Miagava-Vase*' were the object of our study, being highly competitive with frost-resistant '*Unshiu*'. However, some biological features of dwarf cultivars are significantly different, and, therefore, their biological potential, their adaptability, productivity and fruit quality parameters in terms of the region, as well as probable use of biogenic microelements as regulators and listed characteristics represent great interest.

The role of microelements is extremely high, not only in various physiological (photosynthesis, respiration, synthesis of proteins), but also in biochemical processes that underlie fructification **(Arnon, 1962)**. Despite the fact that research of mineral nutrition physiology has been developed for more than 100 years, the main issues of controlling microelemental plant nutrition processes are relevant today **(Sennovskaya, 2006; Shchukin et al., 2006; Mohseni et al., 2006; Kashina et al., 2008)**. This is not only due to the complexity of the given issues under study, but due to the change in varietal composition as well as increasing demands on their productivity and product quality.

There is evidence that microelements are localized in organs and parts of plants, most rich in vitamins, in turn, plants rich in vitamins contain more micronutrients, especially manganese. Zinc has a positive effect on the accumulation of vitamin C in plants **(Chvapil, 1973; Stoyanova and Doncheva, 2002)** and Cu **(Inmaculada, 2005)**. Currently it is found that microelements are part of many enzymes that speed up biochemical reactions of synthesis, decomposition and metabolism of organic substances **(Volodko, 1983; Anspok, 1990)**. The data on

the occurrence of Cu and Zn in the composition of some enzymes made their role clear in redox processes. A major achievement is the definition of microelements in nitrogen metabolism **(Salyaev at al., 2003; Rak at al., 2005)**. The scientific paper informs about the way microelements influence on the movement and redistribution of mineral elements in plants **(Epstein, 1956; Khomenko at al., 1974; Rybchenko, 1975; Salyaev at al., 2003; Mingkui Zhang at al., 2006)**. A positive influence of microelements on plants ability to withstand adverse conditions was proved **(Bogomaz and Korshuk, 1967; Bozhenko, 1976; Serga, 1998; Gudkovskiy, et al., 2005)**. Application of essential micronutrients, among other agro-technical measures, leads to higher yields and improves the quality of many crops **(Belous, 2006, 2013; Poschenrieder, 2008; Khalid and Khalid, 2012)**. Under their influence, plants can use nitrogen, phosphorus, potash and other fertilizers better **(Rinkis and Nollendorf, 1982)**. Thus, the targeted use of microelements as selective regulators of metabolism is one of the ways to increase the productivity of fruit, vegetable and field crops.

Along with this, there are much less common experimental data on the effect of microelements on subtropical plants, especiallyon tangerines. There is a large prospect in studies to identify the value of microelements for plants belonging to subtropical crops group (mainly tangerine); moreover, these issues still remain poorly studied.

MATERIAL AND METHODOLOGY

The objects of our study were mature fruits of dwarf tangerine *Citrus reticulata* '*Miagava Vase'*. *Poncirus trifoliata* (L.) Raf. served rootstock. Comprehensive research of the effect of microelements on plants functional state was carried out on the plantation (1986) from 1998 till 2008 (the data presented in the paper include three years' period). Soils in pilot area were brown forest soil, slightly unsaturated: it had about 12% humus, fulvic acids exceeds the volume of humic and this soil characterized as sub-acid.

The experiment was laid in the open ground, using randomization method by 4-fold repetition in a covering culture. The plot area was 0.25 hectares and the placement of the plants by scheme 2500 trees per 1 ha correspond to the feeding area 4x1 m^2. Each account of the plot is protected with shelterbelt against accidental damage from strip technique, which also serves to eliminate the interference of neighboring microfertilizers. The experimental scheme included five options: control (spraying with water); H_3BO_3 - at a concentration of 0.06%; $MnSO_4$ H_2O – 0.4%; $ZnSO_4$ H_2O – 0.3%; $CuSO_4$ H_2O – 0.06%. Foliar spraying during the growing season was carried out twice: first spraying at finish blossom and second at beginning of coloring fruits. Agricultural technique was common for tangerine **(Gamkrelidze, 1971)**. Laboratory and field studies were conducted at the Institute, using classical methods. Biochemical and other analyses of tangerine fruits were performed according to the State Cultivar Commission **(Lobanov, 1973)**. The content of ascorbic acid was determined by iodometric method; titratable acidity – by titration with 0.1 N NaOH and sugars amount by refractometers method **(Pochinok, 1978)**. Laboratory analyses were made in triple repetitions: ascorbic acid content was determined by iodometric method with 2% HCl, titrated 0.001 N solution of KIO_3; total acidity - titration with NaOH (0.1 $mol.dm^{-3}$) in the presence of phenolphthalein indicator; dry matter - the method of drying the sample at 105 °C to constant weight The amount of sugar was determined by Bertran`s refractometric method. The method is based on the ability of sugars aldehyde group interact with Fehling's reagent and restore CuO to Cu_2O precipitated as a red solid. Organoleptic analysis of fruits was carried out according to the guidelines and 9 experts took part in fruits testing **(Lobanov, 1973)**.

The program STATGRAPHICS Centurion XV, Statpoint Technologies, Inc. USA, and mathematical software package MS Excel 7.0 were applied during processing research materials and evaluating experiment results.

RESULTS AND DISCUSSION

Nowadays the scientists are very interest about to natural antioxidants – flavonoids, anthocyanin's, vitamins (and especially vitamin C), which are able to prevent formation of free radicals in human body that usually cause cardiovascular diseases and cancer. Therefore, of a great interest are fruits that are natural sources of these compounds. Not coincidentally, we investigated the amount of ascorbic acid in tangerine fruits and change in its content under the influence of foliar fertilization with microelements.

The studies had found out that the content of ascorbic acid in tangerine fruits under humid subtropics is quite high (within 44 $mg.100g^{-1}$). Thus, treatments with copper, zinc and boron significantly (at 2.37 – 3.66 $mg.100g^{-1}$) increase the amount of vitamin C in fruit as compared to the control one (NCR_{05} = 0.06%). The tendency of ascorbic acid to increase in the fruits of test plants was recorded throughout the study **(Abilfazova, 2009)**. In addition, we found that the content of ascorbic acid in tangerine depends on harvesting term, fruit location on the crown, light level, etc. Equally important is the fact that if storage makes up 3 months the loss of ascorbic acid is not more than 12%.

One of the main fruit quality indicators, interesting to the consumer, is taste, which is largely determined by the ratio of sugars and titratable acids in fruits. As we can see from Table 1, the total amount of sugars in test plants is about 11.02%. A certain increase in this indicator was observed only under manganese salts foliar application. The content of titratable acids ranges among options from 1.88% (in the option with copper sulphate) to 2.33% (for control), and the differences between the options in this case was insignificant. In general, fruits were different in sweet-sour taste (mainly sourish notes), due to the size of sugar-acid index that made up 5.82 relative units. In the ratio of sugars and titratable acids, i.e. sugar-acid index value we observed some influence of microelement treatments; thus, the smallest value was found in the control option, while under foliar application of zinc, especially of manganese, the control was exceeded.

Apart from the qualitative biochemical parameters we were also interested in the influence of micronutrient fertilizing on tangerine mechanical characteristics.

Figure 1 Tangerine experimental plantation.

Figure 2 Tangerine experimental plantation.

Figure 3 Dwarf tangerine *Citrus reticulate* '*Miagava Vase*' cultivar blossoming.

Figure 4 Dwarf tangerine *Citrus reticulate* '*Miagava Vase*' cultivar on *Poncirus trifoliata* (L.) Raf. rootstock.

Figure 5 Dwarf tangerine *Citrus reticulate* '*Miagava Vase*' cultivar fruits.

Figure 6 Dwarf tangerine *Citrus reticulate* '*Miagava Vase*' cultivar fruits. Control – water spraying.

Figure 7 Dwarf tangerine *Citrus reticulate* '*Miagava Vase*' cultivar fruits. Spraying - H_3BO_3 (0.06%).

Figure 8 Dwarf tangerine *Citrus reticulate* '*Miagava Vase*' cultivar fruits. Spraying - $MnSO_4$ (0.4%).

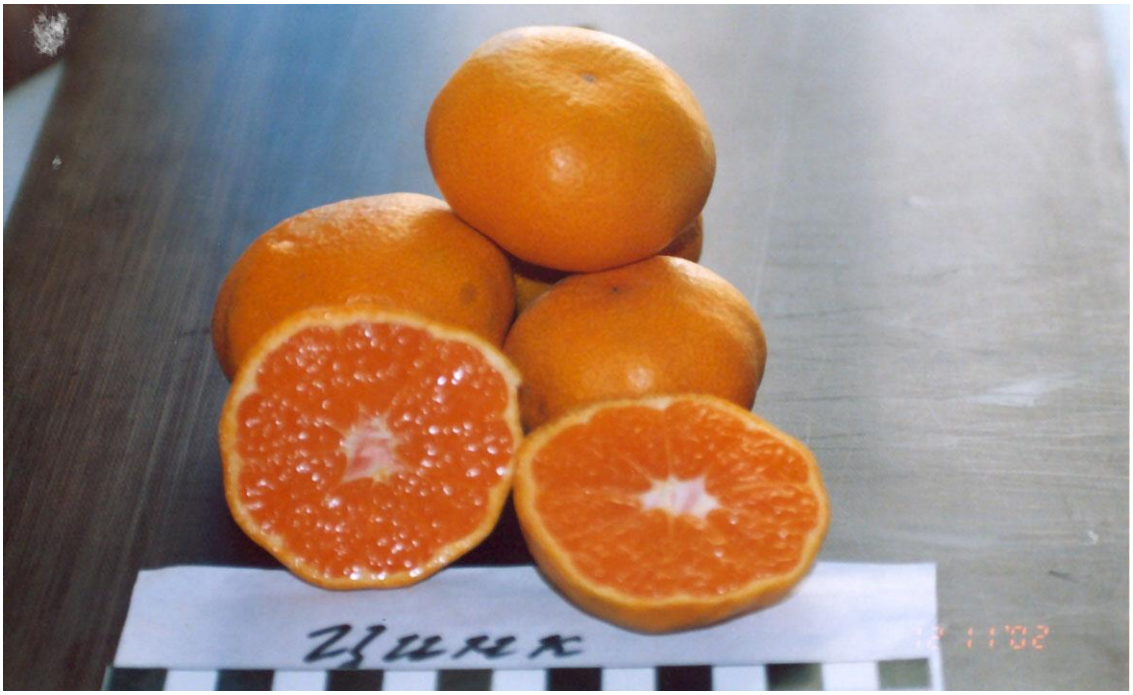

Figure 9 Dwarf tangerine *Citrus reticulate* '*Miagava Vase*' cultivar fruits. Spraying - $ZnSO_4$ (0.3%).

Figure 10 Dwarf tangerine *Citrus reticulate* '*Miagava Vase*' cultivar fruits. Spraying - $CuSO_4$ (0.06%).

Table 1 Biochemical composition of tangerine fruits, average for three research years.

Option	Ascorbic acid ($mg.100g^{-1}$)	Titratable acidity (%)	Amount of sugars (%)	Sugar-acid index (relative units)
Control (treatment with water)	41.14 ±4.68	2.33 ±0.85	10.83 ±1.66	5.14 ±2.08
H_3BO_3 (0.06 %)	43.51 ±2.15	2.18 ±0.72	10.70 ±1.56	5.46 ±2.64
$MnSO_4 \cdot H_2O$ (0.4 %)	43.11 ±3.63	1.95 ±0.50	11.87 ±1.27	6.43 ±2.01
$ZnSO_4 \cdot H_2O$ (0.3 %)	43.99 ±3.75	1.96 ±0.60	10.97 ±1.37	6.14 ±2.58
$CuSO_4 \cdot H_2O$ (0.06 %)	44.80 ±4.63	1.88 ±0.40	10.71 ±1.34	5.91 ±1.67

Table 2 Mechanical composition of tangerine fruits, average for three research years (based on 100 g of weight).

Option	Average fruit weight (g)	Dry substance (%)	Weight from total weight of fruit (%)		Output of juice (%)
			Pulp	Peel	
Control (treatment with water)	66.70 ±4.11	12.73 ±2.91	77.7 ±0.01	22.3	68.40 ±3.21
H_3BO_3 (0.06%)	84.11 ±1.49	12.83 ±3.69	79.0 ±0.03	21.0	74.03 ±1.72
$MnSO_4 \cdot H_2O$ (0.4%)	73.20 ±7.17	11.70 ±3.87	80.8 ±0.01	19.2	71.93 ±3.82
$ZnSO_4 \cdot H_2O$ (0.3 %)	70.53 ±6.18	13.93 ±5.25	77.9 ±0.00	22.1	71.27 ±5.92
$CuSO_4 \cdot H_2O$ (0.06%)	76.52 ±1.16	12.43 ±3.54	78.3 ±0.03	21.7	75.73 ±1.40

The studies had shown that despite differences in climatic characteristics of research years and annual adverse periods, foliar feeding with microfertilizers had a positive effect not only on the accumulation of assimilates in the fruit, but also on the preservation of their presentation. Thus, high air temperature (above 30 °C) in August was often replaced by long heavy rains with a sharp decrease to +20 to +23 °C in early September. This tends to promote stress cracking of fruits, which was not observed in plants with foliar embodiments. In addition, the average fruit weight ranged from 70.5 to 84.1 g, and heavier fruits were in options with boric acid and manganese treatments, slightly above the control in 1.3 times (Table 2). A similar influence of foliar application of microelements on fruit weight was noted by other researchers **(Bedrikovskaya, 1954**; **Naqvi, Alam and Mumtaz, 1990; Lafer, 2008)**.

But just fruit weight cannot be an indicator of their quality, because large weight may be due to the thick peel. Therefore, every year we took into account the percentages of pulp and peel. When these indicators had a similar value on all options (about 4/1), a little thinner-peeled fruits were recorded among options treated with manganese (19.2%) and boron (21.0%), in which the proportion of edible fruit was much more, compared to inedible. This fact was linked to another indicator which characterizes fruits and represents consumer interest – output of juice from the pulp. Thus, treatments with boron and copper sulphate contributed to more juice output from the pulp, reaching 74 and 76%, respectively, which is 3 – 7% higher than the control option (Table 2).

The accumulation of dry mater in tangerine fruits made up 12.7% on the average for all the options. At the same time, higher accumulation of assimilates was recorded in the option with foliar application of zinc – 13.93%.

As a final assessment, we carried out fruit degustation, which showed that fruits in all options had a good taste and market quality, therefore they received the highest evaluation score corresponding to 4.6 points by a 5-point scale.

At introduction of microelements we were testing their contents, and not only in fruits, but also in leaves. Foliar spraying by manganese caused to increasing this element (by 2.4 point) in leaves. Besides, introduction of manganese promoted accumulation of boron by leaves, contents of element on 15.08 mg/kg exceeded control. Processing of zinc and copper stimulated accumulation of boron and iron at plants (Table 3).

Foliare spraying by microelements influenced accumulation of elements at tangerine pulp and peel (Table 4).

Researches showed that foliar spraing of zinc and manganese influenced strengthening of boron accumulation into fruits. Between introduction of boron and accumulation into fruits of zinc, copper and iron was synergetic effect. Manganese showed antagonistic action in relation to zinc and copper, and zinc – reduced copper and iron accumulation.

The revealed regularities are described by such equations of regression:

$Cu = 13.04 - 3.08\ Zn$; $R^2 = 0.29$;

$Zn = 6.16 + 1.35\ Mn + 5.54\ Zn + 5.40\ Cu$; $R^2 = 0.97$;

$Mn = 1.23 + 1.14\ Mn$; $R^2 = 0.92$;

$FE = 0.32 - 0.52\ Cu - 0.89 Zn - 0.92\ Mn$; $R^2 = 0.31$.

Table 3 Contents microelements at tangerine leaves, average for three research years (mg.kg^{-1} of dry weight).

Option	Cu Optimum 5 – 10	Zn Optimum 25 – 100	Mn Optimum 25 – 100	B Optimum 50 – 170	Fe Optimum 60 – 120
Control (treatment with water)	12.12 ±1.6	33.86 ±14.4	27.76 ±3.8	38.70 ±3.04	43.30 ±10.2
H_3BO_3 (0.06%)	11.92 ±1.5	35.12 ±14.6	28.61 ±4.4	51.90 ±9.0	47.04 ±9.6
$MnSO_4 \cdot H_2O$ (0.4%)	11.86 ±1.6	35.20 ±14.6	65.42 ±12.6	53.78 ±14.2	46.78 ±10.7
$ZnSO_4 \cdot H_2O$ (0.3%)	11.34 ±2.1	57.50 ±13.1	29.04 ±3.7	44.92 ±6.5	47.12 ±11.9
$CuSO_4 \cdot H_2O$ (0.06%)	18.02 ±4.1	36.46 ±11.7	27.70 ±2.7	44.57 ±7.5	47.68 ±10.8
SSD_{05}^{*}	1.94	3.71	4.97	3.78	2.91

Note: *Smoller significant difference, confidence level – 95%.

Table 4 Contents microelements at tangerine pulp and peel, average for three research years (mg.kg^{-1} of dry weight).

Option	Pulp					Peel				
	B	Mn	Zn	Cu	Fe	B	Mn	Zn	Cu	Fe
Control (treatment with water)	14.6	7.2	2.13	1.27	25.3	33.4	6.5	16.1	12.3	17.5
H_3BO_3 (0.06%)	15.2	6.6	2.32	1.29	30.0	35.1	6.1	16.5	9.9	17.3
$MnSO_4 \cdot H_2O$ (0.4%)	16.3	10.2	1.93	1.05	23.2	37.8	13.8	16.8	11.2	14.4
$ZnSO_4 \cdot H_2O$ (0.3%)	16.2	7.8	2.56	1.24	23.4	38.3	7.0	35.0	9.5	14.8
$CuSO_4 \cdot H_2O$ (0.06%)	15.5	6.4	2.51	1.36	18.0	35.0	6.0	19.4	13.8	11.8
SSD_{05}^{*}	0.7	0.7	0.4	0.2	3.8	1.1	0.5	1.3	1.0	1.4

The analysis showed, that introduction of microelements didn't lead to chemical pollution of fruits pulp, because maximum permissible concentration of such dangerous elements as copper and zinc are much higher (maximum concentration limit Cu – 5.00 mg.kg^{-1} and Zn – 10.00 mg.kg^{-1}). These elements collect to a peel and don't come to pulp.

CONCLUSION

Describing the results of biochemical parameters and composition of fruits, we can claim that dwarf tangerines of '*Miagava Vase*' displayed response to foliar application of boron, manganese, zinc and copper. Microelements, as a whole, had a positive effect on the biochemical indicators of quality, which resulted in higher amount of sugar, as well as accumulation of ascorbic acid and sugar-acid index. At the same time, foliar application of microelements had a certain effect on market quality: the average fruit weight increased together with a high juice output. Such tendencies caused the necessity to study complex influence of microelements on the functional state of dwarf tangerine ('*Miagava Vase'*) for a long-term; and that is what exactly was being undertaken by us for ten years (1998 – 2008). As result, our researches influence of foliar spraing microelements at accumulation it's into fruits. Studies were shown prospectively in foliar treatment with micronutrients, which allowed us developed microelements use recommendations as a control method on dwarf tangerine plantations in the humid subtropics of Russia.

REFERENCES

Abilfazova, Yu. S. 2009. The value of vitamin C. In *Matherial VIII International Symposium. New and nonconventional plants and prospects of their use*. Moscow : RUDN University, p. 4-6. ISBN: 978-5-209-04047-7.

Anspok, P. I. 1990. *Microelements: Handbook*. 3rd ed. Leningrad: Agropromizdat. 272 p. ISBN: 5-10-001085-1.

Arnon, D. 1962. Role of microelements in plant nutrition. In *Arnon, D. Microelements*. Mockow: Engineering Literature, p. 9-47.

Bedrikovskaya, M. P. 1954. The effect of fertilizers on yield and quality of tangerine fruits. *Subtropical Crops*, vol. 2, p. 79-85.

Belous, O. 2006. *Microelements at the tea plantation of subtropical Russia*. Krasnodar: Kuban State Agrarian University. 164 p.

Belous, O. 2013. Influence of microelements on biochemical parameters of tea. *Potravinarstvo*, vol. 7, Special no., p. 149-152.

Bogomaz, Z. I., Korshuk, T. P. 1967. *Influence of microelements on physiological and biochemical processes and frost resistance of peach seedlings.* Kiev : Naukova dumka. p. 86-96.

Bozhenko, V. G. 1976. Microelements and the problem of plants resistance to adverse environmental conditions. In Bozhenko, V.G.: *Physiological role and practical application of micronutrients*. Riga : Zinatne. p. 110-123.

Chvapil, M. 1973. New aspects in the biological role of zinc. A stabilization of macromolecules and biological membranes. *Life Science*, vol. 13. no. 8, p. 1041-1049. http://dx.doi.org/10.1016/0024-3205(73)90372-X

Epstein, E. 1956. Mineral nutrition of plants. Mechanisms of uptake and transport. *Annual Review Plant Physiology*, vol. 7, p. 1-24. http://dx.doi.org/10.1146/annurev.pp.07.060156.000245

Gamkrelidze, I. D. 1971. *The system of fertilizer for citrus orchards.* Mockow : Kolos: 216 p. http://search.rsl.ru/ru/record/01007241093

Gogia, V. T. 1984. *Biochemistry of subtropical plants*. Mockow : Kolos, 288 p. http://www.lib.unn.ru

Gudkovskiy, V. A., Kashirskaya, N. Y, Tsukanova, Y. M. 2004. Increased stress tolerance of apple plants using micronutriental treatments in combination with elicitors. *Belgorod Agromir*, vol. 2, no. 14, p. 16-22.

Gudkovskiy, V. A., Kashirskaya, N. Y, Tsukanova, Y. M. 2005. Oxidative damage to fruit trees (the main stress factors, types of damage and ways to enhance stability). In *Scientific bases of horticulture*. Voronezh : Russian Research Institute of Horticulture, pp. 9-31.

Inmaculada, Y. 2005. Copper in plants. *Brazilian Journal of Plant Physiology*, vol. 17, no. 1, p. 145-156. http://dx.doi.org/10.1590/S1677-04202005000100012

Kashina, V. A., Semenova, Y. A. 2005. Redistribution of microelements in the system soil - soybean plants. Environmental problems of the Verchneye Priamurye: science materials. *Blagoveshchensk*, vol. 8, p. 89-96.

Khalid, A. K. 2012. Effect of NP and foliar spray on growth and chemical compositions of some medicinal. Apiaceae plants grow in arid regions in Egypt. *Journal of Soil Science and Plant Nutrition*, vol. 12, no. 3, p. 581-596. http://dx.doi.org/10.4067/s0718-95162012005000018

Khomenko, A. D. 1974. *Transport of nutrients and plant productivity.* Kiev : Naukova dumka, 93 p. http://www.lib.unn.ru/php

Kuprienko, N. P., Stepuro, M. F. 2008. The effect of foliar application based on liquid compound fertilizer Basfoliar on yield of vegetable crops. *Belarussiya agriculture*, vol. 71, no. 3, p. 54-57.

Lafer, G. 2008. Effects of different bioregulator applications on fruit set, yield and fruit quality of 'Williams' pears. *Acta Horticulure*, vol. 800 p. 183-188. http://dx.doi.org/10.17660/ActaHortic.2008.800.19

Lobanov, G. A. 1973. *Methods of State Cultivar Testing*. Michurinsk : Vniissok. 492 p.

Metlitskiy, L. V. 1955. *Citrus fruits.* Mockow : Pishchepromizdat: 196 p.

Mingkui, Z., Cui, Z., Changyong, H. 2006. Relationship Between Extractable Metals in Acid Soils and Metals Taken Up by Tea Plants. *Communications in Soil Science and Plant Analysis*, vol. 37, no. 3-4, p. 347-361. http://dx.doi.org/10.1080/00103620500440095

Mohseni, S., Ghanbari, A., Ramzanpoor, M. R., Mohseni, M. 2006. The Effects of Levels and Methods of Application of Zinc Sulfate and Boric Acid on the Yield. Quality and Nutrients Uptake of two Grain Maize Hybrids. *Iran Journal of Agriculture Science*, vol. 37, no. 1, p. 4-16.

Naqvi, S., Alam, S., Mumtaz, S. 1990. Effect of cobalt and silver ions and naphthaleneacetic acid on fruit retention in mango (*Mangifera indica.* L.). *Australian Journal of Experimental Agriculture*, vol. 30, p. 433-435. http://dx.doi.org/10.1071/EA9900433

Pochinok, K. N. 1976. *Methods of plants biochemical analysis.* Kiev : Naukova dumka. p. 43-49.

Poschenrieder, Ch., Allue, J., Tolra, R., Llugany, M., Barcelo, J. 2008. Microelements and Plant. Secondary Metabolism: Quality and Efficacy of Herbal Products. Microelements: Nutritional Benefits. In Prasad, M. N. V. *Environmental Contamination and Health Implications*. New jersey : John Wiley & Sons, Inc., 119 p. ISBN: 9780470180952.

Rak, M. V., Safronovskaya, G. M., Dembitskiy, M. F. 2004. Appearance of microelements in plants under oats foliar feeding cultivated in sod-podzolic sandy loam soil. *Soil Science and Agricultural chemistry*, no 2, p. 25-27.

Rinkis, G. Y., Nollendorf, V. F. 1982. *Balanced treatment with macro-and micronutrients.* Riga : Zinatne. 304 p. http://biblus.ru/Default.aspx?book=1b19j4e1b0

Rybchenko, O. I. 1975. Nutrition elements and their distribution in the organs of perennial plants. *Physiology and biochemistry of cultivated plants*, vol. 2, p. 190.

Salyayev, R. K., Dudareva, L. V., Lankevich, S. V., Yekimova, V. G., Sumtsova, V. M. 2003. Effect of low-intensity laser radiation on the lipid peroxidation in wheat callus culture. *Russian Journal of Plant Physiology*, vol. 50, no 4, p. 498-500. http://dx.doi.org/10.1023/A:1024720707041

Sennovskaya, T. V. 2006. Influence of trace elements of boron, manganese and zinc on efficiency of black currants. *Fruit and berry-culture of Russia*. vol. 17, p. 308-328. Published by: Russian Selection and Technology Institute of Horticulture and Nursery Breeding. Available at: http://www.cnshb.ru/jour/j_as.asp?id=81325

Serga, O. 1998. Reaction of plants to water and high temperature stresses. In *Proceedings of the 11th FESPP Congress*. Varna : Bulgarian Journal of Plant Physiology, vol. XXIV. Special issue: 189 p. http://www.bio21.bas.bg/ipp/gapbfiles/content.html

Shchukin, V. B., Gromov, A. A., Shchukina, N. V. 2006. Influence of foliar fertilization with microelements on photosynthetic parameters of winter wheat planting. *Orenburg news*, no. 4, p. 61-65. Published by: Orenburg State Agrarian University.

Stoyanova, Z., Doncheva, S. 2002. The effect of zinc supply and succinate treatment on plant growth and mineral uptake in pea plant. *Brazilian Journal of Plant Physiology*, vol. 14, no. 2, p. 111-116. http://dx.doi.org/10.1590/S1677-04202002000200005

Volodko, I. K. 1983. *Microelements and plant resistance to adverse environmental factors*. Minis : Science and Technology, 112 p. http://catalog.gsu.by/opac/index.php?url=/notices/index/IdNotice:51266

Vorontsov, V. V., Lavriychuk, I. I., Zagaynyi, S. A., Lozhenitsin, I. P., Goletiani, T. G., Ksenofontova, D. V. 1979. *Methodical instructions on the technology of growing dwarf*

tangerine in the subtropical areas of Krasnodar region. Sochi : Nauka. 60 p.

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SCIENTIFIC BASIS OF USE OF FRUITS *CORIANDRUM SATIVUM* L. IN FOOD TECHNOLOGIES

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ABSTRACT

Today in the world recognized the need for environmentally friendly products for a healthy food and quality life. Products with natural ingredients, including flavoring become very popular. Coriander is one of herbs that functions as both, spice as well as herbal medicine. *Coriandrum sativum* L. is a major aromatic crop in Ukraine. The plants of Coriandrum sativum contain the essential oils and other compounds in the seeds and leaves and have an important role as flavorings. The main objective was to investigate possibility effective utilization of coriander essential oil in national economy of Ukraine. It was necessary to study the chemical compounds of coriander fruits by instrumental analysis and odor by sensory analysis with following creating new aroma compositions. Search had been carried out throughout 2009 – 2014 years. The aerial parts of aromatic plants were harvested at the plots of National Botanical Garden of National Academy of the Sciences of Ukraine. Essential oil was obtained by hydro distillation procedure in National University of food technology. Main and specific components of essential oils from seeds coriander were characterized. Qualitative structure of essential oils was determined by the gas-liquid chromatography method on the chromatograph Agilent Technologies 6890 with mass-spectrometric detector 5973. The run of components was done using Device of Fractional Distillation. Linalool, limonene, geranyl acetate, d-camphor, myrcene and geraniol were found as the major components. In the composition of essential oils each component has its own flavor, the combination of which determines the flavor of the oil. We investigated the possibility of target separation of essential oils of coriander fruits into fractions of different flavor. The article presents the results of research sequential processing fruits *Coriandrum sativum* to obtain a series of natural flavors. Principles and laws of the vacuum distillation were used for directional control of the process distillation of complex mixtures of hydrocarbons on the distillation column. Mode of selection process the fractionation of essential oils allowed changing the component composition of the fractions and to provide more variety of flavors. Monitoring of the fractioning process allows concentrating the key aromatic components and receiving highly concentrated flavors of original pure notes. Combinations of the individual fractions with a specific weight have been created. We obtained some fractions which can be used as flavorings in food industries. Highly concentrated flavor "Coriander fragrance" was developed with fraction № 3 of essential oil coriander. "Coriander fragrance" can be used instead of the aromatic spirits of coriander seeds in liquors and spirits production for producing bitters, liquors and other drinks, e.g. vodkas "Gorilka", "Chernihivska", "Starokyivska". The flavors "Coriander fragrance" was used in processing for "Lollipop" candies, vodka special "Captain", dessert drink "Married couple".

Keywords: *Coriandrum sativum*; distillation; essential oil; fraction; flavor

INTRODUCTION

Today in the whole world there is an urge for a healthy and quality life. Due to this fact, products with natural components, including aromatic ones, are becoming more popular. As a result, the demand for the products with natural flavors is constantly increasing. Coriander (*Coriandrum sativum* L.), a member of the *Apiaceae* family, is among most widely used plant, possessing nutritional as well as medicinal properties. Many species of this genus can be used by medical practitioners and like as flavour of the food products.

The sources for natural flavor substances are aromatic and essential oil crops. The main essential oil crop in Ukraine is *Coriandrum sativum L.*, it occupies up to 80% of space for essential-oil plants, extensively cultivated in India, Russia, Central Europe, in some countries of Asia and Africa. So as essential oils and others flavors in food stuffs are used in small dozes, very important is exact to know their chemical compound at drawing up of compounding. From fruits of *Coriandrum sativum* 1,2% of essential oil, 17% of fatty oil and 65% of oilseed meal, which is a valuable source of secondary raw materials, are obtained. The leaves and fruits are highly fragrant and contain nutrients like fat, proteins, vitamins, minerals etc. Essential oil of *Coriandrum sativum* contains basic "key" components with floral geraniol and herbal linalool flavors. Oil contains a significant quantity of these components, and they have a wide interval of boiling points. Wide physiological properties of oil should be noted **(Matasyoh et al., 2009)**.

In Ukraine coriander is among most widely used medicinal and food plant. The leaves, seeds Coriandrum sativum and essential oils from them used as a spice for food industry. The dried fruits are extensively employed in Ukraine as a condiment, especially for flavouring of sauces and marinades, meat and fish products, bakery and confectionery items **(Korablova and Rakhmetov, 2012)**.

Research of **Peter (2004)** supports that some of these foods, as part of an overall healthful diet, have the potential to delay the onset of many age-related diseases, so there is urgent need to explore the role of these compounds. Coriander leaves are used as parsley like garnish with a fresh fragrance that is vital in, soups, and meat dishes because these are rich in vitamin A, B2 (riboflavin), C and dietary fiber. Salads are incredibly beneficial for weight conscious persons due to their lofty vitamins and fiber contents. The dried seeds contribute to pleasantly aromatic spice that is much used in stews, cuisine, sweet breads, sausages and cakes**.**

There is experience of using of seeds and essential oil *Coriandrum sativum* in different areas of industry **(Khan and Abourashed, 2011)** and medicine **(Garnik et al., 2003)**. An essential oil from leaves and fruits *C. sativum* L. showed biological, antifungal and antimicrobial activity **(Soares et al., 2012; Delaquis et al., 2002; Mandal and Mandal, 2015; Petrová et al., 2015)**, anti-hyperglycemic and diuretic activity **(Bhat, 2014)**.

We took into consideration the experience of medicine, perfume and cosmetic industry regarding fractional distillation of complex organoleptic blends, including those of natural origins **(Patent no. 20100197801, 2013)**.

Such actions are directed to purposefully single out one or several components as a source of flavor, or derivative valuable biologically active components of medicaments, or prophylactic remedies **(Krichkovskaya et al., 2008; Roshina, 2010; Brindza et al., 2013)**. In food productions, this technological method has not been used before.

Fruit and vegetable processing industry is very important for Ukraine regarding both the internal and the external market. The main objective was to investigate possibility effective utilisation of coriander essential oil in national economy of Ukraine. It was necessary to study the chemical compounds of coriander fruits by instrumental analysis and odor by sensory analysis with following creating new aroma compositions.

MATERIAL AND METHODOLOGY

In the researches, there was used three samples of the aromatic raw material collected in Kyiv region in 2008 – 2012 on the plots of National Botanical Garden named after N. N. Gryshko. The samples of *Coriandrum sativum* were examined according to the branch standards methods **(DSTU 4654: 2006)** upon the organoleptic and physical-chemical indications.

Essential oil from freshly ground coriander was allocated by distillation method **(Bondarenko, 2010)**.

The ground materials (200 g) were placed in a 1000 mL round bottom flask containing 250 mL distilled water to which two drops of antifoam were added.

The device also consisted a fridge, an essential oil catcher, and a bain-marie with saturated solution of calcium chloride. The contents were subjected to distillation for 2 h. The collected essential oil was dried over anhydrous Na_2SO_4.

Qualitative structure of essential oils was determined by the gas-liquid chromatography method on the chromatograph Agilent Technologies 6890 with mass-spectrometric detector 5973. The chromatographic column is capillary INNOWAX with an internal diameter of 0.25 mm and length of 30 m. The gas-carrier's speed (helium) is 1.2 $mL.min^{-1}$. Temperature of the thermostat was linearly programmed from 50 to 250 ºC with speed 4 $ºC.min^{-1}$. For identification of components there was used a library of mass-specters NIST 05 and WILEY 2007 with a general quantity of specters more than 470000 combined with the programs for identification AMDIS and NIST **(NIST/EPA/NIH 1994)**. The identification of the components under study was made at the mass-specters and component's retention times.

Fractioning of essential oil *Coriandrum sativum* was performed on a pilot universal automatic facility – DFD (Device of Fractional Distillation). This chromatographic method of investigation was developed in the research laboratory of the National University of Food Technologies **(Frolova et al., 2004)**. Short technical description: column type – three-section; number of real plates, pcs – 20; number of side-bars, pcs – 3; diameter of refractive part, mm – 30; head type – full condensation; regulation of the reflux ratio and temperature in a cube - from the control unit; control of temperature – automatic. Facility elements are made of inert material – heat-resistant glass produced by Simex.

RESULTS AND DISCUSSION

In the laboratory conditions, using water distillation on granulated fruitage of *Coriandrum sativum* there were received samples of essential oil. The research results of the quality indicators of essential oil *Coriandrum sativum* were compared with the basic norms (Table 1).

Obviously, the quality indicators of essential oil of Coriandrum sativum not exceed base values. Number of substandard and cracked fruit and mericarps was less permissible on the 24% and 22%. The dynamics of process distillation of essential oil was studied using the method of fractional distilled.

There was conducted fixing of the essential oil and by-products outflow during certain periods of time. Further, the outflow of essential oil on every distillation stage was calculated compared to its total output in each experiment (Table 2). After completion of the distillation 13.49% of the essential oil left in distilled water and 0.73% was irretrievably lost. Quality indicators of the received samples were checked for conformity with normative documents of Ukraine and are presented in Table 3.

The investigated qualitative composition and the mass

Table 1 Quality indicators of *Coriandrum sativum* fruits (%).

Quality indicator	Norms of quality indicators	Investigation results
Humidity	13.0	10.8 ±0.05
Waste	2.0	1.5 ±0.01
Substandard fruits and mericarps	10.0	7.6 ±0.05
Cracked mericarps	15.0	11.7 ±0.05
Admixtures of other aromatic plants	Is not acceptable	Not detected

Table 2 Material balance of receiving the *Coriandrum sativum* essential oil in the laboratory experiment.

Loaded			
Name of the raw material	**Mass (g)**	**Essential oil**	
		Content (%)	**Mass in the raw material (g)**
Ripe fruits of Coriandrum sativum	200	0.52	1.04

Received				
Name of the product	**Volume (mL)**	**Essential oil**		
		Content (%)	**Mass (g)**	**Outflow (%)**
Essential oil of *Coriandrum sativum*	-	-	0.890	85.78
Distilled water	1815.00	0.005	0.097	9.36
Depleted material	385.00	0.011	0.043	4.13
Irretrievable losses	-	-	0.008	0.73

Table 3 Physical-chemical index of quality of essential oil *Coriandrum sativum*.

External appearance	**Thickness at 20 °C ($g.cm^{-3}$)**	**Deflection indicator at 20 °C**	**Acid index (mg KOH) not more**	**Scent**
Yellow easily moveable liquid	0.870	1.460	1.35	Savory, fragrant with a floral tone

Table 4 Composition of essential oil *Coriandrum sativum.*

Reference compound	**Contents in essential oil (%)**	**Reference compound**	**Contents in essential oil (%)**
β- pinene	7.04 ±0.05	linalool	67.30 ±005
camphen	1.30 ±0.02	d- camphor	2.90 ±0.02
myrcene	2.49 ±0.03	linalylacetate	0.22 ±0.01
limonene	3.13 ±0.05	l-borneol	0.13 ±0.01
1,8-cineole	10.56 ±0.05	δ-terpineol	0.32 ±0.02
β-phellandrene	0.25 ±0.01	geraniol	1.42 ±0.05
n-cymene	0.17 ±0.05	geranilacetate	2.90 ±0.05

Table 5 Modes of fractionation of essential oil *Coriandrum sativum.*

No of fraction	**Temperature (°C)**			**Reflux ratio**	**Pressure (kPa)**
	In the cube	**In the column**	**Distillation waters**		
First	85…91	57…59	29…32	1:7	1.97
Second	90…94	57…66	33…49	1:8	1.32
Third	94…97	66…79	54…58	1:10	1.32
Fourth	97–114	79–94	60–76	1:10	1.32
Distillation residue	122				0.92

ratio of the components of the samples of essential oil *Coriandrum sativum* are shown in Table 4.

Modes of fractional distillation were based upon theoretical calculations, according to which the essential oil was considered as a sum of binary mixtures **(Ukrainec' and Frolova, 2009)**. Model calculations were adequately coordinated with the results of real distillations. The optimized operating modes of distillation of essential oil Coriandrum sativum are listed in Table 5.

On the results of distillations 4 fractions and a distillation residue were received. Alternately, as the accumulation process is going on, all fractions are collected in sealed glass capacities. In Table 6 the material balance of the essential oil distillation in DFD (Device of Fractional Distillation) is shown (calculated as 3.5 kg per 1 charge).

Changing the content of essential oil components in the different fractions has a significant effect on the sensory characteristics of odor, because each component has its original flavor and odor. The major descriptors of *Coriandrum sativum* odor were pleasant, herbal, green, cooling, earthy, rose-like, sweet and floral aroma with turpentine note.

Table 6 Material balance distillation of coriander essential oil with DFD.

Loaded		Received		
Name	**Weight (kg)**	**Name of the received product**	**Product weight**	
			kg	**% from initial.**
Essential oil Coriandrum sativum	3.5	Fraction 1	0.184	5.30
		Fraction 2	0.252	7.20
		Fraction 3	0.400	11.40
		Fraction 4	2.118	61.20
		Distillation residue	0.441	12.50
		Losses	0.105	2.40

Table 7 The composition of fractions essential oil of *Coriandrum sativum*.

No of fractions	Blend composition of fractions	Aromatic properties
Essential oil of *Coriandrum sativum*		Yellow liquid with a savory floral note
1	α -pinene, camphene, myrcene	Liquid of intense yellow color with a pine note
2	β –phellandrene, n–cimol, d-limonene	Liquid of intense yellow color with a well scented lemon note
3	borneol, camphor	Liquid of yellow color with a sharp camphoraceous scent
4	l-linalool, linalyl acetate	Liquid of slightly yellow color with a specific note of lily of the valley
Distillation residue	terpineol, geraniol, geranylacetat	Liquid of slightly yellow color with a rose fragrance

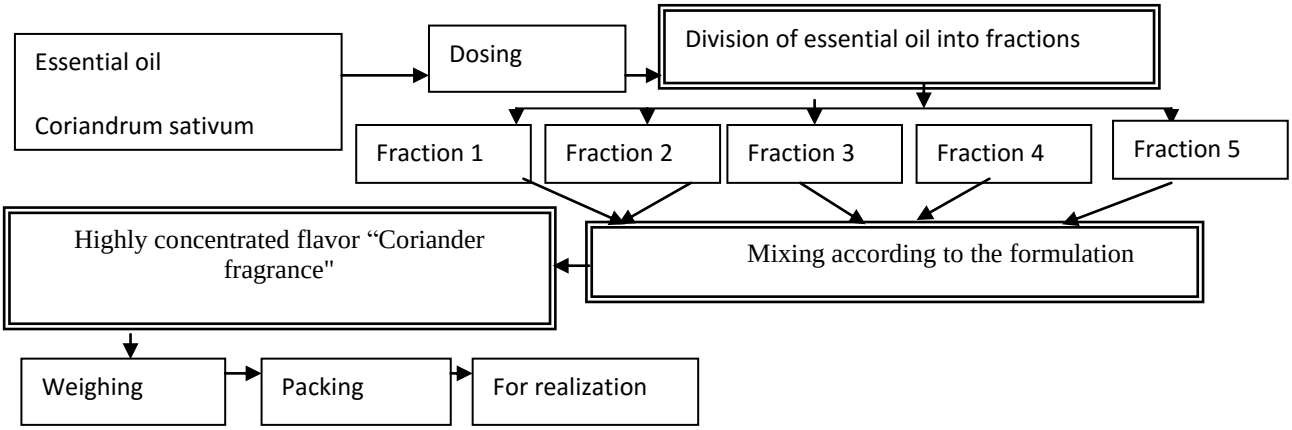

Figure 1 The scheme of receiving a highly concentrated flavor “Coriander fragrance”.

Table 8 Organoleptic indicators of special vodka “Captain”.

Indicator	Value
External appearance	Transparent liquid
Color	Colorless
Taste	Warm, a bit savory, typical of vodka
Scent	Harmonious accord of honey with field flowers

The given data show that the outflow of the fractions constitutes 97.6% from the initial raw material. Losses are estimated at 2.4% (incomplete capture of essential low-boiling components, losses occurring during column flooding). From each fraction, after thorough mixing, the average sample of the product was selected. A qualitative composition was defined in it by means of gas chromatography, and aromatic properties – by generally accepted and standardized methods (State standard 2729-94) (Table 7).

Descriptors and reference compounds were used for sensory profiling of essential oil.

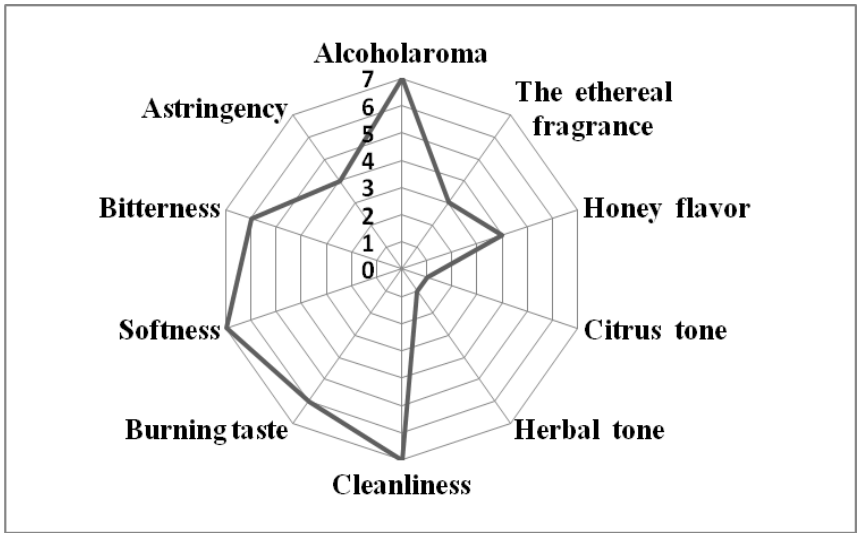

Figure 2 Sensory profilogram of flavor vodka special "Captain".

Every received fraction of *Coriandrum* essential oil is an independent highly concentrated natural flavor agent with stable physicochemical characteristics. It should be noted that changing the process modes leads to changes in the fraction structure.

Besides that, fractioning allows to exclude from fraction constitution those components that worsen organoleptic, physical, chemical and functional properties of a flavor including solubility in liquid mediums, and the expiration date. It is harder to single out such components using other methods **(Sarker et al., 2005)**.

It is advised to use fraction 3 of essential oil *Coriandrum sativum* for "Lollipop" candies with original savory scent. Besides a nice taste and scent, lollipops are advised to be used for prophylaxis of bronchial diseases. The formulation of aromatic component of "Lollipop" candies consists of 10% alcohol solvent: 3 fractions of coriander essential oil – 59%; 3 fractions of fennel essential oil – 29%; 4 fractions of cat mint essential oil – 6%; 2 fractions of clary sage essential oil – 6%.

During the development of flavor for non-alcoholic drinks of immunostimulatory action with a harmonious flavor of lemon floral tone, 10% alcohol solution components were used: 4 fractions of coriander essential oil – 62%; 3 fractions of fennel essential oil – 31%; 5 fractions of cat mint essential oil – 6%.

Apart from receiving flavored products that have formulations which involve fractions of various essential oils, model combining of fractions of essential oil *Coriandrum sativum* was used, and as a result there was developed a formulation of a highly concentrated flavor – "Coriander fragrance".

The scheme of receiving the flavoring is shown in Figure 1.

To receive flavor "Coriander fragrance" fractions of essential oil Coriandrum sativum were mixed in the following quantities, g: the first fraction – 0.81 g, the second one – 1.7 g, the third fraction – 4.37 g, the fourth fraction – 36.61 g, the fifth fraction – 1.70 g, which corresponds to the mass ratio 1:2:3:4=1.0 : 2.1 : 5.4 : 44.4 : 2.1.

The flavor "Coriander fragrance" is a slightly yellow liquid with a specific pure scent of coriander. Floral, rose-like, pleasant, green, herbal, cooling, earthy, spicy, sweet and were the major descriptors of coriander aroma.

The developed flavor can be advised for usage in the production of vodka, brandy, whisky, rum, and other strong alcoholic as well as low alcoholic and non-alcoholic drinks. "Coriander fragrance" can be used instead of the aromatic spirits of coriander seeds in liquors and spirits production for producing bitters, liquors, and other drinks, e.g. "Gorilka", "Chernihivska", "Starokyivska".

Together with a team of authors there was developed a formulation of special vodka "Captain" **(Patent Ukrainy. *Gorilka osoblyva «Kapitan*, 2011)** which by its aromatic palette has a harmonious combination of a honey citrus scent with the fragrance of field flowers.

In table 8 the quality characteristics of vodka "Captain" are shown.

In the formulation, a composition of flavors "Coriander fragrance" and "Fennel - elite fragrance" are used (1.6 : 0.9).

Adding of orange and star-anise essential oils creates a taste impression of an exotic citrus (1.63 : 0.10). Orange oil can be substituted with aromatic spirit, and star-anise essential oil - with the anise one. The expiration date of the drink is 6 months.

Figure 2 shows the sensory profilogram of flavor vodka special "Captain".

Dessert drink "Married couple" is made by mixing rectified ethyl spirit of the highest cleaning, drinking water, citric acid, sugar in the form of 65,8% sugar syrup, apple fortified juice, flavors, lemon essential oil **(**State Intellectual Property Service of Ukraine **Patent of Ukraine. *Napii desertnii «Podruzhzhya»*)**.

CONCLUSION

Coriandrum sativum fruits are the main industrial essential-oil raw material in Ukraine. Its consecutive processing by means of physical procedures into the essential oil, aromatic fractions extenda natural flavors assortment of a savory floral note. Monitoring of the fractioning process allows concentrating the key aromatic components and receiving highly concentrated flavors of original pure notes. Results of the study indicate the possibility to combine the components of the fractions for use in the product to provide the desired flavor notes. The flavors not only give products a special scent, but also are

characterized by the orientation of the physiological action; saturation, and improved stability. Application flavour enables to expand assortment of developed products to improve them tasting properties.

REFERENCES

Bedi, S., Tanuja, Vyas, S. P. 2010. *A handbook of aromatic and essential oil plants (Cultivation, chemistry, processing and uses)*. Jodhpur : Agrobios, 598 p. ISBN 8177543415 / ISBN 13: 9788177543414.

Bhat, S., Kaushal, P., Kaur, M., Sharma, H. K. 2014. Coriander (*Coriandrum sativum* L.): Processing, nutritional and functional aspects. *African Journal of Plant Science*, vol. 8, no. 1, p. 25-33. http://dx.doi.org/10.5897/AJPS2013.1118

Bondarenko, E. Y. 2010. *Improving the technology of processing fruits coriander by steam distillation method using physicochemical methods* : dissertation theses. Krasnodar, Russia: Kuban State University of Technology. 26 p.

Brindza, J., Kucelová, L., Sinica, A., Stehlíková, B., Brindza, J., Kucelová, L., Sinica, A., Stehlíková, B., Čuláková, M. 2013. Morphological, biochemical and sensory characteristics of black mulberry fruits (*Morus nigra* L.), *Potravinarstvo*, vol. 7, no. 1, p. 36-44. http://dx.doi.org/10.5219/234

Chernogorod, L. B., Vinogradov, B. A. 2006. Essential oils of some species of the genus *Achillea* L., containing fragranol. *Plant resources*, vol. 42, no. 2, p. 61-68.

Delaquis, P. J., Stanish, K., Girard, B., Mazza, G. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *International Journal of Food Microbiology*, vol. 74, no. 1-2, p. 101-109. http://dx.doi.org/10.1016/S0168-1605(01)00734-6

DSTU 4654: 2006. *Oliya efirna koriandrova – Requirements.*

DSTU 2729-1994. *Essential oils and products of essential oil production – Requirements.*

Frolova, N. E., Usenko, V. O., Chepel', N. V., Kubrak, T. V. 2004. Development of method of chromatographic analysis of essential oils with using capillary columns. *Harchova promislovist'*, vol. 3, p. 80-82.

Garnik, T. P., Dudchenko, L. G., Shuraeva, T. K., Barsukov, A. V. 2003. Medical-biological aspects using of essential oils. *Fitoterapiya. Chasopis*, vol. 1-2, p. 52-55.

Khan, I. A., Abourashed, E. A. 2010. *Leung's encyclopedia of common natural ingredients: used in food, drugs and cosmetics*. 3rd ed. Hoboken, New Jersey : John Wiley and Sons, 845 p. ISBN 978-0-471-46743-46744.

Korablova, O. A., Rahmetov, D. B. 2012. *Useful plants in Ukraine. From the introduction to using.* Kyiv : Fitosociocentr. 171 p. ISBN 978-966-306-160-2.

Krichkovskaya, L. V., Chernyshov, S. I., Donchenko, G. V., Zhukov, V. I., Staroverov, V. N., Naumenko, E. N. 2008. The biologically active oils and products based on them (essential oils, carotenoids, and others.). Biology. Biochemistry. Application. Methods for determination. Harkov : Model' Vselennoi. 267 p. ISBN 978-966-7875-26-8.

Mandal, S., Mandal, M. 2015. Coriander (*Coriandrum sativum* L.) essential oil: Chemistry and biological activity. *Asian Pacific Journal of Tropical Biomedicine*, vol. 5, no. 6, p. 421-428. http://dx.doi.org/10.1016/j.apjtb.2015.04.001

Matasyoh, J. C., Maiyo, Z. C., Ngure, R. M., Chepkorir, R. 2009. Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum*. *Food Chemistry*, vol. 113, no. 2, p. 526-529. http://dx.doi.org/10.1016/j.foodchem.2008.07.097

Nadeem, M, Anjum, F. M., Khan, M. I., Tehseen, S., El-Ghorab, A., Sultan, J. I. 2013. Nutritional and medicinal aspects of coriander (*Coriandrum sativum* L.). *British Food Journal*, vol. 115, no. 5, p. 743-755. http://dx.doi.org/10.1108/00070701311331526

NIST/EPA/NIH Mass Spectral Data Base. 1994. PC Version 4.5, U.S. Gaithersburg, MD. *Natural Products Isolation, Methods in Biotechnology*. 2006. vol. 20, 2 nd ed. Edited by: S. D. Sarker, Z. Latif, and A. I. Gray. Totowa, NJ : Humana Press Inc., p. 529. ISBN-1-59259-955-9.

Peter, K. V. 2004. *Hand Book of Herbs and Spices.* Cambridge : CRC Press,Woodhead Publishing Ltd. vol. 2, 360 p. ISBN: 978-0-85709-039-3.

Petrová, J., Terentjeva, M., Puchalski, C., Hutková, J., Kántor, A., Mellen, M., Čuboň, J., Haščík, P., Kluz, M., Kordiaka, R., Kunová, S., Kačániová, M. 2015. Application of Lavender and Rosemary essential oils improvement of the microbiological quality of chicken quarters. *Potravinarstvo*, vol. 9, no. 1, p. 530-537. http://dx.doi.org/10.5219/550

Roschina, N. N. 2010. Applied aspects of using of essential oils and terpenes in food. *Vestnik Altaiskogo gos. tehn. un-ta*, vol. 38, p. 387.

Soares, B. V., Morais, S. M., Fontenelle, R. O. S., Queiroz, V. A., Vila-Nova, N. S., Pereira, C. M. C., Brito, E. S., Neto, M. A. S., Brito, E. H. S., Cavalcante, C. S. P., Castelo-Branco, D. S. C. M., Rocha, M. F. G. 2012. Antifungal activity, toxicity and chemical composition of the essential oil of *Coriandrum sativum* L. fruits. *Molecules*, vol. 17, no. 7, p. 8439-8448. http://dx.doi.org/10.3390/molecules17078439 PMid:22785271

State Intellectual Property Service of Ukraine. *Gorilka osoblyva «Kapitan»*. Patent owner: National university of food technology. MPK C12 G3/06. Ukraine. Patent no. 93835. 2011-03-10.

State Intellectual Property Service of Ukraine. *Napii desertnii «Podruzhzhya».* Patent owner: National university of food technology. MPK C12 G3/00. Ukraine. Patent no. 56195. 2011-01-10.

McLafferty, F. W., Stauffer, D. B. 1989. *The Wiley/NBS Registry of mass spectral data.* New York: John Wiley & Sons.

Ukrainec', A. I., Frolova, N. E. 2009. The theoretical justification settings fractional distillation of essential oils. *Harchova Nauka i Tehnologiya*, vol. 2, no. 7, p. 54-58.

US Patent and Trademark Office. Volatile distillate by-product of mint oil that promotes absorption and/or bioavailability of compounds of bio-medical and nutritional interest. Patent owner: Finley, J. W., Finley, J. W., Mezine, I., Kehrer, J. M., Justice, R. J., Int. Cl. A61K31/122. US. Patent no. 20100197801, 2009-10-28.

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FRUIT AND VEGETABLE INTAKE AMONG COLLEGE STUDENTS IN NITRA – COMPARATIVE STUDY

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ABSTRACT

The aim of the study was to collect and analyse the frequency of fruit (fresh, dried, canned and nuts) and vegetable (fresh, tinned, legumes, soya) consumption in the group of 242 respondents aged 19 – 22 years-students of Constantine the Philosopher University in Nitra; to evaluate differences according to field of study and language in which they study (Hungarian or Slovak) by questionnaire method. On the base of collected data it can be concluded that in general the consumption of fresh fruits and vegetables can be considered as very low (only once a day) together with canned and dried fruit (nuts) and tinned vegetable (rarely). Furthermore, the majority of respondents took legumes only 1 – 3 times a week or rarely and soya had never been consumed. The statistically significant differences between college students of PEEH and the rest of assayed group of students had not been confirmed so the higher level of knowledge in health has not been connected with the higher consumption of fruits and vegetables. On the other hand, statistically significant differences have been proved between the following assayed groups of university students: RTH ↔ RTS (χ 7.90, p <0.05), J ↔ RTH (χ 9.99, p <0.05), J ↔ RTS (χ 10.00, p <0.05), J ↔ PEES – SK (χ 9.91, p <0.05). Statistically significant differences were assayed also in consumption of dried fruits or nuts among the following field of study: J ↔RTS (χ 9.48, p <0.01), RTH ↔ RTS (χ 12.57, p <0.05), RTS↔PEES (χ 8.19, p <0.01). Consumption of fresh vegetables was statistically different between the students J↔RTS (χ 9.95, p <0.05) and RTS ↔PEES (χ 8.19, p <0.01).

Keywords: fruit consumption; vegetable consumption; college student; nut; soya; dietary habit

INTRODUCTION

Western countries lifestyle increase the risk for premature development of chronic diseases (cardiovascular, diabetes, metabolic syndrome, osteoporosis, cancer) **(Henauw et al., 2007)**. Poor dietary habits, sedentary leisure time spending and a lack of physical activity are lifestyles that – once installed – have a strong tendency to track from childhood into adulthood and then become extremely resistant to modification **(Koplan et al., 2005)**. This fact has led to increasing interest in studying the dietary habits of pupils, students and most often adolescents **(Babinska et al., 2007, 2008 and Bašková, 2011)**. Unfortunately, the population of college students who present connection between the students and adults has been much less studied in last years than other population groups. College students differ in irregular eating patterns, frequent snacking and frequent skipping of meals, consumption of fast foods **(Juríková and Viczayová, 2014)**, overuse consumption of energetic drinks and caffeine **(Balla et al., 2013)**. According to results of marketing study of **Pap et al. (2012)** 1/3 of students of secondary schools have changed eating habits towards unhealthy foods at university. The most comprehensive study including 3172 respondents from Slovakia has been provided by **Stefanikova et al. (2003).** They examined the changes in eating habits of college students of medicines during 1992 – 2002. The result of the study has confirmed the positive increase in consumption of legumes (including soya) by 32%, on the other hand decrease in consumption of fruit by 25%. Comparative study of **Kimáková et al. (2011)** of eating habits of college students of medicine and lawyers aged 21-26 years had not proved the statistically significant differences between them and focused on high consumption of fast food resulted in problems with digestion.

The regular intake of fruit and vegetables is given by socio – demographic variables (age, gender and socio – economic status) **(Aranceta et al., 2003; Moreno et al., 2008 a,b; Currie et al., 2008)**, family background **(Bere et al., 2008; Friel et al., 2005; Cooke et al., 2004)**. Knowledge level about health promoting effect of fruit and vegetables is considered to be the most important factor that positively influences the regular consumption of fruit and vegetables **(Cooke et al., 2004)**. Certainly, the school play significant role in formation of positive attitude to regular consumption of fruit and vegetables **(Story et al., 2008)** by programmes and activities **(French and Wechsler, 2004)**.

Understanding the factor of education and defining population of college students with the least healthy food habits (at greatest risk) has great importance for the development of relevant interventions, programmes and policies.

MATERIAL AND METHODOLOGY

The questionnaire was designed to determine intake frequency of fruits and vegetables among 242 respondents – students of Constantine the Philosopher University in Nitra during 2013 – 2014 years. The university students aged 19 – 22 year had the following distribution according to field of study: Pre-school and elementary education in Hungarian language (PEEH n = 32), Pre-school and elementary education in Slovak language (PEES n = 55), Journalism (J = 38), Regional Tourism in Hungarian language (RTH n = 45) and Regional Tourism in Slovak language (RTS n = 72). Students were asked for the filling the questionnaire in fruit (fresh, dried or canned) and vegetable consumption (fresh, tinned, legumes and potatoes) and chose the frequency: 3 and more times a day, daily, 1 – 3 times a week, rarely or never. Because of the fact that 200 asked students were female gender, statistical evaluation of frequency of fruit, vegetable and nuts consumption was provided only according to field of study and language of study (Hungarian – Slovak) by the method of χ square statistic on the level of probability p = 0.05 resp. 0.01 in the statistical programme STATGRAPHIC. Among the assayed groups only the students of PEEH have incorporated the health education into the study programme as subjects: Health education, Nutrition of children, Human and environment, Movement and Health. So we supposed that there would be statistically significant differences between students of PEEH and students of the rest field of study.

RESULTS AND DISCUSSION

Our study was aimed at mapping of consumption of fruits and vegetables of 242 college students on the base of field of study and evaluate if health education had statistically significant importance on the regular consumption of fruits and vegetables.

Consumption of fruit (fresh or processed) is 51.9 kg/capita/year that is lower by 53.7% in comparison with recommended value (96.7 kg/capita/year) **(Habánová, 2012)**.The majority of university students indicated to eat fruit in fresh form only once a day (RTH 28.9% – RTS 47.2%) (Figure 1).

This finding is in accord with study of **Kimáková et al. (2011**) in which the 25.3% medicinal students and 27.2% lawyers and 61% students of Slovak Agriculture University **Sramkova (2001)** consumed fruit only once a day. More negative trend has been confirmed in study **Fatrcova-Šramková et al. (2010)** evaluated the eating habits of Slovak population in which 62% of women consumed the fresh fruit only three times a weeks. Similarly, in the study evaluated 145 college students of Slovak Agriculture University 34.6% consumed fresh fruit only 1 – 3 times a week **(Kopčeková and Kolesárová, 2009)**. Moreover, the consumption of canned and dried fruit is lower with prevailing frequency only rarely (RTH 46.7% - PEEH 53.1%; PEES 31% - PEEH 59.4%) (Figure 2 and Figure 3).

Consumption of vegetables (fresh or processed) in Slovakia represents 101.5 kg/capita/year that is lower value by 20.4% than recommended amount **(Habánová, 2012)**. On the basis of analysed dates this trend has been confirmed too, only the students of RTS ate 3- more portion a day (40.3%). The majority of students preferred consumption only once a day (J 22.2% - PEEH 50.0 %) (Figure 4).

Results are in accord with study of **Kimakova et al. (2011)** in which the 24% of medicinal students and 17.5% lawyers consumed the fresh vegetable only once a day. The study of **Fatrcova-Šramkova et al. (2010)** also confirmed that the adult Slovak population aged 25 – 75 years preferred to consume fresh vegetable only once a

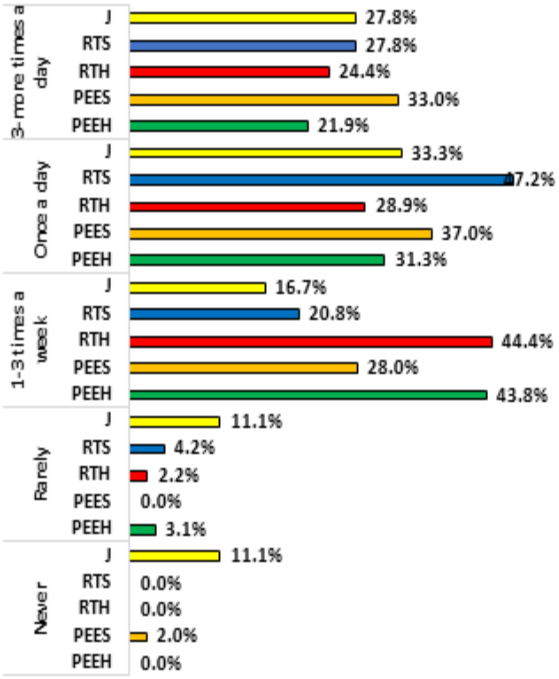

Figure 1 Evaluation of frequency of fresh fruit consumption among college students.

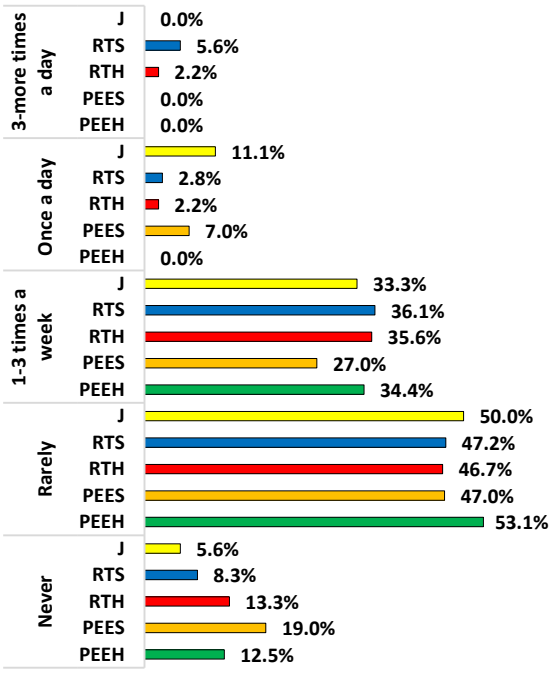

Figure 2 Evaluation of frequency of canned fruit consumption among college students.

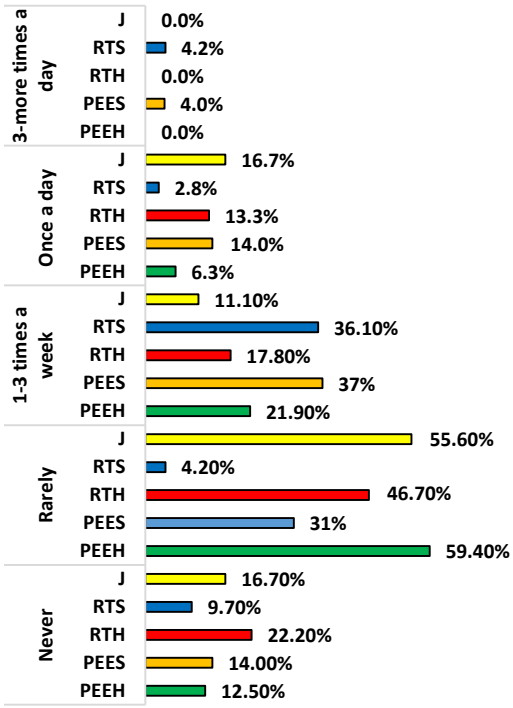

Figure 3 Evaluation of frequency of dried fruit consumption among college students.

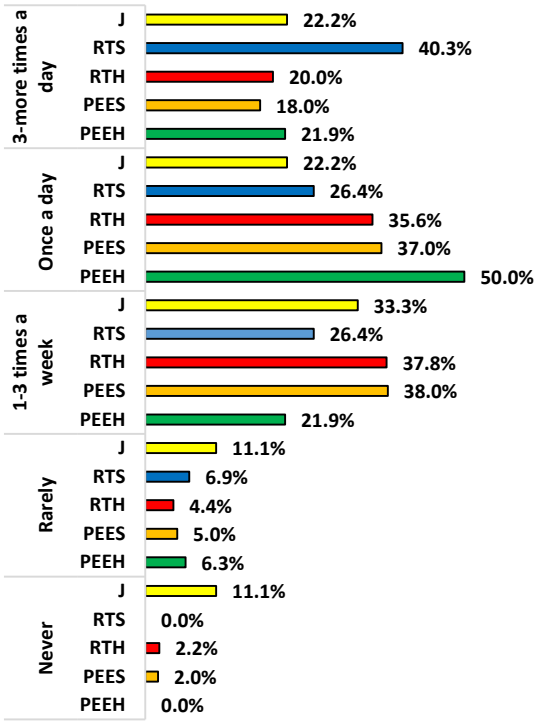

Figure 4 Evaluation of frequency of fresh vegetable consumption among college students.

week (57.47%). In the study of **Kopčeková and Kolesárová (2009)** examined 145 college students of Slovak Agriculture University and found out that the prevalence of them 36.6% consumed fresh vegetable 1 – 3 times a week that is in accordance with assayed students of PEES and PEEH. Tinned vegetable was consumed in lower amount, the majority of students chose answer only rarely (RTS 41.7% – J 61.1%) (Figure 5).

Cognizance of healthy food have been associated with positive attitudes towards healthy eating habits but there has not been significant differences between the people

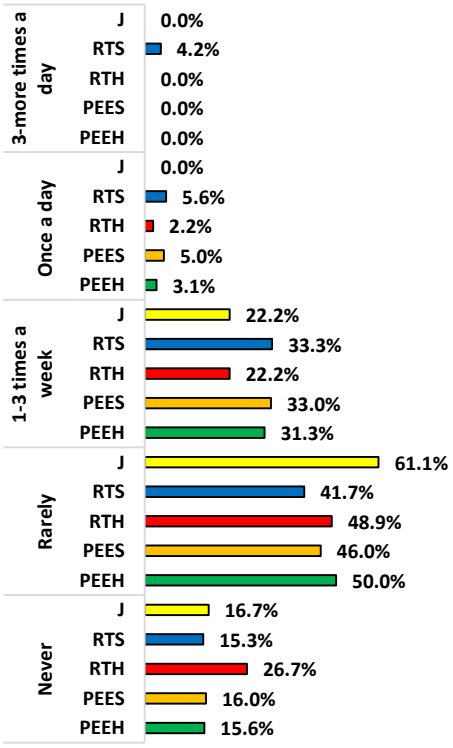

Figure 5 Evaluation of frequency of tinned vegetable consumption among college students.

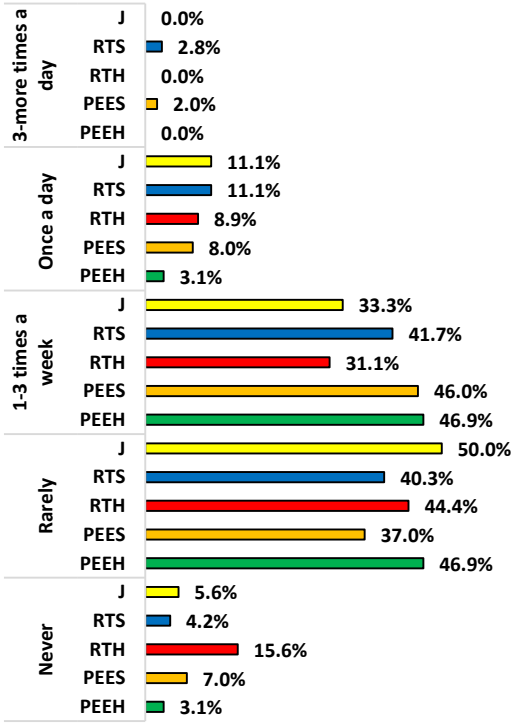

Figure 6 Evaluation of frequency of legumes consumption among college students.

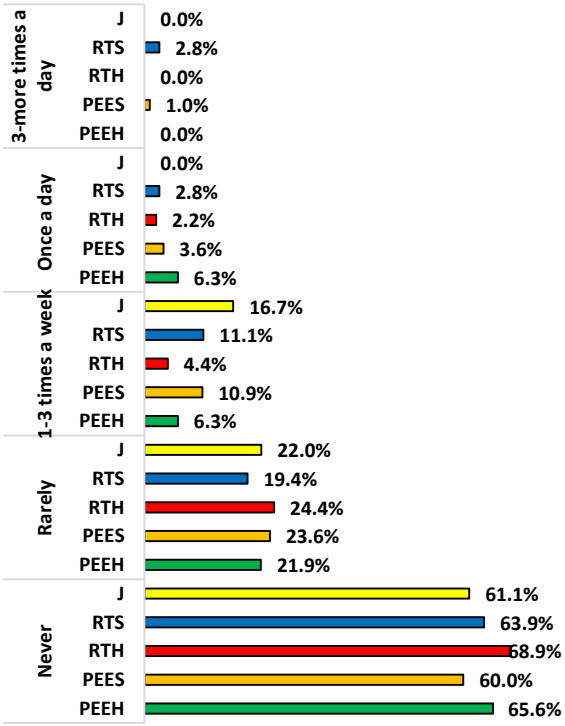

Figure 7 Evaluation of frequency of soy based products consumption among college students.

with higher and lower levels of education **(Herath et al., 2008; Urala and Lähteenmäki, 2007; de Jong et al., 2003; Verbeke, 2005)**. In the same way, the results of statistical evaluation pointed to the fact that there had not been significant differences in fruit consumption (fresh, dried or canned) and nuts, vegetable (fresh, tinned, legumes) among students with field of study PEEH ↔ J, PEEH ↔ RTH, PEEH ↔ PEES and RTH ↔ PEES. So the hypothesis that the students of PEEH (have cognizance of healthy eating) will have consumed higher amount of fruit and vegetable and distinguished from another group of students can be refused. Our results are in accord with study of **Barath et al. (2014)** who were not noticed significant differences in fruit and vegetable consumption between the students of PEEH and PEES. In the similar way, **Kimáková et al. (2011)** has not found statistically significant differences in fruit and vegetable consumption between the medical students and lawyers. This fact can be given by trend to choose food according to taste preference. Children have not consumed enough fruit and vegetable despite the fact that they have cognizance of positive effect of consumption of fruit and vegetable as it has been proved by HELENA study. The young people understood the importance of healthy eating and knew they did not always eat as well as they should. To them, the problem with 'healthy' food including fruit and vegetable was that it is boring and does not taste very nice **(Gilbert et al., 2007)**. In another marketing study realised in Hungary by **Pap et al. (2012)** 75% of respondents (college students) had cognizance of health promoting effect of fruit and vegetable but they preferred to consume sweets or sugared drinks. By contrast, statistically significant differences in fresh fruit and vegetable consumption have been proved between the following assayed groups of university students: RTH ↔ RTS (χ 7.90, p <0.05), J ↔ RTH (χ 9.99, p <0.05), J ↔ RTS (χ 10.00, p <0.05), J ↔ PEES – SK (χ 9.91, p <0.05).

Statistically significant differences were assayed also in consumption of dried fruits or nuts among the following field of study: J ↔RTS (χ 9.48, p <0.01), RTH ↔ RTS (χ 12.57, p <0.05), RTS↔PEES (χ 8.19, p <0.01). Consumption of fresh vegetables was statistically different between the students J↔RTS (χ 9.95, p <0.05) and RTS ↔PEES (χ 8.19, p <0.01).

In comparison with acceptable interval of rational consumption 2.1 – 3.2 kg it is necessary to increase the consumption of leguminous plants at least by 0.5 kg because in Slovakia the consumption of legumes has been very low in the long term **(Habánová, 2012)**. According to achieved dates (Figure 6) the consumption of legumes was not efficient only1-3 times a week PEEH (46.9%) or rarely (PEES 37% – J 50.0%). Similarly, **Fatrcova-Šramková and Gregušová (2009)** examined the eating habits of 392 pupils from elementary schools in Nitra found out that 1/4 of girls and 1/5 of boys consumed legumes only twice a week. Our findings are also in accord with study of **Fatrcova-Šramkova et al. (2010**) in which from 400 adults aged 25 – 75 years only 35% consumed legumes once a week. This negative trend was confirmed by **Jurkovičova (2005**) within study in Slovakia found out that 1/3 respondents consumed legumes rarely or never. According to study of eating habits of pupils of primary school the biggest problem was recognized in consumption of legume in Czech Republic **(Tláskal et al., 2012)** that can be given by their indistinctive taste.

On the basis of collected data we can conclude that the frequency of soya consumption is very low (Figure 7). The majority of respondents chose answer that they have never consumed soya (PEES 60% – RTH 68.9%) or rarely (19.4% RTS – 24.4% RTH). Our results are in contrast with study of **Stefanikova et al. (2003**) examined the increase in legumes and soya consumption among college students during ten years. **Čurlej et al. (2015)** studied the phytoestrogens dietary intake from Middle – North Slovakia region and found out that no respondents aged 50 – 60 years old utilised soya as important source of phytoestrogens. The statistically significant differences in soya consumption were confirmed between PEEH ↔ RTS (χ 8.82, p <0.01); RTH ↔ RTS – SK (χ 12.56, p <0.05).

CONCLUSION

The frequency of fruits and vegetables was measured by questionnaires completed by college students aged 19 – 22 years old at Constantine the Philosopher University in Nitra. Based on achieved results, fruits and vegetables were the most popular in fresh form but they did not meet the recommended daily requirement of five or more portion. Generally, the majority of participated students consumed only one portion of fresh fruit and one portion of fresh vegetable a day. The legumes and dried fruit with nuts have been consumed only rarely and soy hasn't ever. This trend can be considered as negative in eating habits of college students. There has not been proved statistically significant differences among students of PEEH and the rest of evaluated field of study, so in our study the significance of higher level of knowledge of this group of students has not been confirmed. So we can conclude that incorporation of subjects in healthy lifestyle in the group

of university students is not sufficient enough. The health education must present continual process from childhood with support of family followed by preschool and school education. Unfortunately the health education has not been incorporated into state education programme as obligatory subject in Slovakia. So there has not been adequate space for systematic and regular acquirement of healthy behaviour. Similarly, the significance influence of language in which they studied the programme has not been confirmed expressly. According to statistic evaluation there has been proved statistically significant differences between the following assayed groups of college students: RTH ↔ RTS (χ 7.90, $p < 0.05$), J ↔ RTH (χ 9.99, $p < 0.05$), J ↔ RTS (χ 10.00, $p < 0.05$), J ↔ PEES – SK (9.91, $p < 0.05$).

Statistically significant differences were assayed also in consumption of dried fruits or nuts among the following field of study: J ↔RTS (χ 9.48, p <0.01), RTH ↔ RTS (χ 12.57, p <0.05), RTS↔PEES (χ 8.19, p <0.01). Consumption of fresh vegetable was statistically different between the students J↔RTS (χ 9.95, p <0.05) and RTS ↔PEES (χ 8.19, p <0.01).

REFERENCES

Aranceta, J., Pèrez-Rodrigo, C., Ribas, L., Serra-Majem, L. 2003. Sociodemographic and lifestyle determinants of food patterns in Spanish children and adolescents: the enKid study. *European Journal of Clinical Nutrition*, vol. 57, suppl. 1 no., p. 40-44. http://dx.doi.org/10.1038/sj.ejcn.1601813 PMid:12947451

Babinská, K., Vitáriušová, E., Hlavatá, A., Rosinský, J., Babinská, K. M., Košťálová, P., Pribilincová, Z., Kovács, L. 2008. Stravovacie návyky žiakov základných škôl na Slovensku. In: *Nové trendy vo výžive detí*. Bratislava: LF UK v Bratislave, s. 17-28. ISBN 978-80-223-2430-4.

Babinská, K., Vitáriušová, E., Rosinský, J., Babinská, K., Košťálová, P., Hlavatá, A., Pribilincová, Z., Kovács, L. 2007. Stravovací režim školákov na Slovensku. *Pediatria pre prax*, vol. 4, p. 217-220.

Balla, Š., Juríková, T., Rop, O., Mlček, J. 2013. Hodnotenie konzumácie vybraných zdrojov antioxidantov rastlinného pôvodu školákov z Nitrianskeho kraja, 1. vyd., Nitra: FSŠ, Europica variatas č. 9, 360 p. ISBN 978-80-558-0441-5.

Bašková, L. 2011. Konzumácia zeleniny In: *Sociálne determinanty zdravia školákov*, HBSC-Slovensko 2009-2010, 2011, 32 p.

Bašková, L. 2011. Konzumácia ovocia In: *Sociálne determinanty zdravia školákov*, HBSC-Slovensko 2009-2010, 31 p.

Bere E, Brug, J., Klepp, K. I. 2008. Why do boys eat less fruit and vegetables than girls? *Public Health Nutrition*, vol. 11, no. 3, p. 321-325. http://dx.doi.org/10.1017/S1368980007000729 PMid:17666125

Cooke, L. J., Wardle, J., Gibson, E. L., Sapochnik, M., Sheiham, A., Lawson, M. 2004. Demographic, familial and trait predictors of fruit and vegetable consumption by pre-school children. *Public Health Nutrition*, vol. 7, no. 2, p. 295-302. http://dx.doi.org/10.1079/PHN2003527 PMid:15003137

Čurlej, J., Židek, R., Belej, Ľ., Zajác, P., Čapla, J. 2015. Phytoestrogens dietary intake and health status of retiree from middle – north Slovakia region, *Potravinarstvo*, vol. 9, no. 1, p. 573-579. http://dx.doi.org/10.5219/572

Currie, C., Molcho, M., Boyce, W. Holstein, B., Torsheim, T., Richter, M. 2008. Researching health inequalities in adolescents: the development of the Health Behavior in School-Aged Children (HBSC) family affluence scale. *Social Science and Medicine*, vol. 66, no. 6, p. 1429-1436. http://dx.doi.org/10.1016/j.socscimed.2007.11.024 PMid:18179852

De Henauw, S., Gottrand, F., De Bourdeaudhuij, I., Gonzalez-Gross, M., Leclercq, C., Kafatos, A., Molnar, D., Marcos, A., Castillo, M., Dallongeville, J., Gilbert, C., Bergman, P., Widhalm, K., Manios, Y., Breidenassel, C., Kersting, M., Moreno, L. A. 2007. Nutritional status and lifestyles of adolescents in a public health perspective. The HELENA Project - Healthy Lifestyle in Europe by Nutrition in Adolescence. *Journal of Public Health*, vol. 15, no. 3, p. 187-197. http://dx.doi.org/10.1007/s10389-007-0107-3

De Jong, N., Ocké, M. C., Branderhorst, H. A. C., Friele, R. 2003. Demographic and lifestyle characteristics of functional food consumers and dietary supplement users. *British Journal of Nutrition*, vol. 89, p. 273-281. http://dx.doi.org/10.1079/BJN2002772 PMid:12575912

Fatrcová-Šramková, K., Chlebo, P., Dudriková, P. 2010. Risk in Nutrition Habits of Slovak Population, *Ecological Chemistry and Engineering*, vol. 17, no. 1, p. 21-33.

Fatrcová-Šramková, K., Gregušová, A. 2009. Negatives in nutritional habits of students from secondary schools (In Slovak), *Potravinarstvo*, vol. 3, no. 3, p. 67-70.

French, S. A., Weschsler, H. E. 2004. School-based research and initiatives: fruit and vegetable enviroment, policy, and pricing workshop. *Preventive Medicine*, vol. 39, suppl. no 2, p. 101-107.

Friel, S, Newell, J., Kelleher, C. 2005. Who eats four or more servings of fruit and vegetables per day? Multivariate classification tree analysis of data from the 1998. Survey of Lifestyle, Attitudes and Nutrition in the Republic of Ireland. *Public Health Nutrition*, vol. 8, no. 2, p. 159-169. http://dx.doi.org/10.1079/PHN2004670 PMid:15877909

Gilbert, C. C., Sanchez, M. J., Lehoux, C., Hegyi, A., Astrom, A., Hall, G., Merino, G., Masson, A., Fontaine, L., Kuti, T. 2007. Qualitative research investigating food choices and preferences of adolerscents in Europe. Abstract and poster on behalf of the Helena study group. [online] 2016-06-07. [cit. 2016-06-07] Available at: www.helenastudy.com.

Habánová, M. 2012. Nutričná epidemiológia, 1.ed., Nitra: SPU, 158 p. ISBN 978-80-552-0872-5.

Herath, D., Cranfield, J., Henson, S. 2008. Who consumes functional foods and nutraceuticals in Canada? Results of cluster analysis of the 2006 survey of Canadians' Demand for Food Products Supporting Health and Wellness. *Appetite*, vol. 51, no. 2, p. 256-265. http://dx.doi.org/10.1016/j.appet.2008.02.018 PMid:18417254

Juríková, T., Viczayová, I. 2014. New trend in nutrition and healthy lifestyle of people In: *New trend in nutrition and healthy lifestyle of people*, 1. ed., Nitra: UKF, 422 p. ISBN 978-80-558-0629-7.

Jurkovičová, J. 2005. Vieme zdravo žiť? Bratislava: Univerzita Komenského, 166 p. ISBN 80-223-2132-X.

Kimáková, T., Onačilová, E., Zeisbergerová, K. 2011. Porovnanie životného štýlu poslucháčov lekárskej a právnickej fakulty UPJŠ v Košiciach, Škola a zdraví 21, Výchova ke zdravotní gramotnosti, Masarykova univerzita [online] 2016-06-07. [cit. 2016-06-07] Available at:

http://www.ped.muni.cz/z21/knihy/2011/39/texty/cze/26_kimakova_onacillova_zeisbergova_cze.pdf.

Kopčeková, J., Kolesárová, A. 2009. Prieskum konzumácie prírodných zdrojov antioxidantov v náhodne vybranej skupine univerzitných študentov. In *Antioxidanty 2009*: proceeding from the 1.st international scientific conference, 6. May 2009. SPU Nitra, Slovakia. 2009.

Koplan, J. P., Liverman, C. T., Kraak, V. A, 2005. Committee on Prevention of Obesity in Children and Youth Preventing childhood obesity: health in the balance. NationalAcademies, Washington, D.C. ISBN: 0-309-09196-9.

Rey-López, J. P., Vicente-Rodríguez, G., Répásy, J., Mesana, M. I., Ruiz, J. R., Ortega, F. B., Kafatos, A., Huybrechts, I., Cuenca-García, M., León, J. F., González-Gross, M., Sjöström, M., de Bourdeaudhuij, I., Moreno, L. A. 2011. Food and drink intake during television viewing in adolescents: the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study. *Public Health Nutrition*, vol. 14, no. 9, p. 1563-1569. http://dx.doi.org/10.1017/S1368980011000383 PMid:21338558

Moreno, L. A., De Henauw, S., González-Gross, M., Kersting, M., Molnár, D., Gottrand, F., Barrios, L., Sjöström, M., Manios, Y., Gilbert, C. C., Leclercq, C., Widhalm, K., Kafatos, A., Marcos, A., HELENA Study Group. 2008. Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. *International Journal of Obesesity*, vol. 32, suppl. no. 5, p. 4-11. http://dx.doi.org/10.1017/S1368980007000535 PMid:17617932

Moreno, L. A., González-Gross, M., Kersting, M., Molnár, D., de Henauw, S., Beghin, L., Sjöström, M., Hagströmer, M., Manios, Y., Gilbert, C. C., Ortega, F. B., Dallongeville, J., Arcella, D., Wärnberg, J., Hallberg, M., Fredriksson, H., Maes, L., Wildham, K., Kafatos, A. G., marcos, a., HELENA Study Group. 2008. Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. *Public Health Nutrition*, vol. 11, no. 3, 288-299.

Pap-Váry, A. F., Schwang, Z., 2012. Egészségtelen főiskolás évek? Élelmiszervásárlási és fogyasztási szokások. A SJE Nemzetközi tudományos konferenciája, Művelődés – Identitás – Egészség, Komárom, 2012, 13.– 14. September, p. 715-746.

Šramková, K. 2001. Stravovanie a životný štýl vysokoškolských študentov, *Výživa a zdravie*, vol .46, no. 2, 2001, p. 28-36.

Štefániková, Z., Jurkovičová, J., Ševčíková, Ľ., Sobotová, Ľ., Sekretár, S., Ághová, Ľ. 2003. Aktuálne poznatky o životnom štýle vysokoškolákov z aspektu kardivaskulárneho rizika. *Vojenské zdravotnícke listy*, vol. LXXII, no. 4, p. 150-153.

Story, M., Kaphingst, K. M., Robinsos, B., Glanz, K., 2008. Creating healthy food and eating enviroments: policy and enviromental approaches. *Annual Review of Public Health*, vol. 29, p. 253-272. http://dx.doi.org/10.1146/annurev.publhealth.29.020907.090926 PMid:18031223

Tláskal, P., Hrstková, H., Schwartz, J., Fiala, J., Strosserová, A., Packová, A., Lepší, V., Baliková, M., Peterková, M. 2012. Výživové zvyklosti českých školních dětí, 1. Část: Výběr potravin a vitamíny, *Výživa a potraviny*, vol. 67, p. 58-60.

Urala, N., Lähteenmäki, L. 2007. Consumers' changing attitudes towards functional foods. *Food Quality and Preference*, vol. 18, p. 1-12. http://dx.doi.org/10.1016/j.foodqual.2005.06.007

Verbeke, W. 2005. Consumer acceptance of functional foods: Socio-demographic, cognitive and attitudinal determinants. *Food Quality and Preference*, vol. 16, p. 45-57. http://dx.doi.org/10.1016/j.foodqual.2004.01.001

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NUTRITION INTERVENTIONS IN PATIENTS WITH CROHN'S DISEASE

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ABSTRACT

Crohn's disease is a chronic non-specific inflammatory bowel disease of any part of the digestive tract. The seriousness of the disease requires a multi-disciplinary approach when providing patients with secondary and tertiary care. Patients also have specific problems from the nursing perspective that require intervention of nurses, e.g. in the area of nutrition. The role of a nurse in a specific community lies in supporting public health in the field of prevention, health education, group educational activities and care of the acutely or chronically ill. The regulation tool of nursing practice when providing community care is the documented form of nursing data expressed by means of expert terminology. The Omaha System is a standardised terminology for multi-disciplinary teams providing community care. The objective of the research is to draw attention to the possibility of using standardised terminology of the Omaha System when supporting public health in patients with Crohn's disease with nutrition problems. The research was divided into 3 stages: in the first stage we assessed the nutrition problem in 100 patients dispensarised in gastroenterology counselling centres using a form from the Omaha System. Out of these, identified 42 patients suffered from Crohn's disease and had problems with nutrition; in the second stage we chose interventions for nutrition from the Intervention Scheme of the Omaha System: their efficiency in patients was assessed by a nurse/nutritionist in the third stage of the research when the patients came to the gastroenterology counselling centre using Problem Rating Scale for Outcomes. When comparing the initial and final nutrition assessment with socio-demographic indicators we found a statistically significant difference (p = 0.000) between the status assessment where women scored a more remarkable advance than men when comparing the initial and the final assessment. With respect to age groups, education and jobs, no statistically significant differences were found (p >0.05). Nutrition interventions, according to the Omaha System, are linked to administering enteral and parenteral nutrition, monitoring of nutrition condition and education, management and consultancy during the diet that is individual and dependent on various factors.

Keywords: Crohn's disease; The Omaha System; Nutrition; Interventions; Outcomes

INTRODUCTION

Crohn's Disease **(CD)** is a chronic non-specific inflammation of any part of the digestive tract of segmental or plurisegmantal nature affecting the digestive tract wall transmurally in all its layers **(Zbořil, Prokopová, 2010; Dítě et al., 2010)**. This chronic disease is characterised by periods of inflammation deterioration (relapses) and appeasements (remissions). Moreover, it is incurable by drug treatment or surgically **(Dítě et al., 2010)**. It onsets mainly at a young age **(Dítě et al., 2010; Baumgart, 2012**).

With respect to the serious nature of the disease, its incidence and prevalence, etiopathogenesis, variation of the clinical screening and treatment and changes to life quality, patients need a multi-disciplinary approach when provided with care. Dispensarisation in a gastroenterology counselling centre provides these patients with secondary and tertiary care in a joint effort of a physician – gastroenterologist and a nurse or nutritionist. The community of patients with Crohn's disease has specific problems from the nursing point of view, as these problems require specific intervention of nurses. When providing this type of community care, a new role of a community nurse is being formed in this context. It is an autonomous role of nurses who work in a specific community in the area of prevention, health education, health care, group educational activities and care of the acutely or chronically and incurably ill **(Boledovičová, Zrubcová et al, 2009; Sikorová, 2012)**. As nursing care is associated with medical care, nurses use expert terminology, too **(Von Krogh et al., 2005)**. According to **Vörösová et al. (2015a),** the development and use of a standardised language in nursing characterises the new era of nursing. Implementation of standardised terminology into nursing via classification systems defines nursing work and makes it more visible through documentation. The regulation tool of nursing practice is the documented form of nursing data, ensuring quantified and statistically processable valid records of nursing diagnostics, care planning and nursing care effect assessment **(Vörösová et al., 2007)**. One of the best known terminologies for community nursing is the Omaha System **(Martin, 2005)**.

The Omaha System

The Omaha System is a research developed and evidenced comprehensive taxonomy or classification

designed for care documentation, from admittance to discharge **(The Omaha System, 2009)**. It is a working frame for a multidisciplinary team providing community care.

According to **Martin (2005),** this classification system contains three interlinked components/parts: Problem Classification Scheme, Intervention Scheme a Problem Rating Scale for Outcomes. This comprehensive classification gives a clear picture of client's needs/problems, the care/interventions provided and allows for measuring and assessing results of the care provided. Its main contribution is simplicity and comprehensiveness.

At present, it is used mainly in the USA, especially in community care, while in the Czech nursing is focused on support and maintenance of individuals', families' and communities' health, disease prevention, population ageing and advance of institutionalised care into the environment of communities. In the future, it is assumed to use the Omaha system in the Czech Republic.

Problem classification scheme

The Problem Classification Scheme of the Omaha System offers a structure, terms and system for standardised assessment of needs/problems. It is divided into domains, problems, modifiers, signs and symptoms **(Martin, 2005)**.

Under the four domains (environmental, psycho-social, physiological and health-related behaviours) there are 42 needs/problems with defined signs/symptoms for the current problem or risk factors indicating the occurrence of a possible problem. These problems are linked with proposed corresponding medical diagnoses from the International Classification of Diseases (ICD), where the occurrence of the relevant problem may be expected. Examples of problems in patients with Crohn's disease are shown in Table 1.

We can also use the Problem Classification Scheme for assessing etiopathogenetic factors of Crohn's disease, which include e.g. external environment factors, mental stress and smoking **(Lukáš, 2014; Baumgart, 2012)**, or clinical symptoms of Crohn's disease, which are quite diverse and conditioned by localisation, the disease extent and nature of local inflammatory changes **(Zbořil, Prokopová, 2010)**. We can also assess symptoms of different forms of the disease (ileitis and ileocolic, perianal, small and large intestine diseases, atypical localisation) as well as occurrence of intestinal and extraintestinal complications **(Dítě et al., 2010)**. Greenstein's classification is recommended for assessment of the development and course of the disease **(Zbořil, Prokopová, 2010)**, while the clinical Crohn's Disease Activity Index (CDAI) or Harvey-Bradshaw Index are recommended for assessment of the disease activity **(Freeman, 2008)**. The Problem Classification Scheme may also be used for assessment of individual symptomatic response and tolerance to drug treatment (using e.g. corticoids, biological treatment, immunosuppressants) or nutritional therapy **(Kužela, Zakuciová, 2012)**. Impartial interpretation of the nutrition condition may be complemented with a scale for approximate nutritional assessment (Mini Nutritional Assessment (MNA)) and Nutritional screening.

Intervention scheme

The Intervention Scheme is a comprehensive classification of activities designed for a specific problem in relation to primary, secondary and tertiary prevention/care. Interventions are intended for a specific problem and they are formulated in the system as targets for four possible categories **(Martin, 2005)**.

The intervention categories Teaching, Guidance and Counseling include the provision of information, predicting client's problems and supporting activities and clients' responsibility for themselves, assistance when taking a decision and solving the problem **(Martin, 2005)**. A nurse gives patients with Crohn's disease information concerning e.g. nutrition, rental of health equipment necessary for enteral nutrition administration, preparation for radio diagnostic, endoscopic or laboratory examinations, post-examination care, monitoring of disease relapses, specificities of the drug treatment, possibilities of maintaining the disease remission by leading a healthy life style, the importance of regular medical care in a gastroenterology office, supportive groups, etc. In case of needs, a nurse educates patients about other possibilities of care provided by homecare agencies, etc.

Table 1 Problem Classification Scheme **(Martin, 2005)**.

Domain	Problem	Signs/Symptoms of Actual
Environmental	Income	low/no income, uninsured medical expenses, difficulty with money management, able to buy only necessities, difficulty buying necessities, other
Psychosocial	Role Change	involuntary role reversal, assumes new role, loses previous role, other
Physiological	Bowel function	abnormal frequency/consistency of stool, painful defecation, decreased bowel sounds, blood in stools, abnormal color, cramping/abdominal discomfort, incontinent of stool, other
Health-Related Behaviors	Nutrition	overweight: adult BMI 25,0 or more, underweight: adult BMI 18,5 or less, lacks estabilished standards for daily caloric/fluid intake, exceeds estabilished standards for daily caloric/fluid intake, unbalanced diet, improper feeding schedule for age, does not follow reccomended nutrition plan, unexplained/progressive weight loss, unable to obtain/prepare food, hypoglycemia, hyperglycemia, other

The intervention category concerning Treatments and Procedures contains practical procedures such as biological material sampling, drug administration, etc.

The Case Management category focuses on the coordination of multidisciplinary care. Nutrition care is actually a frequent type of intervention in the area of nutrition in patients with Crohn's disease. According to **Vrzalová et al., (2011)**, nutritional support and education of the ill about dietary measures and possibilities of artificial nutrition is an inseparable part of comprehensive care of a patient with this type of non-specific bowel inflammation. According to **Zbořil (2015)**, a good nutritional condition has positive impact on the overall condition of a patient, who then copes better with possible acute onset of the disease. It is also important in the prevention of disease complication occurrence, when maintaining remission or during the perioperational period.

The last category of interventions – Surveillance includes activities such as detection, measuring, critical analysis, analysis of the condition of an individual, family, community with respect to the given conditions **(Martin, 2005)**. When monitoring the disease dynamics, patients are exposed to screening tests. At present, the test that is standard (and the least stressful for patients) is the MR enteroclysis, colonoscopy with subsequent histomorphological assessment of samples. Equally important are esofagogastroduodenoscopy, ultrasound imaging and laboratory diagnostics **(Konečný, Ehrmann, 2014; Zbořil, 2013)**. Nursing intervention when monitoring the disease include monitoring of physical signs/symptoms of the disease as well as monitoring of the behavioural component – patients' compliance, their discipline when taking care of themselves, coping abilities and coping with stress or changes in behaviour in connection with the disease. Other nursing interventions concerning nutrition is monitoring of individual patient's tolerance to meals, monitoring of laboratory indicators of the nutrition condition, monitoring of the nutritional screening and fluid balance, etc.

Problem rating scale for outcomes

The Problem Rating Scale for Outcomes of the Omaha System represents a systematic framework designed for measuring (quantification, impartial interpretation) of a client's advancement in relation to the identified specific problem as well as in the efficiency of the planned interventions. It assesses 3 dimensions – knowledge, behaviour and status using the Likert scale with five points (Table 2). The objective of the subscale assessing knowledge is to determine the extent of clients' abilities to understand, remember and interpret the information obtained. The subscale assessing behaviour focuses on the impartial interpretation of clients' reactions or activities. The objective of the subscale assessing the status is to determine how clients' condition has improved, stabilised or worsened on the basis of subjectively and objectively reported characteristics (signs/symptoms) **(Martin, 2005)**. The main indicators for assessment of the status are clinical symptoms, disease activity, laboratory indicators, screening method results, assessment of symptomatic response and treatment tolerance. From the nursing perspective, we can objectively assess data on patients with Crohn's disease by using evaluation tools which are – with respect to the terminological correctness of their translation from the original – used in clinical practice only sporadically **(Vörösová et al., 2015b)**.

The objective of this study is to sum up findings on standardised terminology of the Omaha System. The objective of the research is to draw attention to the possibility how the standardised terminology of the Omaha System could be used for the support of public health in a community of patients with Crohn's disease who have a nutrition problem.

MATERIAL AND METHODOLOGY

Sample

The research set consisted of 100 patients with gastroenterological disease, dispensarised in the gastroenterology counselling centre, out of which 42 identified patients (42%) with Crohn´s disease had problems with nutrition within nursing assessment. 24 patients were male and 18 patients were female, 14 patients were under 35 years of age, 16 patients were between 36 and 55, 8 patients were between 56 and 65 and 4 patients were over 66 years of age.

Three patients had primary education, 5 patients had vocational education, 23 patients had secondary and 11 patients had tertiary education. More than half of the patients were employed (n = 28), others were retired (n = 10) or on disability pension (n = 4).

Method

Data were collected using the Omaha System **(Martin, 2005)**. The research was divided into 3 stages. In the first stage we assessed the problem with nutrition in 42 intentionally selected patients with Crohn's disease.

Assessment was performed using the documentation form of the Omaha System **(Bowles, 2000)** with the consent of its author, Kathryn Bowles from University of Pennsylvania. In the second research stage we chose interventions in nutrition from the Intervention Scheme of the Omaha System: their efficiency was assessed in patients by a nurse/nutritionist during the third research stage when they visited the gastroenterology counselling centre using a Problem Rating Scale for Outcomes.

Table 2 Problem Rating Scale for Outcomes **(Martin, 2005)**.

	Knowledge	Behavior	Status
1	No knowledge	Not appropriate behavior	Extreme signs/symptoms
2	Minimal knowledge	Rarely appropriate behavior	Severe signs/symptoms
3	Basic knowledge	Inconsistently appropriate behavior	Moderate signs/symptoms
4	Adequate knowledge	Unusually appropriate behavior	Minimal signs/symptoms
5	Superior knowledge	Consistently appropriate behavior	No signs/symptoms

Research objectives

1. Identifying nutrition problem symptoms in patients with Morbus Crohn according to the Omaha System,
2. Mapping nutrition problem interventions in patients with Morbus Crohn according to the Omaha System,
3. Assessing nutrition problem results in patients with Morbus Crohn according to the Problem Rating Scale for Outcomes of the Omaha System,
4. Finding the statistical differences between the values of initial and final assessment of nutrition and socio-demographic indicators (age, sex, education and job).

The data were collected between June and December 2015.

Statistical analysis

The statistical analysis was performed using the SPSS 22.0 software and its results were analysed using non-parametric comparison tests with significance value 0.05: Wilcoxon signed-rank test, Mann-Whitney U test and Kruskal-Wallis test.

RESULTS AND DISCUSSION

The research results are presented in Tables 3 – 8. Knowledge, behaviour and status were assessed by a nurse from gastroenterology counselling centre / nutritionist using 5 point Likert scale (Table 2) with the interval of several weeks. Between assessment of initial and final knowledge there is a difference (Z = 5.82, p <0.001) with high factual significance (r = 0.63). Initial knowledge was assessed as basic (Mdn = 3), while final knowledge as adequate (Mdn = 4). Between behaviour assessment at the admittance and discharge there is a difference (Z = 4.824, p <0.001) with high factual significance (r = 0.53). Initial behaviour was assessed as inconsistently appropriate behaviour (Mdn = 3), while final behaviour as usually appropriate (Mdn = 4). Between the assessment of the initial and final status there is a difference (Z = 5.557, p <0.001) with high factual significance (r = 0.61). Symptoms of the initial status were moderate (Mdn = 3), while final status symptoms were minimal (Mdn = 4) (Table 3, Table 4).

The difference between initial and final assessment of knowledge, behaviour and status was compared with sex, age, education and job.

There is no statistically significant difference between knowledge (U = 181, p >0.05) and behaviour (U = 167, p >0.05) assessed at the beginning and at the end after several weeks.

We found statistically significant difference (U = 88, p <0.001) with high factual significance (r = 0.61) in the case of the difference of status assessment. Women scored a more significant advance (M rank = 28.61, n = 18) in the initial and final assessment of status than men (M rank = 16.17, n = 24) (Table 5).

With respect to age groups (categories: 35 years of age, between 36 – 55 years, between 56 – 65 years, over 66 years of age) we found no statistically significant difference in the advance of knowledge assessment ($\chi^2(3) = 2.134$, p >0.05), in the advance of behaviour ($\chi^2(3) = 0.197$, p >0.05) as well as in the advance of status ($\chi^2(3) = 6.098$, p >0.05) when comparing the initial and final assessment (Table 6).

With respect to patient education (categories: primary education, vocational education, secondary education, tertiary education), there was no statistically significant difference in the advance of knowledge ($\chi^2(3) = 0.167$, p >0.05), behaviour ($\chi^2(3) = 5.776$, p>0.05) and status ($\chi^2(3) = 4.919$, p >0.05) when comparing the initial and final assessment (Table 7).

With respect to jobs (categories: employed, retired and disability pension), we found no statistically significant difference between the advance of knowledge ($\chi^2(2) = 4.108$, p >0.05), behaviour ($\chi^2(2) = 0.253$, p >0.05) and status ($\chi^2(2) = 5.187$, p >0.05) when comparing the initial and final assessment (Table 8).

The main objective of the research is to draw attention to the possibility how the standardised terminology of the Omaha System could be used for the support of public health in the specific group of patients with chronic digestive tract disease with a specific problem – nutrition.

The relation between diet, etiology and symptoms of non-specific bowel inflammations is discussed in the study by **Rajendran and Kumar (2010)**. The nutrition problem is related to Crohn's disease, it influences its symptoms **(Yamamoto, 2013)**, course and life quality **(Owczarek et al., 2016)**. Nutrition problems such as malnutrition (with occurrence in more than 85% patients) and weight loss (affecting over 80% patients) are common. Occurrence of nutrition deficits is also detected partially in relation to anaemia and osteoporosis. They can be caused by radical

Table 3 Description of knowledge, behaviour and status during the initial and final assessment.

	Knowledge Rating		Behaviour Rating		Status Rating	
	Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**
Median	3	4	3	4	3	4
Mode	3	4	3	4	3	4
Minimum	2	3	2	3	1	3
Maximum	4	5	4	5	4	5

Table 4 The difference in knowledge, behaviour and status during the initial and final assessment.

	Knowledge	**Behaviour**	**Status**
Z	5.82	4.824	5.557
p	0.000	0.000	0.000

Note: Z – statistics, p – significance value.

Table 5 The difference between initial and final assessment of knowledge, behaviour and status with respect to sex.

	Knowledge	Behaviour	Status
U	181	167	88
p	0.283	0.18	0.000

Note: U – Mann-Whitney U test, p – significance value.

dietary restrictions introduced by patients themselves with the intention to mitigate symptoms, or inappropriate nutritional recommendations. Side effects of chronic disease treatment may include iron, calcium, vitamin B12, folic acid, zinc, magnesium or vitamin A deficiency **(Owczarek et al., 2016; Donnellan et al., 2013; Basson, 2012)**. Occurrence of intestinal stenosis may limit patients in eating food containing fibre **(Lomer, 2011)**.

Nutrition is in the Health – Related Behaviors Domain of the Omaha System **(Martin, 2005)**. It is defined as „select, consume, and use food and fluids for energy, maintenance, growth, and health" **(Martin, 2005)**.

The occurrence of nutrition problem may be expected in connection with gastrointestinal system diseases **(Pokorná, Maixnerová, 2013)**. This problem occurred in 42 patients, mostly in the 36 – 55 years of age category. When identifying nutrition problem symptoms in patients with Morbus Crohn according to the Omaha System, we found unbalanced diet, often linked to individual tolerance or patient's "fear" of intolerance and possible risk of disease relapse. Alarming symptoms such as unsuitable eating habits or non-observing the recommended diet did not occur at all, which can be regarded as a positive. Most patients had BMI 25 or less. Likelihood of the nutrition problem occurrence may relate to the disease course and development, the disease form or activity or intestinal complication occurrence. With respect to the nutrition problem occurrence, we may conclude it is a topical and specific problem of the community of patients with Crohn's disease. Assessment of the nutrition condition and consultations with a subsequent nutritional recommendation are thus the basis for the care management concerning these clients **(Lomer, 2011; Pokorná and Chudobová, 2011**). Identifying the nutrition problem, particularly its symptoms according to the Omaha System, allows for a rational approach when planning specific nutrition recommendations for patients.

When mapping the connection between the nutrition problem and intervention in the area of nutrition in patients with Morbus Crohn according to the Omaha System, the total of 131 interventions have been documented. The documentation tool was the Intervention Scheme of the Omaha System. The highest number of interventions (35%) was documented under the Treatments and Procedures category and related to parenteral and enteral food administration. Enteral nutrition has come to the fore in the recent years, as it allows for food intake in a more natural way. Its stimulation effects on the immune system, intestinal microflora excess reduction and its positive impact on peristalsis have been proved. In comparison with parenteral nutrition, it is easier to administer and is accompanied with lower incidence of complications **(Novotná, 2013)**. Enteral nutrition preparations are defined in nutrition terms, low-osmolar, usually residue-free, lactose-free and do not contain gluten. They are typically administered orally (sipping) as supplement nutrition with a diet when the food patients eat does not cover their energetic needs. In specific cases enteral nutrition may be administered via a probe **(Novotná, 2013)**. The second most numerous category comprised interventions under the Surveillance category. In the case of respondents with nutrition problems, these concerned particularly monitoring of the signs/symptoms of the nutrition problem, evaluation of the nutrition condition, laboratory indicators and performance of screening tests (e.g. nutrition screening, nutrition risk assessment and the likes, enteral nutrition monitoring, etc.). Each member of the multidisciplinary team, whether it is a nurse, nutritionist or physician nutrition specialist, has an important role. A nurse may draw attention to a nutrition problem on the grounds of medical records, observation, physical examination, nutrition screening or observation of daily food intake and fluid balance. Nutritionists are guarantors of curative nutrition and corresponding patient education doing their work on the basis of diagnoses and in accordance with physician's instructions. At present, there are nutrition teams providing nutrition care in hospitals. In community care, such a comprehensive approach is not common and our study is one of the first to address the issue in Czech Republic. Diet management was the objective of interventions under the Teaching, Guidance and Counseling category. It is important to emphasise that the composition of a diet for patients with Morbus Crohn is individual, depending on the course of the disease, complication occurrence and farmacotherapy **(Owczarek et al., 2016)**. A study focused on the review of dietary recommendations by patients themselves points to insufficient sources of relevant information for patients concerning their nutrition and disease and to the need to establish so-called evidence-based dietary guidelines for patients with IBD (Inflammatory Bowel Disease (IBD)) **(Hou, Lee, Lewis, 2014)**. Another study recommends as IBD adjunctive treatment so-called anti-inflammatory diet with limited saccharide intake including consumption of prebiotics, probiotics and modified fatty acids **(Olendzki et al., 2014)**. A strict diet is generally suitable for inducing remission and an individual diet limiting individually harmful substances is suitable for inducing long-term remission **(Lee et al., 2015)**. A review by **Shah et al., (2015)** presents interesting findings from key studies assessing evidence in the case of most frequent diets currently used in CD treatment. Limited studies of low-fibre diet do not account for the current practice from the perspective of fibre reduction in the active stage of the disease or in stenosis occurrence. A study of high-fibre diet did not evidence benefit in clinical results of active CD. No study describes the use of this diet during the remission stage. There are a low number of studies focusing on vegetarian diets. Only one study **(Chiba et al., 2010)** evidenced a positive effect as a maintenance treatment of CD, but it contained a small research population (n = 22) and lacked in endoscopic or

Table 6 The difference between initial and final assessment of knowledge, behaviour, and status with respect to age.

	Knowledge	Behaviour	Status
Chi-Square	2.134	0.197	6.098
df	3	3	3
p	0.545	0.978	0.107

Note: df – degrees of freedom, p – significance value.

Table 7 The difference between initial and final assessment of knowledge, behaviour, and status with respect to education category.

	Knowledge	Behaviour	Status
Chi-Square	0.167	5.776	4.919
df	3	3	3
p	0.983	0.123	0.178

Note: df – degrees of freedom, p – significance value.

Table 8 The difference between initial and final evaluation of knowledge, behavior, and status with respect to job.

	Knowledge	Behaviour	Status
Chi-Square	4.108	0.253	5.187
df	2	2	2
p	0.128	0.881	0.075

Note: df – degrees of freedom, p – significance value.

histological findings. In the case of lactose malabsorption during a lactose-free diet it is not necessary to exclude lactose completely, but rather reduce its intake to tolerance level. There are no studies that would assess the lactose effect on IBD activity. Only a limited number of uncontrolled studies points to symptom improvement, but with inconsistent changes in inflammatory markers in specific saccharide diet. Evidence from the perspective of functional symptom reduction exists in so-called Low-FODMAP diet (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP)). Up to now, the effect of gluten reduction diet has not been supported with evidence **(Shah et al., 2015)**. Based on selected interventions in nutrition has changed in the assessed areas (knowledge, behaviour, status) in all patients.

The last research objective was to find any statistical differences between the values of initial and final nutrition problem assessment and socio-demographic indicators (age, sex, education and job). The values measured at the initial nutrition assessment concerning knowledge, behaviour and especially status were low, which indicated the occurrence of a topical problem that required high priority solution. Subscale for status assessment evidenced severe nutrition problem signs/symptoms especially in women. Average values of the nutrition problem results at the initial assessment of behaviour scored lower values than in men. Knowledge of both sexes was assessed as "basic".

The final assessment of nutrition results confirms that implementation of selected interventions from the Intervention Scheme of the Omaha System reduced the problem. When assessing the status, we identified minimal signs/symptoms in respect of disease chronicity in both sexes that required above all observance of the treatment regimen (e.g. dietotherapy). Adequate knowledge and usually appropriate behaviour are a significant factor of relapse prevention and remission maintenance. When comparing the initial and final nutrition problem assessment with socio-demographic indicators we found a statistically significant difference with high factual significance only in the case of comparing the status when women scored a greater advance between the initial and final status assessment than men ($p = 0.000$). With respect to age groups, education and jobs, no statistically significant differences were found. Taking into account this finding, we recommend this to be verified on a larger respondent population.

The Problem Rating Scale for Outcomes may be used for monitoring client's progress, assessment of nutrition care efficiency or when determining if there is a need to make changes in nutrition or other aspects of care.

CONCLUSION

Assessment of the nutrition condition together with subsequent nutritional recommendation is the basis of patient care management of Crohn's disease. Identification of the nutrition problem and its symptoms according to the Omaha Systems allows for rational approach in planning specific nutrition recommendations. According to the Omaha System, interventions in the field of nutrition are linked to enteral and parenteral nutrition administration, monitoring of the nutrition condition and to education, management and consultancy during the diet that is individual and dependent on various factors. When assessing the efficiency of selected interventions using a Problem Rating Scale for Outcomes the nutrition problem was considerably reduced. When comparing the initial and final nutrition problem assessment with socio-demographic indicators, we found a statistically significant difference with high factual significance only in the case of difference between the status assessment, when women scored a greater advance between the initial and final status assessment than men. In respect of age groups,

education and jobs, no statistically significant differences were found, in all patients there is a shift between initial and final assessment of knowledge, behaviour and status.

REFERENCES

Basson, A. 2012. Nutrition management in the adult patient with Crohn's disease. *South African Journal of Clinical Nutrition*, vol. 25, no. 4, p. 164-172. http://dx.doi.org/10.1080/16070658.2012.11734423

Baumgart, D. C. 2012. *Crohn's Disease and Ulcerative Colitis. From Epidemiology and Immunology to a Rational Diagnostic and Therapeutic Approach.* New York, USA : Springer. 754 p. ISBN-13:978-1461409977. http://dx.doi.org/10.1007/978-1-4614-0998-4

Boledovičová, M., Zrubcová, D. et al. 2009. *Sestra a pôrodná asistentka v komunitnej starostlivosti.* Nitra, Slovakia : FSVaZ UKF. 400 p. ISBN: 978-80-8094-576-3.

Bowles, K. H. 2000. Patient Problems and Nurse Interventions during Acute Care and Discharge Planning. *Journal of Cardiovascular Nursing*, vol. 14, no 3, p. 29-41. http://dx.doi.org/10.1097/00005082-200004000-00006 PMid:10756472

Dítě, P. et al. 2007. *Vnitřní lékařství.* 2nd ed. Praha : Galén. 586 p. ISBN: 80-7262-496-6. PMCid:PMC2222980

Dítě, P., Lukáš, M., Lata, J. 2010. Idiopatické střevní záněty. In Češka, R. et al. *Interna.*Praha : Triton, p. 400-404. ISBN: 978-80-7387-629-6.

Donnellan, C. F., Yann, L. H., Lal, S. 2013. Nutritional management of Crohn's disease. *Therapeutic Advances in Gastroenterology*, vol. 6, no. 3, p. 231-242. http://dx.doi.org/10.1177/1756283X13477715

Freeman, H. J. 2008. Use of the Crohn's disease activity index in clinical trials of biological agents. *World Journal of Gastroenterology*, vol. 14, no. 26, p. 4127-4130. http://dx.doi.org/10.3748/wjg.14.4127

Hou, J. K., Lee, D., Lewis, J. 2014. Diet and inflammatory bowel disease: review of patient – targeted recommendations. *Clinical Gastroenterology and Hepatology*, vol. 12, no. 10, p. 1592-600. http://dx.doi.org/10.1016/j.cgh.2013.09.063

Chiba, M., Abe T., Tsuda, H., Sugawara, T., Tsuda, S., Tozawa, H., Fujiwara, K., Imai, H. 2010. Lifestyle – related disease in Crohn's disease: relapse prevention by a semi-vegetarian diet. *World Journal of Gastroenterology*, vol. 16, no. 20, p. 2484-2495. http://dx.doi.org/10.3748/wjg.v16.i20.2484 PMid:20503448

Konečný, M., Ehrmann, J. 2014. Pokroky v diagnostic a léčbě nespecifických střevních zánětů. *Vnitřní Lékařství*, vol. 60, no. 7-8, p. 625-629. PMid:25130640

Kužela, L., Zakuciová, M. 2012. Racionálna liečba chronických nešpecifických zápalov čreva. Štandardný diagnostický a terapeutický postup. *Metodický list racionálnej farmakoterapie a liekovej politiky MZ SR*, vol. 15, no. 5-7, p. 2-8.

Lee, D., Albenberg, L., Compher, C., Baldassano, C., Baldassano, R., Piccoli, D., Lewis, J. D., Wu, G. D. 2015. Diet in the pathogenesis and treatment of inflammatory bowel disease. *Gastroenterology*, vol. 148, no. 6, p. 1087-106. http://dx.doi.org/10.1053/j.gastro.2015.01.007 PMid:25597840

Lukáš, M. 2014. Etiologie a patogeneze idiopatických střevních zánětů. *Vnitř Lékařství*, vol. 60, no 7-8, p. 640-644. PMid:25130643

Lomer, M. C. 2011. Dietary and Nutritional considerations for inflammatory bowel disease. *Proceedings of Nutrition Society*, vol. 70, no. 3, p. 329-35. http://dx.doi.org/10.1017/S0029665111000097

Martin, K. S. et al. 2005. The Omaha System. A Key to Practice, Documentation, and Information Management. St. 2nd ed. St. Louis : Elsevier. 457 p. ISBN 978-0-7216-0130-4.

Novotná, H. 2013. Podávání enterální výživy – péče o pacienta. In Holubová, A., Novotná, H., Marečková, J. *Ošetřovatelská péče v gastroenterologii a hepatologii.* Praha : Mladá fronta, p. 193-201. ISBN: 978-80-204-2806-6.

Olendzki, B. C., Silverstein, T. D., Persuitte, G. M., Ma, Y., Baldwin, K. R., Cave, D. 2014. An anti-inflammatory diet as treatment for inflammatory bowel disease: a case series report. *Nutrition Journal*, vol. 13, no. 5. http://dx.doi.org/10.1186/1475-2891-13-5

Owczarek, D., Rodacki, T., Domagala-Rodacka, R., Cibor, D., Mach, T. 2016. Diet and nutritional factors in inflammatory bowel disease. *World Journal of Gastroenterology*, vol. 22, no. 3, p. 895-905. http://dx.doi.org/10.3748/wjg.v22.i3.895 PMid:26811635

Pokorná, A., Maixnerová, L. 2013. Sleodvání nutričního stavu seniorů za hospitalizace. *Prakt. Lékařství.*, vol. 93, no. 5, p. 221-225.

Pokorná, A., Chudobová, L. 2011. Effectiveness of the Standardised Nutritional Support of Senior Citizens in the Institutional Care. *Journal of US-China Medical Science*, vol. 8, no. 9, p. 567-570.

Rajendran, N., Kumar, D. 2010. Role of diet in the management of inflammatory bowel disease. *World Journal of Gastroenterology*, vol. 16, no. 12, p. 1442-1448. http://dx.doi.org/10.3748/wjg.v16.i12.1442 PMid:20333783

Shah, N. D., Parian, A. M., Mullin, G. E., Limketkai, B. N. 2015. Oral diets and nutrition support for inflammatory bowel disease: what is the evidence? *Nutrition in Clinical Practice*, vol. 30, no. 4, p. 462-473. http://dx.doi.org/10.1177/0884533615591059 PMid:26084506

Sikorová, L. 2012. *Dětská sestra v primární a komunitní péči*. 1st ed. Praha : Grada Publishing. 184 p. ISBN: 978-80-247-3592-4.

The Omaha System, 2009. Solving the Clinical Data-Information Puzzle [online] [cit.2014-07-22] Available at: http://www.omahasystem.org/

Von Krogh, G., Dale, C., Nåden, D. 2005. Framework for Integrating NANDA, NIC, and NOC Terminology in Electronic Patient Records. *Journal of Nursing Scholarship*, vol. 37, no. 3, p. 275-281. http://dx.doi.org/10.1111/j.1547-5069.2005.00047.x PMid:16235870

Vörösová, G. et al. 2007. *Klasifikačné systémy a štandardizácia terminológie v ošetrovateľstve*. 1st ed. Martin : Osveta. 112 p. ISBN 978-80-8063-242-7.

Vörösová, G., Solgajová, A., Archalousová, A. 2015a. *Standardizovaná terminologie pro ošetřovatelskou diagnózu.* 1st ed. Praha : Grada Publishing. 144 p. ISBN: 978-80-247-5304-1.

Vörösová, G., Solgajová, A., Archalousová, A. 2015b. *Ošetřovatelská diagnostika v práci sestry.* 1st ed. Praha : Grada Publishing. 208 p. ISBN: 978-80-247-5538-0.

Vrzalová, D., Konečný, M., Ehrmann, J. 2011. Enterální a parenterální výživa u pacientů s nespecifickými střevními záněty. *Medicína pro Praxi*, vol. 8, no. 7-8, p. 337-338.

Yamamoto, T. 2013. Nutrition and diet in inflammatory bowel disease. *Current Opinion in Gastrenterology*, vol. 29, no. 2, p. 216-21. http://dx.doi.org/10.1097/MOG.0b013e32835b9a40

Zbořil, V., Prokopová, L. 2010. Idiopatické střevní záněty – Crohnova nemoc. In Bureš, J., Vaňásek, T. et al. *Gastroenterologie*. Praha : Galén, p. 99-102. ISBN: 978-80-7262-692-2.

Zbořil, V. 2013. Endoskopické metody v racionální diagnostice a terapii idiopatických střevních zánětů. *Časopis lékářů českých*, vol. 152, no 6, p. 280-283.

Zbořil, V. 2015. Nutriční postupy [online] 2015-01-23. [cit. 2015-02-01] Available at: http://www.crohnovanemoc.cz/zakladni-lecebne-metody/nutricni-postupy/dieta.html

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EFFECT OF *RANA GALAMENSIS*–BASED DIET ON THE ACTIVITIES OF SOME ENZYMES AND HISTOPATHOLOGY OF SELECTED TISSUES OF ALBINO RATS

Basiru Olaitan Ajiboye, Nasir Olarewaju Muhammad, Oluwafemi Adeleke Ojo

ABSTRACT

The effect of *Rana galamensis*-based diet on the activities of some enzymes and histopathology of selected tissues of albino rats was investigated for eight weeks. A total of sixteen albino rats weighing between 29.15 and 26.01g (21 days old) were divided into two groups. The first group contains animals fed on casein–based diet (control); the second group was fed on *Rana galamensis*–based diet. The animals were fed with their appropriate diet on daily basis and on the eight weeks of the experiment the animals were sacrificed using diethyl ether as anesthesia, blood was collected by cardiac puncture and organs of interest were harvested. Thereafter, organ to body weight ratio, some biochemical parameters and histopathology examination were carried out. There was no significant difference (p >0.05) in the organ to body weight ratio of the animals fed on control and *Rana galamensis*–based diets. Also, there was no significant different (p >0.05) in the activities of all the enzymes (ALP [alkaline phosphatase], AST [asparate transaminase], ALT [alanine transaminase], and γGT [gamma glutamyl transferase]) investigated in the selected tissues and serum of rats fed on *Rana galamensis*–based diet when compared with the control. In addition, histological examinations of hepatocyte's rats fed on *Rana galamensis*– based diet show normal architecture structure when compared with the control. The insignificant different in the activities of all the enzymes studies (ALP, AST, ALT and γGT) indicated no organ damage, supported by the normal histology studies. The obtained results may imply that *Rana galamensis* is safe for consumption.

Keywords: *Rana galamensis*, ALP, AST, ALT, γGT, histological examination

INTRODUCTION

Food has been one of the most important sources of life to both human and livestock, that is "no food no life". Foods are substances which are capable of producing energy, promoting growth, and repairing of tissues **(Naik, 2011)**. The chemical components of food which perform these functions are called nutrients **(Murray et al., 2009)**. One of the important classes of food or nutrient is protein. The biological roles of this macronutrient in the system includes cells growth, enzymes, antibodies (also called immunoglobulin) which are specific protein produced by specialized cells of the immune system in response to foreign antigen, transport materials from one place to another in the body (for example transport of iron from the liver to the marrow), hormones or regulatory protein (for example insulin and glucagons) and to provide energy during starvation **(Naik, 2011)**. They are the basis of many animals bodies structure (for examples muscle, skin and hair). Inadequate intakes of protein cause protein-energy malnutrition **(Harvey and Ferrier, 2010)**.

Most people cannot afford the exorbitant prices of egg, milk, meat and fish **(Oloyede and Fowomola, 2003)**. Thus, it is important for the nutritionist to search for alternative source of high protein quality. One of the most readily available and cheap sources of protein is *Rana galamensis* **(Muhammad and Ajiboye, 2010)**. *Rana galamensis*, otherwise known as edible toad, belongs to the family of *Ranidae*, which has widest distribution of any frog family, and the class amphibian. Its common name is galam white-lipped frog. *Rana galamensis* is abundant throughout most of the continents except Antartica. In Africa they are found in savannah region of West Africa, South Africa and East Africa **(Ajiboye and Muhammad, 2015; Ajiboye et al., 2014)**. In Nigeria, they are found especially in Lagos State, Ogun State, Oyo State, Kwara State, Osun State, Ondo State, Ekiti State, ZariaCity and Benin City **(Muhammad and Ajiboye, 2010)**. They are strongly aquatic species in savannah area, where they live in and around permanent lakes, rivers, ponds and swamps **(Ajiboye et al., 2014)**. But information on the effect of *Rana galamensis*–based diet on the activity of some enzymes and histopathology of selected tissues of albino rats is still very scanty in literature. Therefore, the objective of this study was to determine effect of *Rana galamensis*–based diet on the activity of some enzymes and histopathology of selected tissues of albino rats.

MATERIAL AND METHODOLOGY

Rana galamensis

These were purchase from 'Oja Tuntun' market, in Ilorin, North Central, Nigeria. They were then authenticated at the Department of Zoology, University of Ilorin, Kwara State, Nigeria.

Albino rats

The albino rats used for this research were inbred in the Animal Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. This was approved by Animal Ethical Committee of the same University.

Kits and Chemicals

AST, ALT and GGT kits were obtained from Randox Laboratories Ltd, UK while ALP and all other chemicals were obtained from Quinica Aplicada (QCA), S. A Spain.

Formulated Diets

The diets were composed as earlier reported by **Ajiboye and Muhammad (2015)** and **Ajiboye et al. (2014)**.

Animal's Treatment

Sixteen weanling albino rats (weighing between 29.15 and 26.01 g) which were twenty one days old were used in this study, divided into two groups. The first group was placed on casein-based diet which serves as the control while the second group was placed on *Rana galamensi*s-based diet which serves as the tested. Each group comprises of eight rats. The animals were fasted for twelve hours and then acclimatized for a week in the laboratory to normalize them for the experiment before being placed on the different diets.

The animals in each group were housed in metal cage. The diet and water were fed to the animals *ad libitum* throughout the period of 8 weeks and sanitary measures were ensured.

Serum and Tissues Homogenates Preparation

At the end of eight week the animals were sacrificed by anaesthetizing them in a jar containing cotton wool that had been soaked in diethyl ether. The anaesthetized animals were then sacrificed by cutting the jugular vein. The blood was collected into clean, dry glass beaker and allowed to coagulate for 1 hour, using pasture pipette. The serum was removed from the clot and collected into centrifuge tube. Clear serum was then obtained by centrifugation at 1,500 g for 15 minutes. The samples were kept frozen and used for analyses within 24 hours **(Akanji, 1986)**. The animals were quickly dissected after sacrifice and organs such as liver, heart, kidney, stomach and small intestine were removed quickly into ice – cold 0.25 M sucrose solution to maintain the integrity of the organs. Each organ was homogenized separately in ice – cold 0.25 M sucrose solution (×6 dilution) using mortar and pestle by cutting a known weight of the tissues finely with a clean scissors and homogenized. All operations were carried out at 0 °C to 4 °C. The homogenates were stored in the freezer and used for analyses within 24 hours while the organs (liver) for histological studies were immersed in 10% formalin.

Determination of Organ to Body Weight Ratio

This was determined using **Drury and Wallington (1973)** formular:

Percentage of organ to body weight ratio = weight of organ/weight of animal X 100%.

Determination of Enzymes Activities

Alkaline phosphates (EC 3.1.3.1) activities were determined according to the methods described by **Wright et al. (1972)** Aspartate transaminase (EC 2.6.1.1) and Alanine transaminase (EC 2.6.1.2) activities were determined as described by **Reitman and Frankel (1957**) while gamma-glutamyl transferase (EC 2.3.2.2) was estimated by method described by **Persijin (1976)**.

Histological Examination

This was done as described by **Krause (2001)**; the photomicrographs were observed using the Leitz, DIALUX research microscope at X 400 magnification.

Statistical Analysis

Statistical analysis was carried out using the students' t-test **(Adamu and Johnson, 1997)**.

RESULTS AND DISCUSSION

Effect of Rana galamensis-Based Diet on Organ to Body Weight Ratio

The percentage of organ to body weight ratio of the selected rat's organs is presented in Table 1. There was no significant difference (p >0.05) in the organ to body weight ratio of the animals fed on control and *Rana galamensis*–based diet.

This suggests that *Rana galamensis* supports normal growth of the organs, thereby causing no inflammation or constriction of the organs studied **(Moore and Dalley, 1999)**. **Akanji and Ngaha (1989)** reported that biochemical parameters like tissue enzyme can indicate tissue or cellular damage long before structural damage can be picked up by conventional histological techniques.

Effect of Rana galamensis-Based Diet on Alkaline Phosphatase (ALP) Activities

The ALP activities in the tissues of the rats fed *Rana galamensis*-based diet is depicted in Table 2. The results shows that there were no significant difference (p >0.05) in ALP activities in comparing the albino rats fed on control (casein-based diet) and *Rana galamensis*-based diet.

ALP is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum **(Shahjahan et al., 2004)**; it is frequently used to assess the integrity of the plasma membrane **(Akanji et al., 1993)**. In all the five tissues studied ALP activities was highest in small intestine followed by kidney and least in the liver. This is in agreement with the report of **Bonting et al. (1960)** that the main mammalian organ, which have very high ALP activities are those involved in active transport mechanisms.

Moreover, ALP is a membrane bound enzyme which is used frequently to detect damage to the plasma membrane. The non-reduction of ALP activities in the tissues which occurred in this study may indicate that there are no likely damages to the plasma membrane, therefore leakage did not occur. This may give an indication that there may not likely be the presence of diseases such as obstructive jaundice, bone diseases, cancer and heart infections which mostly results from increase in serum ALP activities due to leakage from the tissues **(Akanji et al., 2013)**.

Table 1 Changes in organ to body weight ratio (%) of selected tissues of animals fed with *Rana galamensis*-based diet for 8 weeks.

Tissues	Control (casein)	*Rana galamensis*
Liver	3.13 ±0.18	2.81 ±0.11
Heart	0.43 ±0.03	0.36 ±0.01
Kidney	1.03 ±0.09	0.97 ±0.02
Stomach	0.92 ±0.10	0.75 ±0.04

Each value is a mean of eight determinations ±SEM. The values are not significantly different (p >0.05).

Table 2 ALP activity ($IU.L^{-1}$) in the tissues of rats fed Rana galamensis-based diet for eight weeks.

Tissues	Control (casein)	*Rana galamensis*
Liver	11.96 ±2.95	12.02 ±3.07
Heart	12.67 ±1.73	12.68 ±1.25
Kidney	206.09 ±14.75	188.56 ±12.60
Small Intestine	293.52 ±16.35	288.98 ±10.96
Serum	5.75 ±0.52	5.63 ±0.71

Each value is a mean of eight determinations ±SEM. The values are not significantly different (p >0.05).

Table 3 AST activity ($IU.L^{-1}$) in the tissues of rats fed Rana galamensis-based diet for eight weeks.

Tissues	Control (casein)	*Rana galamensis*
Liver	654.67 ±17.67	659.0 ±15.09
Heart	218.63 ±11.09	207 ±10.61
Serum	30.47 ±8.36	33.43 ±8.51

Each value is a mean of eight determinations ±SEM. The values are not significantly different (p >0.05).

Table 4 ALT activity ($IU.L^{-1}$) in the tissues of rats fed *Rana galamensis*-based diet for eight weeks.

Tissues	Control (casein)	*Rana galamensis*
Liver	508.2 ±6.57	504.75 ±6.70
Heart	156.67 ±6.79	158.5 ±6.70
Serum	156.9 ±6.66	150.46 ±3.27

Each value is a mean of eight determinations ±SEM. The values are not significantly different (p >0.05).

Table 5 γGT activity ($IU.L^{-1}$) in the tissues of rats fed *Rana galamensis*-based diet for eight weeks.

Tissues	Control (casein)	*Rana galamensis*
Liver	190.5 ±5.43	182.4 ±4.97
Serum	14.9 ±0.67	14.9 ±0.67

Each value is a mean of eight determinations ±SEM. The values are not significantly different (p >0.05).

Effect of Rana galamensis-Based Diet on Some Transaminases and Transferase Activities

The activities of AST, ALT and γGT in the tissues of the rats fed *Rana galamensis*-based diet is shown in Table 3, table 4 and Table 5. The results shows that there were no significant difference (p >0.05) in the activities of all these transaminases as compared to the control.

AST is one of the enzymes involved in the transfer of anions group to keto acid without formation of ammonia as intermediate. In general, transaminases form an important link between protein and carbohydrate metabolism and are widely distributed in animal tissues **(Yakubu et al., 2008)**. It has been reported that enzymes from damage tissues or disease tissues may become recognizable in the serum, presumably by leakage through altered cell membrane **(Yakubu et al., 2008)**. But in this study, (Table 3) no leakage of AST from tissue was observed showing that there was no alteration in the cytosolic content of the tissues studied. In addition, AST and ALT are widely used for hepatic disorders **(Yakubu et al., 2003)**. Also, high serum level of ALT is an indicator for some form of hepatic diseases. Therefore, the non–significant difference in the serum level of ALT indicates (Table 4) that there is probably absence of any form of hepatic disease **(Abubakar et al., 2010)**.

γGT, the most common enzymatic indicator of hepatobiliary disease, is a membrane-localized enzyme that functions in glutathione metabolism and reabsorption of amino acids from the glomerular filterate and intestinal lumen **(Yakubu et al., 2001)**. It is a group of enzyme called peptidases, which catalyses the hydrolytic cleavage of peptides to form amino acid or smaller peptides. Elevated serum levels of the enzyme are found in association with hepatobiliary and pancreatic disorders, alcohotics and heavy disorders, in myocardial disorders and in diabetics **(Yakubu et al., 2001)**. The non-significance difference in the serum γGT (Table 5) giving an indication that there may not likely be any form of hepatobillary and pancreatic disorders. Also, γGT is more sensitive than ALP, AST and ALT in detecting jaundice **(Mayne, 1998)**. Moreover, the non-significant differences in the ALP, AST, ALT and γGT may be ascribed to the non-toxic of *Rana galamensis* coupled with the present of essential amino acids and minerals in.

Effect of Rana galamensis-Based Diet on Histological examination

Plates 1 to 2 show the histology section of the control and *Rana galamensis* fed rat livers. The results revealed that no significance difference between the two groups. The liver of both animals have normal architecture structure. The photomicrographs show portal tract at the centre with the normal hepatocytes.

This indicate no alterations in the photomicrograph of the tissues studied (Figure 1 and Figure 2) corroborates the results of the enzymes and blood parameters. This shows that *Rana galamensis* is safe for consumption coupled with its high digestibility as reported by **Ajiboye et al. (2014)**.

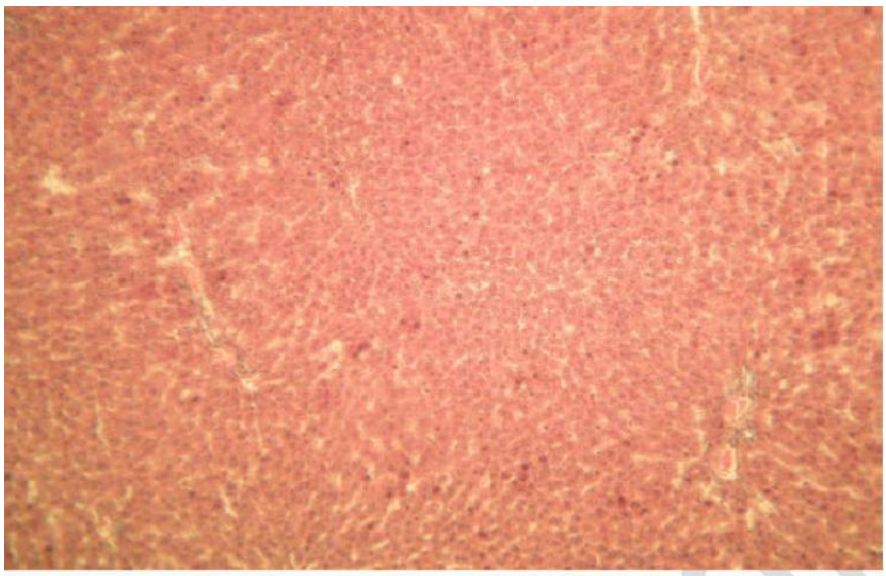

Figure 1 Plate 1: Photomicrograph of the liver of rats fed casein (control) – based diet (Magnification x 400).

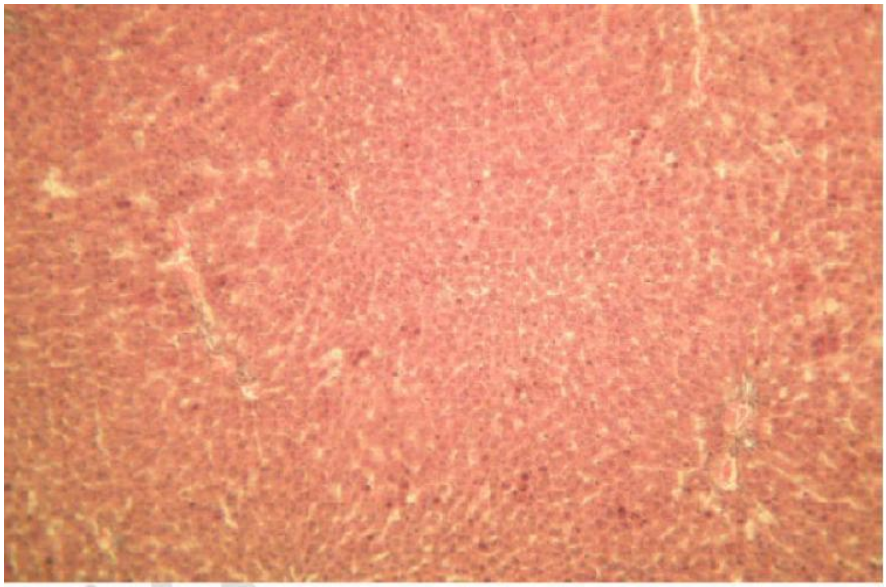

Figure 2 Plate 2: Photomicrograph of the liver of rats fed *Rana galamensis* – based diet (Magnification x 400).

CONCLUSION

The lower activities of all the serum enzymes studies (ALP, GOT, GPT and γGT) indicated no organ damage coupled with the organ to body weight ratio of the animals. This was also supported by the result of liver histological studies. This may probably be attributed to high nutritional status of *Rana galamensis*.

REFERENCES

Abubakar, M. G., Lawal, A., Suleiman, B., Abdullahi, K. 2010. Hepatorenal toxicity studies of sub-chronic administration of calyx aqueous extracts of *Hibiscus sabdariffa* in albino rats. *Bayero Journal of Pure and Applied Sciences*, vol. 3, no. 1, p. 16-19. http://dx.doi.org/10.4314/bajopas.v3i1.58550

Adamu, S. O., Johnson, T. L. 1997. *Statistics for beginners.* Book 1. Ibadan, Nigeria : SAAL Publication. p. 184-199. ISBN:978-1601890917

Ajiboye, B. O., Muhammad, N. O., Abdulsalam, M. S. Oloyede, O. B. 2014. Nutritional evaluation of *Rana galamensis* -based diet in albino rats. *International Journal of Applied Research and Technology*., vol. 3, no. 6, p. 35-40.

Ajiboye, B. O., Muhammad, N. O. 2015. Investigation into the haematological parameters of albino rats fed with *Rana galamensis*–based diet as an alternative source of protein in animal feed. *Journal of Global Agriculture and Ecology*, vol. 2, no. 4, p. 112-116.

Akanji, M. A. 1986. *A comparative biochemical study of the interaction of some trypanocides with rat tissue cellular system* : dissertation thesis. Ile–Ife : University of Ife, p. 34-48.

Akanji, M. A., Ngaha, E. O. 1989. Effect of repeated administration of berenil in urinary enzyme excretion with corresponding tissue pattern in rat. *Pharmacology and Toxicology*, vol. 64, no. 3, p. 272-275. http://dx.doi.org/10.1111/j.1600-0773.1989.tb00645.x PMid:2726690

Akanji, M. A., Olagoke, O. A., Oloyede, O. B. 1993. Effect of chronic consumption of metabisulphite on the integrity of rat cellular system. *Toxicology*, vol. 8, no. 3, p. 173-179. http://dx.doi.org/10.1016/0300-483X(93)90010-P

Akanji, M. A., Yakubu, M. T., Kazeem, M. I. 2013. Hypolipidemic and toxicological potential of aqueous extract of *Rauvolfia vomitoria afzel* root in wistrar rats. *Journal of medical sciences*, vol. 13, p. 253-260. http://dx.doi.org/10.3923/jms.2013.253.260

Bonting, S. L., Pollark, V. E., Muchrcke, R. L., Kack, R. M. C. 1960. Quantitative histochemistry of the nephron II: Alkaline phosphatase activity in man and other species. *Journal of Clinical Investigation*, vol. 39, no. 9, p. 1372-1380. http://dx.doi.org/10.1172/JCI104156 PMid:13802623

Drury, R. A. B., Wallington, E. A. 1973. Tissue Histology. In: *Carleton's Histological Technique*. 4th ed. New York : Oxford University Press, p. 58. ISBN 13: 9780192613103

Bell, G. H., Donald, E. S., Collin, R. P. 1976. *Textbook of Physiology and Biochemistry*. 8th ed., Harcourt Brace : Churchill Livingstone. ISBN-13: 9780443014369.

Harvey, R. A., Ferrier, D. H. 2010. *Lippincott's Illustrated Reviews*. Lippincot William and Wilkins. 5th Edition. ISBN-13: 978-1451175622.

Krause, W. J. 2001. *The art of examining and interpreting histologic preparations. A student handbook.* UK : Partheton Publishing Group. p. 9-10. ISBN 9781850702771.

Mayne, P. D.1998. *Clinical Chemistry in Diagnosis and Treatment*. 6th Edition. UK, London : Arnold International Students. p. 196-205. ISBN-13: 978-0340576472.

Moore, K. L., Dalley, A. F. 1999. *Clinically Oriented Anatomy*. 4th ed. Philadelphia : Lippincoth Williams and Wilkins, a Woiler Klummer Company. p 263-271. ISBN: 9780683061413

Muhammad, N. O., Ajiboye, B. O. 2010. Nutrient composition of *Rana galamensis*. *African Journal of Food Science and Technology*, vol. 1, no. 1, p. 27-30.

Murray, R. K., Bender, D. A., Botham, K. A., Kennelly, P. J., Rodwell, V. W., Weil, P. A. 2009. *Harper's Illustrated Biochemistry*. McGraw Hill Lange. 28th Edition

Naik, P. 2011. *Biochemistry Textbook*. Jaypee Brother Medical Publisher. 3 rd Edition.

Oloyede, O. B., Fowomola, M. A. 2003. Effects of fermentation on some nutrient contents and protein quality of soy bean seeds. *Nigerian Journal of Pure and Applied Science*, vol. 18, p. 1380-1386.

Persijin, J. 1976. Determination of serum gamma glutamyl transferase. *Clinical Chemistry and Biochemistry*, vol. 9, no. 14, p. 421-427.

Reitman, S., Frankel, S. O. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Ameriacn Journal of Clinical Pathology*, vol. 28, no. 1, p. 56-57. http://dx.doi.org/10.1093/ajcp/28.1.56 PMid:13458125

Shahjahan, M., Sabitha, K. E., Mallika, J., Shayamala-Devi, C. S. 2004. Effect of *Solannum trilobtum* against carbon tetrachloride induced hepatic damage in albino rats. *Indian Journal of Medical Research*, vol. 120, no. 3, p. 194-198. PMid:15489557

Wright, P. J., Leathwood, P. D., Plummer, D. T. 1972. Enzymes in rat urine: alkaline phosphatase. *Enzymologia*, vol. 42, no. 4, p. 317-327. PMid:4337755

Yakubu, M. T., Akanji, M., Salau, I. O. 2001. Protective effect of ascorbic acid on some selected tissues of ranitidine-treated rats. *Nigerian Society of Biochemistry and Molecular Biology*, vol. 16, no. 2, p. 177-182.

Yakubu, M. T., Akanji, M. A., Oladiji, A. T. 2008. Effect of oral administration of aqueous extract of *Fadogia agrestis* stem on some testicular function indices of male rats. *Journal of Ethnopharmacology*, vol. 111, no. 2, p. 288-292. http://dx.doi.org/10.1016/j.jep.2007.10.004 PMid:18023305

Yakubu, M. T., Bilbis, L. S., Lawal, M., Akanji, M. A. 2003. Evaluation of selected parameters of rat liver and kidney function repeated administertaion of yohimbine. *Biokemistri*, vol. 15, no. 2, p. 50-56.

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CRAMBE TATARIA SEBEÓK SEEDS AND PLANTS GROWN *IN VITRO* AND *IN VIVO* FATTY ACID COMPOSITION COMPARISON

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ABSTRACT

Methods of *in vitro* conservation offer a number of advantages for endangered species preservation. *Crambe tataria* Sebeók biochemistry study (fatty acid (FA) composition, antioxidant activity (AOA), polyfructan and total soluble protein content) is fairly importaint and could show the potential value of this species in agriculture, Food and Chemical Industry or pharmacology including its use as a source of valuble genetic material and could lead to new promising sources of biofuel discovery. Also, comparison of *in vitro* and *in vivo* cultured plants could point out to the effect of *in vitro* culture methods on plants biochemical composition Fatty acid (FA) content was determined using Gas chromatography-mass spectrophotometry (GC/MS) of fatty acid ethers. Antioxidant activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. Total soluble protein content was measured using Bradford method and polyfructan content determination was based upon ketosugars ability to color in the acidic environment with resorcinol. Plants that were grown under *in vitro* and *in vivo* conditions and seeds were used in this research. Obtained data showed that *C. tataria* plants had high AOA and total soluble protein content along with high total FA content along with high content of α-Linolenic acid and absence of erucic acid. Difference in biochemical composition between plants grown in aseptic and not aseptic conditions was shown.

Keywords: threatened species; *in vitro* culture; GC/MS mass spectrophotometry; fatty acids

INTRODUCTION

A number of *Brassicaceae* family plant species is known to play a significant role in the global oil and biofuel production **(Xiao-qin et al., 2011)**. Very-long-chain FA analysis is an important task for the researches as these acids are components or precursors of numerous specialized metabolites synthesized in specific cell types **(Haslam and Kunst, 2013).** FA is also essential for plant natural processes and is widely used in medicine **(Taylor et al., 2009)**.

Biodiesel is manufactured mostly from vegetable oil (rapeseed, palm and coconut oils). It has some benefits over traditional types of fuel – it isn't toxic, it decomposes under natural conditions in relatively short term, has almost no sulfur and benzene and is obtained from renewable materials. Biodiesel has good environmental properties (lower emissions are produced by the combustion of biodiesel than for diesel) **(Angelovič et al., 2013)**. Taking into account all the benefits of the biofuel its active investigation and application in the USA, Japan, China, Canada and in the EU countries is understandable. International Energy Association predicts the rise of biofuel production up to 150 million tons of the oil energy equivalent till 2030. Ukraine is in beneficial conditions for biofuel production from agricultural materials. Total biodiesel volume that can be manufactured in Ukraine could reach 500 thousand tons that can ensure 60% of our country fuel needs and 10% of petrol needs **(Medvedkova and Trudayeva, 2013)**. Qualitative physico-chemical properties of vegetable oils used for biodiesel production study **(Angelovič at al., 2015)** often excludes potential sources of biofuel from mass production so a search of new effective sources of high quality biofuel is fairly important.

The *Crambe* L. genus belongs to *Brassicaceae* family and consists of about 29 species. These species are annual, biennial or perennial and have diverse application: as vegetable or forage plants, as oilseed, as the source of biofuels (seeds have up to 60% of erucic acid), in Food Industry for making pastry, in paint and varnish industry, in chemical industry **(Prakhova, 2013)**. All studies on *Crambe sp.* were focused on *C. abyssinica* cultures which is proven to be a valuable biofuel source because it doesn't hybridize with any known oilseeds which eliminates gene flow issues **(Gonçalves et al., 2013)**; its seeds have high content of slowly-drying oil with low Iodine number, rich with erucic acid; it has high crop capacity and low demands for soil quality; it is drought-resistant and has short vegetation period **(Askew, 1993)**.

A number of species of *Crambe* L. genus is threatened so they require conservation measures. *In vitro* techniques need a small number of plants and result in a relatively high propagation coefficient even for the species which have problematical *in situ* and *ex situ* reproduction and do not depend on the climatic conditions. These methods provide a long time conservation of plant species outside their natural habitats (in seed banks, under introduction conditions, in *in vitro* collections and cryobanks) with thorough study possibility and can be significant addition

to the global plant biodiversity conservation system **(Belokurova, 2010)**. *In vitro* and *in vivo* cultivated plants biochemistry comparison could lead to better understanding of the aseptic conditions influence on plant secondary metabolites synthesis and accumulation processes and could also estimate the efficiency of *in vitro* culture methods application for fast gaining of raw materials with high content of secondary metabolites.

The aim of this study is to determine *C. tataria* oil FA composition (from seeds and plant grown under *in vitro* and *in vivo* conditions) and study the effect of aseptic conditions on plant biochemical composition.

MATERIAL AND METHODOLOGY

Crambe tataria (*Brassicaceae* family) from the flora of Ukraine was used for this research. *C. tataria* is listed in the Red data book of Ukraine (Vulnerable) **(Didukh, 2009)**. For GC/MS analyses seeds and fresh apical leaves from plants grown under *in vitro* (on hormone-free solid MS medium **(Murashige and Skoog, 1962)** at 24 °C with a 16-h photoperiod and recurrent transplantation on the fresh medium every 30 days) and *in vivo* (plant material was gathered in may (average monthly temperature 21 °C)) conditions were used. *In vivo* plants were grown in M.M. Gryshko National Botanical Garden of National Academy of Sciences of Ukraine and provided by prof. D. B. Rakhmetov. Seeds and *in vitro* plants were obtained from seed bank and *in vitro* collection of The Institute of Cell Biology and Genetic Engineering of National Academy of Science of Ukraine and also provided by research assistant (M. S. Kalista) of National Museum of Natural History of National Academy of Sciences of Ukraine.

Gas Chromatography-Mass Spectrophotometry of fatty acid ethers

FA extraction and methylation were conducted stepwise accordingly to **(Garces and Mancha, 1993)**. 50 mg of seeds and 200 mg of fresh leaves were used for extracts preparation. Seeds samples were ground in pounder and leaves were cut with defatted scissors. Then material was moved to glass tubes with spin caps and teflon gaskets. Reaction mixture which consisted of methanol: toluene: sulfuric acid (volume ratio 44:20:2) was added to the plant material first. Then, 1.7 mL of hexane was added (methanol, toluene, hexane – HPLC-grade, Sigma-Aldrich, Germany; sulfuric acid – chemically pure, Alfarus, Ukraine). Tubes were kept in water bath at 80 °C for 2 hours and then after cooling down to room temperature were gently shaken which led to separation of the liquid into two phases. Upper phase, which contained concentrated methyl fatty acid ethers, was gathered. The acidity of the solution was adjusted to neutral pH with saturated solution of 1M sodium phosphate. FA composition was determined using GC/MS system Agilent 6890N/5973inert (Agilent Technologies, USA) with capillary column DB-FFAP (length – 30 m; inner diameter – 0.25 mm; stationary phase thickness – 0.25 μm). Chromatographic fractionation occurred in gradient mode from 150 °C to 220 °C with a temperature gradient of 2 °C.min^{-1}. Helium was used as a carrier gas with flow rate of 1 mL.min^{-1}. Identification was done using mass spectrum library NIST 02 and standard bacteria methyl fatty acid ethers solution (Supelco). Heptadecanoic acid (C17:0) (chemically pure, ABCR, Germany) was used as an inner standard. All data were expressed as a mean ±SD.

Acil-lipid ω9, ω6 and ω3 desaturases activity and saturation degree estimation

For saturation degree estimation in leaves and seeds the index of saturation (double bound index – DBI) was used **(Lyons, Wheaton and Pratt, 1964)**:

$$DBI = \sum Pjn/100,$$

where: Pj – the amount of FA (mol %), n – the number of double bounds in every unsaturated FA. The unsaturation index (K) – the ratio between total amount of unsaturated FA (UFA) and total amount of saturated FA (SFA) is also used. Acil-lipid ω9, ω6 and ω3 desaturases activity, which catalyzes formation of double bonds into the carbon chain of Oleic (C18:1), Linoleic (C18:2) and α-Linolenic (C18:3) acids respectively was determined by stearic- (SDR), oleic- (ODR) and linoleic- (LDR) desaturases ratio. These ratios were calculated by amount (mol% of total FA content) of C18 components:

$$SDR = (C18:1) / (C18:0 + C18:1)$$
$$ODR = (C18:2 + C18:3) / (C18:1 + C18:2 + C18:3)$$
$$LDR = (C18:3) / (C18:3 + C18:2),$$

where: C18:0, C18:1, C18:2 i C18:3 – mol% amount of Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2) and α - Linolenic (C18:3) acids **(Jaworski and Stumpf, 1974)**.

Antioxidant activity, total soluble protein and polyfructan content

Fresh apical leaves from plants grown under *in vivo* and standard *in vitro* conditions were weighed (200 mg), homogenized with distilled water (0,7 mL) and centrifuged at 10000 g for 10 min. 100 μL of supernatant was taken and mixed with 100 μL of 0.1% alcohol solution of resorcinol (chemically pure, Alfarus, Ukraine) and with 100 μL of concentrated hydrochloric acid (chemically pure, Alfarus, Ukraine). Extraction of polyfructan was held in water bath at +80 °C for 20 min and measured on spectrophotometer (550 nm) (Eppendorf, USA). Calibration was made using fructose **(Maznik and Matvieieva, 2013)**. Antioxidant activity of extracts was measured by DPPH radical scavenging method **(Brand-Williams, Cuvelier and Berset, 1995; Adámková, Kouřimská and Kadlecová, 2015)**. Bradford method was used for total soluble protein determination **(Bradford, 1976)**.

RESULTS AND DISCUSSION

FA can be divided by the unsaturation degree into 2 groups: saturated (SFA) and unsaturated (USFA) (monounsaturated and polyunsaturated). GC/MS of the samples from seeds and leaves grown *in vitro* and *in vivo* showed presence of SFA (Lauric acid (C12:0), Palmitic acid (C16:0) and Stearic acid (C18:0); monounsaturated FA (Oleic acid (C18:1 Δ9, ω 9); polyunsaturated FA (Linoleic acid (18:2 Δ9, 12, ω 6) and α-Linolenic acid

Table 1 *Crambe tataria* seeds and plants grown *in vitro* and *in vivo* total amount of FA, saturation degree, unsaturation index and acil-lipid ω9, ω6 and ω3 desaturases activity estimation[1].

Indexes	*Crambe tataria* samples		
	***In vivo* leaf**	***In vitro* leaf**	**Seed**
FA, mg.g^{-1} ±SD	2.65 ±0.09*	6.09 ±0.51	545.91 ±24.23
SFA, mg.g^{-1} ±SD	0.67 ±0.04*	1.94 ±0.10	16.31 ±1.43
USFA, mg.g^{-1} ±SD	1.95 ±0.11*	4.14 ±0.41	529.59 ±25.63
K ±SD	2.95 ±0.35	2.13 ±0.10	32.72 ±4.60
DBI ±SD	2.02 ±0.08*	1.81 ±0.009*	1.33 ±0.03*
SDR ±SD	0.33 ±0.03*	0.52 ±0.01*	0.98 ±0.008*
ODR ±SD	0.98 ±0.002*	0.94 ±0.009*	0.50 ±0.04*
LDR ±SD	0.80 ±0.017*	0.81 ±0.08*	0.24 ±0.04*

Note: total fatty acids (FA), saturated FA (SFA) and unsaturated FA (USFA) amount; saturation degree (DBI), unsaturation index (K) and acil-lipid ω9, ω6 and ω3 desaturases activity (SDR, ODR and LDR respectively).
* – significance level (p <0.05).

(18:3 Δ9, 12, 15, ω3)). Only seed samples had Paullinic acid (C20:1 Δ13, ω7), Erucic acid (C22:1 Δ13, ω 9) presence. Nervonic acid (C24:1 Δ15, ω 9) was detected only in aseptic plants and Lignoceric acid (C24:0) only in not aseptic plants (Figure 1).

Total FA amount in seed and leaves was quite different (Table 1). *C. tataria* seeds had high content of total FA. Total FA amount in plants grown under in vitro conditions was more than two times higher than FA amount in plants grown *in vivo*. USFA total amount in seeds was 32 times higher than SFA (K – 32.72 ±4.60) but DBI was rather low (DBI – 1.33 ±0.03). Extracts from *C. tataria* plants grown *in vivo* had almost 3 times higher total amount of USFA then SFA (K – 2,95 ±0.35) and *in vitro* leaf samples had 2 times higher USFA amount then SFA (K – 2.13 ±0.10). It shows that aseptic plants had higher ratio of USFA than SFA compared to not aseptic plants. The DBI was higher for *in vivo* cultured plants then for plants grown *in vitro* as well (Table 1).Calculated SDR, ODR and LDR coefficient showed relevant desaturases (ω9, ω6, ω3) activity level. The activity of ω9 – desaturase that provides first double bound input was the lowest among others desaturases activity in aseptic plant samples, and its highest activity was determined in seed samples. ODR and LDR coefficients were very high in leaves of both aseptic and not aseptic plants that indicate high ω6- and ω3-desaturase activity but low in seeds. According to SDR, ODR and LDR coefficient that was calculated ω9-, ω6- and ω3-desaturases activity in plants grown in different conditions and seeds was different. Samples from seeds showed high ω9-desaturase and low ω6- and ω3-desaturases activity while samples from leaves, on the contrary, showed high ω6- and ω3-desaturases and low ω9-desaturase activity (Table 1).

Further FA ethers gas-spectrums of the samples from seeds and leaves study showed that Lauric acid was present in all seed and leaves samples. While seeds and *in vivo* plants had insignificant amount of C12:0 (seeds – 0.37 ±0.04 mol%; *in vivo* grown plants – 0.42 ±0.03 mol%) a part of Lauric acid in leaf samples from aseptic plants was higher (2.84 ±0.09 mol%). Palmitic acid had the biggest share of SFA content. It's amount was low in seeds – 2.80 ±0.11 mol% but leaf samples, on the contrarily, had high and almost equal content of C16:0

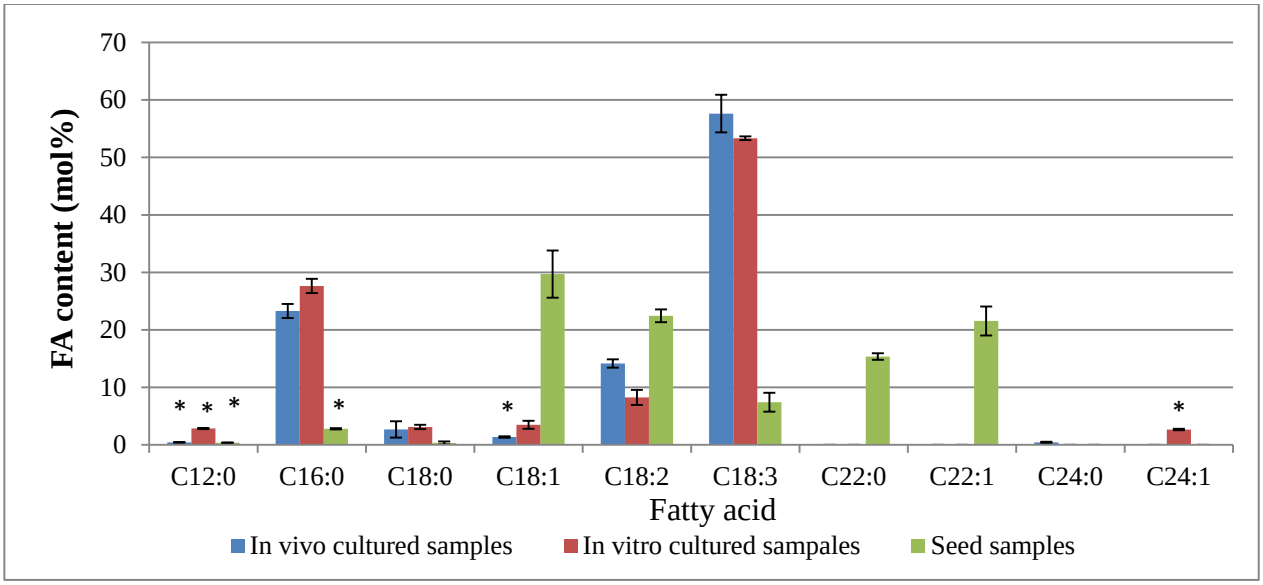

Figure 1 *Crambe tataria* seeds and plants grown *in vitro* and *in vivo* FA content comparison.
* significance level (p <0.05).

(aseptic plants – 27.63 ±1.24 mol% and not aseptic plants – 23.29 ±1.22 mol%) (Figure 1).

Leaf samples had higher amount of C18:0 then seed samples (seeds – 0.33 ±0.26 mol%, aseptic plants – 3.10 ±0.37 mol%; not aseptic plants – 2.68 ±1.41 mol%) though its share in aseptic plants was little higher than in not aseptic ones.

Unsaturated FA have a preventive effect on the many diseases caused by modern lifestyles. It reduces the risk of cardiovascular diseases, modifies blood pressure, improves blood vessels elasticity and reduces the level of cholesterol and blood coagulation **(Frančáková et al., 2015)**. In seeds of studied species the biggest share of all FA had Oleic acid but in leaves its content was low (samples from seeds had 29.71 ±4.10 mol% of C18:1). *In vitro* cultured plants accumulated more than twice as much Oleic acid (3.49 ±0.69 mol%) as plants cultivated in the botanical garden (1.37 ±0.12 mol%) (Figure 1).

Seed samples had high content of monounsaturated omega-9 fatty acids – Gondoic acid (C20:1) and Erucic acid (C22:1) (Figure 1) while leaves from both aseptic and not aseptic plants had no traces of these acids. Those acids are harmful to human and animal health, so their absence in leaves samples indicates the possible use of *Crambe* species green mass as forage crops. At the same time, the use of their seeds as forage crops is not advisable due to high content of C22:1. The Erucic acid content in seed samples (21.54 ±2.52 mol%) was little lower then it was previously reported for *C. tataria* (27.0 – 29.87%) **(Rudloff and Wang, 2011)**. Gondoic acid content in seeds was high (15.35 ±0.56 mol%) compared to previously reported for the same species (7.70 – 21.0%) **(Rudloff and Wang, 2011)**. Some polyunsaturated FA were detected in *C. tataria* seed and leaf samples. Linoleic and α-Linolenic acids are essential fatty acids so foods with high content of these acids should be included in human diet **(Frančáková et al., 2015)**. The biggest amount of C18:2 was detected in *C. tataria* seed samples (22.45 ±1.11 mol%). Though *in vivo* grown plants had quite high amount of this FA (14.15 ±0.72 mol%) *in vitro* cultivated plants had the lowest C18:2 share of all studied samples (8.25 ±1.30 mol%). α-Linolenic acid content had the biggest share among SFA and UNFA in leaf samples from plants cultivated in both *in vitro* and *in vivo* conditions and its amount was approximately equal in both aseptic and not aseptic plants (*in vitro* plants – 53.32 ±0.32 mol% and *in vivo* plants – 57.62 ±3.27 mol%). In seed samples C18:3 content was rather low – 7.42 ±1.64 mol%.

Nervonic acid has a wide range application. It's used for ssymptomatic treatment De-myelinating diseases, Multiple Sclerosis, Parkinson, Schizophrenia, deficiencies associated with neurological disorders (Alzheimer's). Also it has many applications in nutritional markets (senility, memory aid etc.), for treatments of arthritis, liver diseases and obesity and is used as dietary supplements (in baby foods and formulas, in pre-term babies nutrition, in diet for pregnant or lactating women and in enriched energy supplements with neuro-protective effect for high-level training adults/athletes) **(Sandhir, Khan, Chahal and Singh, 1998; Taylor et al., 2010; Sargent, Coupland, and Wilson, 1994)**. Nervonic acid (C24:1) was detected only in aseptic plants and its content was rather low – 2.66 ±0.13 mol% but due to the high value of this FA this data suggest possible use of the studied *C. tataria* species as biotechnological object for C24:1 production.

Antioxidant activity, total soluble protein and polyfructan content

In vivo leaf samples had higher total soluble protein content (6.41 ±1.05 $mg.g^{-1}$ of fresh weight) comparing to *in vitro* samples (4.50 ±0.10 $mg.g^{-1}$). It is interesting that for the most commonly used species of *Brassicaceae* family that were grown *in vivo*, total protein content was lower than for *Crambe* species also grown in not aseptic conditions (4.8 $mg.g^{-1}$ of fresh weight for broccoli) **(Campbell et al, 2012)**.

AOA for *C. tataria* both *in vitro* and *in vivo* samples was very high comparing to the AOA of ascorbic acid solution (1 $mg.mL^{-1}$ – 98.22%). Both aseptic and not aseptic leaf samples showed very high AOA (100%) which is higher than ascorbic solution that was used as a reference.

While AOA and total soluble protein content was roughly equal for both *in vitro* and *in vivo* plants their polyfructan content on the contrary was much different: aseptic plants – 8.17 ±3.98 $mg.g^{-1}$ and not asepticplants – 1.47 ±0.48 $mg.g^{-1}$.

CONCLUSION

GC/MS of the samples from seeds and leaves study reviled an impact of aseptic conditions on the FA composition of studied *C. tataria* species. Quantitative FA analysis shows difference between plants grown in aseptic and not aseptic conditions and also suggests that aseptic conditions benefits to SFA accumulation and increases polyfructan content on the other hand it reduces USFA amount and so as plant material protein content. The study of ω9-, ω6- and ω3- desaturases activity showed that seed samples had high ω9- desaturase activity but very low ω6- and ω3-desaturases activity. Samples from leaves on the contrary showed high ω6- and ω3- desaturases activity and low ω9- desaturase activity.

The dominant FA in leaves samples was α-Linolenic acid and *in vitro* cultured plants had its highest content. The dominant FA of all present in seed samples was Oleic acid. *C. tataria* seeds had rather low content of erucic acid but it still could be as a source of biofuel. On the contrary Erucic acid hasn't been detected in both aseptic and not aseptic plant samples.

Determination of AOA showed very high potential of *C.tataria* as a source of natural antioxidants and could also point out to medical application of this species. Both *in vitro* and *in vivo* plants had almost equal AOA and total soluble protein content, but aseptic plants polyfructan amount was more than 5 times higher comparing to not aseptic plants. Therefore, we can assume that aseptic conditions can benefit to SFA synthesis and increase polyfructan content of *C. tataria* plants.

REFERENCES

Adámková, A., Kouřimská, L., Kadlecová, B. 2015. The effect of drying on antioxidant activity of selected lamiaceae herbs. *Potravinarstvo*, vol 9, no. 1, p. 252-257. http://dx.doi.org/10.5219/474

Angelovič, M., Jablonický, J., Tkáč, Z., Angelovič, M. 2015. Oxidative stability of fatty acid alkyl esters: a review.

Potravinarstvo, vol. 9, no. 1, p. 417-426. http://dx.doi.org/10.5219/500

Angelovič, M., Tkáč, T., Angelovič, M. 2013. Oilseed rape as a feedstock for biodisel production in relation to the enviroment and human health. *Potravinarstvo*, vol. 7, no. 1, p. 101-106. http://dx.doi.org/10.5219/278

Askew, M. F. 1993. Novel oil, fibre and protein crops in UK. A future perspective. Brighton Crop Protection Conference, p. 658.

Belokurova, V. B. 2010. Methods of Biotechnology in system of efforts aimed at plant biodiversity preservation. *Cytology and Genetics*, vol. 44, no. 3, p. 174-185. http://dx.doi.org/10.3103/S0095452710030096

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, vol. 72, p. 248-254.

Brand-Williams, W., Cuvelier M. E., Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft and Technologie*, vol. 28, no. 1. p. 25-30.

Campbell, B., Han, D. Y., Triggs, C. M., Fraser, A. G. Ferguson, L. R. 2012. Brassicaceae: nutrient analysis and investigation of tolerability in people with Crohn's disease in a New Zealand study. *Functional Foods in Health and Disease*, vol. 2, no 2, p. 460-486.

Frančáková, H., Ivanišová, E., Dráb, Š., Krajčovič, T., Tokár, M., Mareček, J., Musilová, J. 2015. Composition of fatty acids in selected vegetable oils. *Potravinarstvo*, vol. 9, no. 1, p. 538-542. http://dx.doi.org/10.5219/556

Garces, R., Mancha, M. 1993. One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Analytical Biochemistry*, vol. 211, no. 1, p. 139-143. http://dx.doi.org/10.1006/abio.1993.1244

Gonçalves, A. C., Rubio, F., Meneghel, A. P., Coelho, G. F., Dragunski, D. C. Strey, L. 2013. The use of Crambe abyssinica seeds as adsorbent in the removal of metals from waters. *Revista Brasileire de Engenharia Agrícola Ambiental*, vol. 17, no. 3, p. 306-311. http://dx.doi.org/10.1590/S1415-43662013000300009

Haslam, T. M., Kunst, L. 2013. Extending the story of very-long-chain- fatty acid elongation. *Plant Science*, vol. 210, p. 93-107. http://dx.doi.org/10.1016/j.plantsci.2013.05.008

Jaworski, J. G. Stumpf, P. K. 1974. Fat metabolism in higher plants. Properties of a soluble stearyl-acyl carrier protein desaturase from maturing Carthamus tinctorius. *Archives of Biochemistry Biophysics*, vol. 162, no. 1, p. 158-165. http://dx.doi.org/10.1016/0003-9861(74)90114-3

Lyons, J. M., Wheaton, T. A., Pratt, H. K. 1964. Relationship between the physical nature of mitochondrial membranes and chilling sensitivity in plant. *Plant Physiology*, vol. 39, no. 2, p. 262-268. http://dx.doi.org/10.1104/pp.39.2.262

Maznik, K. S., Matvieieva, N. A. 2013. Optimization of fructans exstraction from *in vitro* cultivated chicory „Hairy" roots. *Biotechnologia acta*, vol. 3, no 1, p. 83-88.

Medvedkova, I., Trudayeva, T 2013. Biofuel market: problems and prospects. *Mosty*, vol. 6, no. 3.

Murashige, T. Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, vol. 15, p. 473-497. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x

Prakhova, T. Y. 2013. New unconventional oilseed – *Abyssinian Crambe*. *Bulletin of Altai state agricultural university*, vol. 8, no. 106, p. 8-10.

Didukh, Y. P. 2009. *Red data book of Ukraine. Vegetable kingdom*. Globalconsulting, 900 p.

Rudloff, E., Wang, Y. 2011. *Wild Crop Relatives: Genomic and Breeding Resources. Oilseeds*. ed. 5. Crambe : Springer Science & Business Media, 295 p.

Sandhir, R., Khan, M., Chahal, A., Singh, I. 1998. Localization of nervonic acid beta-oxidation in human and rodent peroxisomes: impaired oxidation in Zellweger syndrome and X-linked adrenoleukodystrophy. *Journal of Lipid Research*, vol. 39, no 11, p. 2161-71.

Sargent, J. R., Coupland, K., Wilson, R. 1994. Nervonic acid and demyelinating disease. *Medical Hypotheses*, vol. 42, no 4, p. 237-242. http://dx.doi.org/10.1016/0306-9877(94)90122-8

Taylor, D. C., Guo, Y., Katavic, V., Mietkiewska, E., Francis, T., Bettger, W. 2009. New seed oils for improved human and animal health and as industrial feedstocks: Genetic manipulation of the Brassicaceae to produce oils enriched in nervonic acid. In: Krishnan A. B. 2009. *Modification of Seed Composition to Promote Health and Nutrition*. Madison, Wisconsin, USA : ASA-CSSA-SSSA Publishing, Madison, p. 219-233.

Taylor, D. C., Falk, K. C., Palmer, C. D., Hammerlindl, J., Babic, V., Mietkiewska, E., Jadhav, A., Marillia, E. F., Francis, T., Hoffman, T. Giblin, E. M., Katavic, V., Keller, W. A. 2010. Brassica carinata - a new molecular farming platform for delivering bio-industrial oil feedstocks: case studies of genetic modifications to improve very long-chain fatty acid and oil content in seeds. *Biofuels, Bioproducts & Biorefining*, vol. 4, no. 5, p. 538-561. http://dx.doi.org/10.1002/bbb.231

Xiao-qin, S., Hui, P., Jian-lin, G., Bin, P., Ming-ming, B., Yue-yu, H. 2011. Fatty acid analysis of the seed oil in a germplasm collection of 94 species in 58 genera of brassicaceae. *Chemistry and Industry of Forest Products*, vol. 31, no. 6, p. 46-54.

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SHELF LIFE EXTENSION AND SENSORY EVALUATION OF BIRCH TREE SAP USING CHEMICAL PRESERVATIVES

Maciej Bilek, Vladimír Vietoris, Vojtech Ilko

ABSTRACT

The aim of this study was to assess the stability of the birch tree sap, depending on the addition and concentration of two chemical factors, ie. potassium sorbate and acids: malic, citric or lactic. As in our previous studies we found that the optimal physical parameter to assess the stability of birch sap is turbidity measurement, we used turbidimeter for estimate the effectiveness of shelf life extending. Sensory evaluation was carried out by university sensory panel with 8 skilled people (students and teachers) with pre-selection and basic training of sensory methodology. On the other hand artificial perception measurements were realized by electronic nose. Birch tree sap stability without addition of preservatives, both room temperature and refrigerated, is less than three days. The effectiveness of preservation of birch tree sap depends on the concentration of acids. Independently of storage temperature, samples that received stability during the whole one-month storage period, were those with potassium sorbate and three acids in the highest concentrations, ie. malic acid at 0.3%, citric acid at 0.5% and lactic acid at 0.5%. Unfortunately, concentrations of acids, which allow extension of shelf life at least for one month in a room temperature, are characterized by the worst sensory evaluation rating. Thus, they should be corrected by the use of additives for improving the flavor, such as fruit syrups or herbal extracts. On the other hand, additionally storage in a refrigerated conditions allows one-month-stability for the sample with the highest sensory evaluation rating, ie. with the addition of lactic acid at 0.1% and potassium sorbate, which taste not need to be corrected.

Keywords: birch tree sap; shelf life extending; sensory analysis; beverages; functional food

INTRODUCTION

Birch sap is collected in early spring, usually in the first half of March. It is characterized by pro-health properties, which have been appreciated by the folk medicine. Once, it was recommended, among others, in case of renal disorders, in the anemia, against immunodepressed, in general weakening and in the weakened condition of the skin and hair, in infectious diseases, parasitic infections and even cancer **(Svanberg et al., 2012; Papp et al., 2014; Rastogi et al., 2015)**. This wide range of applications has been confirmed by a number of recent researches conducted, among other, using cell lines **(Moriyama et al., 2008; Peev et al., 2010)**. Very high mineral content in birch tree sap has been demonstrated, including copper, zinc, manganese and magnesium. although it is variable, depending on the individuals **(Bilek et al., 2015b; Bilek et al., 2016b)**. At the same time negligible content of harmful substances in the birch sap has been found, eg. nitrates, considered as one of the most dangerous and the most common factors associated with the products of plant origin **(Bilek et al., 2016c)**.

The results of scientific research, increasing popularity of the behavior based on a folk traditions, as well as popularity of bio-food, low-processed food and independently obtained crops **(Kozelová et al., 2009; Brindza et al., 2011; Kozelová et al., 2013)** contributes to increasing interest in the birch sap **(Godyla, 2014; Stawarczyk, 2015).** Following that, inexperience collectors often acquire significant amounts of sap. Meanwhile, it quickly becomes unsuitable for consumption as a result of the microbiological degradation. This happens after approximately one day at room temperature and after about three days under refrigerated conditions **(Viškelis and Rubinskienė, 2012)**. For this reason, there are many attempts to extend shelf life of the tree saps. The use of pasteurization or thermisation may cause the disappearance of many valuable components of sap, determining the health-promoting properties of this unprocessed raw material **(Yuan et al., 2013)**. In addition, under the influence of the high temperature, decomposition of fructose is observed, resulting in browning of birch sap. Therefore, in the literature, there are several alternatives for thermal methods. Most are based on the use of physical techniques, eg. ultraviolet radiation, ultrafiltration, as well as combination of ultrafiltration and ultraviolet radiation **(Jeong-Jeong et al., 2011, Jeong-Jeong et al., 2013)**. It must be emphasized, however, that the implementation of these arrangements in practice will be associated with the high cost of processing line, as well as with the problem of aseptic bottling. These solutions are also unavailable for the consumers who collect the tree saps own.

The aim of this study was to develop a simple method of extend shelf life of birch tree sap, that would be accessible for any consumers, as well as sensory evaluation of obtained products.

MATERIAL AND METHODOLOGY

Tree sap of silver birch (*Betula pendula* Roth.) was collected in Niwiska village, located on the Kolbuszowa Plateau, accordance with the suggestions of literature **(Yoon et al., 1992)**. Collection has taken place by drilling the hole in the tree trunks at a height of approx. 50 cm, using a drill bit with a diameter of 16 mm, to a depth of 5 cm, on the south side a tree trunk. The tree sap was collected simultaneously from the seven trees. In the evening the collected batches were combined into one, and then frozen at -21 °C.

The birch tree sap was thawed in a water bath on the day of the start of the test. The temperature thereof was controlled and never exceeded 10°C during the thawing. After thawing, the sap was divided into half-liter portions which were preserved as described in the table (Table 1) **(Patent Application P.417738)**.

To preserve the birch sap lactic acid (Chempur, Piekary Slaskie), malic acid (Sigma-Aldrich, St. Louis) and citric acid (Chempur, Piekary Slaskie) was used, as well as potassium sorbate (POCh, Gliwice).

Based on our previous studies **(Bilek et al., 2016a)** we found that the optimal physical parameter to assess the stability of birch sap is turbidity measurement. It was tested using Hanna Instrument HI98703 Turbidimeter.

Sensory analysis

Sensory evaluation was carried out by university sensory panel with 8 skilled people (students and teachers) with pre-selection and basic training of sensory methodology. Samples were stored in regular conditions and served in sequence order. Avoid preservation acids lingering we used demineralized water. All facilities and equipment during experiment were done by ISO EN 8589:2007 standard. For analysis of the results we used non-parametric Friedman's test. Data were processed by R and presented in Table 2 **(R Core Team, 2016)**.

Artificial Perception Measurements

Analysis were realized by electronic nose Heracles II (Alpha MOS, France) in three replications per sample. Data were processed by native software ALPHA soft v.14. For visualization of the results we used PCA technique.

Statistical analysis

Statistical analysis was performed in R-project software, version 3.2.5 (R Foundation for Statistical Computing, Vienna, Austria). We have used One-way ANOVA.

RESULTS AND DISCUSSION

Changes in the turbidity of birch saps preserved are presented in Figures 1 – 6.

To extend the shelf life of birch sap we used the preservatives, ie. citric, malic and lactic acids and the potassium salt of sorbic acid. They are considered one of the safest food additives **(Rogozińska and Wichrowska, 2011)**. We intentionally excluded the use of sodium salt of benzoic acid, posing health risks **(Jędra et al., 2008)**.

Birch tree sap stability without addition of preservatives, both room temperature and refrigerated conditions, is less than three days. Independently of storage temperature, samples that received stability during the whole one-month storage, were those with potassium sorbate and acids in the highest concentrations, ie. malic acid at 0.3%, citric acid at 0.5% and lactic acid at 0.5%. On the other hand, sap containing sorbate but with lower concentration of acids, as well as sap with addition of acids exclusively were much less stable (Figure 1, Figure 2 and Figure 3). And in turn, in refrigerated conditions increased stability has been achieved. For saps with the concentrations at 0.1 and 0.25% of lactic and citric acid shelf-life extension in a monthly test was obtained in refrigerated conditions. Only saps with malic acid concentrations at 0.1% and 0.2% in a reduced temperature were characterized by low stability, ie. about 24 days (Figure 4, Figure 5 and Figure 6). On the other hand, stability of sap preserved solely by the addition

Table 1 A method of preserving and storing the birch tree sap.

Room temperature (21°C)			Refrigerated conditions (4°C)		
Citric acid	0.1% 0.25% 0.5%		Citric acid	0.1% 0.25% 0.5%	
Citric acid	0.1% 0.25% 0.5%	Potassium sorbate 0.03%	Citric acid	0.1% 0.25% 0.5%	Potassium sorbate 0.03%
Lactic acid	0.1% 0.25% 0.5%		Lactic acid	0.1% 0.25% 0.5%	
Lactic acid	0.1% 0.25% 0.5%	Potassium sorbate 0.03%	Lactic acid	0.1% 0.25% 0.5%	Potassium sorbate 0.03%
Malic acid	0.1% 0.2% 0.3%		Malic acid	0.1% 0.25% 0.5%	
Malic acid	0.1% 0.2% 0.3%	Potassium sorbate 0.03%	Malic acid	0.1% 0.25% 0.5%	Potassium sorbate 0.03%

of acids, depending on the type of acid and its concentration, reached from 9 to 12 days stability in refrigerated conditions.

Shelf life extension obtained by the using of potassium sorbate and acids in the highest concentrations may be compared to results reported by other authors. **Jeong-Jeong et al. (2013)** applied to control the turbidity of tree saps spectrophotometry technique by the measuring the absorbance at a wavelength 420 and 590 nm. The results indicate that only birch sap for which both the ultrafiltration and the ultraviolet radiation was used were stable, irrespective of the storage conditions. In this way Jeong-Jeong obtained shelf life extension approx. 20 days, and in turn, our method using two chemical factors, ie. potassium sorbate and the highest concentration of food acids, extended the stability of tree saps at least one month, also irrespective of the temperature of storage conditions.

In the sensory evaluation of birch tree sap with the addition of malic acid statistically significant positively evaluation in the case of overall and harmony has been

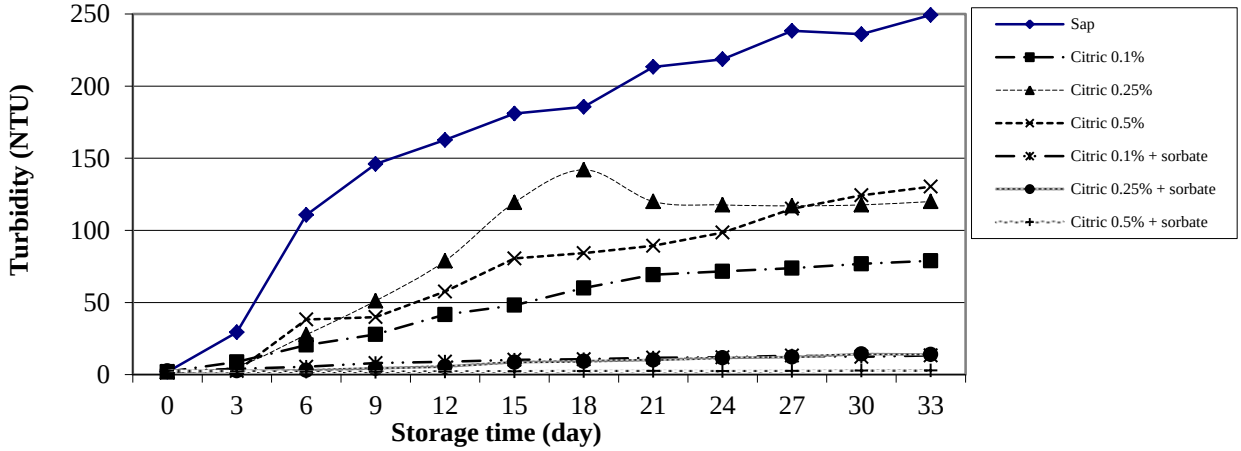

Figure 1 Turbidity changes for birch sap using citric acid-based shelf life extension method (room temperature).

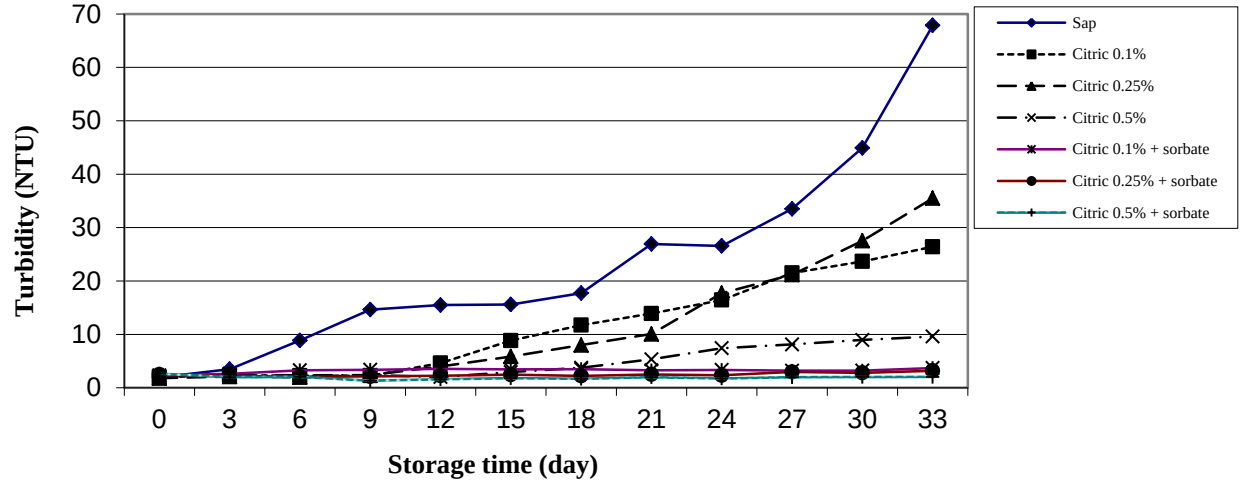

Figure 2 Turbidity changes for birch sap using citric acid-based shelf life extension method (refrigerated conditions).

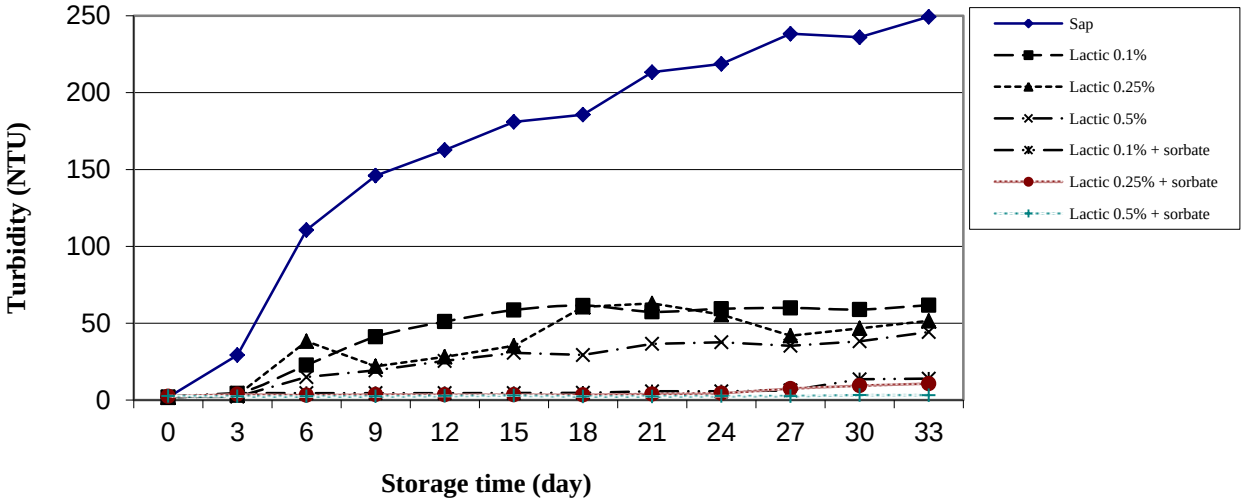

Figure 3 Turbidity changes for birch sap using lactic acid-based shelf life extension method (room temperature).

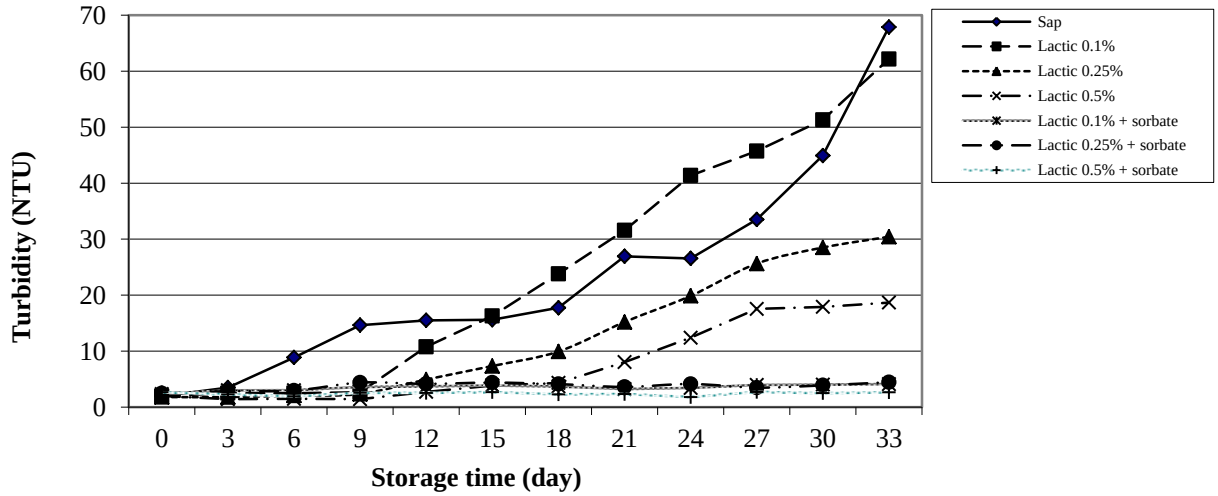

Figure 4 Turbidity changes for birch sap using lactic acid-based shelf life extension method (refrigerated conditions).

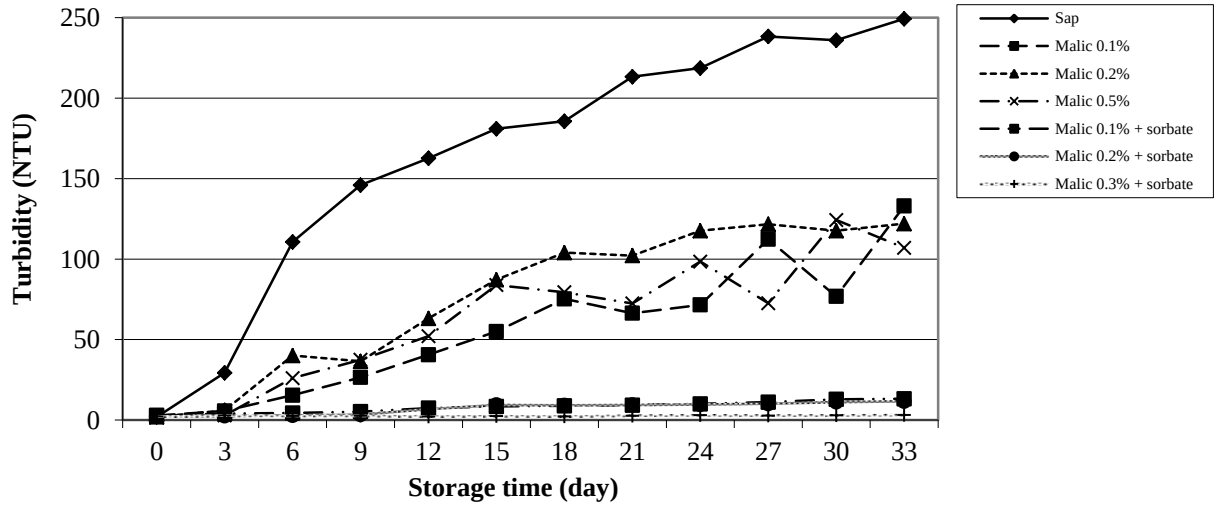

Figure 5 Turbidity changes for birch sap using malic acid-based shelf life extension method (room temperature).

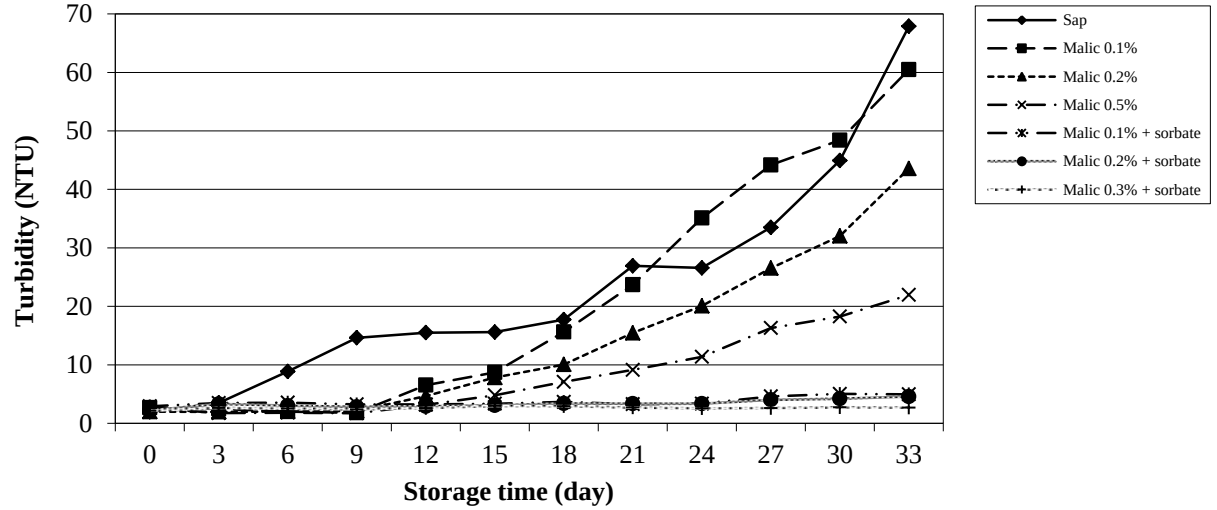

Figure 6 Turbidity changes for birch sap using malic acid-based shelf life extension method (refrigerated conditions).

achieved for the concentrations at 0.1%, and in turn statistically significant negatively evaluation for the sap with addition of malic acid at concentration 0.5%, also for overall and harmony. In the case of sour statistically significant differences have not been reported (Table 2).

In the sensory evaluation of birch tree sap with the addition of citric acid there were no statistically significant differences in the case of sour, and on the other hand for harmony and overall statistically significant negatively evaluation was obtained for the saps with citric acid at the concentration 0.5%.

Table 1 Statistical differences in sensory data of preserved birch tree sap (Sums of Ranks).

Samples		Sour	Harmony	Overall
Citric acid	0.1%	30	22	26
	0.25%	31	30	29
	0.5%	44	58*	55*
Citric acid and potassium sorbate	0.1%	35	22	23
	0.25%	32	27	28
	0.5%	37	48	48
Lactic acid	0.1%	31	23	23
	0.25%	30	35	34
	0.5%	47	56*	58*
Lactic acid and potassium sorbate	0.1%	27	17**	19**
	0.25%	32	29	29
	0.5%	43	50	47
Malic acid	0.1%	27	15**	16**
	0.2%	33	34	31
	0.3%	38	55*	58*
Malic acid and potassium sorbate	0.1%	37	30	28
	0.2%	35	28	29
	0.3%	40	48	48

Note: * Statistically significant negatively evaluation.
** Statistically significant positively evaluation.

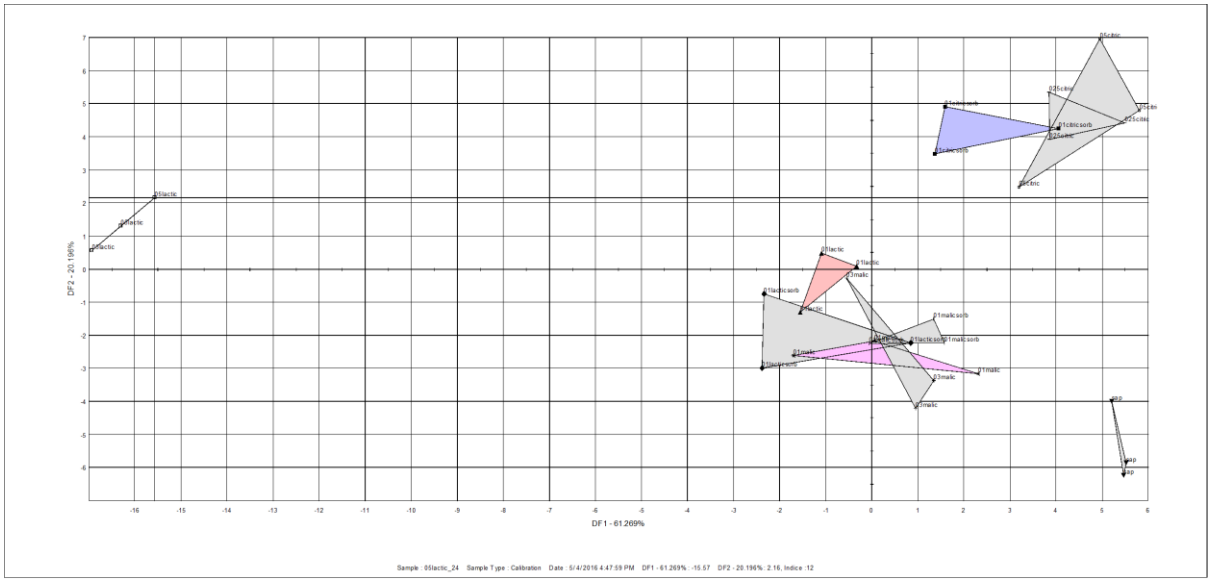

Figure 7 Similarity of the samples classified by electronic nose (own data).

However, in sensory evaluation of tested samples with the lactic acid for sour, there was no statistical differences, while for the harmony and overall there was a statistically significant positively assessment of the sap with the addition of lactic acid in a concentration of 0.1% and with the addition of potassium sorbate, and statistically significant negatively assessment for sap with the addition of lactic acid at 0.5%. Among the samples that were evaluated by the sensory team statistically significantly positively, highest stability has a sample containing sorbate and lactic acid at a concentration 0.1%, ie. approx. 18 days at room temperature and at least a month in the refrigerated condition

How is described in Material and methodology we used for similarity birch saps classification electronic nose. There is relation between aroma and flavour and we would investigate any relations among organoleptic attributes of the samples. We also study interaction between saps characteristics and tasteless, odour less preservation agents (acids). We conclude that prevented birch saps with our selected concentration is possible split to four groups. First group is preserved sap by 0.5 lactid acid (located on opposite quadrant of the plot) typical for very intensive smell of lactic tones. And conservated birch sap was very stable. Another group is represented by pure sap (right bottom corner located). Small triangle of this sample detect small evaporation of volatile compounds (low

interactions). Third banding group are represented samples of citric acid preservation (0.1, 0.25, 0.5). This group is characterized by high evaporation level (triangle size). Last group are combination of low level of lactic and malic acid preservated saps with different level of stability. By sensory tests this groups is prefered to others. All positions of the sample are possible see on Figure 7.

Sensory properties of samples preserved sorbate and the highest concentrations of acids can be improved using functional additives, such as herbal extracts or fruit juices **(Ivanišová et al., 2010)**. They will not only correct the taste but also significantly increased antioxidant capacity, which for birch sap is low **(Bilek et al., 2015a)**. Health benefits will therefore be improved, which is expected by the consumers of food products of plant origin **(Vietoris et al., 2016)**.

CONCLUSION

1. The negative sensory evaluation obtained for the most stable samples could be corrected by the use of additives for improving the flavor such as fruit syrups or herbal extracts. Then, it will be possible to prepare a stable, easy to obtain birch sap-based beverage.

2. We conclude that obtained correlation between sensory preference and increasing ratio of the preservation agents to keep sap stability is low. Investigation of preservation and sensory improvements are just in the beginning. This will be promising way to enlarge usage and awareness about saps application and keep Central European heritage.

REFERENCES

Bilek, M., Pytko, J., Sosnowski, S. 2016a. Shelf life of birch tree sap. *Polish Journal Sustainable Development*, in press.

Bilek, M., Siembida, A., Stawarczyk, K., Cieślik, E. 2015a. Radical scavenging activity of tree saps from podkarpackie region. *Żywność. Nauka. Technologia. Jakość*, vol. 101, no. 4, p. 151-161. http://dx.doi.org/10.15193/ZNTJ/2015/101/063

Bilek, M., Stawarczyk, K., Gostkowski, M., Olszewski, M., Kędziora, K.M., Cieślik, E. 2016b. Mineral content of tree sap from the Subcarpathian region. *Journal of Elementology*, vol. 21, no. 3, p. 669-679. http://dx.doi.org/10.5601/jelem.2015.20.4.932

Bilek, M., Stawarczyk, K., Kuźniar, P., Olszewski, M., Kędziora, K. M., Cieślik, E. 2016c. Evaluation of inorganic anions content in the tree saps. *Journal of Elementology*, vol. 21, no. 4, p. 1277-1288. http://dx.doi.org/10.5601/jelem.2015.20.4.1048

Bilek, M., Stawarczyk, K., Łuczaj, Ł., Cieślik, E. 2015b. Content of selected minerals and inorganic anions in tree saps from Podkarpacie region. *Żywność. Nauka. Technologia. Jakość*, vol. 100, no 3, p. 138-147. http://dx.doi.org/10.15193/zntj/2015/100/046

Bilek, M., Stawarczyk, K., Sosnowski, S. The method of obtaining a beverage with enhanced shelf life and pro-health properties based on tree sap, especially birch tree sap. Republic of Poland, Patent Application P. 417738.

Brindza, J., Vietoris, V., Kucelová, L., Fil, M., Ostrovský, R., Gondová, E. 2011. Morphology and sensory evaluation of traditional products from different landraces of pumpkin (Cucurbita spp.). *Potravinarstvo*, vol. 5, no. 2, p. 1-8. http://dx.doi.org/10.5219/137

Godyla, S. 2015. Consumers' attitude to the birch tree sap. *Think*, vol. 20, p. 7-16.

Ivanišová, E., Fikselová, M., Vietoris, V., Mellen, M. 2009. Antioxidant effects of herbal extracts and their food application. *Potravinarstvo*, vol. 3, no. 4, p. 34-37. http://dx.doi.org/10.5219/75

Jędra, M., Starski, A., Gawarska, H., Sawilska-Rautenstrauch, D. 2008. Occurrence of benzene in beverages. *Bromatologia i Chemia Toksykologiczna*, vol. 41, no. 3, p. 382-388.

Jeong, J. S., Jeong, H. S., Woo, S. H., Shin Ch. S. 2013. Consequences of ultrafiltration and ultraviolet on the quality of white birch (*Betula platyphylla* var. *japonica*) sap during storage. *Australian Journal of Crop Science*, vol. 7, no. 8, p. 1072-1077.

Jeong, J. S., Lee, C. H., Kim, H. Y., Hwang, I. G., Shin, C. S., Park, E. S., Lee, J., Jeong, H. S. 2011. Characeteristic of Goroshoe (Acer mono Max.) Sap with Different Collection Times After Ultra Filtration. *The Korean Society of Food Science and Nutrition*, vol. 40, no 5, p. 753-758. http://dx.doi.org/10.3746/jkfn.2011.40.5.753

Kozelová, D., Vietoris, V., Fikselová, M. 2013. Quality and availability of organic foods by Slovak consumers. *Potravinarstvo*, vol. 7, no. 1, p. 146-150. http://dx.doi.org/10.5219/306

Kozelová, D., Zajác, P., Matejková, E., Zeleňáková, L., Lopašovský, L., Mura, L., Čapla, J., Vietoris, V. 2011. Perception of bio-food labeling by consumers in Slovakia. *Potravinarstvo*, vol. 5, no. 1, p. 33-38. http://dx.doi.org/10.5219/107

Moriyama, M., Naru, E., Misaki, Y., Hayashi, A. 2009. Effect of birch (*Betula platyphylla Sukatchev* var. *japonica Hara*) sap on cultured human epidermal keratinocyte differentiation. *International Journal of Cosmetic Science*, vol. 31, p. 159-161. http://dx.doi.org/10.1111/j.1468-2494.2008.00466_3.x

Papp, N., Czégényi, D., Hegedűs, A., Morschhauser, T., Suave, C. L., Cianfaglione, K., Pieroni, A. 2014. The uses of Betula pendula Roth among Hungarian Csángós and Székelys in Transylvania, Romania. *Acta Societatis Botanicorum Poloniae*, vol. 83, no. 2, p. 113-122, http://dx.doi.org/10.5586/asbp.2014.011

Peev, C., Dehelean, C., Mogosanu, C., Feflea, F., Corina, T. 2010. Spring drugs of Betula pendula Roth.: Biologic and pharmacognostic evaluation. *Studia Univ. VG, SSV*, vol. 20, no. 3, p. 41-43.

R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria [online] s.a. [cit. 2016-04-06] Available at: https://www.R-project.org/

Rastogi, S., Pandey, M. M., Rawat, A. K. S. 2015. Medical plants of the genus Betula – Traditional uses and a phytochemical-pharmacological review. *Journal of Etnopharmacology*, vol. 159, p. 62-83, http://dx.doi.org/10.1016/j.jep.2014.11.010

Rogozińska, I., Wichrowska, D. 2001. The most popular food shelf life additives using in modern food technology. *Inżynieria i Aparatura Chemiczna*, vol. 50, no. 2, p. 19-21.

Stawarczyk, M. 2015. Tree saps. *Aptekarz Polski*, vol. 102, no. 2, p. 17-21.

Svanberg, I., Sõukand, R., Łuczaj, Ł., Kalle, R., Zyryanova O., Dénes, A., Papp, N., Nedelcheva, A., Šeškauskaitė, D., Kołodziejska-Degórska, I., Kolosova, V. 2012. Uses of tree saps in northern and eastern parts of Europe. *Acta Societatis Botanicorum Poloniae*, vol. 81, no. 4, p. 343-357. http://dx.doi.org/10.5586/asbp.2012.036

Vietoris, V., Kozelová, D., Mellen, M., Chreneková, M., Potclan, J. E., Fikselová, M., Kopkáš, P., Horská, E. 2016.

Analysis of Consumer Preferences at Organic Food Purchase in Romania. *Polish Journal of Food and Nutritional Sciences*, vol. 66, no. 2, p. 139-146. http://dx.doi.org/10.1515/pjfns-2015-0028

Viškelis, P., Rubinskienė, M. 2011. The chemical composition of birch sap. Sodininkystė ir Daržininkystė, vol. 30, no. 3-4, p. 75-81.

Yoon, S. L., Jo, J. S., Kim, T. O. 1992. Utilization and Tapping of the Sap from Birches and Maples. *Mokchae Konghak*, vol. 20, no. 4, p. 15-20.

Yuan, T., Li, L., Zhang, Y., Seeram, N. P. 2015. Pasteurized and sterilized maple sap as functional beverages: Chemical composition and antioxidant activities. *Journal of Functional Foods*, vol. 5, no. 4, p. 1582-1590. http://dx.doi.org/10.1016/j.jff.2013.06.009

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EFFECT OF HYDRATED APPLE POWDER ON DOUGH RHEOLOGY AND COOKIES QUALITY

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ABSTRACT

Dietary fiber is a group of food components, which are resistant to human enzymatic digestion. The incorporation of dietary fiber obtained from various sources of fruit and vegetable by-products into the cereal based products such as bread, rolls, cookies, muffins, crackers, cakes and pasta is of growing interest for the food industry. The replacement of wheat flour by dietary fiber from various sources can change physicochemical, textural and organoleptic characteristic of bakery products. Apple pomace is the main by-product produced in the apple fruit processing industry. It is a rich source of carbohydrate, pectin, crude fiber, and minerals. The dietary fiber content in apple pomace ranges between 33 – 35%. The influence of hydrated commercial dietary fiber on wheat dough rheology (5, 10 and 15% flour replacement) and physical and sensory properties of cookies was examined. It was found that addition of HAP significantly increased the rheological properties of dough such as water absorption (from 58.0% to 75.3%), dough stability (from 6.7 min to 11.6 min) and prolonged dough development time (from 3.5 min to 11.0 min) and reduced the mixing tolerance index (from 34.7 BU to 11.9 BU). It was also concluded that hydrated apple powder addition reduced physical properties of cookies such as volume (from 10.4 cm^3 to 8.0 cm^3), diameter (from 4.7 cm to 4.2 cm), volume index (from 1.35 cm to 1.10 cm) and porosity (from 0.32 to 0.24). Sensory properties (taste, odour, stickiness, firmness and density) of cookies were also analysed. Cookies with addition of hydrated apple powder had fruity taste and odour and showed high overall acceptance. From this study resulted that hydrated apple powder can be used as potentially source of dietary fiber in cookie formulation. Moreover, addition of apple pomace inhibits the use of any other flavouring ingredients because has a pleasant fruity flavour.

Keywords: hydrated dietary fiber; rheology; cookie; apple

INTRODUCTION

Because of the rising interest in functional food, especially bioactive substances, food producers look for new sources and carriers of those substances. Consumers search products which would allow them to maintain their physical and mental fitness. Among many bioactive substances to be found in food – such as antioxidants, plant sterols, pro- and prebiotics and vitamins – a crucial role is played by dietary fibre (DF) **(Górecka et al., 2010)**.

The benefits associated with an adequate intake of DF include the regulation of intestinal transit and prevention or treatment of diabetes, cardiovascular disease and colon cancer. DF reduces the risk of hyperlipidaemia, hypercholesterolaemia and hyperglycaemia via mechanisms that modulate food ingestion and influence the digestion, absorption and metabolism of nutrients **(Macagnan et al., 2015)**.

DF as a class of compounds includes a mixture of plant carbohydrate polymers, both oligosaccharides and polysaccharides, e.g., cellulose, hemicelluloses, pectic substances, gums, resistant starch, inulin, that may be associated with lignin and other non-carbohydrate components (e.g., polyphenols, waxes, saponins, cutin, phytates, resistant protein) **(Kohajdová et al., 2011a)**.

Many fiber sources have been identified and are being used in various baked products **(Masoodi et al., 2002)**. Traditionally, consumers have chosen foods such as whole grains, fruits and vegetables as sources of dietary fiber. Recently, food manufacturers have responded to consumer demands for foods with higher fiber content by developing products in which high-fibre ingredients are used **(Almedia et al., 2013)**. The by-products of fruits from industrial applications are potential sources of DF that can be incorporated into food products. In addition, fruit fibres have significant amounts of secondary compounds associated with them, such as polyphenols with high biological activity and other bioactive compounds **(Macagnan et al., 2015)**.

DF can also impart some functional properties to foods, e.g., increase water holding capacity, oil holding capacity, emulsification and/or gel formation. That dietary fibre incorporated into food products (bakery products, dairy, jams, meats, soups) can modify textural properties, avoid synaeresis (the separation of liquid from a gel caused by contraction), stabilise high fat food and emulsions, and improve shelf-life **(Karovičová et al., 2015),** colour, aroma and reduce energy of the final product **(Bilgiçli et al., 2007)**.

Dietary fibre is classified as soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). The SDF/IDF ratio close to 1:2 indicates fibre as suitable for use as food ingredient Fruit fibres have better quality due to higher total and soluble fibre content **(Kołodziejczyk et al., 2007)**.

Apples are good sources of fiber with a well-balanced proportion between soluble and insoluble fraction **(Figuerola et al., 20005)**. Apple pomace (AP) from straight pressing, the primary by-product of the apple juice industry, is rich in cell wall material and is an interesting source of pectins and fibres **(Massiot and Renard, 1997)** and can be considered as a raw material for direct preparation of dietary fiber. AP consists of a heterogeneous mixture of peel, seeds, calyx, stem and pulp. AP can represent about 20 – 40% of the weight of processed fruits, depending on the technology used in the extraction of juice **(Macagnan et al., 2015)**. Dried AP is considered as a potential food ingredient **(Kohajdová et al., 2009, Sudha et al., 2007)**. AP is constituted by simple sugars (glucose, fructose, and sucrose) **(Mirabella et al., 2014)** and is a rich source of carbohydrates total dietary fibre including cellulose, hemicellulose, lignin, pectin, and galacturnic acid and minerals such as calcium, magnesium, zinc, iron, and copper. AP is also a good source of phytochemicals primarily phenolic acids such as chlorogenic, protocatechuic, and caffeic acid and flavonoids, e.g. flavanols and flavonols **(Kohajdová et al., 2014)**. Addition of AP in cake making can avoid the application of other flavouring ingredients as the cakes prepared with AP had pleasant fruity flavour **(Kohajdová et al., 2011a, Sudha et al., 2007)**.

Among foods enriched in fibre, the most known and consumed are breakfast cereals and bakery products such as integral breads and cookies **(Dhingra et al., 2012)**. Cookies have been suggested as a good way to use composite flours as they are ready-to-eat, provide a good source of energy, and are consumed widely throughout the. The term cookies, or biscuits as they are called in many parts of the world, refers to a baked product generally containing the three major ingredients flour, sugar and fat. These are mixed together with other minor ingredients to form dough **(Zucco et al., 2011)**. A variety of fibers from plant sources have been used in cookies to improve the texture, color and aroma with a reduced energy of the final product **(Gupta et al., 2011)**.

In the recent years, several studies have shown potential use of AP in cereal based products such as bread, cakes, cookies, biscuits and muffins **(Chen et al., 1988; Kohajdová et al., 2011a, 2014; Masoodi et al., 2002; Sudha et al. 2007; Vitali et al., 2009; Rupasinghe et al., 2009; Kučerová et al., 2013)**.

The aim of the present study was to determine the potential use of commercial apple powder, through a systematic study of the influence of hydrated apple powder on the rheological properties of wheat dough and the final quality of cookies. The sensory evaluation of cookies was also performed.

MATERIAL AND METHODOLOGY

Material

Fine wheat flour, commercial apple DF powder (Country life, s.r.o., Beroun, Czech Republic) and other ingredients (sugar, shortening, salt and baking powder) were purchased from local market in Slovakia.

Hydrated dietary fiber

Hydrated apple powder (HAP) was prepared according to method of **Chen et al. (1988)**. Seven parts of distilled water were added to one part of commercial apple powder to hydrate for 12 h. The excess water was decanted and discarded before rheological determination of and baking.

Chemical analysis

The chemical composition of commercial apple powder and fine wheat flour was presented in previous study by **Kohajdová et al. (2011a)**. The commercial apple powder contained 46.1% of total dietary fiber, of which 20.4% was content of pectin.

Rheological characteristics

The Farinographic parameters of dough (water absorption, dough development time, dough stability, degree of softening, mixing tolerance index) were determined using Farinograph Brabender (Duisburg, Germany) according to method ISO 5530-1: 2013.

Cookie formulation

The cookies were prepared according to modified formula described by **Tyagi et al. (2007)**. The control formula included: 100 g wheat flour, 53 g sugar, 26.5 g shortening, 1.1 g sodium bicarbonate, 0.89 g sodium chloride and 12 cm^3 water. Wheat flour was replaced by hydrated apple powder at level 5, 10, and 15%. The dough was rolled out to a height of 2 mm and cut into round shape with diameter of 40 mm using cookie cutter. The cookies were baked at 180 °C in oven (Mora, Slovakia) for 8-9 min. The cookies were cooled for 30 min and packed in polyethylene bags.

Cookies physical properties

Volume index of cookies was measured according to the method of **Turabi et al. (2008)**. Cookies were cut vertically through the center and the heights of the cookies were measured at three different points (B, C, and D) along the cross-sectioned cookie using the template. According to this method volume index was determined by the following formula: Volume index = B + C + D, where C is the height of the cookie at the center point and B and D are the heights of the cookie at the points 2.5 cm away from the center towards the left and right sides of the cookie, respectively.

Methods of **Shittu et al. (2007)** were used for determination of cookies weight, volume, specific volume and porosity. The weights of samples (W_1) (accuracy 0.001) were determined after sufficient cooling and the cookie volumes were determined using rapeseed displacement method (V_1). The samples were milled and sieved to 100 mesh size and the underflow was weighed (W_2). The sample was then poured into a 20 cm^3 measuring cylinder (accuracy = 0.5 cm^3) and tapped 10 times. The volume occupied by the cookie sample was determined (V_2). The data were used to determine the crumb (ρ_c) and solid density (ρ_s) as follows: $\rho_c = W_1/V_1$ and $\rho_s = W_2/V_2$. The porosity ε_c was calculated as: $\varepsilon_c = 1 - (\rho c/\rho s)$ **(Shittu et al., 2007)**.

Thickness and diameter of cookies were measured with a calliper in each cookie at three different places.

Sensory evaluation of cookies

The sensory evaluation of cookies was assessed in eight criteria (descriptors) of quality by five point hedonic scale which ranged from 5 = most liked to 1 = most disliked. The panel was made up of staff and students of the Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia. The overall acceptability of cookies was determined using 100 mm graphical non-structured abscissas with the description of extreme points (minimal or maximal intensity, from 0 to 100%) according to the method described by **Kohajdová et al. (2011a)**.

Statistical analysis

All analyses were carried out in triplicate and average values were calculated. The results were expressed as mean ± standard deviation. One-way analysis of variance and Fisher's least significant differences procedure were applied to the data to establish the significance of the differences between the samples at the level of p = 0.05. Statgraphic Plus, Version 3.1 (Statistical Graphic Corporation, Princeton, NY, USA) was used as the statistical analysis software.

RESULTS AND DISCUSSION

The mixing process is the crucial operation in bakery industry by which the wheat flour, water, and additional ingredients are changed through the mechanical energy flow to coherent dough. It is well known that dough properties can be affected by many features with different significance, therefore the dough development and processing optimization towards best quality bakery products is quite a difficult problem **(Bojňanská et al., 2014)**. The effect of the application of HAP on farinographic parameters of dough is presented in Table 1. It was concluded that incorporation of HAP significantly increased the water absorption from 58.0% (control) to 74.5% (15% of HAP). The increase of water absorption could be explained by the important number of hydroxyl groups existing in the fiber structure, which allow more water interactions through hydrogen bonding **(Borchani et al., 2001)**. Similar increase of WA was recorded by **Kohajdová et al. (2012)** and **Ajila et al. (2008)** when the carrot pomace powder and mango peel powder was incorporated to dough.

The dough development time (DDT) is the time from water addition to the flour until the dough reaches the point of the greatest torque. During the mixing phase, water hydrates the flour components and the dough is developed **(Lei et al., 2008)**. It was observed that addition of HAP significantly prolonged DDT from 3.5 min (control) to 11 min (15% of HAP). The same effect on the DDT was reported by several authors when the hydrated apple fiber **(Chen et al., 1988)**, laboratory prepared apple fiber **(Kohajdová et al., 2014)** or commercial apple wheat, potato and bamboo fiber were added to wheat dough **(Kučerová et al., 2013)**. An increase in the DDT indicates that an increase in fiber content in the blends has slowed the rate of hydration and development of gluten **(Sudha et al., 2007)**.

The dough stability (DS) gives some indication of the tolerance of the flour to mixing **(Lei et al., 2008)**. It was found that addition of HAP significantly increased DS from 6.7 min (control) to 11.6 min (15% of HAP). Recently, **Kohajdová et al. (2011a, 2014)** also reported an increase in DS after addition of apple dietary fiber. It can be explained by higher interaction of DF, water and flour proteins **(Kohajdová et al., 2014)**.

Also it was concluded that MTI was significantly reduced by the addition of HAP. Similar effect to MTI was also observed by **Kohajdová et al. (2013)** and **Nassar et al. (2008)** after addition of grapefruit dietary fiber, orange peel and pulp to wheat flour. Reduction in MTI could be explained by the interactions between fibres and gluten **(Kohajdová et al., 2014)**.

The physical properties of cookies with HAP are shown in Table 2. It was showed that addition of HAP significantly reduced the volume of cookies (Figure 1). It can be attributed to the dilution of gluten and also to the

Table 1 Effect of the different level of HAP on rheological parameters of dough.

	Water absorption (%)	Dough development time (min)	Dough stability (min)	Mixing tolerance index (BU)
Control	58.0 ±0.7	3.5 ±0.2	6.7 ±0.3	34.7 ±1.3
5% HAP	66.1 ±1.2*	10.3 ±0.5*	10.8 ±0.6*	30.8 ±0.2*
10% HAP	72.5 ±1.2*	10.4 ±0.4*	11.0 ±0.4*	30.3 ±1.3*
15% HAP	75.3 ±0.7*	11.0 ±0.5*	11.6 ±0.5*	10.9 ±0.5*

NOTE: * denotes a statistically significant difference at $p = 0.05$ level, HAP- hydrated apple powder.

Table 2 Effect of hydrated apple powder on physical parameters of cookies.

	Diameter (cm)	Volume (cm^3)	Porosity	Volume index (cm)
control	4.7 ±0.2	10.3 ±0.5	0.32 ±0.01	1.35 ±0.01
5% HAP	4.6 ±0.1	9.5 ±0.3*	0.32 ±0.01	1.33 ±0.05
10% HAP	4.4 ±0.0*	8.6 ±0.4*	0.27 ±0.01*	1.28 ±0.04
15% HAP	4.2 ±0.1*	8.0 ±0.3*	0.24 ±0.01*	1.10 ±0.05*

NOTE: * denotes a statistically significant difference at $p = 0.05$ level, HAP- hydrated apple powder.

Table 3 Sensory evaluation HAP enriched cookies.

	grain taste	sweet taste	fruity taste	grain odour	fruity odour
control	5.0 ±0.0	4.9 ±0.1	1.0 ±0.1	5.0 ±0.0	1.0 ±0.1
5% HAP	3.7 ±0.1*	4.0 ±0.2*	2.5 ±0.1*	3.7 ±0.1*	3.0 ±0.1*
10% HAP	2.5 ±0.1*	3.4 ±0.2*	3.9 ±0.1*	3.3 ±0.1*	3.7 ±0.1*
15% HAP	2.0 ±0.1*	2.5 ±0.1*	4.5 ±0.1*	2.4 ±0.1*	4.1 ±0.1*

NOTE: * denotes a statistically significant difference at $p = 0.05$ level, HAP- hydrated apple powder.

Table 4 Overall acceptance and textural characteristics of cookies.

	firmness	stickiness	density	overall acceptance
control	4.9 ±0.1	1.1 ±0.1	4.9 ±0.1	99.9 ±0.1
5% HAP	4.6 ±0.1*	1.3 ±0.1*	4.6 ±0.1	96.3 ±0.4
10% HAP	4.5 ±0.1*	2.5 ±0.1*	4.0 ±0.1*	92.8 ±0.7*
15% HAP	4.2 ±0.1*	2.7 ±0.1*	3.5 ±0.1*	90.6 ±0.5*

NOTE: * denotes a statistically significant difference at $p = 0.05$ level, HAP- hydrated apple powder.

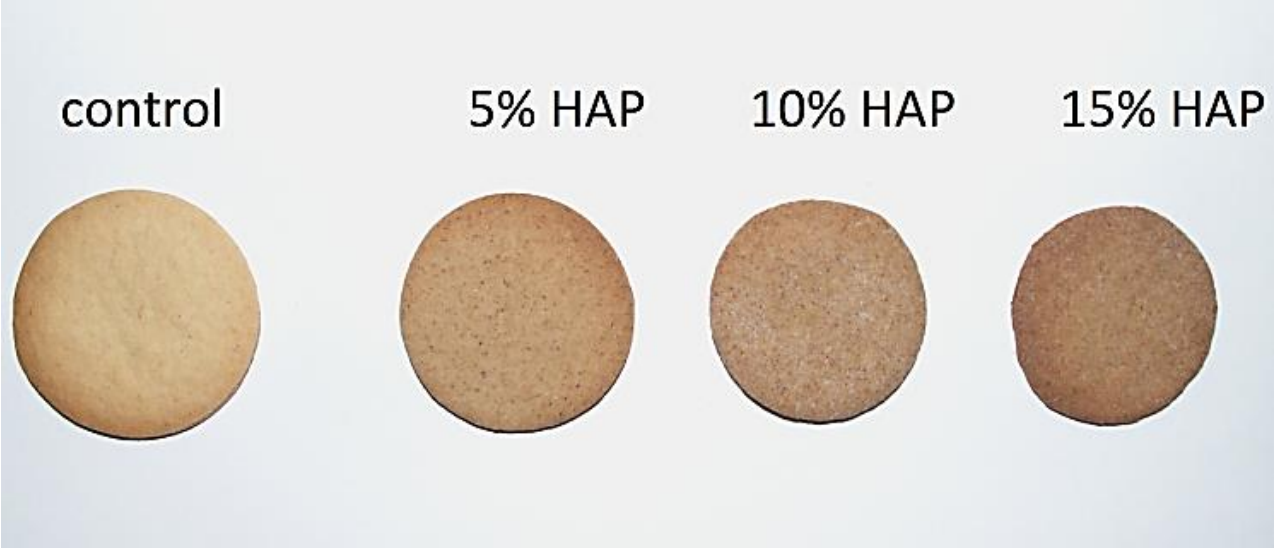

Figure 1 Cookies with addition HAP. NOTE: HAP- hydrated apple powder.

interaction of gluten, DF components, and water **(Kohajdová et al., 2014)**. Hydration of apple fiber before addition to wheat flour can partially alleviate the detrimental effects on bread loaf volume **(Chen et al., 1988)**.

Moreover, it was observed that volume index of cookies decreased significantly when the level of HAP reached 15%. The same effect was reported by **Masoodi et al. (2002)** after addition of apple pomace to cake formula.

From results also concluded that higher level of HAP (10 and 15% of HAP) significantly reduced diameter of cookies. This finding is in agreement with those described by **El-Sharnouby et al. (2012)**, **Larrea et al. (2005) and Chen et al. (1988)** for wheat bran and date powder, orange pulp and apple fiber incorporated cookies and biscuits.

Results also showed that cookies with higher level (10 and 15%) of HAP had significant lower porosity (0.27 and 0.24 respectively) as control sample. This decreased porosity is related to the incorporation of air as smaller bubbles during the kneading process, giving rise to a dough with greater stability that achieves greater expansion in the subsequent processes **(Martínez et al., 2014)**.

The effects of HAP on the sensory properties of cookies are presented in Table 3. Results showed that addition of HAP significantly increased fruity taste and fruity odour and reduced grain taste and odour of cookies. Similar effect of increasing fruity taste and odour was noticed after addition of raspberry pomace **(Górecka et al., 2010)**, apple dietary fiber **(Kohajdová et al., 2011a)** and grapefruit dietary fiber **(Kohajdová et al., 2013)** to cookies and biscuits. It was also observed that incorporation of HAP at level 5% had no significant effect on overall acceptability (Table 4).

Incorporation of HAP to cookies also resulted in the significant decrease of density and firmness. Moreover, the stickiness was significantly increased after increasing flour substitution by HAP. The same result was reported by **Kohajdová et al. (2011a)** in cookies with addition of commercial apple fiber.

CONCLUSION

The additions of hydrated commercial apple powder significantly affect the rheological properties of wheat dough in various ways. It was concluded that WA was increased, DS and DDT were prolonged and MTI was decreased. Physical characteristics of cookies (diameter, volume, volume index and porosity) were significantly decreased with the increasing level of HAP. Sensorical parameters were also affected significantly. Furthermore, it was observed that cookies with 5% HAF were the most acceptable for assessors.

The result of this study showed that cookies with acceptable physical, textural and sensory properties can be developed by incorporating hydrated apple powder in wheat flour at level 5%. Moreover, it was reported that the addition of apple pomace avoids the use of any other flavouring ingredients because has a pleasant fruity flavour.

REFERENCES

Ahmed, J. 2015. Effect of barley β-glucan concentrate on oscillatory and creep behavior of composite wheat flour dough. *Journal of Food Engineering*, vol. 152, p. 85-94. http://dx.doi.org/10.1016/j.jfoodeng.2014.11.018

Ajila, C. M., Leelavathi, K., Prasada Rao, U. J. S. 2008. Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. *Journal of Cereal Science*, vol. 48, no. 2, p. 319-326. http://dx.doi.org/10.1016/j.jcs.2007.10.001

Almedia, E. L., Chang, Y. K., Steel, C. J. 2013. Dietary fibre sources in bread: Influence on technological quality. *LWT - Food Science and Technology*, vol. 50, no. 2, p. 545-553. http://dx.doi.org/10.1016/j.lwt.2012.08.012

Anil. M. 2007. Using of hazelnut testa as a source of dietary fiber in breadmaking. *Journal of Food Engineering*, vol. 80, no. 1, p. 61-67. http://dx.doi.org/10.1016/j.jfoodeng.2006.05.003

Arun, K. B., Persia, F., Aswathy, P. S. P., Chandran, J., Sajeev, M. S., Jayamurthy, P., Nisha, P. 2015. Plantain peel - a potential source of antioxidant dietary fibre for developing functional cookies. *J. Food Sci. Technol.*, vol. 52, no. 10, p. 6355-6364. http://dx.doi.org/10.1007/s13197-015-1727-1 PMid:26396380

Bilgiçli, N., İbanoğlu, Ş., Herken, E. N. 2007. Effect of dietary fibre addition on the selected nutritional properties of cookies. *Journal of Food Engineering*, vol. 78, no. 1, p. 86-89. http://dx.doi.org/10.1016/j.jfoodeng.2005.09.009

Bojňanská, T., Tokár, M., Frančáková, H. Changes of the dough rheological properties influenced by addition of potato fibre. *Potravinarstvo*, vol. 8, no. 1, p. 161-166. http://dx.doi.org/10.5219/371.

Borchani, Ch., Masmoudi, M., Besbes, S., Attia, H., Deroanne, C., Blecker, Ch. 2011. Effect of date flesh fiber concentrate addition on dough performance and bread quality. *Journal of Texture Studies*, vol. 42, no. 4, p. 300-308. http://dx.doi.org/10.1111/j.1745-4603.2010.00278.x

Dhingra, D., Michael, M.,Rajput, H., Patil, R. T. 2012. Dietary fibre in foods: a rewiew. *Journal of Food Science and Technology*, vol. 49, no. 3, p. 255–266. http://dx.doi.org/10.1007/s13197-011-0365-5

El-Sharnouby, G. A., Aleid, A. M., Al-Otaibi, M. M. 2012. Nutritional Quality of Biscuit Supplemented with Wheat Bran and Date Palm Fruits (*Phoenix dactylifera* L.), *Food and Nutrition Sciences*, vol. 3, no. 3, p. 322-328. http://dx.doi.org/10.4236/fns.2012.33047

Fernández-Ginés, J. M., Fernández-López, J., Sayas-Barberá, E., Sendra, E., Pérez-Álvarez, J. A. 2004. Lemon albedo as a new source of dietary fiber: Application to bologna sausages. *Meat Science*, vol. 67, no. 1, p. 7-13. http://dx.doi.org/10.1016/j.meatsci.2003.08.017

Gómez, M., Ronda, F., Blanco, C. A., Caballero, A., Apesteguía, A. 2003. Effect of dietary fibre on dough rheology and bread quality. *European Food Research and Technology*, vol. 216, no. 1, p. 51-56. http://dx.doi.org/10.1007/s00217-002-0632-9

Gupta, M., Bawa, A. S., Abu-Ghannam, N. 2011. Effect of barley flour and freeze–thaw cycles on textural nutritional and functional properties of cookies. *Food and Bioproduct Processing*, vol. 89, no. 4, p. 520-527. http://dx.doi.org/10.1016/j.fbp.2010.07.005

Chen, H., Rubenthaler, G. L., Leung, H. K., Baranowski, J. D. 1988. Chemical, Physical, and Baking Properties of Apple Fiber Compared with Wheat and Oat Bran. *Cereal Chemistry*, vol. 65, no. 3, p. 244-247. [cit. 2016-02-06] Available at: http://www.aaccnet.org/publications/cc/backissues/1988/documents/65_244.pdf.

ISO 5530-1: 2013. *Wheat flour -- Physical characteristic of doughs -- Part 1: Determination of water absorption and rheological properties using a farinograph.*

Jurasová, M., Kohajdová, Z., Karovičová, J. Dietary fiber: Definition, sources and extraction. *Potravinarstvo*, vol. 5, no. 2, p. 22-26. http://dx.doi.org/10.5219/125

Karovičová, J., Kohajdová, Z., Minarovičová, L., Kuchtová, V. 2015. The Chemical Composition of Grape Fibre. *Potravinarstvo*, vol. 9, no. 1, p. 56-57. http://dx.doi.org/10.5219/428

Kohajdová, Z., Karovičová, J., Jurasová, M., Kukurová, K. 2011a. Effect of the addition of commercial apple fibre powder on the baking and sensory properties of cookies. *Acta Chimica Slovaca*, vol. 4, no. 2, p. 88-97. [cit. 2016-02-05] Available at: http://www.acs.chtf.stuba.sk/papers/acs_0095.pdf.

Kohajdová, Z., Karovičová, J., Šimková, S. 2009. Use of Apple fibre in bakery products. *Acta fytotechnica et zootechnica*, vol. 12, p. 286-290.

Kohajdová, Z., Karovičová, J., Jurasová, M., Kukurová, K. 2011b. Application of citrus dietary fibre preparations in biscuit production, *Journal of Food and Nutrition Research*, vol. 50, no. 3, p. 182-190. Available at: http://web.b.ebscohost.com/ehost/pdfviewer/pdfviewer?sid=cf25913f-ce38-4a6a-8de1-0cca7196cf67%40sessionmgr102&vid=2&hid=106

Kohajdová, Z., Karovičová, J., Jurasová, M. 2012. Influence of carrot pomace powder on the rheological characteristics of wheat flour dough and on wheat rolls quality. *Acta Scientiarum Polonorum, Technologia Alimentaria*, vo.11, no.4, p. 381-387. Available at: http://www.food.actapol.net/pub/7_4_2012.pdf.

Kohajdová, Z., Karovičová, J., Magala, M., Kuchtová, V. 2014. Effect of apple pomace powder addition on

farinographic properties of wheat dough and biscuits quality. *Chemical papers*, vol. 68, no. 8, p. 1059-1065. http://dx.doi.org/10.2478/s11696-014-0567-1

Kołodziejczyk, K., Markowski, J., Kosmala, M., Król, B., Płocharski, W. 2007. Apple pomace as a potencial source of nutraceutical products. *Polish Journal of Food and Nutrition sciences*, vol. 57, no. 4, p. 291-295.

Kučerová, J., Šottníková, V., Nemodová, Š. 2013. Influence of Dietary Fibre Addition on the Rheological and Sensory Properties of Dough and Bakery Products. *Czech Journal of Food Science*, vol. 31, no. 4, p. 340-346. Available at: http://www.agriculturejournals.cz/publicFiles/97017.pdf.

Larrea, M. A., Chang, Y. K., Martinez-Bustos, F. 2005. Some functional properties of extruded orange pulp and its effect on the quality of cookies. *LWT - Food Science and Technology*, vol. 38, no. 3, p. 213-220. http://dx.doi.org/10.1016/j.lwt.2004.05.014

Lei, F., Ji-Chun, T., Cai-Ling, S., Chun, L. 2008. RVA and Farinograph Properties Study on Blends of Resistant Starch and Wheat Flour. *Agricultural Sciences in China*, vol. 7, no. 7, p. 812-822. http://dx.doi.org/10.1016/S1671-2927%2808%2960118-2

Macagnan, F. T., Santos, L. R., Roberto, B. S., De Moura, F. A., Bizzani, M., Da Silva, L. P. 2015. Biological properties of apple pomace, orange bagasse and passion fruit peel as alternative sources of dietary fibre. *Bioactive Carbohydrates and Dietary Fibre*, vol. 6, no. 1, p. 1-6. http://dx.doi.org/10.1016/j.bcdf.2015.04.001

Martínez, M. M., Díaz, Á. Gómez, M. 2014. Effect of different microstructural features of soluble and insoluble fibres on gluten-free dough rheology and bread-making. *Journal of Food Engineering*, vol. 142, p. 49-56. http://dx.doi.org/10.1016/j.jfoodeng.2014.06.020

Masoodi, F. A., Sharma, B., Chauhan, G. S. 2002. Use of apple pomace as a source of dietary fiber in cakes. *Plant Foods for Human Nutrition*, vol. 57, p. 121-128. http://dx.doi.org/ 10.1023/A:1015264032164

Mirabella, N., Castellani, V., Sala, S. 2014. Current options for the valorization of food manufacturing waste: a review. Journal of Cleaner Production, vol. 65, p. 28-41. http://dx.doi.org/10.1016/j.jclepro.2013.10.051

Rupasinghe, H. P. V., Wang, L. X., Pitts, N. L., Astatkie, T. 2009. Baking and sensory characteristics of muffins incorporated with apple skin powder. *Journal of Food Quality*, vol. 32, no. 6, p. 685-694. http://dx.doi.org/10.1111/j.1745-4557.2009.00275.x

Shiau, S.-Y., Wu, M.-Y., Liu, Y.-L. 2015. The effect of pineapple core fiber on dough rheology and the quality of mantou. *Journal of Food and Drug Analysis*, vol. 23, no.3, p. 493-500. http://dx.doi.org/ http://dx.doi.org/10.1016/j.jfda.2014.10.010

Shittu, T. A., Raji, A. O., Sanni, L. O. 2007. Bread from composite cassava-wheat flour: I. Effect of baking time and temperature on some physical properties of bread loaf. *Food Research International*, vol. 40, no. 2, p. 280-290. http://dx.doi.org/10.1016/j.foodres.2006.10.012

Singh, M., Liu, S. X., Vaughn, S. F. 2012. Effect of corn bran as dietary fiber addition on baking and sensory quality. *Biocatalysis and Agricultural Biotechnology*, vol. 1, no. 4, p. 348-352. http://dx.doi.org/10.1016/j.bcab.2012.02.005

Sowbhagya, H. B. Suomya, C., Indrani, D., Srinivas, P. 2015. Physico-chemical characteristics of – chilli spent residue and its effect on the rheological, microstructural and nutritional qualities of bread. *J. Food Sci. Technol.*, vol. 52, no. 11, p. 7218-7226. http://dx.doi.org/10.1007/s13197-015-1824-1

Struck, S., Gundel, L., Zahn, S., Rohm, H. 2016. Fiber enriched reduced sugar muffins made from iso-viscous batters. *LWT – Food Science and Technology*, vol. 65, p. 32-38. http://dx.doi.org/10.1016/j.lwt.2015.07.053

Sudha, M. L., Baskaran, V., Leelavathi, K. 2007. Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. *Food Chemistry*, vol. 104, no. 2, p. 686-692. http://dx.doi.org/10.1016/j.foodchem.2006.12.016

Turabi, E., Gulum, S., Sahin, S. 2008. Rheological properties and quality of rice cakes formulated with different gums and an emulsifier blend. *Food Hydrocolloids*, vol. 22, no. 2, p. 305-312. http://dx.doi.org/10.1016/j.foodhyd.2006.11.016

Tyagi, S. K., Manikantan, M. R., Oberoi, H. S., Kaur, G. 2007. Effect of mustard flour incorporation on nutritional, textural and organoleptic characteristics of biscuits. *Journal of Food Engineering*, vol. 80, no. 4, p. 1043-105. http://dx.doi.org/10.1016/j.jfoodeng.2006.08.016

Vitali, D., Dragojević, I. V., Šebečić, B. 2009. Effects of incorporation of integral raw materials and dietary fibre on the selected nutritional and functional properties of biscuits. *Food Chemistry*, vol. 114, no. 4, p. 1462-1469. http://dx.doi.org/10.1016/j.foodchem.2008.11.032

Zucco, F., Borsuk, Y., Arntfield, S. D. 2011. Physical and nutritional evaluation of wheat cookies supplemented with pulse flours of different particle sizes. *LWT - Food Science and Technology*, vol. 44, no. 10, p. 2070-2076. http://dx.http://dx.doi.org/10.1016/j.lwt.2011.06.007

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POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY OF FRUIT AND VEGETABLE BEVERAGES PROCESSED BY DIFFERENT TECHNOLOGY METHODS

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ABSTRACT

The purpose of the natural drinks production is the preservation of biologically active compounds in maximal amount in prepared drinks. The issue is the loss of these substances due to conventional conservation methods, such as pasteurization. Pascalization, a conservation method using high pressure, performs a new trend in conservation. According to available research, it causes only a minimal loss of bioactive compounds. Influence of conservation technology of fruit and vegetable beverages on the content of bioactive substances – polyphenols, flavonoids and on their antioxidative activity has been investigated. Their content has been compared in fresh juice samples, in samples conserved by pasteurization and after the appliance of high pressure treatment – pascalization (HPP). HPP has a positive effect on total antioxidative capacity of juices – broccoli with apple (increase of the amount from 189.12 mg.100 mL^{-1} to 217.12 mg.100 mL^{-1}) and beetroot and on total polyphenol content within all samples of beverages except from carrot juice. Decrease of the amounts of flavonoids has been observed within all beverages. For drinks after pasteurization treatment there is evident the decrease of total polyphenols content and total antioxidant activity, besides carrot juice, where the antioxidant capacity value had increased from 37.24 to 43.14 mg.100 mL^{-1}. The flavonoid content of fruit and vegetable juices after heat treatment had increased only in the juice prepared from broccoli with apple (from 40.71 mg.100 mL^{-1} to 45.14 mg.100 mL^{-1}), the content in other juices had decreased. However, the decrease of the flavonoid content is lower after heat treatment in comparison to HPP, except the samples of cabbage juice with apple. With the exception of flavonoids, HPP has been proved as a gentle conservation technology enabling preserving higher amounts of bioactive substances with antioxidative properties if compared with the heat treatment. For the samples treated by HPP there was observed statistically significant difference in comparison with fresh juice in all factors mentioned above (p <0.05).

Keywords: beverages; pasteurization; pascalization; polyphenols; antioxidative activity

INTRODUCTION

Fruit and vegetable contain a significant amount of biologically active substances able to lower a risk of any type of cancer or other civilization diseases **(Mendelová et al., 2016; Jakubcova et al., 2014)**. These bioactive substances are engaged in biochemical processes and are significant parts of the immune system **(Turati et al., 2015; Paliyath, 2011)**.

One of the postulate held by supporters of natural beverages is the preservation of the maximal amount of biologically active substances in processed juices. The issue is the loss of these substances due to conventional conservation methods, such as pasteurization. Pascalization, a conservation method using high pressure, performs a new trend in conservation. According to available research, it causes only a minimal loss of volatile compounds, pigments, vitamins, antioxidant compounds, and mineral substances **(Norton and Sun, 2008; Ramirez et al., 2009)**.

Conservation by high pressure (100 – 1000 MPa), high pressure processing (HPP), is a relatively new conservative method enabling preserving food natural properties and content of nutritional substances with health benefits using non-thermal processing to paralyze harmful pathogens and spoilage microorganisms by instantaneously transmitting isostatic pressure **(Bala et al., 2008; Oey et al., 2008)**. Conventional conservational processes use either physical or chemical means and thus they influence sensoric properties of raw materials and content of bioactive substances **(VSCHT, 2014)**.

HPP has been studied in several fruit juices, such as blueberry juice **(Barba, et al., 2013)**, tomato puree **(Rodrigo et al., 2007)**, apple juice **(Baron et al., 2006; Valdramidis et al., 2009)** and pomegranate juice **(Chen et al., 2013; Varela-Santos et al., 2012)**.

The aim of this work was to observe contents of important biologically active substances in fruit and vegetable juices and to compare their contents in fresh juice, in juice treated by pasteurization and pascalization.

MATERIAL AND METHODOLOGY

As the source of material to determine antioxidative activity, total polyphenols and flavonoids, 100% natural beverages, cold-pressed juices of one species or a mixture

of different fruit or vegetable species, were used. They are without an addition of water, sugar, aroma or any other additives. Vegetable and fruit juices were treated by high pressure (600 MPa) and pasteurization (the temperature of 70 °C for 10 minutes) or they were used as fresh juice to compare with the processed juices. The samples were analyzed immediately after pressing in five repetitions.

The samples content was as follows: beetroot 100%, carrot 100%, broccoli with apple (apple juice 50%, broccoli juice 30%, orange juice 18%, lime juice 2%), cabbage with apple (apple juice 64 %, cabbage juice 32%, lemon juice 4%).

Total phenolic content assay

To measure total phenolic content (TPC) Folin-Ciocalteau reagent was used. 500 μL of the juice was taken and mixed with water in a 50 mL volumetric flask. Thereafter, 2.5 mL of Folin-Ciocalteau reagent and 7.5 mL of 20% solution of Na_2CO_3 were added. The resulting absorbance was measured by LIBRA S6 spectrophotometer (Biochrom Ltd., Cambridge, UK) at the wavelength of 765 nm. Water was used as reference **(Thaipong et al., 2006)**. The results were expressed as milligrams of gallic acid (GAE) per 100 mL.

Total flavonoid content assay

Total flavonoid content (TFC) was determined by using 300 mL of juice mixed with 3.4 mL of 30% ethanol, 0.15 mL of $NaNO_2$ (c = 0.5 mol.dm^{-3}) and 0.15 mL of $AlC1_3.6H_20$ (c = 0.3 mol.dm^{-3}) as is described by **Park et al., (2008)**. The mixture was measured at the wavelength of 506 nm by LIBRA S6 spectrophotometer (Biochrom Ltd., Cambridge, UK). Total flavonoid content was calculated from a calibration curve by using rutin as the standard. The results were expressed milligrams of rutin per 100 mL.

Total antioxidant capacity assay

determine total antioxidant capacity (TAC) DPPH (1,1-diphenyl-2picrylhydrazyl) assay was used according to the study by **Brand-Williams et al. (1995)**. The stock solution was prepared by dissolving 24 mg of DPPH with 100 mL of methanol and then stored at -20 °C until needed. The absorbance of DPPH radical without juice was measured daily. The sample solution was obtained by mixing 10 mL of the stock solution with 45 mL of methanol to obtain the absorbance of 1.1 ±0.02 units at 515 nm using the spectrophotometer LIBRA S6 (Biochrom Ltd., Cambridge, UK). The juice (150 μL) was allowed to react with 2.850 μL DPPH solution for 1 hour in the dark. Then, the absorbance was taken at 515 nm. Antioxidant capacity was calculated as a decrease of the absorbance value using the formula:

Antioxidant capacity (%) = (A0 - Ai/A0) x 100%, where A is the absorbance of a blank (without the sample) and Ai is the absorbance of the mixture containing the sample. Calculated antioxidant capacity was converted using a calibration curve of the standard and expressed in ascorbic acid equivalents (AAE) **(Rupasinghe et al., 2006)**.

Statistical analysis

The data were analyzed using Adstat v.1.25 (TriloByte) and expressed as means ± standard deviations. Any significant differences between samples were determined by one-way analysis of variance, considering differences significant at p <0.05. This statistical analysis was performed with Statistica v.1.25 (StatSoft).

RESULTS AND DISCUSSION

Antioxidant capacity

Results of the amounts of antioxidant capacity are shown in Table 1.

Antioxidative activity in fresh juice of broccoli and apple was found to be 189.12 ±4.2 mg.100 mL^{-1}. It decreased after the thermal treatment by pasteurization from 189.12 ±4.2 mg.100 mL^{-1} to 170.87 ±5.21 mg.100 mL^{-1}. Pasteurization treatment caused an expected fall on antioxidative activity. However, after pascalization, treatment by high pressure, antioxidative activity rose 189.12 ±4.2 mg.100 mL^{-1} to 217.12 ±7.26 mg.100 mL^{-1}. Therefore the impact of pascalization on antioxidative capacity is positive.

Antioxidative activity in fresh juice of cabbage with apple was determined in the value of 163.32 ±2.65 mg.100 mL^{-1}. After the heat treatment by pasteurization antioxidative activity declined from 163.32 ±2.65 mg.100 mL^{-1} to 159.11 ±4.23 mg.100 mL^{-1}. An anticipated drop in antioxidative activity manifested again. Surprisingly, pascalization induced a relatively distinctive decrease in antioxidative activity compared with fresh juice, specifically from 163.32 ±2.65 mg.100 mL^{-1} to 109.54 ±5.65 mg.100 mL^{-1}.

Antioxidative activity of fresh beetroot juice was 168.54 ±7.84 mg.100 mL^{-1}. It remained at almost the same value as in fresh juice after pasteurization, specifically at 168.01 ±5.26 mg.100 mL^{-1}. After the treatment of pascalization, it increased from 168.54 ±7.84 mg.100 mL^{-1} to 186.77 ±9.45 mg.100 mL^{-1} which could be considered as a positive result of the experiment.

In fresh carrot juice antioxidative activity was 37.24 ±4.23 mg.100 mL^{-1} which is quite lower in comparison with other juices. After the heat treatment it rose from 37.24 ±4.23 mg.100 mL^{-1} to 43.14 ±0.56 mg.100 mL^{-1} compared with fresh juice. A slight decrease of antioxidative activity from 37.24 ±4.23 mg.100 mL^{-1} to 36.78 ±5.26 mg.100 mL^{-1} was caused by pascalization.

The treatment of pascalization should lead to maintenance of antioxidative activity within the examined matter while pasteurization should cause a decrease of antioxidative activity in comparison with an unprocessed sample.

It has been proved also by already quoted references. **Patras et al. (2009a)** established a higher antioxidative activity in the sample treated by HPP than in the sample after the heat treatment in strawberry and blackberry puree. **McInerney et al. (2007)** studied the activity and other parametres in vegetable. It has been proved that antioxidative activity has not changed after pascalization. In other studies **Patras et al. (2009b)** observed antioxidative activity in tomato and carrot puree. The

Table 1 Determination of antioxidative capacity in fresh and treated beverages.

Antioxidative capacity (DPPH assay) (mg.100 mL^{-1})			
	Fresh juice	**Pasterization**	**Pascalization**
Broccoli with apple	189.12 ±4.2^{a}	170.87 ±5.21^{b}	217.12 ±7.26^{c}
Cabbage with apple	16.,32 ±2.65^{a}	159.11 ±4.23^{a}	109.54 ±5.65^{b}
Beetroot 100%	168.54 ±7.84^{a}	168.01 ±5.26^{a}	186.77 ±9.45^{b}
Carrot 100%	37.24 ±4.23^{a}	43.14 ±0.56^{b}	36.78 ±5.26^{a}

The different superscripts in rows indicate statistically significant differences between data groups (statistically tested on level of significance $\alpha = 0.05$).

Table 2 Determination of total polyphenols in fresh and processed beverages.

Total polyphenols (mg.100 mL^{-1})			
	Fresh juice	**Pasteurization**	**Pascalization**
Broccoli with apple	174.55 ±7.23^{a}	142.97 ±8.14^{b}	183.74 ±4.21^{c}
Cabbage with apple	105.12 ±4.21^{a}	102.32 ±3.33^{a}	114.84 ±6.54^{b}
Beetroot 100%	126.41 ±1.21^{a}	95.47 ±2.39^{b}	154.1 ±4.02^{c}
Carrot 100%	35.2 ±0.15^{a}	26.87 ±0.89^{b}	33.32 ±2.04^{ab}

The different superscripts in rows indicate statistically significant differences between data groups (statistically tested on level of significance $\alpha = 0.05$).

activity was even significantly higher within the matter treated by high pressure (400-600 MPa) than in the samples without any processing or after the heat treatment. Similar conclusions are brought by **Chen et al. (2015)** stating a slight rise of antioxidant capacity after HPP and decrease after the heat treatment in papaya beverage.

Total phenolic content

The values of total phenolic contents are shown in Table 2.

In fresh juice of broccoli with apple the content of polyphenols was 174.55 ±7.23 mg.100 mL^{-1}. Polyphenolic content decreased after the heat treatment of pasteurization from 174.55 ±7.23 mg.100 mL^{-1} to 142.97 ±8.14 mg.100 mL^{-1} which responds to literature data. The treatment of high pressure, pascalization, caused an increase of polyphenol content from to 183.74 ±4.21 mg.100 mL^{-1} which brings a positive contribution of the experiment.

Polyphenolic content in fresh juice made from cabbage with apple was 105.12 ±4.21 mg.100 mL^{-1}. It slightly fell from 105.12 ±4.21 mg.100 mL^{-1} to 102.32 ±3.33 mg.100 mL^{-1} after the heat treatment which had been anticipated. The treatment of high pressure induced a growth of polyphenolic content from 105.12 ±4.21 mg.100 mL^{-1} to 114.84 ±6.54 mg.100 mL^{-1}.

Polyphenolic content in beetroot fresh juice was 126.41 ±1.21 mg.100 mL^{-1}. It fell after the heat treatment of pasteurization to 95.47 ±2.39 mg.100 mL^{-1}. The treatment by high pressure caused an increase in polyphenolic content from 126.41 ±1.21 mg.100 mL^{-1} to 154.1 ±4.02 mg.100 mL^{-1} which has proved the hypothesis.

Polyphenolic content in carrot fresh juice was determined to be 35.2 ±0.15 mg.100 mL^{-1}. Pasteurization reduced it from 35.2 ±0.15 mg.100 mL^{-1} to 26.87 ±0.89 mg.100 mL^{-1}. Pascalization caused a slight fall in polyphenolic content to 33.32 ±2.04 mg.100 mL^{-1}. While it was still a higher amount than the value after the heat treatment, it may be considered as positive.

Pascalization should maintain the amount of polyphenolic content in processed material, while pasteurization could cause the fall of polyphenolic content when compared with non-processed matter which is also stated by **Patras et al. (2009a)**.

The expectation of the fall of polyphenolic content after the heat treatment has been proved by this study. **Chipurura et al. (2010)** confirmed the decline of polyphenolic content after the heat treatment as well.

Even though a slight decrease of polyphenolic content in carrot juice was performed after pascalization, there was a growth of polyphenolic content in all the other tested samples. **Patras et al. (2009b)** examined carrot and tomato puree after the heat treatment and pascalization. Regarding the heat treatment, the fall of polyphenolic content was measured in both carrot and tomato puree. The value of polyphenolic content after HPP depended on the applied pressure. In carrot puree it declined with a growing pressure. With the pressure between 400 and 500 MPa the content was higher than in fresh juice and it slightly dropped with the applied pressure higher than 600 MPa. In tomatoes it grew with the increasing pressure. Compared

Table 3 Determination of total flavonoids in fresh and processed beverages.

	Total flavonoids (mg.100 mL^{-1})		
	Fresh juice	**Pasteurization**	**Pascalization**
Broccoli with apple	40.71 ±0.15^{a}	45.14 ±0.08^{b}	30.21 ±0.54^{c}
Cabbage with apple	7.21 ±0.04^{a}	5.69 ±0.56^{b}	6.53 ±0.99^{ab}
Beetroot 100%	45.25 ±2.14^{a}	43.98 ±1.02^{a}	36.77 ±0.36^{b}
Carrot 100%	9.74 ±0.14^{a}	6.65 ±0.09^{b}	5.29 ±0.09^{c}

The different superscripts in rows indicate statistically significant differences between data groups (statistically tested on level of significance α = 0.05).

with fresh juice, it was lower at the pressure of 400 MPa and higher at 500 MPa.

Chen et al. (2015) brought similar conclusions with papaya beverage performing a slight growth in total polyphenols after HPP and a fall after the thermal treatment. On the other hand, **Barba et al. (2010)** stated that no significant changes in total phenolics were observed between HHP treated and thermally processed vegetable beverage and unprocessed beverage.

Total flavonoid content

The results of total flavonoids are shown in Table 3.

Flavonoid content in fresh juice from broccoli with apple was 40.71 ±0.15 mg.100 mL^{-1}. It surprisingly rose to 45.14 ±0.08 mg.100 mL^{-1} after the heat treatment. Pascalization caused a lower flavonoid content of 30.21 ±0.54 mg.100 mL^{-1} which was a significant reduction.

Flavonoid content in fresh juice made from cabbage with apple was 7.21 ±0.04 mg.100 mL^{-1}. It decreased to the value of 5.69 ±0.56 mg.100 mL^{-1} after the heat treatment. Pascalization caused a smaller decline of flavonoid content to the level of 6.53 ±0.99 mg.100 mL^{-1}.

In fresh beetroot juice flavonoid content was found to be 45.25 ±2.14 mg.100 mL^{-1}. It expectedly fell to the value of 43.98 ±1.02 mg.100 mL^{-1} after the heat treatment of pasteurization. Pascalization, technology using high pressure, induced a significant decline in flavonoids to the amount of 36.77 ±0.36 mg.100 mL^{-1} albeit a smaller decline was expectable due to a gentle processing method of pascalization.

Flavonoid content in fresh carrot juice was determined to be 9.74 ±0.14 mg.100 mL^{-1}. Not surprisingly, it decreased to the amount of 6.65 ±0.09 mg.100 mL^{-1}. Similarly as in the experiment with the beetroot juice, flavonoid content also fell after pascalization to the value of 5.29 ±0.09 mg.100 mL^{-1}.

Pascalization processing should result in the maintenance of flavonoid content while after pasteurization a drop in flavonoid content is expected in comparison with unprocessed material. It has been supported by many studies, also by **Chen et al. (2011)** who examined phenolic and flavonoid content and antioxidative activity in soya milk. After pascalization higher flavonoid content was reached than in fresh or in heat-treated matter **(Chen et al., 2011)**.

As flavonoids belong to the group of polyphenolic compounds, it could be expected to gain similar measured results as within total polyphenols. It may be due to a higher sensitivity of flavonoid fraction for high pressure treatment.

CONCLUSION

Determination of antioxidative activity by DPPH assay has shown a decline in the activity in most of the samples after the heat treatment. Pascalization led to the growth of antioxidative activity in broccoli and beetroot juice, in carrot juice the activity was lower even though it was a little bit higher than in pasteurized juice. In juice made from cabbage with apple antioxidaive activity decreased after pascalization as well.

Regarding polyphenolic content in studied samples, it decreased after pasteurization in all the samples without an exception. Pascalization caused a slight drop in polyphenolic content in carrot juice and an increase in all other juices.

Flavonoid content in fruit and vegetable juices was higher after pasteurization in juice from broccoli with apple. In the other samples it decreased. After the treatment by high pressure the amount of flavonoids fell a little in cabbage and apple juice. In the other samples it fell more significantly than after pasteurization.

With the exception of flavonoids, hypotheses stating that HPP is a gentle conservation method enabling maintaining a higher amount of bioactive and antioxidative substances than the heat treatment.

REFERENCES

Bala, B., Farkas, D., Turek, E. J. 2008. Preserving foods through high-pressure processing. *Food Technology*, vol. 11, p. 32-38.

Barba, F. J., Esteve, M. J., Frigola, A. 2010. Ascorbic acid is the only bioactive that is better preserved by high hydrostatic pressure than by thermal treatment of a vegetable beverage. *Journal of Agricultural and Food Chemistry*, vol. 58, no. 18, p. 10070-10075. http://dx.doi.org/10.1021/jf1019483 PMid:20735131

Barba, F. J. Esteve, M. J. Frigola, A. 2013. Physicochemical and nutritional characteristics of blueberry juice after high pressure processing. *Food Research International*, vol. 50, no. 2, p. 545-549. http://dx.doi.org/10.1016/j.foodres.2011.02.038

Baron, A., Dénes, J., Durier C. 2006. High-pressure treatment of cloudy apple juice, *LWT - Food Science and Technology*. vol. 39, no. 9, p. 1005-1013.

Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, vol. 28, no. 1, p. 25-30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5

Chen, D. Xi, H. Guo, X., Qin, Z. Pang, X., Hu, X., Liao, X., Wu, J. 2013. Comparative study of quality of cloudy pomegranate juice treated by high hydrostatic pressure and high temperature short time. *Innovative Food Science and Emerging Technologies*, vol. 19, p. 85-94. http://dx.doi.org/10.1016/j.ifset.2013.03.003

Chen, D., Pang, X., Zhao, J., Gao, L., Liao, X., Wu, J., Li, Q. 2015. Comparing the effects of high hydrostatic pressure and high temperature short time on papaya beverage. *Innovative Food Science and Emerging Technologies*, vol. 32, p. 16-28. http://dx.doi.org/10.1016/j.ifset.2015.09.018

Chen, F., Ma, S., Xu, Y., Ma, Y. 2011. Effects of High Pressure Processing on Total Phenolic, Flavonoid Content and Antioxidant Activity of Soymilk. *Soyabean Science*, vol. 30, no. 2, p. 310-313.

Chipurura, B., Muchuweti, M., Manditseraa, F. 2010. Effects of Thermal Treatment on the Phenolic Content and Antioxidant Activity of Some Vegetables. *Asian Journal of Clinical Nutrition*, vol. 2, no. 3, p. 93-100. http://dx.doi.org/10.3923/ajcn.2010.93.100

Jakubcova, Z., Zeman, L., Mares, P., Mlcek, J., Jurikova, T., Dostalova, L., Mrazkova, E., Mrkvicova, E., Balla, S., Sochor, J. 2014. Effect of chamomile supplements to feeding doses on antimicrobial parameters in poultry. *Potravinarstvo*, vol. 8, no. 1, p. 228-232. http://dx.doi.org/10.5219/383

McInerney, J. K., Seccafien, C. A, Stewart, C. M., Bird, A. R. 2007. Effects of high pressure processing on antioxidant activity, and total carotenoid content and availability, in vegetables. *Innovative Food Science & Emerging Technologies*, vol. 8, no. 4, p. 543-548. http://dx.doi.org/10.1016/j.ifset.2007.04.005

Mendelová, A., Mendel, L., Czako, P., Mareček, J. 2016. Evaluation of carotenoids, polyphenols content and antioxidant aktivity in the sea buckthorn fruit juice. *Potravinarstvo*, vol. 10, no. 1, p. 59-64. http://dx.doi.org/10.5219/551

Norton, T., Sun, D. W. 2008. Recent advantages in the use of high pressure as an effective processing technique in the food industry. *Food and Bioprocess Technology*, vol. 1, no. 1, p. 2-34. http://dx.doi.org/10.1007/s11947-007-0007-0

Oey, I., Lille, M., Van Loey, A., Hendrickx, M. 2008. Effect of high pressure processing on colour, texture and flavour of fruit and vegetable-based food products: a review. *Trends in Food Science and Technology*, vol. 19, no. 6, p. 320-328. http://dx.doi.org/10.1016/j.tifs.2008.04.001

Paliyath, G., Bakovic, M., Shetty, K. 2011. *Functional Foods, Nutraceuticals and Degenerative Disease Prevention*, John Wiley & Sons. 500 p. ISBN 9780470960820. http://dx.doi.org/10.1002/9780470960844

Park, Y. S., Jung, S. T., Kang, S. G., Heo, B. G., Arancibia-Avila, P., Toledo, F., Drzewiecki, J., Namiesnik, J., Gorinstein, S. 2008. Antioxidants and protein in ethylene-treated kiwifruits. *Food Chemistry*, vol. 107, no. 2, p. 640-648. http://dx.doi.org/10.1016/j.foodchem.2007.08.070

Patras, A., Brunton, N. P., Da Pieve, S., Butler, F. 2009a. Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science and Emerging Technologies*, vol. 10, no. 3, p. 308-313. http://dx.doi.org/10.1016/j.ifset.2008.12.004

Patras, A., Brunton, N. P., Da Pieve, S., Butler, F., Downey, G. 2009b. Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées. *Innovative Food Science and Emerging Technologies*, vol. 10, no. 1, p. 16-22. http://dx.doi.org/10.1016/j.ifset.2008.09.008

Ramirez, R., Saraiva, J. A., Perez Lamela, C., Torres, J. A. 2009. Reaction kinetics analysis of chemical changes in pressure-assisted thermal processing. *Food Engineering Reviews*, vol. 1, no. 1, p. 16-30. http://dx.doi.org/10.1007/s12393-009-9002-8

Rodrigo, D., Van Loey, A., Hendrickx, M. 2007. Combined thermal and high pressure color degradation of tomato puree and strawberry juice. *Journal of Food Engineering*, vol. 79, no. 2, p. 553-560. http://dx.doi.org/10.1016/j.jfoodeng.2006.02.015

Rupasinghe, H. P. V., Jayasankar, S., Lay, W. 2006. Variation in total phenolic and antioxidant capacity among European plum genotypes. *Scientia Horticulture-Amsterdam*, vol. 108, no. 3, p. 243-246. http://dx.doi.org/10.1016/j.scienta.2006.01.020

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D. H. 2006. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Food Composition and Analysis*, vol. 19, no. 6-7, p. 669-675. http://dx.doi.org/10.1016/j.jfca.2006.01.003

Turati, F., Rossi, M., Peluccohi, C., Levi, F., La Veccia, C. 2015. Fruit and vegetables and cancer risk: A review of southern European studies. *British Journal of Nutrition*, vol. 113, no. 2, p. 102-110. http://dx.doi.org/10.1017/S0007114515000148 PMid:26148912

Valdramidis, V. P., Graham, W. D., Beatie, A., Linton, M. Mckay, A. Fearon, A. M., Patterson, M. F. 2009. Defining the stability interfaces of apple juice: implications on the optimisation and design of high hydrostatic pressure treatment. *Innovative Food Science & Emerging Technologies*, vol. 10, no. 4, p. 396-404. http://dx.doi.org/10.1016/j.ifset.2009.02.006

Varela-Santos, E., Ochoa-Martinez, A., Tabilo-Munizaga, G., Reyes, J. E., Pérez-Won, M., Briones-Labarca, V., Morales-Castro, J. 2012. Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *Innovative Food Science and Emerging Technologies*, vol. 13, p. 13-22. http://dx.doi.org/10.1016/j.ifset.2011.10.009

VSCHT, 2014. Principy konzervace potravin. Konzervace vysokým tlakem. Vysoká škola chemicko-technologická v Praze [online]. 2014-01-25 [cit. 2014-02-

25]. Available at: http://www.vscht.cz/ktk/www_324/studium/KP/konzervace.swf.

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SELECTED TECHNOLOGICAL PROPERTIES AND ANTIBIOTIC RESISTANCE OF ENTEROCOCCI ISOLATED FROM MILK

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ABSTRACT

The aim of this work was to determine counts of enterococci in raw cow milk, to isolate and identify them, to determinate their antibiotic resistance, ability of lactose fermentation, proteolytic and lipolytic activity in different conditions of cultivation. Counts of enterococci were determined after 48 ±2 h cultivation on Slanetz-Bartley agar at 37 ±1 °C. The counts of enterococci in raw cow milk fluctuated from 1.80 x 10^2 to 1.77 x 10^3 CFU.mL^{-1} with average value 7.25 x 10^2 CFU.mL^{-1}. Species identifications of enterococci isolates were performed using commercial EN-COCCUS test and confirmed by PCR. Majority of tested isolates (85.7%) was included to species *E. faecalis*. Antibiotic resistance was tested on Mueller-Hinton agar using following antimicrobial discs: vancomycin (VA) 30 µg.disc^{-1}, gentamicin (CN) 120 µg.disc^{-1}, erythromycin (E) 15 µg.disc^{-1}, tetracycline (TE) 30 µg.disc^{-1}, ampicillin (AMP) 10 µg.disc^{-1}, teicoplanin (TEC) 30 µg.disc^{-1}. From 13 isolates of enterococci, 1 strain was resistant to vancomycin, 1 strain to tetracycline and 1 to ampicillin, but higher prevalence of intermediate resistance of isolates was determined to tetracycline (5 strains). Ability of lactose fermentation was monitored by change of titratable acidity in UHT milk after 0, 18, 24, 40 and 48 h of cultivation at temperature 25, 30 and 37 °C. The tested strains of enterococci exhibit low milk acidifying ability. Production of proteolytic enzymes was evaluated after cultivation at temperature 7, 25 and 30 °C after 10 days on nutrient agar no. 2 with sterile skim milk (10% w/v) with pH 6.0 and 6.5. Proteolytic activity of tested enterococci strains varied depending on tested temperature and pH. Lipolytic activity was determined similarly like proteolytic activity but on tributyrin agar base with tributyrin (1% w/v). Lipolytic activity of isolated enterococci was very low. The tested strains produced halos with zone in range from 7 to 15 mm regardless of pH, cultivation time and temperature. Some of isolated and tested enterococci strains have shown suitable technological properties, but they have exhibited resistance to antibiotic.

Keywords: enterococci; milk; lactose fermentation; proteolytic and lipolytic activity; antibiotic resistance

INTRODUCTION

Enterococci are Gram-positive, non-sporeforming, catalase-negative, oxidase-negative, facultative anaerobic cocci that occur singly, in pairs, or in chains **(Hollenbeck and Rice, 2012)**. Most enterococcal species are able to grow in the presence of 6.5% NaCl, 40% bile salts, at pH 9.6 **(Ogier and Serror, 2008)**, at 10 and 45 °C and survive for at least 30 min at 60 °C **(Domig et al., 2003)**. Enterococci are ubiquitous bacteria which occur in many different habitats such as in soil, surface water, ocean water, sewage, on plants and in the gastrointestinal tract of animals and humans. Based on their association with the gastrointestinal tract, enterococci often occur in foods of animal origin such as meat, fermented and cooked meat, as well as cheese **(Franz et al., 2011)**.

Enterococci are normal components of the raw milk microbiota **(Giménez-Pereira, 2005)** and pasteurised milk microflora. Due to their psychrotrophic nature, their heat resistance and their adaptability to different substrates and growth conditions, count of enterococci can increase during milk refrigeration and survive after pasteurisation **(Giraffa, 2003)**.

Presence of enterococci in dairy products can have conflicting effects, of either a risk as a foreign or intrusive flora indicating poor hygiene during milk handling and processing (if in excessive numbers), or as a benefit in contributing to produce unique traditional and emerging by-products, in protecting against diverse spoilers, and as probiotics **(Giménez-Pereira, 2005)**.

Enterococci possess intrinsic antibiotic resistance to cephalosporins, ß-lactams, sulphonamides, and to certain levels of clindamycin and aminoglycosides, while acquired resistance exists to chloramphenicol, erythromycin, clindamycin, aminoglycosides, tetracycline, ß-lactams, fluoroquinolones **(Giménez-Pereira, 2005)** and glycopeptide antibiotics (vancomycin and teicoplanin) **(Cariolato et al., 2008)**.

On the other hand certain enterococcal strains are also successfully used as probiotics to improve human or animal health **(Araújo and Ferreira, 2013)**. *Enterococcus* main characteristic is the ability to produce L-lactic acid (lactate) from hexoses by means of homofermentative lactic acid fermentation. Although the main product is lactate, they can also produce significant amounts of acetate, formate **(Rea and Cogan, 2003)**. Acetate and the others are recognised as "flavour compounds" since they are important in determining the taste of many fermented dairy products **(Battelli et al., 2011)**. Enterococci

contribute to texture and aroma development of cheeses also thanks to their proteolytic and lipolytic activities **(Martín-Platero et al., 2009)**.

The aim of this work was to determine counts of enterococci in raw cow milk from milk machines, to isolate and identify them, to determinate their antibiotic resistance, ability of lactose fermentation, proteolytic and lipolytic activity in different conditions of cultivation.

MATERIAL AND METHODOLOGY

Ten samples of raw cow milk were obtained from the milk machines. The counts of enterococci were determined by cultivation on Slanetz-Bartley agar (HiMedia Laboratories, India) at 37 ±1 °C after 48 ±2 h. Suspect colonies of enterococci (n = 38) were incubated on bile esculin azide agar (BEAA) (Biokar Diagnostic, France) at 37 ±1 °C for 24 ±2 h **(STN 56 0100, 1970)** for evaluation their hydrolysis. Then the isolates were identified using optical microscopy, catalase production and by PYR test (Lachema, Czech Republic). Species identification was performed using commercial EN-COCCUS test (Lachema, Czech Republic) and confirmed by PCR **(Kariyama et al., 2000)**.

Antibiotic resistance was tested on Mueller-Hinton agar (HiMedia Laboratories, India) using following antimicrobial discs: vancomycin (VA) 30 μg.disc^{-1}, gentamicin (CN) 120 μg.disc^{-1}, erytromycin (E) 15 μg.disc^{-1}, tetracycline (TE) 30 μg.disc^{-1}, ampicillin (AMP) 10 μg.disc^{-1}, teicoplanin (TEC) 30 μg.disc^{-1} (HiMedia Laboratories, India). The isolates were classified as susceptible, intermediate resistant and resistant according **CLSI criteries (2013)**.

Then lactose fermentation, production of proteolytic and lipolytic enzymes was determined. Enterococci strains were incubated on glucose tryptone yeast extract agar (HiMedia Laboratories, India) at 37 °C for 24 ±2 h. The inoculum was prepared by suspending of enterococcal colonies in saline and by adjusting to equal 0.5 McFarland standard using Densilameter (Lachema, Czech Republic).

Ability of lactose fermentation was monitored by change of titratable acidity in UHT milk (100 mL) with 1 mL of inoculum. It was cultivated at temperature 25, 30 and 37 °C. The titratable acidity was reported immediately after inoculation and after 18, 24, 40 and 48 h of cultivation.

The inoculated UHT milk after 48 h of cultivation at 7, 25 and 30 °C was used for detection of proteolytic activity of enterococci. It was determined using hole diffusion method on nutrient agar no. 2 (HiMedia Laboratories, India) with sterile skim milk (10% w/v), with pH value 6.0 and 6.5. The inoculated UHT milk (30 μL) was applied into the hole in medium. Production of proteolytic enzymes was evaluated after cultivation at temperature 7, 25 and 30 °C after 10 days.

Lipolytic activity was determined similarly like proteolytic activity but on tributyrin agar base (HiMedia Laboratories, India) with tributyrin (1% w/v).

RESULTS AND DISCUSSION

Enterococci are commonly found in raw milk with different flora reported in different countries, reflecting local practices and levels of hygiene. The counts of enterococci in our samples of raw cow milk ranged from 1.80 x 10^2 to 1.77 x 10^3 CFU.mL^{-1} with average value 7.25 x 10^2 CFU.mL^{-1}.

Giménez-Pereira (2005) determined higher values in raw cow milk, counts of enterococci fluctuated from 10^3 to 10^5 CFU.mL^{-1}. In the study of **Fabianová et al. (2010)** were presented counts of enterococci in cistern samples from 1.3 x 10^3 to 2.9 x 10^4 CFU.mL^{-1} and in the samples from storage tank from 2.1 x 10^3 to 3.2 x 10^4 CFU. mL^{-1}. **McAuley et al. (2015)** detected enterococci in 96% of the raw milk samples (detection limit 1 log CFU.mL^{-1}), with counts ranging from <1 to 6.80 log CFU.mL^{-1} with an average of 2.48 log CFU.mL^{-1}; most counts (77.3%) were <3 log CFU.mL^{-1}.

Sources tracking entry of enterococci into raw milk and subsequent transmission into processed products have indicated persistence of particular species of strain types, where the likely source of contamination of these is through milking process and processing equipment, as well

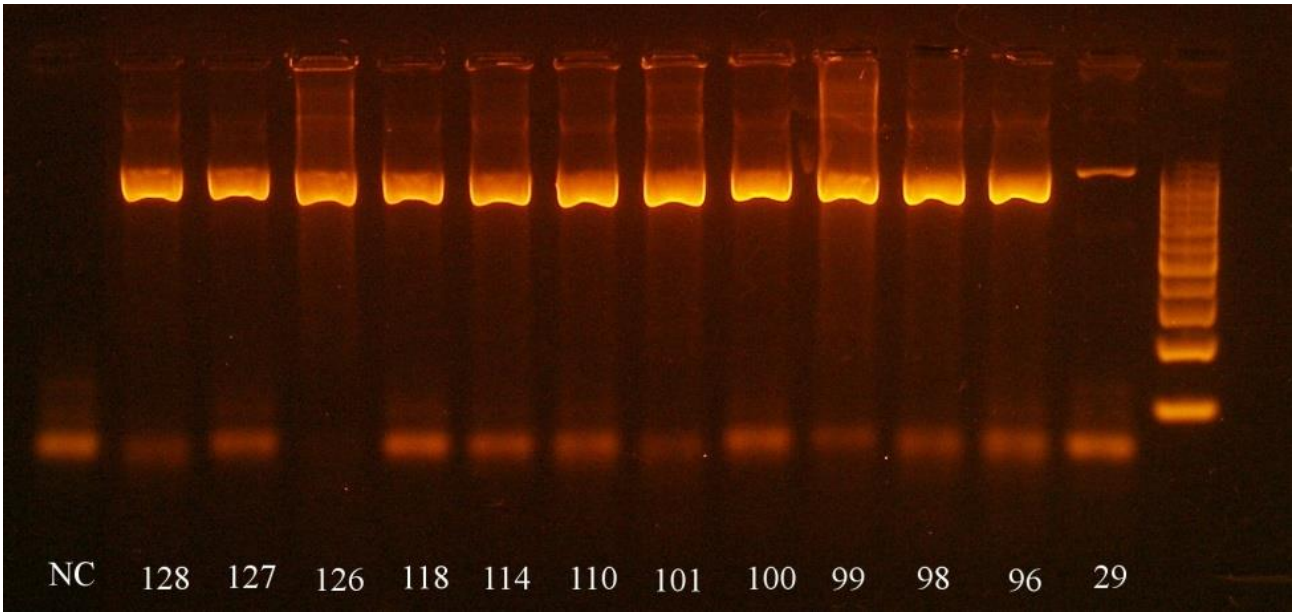

Figure 1 PCR identification of enterococci strains.

Table 1 Evaluation of antibiotic resistance of enterococci isolated from raw cow milk according CLSI (2013).

Number of strain	antibiotics					
	VA	CN	E	TE	AMP	TEC
29	S	S	S	S	S	S
96	R	S	S	I	S	S
98	S	S	S	R	S	S
99	S	S	S	S	S	S
100	S	S	S	I	R	S
101	S	S	S	I	S	S
108	S	S	R	I	S	S
110	S	S	S	S	S	S
114	S	S	S	I	S	S
118	S	S	S	S	S	S
126	S	S	S	S	S	S
127	S	S	S	S	S	S
128	S	S	S	S	S	S

NOTE: VA – Vancomycin (30 µg.disc^{-1}), CN – Gentamicin (120 µg. disc^{-1}), E – Erythromycin (15 µg. disc^{-1}), TE – Tetracycline (30 µg. disc^{-1}), AMP – Ampicillin (10 µg. disc^{-1}), TEC – Teicoplanin (30 µg. disc^{-1}), S – susceptible, I – intermediate resistant, R – resistant.

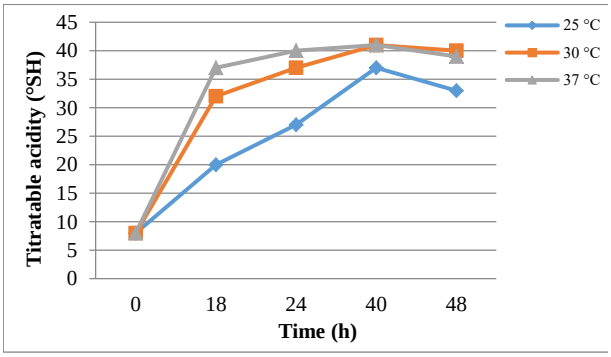

Figure 2 Acidifying activity of strain No. 98 in dependence on time and temperature.

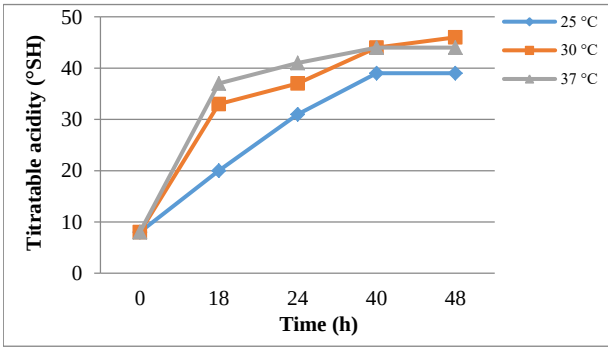

Figure 3 Acidifying activity of strain No. 100 in dependence on time and temperature.

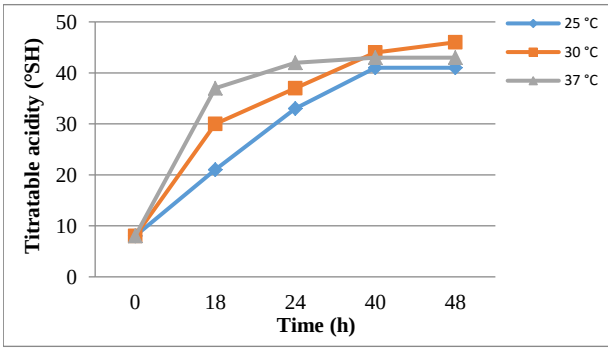

Figure 4 Acidifying activity of strain No. 118 in dependence on time and temperature.

The species identification of enterococci was determined by EN-COCCUS test. Majority of tested isolates (85.7%) was included to species *E. faecalis*. One strain (no 29) was classified as *E. durans* and one strain was not included to *Enterococcus* spp. Results of EN-COCCUS test were confirmed using PCR method (Figure 1).

McAuley et al. (2012) observed also a low prevalence of the more thermoduric species in the raw milk (*E. faecium*, *E. hirae*, and *E. durans*).

Several studies suggest that *E. faecalis* is the dominant species of the genus *Enterococcus* in raw milk. For example, **Fabianová et al. (2010)** determined species *E. faecalis*, *E. faecium*, *E. group III.*, *E. mundtii* and *E. casseliflavus* by EN-COCCUS test in samples of raw cow milk. Species *E. faecalis* represented dominant part of all isolates (56.5%). Also according **McAuley et al. (2015)** *E. faecalis* was the most prevalent species isolated from raw milk, comprising between 61.5 and 83.5% of enterococcal species across each season.

From 13 isolates of enterococci, 1 strain was resistant to vancomycin, 1 strain to tetracycline and 1 to ampicillin, but higher prevalence of intermediate resistance of isolates was determined to tetracycline (5 strains) (Table 1). In the study of **Ruiz et al. (2016)** *E. faecium*, *E. faecalis* and *E. hirae* showed a high percentage of resistance to tetracycline. However, in a research of **Gaglio et al. (2016)**, the strains exhibited high percentages of resistance to erythromycin (52.5%), ciprofloxacin (35.0%), quinupristin-dalfopristin (20.0%), tetracycline (17.5%) and high-level streptomycin (5.0%).

In a study of **Nam et al. (2010)** was detected that 105 of enterococci isolates were more resistant to tetracycline (69.5%) than penicillin (64.7%), erythromycin (57.1%) and cephalotine (44.7%). According to **Trivedi et al. (2011)**, from 250 isolates of enterococci 46% were resistant to cephalotine and 38% to ofloxacine. Low resistance was determined to ampicillin, chloramphenicol, gentamicin and teicoplanin. In this study was shown that strains *E. faecalis* and *E. faecium* were resistant the most frequently. Also in the results of **Valenzuela et al. (2009)**, the strains *E. faecalis* isolated from milk and cheeses were the most frequent species of genus *Enterococcus* resistant to antibiotics.

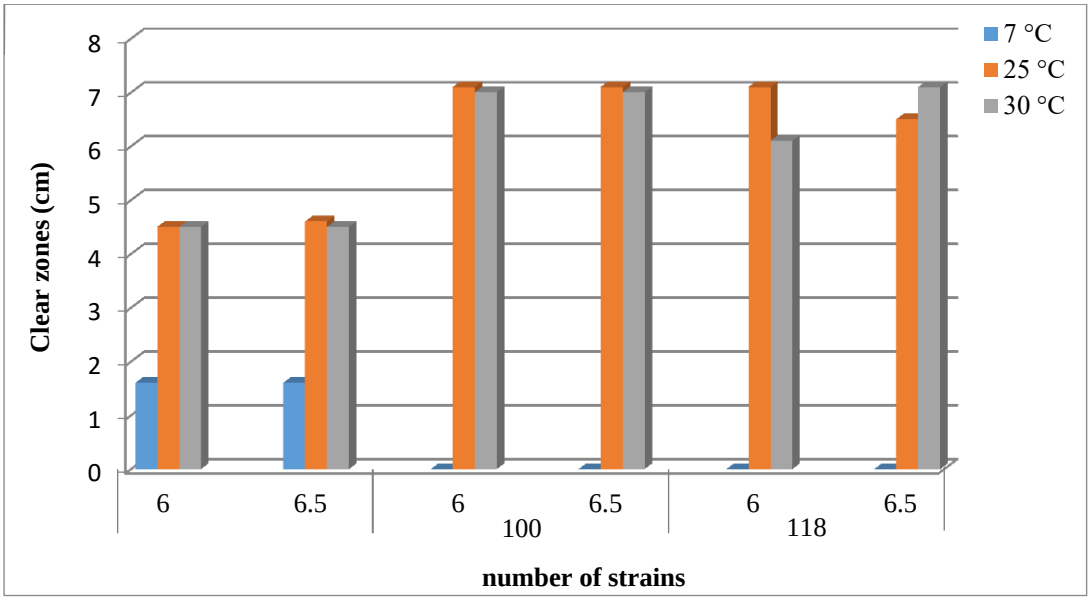

Figure 5 Proteolytic activity of enterococci after 10 days of cultivation at different pH (6.0 and 6.5) and temperature.

Ability of lactose fermentation was evaluated in all identified strains. The most active strains of enterococci (no. 98, 100 and 118 – *E. faecalis*) are shown in Figure 2, Figure 3 and Figure 4, where are presented changes of titratable acidity of inoculated UHT milk in dependence on time and temperature.

The highest increase of titratable acidity was observed after 18 hour of cultivation at temperature 37 °C and the highest values of titratable acidity were observed after 40 hour of cultivation at both temperatures 30 and 37 °C. The lowest values of titratable acidity were reached after cultivation at 25 °C.

In general, enterococci exhibit low milk acidifying ability. According to **Morea et al. (1999)** the pH of milk 24 hour after inoculation with strains of enterococci isolated from Mozzarella

The poor acidifying capacity of enterococci isolated from food of dairy origin was confirmed also by **Durlu-Ozkaya et al. (2001)**, **Morandi et al. (2006)**, **Serio et al. (2010)** and **Aspri et al. (2016)**.

Acidifying activity was weak, while interesting differences were found for proteolytic capability. The proteolytic system of LAB (including genera *Enterococcus*) is essential for the optimal growth in milk through the release of proteolytic enzymes. LAB have a complex system of proteases and peptidases, which allow them to use milk casein as a source of amino acids and nitrogen. Intra- and inter-specific variability in proteolysis is commonly reported for isolates from natural sources **(Franciosi et al., 2009)**.

Proteolytic activity of tested enterococci strains varied depending on tested temperature and pH value. In Figure 5 are shown proteolytic activities of strains no. 98, 100 and 118. The lowest production of proteolytic enzymes was determined in strain no. 98. No proteolytic activity was determined in strains no. 100 and 118 at 7 °C, in contrast with strain no. 98. The highest values of proteolytic activity were determined in strain 100.

Gardini et al. (2001) found out the maximum proteolysis of *E. faecalis* at an incubation temperature 32 – 34 °C. The effect of pH value on this activity was rather weak (at least within the interval of values considered in this investigation). In a study of **Serio et al. (2010)** proteolytic activity was higher at 10 °C than at 30 °C, possibly due to the prolonged incubation time. Especially after 15 days, *E. faecalis* was the most active species. According to **Aspri et al. (2016)** 78% of tested enterococci have shown positive result for proteolytic activity after 4 days of cultivation at 37 °C.

Lipolysis is an important process mainly in cheese ripening due to its role in the development of flavor and texture of the final product. Lipolytic activity of isolated enterococci was very low. Our strains produced halos with zone in range from 7 to 15 mm regardless of pH value, cultivation time and temperature. Limited reports exist on the lipolytic activity of enterococci with *E. faecalis* being the most lipolytic species, followed by *E. faecium* and *E. durans* **(Giraffa, 2003)**. The results obtained in study of **Aspri et al. (2016)** confirmed that enterococci have generally low lipolytic activity, because none of the enterococci tested gave a zone.

CONCLUSION

The results of this study demonstrated that raw cow milk is good source of autochthones enterococci. The tested indigenous strains of *Enterococcus* spp. showed interesting technological properties that could potentially be utilized further by the food industry especially in dairy technology (i.e. fermented dairy products and cheeses). Regarding the values of titratable acidity, the isolated strains are capable of producing a mild acid flavor to the fermented milk product and could be used as adjunct cultures. The proteolytic activity of enterococci as a selection criterion for the production of fermented milks may not be as crucial as it is for say, cheese production, but proteolytic strains could lead to the formation of peptides with bioactive properties during milk fermentation. The low lipolytic activity of tested enterococci can be considered as an advantage, because it may cause only a slight lysis of the milk fat without flavor change of final product. Furthermore, in order to assessment suitability of tested

enterococci as adjunct cultures, their safety profile (e.g. susceptibility to antibiotics, the absence of virulence factors and production of biogenic amines) must be also investigated.

REFERENCES

Araújo, T. F., Ferreira, C. L. L. F. 2013. The genus Enterococcus as probiotic: safety concerns. *Brazilian Archives of Biology and Technology*, vol. 56, no. 3, p. 457-466. http://dx.doi.org/10.1590/S1516-89132013000300014

Aspri, M., Bozoudi, D., Tsaltas, D., Hill, C., Papademas, P. 2016. Raw donkey milk as a source of Enterococcus diversity: Assessment of their technological properties and safety characteristics. *Food Control*, article in press. http://dx.doi.org/10.1016/j.foodcont.2016.05.022

Battelli, T., Silvetti, T., Decimo, M., Brasca, M. 2011. Volatile organic compounds production in milk by *Enterococcus faecalis*. 10th international meeting on mountain cheese 14-15 September 2011, Dronero (CN) Italy, p. 75-76.

Cariolato, D., Andrighetto, C., Lombardi, A. 2008. Occurrence of virulence factors and antibiotic resistances in *Enterococcus faecalis* and *Enterococcus faecium* collected from dairy and human samples in North Italy. *Food Control*, vol. 19, no. 9, p. 886-892. http://dx.doi.org/10.1016/j.foodcont.2007.08.019

CLSI: M 100 – S23. 2013. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-third Informational Supplement, M02-A11. Clinical and Laboratory Standards Institute, USA, 206 p.

Domig, K. J., Mayer, H. K., Kneifel, W. 2003. Methods used for the isolation, enumeration, characterization and identification of *Enterococcus* spp.: 1. Media for isolation and enumeration. *International Journal of Food Microbiology*, vol. 88, no. 2-3, p.147-164. http://dx.doi.org/10.1016/s0168-1605(03)00178-8

Durlu-Ozkaya, F., Xanthopoulos, V., Tunail, N., Litopoulou-Tzanetak, E. 2001. Technologically important properties of lactic acid bacteria isolates from Beyaz cheese made from raw ewes' milk. *Journal of Applied Microbiology*, vol. 91, p. 861-870. http://dx.doi.org/10.1046/j.1365-2672.2001.01448.x

Fabianová, J., Ducková, V., Čanigová, M., Kročko, M. 2010. Presence of Enterococci in cow milk and their antibiotic resistance. *Potravinarstvo*, vol. 4, no. 2, p. 17-21. http://dx.doi.org/10.5219/45

Franciosi, E., Settanni, L., Cavazza, A., Poznanski, E. 2009. Biodiversity and technological potential of wild lactic acid bacteria from raw cows' milk. *International Dairy Journal*, vol. 19, p. 3-11. http://dx.doi.org/10.1016/j.idairyj.2008.07.008

Franz, C. M. A. P., Huch, M., Abriouel, H., Holzapfel, W., Gálvez, A. 2011. Enterococci as probiotics and their implications in food safety. *International Journal of Food Microbiology*, vol. 151, no. 2, p. 125-140. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.08.014

Gaglio, R., Couto, N., Marques, C´., Fatima, M., Lopes, S., Moschetti, G., Pomba, C., Settanni, L. 2016. Evaluation of antimicrobial resistance and virulence of enterococci from equipment surfaces, raw materials and traditional cheeses. *International Journal of Food Microbiology*, vol. 236, p. 107-114. http://dx.doi.org/10.1016/j.ijfoodmicro.2016.07.020

Gardini, F., Martuscelli, M., Caruso, M. C., Galgano, F., Crudele, M. A., Favati, F., Guerzoni, M. E., Suzzi, G. 2001. Effects of pH, temperature and NaCl concentration on the growth kinetics, proteolytic activity and biogenic amine production of Enterococcus faecalis. *International Journal of Food Microbiology*, vol. 64, no. 1-2, p. 105-117. http://dx.doi.org/10.1016/s0168-1605(00)00445-1

Giménez-Pereira, M. L. 2005. Enterococci in milk products: Dissertation Work. New Zealand: Massey University Palmerston North, 123 p.

Giraffa, G. 2003. Functionality of enterococci in dairy products. *International Journal of Food Microbiology*, vol. 88, no. 2-3, p. 215-222. http://dx.doi.org/10.1016/s0168-1605(03)00183-1

Hollenbeck, B. L., Rice, L. B. 2012. Intrinsic and acquiried resistance mechanisms in Enterococcus. *Virulence*, vol. 3, no. 5, p. 421-569. http://dx.doi.org/10.4161/viru.21282

Kagkli, D. M., Vancanneyt, M., Hill, C., Vandamme, P., Cogan, T. M. 2007. Enterococcus and Lactobacillus contamination of raw milk in a farm dairy environment. *International Journal of Food Microbiology*, vol. 114, no. 2, p. 243-251. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.09.016

Karyiama, R., Mitsuhata, R., Chow, J. W., Clewell, D. B., Kumon, H. 2000. Simple a reliable multiple PCR assay for surveillance isolates of vancomycin-resistant Enterococci. *Journal of Clinical Microbiology*, vol. 38, no. 8, p. 3092-3095.

Martín-Platero, A. M., Valdivia, E., Maqueda, M., Martínez-Bueno, M. 2009. Characterization and safety evaluation of enterococci isolated from Spanish goats' milk cheeses. *International Journal of Food Microbiology*, vol. 132, no. 1, p. 24-32. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.03.010

McAuley, M. C., Britz, M. L., Gobius, K. S., Craven, H. M. 2015. Prevalence, seasonality, and growth of enterococci in raw and pasteurized milk in Victoria. *Australia. Journal of Dairy Science*, vol. 98, p. 8348-8358. http://dx.doi.org/10.3168/jds.2015-9335

McAuley, M. C., Gobius, K. S., Britz, M. L., Craven, H. M. 2012. Heat resistance of thermoduric enterococci isolated from milk. *International Journal of Food Microbiology*, vol. 154, no. 3, p. 162-168 http://dx.doi.org/10.1016/j.ijfoodmicro.2011.12.033

Morandi, S., Brasca, M., Andrighetto, Ch., Lombardi, A., Lodi, R. 2006. Technological and molecular characterization of enterococci isolated from north–west Italian dairy products. *International Dairy Journal*, vol. 16, p. 867-875. http://dx.doi.org/10.1016/j.idairyj.2005.09.005

Morea, M., Baruzzi, F., Cocconcelli, P. S. 1999. Molecular and physiological characterization of dominant bacterial populations in traditional Mozzarella cheese processing. *Journal of Applied Microbiology*, vol. 87, p. 574-582. http://dx.doi.org/10.1046/j.1365-2672.1999.00855.x

Nam, H. M., Lim, S. K., Moon, J. S., Kang, H. M., Kim, J. M., Jang, K. C., Kim, J. M., Kang, M. I., Joo, Y. S., Jung, S. C. 2010. Antimicrobial resistance of enterococci isolated from mastic bovine milk samples in Korea. *Zoonoses and public health*, vol. 57, no. 7-8, p. 59-64. http://dx.doi.org/10.1111/j.1863-2378.2009.01307.x

Ogier, J. C., Serror, P. 2008. Safety assessment of dairy microorganisms: The Enterococcus genus. *International Journal of Food Microbiology*, vol. 126, no. 3, p. 291-301. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.08.017

Rea, M. C., Cogan, T. M. 2003. Glucose prevents citrate metabolism by enterococci. *International Journal of Food Microbiology*, vol. 88, no. 2-3, p. 201-206. http://dx.doi.org/10.1016/s0168-1605(03)00181-8

Ruiz, P., Perez-Martin, F., Sesena, S., Palop, M. L. 2016. Seasonal diversity and safety evaluation of enterococci

population from goat milk in a farm. *Dairy Science & Technology*, vol. 96, no. 3, p. 359-375. http://dx.doi.org/10.1007/s13594-015-0273-y

Serio, C., Chaves-López, A., Paparella, A., Suzzi, G. 2010. Evaluation of metabolic activities of enterococci isolated from Pecorino Abruzzese cheese. *International Dairy Journal*, vol. 20, no. 7, p. 459-464. http://dx.doi.org/10.1016/j.idairyj.2010.02.005

STN 56 0100, 1970. Mikrobiologické skúšanie požívatín, predmetov bežnej spotreby a prostredia potravinárskych prevádzok - Microbiological examination of foodstuffs, articles of current use and environment of food establishments. Bratislava: ÚNMS, 239 p.

Trivedi, K., Cupáková, S., Karpišková, R. 2011. Virulence factors and antibiotic resistance in enterococci isolated from food-stufs. *Veterinarni Medicina*, vol. 56, no. 7, p. 352-357. http://dx.doi.org/10.17221/vetmed

Valenzuela, A. S., Omar, N. B., Abriouel, H., López, R, L., Ortega, E., Cañamero, M. M., Gálvez, A. 2008. Risk factors in enterococci isolated from foods in Morocco: Determination of antimicrobial resistance and incidence of virulence traits. *Food and Chemical Toxicology*, vol. 46, no. 8, p. 2648-2652. http://dx.doi.org/10.1016/j.fct.2008.04.021

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PERCEPTION OF WINE LABELS BY GENERATION Z: EYE-TRACKING EXPERIMENT

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ABSTRACT

Product quality is the result of an involved technological process. For the customer, product quality is not easy to grasp and the decision to buy the product is more influenced by the customer's perception of quality than by quality itself. It is therefore the result of many factors making an impression on the customer, their personal taste and the mood of the moment. The role of marketing is to understand the factors that have a customer impact. We need to identify the factors the customer is aware of and is able to communicate. Yet there are also a number of factors at play that affect the customer without their being aware of it. The aim of the paper is to get to know customer behaviour not just through the factors the customer communicates (answering questions) but to seek new methods that allow an objective examination of the customer's stimulus response, in our research case, using eye-tracking technology. The research study was conducted by way of an experiment with concurrent questioning in June 2016. There were 44 respondents taking part in the experiment, aged from 19 to 25 (Generation Z). The experiment set out to identify the importance of various visual attributes of a bottle of white wine, using a total of 7 stimuli. The experiment was carried out using the method called A/B testing, whereby one half of the respondents (A) was shown the original version of the stimulus and the second half (B) the modified stimulus. The eye-tracking research was carried out using remote eye-tracker SMI RED 250 at a sampling frequency of 125 Hz. In answering questions, the respondents evaluated the importance of the factors of price, type, awards, the shape and colour of the bottle and information on the label, i.e. information about the producer (maker) of the wine, wine variety, wine-growing region, country of origin, year of vintage and the sugar content indication. The paper concludes with a summary of the respective importance of the individual visual attributes that Generation Z consumers are most influenced by, when purchasing bottled white wine.

Keywords: customer; factor; quality; wine; eye-tracking

INTRODUCTION

Generation Z is the category name given to persons born after 1995 and up to about the year 2010, the onset of the global financial crisis. This generation grew up with the Internet and other modern methods of communication (mobile networks, digital television, etc.). As a result, this generation is characterized by a high degree of mobility, having values in common, shared ideas, virtualization and job preferences tending toward information technology, business, economics and the humanities **(Ilin and Shestova, 2014)**.

The popularity of wine in the Czech Republic is constantly increasing, to the detriment of beer consumption in particular. In 2014, according to the **Czech Statistical Office (2015)** the average annual consumption of wine was 19.5 litres per capita (including children). The wine trade offers consumers a wide range of products of different makes, varieties, regions, labels, wine styles and prices, affecting the purchasing decision process **(Dodd, et al., 2005; Johnson and Bruwer, 2004)**.

To reduce the risk consumers face when choosing wine, various individual attributes of the products are taken into account during decision-making. Some of these attributes, particularly taste and quality, cannot be assessed prior to consumption. Other attributes that also relate to product characteristics may be gleaned by the consumer from the labels – the wine type/variety, the commercial brand, year of vintage, region of origin, awards, production processes, etc. The attributes listed on the label may thus fundamentally determine product choice when buying **(Ling and Lockshin, 2003)**. When choosing a wine, both the bottle and its label thus play an important role. According to **Kotler and Armstrong (2012)** the role of packaging is not only to protect the product but serves as an important marketing tool, since 40 to 70% of purchase decisions are made directly in-store. Due to much competition and reduced visibility on retail shelves, the packaging must attract attention, describe the product and at the same time, sell it. **Wang and Chou (2011)** state that packaging consists of two elements: the first being the structure and shape of the packaging, the second, the external graphic design (colour, typography, decor). The basic function of a label is to identify the given product or brand. Another function is to provide information about the product content, when and where it was made, how it is to be used, etc. And finally, the label serves together with the bottle to promote the product, to support where it is placed in the store and how it connects with customers.

The information provided on a wine label is subject to European Parliament and Council Regulation (EU) No 1308/2013, as well as Commission Regulation (EC) No 607/2009, European Parliament and Council Regulation (EU) No 1169/2011 and other national legislation. Compulsory information to be shown on the label must be in a language easily understood by consumers in the Member States where the foodstuff is put on the market. In the case of wine intended for Czech consumers, the compulsory particulars laid down by the legislation of the Union are to be shown in the Czech language. The compulsory particulars laid down by EU legislation that are deemed a priority for the Czech Agriculture and Food Inspection Authority are the product type, the stated provenance, the name of the bottler or manufacturer/dealer, and for imported wines the name of the importer, and any applicable allergen notice. The last two items may be listed outside of the field of view of the other compulsory particulars. The other compulsory particulars are, for wines with a protected designation of origin (PDO) or protected geographical indication (PGI), the expression PDO/PGI ("CHOP/CHZO" in Czech) and the PDO/PGI name, the actual alcoholic strength by volume in percent, an indication of the sugar content (only sparkling wines, aerated sparkling, sparkling or quality aromatic sparkling), lot number (may be outside the field of view of the other compulsory particulars), the nominal volume and the special rules for certain wines **(Czech Agriculture and Food Inspection Authority, 2015)**. **Scollary (2016)** states that additional information on the label may be added at the manufacturer's discretion to entice the customer to buy. The back label often gives the sensory properties of the wine, the winemaker's notes and recommended foods that go with the particular wine **(Mueller et al., 2010)**. According to the study entitled 'Message on a Bottle: Colours and Shapes in Wine Labels' **(Scollary, 2016)** consumers prefer specifically coloured and shaped printed labelling. The study concludes that an influencing purchase decision factor, apart from label design, price, availability and previous experience, is also the easy pronounceability of the variety name, should the customer be presenting the wine to their friends **(Scollary, 2016)**. The research findings of **Di Vita et al., (2014)** also confirm the conclusions of the studies about strong consumer ties to local products, whereby consumers tend to prefer products from their home region, especially when it comes to agri-food products.

Product quality is very difficult to pin down, and identifying wine quality no less so, determined as it is not only by the basic method of cultivation and processing, but by the consumer's personal tastes. Hence, marketing focuses on customer quality perceptions rather than on objective quality **(Charters and Pettigrew, 2007)**.

The aim of marketing is to get to know the consumer's decision-making process when choosing wines, to identify the factors that most influence the consumer, among the ones the consumer is aware of and is able and willing to identify e.g. in response to questions. Another large group includes those factors that affect the consumer's purchasing without the consumer being consciously aware of them. Therefore, the process of learning about consumer behaviour does not focus solely on the information gained through questioning, but methods that allow the objective evaluation of how marketing initiatives affect consumer response are constantly being sought. One of the techniques that enables monitoring unwitting human response is eye-tracking, following where the eyes point to. It is this eye-tracking technology that has been used in the experiment this paper is concerned with. The aim of the research was to identify by experiment, implemented with the help of eye-tracking technology and supplementary questioning, the significance of each of the visual attributes of bottled wines that have an impact on the 'Generation Z' consumer's wine choice.

MATERIAL AND METHODOLOGY

The research was conducted by way of an experiment with concomitant questioning in June 2016. There were 48 respondents taking part in the experiment, aged from 19 to 25, selected based on their ready availability and their being a relevant representative sample of Generation Z. All the respondents indicated that they were wine buyers.

The findings here interpreted include data from 44 respondents, as in the case of four of the respondents there were significant deviations in the initial eye-tracker calibration or the results of these respondents exhibited high signal loss. Problems with calibration or high signal loss can have a variety of causes. Typically, they can be caused by the respondent having an eye defect or by their position relative to the eye-tracking device, by eye fatigue and the like **(Bojko, 2013)**.

With regard to the quantitative nature of the eye-tracking research, this should comply with the recommendations of **Pernice & Nielsen (2009)**, who considered it appropriate to work with a minimum of thirty respondents.

The experiment was carried out using the method called A/B testing, whereby one half of the respondents (A) was shown the original version of the stimulus and the second half (B) the modified stimulus. The eye-tracking investigation was carried out using remote eye-tracker SMI RED 250 at a sampling frequency of 125 Hz. The eye-tracker was affixed to the bottom edge of a monitor having a diagonal size of 22″ with a 16:10 aspect ratio. The respondents' viewing distance was about 60 cm. The first step in the experiment was to calibrate the eye-tracker to the respondent's sight, using a nine-point auto calibration with subsequent four-point verification. After calibration, the stimuli were presented, in a randomized order. The task of the respondents was to view each individual stimulus and then answer the questions concerning the stimulus shown. A total of seven stimuli were used, each displayed for 10 seconds.

The questioning had two parts. The first questioning was done immediately after the stimulus, whereby the respondents were asked whether they know the wine displayed and, where appropriate, whether they buy it. Furthermore, the respondents had to rate on a ten-point scale how much the packaging and labelling had caught their attention and how upmarket was the impression. The final questions for each stimulus was how much they would be willing to spend on the wine depicted, and whether they would indeed buy the given wine.

The second round of questions came after all the stimuli had been shown, the respondents being asked questions about their purchasing habits when buying white wine. Specifically, the questions concerned the frequency of

buying wine (options: once a week, more than 3 x a month, 2 – 3 x a month, once a month, less than once a month). They rated on a ten-point scale the importance of the factors of price, variety, awards, the shape and colour of the bottle and the label, when buying wine. They further evaluated the importance of the individual bits of information on the label, i.e. information about the producer (maker) of the wine, wine variety, wine-growing region, country of origin, year of vintage and the sugar content indication.

For processing the study results, all the data was submitted to the SMI BeGaze software, used to analyse the eye-tracking data in more detail. The first step carried out was to cleanse the data of respondents with high signal losses or marked signal calibration deviations. Subsequently, using the editor implemented in the BeGaze program, the so-called Areas of Interest (AOI) were created. The Area of Interest (AOI) was created over such parts of the image that were the subject of changes between the A/B testing. The monitored metric for each area of interest was in particular the time spent observing the AOI, referred to as Dwell Time, measured in milliseconds. For illustrating the findings, we also generated what are called 'heat maps' that display data by using the colour spectrum, whereby the greater the intensity of the observation of the image elements the more pronounced is the red colouration. For the analysis of the data obtained and the influence of individual stimuli, the statistical characteristics were supplemented with the paired t-test and the Mann-Whitney test. The data was analysed using IBM SPSS software.

RESULTS AND DISCUSSION

As stimuli for the experiment we used photos of 7 bottles of selected white wines sold in the Czech Republic. Figure 1 shows the individual varieties, where column A shows the original stimulus, while column B shows the stimulus with modified attributes.

Under the eye-tracking investigation, the respective attributes on the wine labels were the Sweetness of the wine, Wine type, Producer, Country of origin, Wine area, Vintage – tracking over the AOI. All the attributes of interest were present only on the labels of stimulus #1 (version A and B), stimulus #2 (version A and B), and stimulus #7 (version A and B). For the other stimuli only some of the monitored attributes were shown on the front label. The individual Dwell Times of observation are given in Table 1. For illustrative purposes the Table also includes the rating of the wine, the Award sticker. The most noticed attribute can safely be considered to be the last-mentioned attribute, i.e. the Award sticker, which, if displayed on the stimulus, received the greatest degree of attention in almost all cases (AVG Dwell Time [ms] 2164.56). The second visual attribute in terms of receiving much attention was the information about the wine Producer (AVG 1425.51 [ms]). Significantly less attention was paid to the attributes of Sweetness of the wine (AVG 293.16 [ms]), Vintage (AVG 270.60 [ms]) and Country of origin (AVG 142.68 [ms]).

To verify the conclusiveness of the investigated influences on observation Dwell Times we used statistical

Figure 1 Stimuli and its modifications used for A/B testing.

hypothesis testing. The results of the individual tests are summarized in Table 2.

Hypothesis #1 assumes that there isn't [sic] a relationship between the observation Dwell Time of the wine variety information and its location on the wine label. This hypothesis was not confirmed at the $\alpha = 0.05$ significance level and so we cannot reject the null hypothesis. From the average Dwell Time, it is evident that a non-significantly higher level of attention went to the manufacturer-used rendition of the Wine type (Mode A).

To verify the relationship between the presence of a secondary label and the price valuation of the wine we used hypothesis #2. The control group in this case worked using a wine bottle with a secondary label depicting a lizard (see 2A in Figure 1), which is associated with the wine name. For the experimental group, this was removed (see 2B in Figure 1). We also do not reject [sic] this hypothesis at the $\alpha = 0.05$ significance level. A non-significantly higher average price valuation was obtained here by the producer's rendition depicting the lizard.

Table 1 Average Dwell Time of selected AOIs.

Stimulus	Award sticker	Producer	Wine type	Wine area	Sweetness of wine	Vintage	Country of origin
1A	**2184.51**	1165.58	1405.65	754.91	334.00	291.32	201.51
1B	**2626.33**	1429.75	967.82	693.76	399.80	327.71	97.85
2A	-	**1866.27**	1036.64	299.77	267.69	297.79	30.26
2B	-	**2107.18**	1162.26	518.21	200.21	246.27	49.01
3A	1242.65	**1622.41**	1025.15	-	-	-	-
3B	**1725.00**	1181.13	672.47	-	-	-	-
4A	-	**1902.44**	1269.53	-	-	-	-
4B	**2060.76**	1476.75	995.00	-	-	-	-
5A	**2357.51**	689.53	2131.64	-	248.65	85.32	-
5B	**2955.39**	818.18	1945.36	-	-	-	-
6A	-	1697.21	**2201.17**	-	561.46	-	-
6B	-	1485.56	**1806.89**	-	610.34	-	-
7A	-	**1322.52**	1019.00	940.73	12.55	299.15	221.70
7B	-	1193.24	1133.41	**1241.90**	4.03	347.31	255.95
Mean	**2164.56**	**1425.51**	**1340.81**	**741.52**	**293.16**	**270.60**	**142.68**

When it comes to Dwell Time, the influence of whether and where on the wine bottle there was an Award sticker was the subject of hypothesis #3. In this case, we accept the alternative hypothesis, and from the values of the averages it is clear that a greater degree of attention went to the design where the sticker was rendered near the top of the label (Mode B = 1912.47 [ms]). The greater attention given to the upper part of the label is also evident from the Heat Map in Figure 2. In the case of the control variant A, the respondents' attention was evidently elsewhere compared to variant B, latching onto other attributes than the Award sticker attribute when placed in the bottom part of the label.

The dependency between the label observation Dwell Time and the presence of the Award sticker was the subject of hypothesis #4. In view of the value of $p = 0.00$, we accept the alternative hypothesis, i.e. that there exists a relationship between the label observation Dwell Time and the presence of an Award sticker. From the values of the averages it is apparent that, with the sticker present, the average label observation Dwell Time was 1826.66 milliseconds less than in the variant rendition without the sticker.

The dependency between the designation of the wine with there being an Award sticker present and the price valuation was tested by hypothesis #5. The hypothesis was applied to the same stimulus (#4) and with regard to the value of p = 0.851 we cannot reject the null hypothesis. In this case, the price differences between the respective renditions are almost negligible, i.e. Mode A = 117.22 CZK, Mode B = 113.55 CZK.

The authors also sought to verify the importance of presenting information about the Vintage, the Wine type and classification, insomuch as replacing this information with a lower rated wine classification text (table wine) will have an effect on the price valuation of the relevant wine (hypothesis #6). In this case, the null hypothesis is rejected at the $\alpha = 0.05$ significance level and we adopt the alternative hypothesis, i.e. that there is a dependency between the generic designation of the wine and this wine's price valuation. The average pricing of the rendition with the original text was 159.05 CZK, while with the text replaced the wine pricing average was 123.82 CZK.

Figure 1 A/B test of award sticker position - heat map.

When testing the impact of the stopper capsule colouring on the stopper observation Dwell Time, (hypothesis #7), in view of the value of p = 0.529 we do not reject the null hypothesis. The average observation time was lower for the experimental group, i.e. Mode B (Dwell Time) = 476.98 [ms] compared to Mode A (Dwell Time) =

569.91 [ms]. From Figure 3 it is clear that the attention of the respondents was for both variations of the stimuli directed at similar attributes of the labels, and alternate stopper coverings made a minimal impression.

To test for any dependency between the colour of the wine bottle and the price valuation influenced by the colour changes, the impact of this change on the valuation of the observed wine was assessed. As with the previous hypothesis, no effect was confirmed to exist and in view of the value of p = 0.897 the null hypothesis cannot be rejected.

During questioning the respondents were asked to rate each of the factors that affect them when buying wine, on a scale from 1 to 10 (1 = least important factor, 10 = most important). From Table 3 it is clear that the respondents ascribe the greatest influence to the information on the wine label and the wine variety. In contrast, the least importance was ascribed by the respondents to the factors of awards and the bottle shape of the wine purchased.

The respondents were subsequently asked about their perceived importance of each of the elements of the label and what importance they ascribe to each of the information items on the labels. From Table 4 it is clear that the highest rating was assigned to the information about the sugar content of the wine. In contrast, the least importance was, on average, ascribed by the respondents to the year of vintage.

Comparing the two preceding tables (Table 3 and 4) with Table 1, which lists the observation times of individual attributes, we find a certain paradox. Although the respondents claim wine awards to be one of the least important attributes, when that is shown on the bottle it gets distinctly the highest level of attention. The importance of the presence of the Award sticker was tested under hypothesis #4, which proved a dependence between the Dwell Time on other attributes and the presence of the Award sticker. Likewise, the attribute of sugar content, which the Generation Z respondents considered the most important, was by contrast among the attributes that received the least amount of attention under the eye-tracking investigation.

Figure 2 A/B test of stopper capsule colouring - heat map.

Table 2 Hypothesis results.

Hypothesis	Mode	Test	Sig. value	Hypothesis accepted
#1	Mode A (Dwell Time) = 1509.82 Mode B (Dwell Time) = 1060.37	Independent-samples t-test	0.056	H0
#2	Mode A (price) = 129.86 Mode B (price) = 109.73	Independent samples Mann-Whitney U test	0.279	H0
#3	Mode A (Dwell Time) = 1159.88 Mode B (Dwell Time) = 1912.47	Independent-samples t-test	0.030	H1
#4	Mode A (Dwell Time) = 8999.18 Mode B (Dwell Time) = 7172.52	Independent-samples t-test	0.00	H1
#5	Mode A (price) = 117.22 Mode B (price) = 113.55	Independent samples Mann-Whitney U test	0.851	H0
#6	Mode A (price) = 159.05 Mode B (price) = 123.82	Independent samples Mann-Whitney U test	0.038	H1
#7	Mode A (Dwell Time) = 569.91 Mode B (Dwell Time) = 476.98	Independent-samples t-test	0.529	H0
#8	Mode A (price) = 142.14 Mode B (price) = 131.50	Independent samples Mann-Whitney U test	0.897	H0

Table 3 Importance of factors when buying wine.

Variable	Mean	Median	Mode	Mode frequency
Label	7.02	7	7	16
Variety	6.77	7	8	10
Price	6.09	6	7	12
Awards	5.84	6	7	11
Bottle shape	5.80	6	6	11

Table 4 Information items on the label, and their importance when buying wine.

Factor	Mean	Median	Mode	Mode freq
Sugar content	8.43	9	9	16
Variety	7.20	8	8	10
Producer	6.95	8	8	17
Country of origin	6.80	8	8	12
Area	5.64	6	5	8
Vintage	5.14	5	5	9

Table 5 Monitored attribute areas taken up.

Stimulus	Producer (%)	Sugar content (%)	Award Sticker (%)
1A/B	7.01	2.14	18.95
2A/B	10.54	1.32	-
3A/B	3.19	-	5.91
4A	8.76	-	-
4B	8.76	-	8.84
5A	4.94	1.07	8.24
5B	4.94	-	8.24
6A/B	4.04	2.71	-
7A/B	5.35	0.54	-

Table 6 Pricing of the respective stimuli.

Stimulus	Modal price A (CZK)	Modal price B (CZK)
1	128.18	106.77
2	129.86	109.73
3	133.73	112.45
4	117.23	113.55
5	159.05	123.82
6	119.59	114.59
7	142.14	131.50

For other attributes, the difference between their observation Dwell Time and their adjudged importance is none too great.

One of the possible causes of the low Dwell Times for the Sweetness of wine AOI, and conversely, the high Dwell Times on the Award sticker AOI may be the space taken up by these attributes on the label/wine bottle. Hence if we compare the areas of the respective information items on the labels, see Table 5, it is clear that the areas taken up by the individual attributes are quite different. For example, in the case of stimulus #7, the area of information about sugar takes up only 0.54% of the stimulus area. One of the factors influencing the observation Dwell Time of the reference attribute is undoubtedly to be considered the very size of the area taken up by this attribute on the label.

Another possible explanation for the low Dwell Times of the Sugar content reference attribute can also be the effect of the attribute's placing on the label. Most of the test labels had the Sugar content information placed in the bottom part of the label, in the case of two stimuli, it was not shown at all. This lends itself to making a clear recommendation for wine producers to optimize their labels for Generation Z consumers: if possible, always visibly show information about the wine sugar content.

If we look at the pricing of the respective stimuli that were part of the supplementary questioning and are shown in Table 6, we see that the highest rating was reached by stimulus #5 in variant A. This was also part of testing hypothesis #6, discussed above. What was the cause of this high rating compared to the other stimuli? In the first place we need to be aware what attributes were displayed for this stimulus. Here, specifically, they were the Producer, Wine type, Sweetness of wine, Vintage, Classification of the wine and furthermore the Award sticker. Comparing the displayed attributes with the attributes for the stimulus, shown under variant B, which left showing only the attributes of Producer, Wine type and the Award sticker and the remaining attributes replaced by the classification: table wine attribute, it can be postulated that the presence of the Award sticker may have had far less an influence, than the absence of the other attributes and their being replaced with a basic classification.

For comparison, we can mention stimulus #4, which was included in the experiment with the aim of testing the significance of the presence of the Award sticker attribute. In the case of this stimulus only the following attributes were present: Producer, Wine type in the control group, the experimental group was, moreover, presented with the Award sticker attribute. On the basis of hypothesis #5 the above assumption is confirmed, about the location of the Award sticker on the bottle without the presence of other appropriate attributes.

As regards the actual position of the Award sticker, for emphasis it is entirely appropriate to locate it at the top of the label, as is common practice with most producers. When placed in the lower part of the label the Award sticker receives less attention, see hypothesis #3.

The authors had set out to compare their results with other studies where eye-tracking technology was used to evaluate wine labels, yet no similar studies focused on performing A/B testing seem to have been published thus far. Eye-tracking technology used to assess boxed wine packaging is present in the write-up by **Moskowitz et. al., (2009)**. The issue of designing labels for the preferences of (Hong Kong) Chinese consumers was dealt with by the paper authored by **Tang et al., (2015)**.

CONCLUSION

The respondents to our research study, which set out to identify the significance of each of the visual attributes of bottled wines that have an impact on Generation Z consumers' wine choices, stated the sugar content to be the most important attribute influencing their wine choice. Yet as shown by the results of our eye-tracking study, this attribute gets less attention compared to other attributes. In contrast, the highest degree of attention among the monitored attributes was obtained by the Award sticker, which, however, the respondents ranked lower down on their factor preference scale. It was found, furthermore, that the presence of the Award sticker attribute itself does not automatically translate to a higher price valuation of such a bottle by respondents and that other among the given attributes must be considered. The position of the sticker on the bottle has a significant effect on the intensity of the attention it gets. The present study has confirmed a greater degree of observation Dwell Time when the sticker is placed in the upper part of the label. Under the experiment we also tested the effect of changes to the colour of stopper capsule and also the colour of the glass bottle itself. Neither one of these attributes was found to have a significant effect on the observation Dwell Time in the case of changing the colour of the capsule, nor on the price valuation in the case of changing the bottle glass colour.

The present study has several limitations, and some follow-up studies would be advisable. With regard to the limited representativeness of the sample used, the first consideration would be to conduct the study with a broader sample of respondents, in order to get more generally applicable findings. Further studies could also work with a larger number of chosen product samples, or greater diversity as may be, whereas this study restricted itself primarily to commonly available bottles of white wine produced in the Czech Republic.

REFERENCES

Bojko, A. 2013. *Eye tracking the user experience: a practical guide to research.* BROOKLYN, NEW YORK: Rosenfeld Media, XIV. 304 p. ISBN 19-338-2010-1.

Charters, S., Pettigrew, S. 2007. The dimensions of wine quality. *Food Quality and Preference*, vol. 18, no. 7, p. 997-1007. https://doi.org/10.1016/j.foodqual.2007.04.003

Czech Statistical Office. 2015. Spotřeba alkoholických nápojů a cigaret na obyvatele za rok (In Czech)) (The consumption of alcoholic drinks and cigarette per inhabitant and year) [online] s.a. [cit. 2015-08-21] Available at: https://www.czso.cz/documents/10180/20562003/270139150 2.pdf/a7362c11-36fe-4c80-9b66-c1b6561193f7?version=1.1

Di Vita, G., Chinnici, G., D'Amico, M. 2014. Clustering attitudes and behaviours of Italian wine consumers. *Quality – Access to Success*, vol. 15, no. 139, p. 54-61.

Ilin, I., Shestova, T. 2014. Z-Generation. *Value Inquiry* Book Series, vol. 276, p. 500-500.

Kotler, P., Armstrong, G. 2012. *Principles of Marketing*. 14th ed. BOSTON: Pearson Prentice Hall, 744 p. ISBN 0132167123.

Ling, B. H., Lockshin, L. 2003. Components of Wine Prices for Australian Wine: How Winery Reputation, Wine Quality, Region, Vintage, and Winery Size Contribute to the Price of Varietal Wines. *Australasian Marketing Journal*, vol. 11, no. 3, p. 19-32. https://doi.org/10.1016/S1441-3582(03)70132-3

Moskowitz, H. R., Reisner, M., Lawlor, J. B., Deliza, R. 2009. *Packaging Research in Food Product Design and Development*. SINGAPORE: John Wiley & Sons, 264 p. ISBN 978-0-8138-1222-9. https://doi.org/10.1002/9781444319330

Mueller, S., Lockshin, L., Saltman, Y., Blanford, J. 2010. Clustering attitudes and behaviours of Italian wine consumers *Food Quality and Preference*, vol. 21, p. 22-32. http://dx.doi.org/10.1016/j.foodqual.2009.07.004

Pernice, K., Nielsen, J. 2009. *How to Conduct Eyetracking Studies*. USA: Nielsen Norman Group, 159 p. Available at: http://www.nngroup.com/reports/how-to-conduct-eyetracking-studies/

Scollary, G. R. 2016. Wine labels. *Chemistry in Australia*. p. 39-39.

SMI. *BeGaze manual: Version 3.3*. Teltow, Germany, March 2013, 228 p. Document number: 091222-P-1400-001-000-A.

Czech Agriculture and Food Inspection Authority. 2015. Označování vína podle platných právních předpisů (In Czech) (Wine labelling according to the valid legislation). [online] s.a. [cit. 2016-07-04] Available at: http://www.szpi.gov.cz/clanek/oznacovani-vina-podle-platnych-pravnich-predpisu.aspx

Stávková, J., Stejskal, L., Toufarová, Z. 2008. Factors influencing consumer behaviour. *Agricultural Economics*, vol. 54, no. 6, p. 27-34.

Tang, V. Ch. M., Tchetchik, A., Cohen. E. 2015. Perception of wine labels by Hong Kong Chinese consumers. *Wine Economics and Policy*. vol. 4, p. 12-21. https://doi.org/10.1016/j.wep.2015.02.002

Wang, R. W. Y., Chou, M. C. 2011. The Comprehension modes of visual elements: How people know about the contents by product packaging. *International Journal of Business Research and Management*, vol. 1, no. 1, p. 1-13.

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GENETIC VARIATION AND RELATIONSHIPS OF OLD MAIZE GENOTYPES (*ZEA MAYS L.*) DETECTED USING SDS-PAGE

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ABSTRACT

The assessment of genetic diversity among the members of a species is of vital importance for successful breeding and adaptability. In the present study 40 old genotypes of maize from Hungary, Union of Soviet Socialist Republics, Poland, Czechoslovakia, Yugoslavia and Slovak Republic were evaluated for the total seed storage proteins using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) through vertical slab unit. The number of total scorable protein bands was twentythree as a result of SDS-PAGE technique but those that were not cosistent in reproducibility and showed occasional variation in sharpness and density were not considered. Out of twentythree polypeptide bands, 6 (31%) were commonly present in all accessions and considered as monomorphic, while 17 (65%) showed variations and considered as polymorphic. On the basis of banding profiles of proteins of different kDa, gel was divided into zones A, B and C. The major protein bands were lied in zones A and B, while minor bands were present in zones C. In zone A out of 10 protein bands, 1 were monomorphic and 9 were polymorphic. In zone B out of 8 protein bands, 3 was monomorphic and 5 was polymorphic and in zone C out of 5 protein bands, 2 were monomorphic whereas 3 polymorphic. The dendrogram tree demonstrated the relationship among the forty registered old maize genotypes according to the similarity index, using UPGMA cluster analysis. The dendrogram was divided into two main clusters. The first one contained eleven genotypes from maize, while the second cluster contained the twentynine genotypes of maize. Similarly the present study of genetic variability in the seed storage polypeptide determined by SDS-PAGE technique proved that it is fruitful to identify genetic diversity among accessions of maize.

Keywords: maize; dendrogram; SDS-PAGE; genetic diversity

INTRODUCTION

Maize (*Zea mays* L.) is an annual, cross-pollinated by wind and the only monoecious among cereal crops to have male and female inflorescences on separate branches of the same plant. It belongs to grass family *Poaceae* (Gramineae) which is leading in importance in the order Poales **(Bremer et al., 2003)**. This family contributes to the world economy, food and industry through valuable crops i.e. wheat, rice and maize **(Mabberley, 2008)**. Being most domesticated with controversy in origin and evolution, there is one school of thoughts that maize is the nearest descendant of Mexican teosinte **(Dowswell et al., 1996)**. There is no doubt that human beings directly or indirectly depend on plants for various purposes for which they domesticated these with the passage of time and flourished with spreading communities, undergone through evolution, passing through various cultivating methodologies throughout the world **(Larik, 1994)**.

Maize seed consists of two types of protein i.e., zein and non-zein protein. The term zein is used for prolamins in maize which is alcohol soluble protein and could be extracted with ethanol **(Lawton, 2006)**. Zein is major seed storage protein of maize **(Freitas et al., 2005)** and consists of one major and three minor classes and these four classes constitute approximately 50 – 70% of maize endosperm **(Vasal, 1999)**. The non-zein protein consists of globulins (3%), glutelins (34%) and albumins (3%). Zein is specific to maize endosperm **(Prasanna et al., 2001)** and not present in any other part of plant.

Proteins are primary gene products of active structural genes; their size and amino acids sequence are the direct results of nucleotide sequences of the genes; hence, any observed variation in protein systems induced by any mutagen is considered a mirror for genetic variations **(Hamoud et al., 2005)**. Variation in theDNAcoding sequences frequently causes variation in the primary conformation of the proteins. Determination of protein molecular weight (MW) via polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) is a universally used method in biomedical research; **(Ranjan et al., 2013)** concluded that electrophoresis (SDS-PAGE) of proteins can be economically used to assess genetic variation and relation in germplasm and also to differentiate mutants from their parent genotypes. Some studies used SDS-PAGE for detection of alterations in protein profiles occurring during exposure to electric field **(Hanafy et al., 2006; Dymek et al., 2012)**.

So far, several investigations on the discrimination between crop genotypes using SDS-PAGE have been carried out by **Yoon et al., (2010)**; **Osman et al., (2013); Iqbal et al., (2014); Iqbal et al., (2014); Khan et al.,**

(2014); AL-Huqail et al., (2015); Gregova et al., (2015); Kačmárová et al., (2016); Socha et al., (2016).

The objectives were to find out the level of genetic variability present in 40 maize germplasm by using the electrophoretic profiles of total seed proteins with different molecular weights through SDS-PAGE.

MATERIAL AND METHODOLOGY

Maize genotypes (40) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the GeneBank in Piesťany, the Slovak Republic (Table 1).

SDS-PAGE was carried out according to the standard reference ISTA method **(Wrighley, 1992)**. Storage proteins were extracted from individually ground seeds using extracting using a buffer composed of 6.25 mL Tris (1.0 mol L^{-1}, pH = 6.8), 10 mL glycerol, 12.05 mL H_2O and 2.0 g SDS, diluted with mercaptoethanol and H_2O in a 17:3:40 (v/v) proportion. The buffer was added to flour in a 1:25 (w/v) proportion. Extraction was performed at room temperature overnight and heating in boiled water for 5 minutes, centrifugation at 5000 x g for 5 min. 10 μL of extracts were applied to the sample wells. The gel (1.0 mm thick) consists of two parts: stacking gel (3.5% acrylamide, pH = 6.8 acrylamide) and resolution gel (10 % acrylamide, pH = 6.8). Staining of gels was performed in a solution of Coomassie Brilliant Blue R250 dissolved in acetic acid and methanol solution. Gel was scanned with densitometer GS 800 (Bio-Rad) and evaluated with Quantity One-1D Analysis Software.

RESULTS AND DISCUSSION

The number of total scorable protein bands was twentythree as a result of SDS-PAGE technique but those that were not cosistent in reproducibility and showed occasional variation in sharpness and density were not considered. Based on these bands forty accessions of maize (Table 1) were screened. Out of twentythree polypeptide bands, 6 (31%) were commonly present in all

Table 1 List of 40 analyzed genotypes of maize.

Genotypes	Country of origin	Year of registration
1. Feheres Sarga Filleres	Hungary	1965
2. Mindszentpusztai Feher	Hungary	1964
3. Zakarpatskaja	Union of Soviet Socialist Republics	1964
4. Przebedowska Burskynowa	Poland	1964
5. Krasnodarskaja	Union of Soviet Socialist Republics	1964
6. Mesterhazy Sarga Simaszemu	Hungary	1964
7. Slovenska biela perlova	Czechoslovakia	1964
8. Zuta Brzica	Yugoslavia	1975
9. Zloty Zar	Poland	1964
10. Slovenska Florentinka	Czechoslovakia	1964
11. Juhoslavska	Yugoslavia	1964
12. Kostycevskaja	Union of Soviet Socialist Republics	1964
13. Mindszentpusztai Sarga Lofogu	Hungary	1964
14. Stodnova	Czechoslovakia	1964
15. Slovenska žltá	Slovak Republic	1964
16. Slovenska krajová velkozrná	Slovak Republic	1964
17. Partizanka	Union of Soviet Socialist Republics	1964
18. Voroneskaja	Union of Soviet Socialist Republics	1964
19. Kocovska Skora	Slovak Republic	1964
20. Milada	Czechoslovakia	1964
21. Moldavskaja	Union of Soviet Socialist Republics	1964
22. Bučiansky Konský Zub	Slovak Republic	1964
23. Hodoninský konský zub žltý	Czechoslovakia	1964
24. M Silokukurica	Hungary	1964
25. Valticka	Czechoslovakia	1964
26. Przebedowska Biala	Poland	1964
27. Toschevska	Slovak Republic	1964
28. Šamorinsky konský zub	Hungary	1964
29. Wielkopolanka	Poland	1964
30. Czechnicka	Poland	1964
31. Manalta	Czechoslovakia	1964
32. Zlota gorecka	Poland	1964
33. Celchovicka ADQ	Czechoslovakia	1964
34. Belaja mestnaja	Union of Soviet Socialist Republics	1964
35. Bučanská žltá	Slovak Republic	1964
36. Iregszemeseil 2 hetes	Hungary	1964
37. Dnepropetrovskaja	Union of Soviet Socialist Republics	1964
38. Bezuncukskaja	Union of Soviet Socialist Republics	1964
39. Mikulická	Czechoslovakia	1964
40. Aranyozon sarga lofogu	Hungary	1964

accessions and considered as monomorphic, while 17 (65%) showed variations and considered as polymorphic. The size of the protein bands obtained through SDS-PAGE ranged from 20 to 140 kDa.

On the basis of banding profiles of proteins of different kDa, gel was divided into zones A, B and C (Figure 1). The major protein bands were lied in zones A and B, while minor bands were present in zones C. It was noted that different accessions of maize showed more diversity in seed storage proteins in minor bands in comparison to major bands. In zone A out of 10 protein bands, 1 were monomorphic and 9 were polymorphic. In zone B out of 8 protein bands, 3 was monomorphic and 5 was polymorphic and in zone C out of 5 protein bands, 2 were monomorphic whereas 3 polymorphic. By considering these facts zone A and B were more polymorphic.

The dendrogram tree (Figure 2) demonstrated the relationship among the forty registered old maize genotypes according to the similarity index, using UPGMA cluster analysis. The dendrogram was divided into two main clusters. The first one contained eleven genotypes from maize, while the second cluster contained the twentynine genotypes of maize. In Cluster I was separated unique genotype Kostycevskaja (Union of Soviet Socialist Republics) from other 10 genotypes (Figure 2). One genotypes in cluster I is from Hungary (Mindszentpusztai Sarga Lofogu) and one genotypes is from Yugoslavia (Juhoslavska), two genotypes are from Union of Soviet Socialist Republics and Czechoslovakia and four genotypes are from Slovak Republic. Cluster I not contained genotype from Poland. Cluster II contained eight genotypes from Hungary (27.6%), six genotypes from Poland (20.7%), six genotypes of maize from Union of Soviet Socialist Republics (20.7%), five genotypes from Czechoslovakia (17.2%), three genotypes from Slovak Republic (10.3%) and one genotype of maize is from Yugoslavia (3.4%) (Figure 2).

Similarly the present study of genetic variability in the seed storage polypeptide determined by SDS-PAGE technique proved that it is fruitful to identify genetic diversity among accessions of maize.

Similar results were detected by other authors **(Yoon et al., 2010; Osman et al., 2013; Iqbal et al., 2014; Iqbal et al., 2014; Khan et al., 2014; AL-Huqail et al., 2015)** and these results presented a high level of polymorphism of old maize genotypes detected by SDS-PAGE.

Osman et al., (2013) study genetic relationship between some species of Zea mays using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of seed proteins.

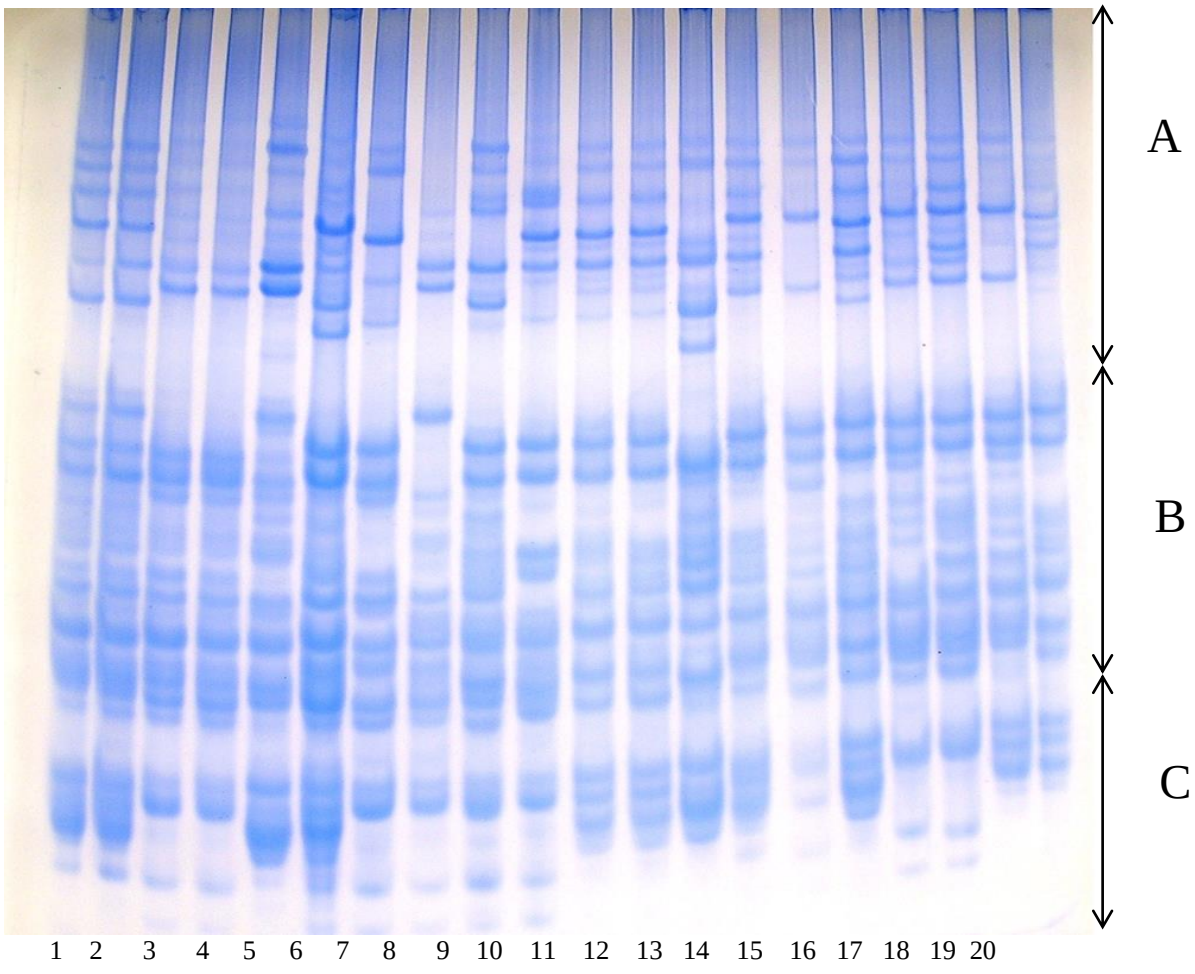

Figure 1 Protein profile showing total seed storage proteins in maize genotypes as a result of SDS-PAGE. Lanes 1 - 20 are maize genotypes (Table 1).

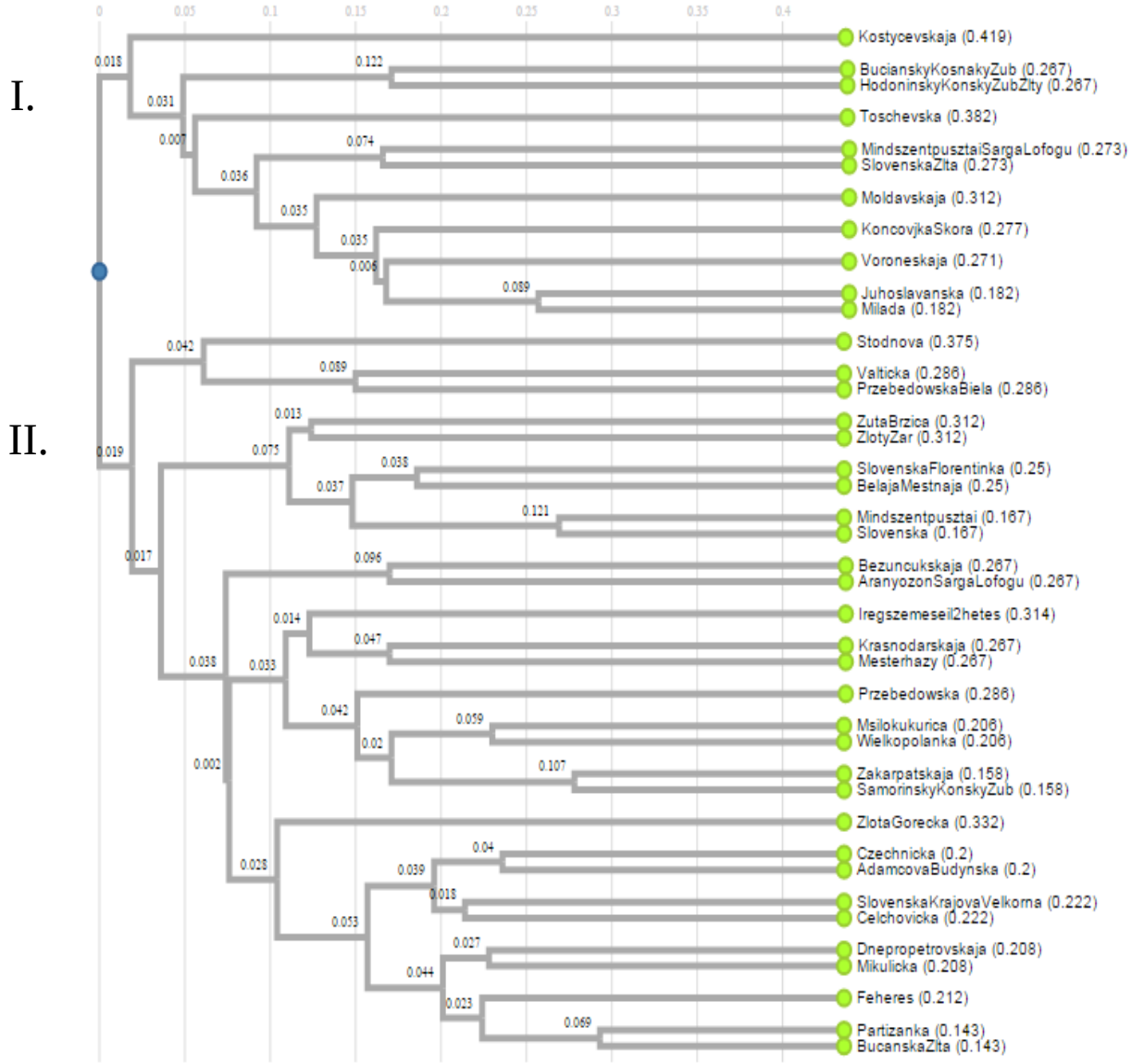

Figure 2 Dendrogram of 40 maize genotypes prepared based on SDS-PAGE markers.

Autors identified 78 bands across the studied species. The number of bands varies from 17 bands in sample number 5 to 6 in sample number 6. **Iqbal et al., (2014)** analyzed 73 genotypes of maize from China, Japan and Pakistan for the total seed storage proteins using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). A total of 18 protein bands were recorded. Among these 7 (39%) were monomorphic and 11 (61%) polymorphic, with molecular weight varied from 10 kDa to 122 kDa. The aim of **Iqbal et al., (2014)** was to estimate the genetic diversity across 83 genotypes of maize of Pakistan and Japanese origin using SDS-PAGE. A total of 18 protein subunits were noted out of which 7 (39%) were monomorphic and 11 (61%) were polymorphic, with molecular weight ranging from 10 to 122 kDa. Coefficients of similarity among the accessions ranged between 0.89 and 1.00. The dendrogram obtained through UPGMA clustering method showed two main clusters: 1 and 2. First cluster contained 9 genotypes, while second cluster contained 74 genotypes. **Khan et al., (2014)** study the variation of zein fraction of seed storage protein in maize by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Variation in terms of absence and presence, intensity and molecular size was observed in zein polypeptides. **AL-Huqail et al., (2015)** used SDS-PAGE to detection of 46 polypeptides bands with different molecular weights ranging from 186.20 to 36.00 KDa. It generated distinctive polymorphism value of 84.62%.

CONCLUSION

SDS-PAGE techniques may provide useful information on the level of polymorphism and diversity in old maize genotypes. Forty maize genotypes originated from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the Gene Bank in Piesťany, the Slovak Republic were very closely related. The dendrogram was divided into two main clusters. The first one contained eleven genotypes from maize, while the second cluster contained the twentynine genotypes of maize. Result from this study show that protein markers are powerful and efficient in

characterising and identifying of old maize genotypes in addition to their usefulness in phylogenetic studies.

REFERENCES

AL-Huqail, A. A., Abdelhaliem, E. 2015. Evaluation of Genetic Variations in maize Seedlings Exposed to Electric Field Based on Protein and DNA Markers. *BioMed Research International*, vol. 2015, p. 1-15. http://dx.doi.org/10.1155/2015/874906

Bremer, K., Bremer, B., Thulin, M. 2003. Introduction of Phylogeny and Systematic of Flowering Plants. *Symbolae Botanicae Upsalienses*, vol.33, no. 2, p. 64-73.

Dowswell, C. R., Paliwal, R. L., Cantrell, R. P. 1996. *Maize in the Third World*. University of Michigan : Westview Press, ISBN: 978-0813389639.

Dymek, K., Dejmek, P., Panarese, V., Vincente, A. A., Wadsö, L., Finnie, C., Galindo, F. G. 2012. Effect of pulsed electric field on the germination of barley seeds. *LWT-Food Science and Technology*, vol. 47, no. 1, p. 161-166. http://dx.doi.org/10.1016/j.lwt.2011.12.019

Freitas, I. R. A., Gananca, F., Santos, T. M., Carvalho, M. A. A., Motto, M., Vieira, M. C. 2005. Evaluation of maize germplasm based on zein polymorphism from the archipelago of Madeira. *Maydica*, vol. 50, p. 105-112.

Gregova, E., Šliková, S., Hozlár, P. 2015. Seed protein electrophoresis for identification of oat registered cultivars. *Potravinarstvo*, vol. 9, no. 1, p. 411-416. http://dx.doi.org/10.5219/513

Hamoud, M. A., El-Shanshory, A. R., Al-Sodany, Y. M., El-Karim, M. S. G. 2005. Genetic diversity among *Ipomoea carnea* jacq. Populations from different habitats types in Nile-Delta region of Egypt. *The Egyptian Journal of Experimental Biology*, vol. 1, p. 1-9.

Hanafy, M. S., Mohamed, H. A. and Abd El-Hady, E. A. 2006. Effect of low frequency electric field on growth characteristics and protein molecular structure of wheat plant. *Romanian Journal of Biophysics*, vol. 16, no. 4, p. 253-271.

Iqbal, J., Shinwari, Z. K., Rabbani, M. A., Khan, S. A. 2014. Genetic variability assessment of maize (*Zea mays* L.) germplasm based on total seed storage proteins banding pattern using SDS-PAGE. *European academic research*, vol. 2, no. 2, p. 2144-2160.

Iqbal, J., Shinwari, Z. K. and Rabbani, M. A. 2014. Investigation of total seed storage proteins of Pakistani and Japanese maize (*Zea mays* L.) through SDS-PAGE markers. *Pakistan Journal of Botany*, vol. 46, no. 3, p. 817-822.

Kačmárová, K., Lavová, B., Socha, P., Urminská, D. 2016. Characterization of protein fractions and antioxidant activity of Chia seeds (*Salvia Hispanica* L.). *Potravinarstvo*, vol. 10, no. 1, p. 78-82 http://dx.doi.org/10.5219/563

Khan, A. H., Khan, N., Minhas, N. M., Ghafoor, A., Rabbani, M. A. 2014. Diversity in seed storage proteins in maize genetic resources: I. variation in alcohol soluble zein protein fraction. *International Journal of Agricurtural and Biological Engineering*, vol. 16, p. 1015-1018.

Larik, A. S. 1994. Genetic resources and their conservation in crop plants. In: *Plant breeding.* (Eds.): M. A. Khan, E. Bashir and R. Bantel. National Book Foundation, Islamabad, p. 235-254.

Lawton, J. W. 2006. Isolation of zein using 100% ethanol. *Cereal Chemistry*, vol. 83, no. 5, p. 565-568. http://dx.doi.org/10.1094/CC-83-0565

Mabberely, D. J. 2008. *Mabberleys Plant-Book: A portable dictionary of plants, their classifications, and uses. 3rd edition.* Cambridge Univ. Press.: VII-XVIII, 1-1021.

Osman, G., Munshi, A., Altf, F. and Mutawie, H. 2013. Genetic variation and relationships of *Zea mays* and *Sorghum species* using RAPD-PCR and SDS-PAGE of seed proteins. *African Journal of Biotechnology*, vol. 12, no. 27, p. 4269-4276. http://dx.doi.org/10.5897/ajb12.2644

Prasanna, B., Vasal, S., Kashun, B. and Singh, N. N. 2001. Quality protein maize. *Current Science*, vol. 81, no. 10, p. 1308-1319.

Ranjan, S., Poosapati, A., Vardhan, H. et al., 2013. Seed storage protein profile of few leguminous grains grown in india using SDSPAGE. *International Journal of Advanced Biotechnology and Research*, vol. 4, no. 4, p. 505-510.

Socha, P., Tomka, M., Kačmárová, K., Lavová, B., Ivanišová, E., Mickowska, B., Urminská, D. 2016. Comparable efficiency of different extraction protocols for wheat and rye prolamins. *Potravinarstvo*, vol. 10, no. 1, p. 139-144 http://dx.doi.org/10.5219/540

Vasal, S. K., 1999. Quality protein maize story. *Proceedings of workshop on improving human nutrition through agriculture: the role of international agricultural research.* p: 1-16. IRRI, Los Banos, Philippines

Yoon, J. W., Jung, J. Y., Chung, H. J., Kim, M. R., Kim, Ch. W., Lim, S. T. 2010. Identification of botanical origin of starches by SDS-PAGE analysis of starch granule-associated proteins. *Journal of Cereal Science*, vol. 52, no. 2, p. 321-326. http://dx.doi.org/10.1016/j.jcs.2010.06.015

Wrigley, C. W. 1992. Identification of cereal varieties by gel electrophoresis of the grain proteins. *Heidelberg: Springer – Verlag*, p. 17-41. http://dx.doi.org/10.1007/978-3-662-01639-8_2

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EFFECT OF THERMAL PASTEURIZATION AND HIGH PRESSURE PROCESSING ON BIOACTIVE PROPERTIES IN STRAWBERRY JUICE

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ABSTRACT

In the current food industry, companies often offer new and revolutionary processing methods that allow to improve food properties. A prominent technology is High Pressure Processing (HPP), a non-thermal technology that arises as an alternative to the traditional thermal pasteurization (TP). With HPP it is possible to obtain food and drinks similar to the raw food while improving important nutritive and functional properties. Since strawberries are very important fruit in the human diet, the aim of this study was to study the effect of HPP and TP on selected qualitative-quantitative parameters of strawberry juices (HPSJ - High Pressure Strawberry Juice/TPSJ - Thermal Pasteurized Strawberry Juice). It seems that strawberries can have a positive effect on human health due to their high content in beneficial nutrients. From monitored parameters, significant differences (p <0.001) were found between juices in the following parameters: antioxidant activity, β-carotene and zeaxanthin content. Higher antioxidant activity (1547.60 ±4.89 mg AA.L^{-1} FM *vs*. 1424.72 ±10.66 mg AA.L^{-1} FM) and zeaxanthin (1.34 ±0.11 μg.mL^{-1} FM *vs.* 0.89 ±0.08 μg.mL^{-1} FM) was found in HPSJ, comparatively to TPSJ. The content of β-carotene was higher in TPSJ (156.28 ±2.13 μg.mL^{-1} FM) than in HPSJ (122.02 ±4.28 μg.mL^{-1} FM). Results related to the polyphenols content showed significantly higher values (p >0.01) in HPSJ, compared to TPSJ (1100.04 ±17.16 mg GAE.L^{-1} FM *vs*. 1002.66 ±17.16 mg GAE.L^{-1} FM). The difference in the content of lutein (TPSJ 8.84 ±0.57 μg.mL^{-1} FM; HPSJ 8.17 ±0.13 μg.mL^{-1} FM) was not significant (p >0.05).

Keywords: strawberry; thermal pasteurization (TP); high pressure processing (HPP); juice

INTRODUCTION

Cardiovascular disorders (CVD) are one of the leading causes of death and disability in the world **(Bansilala et al., 2015; Protulipac et al., 2015)** and their prevention is a major public health challenge **(Perk et al., 2012)**. The oxidative modification of the Low-density lipoprotein (LDL) in the vascular wall seems to be a key factor in the development of atherosclerosis that is considered as the main cause of CVD **(Wilson et al., 1998)**. A lower incidence of chronic diseases seems to be linked to the consumption of flavonoid-rich foods, particularly fruits and vegetables **(Dragsted et al., 2006)**. Their consumption is associated with positive effects in prevention of the cardiovascular diseases **(Takachi et al., 2010)**, ischemic stroke **(Joshipura et al., 1999)**, cancer **(Peterson et al., 2003; Riboli et al., 2003; Bosetti et al., 2005; Reis et al., 2012)**, diabetes mellitus - type 2 **(Carter et al., 2010)**, metabolic syndrome **(Esmaillzadeh et al., 2006)** and other diseases. These foods often show anti-inflammatory effects **(Larrosa et al., 2010)**, are normolipidemic and normo-glicemic agents **(Chong et al., 2010; Tarozzi et al., 2010)**, being beneficial against age-related diseases **(Halliwell, 2008)**, and have a significant role in eye health **(Kalt et al., 2010)**. All these effects are related to the inhibition of LDL oxidation **(Cook and Samman, 1996)**.

Besides whole fruits and vegetables, a relevant part of the intake of antioxidant coumpounds such as polyphenolic phytochemicals (including anthocyanins) is supplied by fruit juices, since they are suitable food products in terms of ingestion of health-protective phytochemicals. Bioactive components may even be better absorbed from juices than from plant tissues. Some studies have shown that the intake of berry juices can increase the plasma antioxidant capacity, which suggests an improvement of the antioxidant status and indicates that antioxidant constituents of the juice might decrease lipid oxidation within plasma compartment **(Jakobek et al., 2007)**.

Generally, manufacturers use thermal pasteurization (TP) in the production of juices in order to prevent their microbial spoilage and thus extend the shelf-life. However, it has been shown that TP induces deleterious changes in sensorial and nutritional quality of the processed juices. Furthermore, nowadays, the modern consumers demand minimally processed, high quality healthy drinks, with a fresh taste, free from additives and with the same or more nutrients than the natural raw products **(Considine et al. 2008)**.

High pressure processing (HPP) is an industrially tested technology that offers a more natural, environmentally friendly alternative to the traditional TP for a wide range of food products **(Barbosa-Cánovas et al., 2005)**. Non-thermal technologies are useful, not only to inactivate microorganisms and enzymes, but also to improve extractions' yield of desirable compounds, develop new

ingredients and market foods with novel quality and nutritional characteristic **(Tokuşoğlu and Swanson, 2015)**. HPP, operating at room or refrigeration temperature, is an attractive innovative technology, which involves using cold isostatic high hydraulic pressure (ranges from 100 – 800 MPa) **(Xi, 2006)**. The use of lower temperatures allows a better retention of the food nutritional components as well as their sensory characteristics attributed to "fresh" or "just prepared," **(Barbosa-Cánovas et al., 2005)**, which may lead to the development of new products with novel functional properties **(Mota et al., 2013)**. In high pressure extraction (HPE) process, the cell membranes are destroyed by the use of elevated pressures which leads to a greater number of bioactive compounds extracted out of the cells **(Joo, et al., 2013)**. As a result, HPP has become a post-packaging convenient technology for foods whose quality would otherwise be altered by TP. Moreover, HPP can add a significant shelf-life increase to an existing refrigerated product. In fact, it has the potential to deliver chemical- or additive-free products with minimum impact on shelf life **(Barbosa-Cánovas et al., 2005)**.

The aim of this work was to verify and compare the effects of two different processing technologies on strawberry juices's antioxidant activity, polyphenols, and carotenoid content (β-carotene, zeaxanthin and lutein).

MATERIAL AND METHODOLOGY

Biological material: Both juices (HPSJ and TPSJ) were prepared without the addtion of preservatives and sweeteners, and were not made from concentrate (in ratio 80 – 85 % strawberries: 10 – 15 % water). The origin of strawberries was Portugal and Spain and the juice was kindly supplied by Sumol +Compal (Portugal). Two different processing methodologies were used: in the first one the strawberry juice was processed by HPP (HPSJ - High Pressure Strawberry Juice), whereas in the second it was processed by TP (TPSJ - Thermal Pasteurized Strawberry Juice). The used processing conditions in the HPSJ and TPSJ were the following: 550 MPa, at 20 °C for 2 min and 95 ±2 °C for 1 min, respectively.

TP was performed by the suplier of the juices and HPP was performed at the University of Aveiro. The processed strawberry juices were transported from the University of Aveiro in Portugal to Slovakia in refrigerated conditions. All analyses were realized in the Slovak University of Agriculture in Nitra.

Preparation of the sample: 10 g of the homogenized samples was transferred into a 100 mL Erlenmeyer flask, mixed with 70 mL of distilled water and extracted at a speed of 150 min^{-1}/24 hours. After 24 hours, it was added distilled water to make 100 mL, filtered and used for analysis. The extraction of both juices (HPSJ and TPSJ) was carried out in the same manner for the analysis.

Determination of antioxidant activity: The antioxidant activity in the strawberry juices (both HPSJ and TPSJ) was determined by the FOMO method **(Prieto et al., 1999)**. The principle of the method is the reduction of Molybdenum (VI+) to Molybdenum (V+) by the activity of the reducing component in the phosphorus presence. There is a green Phosphomolybdic complex which color intensity is measured at a wavelength of 695 nm by spectrophotometer. The reductive ability of compounds can be expressed as ascorbic acid (AA) content, which is needed to achieve the same reduction effect. The antioxidant activity was expressed in $mg.L^{-1}$ equivalent of ascorbic acid (AA).

Determination of polyphenols: The polyphenols of juices HPSJ and TPSJ was determined by spectrophotometry at a wavelength of 700 nm using the Folin-Ciocalteu method **(Singleton and Rossi, 1965)** and was measured as the equivalent content of gallic acid and expressed in mg $GAE.L^{-1}$ (gallic acid equivalent). The method is based on the reaction of the Folin–Ciocalteu reagent with polyphenols, with leads to the formation of a blue color product. The intensity of the blue color is proportional to the content of the polyphenols.

Determination of carotenoids: The carotenoid content expressed in β-carotene, zeaxanthin and lutein of the HPSJ and TPSJ juices was measured at a wavelength of 445 nm using a spectrophotometer by method of liquid chromatography HPLC (HPLC Agilent Infinity 1260) with slight modification, as described by **Lachman et al. (2013)** and **Ashokkumar et al. (2015)**.

The results were processed by the statistical package STATISTICA Cz version 10. The differences between the samples were followed by Tukey's HSD test.

RESULTS AND DISCUSSION

Consumers around the world are better educated and more demanding in their identification and purchase of quality health-promoting foods. The food industry and regulatory agencies are searching for innovative technologies to provide safe and stable foods for their clientele. Thermal pasteurization and commercial sterilization of foods provide safe and nutritious foods that, unfortunately, are often heated beyond a safety factor that results in unacceptable quality and nutrient retention. **(Tokuşoğlu and Swanson, 2015)**. Thermal degradation of anthocyanins results in the formation of polyphenolic degradation products; it is not clear if the formation of these components results in an overall reduction in antioxidant activity **(Patras et al., 2010)**. Non-thermal processing technologies offer unprecedented opportunities and challenges for the food industry to market safe, high-quality health-promoting foods. These technologies for food processing provide an excellent balance between safety and minimal processing, between acceptable economic constraints and superior quality, and between unique approaches and traditional processing resources **(Tokuşoğlu and Swanson, 2015)**.

The health benefit of fruit juices has been described, in part, to natural antioxidants which may inhibit the development of major clinical conditions including cardiovascular disease and cancer. Many fruit juices also contain phenolic compounds and carotenoids which have antioxidant potential **(Gardner et al., 2000)**. Antioxidant activity is associated with decreases in DNA damage, reduction in lipid peroxidation, maintenance of the immune function, and prevention of the development of some diseases **(Gropper, Smith and Groff 2005)**. The function of β-carotene and carotenoids is manifested in the antioxidant activity, inhibition of lipid peroxidation and increased resistance of LDL to oxidation, the antioxidant protection against sunlight. Zeaxanthin affects visual acuity, is important for immunity, intercellular

Table 1 Statistical evaluation of average values of monitored parameters in strawberry juices.

Processing method	Antioxidant activity (mg AA.L^{-1} FM)	Polyphenols (mg GAE.L^{-1} FM)	β-carotene (μg.mL^{-1} FM)	Zeaxanthin (μg.mL^{-1} FM)	Lutein (μg.mL^{-1} FM)
HPSJ	1547.60 ±4.89	1100.04 ±17.16	122.02 ±4.28	1.34 ±0.11	8.17 ±0.13
TPSJ	1424.72 ±10.66	1002.66 ±17.16	156.28 ±2.13	0.89 ±0.08	8.84 ±0.57
p-value	0.001	0.01	0.001	0.001	0.062
Significance	++	+	++	++	-

Note: Data are expressed as mean ±*SD* (standard deviation); ANOVA, Tukey's HSD test; FM – fresh mater.

communication and metabolism of arachidonic acid **(Keresteš et al., 2016)**. β-carotene preventively protects against cardiovascular diseases, lung cancer and other cancers of the gastrointestinal tract **(Nemeth-Balogh et al., 2009)**.

Strawberries are the most popular berries in the world, being a nutritious fruit with putative health benefits **(Hossain et al., 2016; Giampieri et al., 2012)**. Bioactive components and anthocyanin compounds of strawberries have antioxidant, anti-inflammatory, antihypertensive, antihyperlipidemic and antiproliferative effects **(Basu et al., 2014)**. Studies showed that strawberries have high concentrations of ascorbic acid **(Larson, 1998; Szajdek and Borowska, 2008)**, anthocyanins **(da-Silva et al., 2007)**, phenolic acids, flavonoids, vitamins, carotenoids **(Kelebek and Selli, 2011)**, ellagic acid **(Häkkinen et al., 2000)**, and can achieve high total antioxidant capacity **(Wang and Lin, 2000)**.

We found that the HPSJ presented significantly (p <0.001) higher antioxidant activity (1547.60 ±4.89 mg AA.L^{-1} fresh matter) than TPSJ (1424.72 ±10.66 mg AA.L^{-1} fresh matter). With the same method, **Mendelová et al., (2016)** found that antioxidant activity of sea buckthorn fruit juice ranged from 45.11 g AA.L^{-1} dry matter to 108.77 g AA.L^{-1} dry matter.

The results showed a significant (p <0.01) higher amount of total polyphenols in the HPSJ (1100.04 ±17.16 mg GAE.L^{-1} fresh matter) than in the TPSJ (1002.66 ±17.16 mg GAE.L^{-1} fresh matter). In another study, the polyphenols conten ranged from 532 to 960 μg GAE.mL^{-1} in fruit citrus juice **(Rekha et al., 2012)**. According to **Rolle et al., (2016)** concentrations of polyphenols were similar between the two types of orange juices, the values of commercial thermally pasteurized juice was 63.3 ±5.85 mg.100mL^{-1} and home-made fresh juices were 62.9 ±5.94 mg.100mL^{-1}.

Statistically, significant differences (p <0.001) were reported in the content of β-carotene and zeaxanthin between the studied juices. The difference in the content of lutein in the juice HPSJ and TPSJ was not statistically confirmed (p >0.05). The content of β-carotene was 156.28 ±2.13 μg.mL^{-1} fresh matter in TPSJ, whereas in HPSJ was found a lower value 122.02 ±4.28 μg.mL^{-1} fresh matter.

In studies focused on the investigation of these ingredients in different kinds of vegetables, fruits, juices and other food products, they were reported different values, which are associated with inconsistent methodologies: β-carotene‘s content in carrots was 192.25 ±19.70 μg.g^{-1} dry matter **(Stinco et al., 2014)**, and **Maurer et al., (2014)** indicated the amount of 53.6 μg.g^{-1} fresh matter. Different amounts of β-carotene was found in apples (0.17 μg.g^{-1} dry matter) **(Delgado-Pelayo et al., 2014)**, canned apricots (170.0 μg.g^{-1} fresh mater) and canned peaches (9.3 μg.g^{-1} fresh matter) **(Campbell and Padilla-Zakour, 2013)**.

On the contrary, the content of zeaxanthin was 0.89 ±0.08 μg.mL^{-1} fresh matter in TPSJ, whereas in HPSJ a higher value was found (1.34 ±0.11 μg.mL^{-1} fresh matter). Also, the Zeaxanthin content found in different fruits was variable: in apples was 0.01 μg.g^{-1} dry matter **(Delgado-Pelayo et al., 2014)**, in canned apricots 2.7 μg.g^{-1} and in canned peaches 1.1 μg.g^{-1} fresh matter **(Campbell and Padilla-Zakour, 2013)**.

The content of lutein was higher in TPSJ than HPSJ (8.84 ±0.57 μg.mL^{-1} fresh matter and 8.17 ±0.13 μg.mL^{-1} fresh matter, respectively). The lutein content found in carrots by **Maurer et al., (2014)** was 1.5 μg.g^{-1} fresh matter, whereas **Stinco et al., (2014)** reported values of 44.11 ±6.11 μg.g^{-1} dry matter. **Delgado-Pelayo et al., (2014)** reported that red apples contain 0.06 μg.g^{-1} dry matter of lutein and canned apricots 0.09 μg.g^{-1} fresh matter of lutein **(Campbell and Padilla-Zakour, 2013)**.

Other studies evaluated the carotenoid content in a variety of fruits and juices. According to **Aschoff et al., (2015)**, the concentration of β-carotene in orange juice was 11.1 ±0.9 μg.100 g^{-1}, lutein 58.9±0.8 μg.100 g^{-1}, and zeaxanthin 47.0 ±1.4 μg.100 g^{-1}, whereas the fresh oranges that were used for the preparation of the juice had higher values (β-carotene 21.1 ±1.6 μg.100 g^{-1}, lutein 61.2 ±1.6 μg.100 g^{-1} and zeaxanthin 49.4 ±3.6 μg.100 g^{-1}). The same tendency was observed in the content of β-carotene and zeaxanthin of peach juice (1.61 ±0.82 μg.g^{-1} and 0.19 ±0.02 μg.g^{-1}) when compared to the fresh peaches (1.98 ±0.62 μg.g^{-1} and 0.27 ±0.12 μg.g^{-1}) **(Giuffrida, 2013)**. **Esteve et al., (2009)** reported that high pressure technology supports a higher extraction of biologically valuable substances of fruits and vegetables because they found higher levels of total carotenoids in orange juice treated by high pressure method (1309.2 ±46.7 μg.100 mL^{-1}) in comparison with the thermally pasteurized juice (1195.4±31.6 μg.100 mL^{-1}).

CONCLUSION

Based on the assessment of the qualitative-quantitative parameters, we found that there were statistically significant differences in the antioxidant activity, polyphenols and zeaxanthin content between HPSJ and TPSJ, where the former presented a clear advanteage. On the contrary, we found that TPSJ was able to retain higher values of β-carotene than HPSJ. Statistically, no significant differences (p >0.05) were detected in the content of lutein in both juices.

REFERENCES

Aschoff, J. K., Kaufmann, S., Kalkan, O., Neidhart, S., Carle, R., Schweiggert, R. M. 2015. In vitro bioaccessibility of carotenoids, flavonoids, and vitamin C from differently processed oranges and orange juices [*Citrus sinensis* (L.) Osbeck]. *Journal of Agricurtural and Food Chemistry*, vol. 63, no. 2, p. 578-587. http://dx.doi.org/10.1021/jf505297t PMid:25539394

Ashokkumar, K., Diapari, M., Jha, A. B., Tar´an, B., Arganosa, G., Warkentin, T. D. 2015. Genetic diversity of nutritionally important carotenoids on 94 pea and 121 chickpea accesions. *Journal of Food Composition and Analysis*, vol. 43, p. 49-60. http://dx.doi.org/10.1016/j.jfca.2015.04.014

Bansilala, S., Castellanoa, J. M., Fustera, V. 2015. Global burden of CVD: focus on secondary prevention of cardiovascular disease. *International Journal of Cardiology*, vol. 201, suppl. 1, p. S1-S7. http://dx.doi.org/10.1016/S0167-5273(15)31026-3

Barbosa-Cánovas, G. V., Tapia, M. S., Cano, M. P. 2005. *Novel Food Processing Technologies*. New York: CRP Press, 720 ps. SBN 9780824753337.

Basu, A., Nguyen, A., Betts, N. M., Lyons, T. J. 2014. Strawberry as a functional food: an evidence- based review. *Critical Reviews in Food Science and Nutrition*, vol. 54, no. 6, p. 790-806. http://dx.doi.org/10.1080/10408398.2011.608174 PMid:24345049

Bosetti, C., Spertini, L., Parpinel, M., Gnagnarella, P., Lagiou, P., Negri, E., Franceschi, S., Montella, M., Peterson, J., Dwyer, J., Giacosa, A., LaVecchia, C. 2005. Flavonoids and breast cancer risk in Italy. Cancer Epidemioligy Biomarkers and Prevention, vol. 14, no. 4, p. 805-808. http://dx.doi.org/10.1158/1055-9965.EPI-04-0838 PMid:15824147

Campbell, O. E., Padilla-Zakour, O. I. 2013. Phenolic and carotenoid composition of canned peaches (*Prunus persica*) and apricots (*Prunus armeniaca*) as affected by variety and peeling. *Food Research International*, vol. 54, no. 1, p. 448-455. http://dx.doi.org/10.1016/j.foodres.2013.07.016

Carter, P., Gray, L. J., Troughton, J., Khunti, K., Davies, M. J. 2010. Fruit and vegetable intake and incidence of type 2 diabetes mellitus: systematic review and meta-analysis. *BMJ.*, vol. 341, p. 1-8. http://dx.doi.org/10.1136/bmj.c4229 PMid:20724400

Chong, M. F. F., Macdonald, R., Lovegrove, J. A. 2010. Fruit polyphenols and CVD risk: a review of human intervention studies. *British Journal of Nutrition*, vol. 104, suppl. 3, p. S28-S39. http://dx.doi.org/10.1017/S0007114510003922 PMid:20955648

Cook, N. C., SAMMAN, S. 1996. Flavonoids—Chemistry, metabolism, cardioprotective effects, and dietary sources. *The Journal of Nutritional Biochemistry*, vol. 7, no. 2, p. 66-76. http://dx.doi.org/10.1016/0955-2863(95)00168-9

Da Silva, F. L., Escribano-Bailon, M. T., Perez-Alonso, J. J., Rivas-Gonzalo, J., Santos-Buelga, C. 2007. Anthocyanin pigments in strawberry. *LWT Food Science and Technology*, vol. 40, no. 2, p. 374-382. http://dx.doi.org/10.1016/j.lwt.2005.09.018

Delgado-Pelayo, R., Gallardo-Guerrero, L., Hornero-Méndez, D. 2014. Chlorophyll and carotenoid pigments in the peel and flesh of commercial apple fruit varieties. *Food Research International*, vol. 65, part B, p. 272-281.

Dragsted, L. O., Krath, B., Ravn-Haren, G., Vogel, U. B., Vinggaard, A. M., Bo Jensen, P., Loft, S., Rasmussen, S. E., Sandstrom, T. B., Pedersen, A. 2006. Biological effects of fruit and vegetables. *The Proceedings of the Nutrition Society*, vol. 65, no. 1, p. 61-67. http://dx.doi.org/10.1079/PNS2005480 PMid:16441945

Esmaillzadeh, A., Kimiagar, M., Mehrabi, Y., Azadbakht, L., Hu, F. B., Willett, W.C. 2006. Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome. *American Journal of Clinical Nutrition*, vol. 84, no. 6, p. 1489-1497. PMid:17158434

Esteve, M. J., Barba, F. J., Palop, S., Frígola, A. 2009. The Effects of Non thermal Processing on Carotenoids in Orange Juice. *Czech J. Food Sci*., vol. 27, special no. p. S304-S306.

Gardner, P. T., White, T. A. C., McPhail, D. B., Duthie, G. G. 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry*, vol. 68, no. 4, p. 471-474. http://dx.doi.org/10.1016/S0308-8146(99)00225-3

Giampieri, F., Tulipani, S., Alvaarez-Suarez, J. M., Quiles, J. L., Mezzetti, B., Battino, M. 2012. The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition*, vol. 28, no. 1, p. 9-19. http://dx.doi.org/10.1016/j.nut.2011.08.009 PMid:22153122

Giuffrida, D., Torre, G., Dugo, P. 2013. Determination of the carotenoid profile in peach fruits, juice and jam. Fruits, vol. 68, p. 39-44. http://dx.doi.org/10.1051/fruits/2012049

Gropper, S. S., Smith, J. L., Groff, J. L. 2005. *Advanced nutrition and human metabolism* (4th ed.). Belmont: Thomson Wadsworth, 608 p.

Halliwell, B. 2008. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and *in vivo* studies? *Archives of Bopchemistry and Biophysics*, vol. 476, no. 2, p. 107-112. http://dx.doi.org/10.1016/j.abb.2008.01.028 PMid:18284912

Häkkinen, S. H., Kärenlampi, S. O., Mykkänen, H. M., Heinonen, I. M., Törrönen, A. R. 2000. Ellagic acids content in berries: influence of domestic processing and storage. *European Food Research and Technology*, vol. 212, no. 1, p. 75-80. http://dx.doi.org/10.1007/s002170000184

Hossain, A., Begum, P., Salma Zannat, M., Hafizur Rahman, Md., Ahsan, M., Nazrul Islam, S. 2016. Nutrient composition of strawberry genotypes cultivated in a horticulture farm. *Food Chemistry*, vol. 199, p. 648-652. http://dx.doi.org/10.1016/j.foodchem.2015.12.056 PMid:26776020

Jakobek, J., Šegura, J., Medvodovič-Kosanovič, M., Novak, I. 2007. Anthocyanin content and antioxidant activity of various red fruit juices. *Deutsche Lebensmittel-Rundschau: Zeitschrift für Lebensmittelkunde und Lebensmittelrecht*, vol. 103, no. 2, p. 58-64.

Joo, H., Kim, C. T., Kim, L. H., Kim, Y. 2013. Anti-obesity effect of hot water extract and high hidrostatic pressure extract of garlic in rats fed a high-fat diet. *Food and Chemical Toxicology*, vol. 55, p. 100-105. http://dx.doi.org/10.1016/j.fct.2012.12.044 PMid:23306787

Joshipura, K. J., Ascherio, A., Manson, J. E., Stampfer, M. J, Rimm, E. B., Speizer, F. E., Hennekens, C. H., Spiegelman, D., Willett, W. C. 1999. Fruit and vegetable intake in relation to risk of ischemic stroke. *Journal of the American Medical Association*, vol. 282, no. 13, p. 1233-1239. http://dx.doi.org/10.1001/jama.282.13.1233 PMid:10517425

Kalt, W., Hanneken, A., Milbury, P., Trembla, F. 2010. Recent Research on Polyphenolics in Vision and Eye Health. *Journal of Agriculture and Food Chemistry*, vol. 58, no. 7, p. 4001-4007. http://dx.doi.org/10.1021/jf903038r PMid:20102149

Kelebek, H., Selli, S. 2011. Characterization of phenolic compounds in strawberry fruits by RP-HPLC-DAD and investigation of their antioxidant capacity. *Journal of Liquid Chromatography & Related Technologies*, vol. 34, no. 20, p. 2495-2504. http://dx.doi.org/10.1080/10826076.2011.591029

Keresteš, J. et al. 2016. *Mlieko vo výžive ľudí*. (In Slovak) *Milk in the human nutrition*. SPU Nitra. Published in: Bratislava : CAD PRESS, 2016, 649 p. ISBN 978-80-88969-72-3.

Konczak, I., Zhang, W. 2004. Anthocyanins-more than nature' s colours 2004. *Journal of Biomedicine and Biotechnology*, vol. 2004, no. 5, p. 239-240.

Lachman, J., Hejtmánková, K., Kotíková, Z. 2013. Tocols and carotenoids of enkorn, emmer and spring wheat varietes: Selection for breading and production. *Journal of Cereal Science*, vol. 57, no. 2, p. 2007-2014. http://dx.doi.org/10.1016/j.jcs.2012.05.011

Larson, R. A. 1998. The antioxidants of higher plants. *Phytochemistry*, vol. 27, no. 4, p. 969-972. http://dx.doi.org/10.1016/0031-9422(88)80254-1

Larrosa, M., González-Sarrías, A., Yáñez-Gascón, M. J., Selma, M. V., Azorín-Ortuño, M., Toti, S., Tomás-Barberán, F., Dolara, P., Espín, J. C. 2010. Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. *The Journal of Nutritional Biochemistry*. vol. 21, no. 8, p. 717-725. http://dx.doi.org/10.1016/j.jnutbio.2009.04.012 PMid:19616930

Maurer, M. M., Mein, J. R., Chaudhuri, S. K., Constant, H. L. 2014. An improved UHPLC-UV method for separation and quantification of carotenoids in vegetable crops. *Food Chemistry*, vol. 165, p. 475-482. http://dx.doi.org/10.1016/j.foodchem.2014.05.038 PMid:25038701

Németh-Balogh, M., Paulovicsová, B., Turianica, I., Pokorná-Juríková, T. 2009. Úloha potravín s vysokým obsahom antioxidantov v prevencii kardiovaskulárnych ochorení. (In Slovak) (The role of food with high antioxidant content in the prevention of cardiovascular diseases) *Slovenský veterinárny časopis*, vol. 34, no. 3, p. 183-185.

Mendelová, A., Mendel, Ľ., Czako, P., Mareček, J. 2016. Evaluation of carotenoids, polyphenols content and antioxidant activity in the sea buckthorn fruit juice. *Potravinarstvo*, vol. 10, no. 1, p. 59-64. http://dx.doi.org/10.5219/551

Mota, M. J., Lopes, R. T, Delgadillo, I., Saraiva, J. A. 2013. Microorganisms under high pressure – Adaptation, growth and biotechnological potential. *Biotechnology Advances*, vol. 31, no. 8, p. 1426-1434. http://dx.doi.org/10.1016/j.biotechadv.2013.06.007 PMid:23831003

Patras, A., Brunton, N. P., O'dnnell, C., Tiwari, B. K. 2010. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science and Technology*, vol. 21, no. 1, p. 3-11. http://dx.doi.org/10.1016/j.tifs.2009.07.004

Perk, J., De Backer, G., Gohlke, H., Graham, I., Reiner, Ž., Verschuren, M., Deaton, C. 2012. European Guidelines on cardiovascular disease prevention in clinical practice. *European Heart Journal*, vol. 33, no. 13, p. 1635-1701. PMid:22555213

Peterson, J., Lagiou, P., Samoli, E., Lagiou, A., Katsouyanni, K., LaVecchia, C., Dwyer, J., Trichopoulos, D. 2003. Flavonoid intake and breast cancer risk: a case-control study in Greece. *British Journal of Cancer*, vol. 89, no. 7, p. 1255-1259. http://dx.doi.org/10.1038/sj.bjc.6601271 PMid:14520456

Protulipac, J. M., Sonicki, Z., Reiner, Z. 2015. Cardiovascular disease (CVD) risk factors in older adults – Perception and reality. *Archives of Gerontology and Geriatrics*, vol. 61, no. 1, p. 88-92. http://dx.doi.org/10.1016/j.archger.2015.04.001 PMid:25944060

Reis, R., Johnston, J., Tucker, K., DeSesso, J. M., Keen, C. L. 2012. Estimation of cancer risks and benefits associated with a potential increased consumption of fruits and vegetables. *Food and Chemical Toxicology*, vol. 50, no. 12, p. 4421-4427. http://dx.doi.org/10.1016/j.fct.2012.08.055 PMid:22981907

Rekha, C., Poornima, G., Manasa, M., Abhipsa, V., Pavithra Devi, J., Vijay Kumar, H. T., Prashith Kekuda, T. R. 2012. Ascorbic Acid, Total Phenols Content and Antioxidant Activity of Fresh Juices of Four Ripe and Unripe Citrus Fruits. *Chemical Science Transaction*, vol. 1, no. 2, p. 303-310. http://dx.doi.org/10.7598/cst2012.182

Riboli, E., Norat, T. 2003. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *American Journal of Clinical Nutrition*, vol. 78, no. 3, p. 559S-569S. PMid:12936950

Rolle, A. C., Braesco, V., Chupin, J., Bouillot, L. 2016. Nutritional Composition of Orange Juice: A Comparative

Study between French Commercial and Home-Made Juices. *Food and Nutrition Sciences*, vol. 7, p. 252-261. http://dx.doi.org/10.4236/fns.2016.74027

Szajdek, A., Borowska, E. J. 2008. Bioactive compounds and health promoting properties of berry fruits: a review. *Plant Foods Human Nutrition*, vol. 63, no. 4, p. 147-156. http://dx.doi.org/10.1007/s11130-008-0097-5 PMid:18931913

Sánchez-Moreno, C., Plaza, L., de Ancos, B., Cano, M. P. 2003. Effect of high-pressure processing on health-promoting attributes of freshly squeezed orange juice (*Citrus sinensis* L.) during chilled storage. *European Food Research and Technology*, vol. 216, no. 1, p. 18-22.

Stinco, C. M., Benítez-González, A. M., Hernanz, D., Vicario, I. M., Meléndez-Martínez, A. J. 2014. Development and validation of a rapid resolution liquid chromatography method for the screening of dietary plant isoprenoids: Carotenoids, tocopherols and chlorophylls. *Journal of Chromatography A*, vol. 1370, p. 162-170. http://dx.doi.org/10.1016/j.chroma.2014.10.044 PMid:25454141

Takachi, R., Inoue, M., Sawada, N. Iwasaki, M., Sasazuki, S., Ishihara, J., Tsubono, Y., Tsugane, S. 2010. Fruits and vegetables in relation to prostate cancer in Japanese men: the Japan Public Health Center-Based Prospective Study. *Nutrition and Cancer*, vol. 62, no.1, p. 30-39. http://dx.doi.org/10.1080/01635580903191502 PMid:20043257

Tarozzi, A., Morroni, M., Merlicco, A., Bolondi, C., Teti, G., Falconi, M., Cantelli-Forti, G., Hrelia, P. 2010. Neuroprotective effects of cyanidin 3-O-glucopyranoside on amyloid beta (25 – 35) oligomer-induced toxicity. *Neuroscience Letters*, vol. 473, no. 2, p. 72-76. http://dx.doi.org/10.1016/j.neulet.2010.02.006 PMid:20152881

Tokuşoğlu, Ö., Swanson, B. G. 2015. *Improving food Quality with Novel Food Processing Technologies*. CRP Press, 3-8 p. eBook ISBN 978-1-4665-0725-8. http://dx.doi.org/10.1201/b17780-3

Wang, S. Y., Lin, H. S. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry*, vol. 48, no. 2, p. 140-146. http://dx.doi.org/10.1021/jf9908345 PMid:10691606

Wilson, P. W. F., D'Agostino, R. B., Levy, D., Belanger, A. M., Silbershatz, H., Kannel, W. B. 1998. Prediction of Coronary Heart Disease Using Risk Factor Categories. *Circulation*, vol. 97, no. 18, p. 1837-1847. http://dx.doi.org/10.1161/01.CIR.97.18.1837 PMid:9603539

Xi, J. 2006. Effect of high pressure processing on the extraction of lycopene in tomato paste waste. *Chemical Engineering & Technology*, vol. 29, no. 6, p. 736-739. http://dx.doi.org/10.1002/ceat.200600024

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EFFECT OF WHEAT AND CORN GERMS ADDITION ON THE PHYSICAL PROPERTIES AND SENSORY QUALITYOF CRACKERS

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ABSTRACT

Crackers are a potential material for the addition of cereal germs as a functional ingredient because they are a popular bakery item. The suitability of cereal germs for crackers production was investigated in this study. The effect of cereal germs incorporation to wheat dough (at level 5, 10 and 15%) on the physical properties (specific volume, volume index, width, thickness and spread ratio) and sensory parameters (appearance, firmness, taste, odor and overall acceptability) of cracker were evaluated. It was shown that wheat and corn germ addition to crackers resulted in decreased specific volume from 1.65 $cm^3.g^{-1}$ (control sample) to 1.52 $cm^3.g^{-1}$ (10% corn germs addition) and volume index 3.20 cm (control sample) to 2.57 cm (15% wheat germ), whereas spread ratio increased from 4.71 (fine wheat flour) to 5.06 (15% corn germ). No significant differences were found between the values obtained for width and thickness for crackers supplemented with 5% wheat germ to control sample. Addition of corn germ and wheat germ at level 15% caused decrease volume index of crackers about 13 and 20%. On the other hand enriched crackers of wheat germ and corn germ at level 15% was increment spread ratio by 5 and 7%. Regarding to sensory properties the overall appearance was affected significantly by addition of wheat and corn germ. Higher addition of wheat and corn germ in the crackers adversely affected firmness, taste and odor of final products. In generally, sensory properties of crackers were markedly affected with addition of cereal germs. The most significant differences were observed in appearance of crackers, when the 15% of wheat or corn germs were added (15 and 30% decreasing of this attribute in compare to control sample, respectively). The results of sensory analysis also showed that the crackers incorporated with wheat germs up to 10% level resulted in products with good acceptability.

Keywords: wheat germ; corn germ; cracker; physicochemical properties; sensory quality

INTRODUCTION

Baking industry is one of the largest organized food industries **(Ganorkar and Jain, 2014)**. Bakery products are daily consumed in large volumes and they provide an intake of dietary fiberand other healthy compounds to consumers **(Ktenioudaki and Gallagher, 2012)**. Demand for health oriented products such as sugar-free, low calorie and high fiber products are increasing. Traditionally, fiber supplementation has focused on the use of milling by-products of cereal grains. All of the milling by-products of wheat, corn, sorghum and other grains, as well as the by-products from the wet milling of corn and wheat, have been investigated as possible fiber supplements **(Sudha et al., 2007; McKee and Lantner, 2000)**. Whole grain foods are rich source of fiber, antioxidants and other nutrients, which have positive influence on human health. Dietary cerealfiberisused more often than fiber from fruits **(Karovičová et al., 2015)** Incorporation of whole grains in foods can reduce their sensory quality and cause less consumer acceptance. As a result, there are challenges in producing whole grain products to maintain their functionality and quality that are equivalent to the traditional products without whole grain incorporation **(Wang et al., 2016)**.

In baked products, the main effects include a decrease in loaf volume or height, textural modifications (increased crumb hardness, loss of crispiness), changes in appearance (colour, surface properties, density) and taste **(Rosa et al., 2015)**.

The milling process of wheat produces large amount of wheat bran and germ as a by-product. During milling, the endosperm is grinded into smaller particles (white flour) while bran and germ are removed. The wheat germ represents about 2.5 – 3.8% of total seed weight **(Bansal and Sudha, 2011)**. Wheat germ, being a by-product of the flour milling industry, is considered a natural source of nutrients due to their low cost **(Tsadik and Emire, 2015)**. Wheat germ is the most vitamin and mineral rich part of the wheat kernel. In the fact, the germ is actually the embryo of the wheat plant **(Tsadik and Emire, 2015)**.
The germ contains about 10 – 15% lipids, 26 – 35% proteins, 17% sugars, 1.5 – 4.5%fiberand 4% minerals, as well as significant quantities of bioactive compounds such as tocopherol, phytosterols, policosanols, carotenoids, thiamin and riboflavin **(Brandolini and Hidalgo, 2012)**. The wheat germ is therefore a unique source of concentrated nutrients, highly valued as food supplement and offers an appropriate medium to convey these benefits to the human diet **(Brandolini and Hidalgo, 2012; Ma et al., 2014)**. The wheat germ is also characterized by a palatable taste due to its high oil and sugar contents and defatted wheat germ is the ideal ingredient for grain based products. Wheat germ usage in bakery products provides some advantages such as crumb softness and air incorporation. The functional qualities of wheat germ include improving the stability texture, nutritional value and flavour ofcereal products **(Hayam et al., 2015; Levent and Bilgiçli 2013)**.

Majzoobi et al., (2012) found that addition of wheat germ above the 15% levels in cake formulation is not suitable for sensory quality. Results showed that all addition levels (10, 20, and 30%) of wheat germ were not negatively affected the taste and odor of cakes.

In recent years, more cereal-based foods have been enriched with wheat germ or its derivates. Many studies are focused to investigation the effects of raw wheat germ, defatted wheat germ or toasted wheat germ on bread **(Rizzello et al., 2011; Sidhu et al., 1999)**, cookies **(Bajaj et al., 1991; Arshad, et al., 2007)**, cakes **(Majzoobi et al. 2012)**, macaroni, noodle **(Ge et al. 2001; Pınarlı et al. 2004)**, biscuits **(Bansal and Sudha, 2011)** and tarhana **(Bilgiçli and Ibanoğlu 2007)**.

Corn or maize (*Zea mays L. ssp. mays*) is a cereal grain that is widely cultivated on the world. A by-product in dry milling process, not applied in food production until recently, is corn mix consisting of corn grain germ and slight amounts of endosperm and seedcoat. Dry milled corn germ is characterized by high nutritional value **(Peksa et al. 2010)**. The corn has been applied as feed for livestock, forage, silage and grain, and it is also used in industry including transformation into plastics, syrups and alcohol for biofuels. In addition, corn is also widely dispersed in human nutrition **(Shi et al., 2016)**.

Typical corn germ contains 48 – 30.7% of oil, 20.5 – 13% of protein, 9.0 – 12% of starch, 2 – 9.7% of ash, 9.2 – 10.5 % of sugar and 3 – 11.2 % of moisture **(Ramirez et al., 2008; Kulakova et al., 1982)**. Corn germs proteins contained a considerable amount of such essential amino acids as lysine, methionine, tryptophane **(Kulakova et al., 1982).**

The corn germ comprises 5 – 14% of the weight of a corn kernel, depending on variety and grain size, is high in protein content, dietary fibre and minerals. The proteins in defatted corn germ flour mostly consist of albumin and globulin, and they are balanced in most of the essential amino acids; lysine, a major limiting amino acid in wheat, accounts for 5 – 6% of the total proteins in defatted corn germ, which is more than twice than that in wheat flour **(Siddiq et al., 2009)**.

Defatted corn germ flour being a low source of nutrients and decreased the price of fortified flour blends or finished product besides improving the nutritional profile.

The defatted corn germ flour offers a good potential for its use as an ingredient in a variety of foods, such as bread, cookies, muffins, cakes **(Arora and Saini, 2016; Han et al., 2010)**. In most countries all over the world continuously growing consumption of snack products has made them a considerable component of a human diet. Therefore, snacks nutritive and energetic value should meet strictly determined requirements. These products are often fortified with wholesome or functional components **(Peksa et al. 2010)**. Crackers are becoming a versatile food, and manufacturers are trying to satisfy the demands of consumer by providing the various products **(Han et al., 2010).** In the present, crackers are enhanced by various healthy ingredients including buckwheat flour **(Sedej et al., 2011)**, rice bran **(Yilmaz et al., 2014)**, pulse flour **(Kohajdová et al., 2011; Han et al., 2010))**, amaranth **(Hozová et al., 1997)**, triticale **(Peréz et al., 2003)**, casava starch **(Saeleaw and Schleining, 2010)**, eggplant flour **(Peréz and Germani, 2007; Hempel et al., 2007)**.

The main objectives of this study were to assess the effect of addition of wheat germ (WG) and corn germ (CG) on the physical properties of crackers (specific volume, volume index, width, thickness and spread ratio) and sensory properties (appearance, firmness, taste, odor and overall acceptability) of crackers.

MATERIAL AND METHODOLOGY

Wheat flour and commercial wheat and corn germs were purchased from local market and health food shop (Slovak Republic).

Cracker preparation: The soda crackers were prepared according to **Han et al., (2010)**. This procedure involves separate mixing of dry ingredients and separate mixing of liquid ingredients (water, oil) including sugar. The dry ingredients are gradually added to mixed liquid ingredients and after 3 – 4 min, the dough begins to form. The mixed dough was resting for 10 min at room temperature and subsequently the dough was laminated and cut to the circular shape. After that the crackers were baked in electric oven (Model 524, Mora, Czech Republic) at 175 °C for 4 min. Before further experiments, the crackers were cooled for 30 min, packed in sealed polypropylene bags and stored at room temperature. Wheat flour was replaced by 5, 10 and 15% addition of wheat germ and corn germin the recipe for cracker. Control sample was crackers which were prepared without the addition of wheat and corn germs.

Determination of physical parameters of crackers: Crackers were evaluated for their physical properties (volume index, specific volume, diameter (width), thickness, spread ratio) parameters were measured. Determination of thickness, width and spread ratio was performed according to the **AACC (1995)** method. Diameter (D): To determine the diameter, six crackers were placed edge to edge. The total diameters of the six crackers were measured in cm by using a ruler. The crackers were rotated at an angle of 90 for duplicate reading. This was repeated once more and average diameter was taken in centimetre. Thickness (T): To determine the thickness, seven crackers were placed on top of one another. The total thickness was measured in centimetres with the help of ruler. This process was repeated twice to get an average value and results were taken in cm. The spread ratio was determined by the formula W/T, where W is the average diameter and T is the average thickness **(Kohajdová et al., 2011)**. Specific volume of crackers was determined using a method described by **Bouaziz et al., (2010)**.

Volume index of sampleswas determinedaccording to **Turabi et al., (2008)**. In this method, cake sample is cut vertically through the centre and the heights of the sample are set at three various points (B, C, D) along the cross-sectioned cakes using the template. Subsequently the index volume was determined in accordance with following formula:

$$\text{Volume index} = B + C + D \quad (1),$$

Where: C represents the height of the cake at the centre point, B and D represent the heights of the cake at the points 2.5 cm away from the centre to the left and also from the centre to the right side of the cake.

Sensory evaluation of crackers: Sensory evaluation was performed by using 9 trained panelists. The panelists were

Table 1 The physical characteristics of crackers incorporated wheat and corn germ.

	Specific volume ($cm^3.g^{-1}$)	Volume index (cm)	Width (T /cm)	Thickness (W /cm)	Spread ratio (W/ T)
Control	1.65 ±0.02	3.20 ±0.03	5.00 ±0.01	1.06 ±0.03	4.71 ±0.03
WG 5%	1.58 ±0.03*	2.74 ±0.01*	5.00 ±0.01	1.06 ±0.01	4.71 ±0.01
WG 10%	1.41 ±0.01*	2.68 ±0.01*	4.96 ±0.01*	1.05 ±0.01*	4.72± 0.01
WG 15%	1.32 ±0.01*	2.57 ±0.08*	4.91 ±0.03*	1.00 ±0.02*	4.94 ±0.02*
CG 5%	1.53 ±0.02*	3.17 ±0.03	5.01 ±0.01	1.08 ±0.01	4.64 ±0.01*
CG 10%	1.52 ±0.01*	2.99 ±0.02*	4.98 ±0.01*	1.04 ±0.01*	4.81 ±0.01*
CG 15%	1.34 ±0.01*	2.80 ±0.06*	4.96 ±0.01*	0.98 ±0.01*	5.06± 0.01*

Note: * indicates a statistically significant differences (p = 0.05), WG - wheat germ, CG – corn germ.

students of Faculty of Chemical and Food Technology, (Slovak University of Technology, Slovak Republic). Panelists evaluated the sensory parameters as the odor, firmness, taste and appearance of the crackers using the 9 point hedonic scale with 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely.

Samples were presented simultaneously **(Kohajdová et al., 2014)**. Overall acceptability of crackers was assessed using 100 mm graphic non-structured line segment with the description of extremes (minimal or maximal intensity, from 0 to 100%) **(Kohajdová, et al., 2011**).

Statistical analysis

Statistical analysis: All analyses were carried out in triplicate and average values were calculated. The results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) and Fisher´s least – significant difference (LSD) multiple range test was applied to data establish the significance of differences at the level of p = 0.05. Statgraphic Plus, Version 3.1 (Statistical Graphic Corporation, Princeton, USA), was used as the statistical software.

RESULTS AND DISCUSSION

The physical characteristics of crackers are presented in Table 1. The specific volume has a great importance in determining the quality because it is generally influenced by the quality of the ingredients used in the formulation of crackers **(Perez and Germani, 2003)**. Incorporation of WG and CG to crackers significantly reduced specific volume and volume index.

Levent and Bilgiçli (2013) found that increasing the amount of differently addition levels (10, 20, and 30%) of WG in cake formulation decreased the volume and the volume index of cake samples. The highest decreasing of volume index was at 15% addition in crackers with CG (2.80 cm). This might be due to the different flour quality of the flours with fiber and in general, addition of fiber into wheat flour has a negative effect on the formation of gluten network due to dilution of gluten protein and fiber – gluten interaction **(Arshad et al., 2007; Wang et al., 2016)**. It was also found that higher addition (10 and 15%) of WG and CG markedly decreased thickness of crackers. Same trends in thickness were also described in study of **Arshad et al., (2007)** for cookies incorporated with various levels of defatted wheat germ flour. No significant differences were found between the values obtained for width of crackers supplemented with 5% WG and CG and the control sample. The results described that addition of WG and CG at a level of 15% significantly increased spread ratio.

Similar increasing in spread ratio was also reported in study **Youssef (2015)** for biscuits fortified of 15% and 20% of WG. The differences in spread ratio between the control sample crackers and the samples with 5%, 10% addition of WG was not significantly. Our results are comparable with finding of **Arshad et al. (2007).** It has been suggested that the spread ratio is affected by available water which can be absorbed either by flour or any other ingredient which absorbs water during the dough mixing. Subsequently, the spread ratio is decreased when other ingredients absorbed the water **(Arshad et al., 2007)**. Crackers with 15% addition of CG had higher spread ratio (5.06) in compare to control sample (4.71). **Sharma Savita et al., (2012)** reported that using corn gluten in cookies concluded in increased the spread ratio. As light increase in spread ratio of crackers was observed at 5 and 10% addition of WG. Another result was observed by **Chen et al., (1988)**. They did not find a significant difference in cookie spread ratio between the control and cookies containing oat and wheat bran. Earlier studies also reported reduction of spread ratio when cereal bran (wheat, rice, and oat) and defatted wheat germ were added (**Sudha et al., 2007; Arshad et al., 2007)**. Significant differences were observed between WG and CG enriched crackers in width, spread ratio and specific volume.

Table 2 Sensory properties of crackers incorporated with corn and wheat germ with cereal germs.

Addition levels / %		Sensory parameter			
		appearance	**taste**	**odor**	**firmness**
Control	0	9.00 ±0.13	8.92 ±0.05	8.76 ±0.13	8.84 ±0.06
WG	5	8.27 ±0.06*	8.15 ±0.10*	8.46 ±0.08*	8.39 ±0.05*
WG	10	8.61 ±0.03*	8.66±0.11*	8.82±0.09	8.78 ±0.21
WG	15	7.71 ±0.07*	7.93 ±0.05*	8.27 ±0.07*	8.08 ±0.07*
CG	5	7.65 ±0.11*	7.36 ±0.08*	7.15 ±0.09*	8.16 ±0.08*
CG	10	6.41 ±0.11*	6.33 ±0.13*	6.71 ±0.04*	7.86 ±0.08*
CG	15	6.01 ±0.17*	5.84 ±0.05*	6.21 ±0.09*	7.37 ±0.17*

Note: * indicates a statistically significant differences (p = 0.05), WG – wheatgerm, CG – corngerm.

Results of sensory evaluation such as appearance, taste, firmness, odor and overall acceptability are presented in Table 2. The overall sensory quality of crackers decreased with increased cereals germ substitution. Taste is an important sensory analysis attribute of any food because has influence on overall acceptability. The differences in taste of WG and CG crackers were statistically significant. With the increasing addition of CG (range from 5% to 15%), the taste of crackers (range from 7.36 to 5.84) was decreased. The taste of cracker, with higher levels of CG, was characterized with higher intensity of bitter taste (results are not shown). **Arshad et al., (2007)** described, that the cookies containing more than 15% defatted wheat germ flour as having an after taste and bean flavour. Also it was found, that the crackers with WG and CG addition had significantly reduced firmness than control sample. The overall acceptability is influenced by all the dominant sensory properties **(Ganorkar and Jain, 2014)**. Cereal germs have beneficial effects on the human health such as diabetes (type2), cardiovascular diseases and some types of cancer as well as improving physical endurance and retarding aging **(Hayam et al., 2015; Brandolini and Hidalgo 2012)**.

Therefore is appropriate to use the cereal germs in the bakery products. On the other hand, the cereal germs incorporated in bakery products represent a problem, because they are sensitive for oxidation and it is reflected in bitter taste of products

The cause oxidation of germs oils is because containing highly unsatured oxidation fatty acids, which started reacting with the oxygen **(Grosh and Laskawy, 1984)**.

In the future, before using corn germs in the product, it would be appropriate to defat them, to avoid the reduction of sensory parameters which would result in the bitter taste

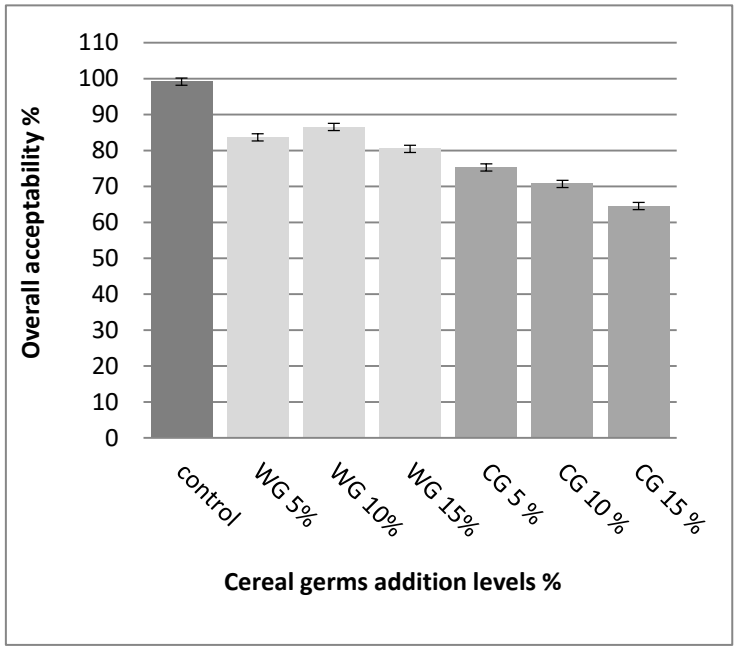

Figure 1 Overall acceptability of crackers incorporated.

of the product.

The overall acceptability of crackers with addition of WG and CG is shown in Figure 1. From the results concluded that, this parameter was the highest at 10% addition of WG to crackers. Moreover it was found, that significant difference were recorded in the overall acceptability between the cereal germs and control sample. Crackers with 15% addition of CG had the lowest overall acceptability (64.56%). It could be caused by unpleasant odour and the bitter taste of the crackers.

CONCLUSION

This study presented effects of addition of different cereals germs (WG and CG) on the physical properties and sensory parameters of crackers. It has been observed that WG and CG addition at higher level (10 – 15%) significantly affected volume index and specific volume of final products. On the other hand width, thickness and spread ratio were remarkable affected only at 15% substitution level WG and CG.

Moreover, it was resulted that incorporation of WG and CG reduced firmness of crackers. Also, it was observed that overall acceptability of crackers showed significant differences between control sample (fine wheat flour based crackers) and crackers, in which 10 and 15% of fine wheat flour were replaced by wheat and corn germ. The results of sensory analysis showed that the crackers incorporated with WG up to 10% level resulted in products with good acceptability. Adding a CG to bakery products is limiting. Results showed that addition higher levels (10, and 15%) of CG markedly negatively affected the taste and odor of crackers.

REFERENCES

AACC (1995) Approved methods of the American Association of Cereal Chemists. Micro Sugar-Snap Cookie Method (10-52), USA

Arora, A., Saini, C. S. 2016. Development of bun from wheat flour fortified with de-oiled maize germ. *Cogent Food Agriculture*, vol. 2, no. 1, p. 1-9. http://dx.doi.org/10.1080/23311932.2016.1183252

Arshad, M. U., Anjum, F. M., Zahoor, T. 2007. Nutritional assessment of cookies supplemented with defatted wheat germ. *Food Chemistry*, vol. 102, no. 1, p. 123-128. http://dx.doi.org/10.1016/j.foodchem.2006.04.040

Bajaj, M., Kaur, A., Sidhu, J. S. 1991. Studies on the development of nutritious cookies utilizing sunflower kernels and wheat germ. *Plant Foods for Human Nutrition*, vol. 4, no. 4, p. 381-387. http://dx.doi.org/10.1007/BF02310631

Bansal, S., Sudha, M. L. 2011. Nutritional, microstructural, rheological and quality characteristics of biscuits using processed wheat germ. *International Journal of Food Sciences and Nutrition*, vol. 62, no. 5, p. 474-479. http://dx.doi.org/10.3109/09637486.2010.549116

Bouaziz, M. A., Amara, W. B., Attia, H., Blecker, C., Besbes, S. 2010. Effect of the addition of defatted date seeds on wheat dough performance and bread quality. *Journal of texture studies*, vol. 41, no. 4, p. 511-531. http://dx.doi.org/10.1111/j.1745-4603.2010.00239.x

Brandolini, A., Hidalgo, A. 2012. Wheat germ: not only a by-product. *International Journal of Food Sciences and Nutrition*, vol. 63, no. 1, p. 71-74. http://dx.doi.org/10.3109/09637486.2011.633898

Ge, Y., Sun, A., Ni, Y., Cai, T. 2001. Study and development of a defatted wheat germ nutritive noodle. *European Food Research and Technology*, vol. 212, no. 3, p. 344-348. http://dx.doi.org/10.1007/s002170000253

Grosch, W., Laskawy, G. 1984. Contribution of linoleic acid to the bitter taste of poppy seeds (*Papavers omniferum*). *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung*, vol. 178, no. 4, p. 257-259. PMid:6720091

Han, J. J., Janz, J. A. Gerlat, M. 2010. Development of gluten free cracker snacks using pulse flours and fractions. *Food Research International*, vol. 43, no.2, p. 627-633. http://dx.doi.org/10.1016/j.foodres.2009.07.015

Hayam M. Abbas, Ahmed M.S. Hussein, Faten L. Seleet, Hala M. Bayoumi, M. AbdEl-Aziz. 2015. Quality of some Dairy By-products Supplemented with WheatGermas Functional Beverages. *International Journal of Dairy Science*, vol. 10, no. 6, p. 266-277. http://dx.doi.org/10.3923/ijds.2015.266.277

Hempel, S., Jacob, A., Rohm, H. 2007. Influence of inulin modification and flour type on the sensory quality of prebiotic wafer crackers. *European Food Research and Technology*, vol. 224, no. 3, p. 335-341. http://dx.doi.org/10.1007/s00217-006-0326-9

Hozová B, Buchtová V, Dodok L, Zemanovič J. 1997. Microbiological, nutritional and sensory aspects of stored amaranth biscuits and amaranth crackers. *Nahrung*, vol. 41, no. 3, p. 155-158. http://dx.doi.org/10.1002/food.19970410308

Chen, H., Rubenthaler, G. L., Leung, H. K., Baranowski, J. D. 1988. Chemical, physical, and baking properties of apple fiber compared with wheat and oat bran. *Cereal Chemistry*, vol. 65, no. 3, p. 244-247. [cit. 2016-02-08] Available at: http://www.aaccnet.org/publications/cc/backissues/1988/Documents/65_244.pdf

Karovičová, J., Kohajdová, Z., Minarovičová, L., Kuchtová, V. 2015. The Chemical Composition of Grape Fibre. *Potravinarstvo*, vol. 9, no. 1, p. 53-57. http://dx.doi.org/10.5219/428

Kohajdová, Z., Karovičová, J., Magala, M. 2011. Utilisation of chickpea flour for cracker production. *Acta Chimica Slovaca*, vol. 4, no. 2, p. 98-107. [cit. 2016-02-08] Available at: http://www.acs.chtf.stuba.sk/papers/acs_0096.pdf

Kohajdová, Z., Karovičová, J., Magala, M. 2013. Rheological and qualitative characteristics of pea flour incorporated cracker biscuits. *Croatian Journal of Food Science and Technology*, vol.5, no.1, p. 11-17. [cit. 2016-02-08] Available at: http://hrcak.srce.hr/106157

Kohajdová, Z., Karovičová, J., Magala, M., Kuchtová, V. 2014. Effect of apple pomace powder addition on farinographic properties of wheat dough and biscuits quality. *Chemical Papers*, vol. 68, no. 8, p. 1059-1065. http://dx.doi.org/10.2478/s11696-014-0567-1

Ktenioudaki, A., Gallagher, E. 2012. Recent advances in the development of high-fibre baked products. *Trends in Food Science and Technology*, vol. 28, no. 1, p. 4-14. http://dx.doi.org/10.1016/j.tifs.2012.06.004

Kulakova, E. V., Vainerman, E. S., Rogoshin, S. V. 1982. Contribution to the investigation of the corn germ Part I. Corn germ as a valuable source of protein. *Food/Nahrung*, vol. 26, no. 5, p. 451-456. http://dx.doi.org/10.1002/food.19820260508

Lebesi, D. M., Tzia, C. 2011. Effect of the addition of different dietary fiber and edible cereal bran sources on the baking and sensory characteristics of cupcakes. *Food and Bioprocess Technology*, vol. 4, no. 5, p. 710-722. http://dx.doi.org/10.1007/s11947-009-0181-3

Levent, H., Bilgiçli, N. 2013. Quality evaluation of wheat germ cake prepared with different emulsifiers. *Journal of Food Quality*, vol. 36, no. 5, p. 334-341. http://dx.doi.org/10.1111/jfq.12042

Ma, S., Wang, X. X., Zheng, X. L., Tian, S.Q., Liu, C., Li, L., Ding, Y. F. 2014. Improvement of the quality of steamed bread supplementation of wheat germ from milling process. *Journal of Cereal Science*, vol. 60, no. 3, p. 589-594. http://dx.doi.org/10.1016/j.jcs.2014.07.010

Majzoobi, M., Darabzadeh, N., Farahnaky, A. 2012. Effects of percentage and particle size of wheat germ on some properties of batter and cake. *Journal of Agricultural Science and Technology*, vol. 14, no. 4, p. 827-836. Available at: http://jast.modares.ac.ir/article_4854_ bcfc5803741 e6257c9ec6e77d0577869.pdf

McKee, L., Latner, T. A. 2000. Underutilized sources of dietary fiber: A review. *Plant Foods for Human Nutrition*, vol. 55, no. 4, p. 285-304. http://dx.doi.org/10.1023/A:1008144310986

Pęksa, A., Miedzianka, J., Czopek, A. T., Rytel, E. 2010. The quality of fried snacks fortified with fiber and protein supplements. *Potravinarstvo*, vol. 4, no. 2, p. 59-64. http://dx.doi.org/10.5219/54

Perez, P., Germani, R. 2000. Elaboração de biscoitostiposalgado, com alto teor de fibraalimentar, utilizandofarinha de berinjela (*Solanummelongena, L.*) *Food Science and Technology*, vol. 27, no. 2, p. 186-192. Available at: http://www.scielo.br/pdf/cta/v27n1/32.pdf

Pérez, G. T., León, A. E., Ribotta, P. D., Aguirre, A., Rubiolo, O. J., Añón, M. C. 2003. Use of triticale flours in cracker-making. *European Food Research and Technology*, vol. 217, no. 2, p. 134-137. http://dx.doi.org/ 10.1007/s00217-003-0729-9

Pınarlı, İ., İbanoğlu, Ş., Öner, M. D. 2004. Effect of storage on the selected properties of macaroni enriched with wheat germ. *Journal of Food Engineering*, vol. 64, no. 2, p. 249-256. http://dx.doi.org/10.1016/j.jfoodeng.2003.10.005

Ramirez, E. C., Johnston, D. B., McAloon, A. J., Yee, W., Singh, V. 2008. Engineering process and cost model for a conventional corn wet milling facility. *Industrial crops and products*, vol. 27, no. 1, p. 91-97. http://dx.doi.org/10.1016/j.indcrop.2007.08.002

Rosa-Sibakov, N., Poutanen, K., Micard, V. 2015. How does wheat grain, bran and aleurone structure impact their nutritional and technological properties? *Trends in Food Science & Technology*, vol. 41, no. 2, 118-134. http://dx.doi.org/10.1016/j.tifs.2014.10.003

Saeleaw M, Schleining G. 2010. Effect of blending cassava starch, rice, waxy rice and wheat flour on physico-chemical properties off flour mixtures and mechanical and sound emission properties of cassava crackers. *Journal of Food Engineering*, vol. 100, no.1, p.12-24. http://dx.doi.org/10.1016/j.jfoodeng.2010.03.020

Sedej, I., Sakač, M., Mandić, A., Mišan, A., Pestorić, M., Šimurina, O., Čanadanović-Brunet, J. 2011. Quality assessment of gluten-free crackers based on buckwheat flour. *LWT Food Science and Technology*, vol. 44, no. 3, p. 694-699. http://dx.doi.org/10.1016/j.lwt.2010.11.010

Shi, L., Li, W., Sun, J., Qiu, Y., Wei, X., Luan, G., Hu, Y., Tatsumi, E. 2016. Grinding of maize: The effects of fine grinding on compositional, functional and physicochemical properties of maize flour. *Journal of Cereal Science*, vol. 68, p. 25-30. http://dx.doi.org/10.1016/j.jcs.2015.11.004

Sidhu, J. S., Al-Hooti, S. N., Al-Saqer, J. M. 1999. Effect of adding wheat bran and germ fractions on the chemical composition of high-fiber toast bread. *Food Chemistry*, vol. 67, p. 365-371. http://dx.doi.org/10.1016/S0308-8146%2899%2900123-5

Siddiq, M., Nasir, M., Ravi, R., Dolan, K. D., Butt, M. S. 2009. Effect of defatted maize germ addition on the functional and textural properties of wheat flour. *International Journal of Food Properties*, vol. 12, no. 4, p. 860-870. http://dx.doi.org/10.1080/10942910802103028

Singh, M., Liu, S. X., Vaughn, S. F. 2012. Effect of corn bran as dietary fiber addition on baking and sensory quality. *Biocatalysis and Agricultural Biotechnology*, vol. 1, no. 4, p. 348-352. http://dx.doi.org/10.1016/j.bcab.2012.02.005

Sudha, M. L., Vetrimani, R., Leelavathi, K. 2007. Influence of fibre from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. *Food Chemistry*, vol. 100, no. 4, p. 1365-1370. http://dx.doi.org/10.1016/j.foodchem.2005.12.013

Rizzello, C. G., Cassone, A., Coda, R., Gobbetti, M. 2011. Antifungal activity of sour-dough fermented wheat germ used as an ingredient for bread making. *Food Chemistry*, vol. 127, p. 952-959. http://dx.doi.org/10.1016/j.foodchem.2011.01.063

Tsadik, Y. Y. G., Emire, S. A. 2015. Development of Value Added Products from By-products of Ethiopian Wheat Milling Industries. *Journal of Food Processing &Technology*, vol. 6, p. 474. http://dx.doi.org/10.4172/2157-7110.1000474

Turabi, E., Sumnu, G., Sahin, S. 2008. Rheological properties and quality of rice cakes formulated with different gums and an emulsifier blend. *Food Hydrocolloids*, vol. 22, no. 2, p. 305-312. http://dx.doi.org/10.1016/j.foodhyd.2006.11.016

Wang, N., Hou, G. G., Kweon, M., & Lee, B. 2016. Effects of particle size on the properties of whole-grain soft wheat flour and its cracker baking performance. *Journal of Cereal Science*, vol. 69, p. 187-193. http://dx.doi.org/10.1016/j.jcs.2016.03.010

Yılmaz, N., Tuncel, N. B., Kocabıyık, H. 2014. The effect of infrared stabilized rice bran substitution on nutritional, sensory, and textural properties of cracker. *European Food Research and Technology*, vol. 239, no. 2, p. 259-265. http://dx.doi.org/10.1007/s00217-014-2217-9

Youssef, H. M. 2015. Assessment of Gross Chemical Composition, Mineral Composition, Vitamin Composition and Amino Acids Composition of Wheat Biscuits and Wheat Germ Fortified Biscuits. *Food and Nutrition Sciences*, vol. 6, no. 10, p. 845. http://dx.doi.org/10.4236/fns.2015.610088

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THE RELATIONSHIP OF HEAVY METALS CONTENTS IN SOILS TO THEIR CONTENT IN LEGUME SEEDS USED IN FAMOUS TRADITIONAL FOOD IN KURDISTAN REGION-IRAQ

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ABSTRACT

In this work the level of risk heavy metals contents in Cowpea seeds comparison with heavy metal content in soil was studied. For the experiment three cowpea cultivars (brown, red, white) were used. Cowpeas were harvested at full ripeness in Kalak location in Erbil city. The flame AAS (AAS Varian AA Spectr. DUO 240 FS/240Z/UltrAA) was used for the determination of heavy metal contents in soil and plant materials. The soil which cultivated Cowpea, characterized neutral to slit alkali, with a typical content of cations K, Mg and P. Beans and the seeds of faba bean, cowpea and chickpeas boiled with salt eaten in the form of Lablabe, traditionly used heavy sweets such as knafa. Ful, which is fava beans cooked with chickpeas (garbanzo beans) or make soup from fresh cowpea, fresh faba bean, fresh fasoulia, as well as lentil soup (shorbat adas) and different kinds of salad after boiled. Cowpea grain legumes occupy an important place in human nutrition, especially in the dietary pattern of low income groups of people in developing countries. The level risk heavy metal contents in the soil determined was only Cd content was on the level of limit value given for the soil extract by aqua regia as well as Co content was higher than the limit value given for the relationship between soil and plant. All of determined values were lower than critical value extracted by NH_4NO_3 only the maximal available soil content of mobile Pb forms was exceeded but cowpea accumulated seeds in amounts the risky elements contents, with the exception of Ni, did not exceed limit for the maximum levels of chosen risk elements in studied legume. The content of the metals studied with the exception of cadmium, not exceed the maximum permissible value in legumes, as defined in the Codex Alimentarius. The aim of this research, to study or determine the content of risky heavy metals (Cu, Ni, Cr, Pb, and Cd) in the soil and their relationship in selected varieties cowpea seeds cultivar. Faba bean and Fresh bean with tomatoes uses for preparing soup, or a popular snack eaten on boiled and roasted in oil with egg or onion, other legume seeds broad bean, fababean, lentil, pea, chickpea used for different traditional foods in Iraq.

Keywords: cowpea; heavy metals; soil; traditional food

INTRODUCTION

Legume grains have been playing a key role in the traditional diets of human beings throughout the world. They are excellent source of protein, dietary fiber, starch, micronutrients and bioactive compounds with low level of fat **(Chang et al., 2000)**. Grain legumes occupy an important place in human nutrition, especially in the dietary pattern of low income groups of people in developing countries. Legume Grains are normally consumed after processing, which not only improves palatability of foods but also increases the bioavailability of nutrients **(Tharanathan and Mahadevamma, 2003)**. Plant proteins are cheaper than the animal proteins; therefore, the people consume legume seeds worldwide as major source of protein **(Petchiammal et al., 2014)**. Legume grains are a rich source of polyphenols, which have high antioxidant activities **(Cardador Martinez et al., 2002; Troszynska et al., 2002)**. Antioxidant activity has been reported for extracts of legumes such as pea; white, green, red and navy beans; beach pea; lentils; everlasting pea; Jack bean; adzuki bean; and cowpea **(Lopez-Amoros et al., 2006)**. Phenolic compounds, such as phenolic acids, flavonols, flavones, isoflavones, anthocyannins, and condensed tannins, have been identified and characterized in food legumes **(Beninger and Hosfield, 2003; Xu et al., 2007a, b)**. Important biological activities have now been suggested for these bioactive compounds like enhancement of the antioxidant, antimutagenic, anticarcinogenic and anti-hyperglycemic effects, which makes pulses an important crop for human health **(Singh and Basu, 2012)**. Dry beans are widely known for their fiber, mineral and protein contents; however, its nutraceutical value is yet to gain as much attention in the prevention of chronic diseases **(Dinelli et al., 2006)**. Heavy metals are potential environmental contaminants with the capability of causing human health problems if present to excess in the food. They are given special attention throughout the world due to their toxic effects even at very low concentrations **(Das, 1990)**. Several cases of human disease, disorders, malfunction and malformation of organs due to metal toxicity have been reported **(Jarup, 2003)**. Plants have a natural

propensity to take up metals. Some of them like Cu^{2+}, Co^{2+}, Fe^{2+}, Mo^{2+}, Mn^{2+}, and Zn^{2+} are essential plant micronutrients **(Baker et al., 1991)**, while few others like Hg^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} are toxic to plants. However, such toxic effects are even varying from genotype to genotype of the same crop **(Liu et al., 2001)**. The toxic dose depends on the type of ion, ion concentration, plant species, and stage of plant differences by the leafy vegetables are attributed to plant differences in tolerance to heavy metals **(Itanna, 2002)**. Zinc is one of the most important trace elements essential to human, and zinc deficiency is common in most of the legume growing areas of the world. Lead and cadmium are non-essential metals as they are toxic, even in trace **(Gençcelep et al., 2009)**. Food contains many different nutrients that help the body function well. The body cannot produce these nutrients, so they must be obtained from the food we eat. Along with essential nutrients, lentils are good sources of many nonnutrient functional phytochemicals such as phytic acid and tannins **(Vidal-Valverde et al., 1994)**, which are considered among the functional antioxidant ingredients **(Scalbert et al., 2005; Vucenik and Shamsuddin, 2006)**. Soil is a dynamic system which is influenced by various factors, whether natural or anthropic, causing the contamination. Legumes: beans, lentils, soybean Cooking competition. Cowpea, Faba beans, Broad beans are can be eaten while still young, enabling harvesting to begin as early as the middle of spring. Cowpea has found utilization in various ways in traditional and modern food processing in the world. Traditionally in Iraq, cowpeas are consumed as boiled vegetables using fresh Cow pea, bean, faba bean as a snack or soup with tomato, roasted in oil with egg or onion, or processed to make other food products. The nutritional and functional properties of Cow pea flours are comparable to chickpea flour **(Sreerama et al., 2012)**. Due to their favourable flour functionality and their phytochemical-associated health benefits, these flours offer an enormous potential for the production of legume composite flours. Pulses have shown numerous health benefits, e.g. lower glycemic index for people with diabetes, increased satiation and cancer prevention as well as protection against cardiovascular diseases due to their dietary fiber content **(Chillo et al., 2008)**. Legumes in generally can be considered as a therapeutic functional foods due to their significant content of functional proteins and carbohydrates and their extraordinary reserve of secondary metabolites and bioactive constituents that are beneficial for managing and preventing several chronic illnesses in humans **(Fratianni et al., 2014)**. The antioxidant activity of plant polyphenols can retard the development of most major age-related degenerative diseases such as cancers, diabetes, cardiovascular disease, and neurodegenerative diseases **(Lee, 2013; Seo et al., 2012)**. A number of epidemiological studies have correlated the consumption of legumes with high phenolic content to the reduced incidence of diseases such as cancer, ageing, diabetes, and cardiovascular disease **(Kris-Etherton et al., 2002)**. Cholesterol-free legumes in combination with their low sodium content form a good food stuff not only for people living in developing countries but also for those living in industrialized nations **(Sebastiá et al., 2001)**.

The aim of this study was to evaluate the influence of the grown locality on risky metal intake from the soil to the variety Cowpea seeds.

MATERIAL AND METHODOLOGY

Material

Cowpea samples (Three Varity) at full ripeness were provided from Erbil locality Khabat or (Kalak) in Erbil City

Soil

Soil samples were taken by auger tool, depths 20 cm (A horizon). The soil from the same sites, from which the legume samples were taken with the aim to find out the relations between soil traits in grain. Then the pH of soil was determined, the nutrients contents and the risk elements contents in soil. The contents of available nutrients in the solution were determined by Mehlich II method. Contents of risky heavy metals were determined in different soil extracts (aquaregia; c = 1 $mol.dm^{-3}$ NH_4NO_3; c = 2 $mol.dm^{-3}$ HNO_3). Atomic absorption spectrometry analysis was finally used. In this work soils were evaluated according to recent legislative norm valid in Slovakia (Law No. 220/2004 as ammended). By this norm, the limit values of risky elements are considered to be critical values of agricultural soil in relationship to the plant and are also harmonized with EU limits. In the soil the exchangeable reaction (pH/KCl), the contents of available nutrients (K, Mg, P) and mobile forms of Ca according Mehlich II., content of humus by Tjurin method and content of N were determined. Pseudototal content of risk metals including all of the forms besides residual metal fraction was assessed in solution of aqua regia and content of mobile forms of selected heavy metals in soil extract by NH_4NO_3 (c = 1 $mol.dm^{-3}$). Gained results were evaluated according Law 220/2004.

For the experiment three cowpea variants were realized. Risky element contents in dry seeds were determined using the atomic absorption spectrometry. The flame AAS (AAS Varian AA Spectra DUO 240 FS/240Z/UltrAA) was used for the determination of heavy metal contents in soil and plant materials. The content of risky elements (Cd, Pb, Cu, Cr, Ni) in Cowpea seeds were evaluated according to Food codex of the Slovak Republic.

RESULTS AND DISCUSSION

The soil is characterized by low supply of humus. The soil reaction was alkaline. Soil reaction has a major effect on the uptake of many risky elements, the most of them become more available to plants as pH decreases. The neutral soil reaction suitable for the legume cultivation.

Currently, contamination of soil in cultivated fields with toxic heavy metals such as cadmium, copper, nickel and zinc has emerged as a new threat to agriculture **(Singh et al., 2007)**. Excessive intake of either copper or zinc has been reported to be toxic **(Somer, 1974; Graham and Cordano, 1976)**. Cadmium is an unnecessary element for both plants and animals and has toxic effects when its concentration has exceeded a limit.

Table 1 Agrochemical characteristics of the soil in (mg.kg^{-1}) and hums content (%) of the soil surse of diferent color (variety) of Cowpea.

Area sorse/Variety	pH (H2O)	pH (KCl)	Cox (%)	Humus (%)
1 A/brown	8.48	7.39	0.52	0.89
2A/red	8.76	7.43	0.64	1.01
3A/white	8.58	7.32	0.59	0.98

Note: n = 3 (three samples soil).

Table 2 Macroelement contents in the soil in (mg.kg^{-1}) soil surse of diferent color of Cowpea from locality Kalak.

Area/Variety	Macroelements	Ca	Mg	K	P	N
1 A/brown		9260.40	385.80	360.55	31.92	603.00
2A/red		8927.20	382.70	379.35	35.49	56.00
3A/white		8644.50	409.50	321.50	38.80	550.00

Note: n = 3 (three samples of soil).

Table 3 Heavy metals content (mg.kg^{-1}) in soils from Iraq (soil extract by *aqua regia*). of diferent color(variety) of Cowpea.

Area/Variety	Zn	Cu	Co	Ni	Cr	Pb	Cd
1 A/brown	76.20	28.80	22.20	43.00	66.40	17.00	0.62
2A/red	69.80	30.00	21.60	44.20	65.80	18.60	0.70
3A/white	68.60	29.20	20.60	45.70	57.90	18.40	0.64
Limit value*	150	60	15	50	70	70	0.70

Note: **Low No.22/2004, **European Commission (2006*) n = 3 (three samples of soil).

Table 4 Risk elements content in mg·kg^{-1} by NH4NO3 extract (c = 1 mol·dm^{-3}) in the soil of diferent color(variety) of Cowpea.

Area/Variety	Zn	Cu	Co	Ni	Cr	Pb	Cd
1 A/brown	0.67	0.078	0.172	0.265	0.027	0.030	0.08
2A/red	0.58	0.066	0.154	0.237	0.023	0.185	0.066
3A/white	0.74	0.069	0.144	0.267	0.053	0.165	0.072
critical value*	2	1	–	1.50	–	0.10	0.10

Note: **Low No.22/2004*, n = 3 (three samples soil).

Table 5 Nutrients contents (Mehlich II) in cowpea seeds (mg.kg^{-1} DM) Kalak (Khabat) location.

Seeds	K	Na	Ca	Mg	P	N	DM
Brown	19400	87	22400	2480	3834.4	30500	90.90
Red	17920	125	1980	1980	4760.8	34100	90.15
White	18790	108	2128	2530	4390	32200	90.70

Note: n = 3 (three samples of soil).

Table 6. Heavy metal contents (*M* ±S.D.) in cowpea seeds (mg.kg^{-1} DM) Kalak (Khabat) location.

Variant	Cu	Ni	Cr	Pb	Cd
Brown	12.00 ±0.50	3.5 ±0.13	1.92 ±0.60	0.20 ±0.30	0.09 ±0.04
Red	14.03 ±0.80	3.8 ±0.70	2.10 ±0.40	0.20 ±0.20	0.06 ±0.01
White	15.40 ±0.75	2.04 ±0.40	1.80 ±0.04	0.18 ±0.10	0.09 ±0.18
Limit **	15.0	3.0	4.0	0.2	0.1

Note: ***Food Codex of the Slovak Republic.*

Generally, it makes negative effect on their metabolisms by influencing the activity of cellular enzymes **(Yang et al., 1986)**. Cadmium and lead are among the most abundant heavy metals and are particularly toxic. The excessive content of these metals in food is associated with etiology of a number of diseases **(WHO, 1992, 1995)**. Cadmium exposure may cause kidney damage or skeletal damage **(WHO, 1992)**. The soil was only determined Cd content was on the level of limit value given for the soil extract by *aqua regi*a as well as Co content was higher than the limit value given for the relationship between soil and plant (Table 3).

In soil samples the releasable risk elements contents were also determined in the solution of NH_4NO_3 (c = 1 mol.dm^{-3}). All of determined values were lower than critical value (Table 4) only the maximal available soil content of mobile Pb forms was exceeded. In soils with alkali soil reaction these forms are less mobile (soil reaction is one of the factors influencing risk elements toxicity to plants: Heavy metals at supra-optimal concentrations affect the agronomic traits of plants **(Sinha and Gupta, 2005)**. Lead is accumulated in the skeleton and cause renal tubular damage and may also give rise to kidney damage **(WHO, 1995)**. International Agency for

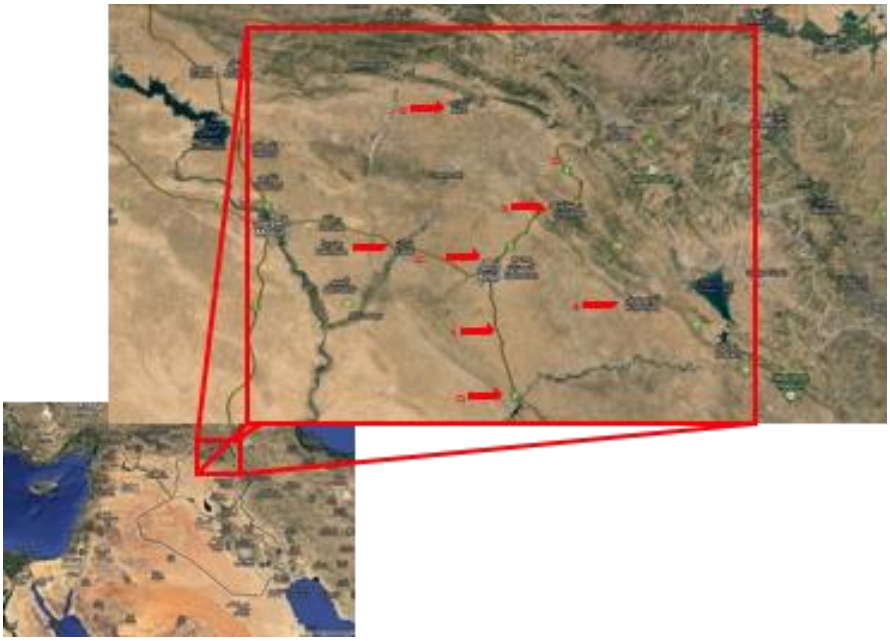

Figure 1 Location Kalak or (Khabat, **خبات**) on the Erbil map is no 6.

Research on Cancer (IARC) classified cadmium and lead as human carcinogen **(IARC, 1993; Steenland and Boffetta, 2000)**.

The heavy metals contents in soil did not exceeded the limit values specified by law 531/1994 – 540 (Decision of the Ministry of Agriculture SR). However, from the point of view of risky metal intake by plants, is important content of accessible, respectively potentially mobilizable forms of heavy metal. And from this perspective soil can be described as relatively uncontaminated. Any of the determination of heavy metals content in the soil below the threshold does not guarantee that the plants growing on this soil will always contain their tolerable amounts. It is therefore crucial in terms of hygiene, whether the heavy metals accumulate in parts of plant used for consumption **(Zrůst 2003)**.

The determination of macro- and trace elements in foodstuffs is an important part of nutritional and toxicological analyses. Cadmium and lead are best known for their toxicological properties. Pb and Cd can be accumulated in biological systems becoming potential contaminants along the alimentary chain. Copper, chromium, iron, and zinc in adequate amounts are essential micronutrients for human health. These elements play an important role in human metabolism, and interest in these elements is increasing together with reports of relationships between trace element status and oxidative diseases. On the other hand, e.g. Cu and Zn are essential micronutrients, they can be risk elements when taken in excess. Legumes are known as zinc accumulators **(Gençcelep et al., 2009)**. Food Codex of Slovak Republic has set a limit for the maximum levels of chosen risk elements in legumes as shown in Table 6. Limits for contaminants in Slovak food commodities are harmonized with EU limits **(Cimboláková and Nováková 2009)**. The risky elements contents, with the exception of Ni, did not exceed limit for the maximum levels of chosen risk elements in studied Cowpea legume. **(Gadd, 1992)** and **(Giller et al., 1998)** postulated that some metals such as Zn, Cu, Ni and Cr are essential or beneficial micronutrients for plants, animals and microorganisms, whereas others, such as Cd, Hg, and Pb have no known biological and/ or physiological functions. However, all these metals could be toxic at relative low concentrations. Nickel is an essential element for plants, in small quantities, has been reported to improve crop yield and quality **(Brown et al., 1990; Atta-Aly, 1999)**. These metals are taken up from soils and bioaccumulated in crops, causing damage to plants when reach high levels and under certain conditions becoming toxic to human and animals fed on these metal enriched plants **(EL-Sokkary and Sharaf, 1996)**. Heavy metals at supra-optimal concentrations affect the agronomic traits of plants **(Sinha and Gupta, 2005)**.

The determined contents of Cr, Cu and Pb (0.1 $mg.kg^{-1}$, 0.7 $mg.kg^{-1}$ and 0.1 $mg.kg^{-1}$ respectively) by **Hicsonmez et al., (2012)** in fababea seeds lower than those determined in our Cowpea cultivar results only Ni content determined by these authors was similar to that in our samples (3.4 $mg.kg^{-1}$ DM), On the other hand**, Haciseferoğullari et al., (2003)** determined higher amounts of Cr, Cu and Pb (11.25 $mg.kg^{-1}$, 18, $mg.kg^{-1}$ and 1.5 $mg.kg^{-1}$ respectively), and a similar Ni content (3.83 $mg.kg^{-1}$) in comparison to our results. **Dalaram et al., (2013)** in fresh fababea seeds determined content of Cr, Cu, Pb (1.54 $mg.kg^{-1}$, 7.5 $mg.kg^{-1}$ and 5.6 $mg.kg^{-1}$ respectively) in comparison our results in Cowpea seeds with the result by **Dalaram et al., (2013)** Cr and Cu content higher but Pb lower. Heavy metal accumulation in plants depends upon plant species, and the efficiency of different plants in absorbing metals is evaluated by either plant uptake or soil-to plant transfer factors of the metals **(Rattan et al., 2005)**.

CONCLUSION

In present study the determined contents of Ni higher than the hygienic limit and content of Cu slightly exceeded the hygienic limit too, the risky elements contents, with the exception of Ni and Cu, did not exceed limit for the

maximum levels of chosen risk elements in studied Cowpea legume. Our results confirmed the low ability to accumulate large amounts of risky metals. The presented results indicate the serious risk heavy metal intake by human organism due the consumption of foodstuffs based on Cowpea. It is permanently necessary to monitor the content of risky heavy metals if it is content a high amount and to apply measures for the minimization of risky metal input into the human food chain. Heavy metal accumulation in plants depends upon plant species, and the efficiency of different plants in absorbing metals is evaluated by either plant uptake or soil to plant transfer factors of the metal. Some metals like Fe, Se, Mn, Co, Zn, Mo and Ni, are essential micronutrient for most of the redox reactions which are fundamental for cellular functions. Some famous and traditional Iraqi foods have some bioactive components related with health benefits, such as polyphenols, lectins, and carbohydrates. Adequate consumption of the foods with high functional content can result in improved health thereby reducing diseases.

REFERENCES

Atta Aly, M. A. 1999. Effect of nickel addition on the yield and quality of parsley leaves. *Scientia Horticulturae*, vol. 82, no. 1-2, p. 9-24. http://dx.doi.org/10.1016/S0304-4238(99)00032-1

Baker, A. J. M., Reeves, R. D., McGrath, S. P. 1991. In situ decontamination of heavy metal polluted soils using crops of metal-accumulating plants-a feasibility study. In Hinchee R. E., Olfenbuttel, R. F. (Eds.). *In Situ Bioreclamation.* Butterworth-Heinemann, Stoneham MA, USA, p. 539-544. http://dx.doi.org/10.1016/B978-0-7506-9301-1.50049-4

Beninger, C. W., Hosfield, G. L. 2003. Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris L*. seed coat color genotypes. *Journal of Agricultural and Food Chemistry*, vol. 51, no. 27, p. 7879-7883. http://dx.doi.org/10.1021/jf0304324

Brown, P. H., Welch, R. M., Madison, J. T. 1990. Effect of nickel deficiency on soluble anion, amino acid, and nitrogen levels in barley. *Plant Soil*, vol. 125, no. 1, p. 19-27. http://dx.doi.org/10.1007/BF00010740

Brown, L., Griffin, P., Hagerman, R., Zmijewski, M. 1987. Security analyst superiority relative to univariate time-series models in forecasting quarterly earnings. *Journal of Accounting and Economics*, vol. 9, no. 1, p. 61-87. http://dx.doi.org/10.1016/0165-4101(87)90017-6

Cardador-Martinez, A. Loacra-pina, G. Oomah, B. D. 2002. Antioxidant activity in common beans (*Phaseolus vulgaris* L.). *Journal of Agricurtural and Food Chemistry*, vol. 50, no. 24, p. 6975-6980. http://dx.doi.org/10.1021/jf020296n

Chang, S. Tan., CH. Frankel, E. N., Barret, D. M. 2000. Low-density lipoprotein antioxidant activity of phenolic compounds and polyphenol oxidase activity in selected clingstone peach cultivars. *Journal of Agricultural and Food Chemistry*, vol. 48, no. 2, p. 147-151. http://dx.doi.org/10.1021/jf9904564 PMid:10691607

Chillo, S., Laverse, J., Falcone, P., Protopapa, M., Del Nobile, A. 2008. Influence of the addition of buckwheat flour and durum wheat bran on spaghetti quality. *Journal of Cereal* Science, vol. 47, no. 2, p. 144-152. http://dx.doi.org/10.1016/j.jcs.2007.03.004

Cimboláková I., Nováková J. 2009. Heavy metals – the important element of the food chain. *Potravinarstvo*, vol. 3, no. 3, p. 14-16. Available at: http://www.potravinarstvo.com/dokumenty/potravinarstvo_no3_2009.pdf

Codex Alimentarius. 2004. *Slovak decree of the Ministry of Agriculture of the Slovak Republic and the Slovak Ministry of Health of 15 March 2004 no. 608/3/2004-100, issuing the chapter of the Slovak Republic Foodstuffs Code governing contaminants in food.* (*Výnos Ministerstva pôdohospodárstva Slovenskej republiky a Ministerstva zdravotníctva Slovenskej republiky z 15. marca 2004 č. 608/3/2004-100, ktorým sa vydáva hlava Potravinového kódexu Slovenskej republiky upravujúca kontaminanty v potravinách*).

Das A. 1990. *Metal ion induced toxicity and detoxification by chelation therapy*. 1st ed. A text book on medical aspects of bioinorganic chemistry, CBS : Delhi, p. 17-58.

Decision of the Ministry of Agriculture SR No. 531/1994 – 540 about the maximum permitted levels of hazardous substances in the soil. Ministry of Agriculture of the Slovak Republic, Bulletin, Vol. XXVI, item I, decision 3, No. 531/1994 (*Rozhodnutie o najvyšších prípustných hodnotách rizikových látok v pôde*. Ministerstvo pôdohospodárstva SR, Vestník, roč. XXVI, čiastka I., rozhodnutie 3, číslo 531/1994).

Dinelli, A., Bonetti, M., Minelli, I., Marotti, P. 2006. Content of flavonols in Italian bean (*Phaseolus vulgaris* L.) ecotypes. *Food Chemistry*, vol. 99, no. 1, p. 105-114. http://dx.doi.org/10.1016/j.foodchem.2005.07.028

EL-Sokkary, I. H. and Sharaf, A. I. 1996. Enrichment of soils and plants irrigated by wastewater by zinc and cadmium. *Egyptian Journal of Soil Science*, vol. 36, no. 1-4, p. 219. http://dx.doi.org/10.1016/j.jff.2013.12.030

Food codex, 2004. *Slovak decree of the Ministry of Agriculture of the Slovak Republic and the Slovak Ministry of Health of 15 March 2004 No. 608/3/2004 – 100, issuing the chapter Slovak Republic Foodstuffs Code governing contaminants in food (Výnos Ministerstva Pôdohospodárstva Slovenskej republiky a Ministerstva Zdravotníctva Slovenskej republiky z 15. marca 2004 No. 608/3/2004 – 100, ktorým sa vydáva hlava Potravinového Kódexu Slovenskej republiky upravujúca kontaminanty v potravinách)*.

Fratianni, F., Cardinalea, F., Cozzolinoa, A, Granesea, T. 2014. Polyphenol composition and antioxidant activity of different grass pea (*Lathyrus sativus*), lentils (*Lens culinaris*), and chickpea (*Cicer arietinum*) ecotypes of the Campania region (Southern Italy). *Journal of Functional Foods*, vol. 7, p. 551-557. http://dx.doi.org/10.1016/j.jff.2013.12.030

Gadd, G. M., 1992. Metals and microorganisms: a problem of definition. *FEMS Microbiology Letters*, vol. 100, no. 1-3, p. 197-204. http://dx.doi.org/10.1111/j.1574-6968.1992.tb05703.x

Gençcelep, H., Uzun, Y., Tun, Çtűrk, Y., Demirel, K. 2009. Determination of mineral content of wild-grown edible mushrooms. *Food Chemistry*, vol. 133, no. 4, p. 1033-1036. http://dx.doi.org/10.1016/j.foodchem.2008.08.058

Giller, K. E., Witter, E. Mcgrath, S. P. 1998. Toxicity of heavy metals to microorganisms and microbial process in agricultural soils: a review. *Soil Bioogy and Biochemistry*, vol. 30, p. 1389-1414. http://dx.doi.org/10.1016/S0038-0717(97)00270-8

Graham, C. G., Cordano, A. 1976. Copper deficiency in human subjects. In Prasad, A. S., Oberleas, D. (Eds.) *Trace Elements in Human Health and Disease: Zinc and Copper*, vol. 1. New York : Academic Press, p. 363.

Haciseferoğullari, H., Gezer I., Bahtiyarca, Y., Mengeş, H. C. O. 2003. Determination of some chemical and physical properties of Sakiz faba bean (*Vicia faba* L. var. major).

Journal of Food engineering, vol. 60, no. 4, p. 475-479. http://dx.doi.org/10.1016/S0260-8774(03)00075-X

Hicsonmez, U., Ozdemir, C., Cam, S., Ozdemir, A., Serap, Erees, F. 2012. Major-minor element analysis in some plant seeds consumed as feed in Turkey. *Natural Science*, vol. 4, no. 5, p. 298-303. http://dx.doi.org/10.4236/ns.2012.45042

IARC, 1993. Cadmium and cadmium compounds. In: *Beryllium, cadmium, mercury and exposure in the glass manufacturing industry*. IARC Monographs on the evaluation of carcinogenic risks to humans. International Agency for Research on Cancer, Lyon, France, vol. 58. p. 119-237.

Ismael S. D. Vollmannová A., Timoracká, M. 2013. Risk of enhanced heavy metal contents in fresh fababean used in traditional food preparation in Iraq. *Potravinarstvo*, vol. 7, Special no., p. 29-32. Available at: http://www.potravinarstvo.com/dokumenty/mc_march_2013/chemicka_bezpecnost_potravin/dalaram.pdf

Itanna, F. 2002. Metals in leafy vegetables grown in Addis Ababa and toxicological amplications. *The Ethiopian Journal of Health Development*, vol. 16, no. 3, p. 295-302. http://dx.doi.org/10.4314/ejhd.v16i3.9797

Jarup, L. 2003. Hazards of heavy metal contamination. *British Medical Bulletin*, vol. 68, no. 1, p. 167-182. http://dx.doi.org/10.1093/bmb/ldg032

Kris-Etherton, P. M., Heckar, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., Griel, A. E., Etherton, T. D. 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine*, vol. 113, no. 9, p. 71-88. http://dx.doi.org/10.1016/S0002-9343(01)00995-0

Lee, C. Y. 2013. Challenges in providing credible scientific evidence of health benefits of dietary polyphenols. *Journal of Functional Foods*, vol. 5, no. 1, p. 524-526. http://dx.doi.org/10.1016/j.jff.2012.10.018

Liu, D. H., Jiang, W. S., Hou, W. Q. 2001. Uptake and accumulation of copper by roots and shoots of maize (*Zeamays* L.). *Journal of Environmental Sciences*, vol. 13, no. 2, p. 228-232. http://dx.doi.org/10.1016/j.biortech.2005.11.028

López-Amorós, M. L., Hernández, T. Estrella, I. 2006. Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis*, vol. 19, no. 4, p. 277-283. http://dx.doi.org/10.1016/j.jfca.2004.06.012

Odedej, I, J. O., Oyeleke, W. A. 2011. Comparative studies on functional properties of whole and dehulled cowpea seed flour (*Vigna unguiculata*). *Pakistan Journal of Nutrition*, vol. 10, no. 9, p. 899-902.

Pandey, K. B., Rizvi, S. I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, vol. 2, no. 5, p. 270-278. http://dx.doi.org/10.4161/oxim.2.5.9498

Petchiammal, C., Waheeta, H. 2014. Antioxidant activity of proteins from fifteen varieties of legume seeds commonly consumed in India. *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 6, suppl. no 2, p. 476-479.

Rattan, R. K., Datta, S. P., Chhonkar, P., K. Suribabu, K., Singh, A., K. 2005. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater-a case study. *Agriculture Ecosystems and Environment*, vol. 109, no. 3-4, p. 310-322. http://dx.doi.org/10.1016/j.agee.2005.02.025

Scalbert, A., Manach, C., Morand, C., Reme'sy, C., Jime'nez, L. 2005. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*, vol. 45, no. 4, p. 287-306. http://dx.doi.org/10.1080/1040869059096 PMID:16047496

Sebastiá, V., Barberá, R., Farré, R., Lagarda, M. J. 2001. Effects of legume processing on calcium, iron and zinc contents and dialysabilities. *Journal of the Science of Food and Agriculture*, vol. 81, no. 12, p. 1180-1185. http://dx.doi.org/10.1002/jsfa.927

Seo, O. N., Kim, G. S., Park, S. Lee., J. H., Kim, Y. H., Lee, W. S., Lee, S. J., Kim, C. Y., Jin, J. S., Choi, S. G., Shin, S. C. 2012. Determination of polyphenol components of *Lonicera japonica* Thunb. using liquid chromatography tandem mass spectrometry: Contribution to the overall antioxidant activity. *Food Chemistry*, vol. 134, no. 1, p. 572-577. http://dx.doi.org/10.1016/j.foodchem.2012.02.124

Singh, D., Nath, K., Sharma, Y. K. 2007. Response of wheat seed germination and seedling growth under copper stress. *Environmental Biology*, vol. 28, no. 2, p. 409-414.

Singh, J., Basu, P. S. 2012. Non-Nutritive Bioactive Compounds in Pulses and Their Impact on Human Health: An Overview. *Food and Nutrition Sciences*, vol. 3, no. 12, p. 1664-1672. http://dx.doi.org/10.4236/fns.2012.312218

Sinha, S., Gupta, A., K. 2005. Assessment of metals in leguminous green manuring plant *Sesbaniacannabina* L. grown on Fly ash amended soil: effect on antioxidants. *Chemosphere*, vol. 61, no. 8, p. 1204-1214. http://dx.doi.org/10.1016/j.chemosphere.2005.02.063

Somer, E. 1974. Toxic potential of trace metals in foods. A review. *Journal of Food Science*, vol. 39, no. 2, p. 215-217. http://dx.doi.org/10.1111/j.1365-2621.1974.tb02860.x

Sreerama, Y. N., Sashikala, V. B., Pratape, V. M., Singh, V. 2012. Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. *Food Chemistry*, vol. 131, no. 2, p. 462-468. http://dx.doi.org/10.1016/j.mrfmmm.2005.03.023

Steenland, K. Boffetta, P. 2000. Lead and cancer in humans: where are we now? *American Journal of Industrial Medicine*, vol. 38, no. 3, p. 295-299. http://dx.doi.org/10.1002/1097-0274(200009)38:3<295::AID-AJIM8>3.0.CO;2-L

Tharanathan, R. N., Mahadevamma, S. 2003. Grain legumes a boon to human nutrition. *Trends in Food Science and Technology*, vol. 14, no.12, p. 507-518. http://dx.doi.org/10.1016/j.tifs.2003.07.002

Troszyńska, A., Ciska, E. 2002. Phenolic compounds of seed coats of white and colored varietes of pea and their total antioxidant activity. *Czech Journal of Food Science*, vol. 20, no. 1, p. 15-22.

Vidal-Valverde, C. Frias, J. Estrella, I., Gorospe, M. J. Ruiz, R. Bacon, J. 1994. Effect of processing on some antinutritional factors of lentils. *Journal of Agricultural and Food Chemistry*, vol. 42, no. 10, p. 2291-2295. http://dx.doi.org/10.1021/jf00046a039

Vucenik, I., Shamsuddin, A., M. 2006. Protection against cancer by dietary IP6 and inositol. *Nutrition and Cancer*, vol. 55, no. 2, p. 109-125. http://dx.doi.org/10.1207/s15327914nc5502_1 PMid:17044765

WHO. 1992. International Programme on Chemical Safety (IPCS) 1992. Cadmium. Environmental Health Criteria 134. World Health Organisation. Geneva.

WHO. 1995. Lead. Environmental Health Criteria, vol. 165, Geneva. Available at: http://www.inchem.org/documents/ehc/ehc/ehc165.htm

Wu, Leung., W. T., Butrum, R. 1972. Proximate composition, mineral and vitamin contents of East Asian foods. In: *Food composition table for use in East Asia*. Bethesda, M. D: Food and Agriculture Organization (FAO)

and United State Department of Health, Education and Welfare, p. 185-187.

Xu, B. J., Yuan, S. H., Chang, S. K. C. 2007a. Comparative studies on the antioxidant activities of nine common food legumes against copper-induced human low-density lipoprotein oxidation in vitro. *Journal of Food Science*, vol. 72, no. 7, p. 522-527. http://dx.doi.org/10.1111/j.1750-3841.2007.00464.x

Xu, B. J., Yuan, S. H., Chang, S. K., C. 2007b. Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *Journal of Food Science*, vol. 72, no. 2, p. 167-177. http://dx.doi.org/10.1111/j.1750-3841.2006.00261.x

Yang, G. F., Liu, Q. S., Zhang, C. G., Shang, S. H., Jing, Q. N., Xu, H. X. 1986. The effect of lead cadmium mineral oil in soil on soil microorganism and soil enzyme. In Xia Z. L. (Ed.) *Study of Soil Environmental Capacity*. Beijing : Meteorological Publishing House, p. 157-164.

Zrůst, J. 2003. The risk of potato growing in soils contaminated with heavy metals (In Czech: Riziko pěstování brambor v půdáchfytosanitární a životního prostředí). Vědecký výbor fytosanitární a životní- ho prostředí, Praha, Czeh Republic, 36 p.

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RISK OF AGRICULTURAL PRODUCTION IN RUSSIAN OREL REGION

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ABSTRACT

The paper evaluates the risk of agricultural farms in Russian Orel region by using the modified Markowitz portfolio theory. We analyse individual farm data of agricultural animal and crop production with respect to yield, price and revenue agricultural risk. Farms included in the analyses represent four organizational legal forms and the range of agricultural products produced by these farms is wide. Therefore the research focused on the grain and milk production only. Over the period 2010 to 2014 the effects of Russian ban on import of agricultural products from EU can be observed in form of increased price level of individual commodity prices. Risk and return are negatively related and investors are comparing the risk with profitability. The same stands for farmers. They select the type of production based on expected return. The result show that the systemic yield, price and revenue risk of grain production is higher when compared to milk production. This is due the nature of animal and crop production. Climate and weather risk has much lower effect on animal production when compared to crop production. Therefore the overall risk of crop production is higher. But farmers consider the risk not isolated but in relation to profitability. The profitability of crop production is higher as in Orel region more than 90% of agricultural production is not animal related and farms are profitable with and also without subsidies. Our empirical study shows that in case of equal expected profitability animal production is more profitable for the farmer as it is linked to lower yield, price and revenue risk.

Keywords: Markowitz; profitability; risk; production; price; yield

INTRODUCTION

In the last years the agro-industrial complex of the Russian Federation faces serious restructuring, namely the transformation into an independent sector of the economy. These changes are due to a number of macroeconomic factors, such as: reduction of oil prices, depreciation of the national currency, the growth of the consumer price index, a ban on the import of consumer goods and raw materials, increase in the refinancing rate and many other effects of economic and political sanctions on Russian economy. Generaly in many transition economies agricultural production is adapting to domestic demand influenced by the lower purchasing power of population and by changes that occurred in the structure of consumption and in consumer behaviour of the population **(Michalski, 2015)**. Crop and also milk production is changing due to globalization **(Mura et al., 2012)**. Milk production exports increases and country competitiveness in exporting milk changes over time **(Mura, 2011)**. One of the reason are also changes in the consumption and consumer preferences **(Kubicová and Habánová, 2012)**.

All the changes in Russian economy contribute to a series of macro-economic structural changes in agriculture, such as:

- Providing food security by reducing dependence on imported goods and services;
- Increasing profitability and efficiency of agrarian sector of the economy, by means of increasing the volume of production and sales of agricultural products;
- Improving the quality of products, due to the modernization (machinery and technology);
- Reducing the dependence of the Russian economy on raw materials industry and the influx of capital in other areas of production, including agriculture;
- Increasing level of state support of agricultural producers;
- Implementation of the results of science and research in production, processing and storage of agricultural products.

Each of the presented changes can improve the agricultural economy and the Russian economy as a whole. However, success of each of these factors is only possible in the complex of all the others.

One of the aspect which influences the success of a farm is the risk management. Farms are generally affected by various types of risks. In the paper we focus on the risk of Russian agricultural farms engaged in primary production in Orel region.

Risk generally refers to deviation of the evaluated indicator, and its level depends on the volatility over a certain period. Risk in agriculture has been a matter of worldwide concern since 1933, when the concept of risk analysis had been introduced **(Hardaker et al., 2004)**. Agriculture is a sector facing particularly large risks, resulting mainly from natural factors outside the control of farmers. The sources of risks, that are relevant in agriculture have different characteristics, and can be classified in very different ways **(Huirne et al., 2000; Holzman and Jorgensen, 2001)**. Production or yield risk occurs because agriculture is affected by many uncontrollable events that are often related to weather, including excessive or insufficient rainfall, extreme temperatures, hail, insects, and diseases **(Miller et al.,**

2004). For crops, common causes of yield risk include weather events (drought, excess moisture, hail, freeze and flooding), crop pests and disease. Livestock production losses are much less frequent than crop production losses, and tend to be due to disease outbreaks, weather-related perils or predators. Production risk is likely to grow, due to climate change and globalisation **(Kahn and Zaks, 2009; Heymann, 2007)**. Price risk refers to variability in output and input prices. Variability in fuel prices and in fertilizer prices appear to be the main components of input price variability in crop production, partly because fuel and fertilizer amount to most of the input costs in conventional agriculture, and partly because, as commodities themselves, they are subject to price fluctuations. This variability is expected to increase, in line with increased volatility of energy prices. In the livestock sector, input costs amount predominantly to feed costs **(Kimura et al., 2010)**. Output price risk arises due to the biological lag inherent in agricultural production. During this period, output prices may change dramatically in response to shocks in supply and demand. This may put farmers in a difficult situation if commodity prices decrease drastically during the production and marketing cycle, as observed also during the food commodity price spike in 2007/2008. Price and production risk are two important components of revenue risk. Unpredictable variations in farm revenues can reduce the ability of farm businesses to invest in order to improve productivity and profitability, and consequently affect the future economic welfare of those working in agriculture.

Direct sources of risk not only for Russian agriculture are the climate change, price fluctuations and foreign exchange markets, the violation of the organization of technological operations, negative epidemiological situation and many other factors.

Within this concept, there are many different approaches for assessing the impact of risk on the activities of the organization. Talking about the systematic risk which refers to the general level and not individual or farm level the concept of diversification is applied. One of them is the Markowitz portfolio theory. Its essence lies in the fact that the risk is a standard measurement of the medium-dispersion model and the standard deviation of return on the company's shares **(Markowitz, 1952)**.

The risk analysis of agriculture, using the Markowitz approach or Single index model, has been applied to a number of studies. They mainly focused on the certain part of agriculture production, for example, **Peterson and Leuthold (1987)** used the portfolio approach to examine the cattle feeding problem, **Sanchirico et al., (2005)** use portfolio theory to develop optimal management of fisheries, **Gempesaw et al., (1988)** applied the model to Delaware farm sector market portfolio or in more recent study **Libbin et al., (2004)** applied the Markowitz portfolio model directly to a series of New Mexico farms and many other studies could be mentioned.

This paper is the extension of our previous study **(Tóth et al., 2014)** and we focus on the study of yield, price, and revenue risk. The main purpose is to evaluate the above-mentioned risks of Russian agricultural farms in the Orel region over the period 2010 – 2014. We use the alternative approach based on the Markowitz portfolio theory.

MATERIAL AND METHODOLOGY

The data used for the analysis are individual data of agricultural farms of Orel Region (Russia) for the period 2010 – 2014. Orel region is located in western part of Russian federation with total area of 24 700 km^2. Farms included four organizational and legal forms: agricultural cooperatives, partnerships, limited liability companies and joint stock companies. Since the range of agricultural products produced by these farms is wide, the selection criterion was the obligatory presence of the product in all years. We focus on grain and milk production only.

The modified Markowitz portfolio theory approach was used to assess the agricultural enterprises of Orel region (Russia), namely to assess the yield risk, price risk and revenue risk in the Orel region of farm i. Yield risk is measuring the volatility of tons over the observed period. Price risk is focusing on the volatility of prices of the agricultural commodity. Revenue risk combines the production with prices and measures the volatility of the revenue from hectare (grain) or head (milk).

$$Yield^{\,i} = \frac{Production\ output}{Sowing\ area\ (or\ livestock\ animals)}$$

$$Price^{\,i} = \frac{Revenues\ from\ sales}{Production\ output}$$

$$Revenue^{\,i} = Yield * Price$$

The modified Markowitz portfolio theory approach was used to estimate the total yield, price, revenue risk. The calculation is based on the average value EX of the evaluated indicator X (Yield, Price, Revenue) of individual farm i:

$$EX_i = \sum_{i=1}^{t} X_i \cdot d_i$$

Where Xi is indicator of farm "i", di is a weight of Xi over the observed period (5 years, di = 0.20), t is number of years in observed period, i, j are individual farms. The individual risk of each farm (σ_i) is calculated using the standard deviation.

$$\sigma_i = \sqrt{\sum_{i=1}^{t} (X_i - EX_i)^2 \cdot d_i}$$

Where σi is standard deviation of the individual indicator X (individual farm risk), Xi is individual farm indicator, EXi is average individual farm indicator.

The portfolio risk (σ_p) is determined by three variables: weight of the individual farm in portfolio (wi), standard deviation of individual risk (σi), and covariance, relation between the Xi and Xj (σij). To take into account market portfolio of all agriculture farms, the weight wi of each farm is determined by farm market share on the specific market in Orel Region. The covariance represents the relationship between returns and Σ covariance matrix. The portfolio risk is then measured according to eq. for σ_p.

$$\sigma_{ij} = \frac{1}{n} \sum_{i=1}^{n} (X_i - EX_i)(X_j - EX_j)$$

$$\Sigma = \begin{bmatrix} \sigma_{11}\,\sigma_{12} & \sigma_{13} & \dots & \sigma_{1k} \\ \sigma_{21}\,\sigma_{22} & \sigma_{23} & \dots & \sigma_{2k} \\ \sigma_{31}\,\sigma_{32} & \sigma_{33} & \dots & \sigma_{3k} \\ & \cdots & & \\ \sigma_{k1}\,\sigma_{k2} & \sigma_{k3} & \dots & \sigma_{kk} \end{bmatrix}$$

$$\sigma_p = \sqrt{\sum_{i=1}^{n} w_i^2 \cdot \sigma_i^2 + \sum_{i=1}^{n} \sum_{j=1}^{n} w_i \cdot w_j \cdot \sigma_{ij}}$$

Where wi is an individual weight of i-farm in a portfolio and n is number of farms.

The expected portfolio yield, price and revenue is estimated by the multiplication of k x 1 vector of individual weight of farm in portfolio (w) and k x 1 vector of corresponding individual expected indicator (the sum of multiplication of each farm´s expected X and its share in the market).

$$EX_p = \sum_{i=1}^{n} EX_i \cdot w_i$$

Where EXp is expected portfolio yield, price and revenue and EXi is the average yield, price and revenue of individual farm. Finally to compare the relative extent of the risk coefficient of variation was used.

$$CV_p = \frac{\sigma_p}{EX_p}$$

Markowitz portfolio theory has several assumptions describing the behaviour of rational investor. The paper does not focus on the investment choice and decision making process of investor, as well as the efficient frontier modelling, but uses the theory as a tool to collect individual farms into common portfolio for risk assessment. Therefore the non-compliance of the assumptions of theory is not considered to have a negative effect on the results.

RESULTS AND DISCUSSION

Agro-industrial complex of the Russian Federation is a complex economic structure whose primary purpose is the production, storage, transportation and marketing of agricultural products. It consists of three units:

- Organizations involved in the production of capital goods (fixed assets, raw materials, etc.), required for the production of agricultural products;
- Organizations that are directly involved in agricultural production (production of livestock and crop production);
- Processing organization.

The main part of risk is linked to farms directly involved in the production of goods, as they are facing a variety of climatic and economic risks. Therefore, research of agricultural risks should be carried out on the example of such a farms. Table 1 shows the dynamics of the financial performance of farms in the Orel region.

The table 1 reflects the decrease in the number of farms and the relative stable share of profitable farms with an average of 76.8% in the analyzed period. Financial indicators show the increase in average profitability and also the ability to generate profit without subsidies in last three years. One of the reason for the increase in 2014 is the ban on agricultural imports from EU and other countries. The structure of agricultural production in Orel region is presented in Table 2. Agricultural crop production in Orel region is focused on grain and sugar beet production. These two crops amount to 90% of the total agricultural production. Animal production is less than 10% and it is dominated by milk production. For our analysis we selected grain and milk production with the aim to compare risk and profit of crop and animal production in Russian Orel region.

Table 1 The dynamics of financial performance of farms in Orel region.

Indicators	**Years**				
	2010	**2011**	**2012**	**2013**	**2014**
Farms	214	186	197	171	173
- Out of them profitable	151	148	157	123	140
Share of profitable on all, %	71	80	80	72	81
Return on costs, %	11.8	15.9	25.5	14.2	23.9
Net profit margin(profit/assets), %	5.0	10.1	17.7	10.4	19.1
Net profit margin without subsidies, %	(7.3)	(3.0)	8.9	0.6	11.8

Source: Territorial body of the Federal State Statistics Service of the Orel region.

Table 2 Structure of agricultural production Orel Region, %.

Types of products	**2010**	**2011**	**2012**	**2013**	**2014**
Grain	55.40	40.50	43.88	47.24	57.33
Beet sugar	31.68	50.10	46.80	44.65	34.98
Sunflower seeds	0.57	1.88	1.57	2.03	1.81
Potatoes	0.67	1.05	1.91	1.30	1.21
Vegetables	0.18	0.18	0.13	0.14	0.12
Fruits and berries	0.07	0.03	0.07	0.03	0.06
Cattle and poultry	2.32	1.41	1.31	1.20	1.42
Milk	6.04	4.00	3.61	2.88	2.54
Eggs	1.83	0.59	0.51	0.45	0.45
Wool	0.13	0.15	0.10	0.07	0.04
Honey	1.11	0.12	0.10	0.02	0.04

Source: Territorial body of the Federal State Statistics Service of the Orel region.

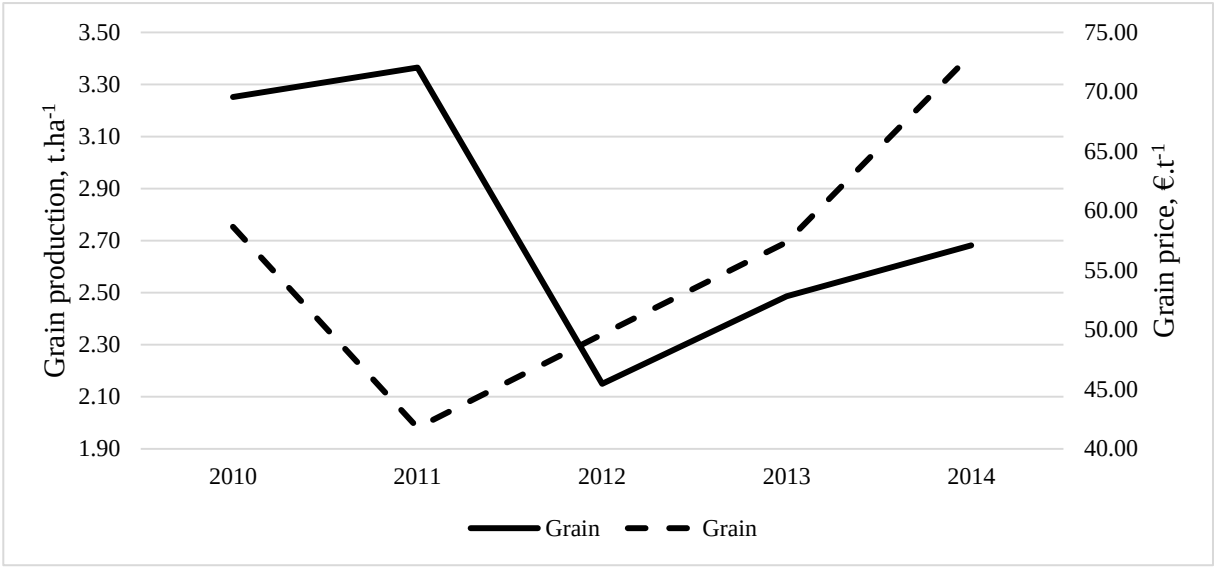

Figure 1 Grain production and price in Orel region.
Source: own processing.

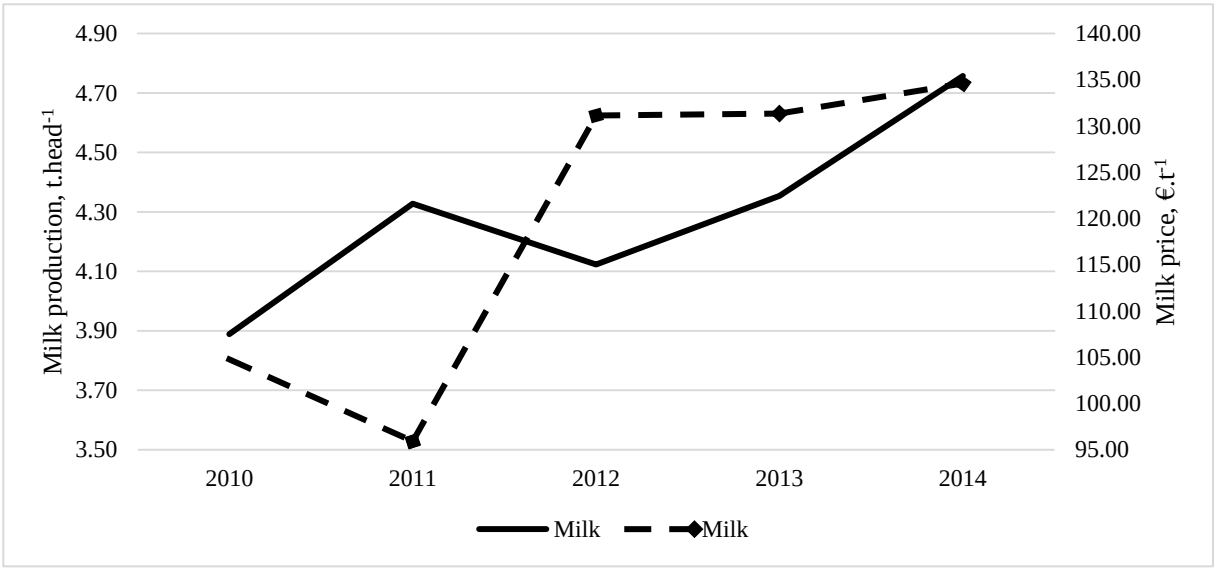

Figure 2 Milk production and price in Orel region.
Source: own processing.

Risk of Russian farms in Orel region

To assess the risks of farms in the Orel region we selected farms operating in each year of the observed period producing grain or milk in each year (2010 – 2014). We included 40 farms in the calculation of grain related risk and 32 farms in the calculation of milk related risk.

The average price and yield developments of grain and milk in Orel region are in figure 1 and 2. Both types of production are volatile with respect to ton per ha, ton per head and with respect the price. For the price we can see an increase in case of milk and grain from 2013 to 2014. Year 2014 was in Russia specific for the ban on agricultural imports from EU and Russian farms were benefitting in form of higher prices of agricultural commodities.

The differences in risk between milk and grain production were reflecting the individual changes in yields, prices and were cumulated by the Markowitz portfolio theory. The results measure the volatility on the level of systematic risk.

Direct calculation of each type of the risk using the Markowitz portfolio theory was performed in ton per hectare in case of grain and in tons per head in case of milk (Table 3). The methodology decreases the individual farm risk to the level of systematic or so called market risk. Based on the results it is possible to compare the yield, price and revenue risk between crop (represented by grain) production and animal (represented by milk production) production in the Orel region. The yield risk of grain in Orel region was measured by the volatility per hectare (Table 3). The average yield in ton was 2.87ton per ha with risk 0.56 ton per ha in the whole Orel region. Milk production is less risky with the results 4.21 ton per head and risk 0.44 ton per head. The best indicator to evaluate the relative size of the risk is variation coefficient. We can conclude that the risk of grain yield was 19.5% while the

Table 3 Risks of farms in the Orel region (Russia).

Risk type		Grain	Number of farms	Milk	Number of farms
Yield risk	Average yield	2.87		4.21	
(in tons/ha or tons/head)	Risk	0.56	40	0.44	32
	Variation coefficient	0.195		0.105	
Price risk	Average price	56.03		119.03	
(in €/ton)	Risk	10.09	40	17.79	32
	Variation coefficient	0.180		0.149	
Revenue risk	Average revenue	160.06		494.63	
(in €/ha or €/head)	Risk	39.72	40	94.55	32
	Variation coefficient	0.248		0.191	

Source: own processing.

risk of milk production 10.5% over the observed period. This is due the nature of animal and crop production. Climate and weather risk has much lower effect on animal production when compared to crop production.

Price risk was measured as the volatility of grain and milk price over the observed period not in individual farm but in the whole Orel region represented by 40 or 32 farms respectively. Grain price fluctuations were higher when compared to milk price. Measured in absolute measures the price risk of grain was 10.09€ per ton with the average 56.03€ per ton. So the relative volatility of grain price was 18% while the relative price volatility of milk was only 14.9%.

The revenue risk covers the volatility of production and price risk. Generally the revenue risk is lower as the sum of yield and price risk as in many cases the correlation is negative. In years of low yields the price is increasing and vice versa. Based on our results we can conclude that crop revenues are more volatile when compared to animal revenues. Grain revenue relative risk was 24.8% over the observed period in Orel region. Milk revenues are less volatile. Measured by variation coefficient the risk was 19.1% in the observed period.

CONCLUSION

Agricultural production is linked to risk. Some of the risks are common with other sectors in the economy and some are unique. Climate and weather related risk have a strong effect on agricultural production. In the paper we focused on the differences in risk between crop and animal production in Orel region over the period 2010-2014. Based on our results we can conclude that the Russian ban on agricultural imports from EU and other countries in 2014 had a positive effect on price development of grain and milk. Prices increased and farmers profitability also. There are differences in relative power of risk between crop production represented by grain and animal production represented by milk. Based on individual data we compared the yield, price and revenue risk in the whole Orel region. We can conclude that grain production is linked to higher yield, price and revenue risk when compared to milk production. Farmers same like investors are not evaluating risk individually. Risk and return are negatively related and investors are comparing the risk with profitability. The same stands for farmers. They select the type of production based on expected return. But the risk is hard to be evaluated individually. Our empirical study shows that in case of equal expected profitability animal production is more profitable for the farmer as it is linked to lower yield, price and revenue risk.

REFERENCES

Gempesaw C. M., Tambe A. M., Nayga R. M., Toensmeyer U. C. 1988. The Single Index Market Model in Agriculture. Northeast Journal of Agricultural and Resource Economics, vol. 17, p. 147-155.

Hardaker J., Huirne R., Anderson J., Lien G. 2004. *Coping with risk in agriculture*. CABI Publishing, ISBN 0-85-199973-5. http://dx.doi.org/10.1079/9780851998312.0000

Heymann, E. 2007. *Climate change and sectors: Some like it hot : working paper*. Frankfurt : Deutsche Bank Research.

Holzman R., Jorgensen S. 2001. Social Risk management: A New conceptual framework for social protection, and beyond. *International Tax and Public Finance*, vol. 8, no. 4, p. 529-556. http://dx.doi.org/10.1023/A:1011247814590

Huirne R., Meuwissen M., Hardaker B., Anderson J. 2000. Risk and risk management in agriculture: an overview and empirical results. *International Journal of Risk Assessment and Management*, vol. 1, no. 1-2, p. 125-136. http://dx.doi.org/10.1504/IJRAM.2000.001491

Kahn, B. M., Zaks, D. 2009. *Investing in agriculture: Far-Reaching challenge, significant opportunity : working paper*. Frankfurt : Deutsche Bank Climate Change Advisors.

Kimura, S., Antón, J., Lrthi, C. 2010. Farm Level Analysis of Risk and Risk Management Strategies and Policies Cross Country Analysis : OECD Food. *Agriculture and Fisheries Papers*, no. 26, 55 p.

Kubicová, Ľ., Habánová, M. 2012. Development of milk consumption and marketing analysis of its demand. *Potravinarstvo*, vol. 6, no. 4, p. 66-72. http://dx.doi.org/10.5219/236

Libbin J. D., Kohler J. D., Hawkes J. M. 2004. Does Modern Portfolio Theory Apply to Agricultural Land Ownership? Concepts for Farmers and Farm Managers.

Journal of the American Society of Farm managers and Rural Appraisers, vol. 67, p. 85-96.

Markowitz H. M. 1952. Portfolio Selection. *Journal of Finance*, vol. 7, no. 1, p. 77-91. http://dx.doi.org/10.1111/j.1540-6261.1952.tb01525.x

Michalski G. 2015. Agency Costs in Small and Medium Wood Industry Enterprises with Full Operating Cycle and Cash Levels. *Procedia Economics and Finance*, vol. 34, p. 461-468. http://dx.doi.org/10.1016/S2212-5671(15)01655-X

Miller, A., Dobbins, C., Pritchett, J., Boehlje, M., & Ehmke, C. 2004. Risk management for farmers. Working paper, 04-11.

Mura, L. 2011. Position of competitiveness of Slovak agri-food sector within the V4 countries. *Potravinarstvo*, vol. 5, p. 170-175. Available online: http://www.potravinarstvo.com/dokumenty/mc_februar_2011/pdf/2/mura.pdf

Mura, L., Buleca, J., Zeleňáková, L., Qineti, A., Kozelová, D. 2012. An analysis of selected aspects of international business in Slovak diaries in EU framework. *Mljekarstvo*, vol. 62, no. 3, p. 219-226.

Peterson P. E., Luethold R. M. 1987. A portfolio approach to optimal hedging for a commercial cattle feedlot. *Journal of Futures Markets*, vol. 7, no. 4, p. 443-457. http://dx.doi.org/10.1002/fut.3990070407

Sanchirico J. N., Smith M. D., Lipton D. W. 2005. Ecosystem Portfolios: A Finance-Based Approach to Ecosystem Management. In AERE Workshop 2005: Natural Resources at Risk, Jackson : WY, p. 1-33.

Tóth M., Lančarič D., Piterková A. and Savov R. 2014. Systematic risk in Agriculture: A case of Slovakia. *Agris on-line Papers in Economics and Informatics*, vol 6, p. 185-193.

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START CODON TARGETED (SCOT) POLYMORPHISM REVEALS GENETIC DIVERSITY IN EUROPEAN OLD MAIZE (*ZEA MAYS* L.) GENOTYPES

Martin Vivodík, Zdenka Gálová, Želmíra Balážová, Lenka Petrovičová

ABSTRACT

Maize (*Zea mays* L.) is one of the world's most important crop plants following wheat and rice, which provides staple food to large number of human population in the world. It is cultivated in a wider range of environments than wheat and rice because of its greater adaptability. Molecular characterization is frequently used by maize breeders as an alternative method for selecting more promising genotypes and reducing the cost and time needed to develop hybrid combinations. In the present investigation 40 genotypes of maize from Czechoslovakia, Hungary, Poland, Union of Soviet Socialist Republics, Slovakia and Yugoslavia were analysed using 20 Start codon targeted (SCoT) markers. These primers produced total 114 fragments across 40 maize genotypes, of which 86 (76.43%) were polymorphic with an average of 4.30 polymorphic fragments per primer and number of amplified fragments ranged from 2 (SCoT 45) to 8 (SCoT 28 and SCoT 63). The polymorphic information content (PIC) value ranged from 0.374 (ScoT 45) to 0.846 (SCoT 28) with an average of 0.739. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. The hierarchical cluster analysis showed that the maize genotypes were divided into two main clusters. Unique maize genotype (cluster 1), Zuta Brzica, originating from Yugoslavia separated from others. Cluster 2 was divided into two main clusters (2a and 2b). Subcluster 2a contained one Yugoslavian genotype Juhoslavanska and subcluster 2b was divided in two subclusters 2ba and 2bb. The present study shows effectiveness of employing SCoT markers in analysis of maize, and would be useful for further studies in population genetics, conservation genetics and genotypes improvement.

Keywords: Dendrogram; Maize; Molecular markers; SCoT analysis

INTRODUCTION

Maize (*Zea mays* L.) is one of the world's most important crop plants following wheat and rice, which provides staple food to large number of human population in the world **(Ahmad et al., 2011; Iqbal, et al., 2015).** Determining genetic diversity can be based on agronomic, morphological, biochemical, and molecular types of information, among others **(Goncalves et al., 2009)**. Molecular characterization is frequently used by maize breeders as an alternative method for selecting more promising genotypes and reducing the cost and time needed to develop hybrid combinations **(Garcia et al., 2004)**. In recent years, a number of molecular markers have been employed for genetic diversity evaluation, genetic mapping, and quantitative trait locus analysis. These types of molecular techniques included random amplified polymorphic dna (RAPD) **(Štefúnová et al., 2015)**, amplified fragment length polymorphism (AFLP) **(Molin et al., 2013)**, inter-simple sequence repeat (ISSR) **(Idris et al., 2012; Žiarovská et al., 2013)** and simple sequence repeats (SSR) **(Shehata et al., 2009)**.

Recently, a simple novel DNA marker technique namely start codon targeted (SCoT) polymorphism, was developed by **Collard and Mackill (2009)**. Primers for SCoT marker analysis were designed from the conserved region surrounding the translation initiation codon, ATG **(Joshi et al., 1997; Sawant et al., 1999)**. Single 18-mer oligonucleotides were used as both forward and reverse primer for PCR, and the annealing temperature was set at 50 °C. The amplicons were resolved using standard agarose gel electrophoresis. Suitability of SCoT markers for the construction of genetic maps, fingerprinting and phylogenetic studies has been proved by many authors in many crops, such as tomato **(Shahlaei et al., 2014)**, citrus **(Mahjbi et al., 2015)**, date palm **(Al-qurainy et al., 2015)**, castor (**Kallamadi et al., 2015)** and mango **(Gajera et al., 2014)**.

The goals of this study were to examine the effectiveness of scot markers for analysis of genetic diversity of maize and to study genetic relationships among 40 maize accessions originating from various geographic regions of europe.

MATERIAL AND METHODOLOGY

Plant material: Forty genotypes of old maize lines originating from six different geographical areas (Table 1) (CZE - Czechoslovakia, HUN - Hungary, POL - Poland, SUN – Union of Soviet Socialist Republics, SK – Slovakia, YUG - Yugoslavia) of Europe were obtained from the Gene Bank Praha-Ruzyně (Czech Republic) and from the Gene Bank in Piešťany (Slovakia). Genomic DNA was isolated from the 14 days leaves with GeneJET Plant Genomic DNA Purification Mini Kit.

Table 1 List of 40 analyzed genotypes of maize.

Genotypes	Country of origin	Year of registration
1. Feheres Sarga Filleres	Hungary	1965
2. Mindszentpusztai Feher	Hungary	1964
3. Zakarpatskaja	Union of Soviet Socialist Republics	1964
4. Przebedowska Burskynowa	Poland	1964
5. Krasnodarskaja	Union of Soviet Socialist Republics	1964
6. Mesterhazy Sarga Simaszemu	Hungary	1964
7. Slovenska biela perlova	Czechoslovakia	1964
8. Zuta Brzica	Yugoslavia	1975
9. Zloty Zar	Poland	1964
10. Slovenska Florentinka	Czechoslovakia	1964
11. Juhoslavska	Yugoslavia	1964
12. Kostycevskaja	Union of Soviet Socialist Republics	1964
13. Mindszentpusztai Sarga Lofogu	Hungary	1964
14. Stodnova	Czechoslovakia	1964
15. Slovenska žltá	Slovak Republic	1964
16. Slovenska krajová velkozrná	Slovak Republic	1964
17. Partizanka	Union of Soviet Socialist Republics	1964
18. Voroneskaja	Union of Soviet Socialist Republics	1964
19. Kocovska Skora	Slovak Republic	1964
20. Milada	Czechoslovakia	1964
21. Moldavskaja	Union of Soviet Socialist Republics	1964
22. Bučiansky Konský Zub	Slovak Republic	1964
23. Hodoninský konský zub žltý	Czechoslovakia	1964
24. M Silokukurica	Hungary	1964
25. Valticka	Czechoslovakia	1964
26. Przebedowska Biala	Poland	1964
27. Toschevska	Slovak Republic	1964
28. Šamorinsky konský zub	Hungary	1964
29. Wielkopolanka	Poland	1964
30. Czechnicka	Poland	1964
31. Manalta	Czechoslovakia	1964
32. Zlota gorecka	Poland	1964
33. Celchovicka ADQ	Czechoslovakia	1964
34. Belaja mestnaja	Union of Soviet Socialist Republics	1964
35. Bučanská žltá	Slovak Republic	1964
36. Iregszemeseil 2 hetes	Hungary	1964
37. Dnepropetrovskaja	Union of Soviet Socialist Republics	1964
38. Bezuncukskaja	Union of Soviet Socialist Republics	1964
39. Mikulická	Czechoslovakia	1964
40. Aranyozon sarga lofogu	Hungary	1964

SCoT amplification: A total of 20 SCoT primers developed by **Collard and Mackill (2009)** were selected for the present study (Table 2). Each 15-μL amplification reaction consisted of 1.5 μL (100 ng) template DNA, 7.5 μL Master Mix (Genei, Bangalore, India), 1.5 μL 10 pmol primer, and 4.5 μL distilled water. Amplification was performed in a programmed thermocycler (Biometra, Germany) using the following program: 94 °C for 3 min; 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min; a final extension at 72 °C for 5 min. Amplified products were separated in 1.5% agarose in 1 × TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system UVP PhotoDoc-t® camera system. A dendrogram was constructed based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA). For the assessment of the polymorphism between genotypes maize and usability SCoT markers in their differentiation we used polymorphic information content (PIC) **(Weber, 1990)**.

RESULTS AND DISCUSSION

In this work, all 20 SCoT primers used for analysis of 40 European old maize genotypes produced amplification products and all resulted in polymorphic fingerprint patterns. Twenty primers produced 114 DNA fragments (Figure 1) with an average of 5.7 bands per primer (Table 2). Out of the total of 114 amplified fragments, 86 (76.43 %) were polymorphic, with an average of 4.30 polymorphic bands per primer. From these twenty primers, primers SCoT 28 and SCoT 63, respectively, were the most polymorphic, where 8 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (2) was detected by primer SCoT 45. To determine the level of polymorphism in the analysed group of maize genotypes, polymorphic information content (PIC) was calculated (Table 2).

Table 2 Statistical characteristics of the SCoT markers used in maize.

SCoT Primers	Primer sequence (5´ – 3´)	TNoB	NoPB	PoPB	PIC
SCoT 6	CAACAATGGCTACCACGC	5	4	80.00	0.729
SCoT 8	CAACAATGGCTACCACGT	4	4	100.00	0.652
SCoT 9	CAACAATGGCTACCAGCA	6	4	66.66	0.780
SCoT 12	ACGACATGGCGACCAACG	7	5	71.43	0.715
SCoT 23	CACCATGGCTACCACCAG	7	5	71.43	0.816
SCoT 26	ACCATGGCTACCACCGTC	5	4	80.00	0.714
SCoT 28	CCATGGCTACCACCGCCA	8	5	62.50	0.846
SCoT 29	CCATGGCTACCACCGGCC	6	4	66.66	0.810
SCoT 30	CCATGGCTACCACCGGCG	7	6	85.71	0.825
SCoT 36	GCAACAATGGCTACCACC	7	7	100.00	0.812
SCoT 40	CAATGGCTACCACTACAG	6	5	83.33	0.731
SCoT 44	CAATGGCTACCATTAGCC	4	2	50.00	0.710
SCoT 45	ACAATGGCTACCACTGAC	2	2	100.00	0.374
SCoT 54	ACAATGGCTACCACCAGC	5	3	60.00	0.717
SCoT 59	ACAATGGCTACCACCATC	6	3	50.00	0.794
SCoT 60	ACAATGGCTACCACCACA	6	3	50.00	0.790
SCoT 61	CAACAATGGCTACCACCG	6	5	83.33	0.808
SCoT 62	ACCATGGCTACCACGGAG	4	4	100.00	0.618
SCoT 63	ACCATGGCTACCACGGGC	8	7	87.50	0.832
SCoT 65	ACCATGGCTACCACGGCA	5	4	80.00	0.697
Average		**5.70**	**4.30**	**76.43**	**0.739**
Total		**114**	**86**	**-**	**-**

Note: TNoB – Total number of bands, NoPB – Number of polymorphic bands, PoPB – Percentage of polymorphic bands (%), PIC- Polymorphic information content.

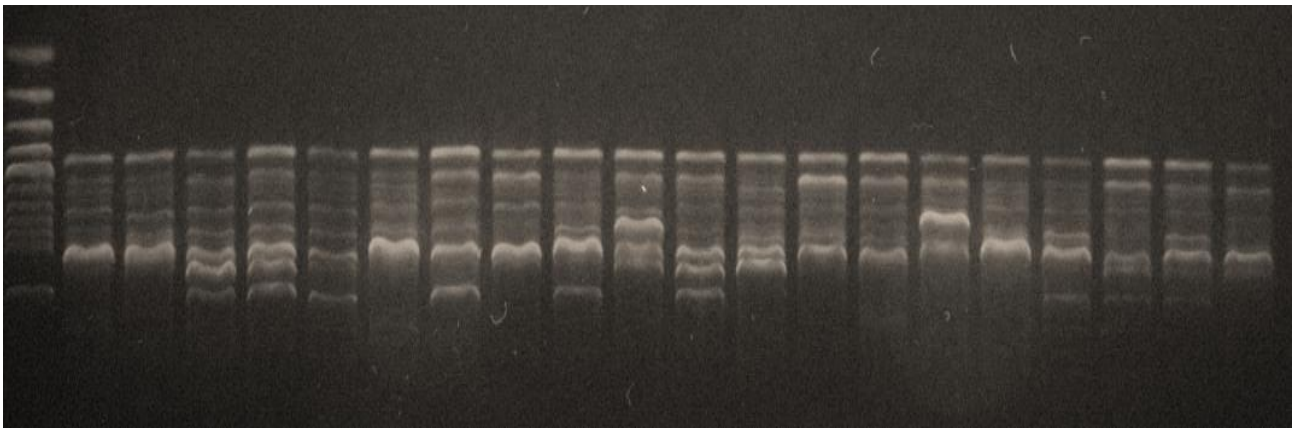

Figure 1 PCR amplification products of 20 genotypes of maize produced with SCoT 54 primer. Lane M is 1-kb DNA ladder and lanes 1-20 are maize genotypes.

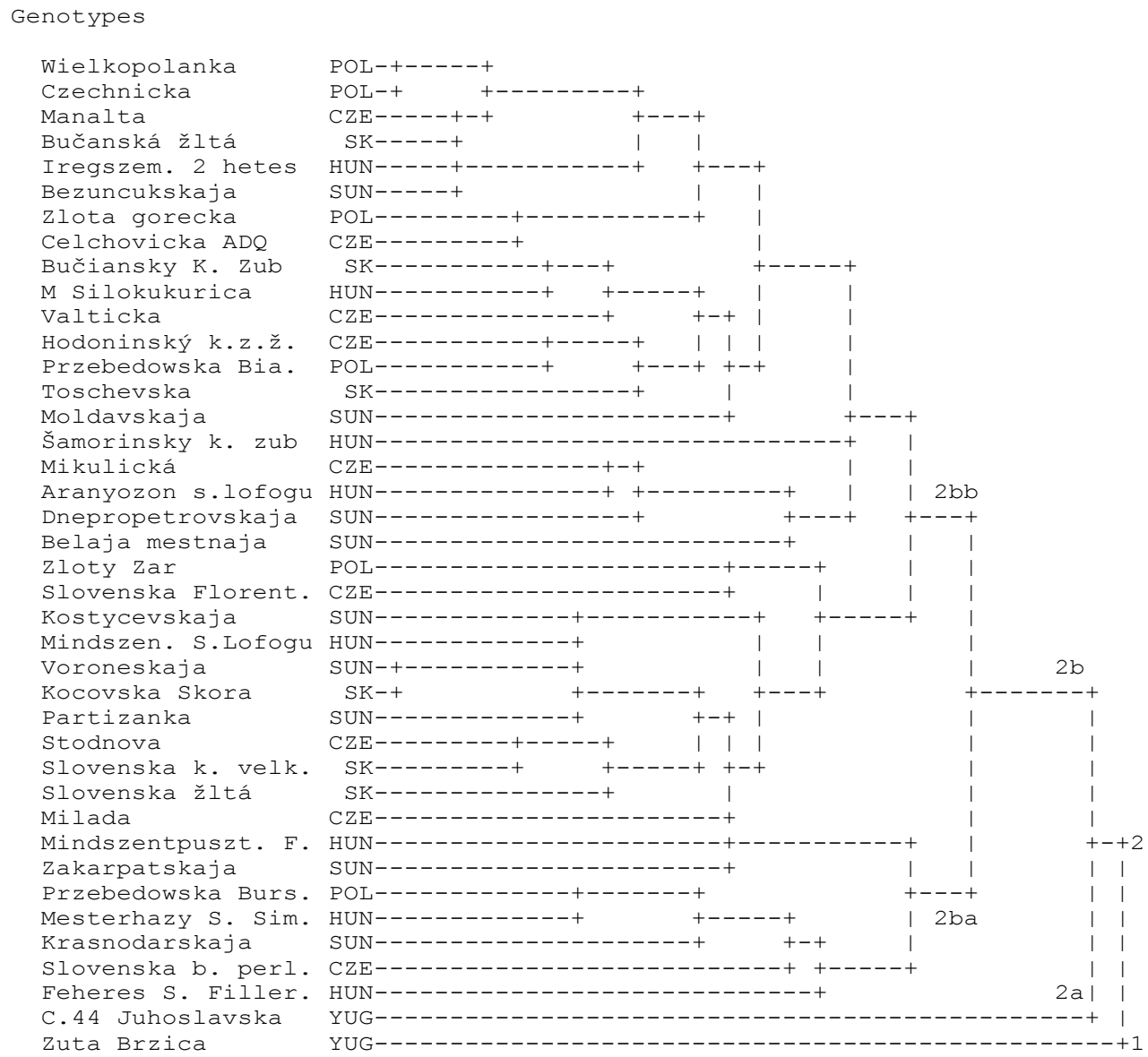

Figure 2 Dendrogram of 40 maize genotypes prepared based on 20 SCoT markers. CZE – Czechoslovakia, HUN – Hungary, POL – Poland, SUN – Union of Soviet Socialist Republics, SK – Slovakia, YUG – Yugoslavia.

The polymorphic information content (PIC) value ranged from 0.374 (SCoT 45) to 0.846 (SCoT 28) with an average of 0.739. The dendrogram of 40 maize genotypes based on SCoT markers using UGMA algorithm was constructed (Figure 2). The hierarchical cluster analysis divided maize genotypes into two main clusters. Unique maize genotype Zuta Brzica, originated from Yugoslavia (cluster 1), separated from others. Cluster 2 containing 39 genotypes was divided into two main subclusters (2a and 2b). Subcluster 2a contained one Yugoslavian genotype Juhoslavanska and subcluster 2b was divided in two subclusters 2ba and 2bb. In the subcluster 2ba were grouped 7 genotypes from Hungary (42.87%), Poland (14.29%), Czechoslovakia (14.29%) and Union of Soviet Socialist Republics (28.58%). Subcluster 2bb of 31 genotypes included genotypes of Polish origin (16.15%), Union of Soviet Socialist Republics origin (22.61%), Slovakia origin (19.38%), Czechoslovak origin (25.84%) and Hungarian origin (16.15%). Two genotypes of 2bb subcluster (Czechnicka and Wielkopolanka) from Poland and two genotypes (Voroneskaja and Kocovska Skora) from Union of Soviet Socialist Republics and Slovakia, respectively, were genetically the closest. We can assume that they have close genetic background.

Level of polymorphism in analysed maize genotypes was determined by calculated polymorphic information content (PIC) (Table 2). The PIC values ranged from 0.374 (SCoT 45) to 0.846 (SCoT 28) with an average of 0.739. Similar values of PIC were detected by other authors **(Luo et al., 2012; Arya et al., 2014; Gajera et al., 2014; Que et al., 2014; Gao et al., 2014; Fang-Yong et al., 2014; Jiang et al., 2014; Huang et al., 2014; Satya et al., 2015)** and these values presented a high level of polymorphism of genotypes detected by SCoT markers**. Huang et al., (2014)** assessed the genetic diversity of six Hemarthria cultivars using seven SCoT primers, which together amplified 105 bands with an average of 15 bands per sample. Start codon-targeted markers were utilized by **Gajera et al., (2014)**

who used 19 SCoT markers for characterization and genetic comparison among 20 mango cultivars. These primers produced total 117 loci across 20 cultivars, of which 96 (79.57 %) were polymorphic. In the study **Que et al., (2014)**, used 20 start codon targeted (SCoT) marker primers to assess the genetic diversity among 107 sugarcane accessions within a local sugarcane germplasm collection. These primers amplified 176 DNA fragments, of which 163 were polymorphic (92.85%). The aim of **Gao et al., (2014)** was to estimate the genetic diversity across 43 varieties of Lycoris. Of 57 SCoT primers screened, 23 SCoT primers were identified to be high polymorphism. **Fang-Yong et al., (2014)** assessed the genetic diversity of 31 germplasm resources of Myrica rubra from Zhejiang Province, the major gathering site and the largest producer of M. rubra in China using start codon-targeted polymorphism (SCoT) markers. Authors used 38 primers to perform PCR amplification of 31 genotypes, from which 298 reproducible bands were obtained, including 251 polymorphic bands (84.23%). **Satya et al., (2015)** used 24 start codon targeted (SCoT) markers to assess genetic diversity and population structure of indigenous, introduced and domesticated ramie (Boehmeria nivea L. Gaudich.). **Jiang et al., (2014)** used start codon-targeted (SCoT) markers to analyze the diversity and genetic relationships among 95 orchardgrass accessions. In total, 273 polymorphic bands were detected with an average of 11.4 bands per primer. In the study **Zhang et al., (2015)** used SCoT markers to study the genetic diversity and relationships among 53 Elymus sibiricus accessions.

Studies of genetic diversity across individuals of plant have been realized by different PCR-based DNA marker methods: random amplified polymorphic DNA (RAPD) **(Molin et al., 2013; Balážová et al., 2016; Kuťka Hlozáková et al., 2016)**, simple sequence repeat (SSR) **(Terra et al., 2011; Molin et al., 2013; Gálová et al., 2015; Balážová et al., 2016)**, amplified fragment length polymorphism (AFLP) **(Molin et al., 2013),** inter-simple sequence repeat (ISSR) **(Žiarovská et al., 2013; Molin et al., 2013)**. These methods are technically simple, fairly cheap and generate a relatively large number of markers per sample. **Molin et al., (2013)** pointed that in general, a higher number of investigated accessions and more varied genetic background result in a higher expected polymorphic rate. Start codon targeted polymorphism (SCoT) is a simple and novel marker system first described by **Collard and Mackill (2009)**, which is based on the short conserved region flanking the ATG translation start codon in plant genes. The higher primer lengths and subsequently higher annealing temperatures ensure higher reproducibility of SCoT markers, compared to RAPD markers **(Rajesh et al., 2015)**. **Gorji et al., (2011)** presented that SCoTs markers were more informative and effective, followed by ISSRs and AFLP marker system in in fingerprinting of potato varieties.

CONCLUSION

The present work is the first report on genetic variability of maize using SCoT markers. In summary, SCoT marker analysis was successfully developed to evaluate the genetic relationships among the genus maize accessions originated from various regions. The hierarchical cluster analysis showed that the maize genotypes were divided into 2 main clusters. One maize genotype Zuta Brzica, origin from Yugoslavia (cluster 1), was separated from others. Cluster 2 was divided into two main clusters (2a and 2b). Four genotypes of 2bb subcluster (Czechnicka and Wielkopolanka) from Poland and two genotypes (Voroneskaja and Kocovska Skora) from Union of Soviet Socialist Republics and Slovakia, respectively, were genetically the closest. Polymorphism revealed by SCoT technique was so abundant and could be used for molecular genetics study of the maize accessions, providing high-valued information for the management of germplasm, improvement of the current breeding strategies, and conservation of the genetic resources of maize species.

REFERENCES

Ahmad, S., Khan, S., Ghaffar, M., Ahmad, F. 2011. Genetic diversity analysis for yield and other parameters in maize (*Zea mays* L.) genotypes. *Asian Journal Agriculture Science*, vol. 3, no. 5, p. 385-388.

Al-Qurainy, F., Khan, S., Nadeem, M. and Tarroum, M. 2015. SCoT marker for the assessment of genetic diversity in Saudi Arabian date palm cultivars. *Pakistan Journal of Botany*, vol. 47, no. 2, p. 637-643.

Arya, L., Narayanan, R. K., Verma, M., Singh, A. K., Gupta, V. 2014. Genetic diversity and population structure analyses of *Morinda tomentosa* Heyne, with neutral and gene based markers. *Genetic Resources and Crop Evolution*, vol. 61, no. 8, p. 1469-1479. http://dx.doi.org/10.1007/s10722-014-0168-4

Balážová, Ž., Vivodík, M., Gálová, Z. 2016. Evaluation of molecular diversity of central European maize cultivars. *Emirates Journal of Food and Agriculture*, vol. 28, no. 2, p. 93-98.

Balážová, Ž., Petrovičová, L., Gálová, Z., Vivodík, M. 2016. Molecular characterization of rye cultivars. *Potravinarstvo*, vol. 10, no. 1, p. 54-58. http://dx.doi.org/10.5219/522

Collard, B. C. Y. and Mackill, D. J. 2009. Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter*, vol. 27, p. 86-93. http://dx.doi.org/10.1007/s11105-008-0060-5

Fang-Yonga, Ch. and Ji-Honga, L. 2014. Germplasm genetic diversity of *Myrica rubra* in Zhejiang Province studied using inter-primer binding site and start codon-targetedpolymorphism markers. *Scientia Horticulturae*, vol. 170, p. 169-175. http://dx.doi.org/10.1016/j.scienta.2014.03.010

Gajera, H. P., Bambharolia, R. P., Domadiya, R. K., Patel, S. V., Golakiya, B.A. 2014. Molecular characterization and genetic variability studies associated with fruit quality of indigenous mango (*Mangifera indica* L.) cultivars. *Plant Systematics and Evolution*, vol. 300, no. 5, p. 1011-1020. http://dx.doi.org/10.1007/s00606-013-0939-y

Gao, Y. H., Zhu, Y. Q., Tong, Z. K., Xu, Z. Y., Jiang, X. F., Huang, CH. H. 2014. Analysis of genetic diversity and relationships among genus *Lycoris* based on start codon targeted (SCoT) marker. *Biochemical Systematics and Ecology*, vol. 57, p. 221-226. http://dx.doi.org/10.1016/j.bse.2014.08.002

Gálová, Z., Vivodík, M., Balážová, Ž., Kuťka Hlozáková, T. 2015. Identification and differentiation of *Ricinus communis* L. using SSR markers. *Potravinarstvo*, vol. 9, no. 1, p. 556-561. http://dx.doi.org/10.5219/516

Goncalves, L. S., Rodrigues, R., do Amaral Junior, A. T., Karasawa, M., Sudre, C. P. 2009. Heirloom tomato gene bank: Assessing genetic divergence based on morphological,

agronomic and molecular data using a Ward-modified location model. *Genetics and Molecular Research*, vol. 8, no. 1, p. 364-374. http://dx.doi.org/10.4238/vol8-1gmr549 PMid:19440972

Gorji, A. M., Poczai, P., Polgar, Z., Taller, J. 2011. Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. *American Journal of Potato Research*, vol. 88, no. 3, p. 226-237. http://dx.doi.org/10.1007/s12230-011-9187-2

Huang, L., Huang, X., Yan, H., Yin, G., Zhang, X., Tian, Y., Zhang, Y., Jiang, X., Yan, Y., Ma, X., Peng, Y., Zhou, J., Nie, G. 2014. Constructing DNA fingerprinting of *Hemarthria* cultivars using EST-SSR and SCoT markers. *Genetic Resources and Crop Evolution*, vol. 61, no. 6, p. 1047-1055. http://dx.doi.org/10.1007/s10722-014-0107-4

Idris, A. E., Hamza, N. B., Yagoub, S. O., Ibrahim A. I. A. and El-Amin, H. K. A. 2012. Maize (*Zea mays* L.) Genotypes Diversity Study by Utilization of Inter-Simple Sequence Repeat (ISSR) Markers. *Australian Journal of Basic and Applied Sciences*, vol. 6, no. 10, p. 42-47.

Iqbal, J., Shinwari, Z. K., Rabbani, M. A. and Khan, S. A. 2015. Genetic divergence in maize (*Zea mays* L.) germplasm using quantitative and qualitative traits. *Pakistan Journal of Botany*, vol. 47, no. SI, p. 227-238.

Jiang, L. F., Qi, X., Zhang, X. Q., Huang, L. K., Ma, X. and Xie, W. G. 2014. Analysis of diversity and relationships among orchardgrass (*Dactylis glomerata* L.) accessions using start codon-targeted markers. *Genetics and Molecular Research*, vol. 13, no. 2, p. 4406-4418. http://dx.doi.org/10.4238/2014.June.11.4

Joshi, C. P., Zhou, H., Huang, X., Chiang, V. L. 1997. Context sequences of translation initiation codon in plants. *Plant Molecular Biology*, vol. 35, no. 6, p. 993-1001. http://dx.doi.org/10.1023/A:1005816823636 PMid:9426620

Kallamadi, P. R., Ganga Rao Nadigatlab, V. P. R., Mulpurib, S. 2015. Molecular diversity in castor (*Ricinus communis* L.). *Industrial Crops and Products*, vol. 66, p. 271-281. http://dx.doi.org/10.1016/j.indcrop.2014.12.061

Kuťka-Hlozáková, T., Gálová, Z., Gregová, E., Vivodík, M., Balážová, Ž., Miháliková, D. 2016. RAPD analysis of the genetic polymorphism in European wheat genotypes. *Potravinarstvo*, vol. 10, no. 1, p. 1-6. http://dx.doi.org/10.5219/520

Luo, C., He, X. H., Chen, H., Hu, Y., Ou, S. J. 2012. Genetic relationship and diversity of *Mangifera indica* L.: revealed through SCoT analysis. *Genetic Resources and Crop Evolution*, vol. 59, no. 7, p. 1505-1515. http://dx.doi.org/10.1007/s10722-011-9779-1

Mahjbi, A., Baraket, G., Oueslati, A., Salhi-Hannachi, A. 2015. Start Codon Targeted (SCoT) markers provide new insights into the genetic diversity analysis and characterization of Tunisian Citrus species. *Biochemical Systematics and Ecology*, vol. 61, p. 390-398. http://dx.doi.org/10.1016/j.bse.2015.07.017

Molin, D., Coelho, C. J., Máximo, D. S., Ferreira, F. S., Gardingo, J. R. and Matiello, R. R. 2013. Genetic diversity in the germplasm of tropical maize landraces determined using molecular markers. *Genetics and Molecular Research*, vol. 12, no. 1, p. 99-114. http://dx.doi.org/10.4238/2013.January.22.8 PMid:23359029

Rajesh, M. K., Sabana, A. A., Rachana, K. E., Rahman, S., Jerard, B. A., Karun, A. 2015. Genetic relationship and diversity among coconut (*Cocos nucifera* L.) accessions revealed through SCoT analysis. *Biotechnology*, vol. 5, no. 6, p. 999-1006. http://dx.doi.org/10.1007/s13205-015-0304-7

Que, Y., Pan, Y., Lu, Y., Yang, C., Yang, Y., Huang, N. and Xu, L. 2014. Genetic Analysis of Diversity within a Chinese Local Sugarcane Germplasm Based on Start Codon Targeted Polymorphism. *Biomed Research International*, vol. 2014, p. 1-10. http://dx.doi.org/10.1155/2014/468375

Satya, P., Karana, M., Jana, S., Mitraa, S., Sharma, A., Karmakar, P. G., Rayb, D. P. 2015. Start codon targeted (SCoT) polymorphism reveals genetic diversity in wild and domesticated populations of ramie (*Boehmeria nivea* L. Gaudich.), a premium textile fiber producing species. *Meta Gene*, vol. 3, p. 62-70. http://dx.doi.org/10.1016/j.mgene.2015.01.003

Sawant, S. V., Singh, P. K., Gupta, S. K., Madnala, R., Tuli, R. 1999. Conserved nucleotide sequences in highly expressed genes in plants. *Journal of Genetics*, vol. 78, no. 2, p. 123-131. http://dx.doi.org/10.1007/BF02924562

Shahlaei, A., Torabi, S., Khosroshahli, M. 2014. Efficiacy of SCoT and ISSR marekers in assesment of tomato (*Lycopersicum esculentum* Mill.) genetic diversity. *International Journal of Biosciences*, vol. 5, no. 2, p. 14-22. http://dx.doi.org/10.12692/ijb/5.2.14-22

Shehata, A. I., Al-Ghethar, H. A., Al-Homaidan, A. A. 2009. Application of simple sequence repeat (SSR) markers for molecular diversity and heterozygosity analysis in maize inbred lines. *Saudi Journal of Biological Sciences*, vol. 16, no. 2, p. 57-62. http://dx.doi.org/10.1016/j.sjbs.2009.10.001 PMid:23961043

Štefúnová, V., Bežo, M., Žiarovská, J., Ražná, K. 2015. Detection of the genetic variability of *Amaranthus* by RAPD and ISSR markers. *Pakistan Journal of Botany*, vol. 47, no. 4, p. 1293-1301.

Terra, T. F., Wiethölter, P., Almeida, C. C., Silva, S. D. A. et al. 2011. Genetic variability in maize and teosinte populations estimated by microsatellites markers. *Ciência Rural*., vol. 41, no. 2, p. 205-211. http://dx.doi.org/10.1590/S0103-84782011005000005

Weber, J. L. 1990. Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics*, vol. 7, no. 4, p. 524-530. http://dx.doi.org/10.1016/0888-7543(90)90195-Z

Zhang, J., Xie, W., Wang, Y. and Zhao, X. 2015. Potential of Start Codon Targeted (SCoT) Markers to Estimate Genetic Diversity and Relationships among Chinese *Elymus sibiricus* Accessions. *Molecules*, vol. 20, no. 4, p. 5987-6001. http://dx.doi.org/10.3390/molecules20045987

Žiarovská, J., Ražná, K. and Labajová, M. 2013. Using of inter microsatellite polymorphism to evaluate gamma-irradiated Amaranth mutants. *Emirates Journal of Food and Agriculture*, vol. 25, no. 9, p. 673-681. http://dx.doi.org/10.9755/ejfa.v25i9.15879

Žiarovská, J., Senková, S., Bežo, M., Ražná, K., Masnica, M., Labajová, M. 2013. ISSR markers as a tool to distinguish Idt and SSS populations of Zea mays L. *Journal of Central European Agriculture*, vol. 14, no. 2, p. 489-499. http://dx.doi.org/10.5513/jcea01/14.2.1227

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THE EFFECT OF POST-HARVEST TREATMENT ON THE QUALITY OF SWEET CHERRIES DURING STORAGE

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ABSTRACT

Cherries are a traditional commodity grown in the Czech Republic. Placing into a cold room is essential for the fruit to be preserved in the long term. Even if optimum storage conditions are followed, the shelf life is relatively short. This study observed the effect of packing cherries into the Xtend polymer wrap on slowing down the degradation of the fruit during the storage period. The experiment was conducted using 4 varieties of the sweet cherry (*Prunus avium* L.) from the identical site (Stošíkovice, Czech Republic) - 'Vanda', 'Kordia', 'Sweetheart' and 'Regina'. Part of the fruit was stored at 20 °C for 7 days (conditions in retail chains) and other part of the fruit was stored at 1 °C for 50 days, first half of fruit was stored in Xtend polymer wrap and second half in the normal air conditions. Changes were also investigated in fruit quality parameters (soluble solids, titratable acidity, weight loss, peel firmness and respiration intensity) under the shelf life conditions when the fruit was placed at the distribution temperature of 20 °C after removal from the store and analysed after 5 and 10 days. Packed fruit exhibited significantly lower weight loss than unpacked fruit. Unpacked fruits showed visible signs of wilting and it is connected to the water loss and loss of turgidity of fruit. Soluble solids content and titratable acidity reduced generally less in unpacked fruit, which was probably related to the higher weight loss in this variant. Between the packaged and control fruit firmness was not statistically significant. Carbon dioxide production characteristic the intensity of respiration was typically higher at 1 °C for fruit packed in the Xtend film. This fruit, however, largely responded by reducing the intensity of respiration when removed from the store and placed at 20 °C, whereas in unpacked fruit there was a several-fold increase in carbon dioxide production under such conditions.

Keywords: *Prunus avium* L.; sweet cherries; Xtend; carbon dioxide; weight loss

INTRODUCTION

In the territory of the Czech Republic, growing sweet cherries (*Prunus avium* L.) has had a long tradition from a historical perspective. The first mention dates from the 14th century. According to 2015 statistics, the production of cherries in the country was more than 9,900 tonnes throughout the total area of 1,429 hectares. Health benefits of cherry fruit are relatively high, mainly due to the content of phenolic compounds, such as procyanidins, anthocyanins and phenolic acids **(Liu et al., 2011; Usenik et al., 2010)**. With the high antioxidant capacity, consumption of cherries has been shown to reduce the risk of cancer **(Kang et al., 2003)** and other diseases **(Jacob et al., 2003)**. The storing potential of cherries is limited and the shelf life is relatively short. Fruit is sensitive to deformation during post-harvest operations and features relatively quick reduction of the flesh firmness **(Ceponis et al., 1987)**. Low temperature is the basic parameter for storing; it keeps the fruit firm longer and reduces the degradation of the colour **(Shick, Toivonen, 2002)**. Reported as recommended conditions to store cherries is the temperature range 0 to 2 °C and the relative humidity of 90% to 95% **(Crisosto et al., 1993; Looney et al., 1996)**. Despite the compliance with the above conditions, the shelf life is shorter compared with other kinds of fruit. The aim of this study was to determine the effect of packing the fruit in the Xtend film on extending the shelf life of cherry fruit while maintaining commercial quality. Films produced using the Xtend technology pass any excess moisture through the walls while keeping the RH value at 90% to 95%. Slowing down the process of biological synthesis and ethylene production in stored fruit, the film can be assumed to have effect on the time of preservation if used.

MATERIAL AND METHODOLOGY

Varieties and storage conditions

The experimental part monitored a total of 4 varieties of the sweet cherry (*Prunus avium* L.) - 'Vanda', 'Kordia', 'Sweetheart' and 'Regina'. The fruit originated from the identical site (Stošíkovice, Czech Republic) and were harvested at the optimum harvest date ('Vanda' - 24 June, 'Kordia' - 30 June, 'Regina' & 'Sweetheart' - 10 July). They were sourced from managed orchards, were undamaged and of adequate quality, with a size tolerance specified for one fruit with a pedicle ('Vanda' 8.9 ±0.3 g, 'Kordia' 10.9 ±0.2 g, 'Regina' 10.75 ±0.25 g, and 'Sweetheart' 9.9 ±0.4) The stalk of each fruit corresponded to the variety.

One third of the fruit was stored at 20 °C for 7 days to simulate conditions of distribution of fruits in retail chains

("shelf life"). The second third of the fruit was stored under refrigerated conditions, i.e. at 1 °C, for 50 days. The surveyed fruit parameters were evaluated after 30 and 50 days. On each sampling, simultaneously, a portion of the fruit was removed from the refrigeration room and placed into 20 °C (shelf life), where the fruit was analysed after 5 and 10 days. The last third of the fruit was stored under the same conditions as the previous part; the fruit, however, was put into a polymer wrap (Xtend) and sealed hermetically.

Weight loss

Each sample for determining the weight loss was made up of 20 pieces of fruit in triplicate. Fruit was weighed on each sampling date as well as under the shelf life conditions. As part of the results the loss was expressed as the percent loss from the initial weight.

Titratable acidity

Total titratable acidity (TTA) was estimated by alkalimeter and expressed in $g.kg^{-1}$ malic acid equivalent of fresh matter, the soluble solids content (SSC) was expressed by the index of refraction (°Bx).

Peel firmness

The determination of peel firmness was done using the TEXAN 2000 device. The fruit was loaded using a punch, a diameter of 5 mm and the loading rate being 8 $mm.min^{-1}$. The evaluation unit for the variety and storage time represented thirty pieces of fruit that were penetrated just from one side of the fruit. The resulting value was expressed in N.

Respiration intensity

The fruit respiration intensity was assessed based on the monitoring of CO_2 production. 200 g of cherries were put into an air-tight container of 1,000 mL; 1 mL sample was taken using a chromatographic syringe (Hamilton Syringe 140 mL HD TCA Analytics AG, Switzerland) once the time of exposure (1 hour) passed. The sprayed volume was determined using a gas chromatography unit (4890D; Agilent Technologies Inc., Wilmington, USA). The measurement of CO_2 production was carried out using the HP Al/KCl column that was connected to a thermal conductivity detector (TCD). Helium was the carrier gas, the flow rate being 1.0 $mL.min^{-1}$. The results were expressed in mg of CO_2 $.kg^{-1}.h^{-1}$.

Statistical analysis

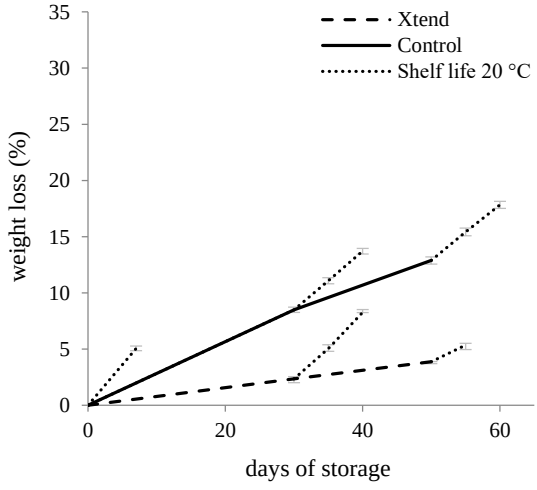
Analysis of variance was conducted and the results were compared using Tukey's multiple range test (p <0.05). Statistical analysis was carried out using the Statistica 12 programme (StatSoft, USA). The obtained results were averaged and standard deviations were calculated.

RESULTS AND DISCUSSION

The loss of weight due to evaporation are clearly distinguished by the variety and ripeness on the tree from which it follows that the early variety 'Vanda' has lost weight up to 9.5% after 7 days of storage at 20 °C; it was demonstrably the largest number compared with other surveyed varieties (Figure 1 – 4). 'Sweetheart' had a weight loss of only 5%, which was significantly the lowest value compared with other varieties. The stalk presence is the essential criterion for minimising losses due to evaporation during the storage of cherry fruit **(Smith and Whiting, 2011)**. For fruit stored under refrigeration conditions differences were observed among different varieties. For 'Vanda', the loss of weight after 30 days was 11.9% when stored in bulk while only 3.2% when wrapped in the Xtend film. Reduction of evaporation was almost ¼ compared with the bulk cherries. The wrapping material had, therefore, a demonstrable effect on lost weight. After removal from the store the loss of weight was identical in both variants under twenty degrees without the use of packaging materials. A further decline was recorded after 50 days of storage, reaching 18.6% of the original weight in case of bulk cherries and 6% when using Xtend. The highest loss of weight after 50 days storage was showed in the fruit of the 'Regina' variety (Figure 2). The weight of unpacked fruit decreased by 23.1% for this variety; when using Xtend, it was only 5.2%. As with the 7-day storage at 20 °C, 'Sweetheart' was the variety to show the least loss of weight after 50 days at 1 °C (12.9% for unpacked fruit, 3.9% for the Xtend wrap).

Soluble solids content in observed varieties ranged from 15.4 for 'Vanda' (Figure 8) to 22.2 °Brix for 'Regina' (Figure 6). 'Kordia' and 'Sweetheart' have an average sugar content of 19.4 °Brix (Figure 7) and 21.88 °Brix (Figure 5), respectively; similar values for these varieties were monitored by authors of other reports **(Harb et al., 2006; Turner et al., 2008)**. During the seven-day storage at 20 °C, the difference between the original and the resulting soluble solids was statistically insignificant; only 'Kordia' had a significantly higher sugar content (0.8 °Brix). For other varieties, there was only a slight increase in sugar content.

For all the varieties, a demonstrable increase in soluble solids was showed only for unpacked fruit after 50 days of storage under refrigerated conditions (1 °C). This was probably due to evaporation of water and concentration of solids. Conversely, for fruit packed using Xtend, where the weight loss was significantly lower, reducing the content of soluble solids was confirmed.



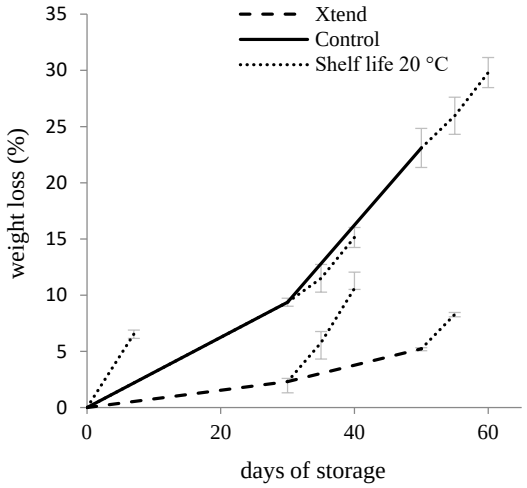


Figure 2 Loss weight for the fruit of the 'Regina' variety when stored at 1 °C, shelf life conditions.

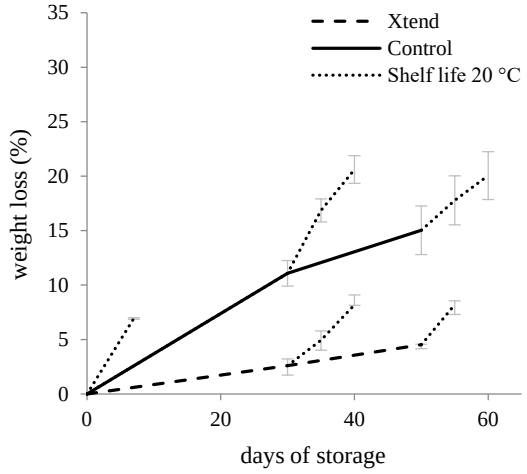


Figure 3 Loss weight for the fruit of the 'Kordia' variety when stored at 1 °C, shelf life conditions.

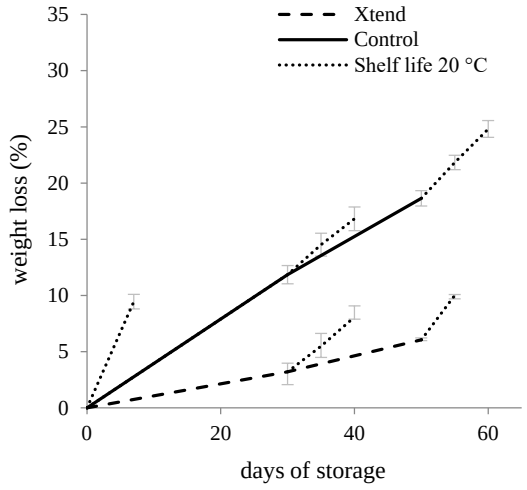


Figure 4 Loss weight for the fruit of the 'Vanda' variety when stored at 1 °C, shelf life conditions.

The reason was probably a negative balance of the rate of ventilating sugars to the cases of the lower loss of weight, which is also confirmed by the results of other studies **(Petersen and Poll, 1999; Ben, 1991)**.

Titratable acidity of the fruit at harvest maturity ranged from a maximum value of 8.1 $g.kg^{-1}$ ('Kordia') up to 5.6 $g.kg^{-1}$ ('Vanda'). During the seven-day storage at 20 °C there was an evidence of decline in titratable acidity observed in the 'Regina' and 'Kordia' varieties (Figure 10 and 11). For 'Sweetheart' and 'Vanda', the latter possessing the lowest acidity, no significant changes were found (Figure 9 and 12). From the results it can be assumed that part of acids are ventilating, causing their decline. In this regard, 'Vanda' and 'Sweetheart' are the varieties that have demonstrably a lesser tendency of ventilating acids compared with 'Regina' and 'Kordia'.

In all the fruit that was stored at 1 °C there was a reduction of titratable acidity after 50 days of storage while the rate of degradation of acids was faster for the fruit packed in Xtend. 'Kordia' was the variety observed to show the greatest loss, when packed fruit contained 2.9 $g.l^{-1}$ and unpacked fruit 1.3 $g.l^{-1}$ less acidity. Fruit that contained lower amounts of titratable

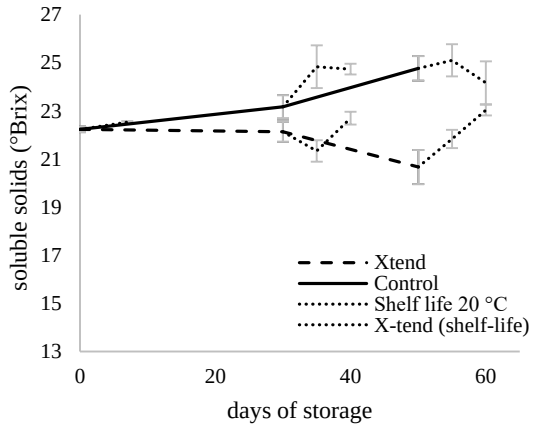


Figure 5 The levels of soluble solids for the fruit of the 'Sweetheart' variety when stored at 1 °C, shelf life conditions.

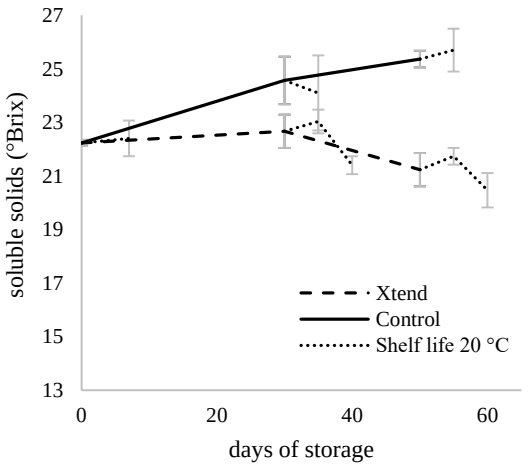


Figure 6 The levels of soluble solids for the fruit of the 'Regina' variety when stored at 1 °C, shelf life conditions.

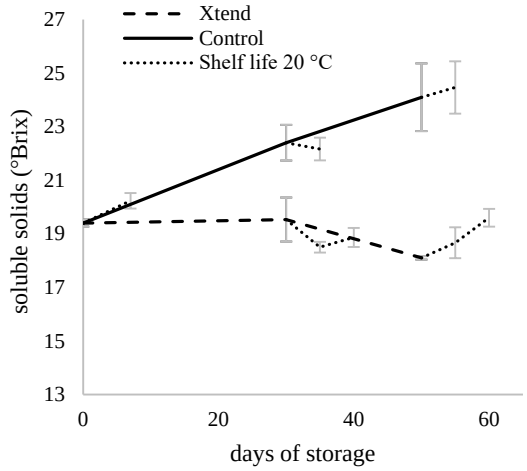


Figure 7 The levels of soluble solids for the fruit of the 'Kordia' variety when stored at 1 °C, shelf life conditions.

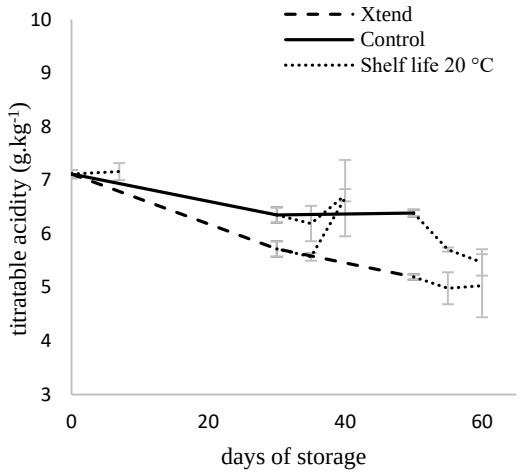


Figure 9 Titratable acidity for the fruit of the 'Sweetheart' variety when stored at 1 °C, shelf life conditions.

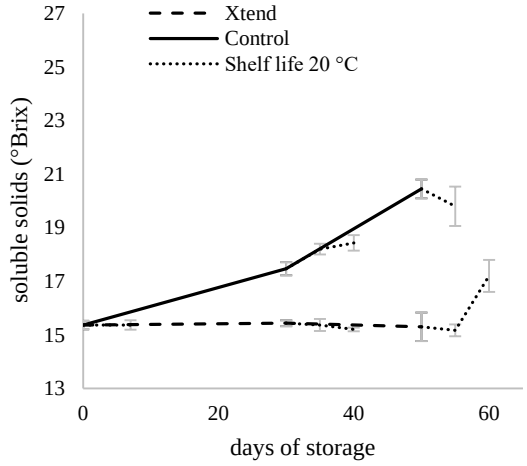


Figure 8 The levels of soluble solids for the fruit of the 'Vanda' variety when stored at 1 °C, shelf life conditions.

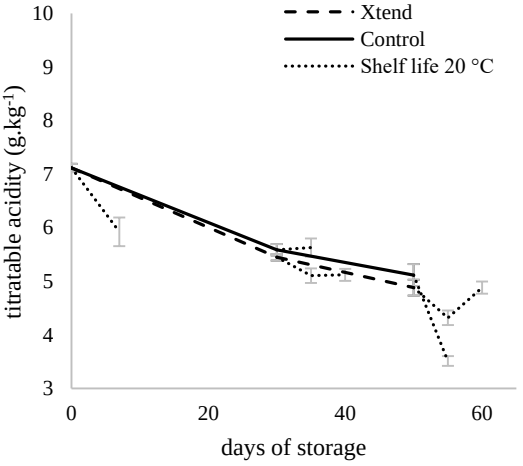


Figure 10 Titratable acidity for the fruit of the 'Regina' variety when stored at 1 °C, shelf life conditions.

acids at harvest time showed a lower rate of degradation during storage. The 'Vanda' fruit contained 5.6 $g.l^{-1}$ titratable acids at the beginning of storage. After 50 days of storage this value decreased by 0.5 $g.l^{-1}$ for unpacked fruit and by 1.5 $g.l^{-1}$ when using the film.

Differences between the fruit firmness at harvest and at 20 °C after 7 days were not statistically significant. 'Sweetheart' exhibited the lowest fruit firmness (Figure 13); the highest firmness after harvest and after seven-day storage at the distribution temperature was seen in the 'Regina' variety (Figure 14). Average values ranged from 20.4 N ('Sweetheart') to the firmest fruit (25.1 N) of 'Kordia' (Figure 15). The standard deviation at unequal diameter ranged from 2.4 to 5.4 N. At harvest, firmness ranged from 20.4 to 25.1 N. After 7 days of storage, 'Regina' was the firmest variety (24.3 N), other varieties ranging from 21.2 to 23.6 N.

Packed and unpacked samples did not significantly differ in terms of fruit firmness when stored at 1 °C. During a prolonged storage period (50 days + shelf-life) fruit unpacked in the film showed more significant weight loss; evaporation of water was causing wilting of the fruit, which also corresponded to some extent of higher firmness. When wrapped in a film, fruit should keep its firmness and green colour of the stalk **(Padilla-Zakour et al., 2004)**. After removal of the fruit from the store into the shelf life mode, however, any significant effect of using Xtend on changed firmness was not observed.

A semipermeable film, Xtend exhibits a constant permeability to physiological gases. During harvest ripeness, which coincides with the value of respiration intensity, the varieties in Figure 17 are shown by maturation on the tree; i.e., the 'Vanda' variety ripens about 7 days sooner than 'Kordia' and about 16 days sooner than 'Sweetheart' and 'Regina'. In addition, 'Regina' and 'Sweetheart' exhibit a maturity difference of four days in favour of 'Regina'. The degree of ripening on the tree by the criterion of respiration intensity is not demonstrable. During subsequent storage at 20 °C, there was reduced

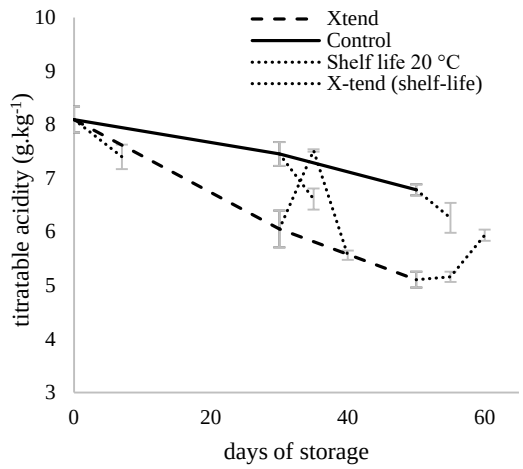


Figure 11 Titratable acidity for the fruit of the 'Kordia' variety when stored at 1 °C, shelf life conditions.

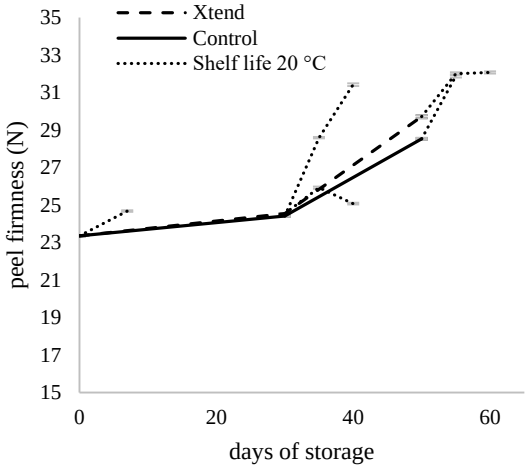


Figure 14 Peel firmness for the fruit of the 'Regina' variety when stored at 1 °C, shelf life conditions.

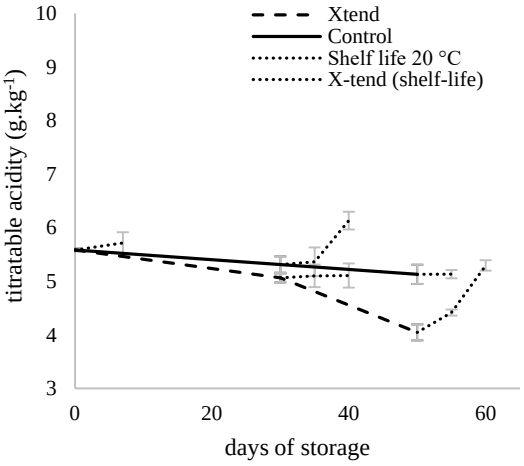


Figure 12 Titratable acidity for the fruit of the 'Vanda' variety when stored at 1 °C, shelf life conditions.

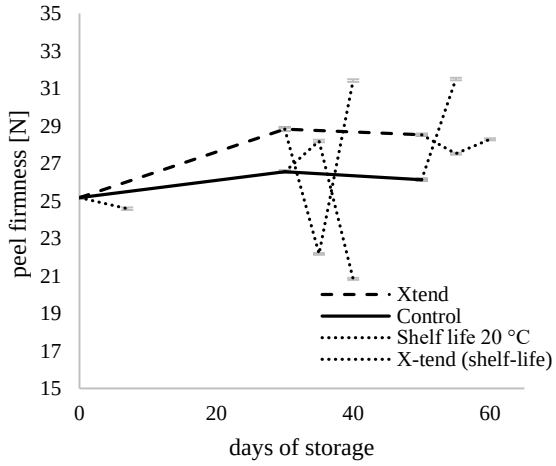


Figure 15 Peel firmness for the fruit of the 'Kordia' variety when stored at 1 °C, shelf life conditions.

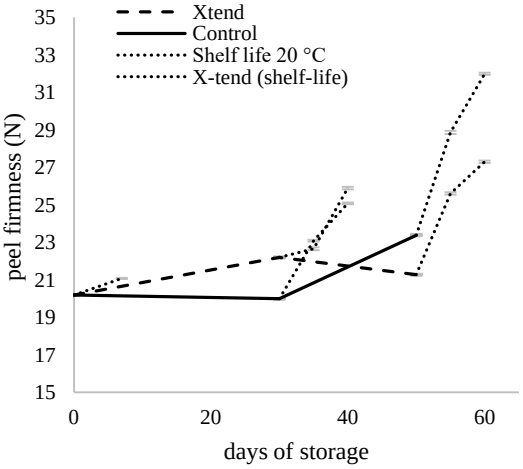


Figure 13 Peel firmness for the fruit of the 'Sweetheart' variety when stored at 1 °C, shelf life conditions.

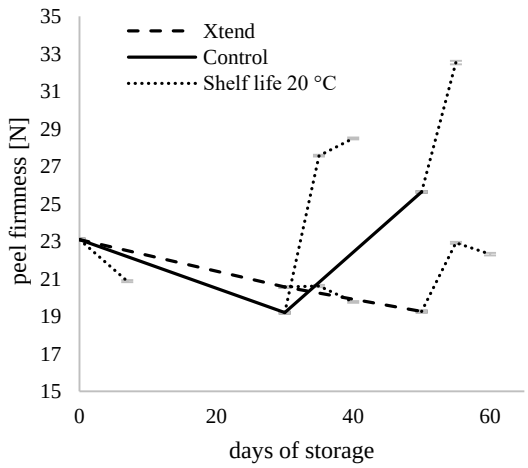


Figure 16 Peel firmness for the fruit of the 'Vanda' variety when stored at 1 °C, shelf life conditions.

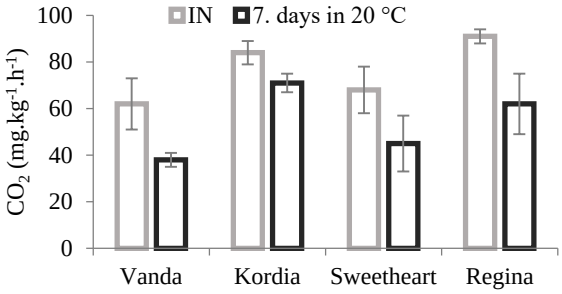


Figure 17 Changes in CO_2 production for cherries stored at 20 °C for 7 days.

respiration intensity in all of the varieties after 7 days; this has a direct relationship to climacteric decline and clearly advanced fruit ripening at this temperature. The smallest decrease in respiration intensity was seen in 'Kordia' (CO_2 production reduced by 17%), 'Regina' (the initial respiration intensity reduced by 32%), 'Vanda' (38% reduction), and 'Sweetheart' (reduction of 34%). Statistical significance is high in all cases.

Since high respiration intensity increases the risk of damage to the fruit during handling, it is advisable to place the fruit to a temperature of 4 – 6 °C after harvest **(Crisosto et al., 1993)**. During storage under refrigerated conditions, respiration intensity was measured on day 30 and day 50. The amount of produced CO_2 was measured immediately after unpacking the air-tight wrap. During 50-day storage a nearly linear rise was observed in Xtend in terms of CO_2 production except for 'Kordia' where a distinct decline occurred for respiration intensity between day 30 and day 50 of storing. Under identical conditions, the monitored varieties packed in Xtend feature intensity of respiration in the following order: 'Vanda' and 'Sweetheart', less 'Regina' and 'Kordia' (Figure 18 – 21). For unpacked fruit, however, this trend was not maintained and the highest CO_2 production was observed for 'Regina', 'Sweetheart', 'Kordia' and 'Vanda' (the lowest level) on day 50 of storage.

After the relocation of the fruit from refrigeration

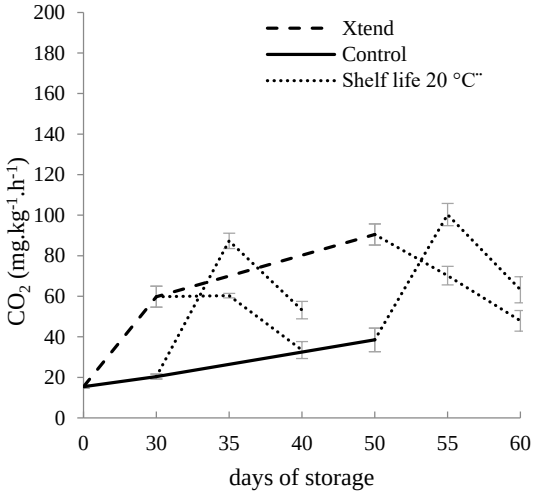


Figure 18 The amount of produced CO_2 for the fruit of the 'Sweetheart' variety when stored at 1 °C, shelf life conditions.

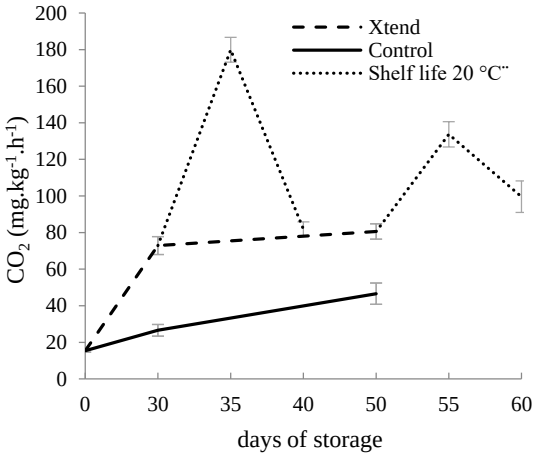


Figure 19 The amount of produced CO_2 for the fruit of the 'Regina' variety when stored at 1 °C, shelf life conditions.

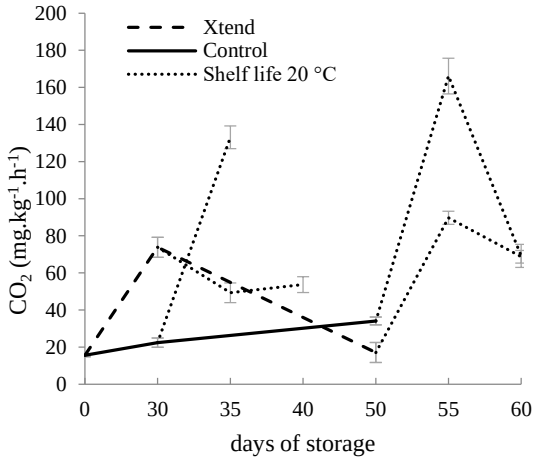


Figure 20 The amount of produced CO_2 for the fruit of the 'Kordia' variety when stored at 1 °C, shelf life conditions.

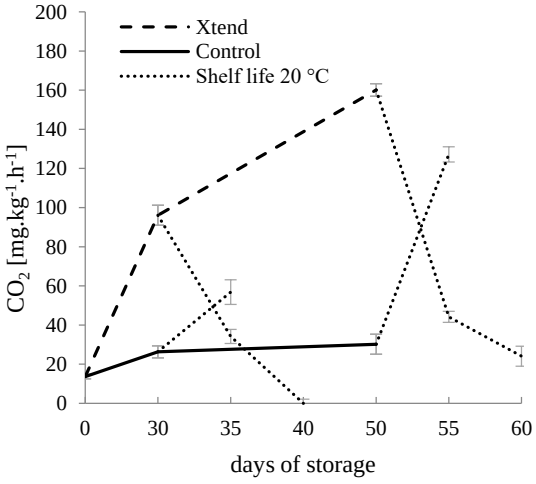


Figure 21 The amount of produced CO_2 for the fruit of the 'Vanda' variety when stored at 1 °C, shelf life conditions.

temperature (1 °C) into the temperature of distribution (20 °C), a high response was seen of fruit to the change in temperature. A different trend was observed in unpacked fruit compared with packed fruit. Fruit which were removed from Xtend reduced the intensity of respiration after placed at 20 °C. Conversely, fruit stored in bulk in the refrigerated room significantly increased the production of CO_2 once placed at higher temperature, which was seen mainly in the initial 5 days. The explanation of opposite waveforms of respiration intensity to identical final temperature arises from the response to a higher temperature in unpacked fruit having a distinct and increasing value due to nothing but the effect of temperature while fruit placed in Xtend were exposed to the higher CO_2 concentration **(Lurie and Weksler, 2008)**.

CONCLUSION

When storing cherries, ambient temperature is undoubtedly an important factor. Higher weight loss in bulk fruit result in reduced quality parameters of stored fruit. Using the Xtend film can partially eliminate wilting of fruit. The evaluation of soluble solids indicates a significant conclusion for storing practice - that is, the fruit does not lose sugar solids even on seven-day storage at the distribution temperature. Testing fruit firmness using a penetration technique is assumed to express the variety's characteristics and flesh reinforcement during storage at 20 °C due to weight loss; such a trend, however, was not observed for all of the varieties during the storage period. At this temperature, the storage of cherries for 7 days is at the limit of consumer quality for two reasons; the first concerns the quality of the stalk, which starts wilting. The second reason involves the fruit which is compact and has the characteristics of consumer quality. Fruit originally packed in an airtight unit with a modified atmosphere adapt through the decreased CO_2 production due to the stress of the higher CO_2 concentration in the fruit and the higher temperature of storage. The lowest values of CO_2 production after 10 days of exposure to a higher temperature are virtually at a comparable level of unpacked fruit. From the viewpoint of the market value of cherry fruit the maximum time that can be applied after removal of fruit from 1 °C to 20 °C is 5 days.

REFERENCES

Ben, J. 1991. Studies on short-term storage of Lutówka sour cherry in a cold store. In Abstracts of the 23rd International. Horticultural Congress, Florence, 27. August–1. September, p. 669.

Ceponis, M. J., Cappellini, R. A., Lightner, G. W. 1987. Disorders in sweet cherry and strawberry shipment market, 1972-1984. *Plant Disease*, vol. 71, no. 5, p. 472-475.

Crisosto, C. H., Crisosto, G. M., Metheney, P. 2003. Consumer acceptance of 'Brooks' and 'Bing' cherries is mainly dependent on fruit SSC and visual skin color. *Postharvest Biology and Technology*, vol. 28, no. 1, p. 159-167. https:/doi.org/10.1016/S0925-5214(02)00173-4

Crisosto, C. H., Garner, D., Doyle, J., Day, K. R. 1993. Relationship between fruit respiration, bruising susceptibility, and temperature in sweet cherries. *Hort. Science*, vol. 28, no. 2, p. 132-135.

Harb, J., Saquet, A., Bisharat, R., Streif, J. 2006. Quality and biochemical changes of sweet cherries cv. Regina stored in modified atmosphere packaging. *Journal of Applied Botany and Food Quality*, vol. 80, no. 2, p. 145-149.

Jacob, R. A., Spinozzi, G. M., Simon, V. A., Kelley, D. S., Prior, R. L., Hess-Pierce, B., Kader, A. A. 2003. Consumption of cherries lowers plasma urate in healthy women. *Journal of Nutrition*, vol. 133, no. 6, p. 1826-1829.

Kang, S. Y., Seeram, N. P., Nair, M. G., Bourquin, L. D. 2003. Tart cherry anthocyanins inhibit tumor development in ApcMin mice and reduce proliferation of human colon cancer cells. *Cancer Letters*, vol. 194, no. 1, p. 13-19. https:/doi.org/10.1016/S0304-3940(02)00583-9

Liu, Y., Liu, X., Zhong, F., Tian, R., Zhang, K., Zhang, X., Li, T. 2011. Comparative study of phenolic compounds and antioxidant activity in different species of cherries. *Journal of Food Science*, vol. 76, no. 4, p. 633-638. https:/doi.org/10.1111/j.1750-3841.2011.02150.x PMid:22417346

Looney, N. E., Webster, A. D., Kupferman, E. M. (eds.). 1996. *Cherries: crop physiology, production and uses*. Wallingford, Oxon, UK: CAB International. ISBN-13: 978-0851989365

Lurie, S., Weksler, A. 2008. Optimizing short term storage of sour cherries. *Acta Horticulturae*, vol. 795, no. 2, p. 799-804. https:/doi.org/10.17660/ActaHortic.2008.795.128

Padilla-Zakour, O. I., Tandom, K. S., Wargo, J. M. 2004. Quality of modified atmosphere packaged ´Hedelfingen´ and ´Lapins´ sweet cherries (*Prunus avium* L.). *Hort. Technology*, vol. 14, no. 3, p. 331-337.

Petersen, M. B., Poll, L. 1999. The influence of storage on aroma, soluble solids, acid and colour of sour cherries *(Prunus cerasus* L.) cv. Stevnsbær. *European Food Research and Technology*, vol. 209, no. 3-4, p. 251-256. https:/doi.org/10.1007/s002170050488

Shick, J. L. and Toivonen, P. M. A. 2002. Reflective traps at harvest reduce stem browning and improve fruit quality of cherries during subsequent storage. *Postharvest Biology and Technology*, vol. 25, p. 117-121. https:/doi.org/10.1016/S0925-5214(01)00145-4

Smith, E. D., Whiting, M. D. 2011. The pedicel's role in postharvest weight loss of two sweet cherry cultivars. *Acta Horticulturae*, vol. 903, no. 2, p. 935-939. https:/doi.org/10.17660/ActaHortic.2011.903.131

Turner, J., Seavert, C., Colonna, A., Long, L. E. 2008. Consumer sensory evaluation of sweet cherry cultivars in oregon, USA. *Acta Horticulturae*, vol. 795, no. 2, p. 781-786. https:/doi.org/10.17660/ActaHortic.2008.795.125

Usenik, V., Fajt, N., Mikulic-Petkovsek, M., Slatnar, A., Stampar, F., Veberic, R. 2010. Sweet cherry pomological and biochemical characteristics influenced by rootstock. *Journal of Agricultural and Food Chemistry*, vol. 58, no 8, p. 4928-4933. https:/doi.org/10.1021/jf903755b PMid:20337477

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TEXTURE QUALITY OF MUSKMELONS (*CUCUMIS MELO* L.) FROM DIFFERENT RETAILERS DURING STORAGE

Alica Bobková, Marek Šnirc, Martin Král, Martina Oštádalová, Jana Pokorná, Tomáš Fekete, Ľubomír Belej

ABSTRACT

The subject of study was to assess and to compare the effect of storage time on flesh firmness and skin strength in muskmelons (*Cucumis melo* L.) obtained from supermarket and marketplace. Texture quality of fruit is considered to be the major determinant for customer preference that depends on harvesting maturity and proper storage conditions of fruit and its fresh-cuts. Changes in flesh firmness and skin strength were measured periodically in stored fresh-cut fruits in both groups for initial, 2nd, 5th and 6th day. Analysis of basic physical parameters revealed that muskmelons from marketplace had higher height and width perimeter and therefore also a higher weight, compared to those ones from supermarkets. Textural analysis pointed out to significant differences (p <0.05) in flesh firmness among initial day group and all tested groups from 2nd, 5th and 6th day in muskmelons from supermarket. In marketplace muskmelon group was observed significant difference (p <0.05) between samples from initial day and 5th day. Within the skin strength, there were demonstrated significant differences (p <0.05) between initial day and most of remaining storage days in both supermarket and marketplace muskmelons groups. The data for flesh firmness and skin strength were used in linear regression analysis, in order to evaluate trends during storage period. The correlation coefficients of linear model describing relationship between storage time and skin strength for the group of supermarket and marketplace muskmelons were r = -0.828 and r = -0.780, respectively, which indicated approximately equal relationship between time and skin strength in both tested groups. A strong inverse correlation (r = -0.816) between time and flesh firmness in the group of supermarket muskmelons was noticed. In the group of marketplace muskmelons, there was observed weaker inverse correlation (r = -0.441) within this relation, compared to commercial ones. The model revealed that the muskmelons from marketplace retailers tend to maintain the flesh firmness for a longer time than did commercial ones. The melon flesh quality was markedly changing during storage period and highly depends on the muskmelon origin.

Keywords: muskmelon fruit; flesh firmness; skin strength

INTRODUCTION

Melon family is an economically important crop that includes wildtypes and numerous varieties, consumed worldwide either as desert fruits, vegetable or sauce ingredients, depending on the type of fruit. Varieties vary widely in fruit size, morphology and taste, as well as vegetative traits and climatic adaptation **(Pitrat et al., 1999)**.

Muskmelon is a beautiful, juicy, tasty and delicious fruit popular for its nutritive and medicinal properties and is one of the popular products in fresh-cut fruit market. The *Cucurbitaceae* family includes squash, pumpkins, cucumbers, Muskmelons, watermelons, and gourds. *Cucumis melo* (Cantaloupe or Muskmelon) is one of the most important cultivated cucurbits, which is native to India and Africa. Muskmelon (*Cucumi smelo* L.) is characterised by number of varieties **(Maran and Priya, 2015; Aguayo et al., 2004; Maran and Priya, 2015; Pitrat et al., 2000)**.

The muskmelons are highly considered for the sweet taste of the flesh, which develop as the fruit reaches full maturity. Volatile compounds, mainly esters, increase with increasing fruit maturity, thus contributing to the desirable sweet aroma of the fruit. Moreover, fruit that remains attached to the plant accumulates sucrose, resulting in a fruit with a sweet taste **(Lignou et al., 2014)**.

Originally, the popularity of melon was due to its refreshing and tasty flesh and pleasant aroma. It was consumed mainly in the summer period as an appetizer, in cold soups or salads, and as a dessert. Increasing interest in muskmelon consumption is associated with its potential human health benefits because contain naturally occurring vitamins, minerals, and pigments, which provide antioxidant activity, anti-inflammatory properties **(Vouldoukis et al., 2004; Ismail et al., 2010)** and anti-diabetic benefits **(Kenny et al., 2013)**.

Muskmelon is an excellent source of vitamin C and a good source of vitamin A, notably through its β-carotene content. In addition, cantaloupe is a good source of

potassium and vitamin B6. Cantaloupes contain carotenoids which are a group of phytochemicals that may contribute to disease prevention because of their antioxidant properties **(Solval et al., 2012)**.

Moreover, it has been reported that seeds of muskmelon boost immunity, reduce cardiovascular risks, help in normalizing blood-fat levels and contain essential nutrients for wound healing **(Yanty et al., 2008)**.

The melon fruit is perishable commodity due to high water content. Minimal processing alters the integrity of the fruit and induces surface damages increasing lightly the tissue respiration and leading biochemical deteriorations such as browning, off-flavour development and texture breakdown decreasing the fresh-cut fruit quality **(Raybaudimassilia et al., 2008)**.

Therefore, high quality of muskmelons and fresh-cuts can be maintained by using product at proper maturity, and controlling deterioration using low temperature and other tools such as modified storage and chemical treatments **(Supapvanich and Tucker, 2012; Oms-Oliu et al., 2008)**.

Fruit quality is a consequence of many biochemical processes that result in changes of its intrinsic properties such as colour, texture, flavour and aroma, together with the exterior appearance (size, colour and shape) and nutritional value. These properties exert a strong influence on producing commercially acceptable melons, and happen to be remarkably different depending on each particular melon cultivar, due to its morphological variability **(Obando et al., 2007)**.

Fruit wholesalers are there for particularly interested in the measurement of fruit texture. All these attributes are based on biochemical, physical and structural components that occur at different levels in the fruit such as turgidity and cell wall composition at cell level; number, size and morphology of cells as well as their cohesion, spatial organization and the distribution of intercellular airspaces at the tissue level. All these components evolved during fruit growth and in postharvest storage **(Harker et al., 2010)**.

Texture is one of the most important quality parameters and is partly responsible for consumer preferences of edible fruit **(Harker and Johnston, 2008)**. Textural characteristics are related to the structure of cell walls and their degradation during the ripening phase. Consumers perceive hardness and juiciness as two factors that the most influence the mouth feel of a fruit **(Toivonen and Brummell, 2008)**. The results of textural analysis can be useful for optimising the cultivation and prolonging shelf life of product during storage **(Bebejová et al., 2014)**.

The use however of grafted plants in commercial melon production is not the one anticipated. This can be attributed partly to the higher cost incurred but also to frequent problems of incompatibility involving inter-specific Cucurbita root stocks, which may eventually lead to plant decline **(Aloni et al., 2010)**.

The subject of study was to evaluate texture quality of muskmelons from different retailers in relation to storage period, and to point out to its importance in logistical chain.

MATERIAL AND METHODOLOGY

Plant material and sample preparation

Ten muskmelons (*Cucumis melo* L.) were obtained from both supermarket and marketplace retailers in June 2015. All analyses were repeated six times. The muskmelons were randomly selected on the basis of uniformity and absence of damage or blemishes and divided into two sub-groups. The first sub-group was assessed in the same day of obtaining (initial day, day 2, 5 and 6).

The fruits from second sub-group were cut into 4 pieces, seeds and placental tissue were removed, and each piece was wrapped into a stretch film and stored in refrigerator for three different time periods (2, 5 and 6 days).

After storage, the fruit were kept in the room temperature (20 °C) 30 min before analysis. It was expected that different point of sale and length of storage would make it possible to vary the textural characteristics of the investigated fruit.

Storage conditions

Cut muskmelons were stored in refrigerator (Zanussi ZRB 36104WA) at 5 ±1 °C temperature and 85 – 90% relative humidity.

Physical parameters

Fresh weight was measured using a balance (KERN PCB 3500-2) and perimeters with plastic tape measure.

Texture analysis

The flesh firmness and skin strength was performed with TA-XT Plus Texture Analyzer (Stable Micro System, Surrey, UK) and samples were examined using a Stable Micro Systems Type (version 5.0, 9.0). The measurements were made at laboratory temperature.

Flesh firmness

The test was performed on sample in cube form (10 mm ×10 mm × 10 mm). The 2 mm cylinder probe penetrated sample into 6 mm distance. The cubes were always penetrated with the original rind-side down. Pre-test speed was 1.5 $mm.s^{-1}$ and then test speed was 1.5 $mm.s^{-1}$, followed by return post-test speed of 10.0 $mm.s^{-1}$.

Skin Strength

Trigger force of 5g has been used to move 2 mm cylinder probe down onto the melon skin. Pre-test speed was 1.5

Table 1 Fresh weight, height perimeter and width diameter of commercial and market group.

Parameters	Supermarket	Marketplace
Fresh weight (g)	1378.3	1781.5
Height perimeter (cm)	48.7	50.3
Width perimeter (cm)	42.5	49.0

$mm.s^{-1}$ and then test speed was 1.0 $mm.s^{-1}$, followed by return post-test speed of 10.0 $mm.s^{-1}$.

Statistical analysis

Linear regression analysis was used to assess evolution of flesh firmness and skin strength during storage period. Differences among groups were analysed with ANOVA. All the computational work, including the graphical presentations, was performed using XLSTAT (Addinsoft, 2014) package program.

RESULTS AND DISCUSSION

Texture represents one of the principal factors defining fruit quality and in melon, as in fruits like tomato strawberry, apple, blueberry or dates, textural characteristics are related to the cell walls' structure and their degradation during the ripening phase. To the consumer, there are two factors that most influence the mouth feel of a fruit or vegetable: hardness and juiciness **(Bianchi et al., 2016)**.

Hardness is a decisive attribute for consumer acceptance, as hardness loss is perceived to be associated with quality loss. It is also a primary quality selection trait used by melon producers to enhance fruit shelf-life during transport and sale **(Bianchi et al., 2016)**.

Texture definition is a sensory consideration, although it can be defined instrumentally. There are two ways to measure texture: sensory and instrumentally. Sensory measurement requires a previously trained panel, despite the existence of studies that employed consumer panels; instrumental measurement uses fundamental, empirical or imitative methods. Fundamental tests, like ultimate strength, Poisson's ratio or Young's modulus, measure viscosity and elasticity; empirical tests, like puncture, shear, and extrusion, measure parameters found to be correlated with sensory texture. Imitative tests are those that imitate with instruments the way food products are subjected in the mouth **(Bianchi et al., 2016)**.

The weight and perimeter parameters of melons from supermarket and group of melons from market place are presented in Table 1. Muskmelons from marketplace have higher height and width perimeter and therefore also a higher weight.

The flesh firmness of melons from supermarket and group of melons from market place are presented in Table 2. There were significant differences in flesh firmness between initial day group and all tested groups from 2nd, 5th and 6th day in melons from supermarket. In marketplace group of melons was observed significant difference between samples from initial day and 5th day. Decreasing of melon firmness or tend to be softened of their flesh was a common pattern for several fruit and it

Table 2 Mean, standard deviation and coefficient of variation of flesh firmness.

	Flesh firmness							
	Supermarket				Marketplace			
	Initial day	2 day	5 day	6 day	Initial day	2 day	5 day	6 day
***M* (g)**	$816.06^{A,B,C}$	$525.56^{A,D,E}$	$339.30^{B,D}$	$387.07^{C,E}$	284.47^{A}	277.61	245.52^{A}	248.03
***SD* (g)**	87.94	60.79	59.36	69.63	29.72	49.09	28.69	48.46
***CV* (%)**	10.78	11.57	17.50	17.99	10.45	17.68	11.69	19.54

Note: Results are shown as mean. Means in same row for group with same letter are significantly different (p <0.05).

Table 3 Mean, standard deviation and coefficient of variation of skin strength.

	Skin strength							
	Supermarket				Marketplace			
	Initial day	2 day	5 day	6 day	Initial day	2 day	5 day	6 day
***M* (g)**	$6376.87^{A,B,C}$	$5659.87^{A,D,E}$	$4143.89^{B,D}$	$4351.35^{C,E}$	$3889.74^{A,B,C}$	$2561.73^{A,D,E}$	$1919.45^{B,D}$	$1909.23^{C,E}$
***SD* (g)**	542.21	324.54	517.84	467.81	311.88	474.78	476.99	465.62
***CV* (%)**	8.50	5.73	12.50	10.75	8.02	18.53	24.85	24.39

Note: Results are shown as mean. Means in same row for group with same letter are significantly different (p <0.05).

Table 4 Fresh weight, height perimeter and width diameter of supermarket and market place group.

	Flesh Firmness		Skin Strength	
	Supermarket	Marketplace	Supermarket	Marketplace
Model	Y = 783.36 + -92.6472 X	Y = 293.63 + -10.3399 X	Y = 6535.43 + -487.803 X	Y = 3750.69 + -410.6611 X
Linear Correlation (*r*)	-0.816	-0.441	-0.828	-0.790
Coefficient of Determination (R^2)	0.666	0.194	0.686	0.623
***p*-value**	1.17992×10^{-6}	0.03	5.74905×10^{-7}	4.5×10^{-6}
Intercept	783.357	293.634	6535.426	3750.688
Slope	-92.647	-10.340	-487.803	-410.661

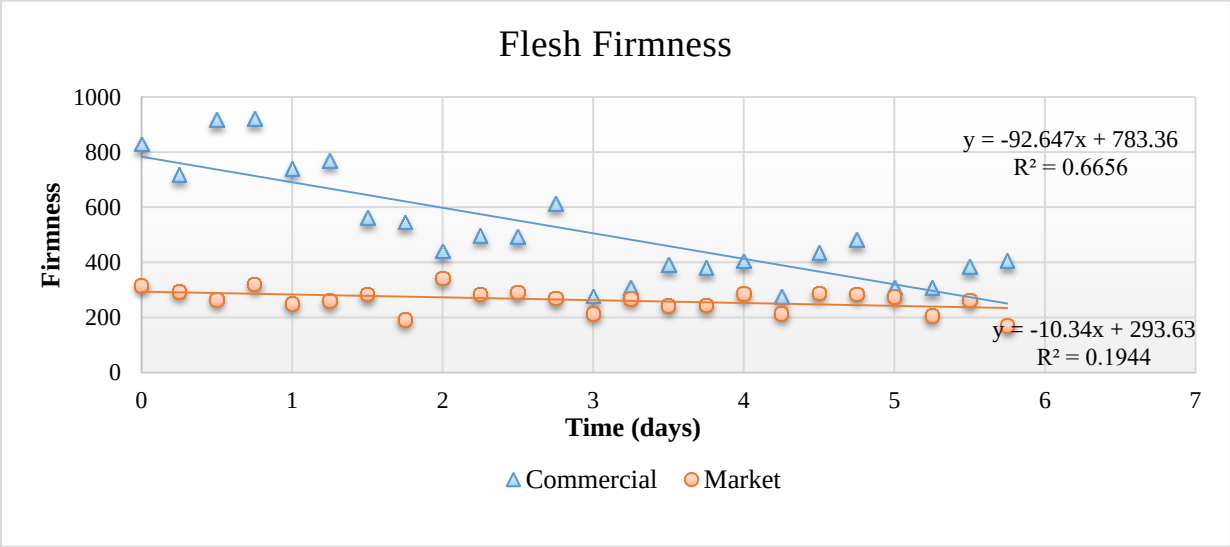

Figure 1 The relationship between time and skin Strength in the group of melons from supermarket and group of melons from marketplace.

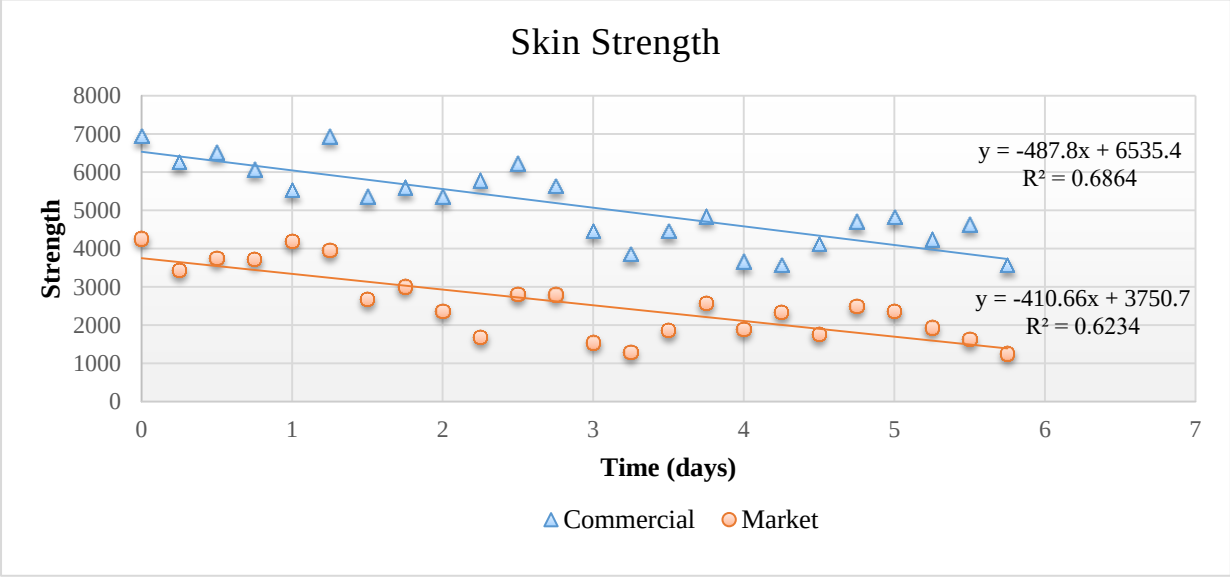

Figure 2 The relationship betweentime and skin Strength in the group of melons from supermarket and group of melons from marketplace.

can be associated with the decline in cell wall strength, cell wall adhesion and turgor changes **(Toivonen and Brummel, 2008)**.

During fruit ripening, there is a decline in turgor which contributes to textural changes **(Shackel et al., 1991; Harker and Sutherland, 1993)**, probably due partly to an accumulation of osmotic solutes in the cell wall space **(Almeida and Huber, 1999)**, and partly to postharvest water loss from the ripening fruit **(Saladie et al., 2007)**.

Degradation of pectine during storage may occur. **Van Buggenhout et al., (2009)** describe that in process-induced textural changes play a role enzymatic and chemical pectin changes: a) enzymatic degradation by the successive demethoxylation and depolymerisation by pectin methylesterase (PME) and polygalacturonase (PG), respectively; b) chemical degradation via a ß-elimination reaction or acid hydrolysis.

The skin strength of melons from supermarket and group of melons from market place are presented in Table 3. Similar trend as flesh firmness in commercial melons was observed for skin strength in both groups. Fresh-cut products are highly susceptible to weight and water loss because of internal tissues are exposed and lack skin or cuticle **(Watada and Qi, 1999)**, and wrapped the fresh-cut products using a plastics film protected skin of fresh-cut pieces and effect of external environment can be reduced.

Ripening can be rapidly initiated by wounding in pre-climacteric fruit **(Starrett and Laties, 1993)**. The rate of softening of fresh-cut fruit pieces is often markedly more rapid than in intact whole fruit **(O'Connor-Shaw et al., 1994)**. Tissue softening is frequently the major problem limiting the shelf-life of fresh-cut products **(Agar et al., 1999)**, which even when refrigerated can become unacceptable in as little as 2 days for tropical fruit such as papaya **(O'Connor-Shaw et al., 1994)**. During ripening naturally increases activity which leads to the degradation of cellulose and hemicellulose in fruit skin a subsequently softening of tissue **(El-Zoghbi, 1994)**.

Figure 3 *Cucumic melo* L. (WORLD CAPITAL, 2016).

The linear regression analysis revealed strong inverse correlation (r = -0.816) between time and flesh firmness in the group of supermarket muskmelons.

In the group of marketplace muskmelons, there was observed weaker inverse correlation (r = -0.441) within this relation, compared to commercial ones (Figure 1, Table 4).

In the group of supermarket muskmelons coefficient of determination (R^2 = 0.666) indicated average strength of relationship between time and flesh firmness, whereas in the group of marketplace muskmelons the model explained a less total variability, as coefficient of determination indicated weak relationship (R^2 = 0.194).

In both tested groups were evidences of correlation with statistical significance. Therefore, the relationship between time and flesh firmness is stronger in the group of muskmelons from supermarket (p <0.01), compared to group of marketplace muskmelons (p <0.05).

In both supermarket and marketplace groups of muskmelons, there was observed strong inverse correlation between time and skin strength, which had value of r = -0.828 and -0.790, respectively. In both groups, the models explained most of the total variability within endogenous variables and relationship above (R^2 = 0.686 and R^2 = 0.623, respectively). In both tested groups were evidences of correlation with statistical significance (p <0.01). The relationship between time and skin strength was approximately equal in both tested groups (Figure 2, Table 4).

CONCLUSION

The flesh firmness and skin strength followed a decreasing linear trend that was storage time dependent. From these results it was possible to understand that at the marketability limit, loss of flesh firmness was more critical in the supermarket group than in market place group. This information should be used to optimise the logistical chain with the aim of increasing the quality of fresh muskmelons on the market while also reducing the related costs.

REFERENCES

Addinsoft. 2014. XLSTAT, Analyse de données et statistique avec MS Excel. Addinsoft, NY, USA.

Aguayo, E., Escalona, V., Rtés, F. 2004. Metabolic Behavior and Quality Changes of Whole and Fresh Processed Melon. *Journal of Food Science*, vol. 69, no. 4, p. SNQ148-SNQ155. http://dx.doi.org/10.1111/j.1365-2621.2004.tb06356.x

Agar, I., Massantini, R., Hess-Pierce, B., Kader, A. 1999. Postharvest CO_2 and Ethylene Production and Quality Maintenance of Fresh-Cut Kiwifruit Slices. *Journal of Food Science*, vol. 64, no. 3, p. 433-440. http://dx.doi.org/10.1111/j.1365-2621.1999.tb15058.x

Almeida, D., Huber, D. 1999. Apoplastic pH and inorganic ion levels in tomato fruit: A potential means for regulation of cell wall metabolism during ripening. *Physiologia Plantarum*, vol. 105, no. 3, p. 506-512. http://dx.doi.org/10.1034/j.1399-3054.1999.105316.x

Aloni, B., Cohen, R., Karni, L., Aktas, H., Edelstein, M. 2010. Hormonal signaling in rootstock–scion interactions. *Scientia Horticulturae*, vol. 127, no. 2, p. 119-126. http://dx.doi.org/10.1016/j.scienta.2010.09.003

Bianchi, T., Guerrero, L., Gratacós-Cubarsí, M., Claret, A., Argyris, J., Garcia-Mas, J., Hortós, M. 2016. Textural properties of different melon (*Cucumis melo* L.) fruit types: Sensory and physical-chemical evaluation. *Scientia Horticulturae*, vol. 201, p. 46-56. http://dx.doi.org/10.1016/j.scienta.2016.01.028

Bebejová, A., Čapla, J., Zajác, P., Belej Ľ., Čurlej, J. Vietoris, V. 2014. Determination of textural properties of different kinds of ketchups of two different rates under different conditions of storage for the determination of their consumal quality. *Potravinarstvo*, vol. 8, no. 1, p. 25-32. http://dx.doi.org/10.5219/323

El-Zoghbi, M. 1994. Biochemical changes in some tropical fruits during ripening. *Food Chemistry*, vol. 49, no. 1, p. 33-37. http://dx.doi.org/10.1016/0308-8146(94)90229-1

Harker, F., Redgwell, R., Hallett, I., Murray, S., Carter, G. 2010. Texture of Fresh Fruit. *Janick/Horticultural Reviews*, vol. 20, p. 121-224. http://dx.doi.org/10.1002/9780470650646.ch2

Harker, F. R., Johnston, J. 2008.Importance of texture in fruit and its interaction with flavor. Brückner, B., Wyllie, S. G. *Fruit and vegetable flavour: Recent advances and future prospects*. Abington, Cambridge, UK: Woodhead Pub. Ltd. 336 p. ISBN 9781845691837.

Harker, F., Sutherland, P. 1993. Physiological changes associated with fruit ripening and the development of mealy texture during storage of nectarines. *Postharvest Biology and Technology*, vol. 2, no. 4, p. 269-277. http://dx.doi.org/10.1016/0925-5214(93)90032-x

Ismail, H., Chan, K., Mariod, A., Ismail, M. 2010. Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts. *Food Chemistry*, vol. 119, no. 2, p. 643-647. http://dx.doi.org/10.1016/j.foodchem.2009.07.023

Kenny, O., Smyth, T., Hewage, C., Brunton, N. 2013.Antioxidant properties and quantitative UPLC-MS analysis of phenolic compounds from extracts of fenugreek (*Trigonella foenum-graecum*) seeds and bitter melon (*Momordica charantia*) fruit. *Food Chemistry*, vol. 141, no. 4, p. 4295-4302. http://dx.doi.org/10.1016/j.foodchem.2013.07.016

Lignou, S., Parker, J., Baxter, C., Mottram, D. 2014. Sensory and instrumental analysis of medium and long shelf-life Charentais cantaloupe melons (*Cucumis melo* L.) harvested at different maturities. *Food Chemistry*, vol. 148, p. 218-229. http://dx.doi.org/10.1016/j.foodchem.2013.10.045

Maran, J., Priya, B. 2015.Supercritical fluid extraction of oil from muskmelon (*Cucumis melo*) seeds. *Journal of the Taiwan Institute of Chemical Engineers*, vol. 47, p. 71-78. http://dx.doi.org/10.1016/j.jtice.2014.10.007

Obando, J., Martínez, J., Roca, M., Alarcón, A., Fernández-Trujillo, J., Hernández, M., Eduardo, I., Arús, P., Monforte, A. 2007. Identification of qtls associated with commercial postharvest quality traits and disorders using a collection of near isogenic lines of melon. *Acta Horticulture*, vol. 763, p. 309-317. http://dx.doi.org/10.17660/actahortic.2007.763.41

O'Connor-Shaw, R., Roberts, R., Ford, A., Nottingham, S. 1994. Shelf Life of Minimally Processed Honeydew, Kiwifruit, Papaya, Pineapple and Cantaloupe. *Journal of Food Science*, vol. 59, no. 6, p. 1202-1206. http://dx.doi.org/10.1111/j.1365-2621.1994.tb14676.x

Oms-Oliu, G., Raybaudi-Massilia Martínez, R., Soliva-Fortuny, R., Martín-Belloso, O. 2008. Effect of superatmospheric and low oxygen modified atmospheres on shelf-life extension of fresh-cut melon. *Food Control*, vol. 19, no. 2, p. 191-199. http://dx.doi.org/10.1016/j.foodcont.2007.03.009

Pitrat, M., Hanelt, P., Hammer, K. 2000. Some Comments on Infraspecific Classification of Cultivars of Melon. *Acta Horticulturae*, vol. 510, p. 29-36. http://dx.doi.org/10.17660/actahortic.2000.510.4

Pitrat, M., Chauvet, M. And Foury, C. 1999. Diversity, History and Production of Cultivated Cucurbits. *Acta Horticulturae*, vol. 492, p. 21-28. http://dx.doi.org/10.17660/actahortic.1999.492.1

Raybaudimassilia, R., Mosquedamelgar, J., Martinbelloso, O. 2008. Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. *International Journal of Food Microbiology*, vol. 121, no. 3, p. 313-327. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.11.010

Saladie, M., Matas, A., Isaacson, T., Jenks, M., Goodwin, S., Niklas, K., Xiaolin, R., Labavitch, J., Shackel, K., Fernie, A., Lytovchenko, A., O'Neill, M., Watkins, C., Rose, J. 2007. A Reevaluation of the Key Factors That Influence Tomato Fruit Softening and Integrity. *Plant Physiology*, vol. 144, no. 2, p. 1012-1028. http://dx.doi.org/10.1104/pp.107.097477

Shackel, K., Greve, C., Labavitch, J., Ahmadi, H. 1991. Cell Turgor Changes Associated with Ripening in Tomato Pericarp Tissue. *Plant Physiology*, vol. 97, no. 2, p. 814-816. http://dx.doi.org/10.1104/pp.97.2.814

Solval, K., Sundararajan, S., Alfaro, L., Sathivel, S. 2012. Development of cantaloupe (*Cucumis melo*) juice powders using spray drying technology. *LWT - Food Science and Technology*, vol. 46, no. 1, p. 287-293. http://dx.doi.org/10.1016/j.lwt.2011.09.017

Starrett, D., Laties, G. 1993. Ethylene and Wound-Induced Gene Expression in the Preclimacteric Phase of Ripening Avocado Fruit and Mesocarp Discs. *Plant Physiology*, vol. 103, p. 227-234. http://dx.doi.org/10.1104/pp.103.1.227

Supapvanich, S., Tucker, G. 2012. The Effect of 1-Methylcyclopropene (1-MCP) on Quality and Cell Wall Hydrolases Activities of Fresh-Cut Muskmelon (*Cucumis melo* var. *reticulatus* L.) During Storage. *Food Bioprocess Technol*, vol. 6, no. 8, p. 2196-2201. http://dx.doi.org/10.1007/s11947-011-0776-3

Toivonen, P., Brummell, D. 2008. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology*, vol. 48, no. 1, p. 1-14.http://dx.doi.org/10.1016/j.postharvbio.2007.09.004

Van Buggenhout, S., Sila, D., Duvetter, T., Van Loey, A., Hendrickx, M. 2009. Pectins in Processed Fruits and Vegetables: Part III-Texture Engineering. *Comprehensive Reviews in Food Science and Food Safety*, vol. 8, no. 2, p. 105-117. http://dx.doi.org/10.1111/j.1541-4337.2009.00072.x

Vouldoukis, I., Lacan, D., Kamate, C., Coste, P., Calenda, A., Mazier, D., Conti, M. and Dugas, B. 2004. Antioxidant and anti-inflammatory properties of a *Cucumis melo* LC. extract rich in superoxide dismutase activity. *Journal of Ethnopharmacology*, vol. 94, no. 1, p. 67-75. https://doi.org/10.1016/j.jep.2004.04.023

Watada, A., Qi, L. 1999. Quality of fresh-cut produce. *Postharvest Biology and Technology*, vol. 15, no. 3, p. 201-205. http://dx.doi.org/10.1016/s0925-5214(98)00085-4

WORLD CAPITAL, 2016. *Cucumis melo* L. Available online: http://worldcapital.gr/wordpress/wp-content/uploads/2014/06/u6712.jpg

Yanty, N., Lai, O., Osman, A., Long, K., Ghazali, H. 2008. Physicochemical Properties of *Cucumis Melo* var. *Inodorus* (Honeydew Melon) Seed and Seed Oil. *Journal of Food Lipids*, vol. 15, no. 1, p. 42-55. http://dx.doi.org/10.1111/j.1745-4522.2007.00101.x

Zhao, X., Guo, Y., Huber, D. J., Lee, J. 2011. Grafting effects on postharvest ripening and quality of 1-methylcyclopropene-treated muskmelon fruit. *Scientia Horticulturae* vol. 130, no. 3, p. 581-587. https://doi.org/10.1016/j.scienta.2011.08.010

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INFLUENCE OF DIFFERENT CURING METHODS ON THE FATTY ACID COMPOSITION IN SAUSAGES PREPARED FROM RED DEER MEAT

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ABSTRACT

These curing agents play a decisive role in obtaining the specific sensory properties, stability and hygienic safety of products such as fermented sausages, ham and, more recently, emulsion type of sausages. The effect of using two different curing agents (sodium chloride and nitrate) on fatty acid compounds in dry-cured deer meat was investigated in our study. The concentration of free fatty acids in the fat depends on the hydrolytic activity of the lipases, the microbial metabolic processes, and the oxidative reactions that work on the free fatty acids released in the lipolysis. The main identified fatty acids in all different types of curing were palmitic acid (16 : 0), oleic acid (c18 : 1 cis-9), stearic acid (C18 : 0). The resulting *n*-6/*n*-3 PUFA ratio in the muscle samples of red deer showed no variation in different types of curing and was beneficially low within the range of 3.9 : 1 and 4.49 : 1. Total free fatty acids, whether saturated, monounsaturated or polyunsaturated fatty acids, did not increased (p >0.05) greatly through the processing of dry-cured deer meat. Also there was no effect of curing method on fatty acids composition in two different muscles *Semitendinosus muscle* (ANOVA, p >0.05, F – 0.003, F crit. – 3.041) and *Triceps brachii* muscle (ANOVA, p >0.05, F – 0.05, F crit. – 3.01). There were found no significant (p >0.05) differences between fatty acids content in sausages prepared by brining in NaCl and Nitrate salt. The present study revealed that game meat can function as a good source of bioactive compounds that are essential for human nutrition.

Keywords: red deer; fatty acids composition; curing

INTRODUCTION

Concentration of fatty acids determines not only nutritional value of the meat, but also its quality, including its shelf life and taste **(Wood et al., 2003; Warren et al., 2008)**. Game meat has characteristic texture and taste: it is generally darker, has a stronger taste and is often tougher than meat from farm animals. Although game meat is considered more difficult to prepare, when purchased raw, current trend towards processed products, such as fermented sausages, is a new aspect to take into account.

Deer (*Cervidae*) belong to the most important species, which are used as a farm animal as well as hunting wild animal. For long-term conservation and development purposes, it therefore appears compulsory to manage wildlife to maintain both species survival and within species genetic diversity **(Franklin, 1980; Maršálková et al., 2014)**.

Deer farming meets the growing interest which consumers show, for meat alternatives to the traditional types **(Volpelli et al., 2003)**: venison is appreciated because it is lean and tasty but, even more, as stressed by **Barry (1994)**, because it fulfils the needs of "a society which is becoming increasingly sensitive to environmental pollution, animal manipulation, and feed additives" **(Belej et al., 2011)**.

In the last 10 years, cured, fermented and dried products made from hunted game species have become more popular and are easily found on the market in European countries. Game meat is distinguished by its characteristic texture and taste: it is generally darker, stronger-tasting and often tougher than that of poultry and farmyard animals. **(Bertolini et al., 2005; Hoffman and Wiklund, 2006; Ruiz et al., 2007)**. Cured, fermented and dried products made from game species have recently appeared on the market. However, their presence remains very restricted, and is highly seasonal. Marketing of venison, for example, is dependent on the timing of the hunting season and on culling quotas. In addition, the quality of game products depends upon the raw meat quality, which is influenced by, among others factors, the period of the year in which the animals are growing, that affects the condition of the pasture and the animal's activity and sexual activity. As an example, the fat reserves of venison are decreased after the mating period in autumn **(Ruiz et al., 2007)**.

In the human diet, meat is seen as a major source of fat, and especially of saturated fatty acids (SFAs), which have been implicated in diseases associated with modern life (various cancers and coronary heart disease), mostly in developed countries. The World Health Organization recommends that the daily fat intake be reduced to 30% of the total energy intake, and that saturated fats should be limited to 10% of this caloric intake **(Krauss et al., 2000)**.

Curing is one of the most ancient meat preservation methods, which contributes positively to the technological and sensory characteristics of the meat. The curing technology is based on the addition of salt, which acts as a preserving agent and is also responsible for causing the physico-chemical and biochemical phenomena that contribute to the development of flavour **(Gil et al., 1999; Vestergaard et al., 2005)**.

Meat is an important source of animal protein but, at the same time, it includes saturated fatty acids, which makes it a potential cause of different cardiovascular diseases and still little is known about influence of age and sex on these parameters in roe deer muscles **(Cygan-Szczegielniak et al., 2011)**.

Wang et al., (2012) found high salt content could promote the formation of aldehydes from fatty acids.

On the other hand, **Corral et al., (2013)** reported that reducing salt in slow fermented sausages had different influences on the generation of different volatile compounds. The changes of lipid composition, fatty acid composition of phospholipids, and free fatty acids were evaluated by one-way analysis of variance techniques where these measurements were as dependent variables and the processing stage as independent variables.

Several food additives are added in food for their preservation to maintain the freshness of food (antioxidants) or to slow down or stop the growth of microorganisms (preservative agents). Nitrites and nitrates are used as preservative agents in meat. Nitrites give a smoked taste, a pinkish color in the meat and protect the consumers against the risk of bacterial deterioration. Their addition is however very limited as, in high dose, it can have risks on human health and the environment. Nitrites may also combine with secondary or tertiary amines to form N-nitroso derivatives. Certain N-nitroso compounds have been shown to produce cancers in a wide range of laboratory animals. Thus, alternatives of nitrates and nitrites are the object of numerous research studies. Alternatives, such as the addition of vitamins, fruits, chemicals products, natural products containing nitrite or spices, which have similar properties of nitrites, are in evaluation. In fact, spices are considered to have several organoleptic and anti-microbial properties **(Gassara et al., 2015)**.

MATERIAL AND METHODOLOGY

Animals and sample collection

The investigations involved 30 red deer (*Cervus elaphus*) coming from Slovak region. Samples (*semitendinosus* muscle and *triceps brachii* muscle) were collected at the deer slaughter plant from 30 (*Cervus elaphus*) males (<1.5 years). These animals had grazed on summer pasture on the west part of Slovakia. The chill after slaughter started at 6 – 8 °C with a reduction to a deep leg temperature of <2 °C overnight, approximately 9 – 15 h after dressing of the carcass. After deboning the following day, samples were vacuum packed, and stored at 2 – 3 °C for 7 days *post mortem* before being frozen at approximately -20 °C. Samples were thawed at 3 – 4 °C for approximately 15 h and used for all measurements.

Curing

Curing of red deer meat was realized by bringing a concentration of 10% NaCl and 10% Nitrate salt. The ratio of brine and the meat was at 3 : 1 in order to ensure the desired diffusion of salt into each sample. Thus, samples were loaded in the cold brine stored at 4 °C for 7 days. After 7 days cold storage, samples were desalted in lukewarm water for 30 minutes and then put into the smoking chamber, where they were dried at about 24 °C for 6 hours. After sufficient drying, the samples were cold smoke three times in two hours. Thus processed samples were subsequently placed in a climatic chamber where the climatic conditions during storage are gradually adjusted until the temperature has not been reached 15 °C, 75% relative humidity and air flow of 0.3 $m.s^{-1}$.

Fatty acids composition

Extracted lipids were dissolved in 5 mL hexane; 1 mL 2M KOH in methanol was added. The tubes were capped and stirred for 30 min to separate into two phases. The upper phase was analyzed using gas chromatography. A 6890 GC with a Multi Mode injector, a 7683B automatic liquid sampler and flame ionization detection (Agilent Technologies, Palo Alto, CA) were used. Separation was performed with a (60 m × 0.25 mm i.d. × 0.15 μm DB-23) column (Agilent 122-2361). The temperature programme was an initial 50 °C with a 1 min hold, ramp 25 $°C.min^{-1}$ to 175 °C, then 2 $°C.min^{-1}$ to 230 °C with a 5 min hold, then 120 $°C.min^{-1}$ to 245 °C with an 8 min hold. Injector temperature was 250 °C. Carrier gas was H2 with a pressure of 238.96 kPa/2.225 $mL.min^{-1}$. Fatty acid analysis was performed by auto injection of 1 μL of each sample at a split ratio of 1 : 10, constant flow mode, velocity 20.4 $cm.s^{-1}$. The flame ionization detector temperature was 280 °C with H2 35 $mL.min^{-1}$, air 350 $mL.min^{-1}$, and N2 make-up gas flow rates of 30 $mL.min^{-1}$, respectively. The run time for a single sample was 32 min.

Statistical analysis

To determinate the effect of curing on fatty acid profile, each detected fatty acids was analyzed using analyses of variance, the content of each fatty acid was the dependent variable, and the kind of curing was the independent variable. Analysis of variance (Single Factor ANOVA) was used to compare the effects of different curing methods on the fatty acids content. The dependent variable was log10 transformed in the order to meet the assumption of homogeneity of variance. A significance level of $\alpha = 0.05$ was applied to the entire statistical analysis.

The basic idea of Principal component analysis (PCA) is to describe the variation of a set of multivariate data in terms of a set of uncorrelated variables, each of which is a particular linear combination of the original variables. The new variables, namely principal components the total number of which equals the number of the original variables in the studied data, are derived in decreasing order of importance so that, for example, the first principle component accounts for as much as possible of the variation in the original data. The second component is chosen to account for as much as possible in the remaining variation subject to being uncorrelated with the first component, and so on. The usual objective of this type of

analysis is to see whether the first few components account for most of the variation in the original data. If so, they can be used to summarize the data with little loss of information. A reduction in dimensionality is thus achieved which might then be useful in visual interpretation of the data represented by two-dimensional graphics. In order to obtain the web, each variable was transformed so that the group having the highest value for a specific variable was set at 1.0 on the radial scale and values for the other two groups were expressed relative to that. All the computational work, including the graphical presentations, was performed using XLSTAT 2014.5.03 (2014) and Tanagra 1.4.50 (2003) package program.

RESULTS AND DISCUSSION

As shown in Table 1, the percentages and standard deviations of free fatty acids with respect to the total free fatty acids (%) extracted from raw meat, meat cured using NaCl and meat cured using nitrate salt. The main identified fatty acids in all different types of curing were palmitic acid (16 : 0), oleic acid (c18 : 1 cis-9), stearic acid (C18 : 0). This profile doesn´t coincides with that found by other authors in pork meat **(Franco et al., 2002; Johansson et al., 2015)**. In addition, the increase of free fatty acids could also be partially associated with increased activities of acid and neutral lipases due to dehydration and salt diffusion **(Vestergaard et al., 2000)**. These results are consistent with other studies that investigated both free-range and farmed red deer **(Purchas et al., 2010; Triumf et al., 2012)**.

These results are in accordance with **Soriano et al., (2006)** who studied free fatty acids in commercial saucissons made with deer meat.

All the examined samples showed a higher content of SFAs and MUFAs than PUFAs. The individual FAMEs measurement can allow to understand the composition of the fatty acids in the acylglycerols fraction of the examined samples **(Johansson et al., 2015)**.

Paleari et al., (2003) obtained a total saturated fatty acid content of 35.5 – 44%, monounsaturated of 30.3 – 45.7%, and polyunsaturated of 16.2 – 19.6%, respectively, in fat extracted from cured products of deer and wild boar from farms. In the present study, the amounts of saturated acids found were higher; however, the polysaturated acid content was lower.

The resulting *n*-6/*n*-3 PUFA ratio in the muscle samples of red deer showed no variation in different types of curing and was beneficially low within the range of 3.9 : 1 and 4.49 : 1. These results are consistent with other studies that investigated the fatty acid profiles of muscle of red deer and showed higher PUFA contents *n*-6/*n*-3 PUFA **(Polak et al., 2008; Purchas et al., 2010; Triumf et al., 2012)**. According to **Demeyer et al., (1974)** and **Soriano et al., (2006)**, in fermented sausages, there is a tendency to hydrolysis of linoleic, oleic, stearic and palmitic acids, probably because of the specific lipolysis developed by microbial lipases, that is dependent on position and structural conformation of fatty acids in glycerides **(Alford et al., 1971; Soriano et al., 2006)**.

The concentration of free fatty acids in the fat depends on

Table 1 Percentages and standard deviations of free fatty acids with respect to the total free fatty acids (%) extracted from raw meat, meat cured using NaCl and meat cured using nitrate salt (mean ±*SD*).

Fatty acid	Curing method					
	Raw		NaCl		Nitrate	
	SM	***TB***	***SM***	***TB***	***SM***	***TB***
C14 : 0	5.40 ±1.01	4.77 ±0.73	5.85 ±1.34	4.57 ±0.84	5.82 ±1.62	4.42 ±0.77
C14 : 1	1.74 ±0.52	1.36 ±0.81	1.76 ±0.69	1.11 ±0.62	2.44 ±1.31	1.14 ±0.65
C15 : 0	0.67 ±0.21	0.82 ±0.14	0.72 ±0.27	0.77 ±0.18	0.70 ±0.24	0.80 ±0.17
C16 : 0	28.4 2±1.70	24.26 ±1.47	29.12 ±1.88	23.80 ±1.27	27.32 ±1.72	23.73 ±1.33
C16 : 1	9.50 ±2.63	6.93 ±3.86	9.41 ±3.45	6.23 ±3.57	11.63 ±5.39	6.51 ±3.70
C17 : 0	0.68 ±0.15	0.81 ±0.21	0.71 ±0.20	0.79 ±0.23	0.63 ±0.21	0.82 ±0.23
C18 : 0	11.71 ±1.20	17.55 ±3.84	12.12 ±2.44	17.84 ±3.58	10.80 ±2.78	17.77 ±3.79
C18 : 1cis n9	19.12 ±1.18	20.78 ±2.70	19.57 ±0.89	19.93 ±2.67	18.67 ±1.04	20.52 ±2.22
C18 : 2cis n6	6.26 ±0.89	6.78 ±1.27	5.27 ±1.86	7.92 ±2.10	5.53 ±2.75	7.36 ±1.48
C18 : 3 n3	1.57 ±0.27	1.90 ±0.43	1.30 ±0.44	2.01 ±0.57	1.32 ±0.56	1.88 ±0.46
C20 : 4 n6	1.91 ±0.22	2.11 ±0.42	1.18 ±0.44	2.16 ±0.72	1.45 ±0.67	1.97 ±0.51
C20 : 5 n3	0.58 ±0.16	0.39 ±0.18	0.35 ±0.17	0.43 ±0.26	0.36 ±0.17	0.37 ±0.20
PUFA	10.44 ±1.45	11.31 ±2.19	8.29 ±2.92	12.85 ±3.48	8.80 ±4.21	11.96 ±2.52
MUFA	30.55 ±2.61	29.10 ±5.71	30.98 ±3.66	27.30 ±5.77	32.90 ±5.76	28.20 ±5.49
SFA	47.05 ±2.42	48.40 ±5.61	48.87 ±2.77	47.95 ±4.91	45.58 ±5.43	47.73 ±5.16
Σn3/Σn6	0.26 ±0.02	0.25 ±0.04	0.25 ±0.04	0.23 ±0.04	0.24 ±0.03	0.23 ±0.04
Σn6/Σn3	3.90 ±0.29	4.11 ±0.91	4.07 ±0.54	4.44 ±0.84	4.25 ±0.51	4.49 ±0.88

Note: SM – *Semitendinosus* muscle; TB – *Triceps brachii* muscle.

the hydrolytic activity of the lipases, the microbial metabolic processes, and the oxidative reactions that work on the free fatty acids released in the lipolysis. These are directly related to the raw material used to prepare the sausages, ingredients, additives and spices added, and the production process **(Soriano et al., 2006)**.

No significant differences were found between the different curing methods for *Semitendinosus* muscle (ANOVA, p >0.05, F – 0.003, F crit. – 3.041) and *Triceps brachii* muscle (ANOVA, p >0.05, F – 0.05, F crit. – 3.01).

The results of the PC analysis are presented in Table 2. The first three PC explain 100% of total variation for percentage fatty acids content. **Laville et al., (1996)** found the first ten PCs analysing 76 morphometric variables from young Charolais bull carcass explained 80% of the total variability of those measurements. However, in rabbits **Hernández et al., (2000)** reported the four first PCs for meat quality explained 62% of the total variation. They analysed meat quality using 23 variables, including pH, meat colour, WHC, cooking loss, fatty acid composition and sensory parameters.

Cañeque (2004) found the first five PCs analysing 20 variables from light lambs explained 77% of total variation. **Šnirc et al., (2016)** reported the first five PC explained more than 85% of total variation for pH, chemical and technological parameters and higher level 86% for textural parameters for meat from farmed red deer and pastured red deer.

Figure 1 shows the loading plot of the measurements of fatty acids content on the first two PCs. The measurements and PCs are interpreted according to the correlations between each parameters and each PC, thus measurements close to each other are positively correlated, measurements separated 180° are negatively correlated, whereas if they are separated by 90° they are independent. The loading plot displays that these measurements are placed far from the origin of the first PC and near each other indicating the high correlation among them (Figure 1).

Table 2 Results from principal component analysis for fatty acids content.

	F1	F2	F3
Eigenvalue	2.9797	0.0164	0.0039
Variability (%)	99.3238	0.5460	0.1301
Cumulative (%)	99.3238	99.8699	100.0000

Note: F1 - Principal component 1; F2 - Principal component 2; F3 - Principal component 3.

The results of present study are in accordance with **Gladwin et al., (2005)** who studied free fatty acids in commercial saucissons made with deer meat. The amounts of these majority acids are similar to those found in our work. The use of salt in production of fermented meat is without alternatives, as it affects fundamental processes in meat conditioning, on microbial performance, hygiene, shelf life, flavour and texture **(Desmond, 2006)**.

Chorizos and saucissons made with deer meat and commercialised in the Spanish market had a higher fat content than those prepared with wild boar meat, and a further two factors that may be differentiating were the protein nitrogen and phosphorus content, which were higher in dry sausages made with wild boar. Chorizos, made with deer or wild boar meat, had higher percentages of polyunsaturated free fatty acids, linoleic and linolenic acids, and lower percentages of the monounsaturated 11-eicosenoic acid, than had the saucissons **(Soriano et al., 2006)**.

Paleari et al., (2006) recorded that cured, fermented and dried products of wild boar and horsemeat showed reduced values of saturated fatty acids (SFA). The products from wild boar, goat and beef had higher values of

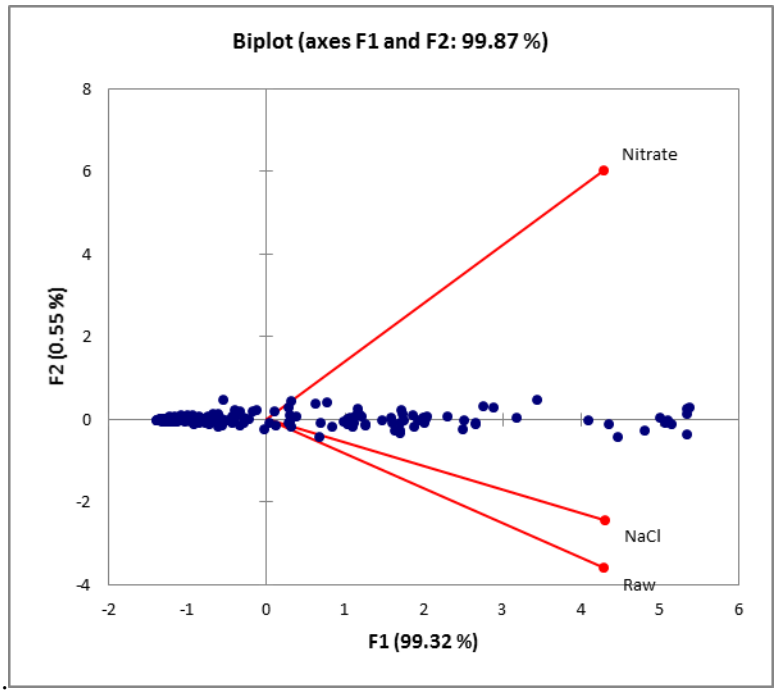

Figure 1 Plot of the first two PC loading vectors. The labels correspond to sample notation given in the Table 1

monounsaturated fatty acids (MUFA). The polyunsaturated fatty acids (PUFA), known to play an irreplaceable dietary role, were found to be highest in the horsemeat product, at an intermediate level in the final product of deer and extremely reduced in the beef product.

CONCLUSION

In this study the effect of different curing methods on the fatty acid composition was evaluated.

The kind of curing salt aren´t significantly affected the fatty acid compounds formation in dry-cured deer meat. The fatty acid compounds were selected based on their presence and contribution of dry-cured meat products.

The different curing agents (sodium chloride and nitrate) were no effect on fatty acids content in dry cured meat products prepared from deer meat muscles. The present study revealed that game meat can function as a good source of bioactive compounds that are essential for human nutrition.

Further research into the relationship between different types of curing on fatty acids profile is needed to determine which compounds could be used as markers of sausage quality.

REFERENCES

Alford, J. A., Smith, J. L., Lilly, H. D. 1971. Relationship of Microbial Activity to Changes in Lipids of Foods. *Journal of Applied Bacteriology*, vol. 34, no. 1, p. 133-146. http://dx.doi.org/10.1111/j.1365-2672.1971.tb02273.x.

Barry, T. N., Wilson, P. R. 1994. Venison production from farmed deer. *The Journal of Agricultural Science*, vol. 123, no. 2, p. 159. http://dx.doi.org/10.1017/s0021859600068416

Belej, Ľ., Barnová, M., Maršálková, L., Golian, J. 2011. The yield of DNA in thermal terated deer meat. *Potravinarstvo*, vol. 5, no. 3, p. 6-10. http://dx.doi.org/10.5219/153

Bertolini, R., Zgrablic, G., Cuffolo, E. 2005. Wild Game Meat: Products, Market, Legislation and Processing Controls. *Veterinary Research Communications*, vol. 29, no. S2, p. 97-100. http://dx.doi.org/10.1007/s11259-005-0029-x

Caneque, V. 2004. Carcass and meat quality of light lambs using principal component analysis. *Meat Science*, vol. 67, no. 4, p. 595-605. http://dx.doi.org/10.1016/j.meatsci.2004.01.002

Corral, S., Salvador, A., Flores, M. 2013. Salt reduction in slow fermented sausages affects the generation of aroma active compounds. *Meat Science*, vol. 93, no. 3, p. 776-785. http://dx.doi.org/10.1016/j.meatsci.2012.11.040

Cygan-Szczegielniak, D., Janicki, B. 2011. Influence of age and sex on the CLA and other fatty acids content in roe deer meat (*Capreolus capreolus* L.). *Folia Biologica*, vol. 59, no. 1, p. 19-24. http://dx.doi.org/10.3409/fb59_1-2.19-24

Demeyer, D., Hoozee, J., Mesdom, H. 1974. Specificity of lipolysis during dry sausage ripening. *Journal of Food Science*, vol. 39, no. 2, p. 293-296. http://dx.doi.org/10.1111/j.1365-2621.1974.tb02878.x

Desmond, E. 2006. Reducing salt: A challenge for the meat industry. *Meat Science*, vol. 74, no. 1, p. 188-196. http://dx.doi.org/10.1016/j.meatsci.2006.04.014

Franco, I., Martínez, A., Prieto, B., Carballo, J. 2002. Total and free fatty acids content during the ripening of artisan and industrially manufactured "Chorizo de cebolla". *Grasas y Aceites*, vol. 53, no. 4, p. 403-413. http://dx.doi.org/10.3989/gya.2002.v53.i4.338

Franklin, I. R. 1980. *Evolutionary change in small populations*. Conservation biology: an evolutionaryecological perspective. In Soule´ M. E., Wilcox, B. A. (ed.); Sunderland (MA : Sinauer Associates). p. 135-149.

Gassara, F., Kouassi, A. P., Brar, S. K., Belkacemi, K. 2015. Green Alternatives to Nitrates and Nitrites in Meat-based Products–A Review. *Critical Reviews in Food Science and Nutrition*, vol. 56, no. 13, p. 2133-2148. http://dx.doi.org/10.1080/10408398.2013.812610

Gil, M., Guerrero, L., Sárraga, C. 1999. The effect of meat quality, salt and ageing time on biochemical parameters of dry-cured *Longissimus dorsi* muscle. *Meat Science*, vol. 51, no. 4, p. 329-337. http://dx.doi.org/10.1016/s0309-1740(98)00129-6

Gladwin, M. T., Schechter, A. N., Kim-Shapiro, D. B., Patel, R. P., Hogg, N., Shiva, S., Cannon, R. O., Kelm, M., Wink, D. A., Espey, M. G., Oldfield, E. H., Pluta, R. M., Freeman, B. A., Lancaster, J. R., Feelisch, M., Lundberg, J. O. 2005. The emerging biology of the nitrite anion. *Nature Chemical Biology*, vol. 1, no. 6, p. 308-314. http://dx.doi.org/10.1038/nchembio1105-308

Hernández, P., Pla, M., Oliver, M. A., Blasco, A. 2000. Relationships between meat quality measurements in rabbits fed with three diets of different fat type and content. *Meat Science*, vol. 55, no. 4, p. 379-384. http://dx.doi.org/10.1016/s0309-1740(99)00163-1

Hoffman, L. C., Wiklund, E. 2006. Game and venison – meat for the modern consumer. *Meat Science*, vol. 74, no. 1, p. 197-208. http://dx.doi.org/10.1016/j.meatsci.2006.04.005

Johansson, M., Belsito, E. L., Gioia, M. L., Leggio, A., Malagrinò, F., Romio, E., Siciliano, C., Tagarelli, A. 2015. GC/MS analysis of fatty acids in Italian dry fermented sausages. *The Open Food Science Journal*, vol. 9, no. 1, p. 5-13. http://dx.doi.org/10.2174/1874256401509010005

Krauss, R. M., Eckel, R. H., Howard, B., Appel, L. J., Daniels, S. R., Deckelbaum, R. J., Erdman, J. W., Kris-Etherton, P., Goldberg, I. J., Kotchen, T. A., Lichtenstein, A. H., Mitch, W. E., Mullis, R., Robinson, K., Wylie-Rosett, J., St. Jeor, S., Suttie, J., Tribble, D. L., Bazzarre, T. L. 2000. AHA Dietary Guidelines : Revision 2000: A Statement for Healthcare Professionals From the Nutrition Committee of the American Heart Association. *Circulation*, vol. 102, no. 18, p. 2284-2299. http://dx.doi.org/10.1161/01.cir.102.18.2284

Laville, E., Martin, V., Bastien, O. 1996. Prediction of composition traits of young Charolais bull carcasses using a morphometric method. *Meat Science*, vol. 44, no. 1-2, p. 93-104. http://dx.doi.org/10.1016/s0309-1740(96)00087-3

Maršálková, L., Židek, R., Pokoradi, J., Golian, J., Belej, Ľ. 2014. Genetic diversity and relatedness among seven red deer (*Cervus elaphus*) populations. *Potravinarstvo*, vol. 8, no. 1, p. 15-19. http://dx.doi.org/10.5219/320

Paleari, M. A., Moretti, V. M., Beretta, G., Mentasti, T., Bersani, C. 2003. Cured products from different animal species. *Meat Science*, vol. 63, no. 4, p. 485-489. http://dx.doi.org/10.1016/s0309-1740(02)00108-0

Polak, T., Rajar, A., Gašperlin, L., Žlender, B. 2008. Cholesterol concentration and fatty acid profile of red deer (*Cervus elaphus*) meat. *Meat Science*, vol. 80, no. 3, p. 864-869. http://dx.doi.org/10.1016/j.meatsci.2008.04.005

Purchas, R. W., Triumf, E. C., Egelandsdal, B. 2010. Quality characteristics and composition of the longissimus muscle in the short-loin from male and female farmed red deer in New Zealand. *Meat Science*, vol. 86, no. 2, p. 505-510. http://dx.doi.org/10.1016/j.meatsci.2010.05.043

Ruiz, A. G., Mariscal, C., Soriano, A. 2007. Influence of hunting-season stage and ripening conditions on nitrogen

fractions and degradation of myofibrillar proteins in venison (*Cervus elaphus*) chorizo sausages. *Meat Science*, vol. 76, no. 1, p. 74-85. http://dx.doi.org/10.1016/j.meatsci.2006.10.015

Šnirc, M., Kral, M., Ošťádalová, M., Golian, J., Tremlová, B. 2016. Application of PCA method for characterization chemical, technological and textural parameters of farmed and pastured red deer. *International Journal of Food Properties.* In Press. http://dx.doi.org/10.1080/10942912.2016.1180532

Soriano, A., Cruz, B., Gómez, L., Mariscal, C., García R. A. 2006. Proteolysis, physicochemical characteristics and free fatty acid composition of dry sausages made with deer (*Cervus elaphus*) or wild boar (*Sus scrofa*) meat: A preliminary study. *Food Chemistry*, vol. 96, no. 2, p. 173-184. http://dx.doi.org/10.1016/j.foodchem.2005.02.019

Triumf, E. C., Purchas, R. W., Mielnik, M., Maehre, H. K., Elvevoll, E., Slinde, E., Egelandsdal, B. 2012. Composition and some quality characteristics of the longissimus muscle of reindeer in Norway compared to farmed New Zealand red deer. *Meat Science*, vol. 90, no. 1, p. 122-129. http://dx.doi.org/10.1016/j.meatsci.2011.06.011

Vestergaard, C. S., Schivazappa, C., Virgili, R. 2000. Lipolysis in dry-cured ham maturation. *Meat Science*, vol. 55, no. 1, p. 1-5. http://dx.doi.org/10.1016/s0309-1740(99)00095-9

Vestergaard, C. S., Risum, J., Adler-Nissen, J. 2005. 23Na-MRI quantification of sodium and water mobility in pork during brine curing. *Meat Science*, vol. 69, no. 4, p. 663-672. http://dx.doi.org/10.1016/j.meatsci.2004.11.001

Volpelli, L. A., Valusso, R., Morgante, M., Pittia, P., Piasentier, E. 2003. Meat quality in male fallow deer (*Dama dama*): effects of age and supplementary feeding. *Meat Science*, vol. 65, no. 1, p. 555-562. http://dx.doi.org/10.1016/s0309-1740(02)00248-6

Wang, J., Jin, G., Zhang, W., Ahn, D. U., Zhang, J. 2012, Effect of curing salt content on lipid oxidation and volatile flavour compounds of dry-cured turkey ham. *LWT - Food Science and Technology*, vol. 48, no. 1, p. 102-106. http://dx.doi.org/10.1016/j.lwt.2012.02.020

Warren, H. E., Scollan, N. D., Enser, M., Hughes, S. I., Richardson, R. I., Wood, J. D. 2008. Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. *Meat Science*, vol. 78, no. 3, p. 256-269. http://dx.doi.org/10.1016/j.meatsci.2007.06.008

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R., Enser, M. 2004. Effects of fatty acids on meat quality: a review. *Meat Science*, vol. 66, no. 1, p. 21-32. http://dx.doi.org/10.1016/s0309-1740(03)00022-6

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EVALUATION OF FAT GRAINS IN GOTHAJ SAUSAGE USING IMAGE ANALYSIS

Ludmila Luňáková, Matej Pospiech, Bohuslava Tremlová, Alena Saláková, Zdeňka Javůrková, Josef Kameník

ABSTRACT

Fat is an irreplacable ingredient in the production of sausages and it determines the appearance of the resulting cut to a significant extent. When shopping, consumers choose a traditional product mostly according to its appearance, based onwhat they are used to. Chemical analysis is capable to determine the total fat content in the product, but it cannot accurately describe the shape and size of fat grains which the consumer observes when looking at the product. The size of fat grains considered acceptable by consumers can be determined using sensory analysis or image analysis. In recent years, image analysis has become widely used when examining meat and meat products. Compared to the human eye, image analysis using a computer system is highly effective, since a correctly adjusted computer program is able to evaluate results with lower error rate. The most commonly monitored parameter in meat products is the aforementioned fat. The fat is located in the cut surface of the product in the form of dispersed particles which can be fairly reliably identified based on color differences in the individual parts of the product matrix. The size of the fat grains depends on the input raw material used as well as on the production technology. The present article describes the application of image analysis when evaluating fat grains in the appearance of cut of the Gothaj sausage whose sensory requirements are set by Czech legislation, namely by Decree No. 326/2001 Coll., as amended. The paper evaluates the size of fat mosaic grains in Gothaj sausages from different manufacturers. Fat grains were divided into ten size classes according to various size limits; specifically, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 5.0, 8.0 and over 8 mm. The upper limit of up to 8 mm in diameter was chosen based on the limit for the size of individual fat grains set by the legislation. This upper limit was not exceeded by any of the products. On the other side the mosaic had the hightest representation of 0.25 mm fat grains.

Keywords: fat grain; Gothaj sausage; image analysis;

INTRODUCTION

Gothaj is a favourite traditional Czech meat product. It is characteristic primarily by its specific mosaic rich in visible fat grains. It is a meat product which is affordable for the majority of consumers. However, consumers are used to a certain standard even in this cheaper assortment of meat products. When purchasing it, the decisive factors are attractiveness as well as previous experience of the customer. As with many other selected meat products, correct recipes and prescribed sensory, physical and chemical properties set by legislation must be followed.

The characteristic size and appearance of lard grains in the mosaic is closely related to the selection of input ingredients, it's mincing and the method of mixing the product. In their work, **Saláková et al. (2013)** analysed fat content as one of the criteria for assessing quality of Gothaj sausage. The study revealed that unlike water content, the fat content in Gothaj has decreased in the last twenty years. Specifically, it was found that the limit set by Decree No. 326/2001 Coll, i.e. 40%, or the limit set by the original quality standard (ČSN 57 7231), i.e. 42%, was not reached by any of the tested samples. Only one of the studied samples reached 32.7% fat content, with the remaining samples scoring even lower.

The filling of soft salamis, which include Gothaj as well, can be processed in two ways: either as homogenous filling with finely cut and ground binder or non-homogenous, containing large-grained core ingredient in the filling. Gothaj is ranked among sausages with large grains of fat in the filling **(Steinhauser et al., 1995)**. The results of sensory evaluation clearly point to the fact that fat in the product has significant influence on its sensory properties. In Gothaj, fat represents one of the core ingredients of the filling and is therefore essential for the appearance of the cut product. Saláková et al. (2013) discovered that currently, the popularity of products with lower fat content is growing among consumers. In a sensory evaluation it was determined that the more visible the lard in the sausage section, the lower the preference of the product.

In terms of quality management of food products, attention is focused on delivering consistent products to the consumer for an affordable price. The ideal method to ensure food safety and quality is regular monitoring food products in all stages of their production and distribution. It is therefore necessary to have effective monitoring systems which can obtain reliable information on the content, composition and safety characteristics of food ingredients and finished products. The processes

associated with quality assurance of food products usually incorporate chemical or mechanical analyses or human senses. Unfortunately, these are time consuming, laborious, costly and destructive methods, or methods requiring specific preparation of the sample. Non-invasive methods have thus been introduced into the process of quality assessment in recent years which are both sufficiently objective and quick. One of these is the use of computer visualisation **(Elmasry and Nakauchi, 2016)**.

As reported by **Brosnan and Sun (2004)**, image analysis is a suitable alternative for the evaluation of meat and meat products. Image analysis can be applied from the early stages of the production process through to the finished products intended for consumers.

Various sensory and physical-chemical parameters as well as the composition and more specific chemical composition are generally assessed not only in meat products, but in other food products as well. Selected quality characteristics, such as color, smell, taste and mincing can be determined in meat and meat products relatively quickly and without complex laboratory analyses. Since determining other, primarily chemical parameters which influence the quality of the food requires special laboratory equipment and work with chemicals, the development of monitoring methods is heading towards an innovative form of instrumental methods for quality management in food businesses. These methods also include digital image analyses **(Zapotoczny, Szczypiński, Daszkiewicz, 2016)**. Also **Pospiech et al. (2009)** confirms that by using image analysis for quantitatively evaluate of meat products are obtained objective and accurate results that are comparable to chemical analysis.

Pork fat does not contain caroteneoids, which makes it usually white **(Steinhauser et al., 1995)**. The fat particles provide sufficiently intense contrast to other ingredients and are thus easily detectable through image analysis. The evaluation of visible fat using image analysis on a section of dry sausage was studied by **Girolami et al. (2014)**. The reasearch assessed the ratio of fat in the analysed portion of the product based on evaluation of the color and fat content of the cut product. The study demonstrated that image analysis is suitable even for meat products such as sausages.

The use of a computer visualisation system for the evaluation of fat in the product was also studied by **Chmiel et al. (2011)**. They specifically focused on the assessment of fat content in chicken and turkey breast and thigh muscles. The analysis was based on assessing color and saturation. When compared to the Soxhlet method, the results showed that image analysis is not suitable for this category of food products. Determination using image analysis was problematic due to the contrast between fat and muscle mass being insufficient.

Image analysis appears to be a suitable method for evaluating quality for food products with sufficient color contrast between the individual components of the product. Such meat products include e.g. salamis and sausages. Evaluation of fat grains using image analysis methods was examined by **Čáslavková et al. (2014)**. The author analysed a cut of Poličan fermented sausage. The suitability of image analysis for the detection of fat in fermented meat products was also confirmed by **Pospiech et al. (2013)**.

The aim of the study was to compare fat grains in Gothaj type products from various manufacturers and evaluate the consistency of their product lots.

MATERIAL AND METHODOLOGY

The object of examination were Gothaj sausages from seven different manufacturers, always in three lots. Samples were taken in the period of October 2014 – January 2015. From each sample, 3 sections were prepared for image analysis at a thickness of 0.5 each. Thus, a total of 63 sections were prepared for the examination.

Constant lighting conditions were established for the taking of images using EASY LIGHT lamps – 3.3 x 28 W and 5000 – 5500 K. The stability of light intensity was monitored using a lux meter (Voltcraft LX – 1108), with light intensity level set to 7290 ±50 lux. Images were captured using EOS 600D camera (Canon, JPN). The images were captured in manual mode with the following settings: exposure time 1/100, aperture F8, image size L, sensitivity ISO 100, data format JPEG, maximum zoom 55 mm, with the additional use of the white balance function. For the contrast between the sample and the background to be sufficient, the pictures were taken on a deep blue background.

The analysis itself was performed in NIS – Elements BR 4.13.00 software (Laboratory imaging, CZE). The program is commonly used to study, scan and archive image structures. The samples can be measured manually or automatically. A scale was attached to each sample when the picture was taken. The scale was calibrated for all the studied images before the analysis itself. For each lots of samples, the size of 1 pixel was determined.

The measuring of the analysed objects in NIS – Elements BR was performed using the fully automated macro function. The macro was defined for the opening of the file, delimiting the region of interest (ROI) and identification of fat grains. Image and binary adjustment, such as double smoothing, hole separation and hole filling, were also included.

Binary transposition of fat grains was performed in HSI color system. The system allows to define a threshold for hue, saturation and intensity of color corresponding to the fat grain contained in the sample matrix. Based on these, the program is capable of automatically marking the boundaries of fat grains. The next step was binarisation, which ensured effective evaluation of fat grains and other components, including the image background (see Fig. 1). The subject of the analysis was the fat grains of all sizes, which were subsequently categorised in size classes: 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 5, 8 and above 8 mm; see the chapter Results and Discussion.

Measured parameters such as length, surface, equivalent diameter and width of fat grains were statistically processed further using UNISTAT software version 6.0. Based on a normality test, the data were evaluated as abnormal.

Lots analysis was performed using a multiple comparisons ANOVA test, Dunnet. Analysis of differences between individual manufacturers was carried out via a non-parametric Kruskal-Wallis One-Way Anova test, Dunnet (UNISTAT software version 6.0, UNISTAT Ltd.).

For the purposes of the analysis, we used the measured length of the objects as the average determined by the Decree, as it is the longest quantity which passes through the object. The length values were then statistically evaluated. Further, the quantity of objects for individual size classes was calculated. Histograms were then created for transparency. We focussed primarily on the length, area, equivalent diameter and width of fat grains (Figure 3).

The representation of ideal fat grains was calculated using the methodology in **Čáslavková et al. (2014)**, called the CNSFT coefficient.

RESULTS AND DISCUSSION

The Gothaj sausage is a meat product of a darker meat-pink color at the cut. The filling is finely processed, the fat mosaic irregularly distributed. Occasional fine-grained collagen particles and small air cavities are acceptable. The size of individual fat grains must be up to 8 mm in diameter; particles of spice may be visible **(Decree, 2001)**. The present study analysed fat grains, i.e. the fatty tissue from the morphological point of view. Manufacturers of Gothaj sausages most commonly use back fat as the core ingredient for their product. Based on the traditional method of producing Gothaj sausages, the core ingredient is mixed into the filing at grain size of 8 mm **(Šedivý, 1998).** The study also included the determination of the size profile of fat grains and the monitoring of whether the limits for fat grain size were exceeded.

The production technology for the majority of meat products is based on mincing and mixing individual components of the product. Due to the constant development of better and more advanced production machinery, the technological procedure of production changes. Mincing leads to reduction in the size of larger meat and fat particles. To create the desired appearance of the mosaic, excessive heating of the product must be avoided, as it could result in softening the fat tissue and smearing of fat particles (Pipek, 1998).

The results of analysis of the quantity of fat grains show that the limit of 8 mm set for the size of fat grains was not exceeded by any of the manufacturers. For the analysis of the size of fat grains, fat tissue was divided into 10 classes; see Table 1.

As shown in Table 1, the size class 5.1 – 8 was represented by one sample only. It is therefore rather an accidental occurrence than the intention of the manufacturer. On the other hand, based on the calculation

Table 1 Distribution of fat grain sizes.

Sample	Class										
	0 – 0.25	**0.26 – 0.5**	**0.26 – 0.75**	**0.76 – 1.0**	**1.1 – 1.5**	**1.6 – 2.0**	**2.1 – 2.5**	**2.6 – 5.0**	**5.1 – 8.0**	**>8.0**	**CNSFT**
B2	3020	1720	522	175	65	4	-	-	-	-	0.73×10^{-3}
B5	2097	801	227	95	61	21	4	-	-	-	7.51×10^{-3}
B6	3461	1628	558	229	195	44	10	5	-	-	9.62×10^{-3}
B7	1841	938	289	114	53	13	3	3	1	-	5.84×10^{-3}
B11	3548	1548	310	65	17	-	-	-	-	-	0
B12	3142	1036	329	128	58	12	1	2	-	-	3.19×10^{-3}
B13	3925	984	242	72	21	4	1	-	-	-	0.95×10^{-3}
SUM	**21034**	**8655**	**2477**	**878**	**470**	**98**	**19**	**10**	**1**	-	

Note: The size of fat grains is measured in cm.

$$CNSFT = \frac{\sum fat\ grain\ length\ of\ category\ (1.6 - 2.0 + 2.1 - 2.50 + 2.6 - 5.0)}{\sum fat\ grain\ length\ of\ all\ categories}$$

Table 2 Lot comparison.

Sample	LOT 1	LOT 2	LOT 3
B2	a	ab	b
B5		a	a
B6	a	a	
B7	a	a	
B11		a	a
B12	a	ab	b
B13	a	ab	b

Note: letters indicate statistically significant difference ($p < 0.05$).

Table 3 Differences between manufacturers.

Sample	B13	B12	B11	B7	B6	B5	B2
B13	-	**	**	**	**	**	**
B12	**	-	-	**	**	-	**
B11	**	-	-	**	**	-	**
B7	**	**	**	-	-	**	-
B6	**	**	**	-	-	**	-
B5	**	-	-	**	**	-	**
B2	**	**	**	-	-	**	-

Note: ** indicate statistically significant difference ($p < 0.05$).

of the sum of occurence frequency for all manufacturers, the greatest representation can be found for fat grains in the 0 – 0.25 category, followed by the 0.26 – 0.5 category, whose frequency is also quite high. Fat grains in the 0 – 0.25 category are at the margin of visibility; therefore, the category plays no part in terms of sensory perception. The high amount of fat grains in this category, however, is indicative of the fat smearing during the incorporation into the product.

Consumers are easily influenced by modern principles which have become rampant in recent years. Therefore, many consumers prefer products with lowest possible fat content. Unfortunately, this attitude is also reflected in purchasing traditional products which are typical by higher fat content. For this reason, the present study evaluates the optimal size of fat grains in the matrix, which for consumers means fat visible macroscopically, i.e. by their naked eye. The resolution capability of the human eye is approximately 0.25 mm. Therefor we chose the value of above 2 mm for the size of fat grains.

To determine which product meets the requirements in terms of the distribution of fat grains best, we used a modified CNSFT ratio coefficient **(Čáslavková, 2014)**. On the basis of the calculation of CNSFT, the best results were achieved by the product from manufacturer B6 (CNSFT = 9.26E-03) which had the largest amount of fat grains approaching the ideal size classes. From among the other tested samples, high CNSFT values were achieved by products from manufacturers B5, B7 and B12. Low CNSFT values in manufacturers B2, B11 and B13 show that their products contained a low number of ideally sized fat grains and a large number of small fat grains **(Table 1)**.

Girolami et al. (2014) evaluated visible fat in a section of salamis and sausages using the image binarisation function, where the color image is converted into a black and white image. The authors also confirm the connection between production process technologies and variability in the appearance of the final product. Similarly, **Saláková et al. (2013)**, based on sensory evaluation the authors came to the conclusion that consumers generally prefer products with lower representation of fat grains.

The size of fat grains naturally has a close relationship to the technology of production of the Gothaj sausage. In our study, we therefore also assessed differences in the representation of fat grain sizes in individual lots of products. The differences between lots are shown in Table 2.

In aggregate, manufacturers B2, B12 and B13 differed in two lots. In other manufacturers, only one out of three lots was different ($p > 0.05$). However, homogeneity of lots which can be evaluated sensorially plays a key role in the consumers' choice of product. Every consumer prefers certain product characteristics; if the products appearance deviates, the consumer is likely to confuse the product with something else, or consider the product poorly made. It is therefore advisable that the manufacturer of standard products monitors the homogeneity of individual lots. An ideal manufacturer should have no differences between lots. Such a case was not found in any of the manufacturers.

Based on the comparison of results, we were also able to confirm the assumption that the finished products also differ from each other. See Table 3 and Figure 2.

Figure 1 Automatic detection of fat grains in the cut appearance using the binarisation function.

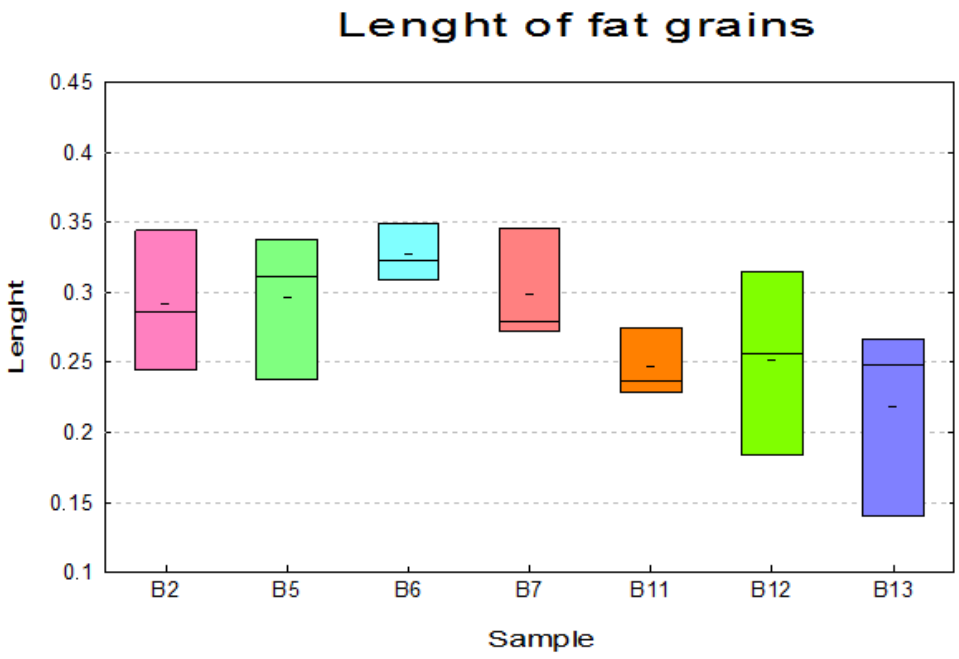

Figure 2 Box diagram for the comparison of similarities between manufacturers based on the length of fat grains.

Product B13 differs the most. The reasons for the differences between manufacturers and lots could be linked to different handling of the raw material added into the cutter as well as heat conditions in the plant or heating caused by intense mincing **(Pipek, 1998)**.

Pork lard is an indispensable material in the production of traditional food products. The production of Gothaj sausage, in which pork lard is a core ingredient, requires the raw lard to be solid and hearty. If that is the case, the lard will be minced properly. It is therefore necessary that the lard is easy to cut, does not smear and does not melt at low temperatures. The viscosity of lard has deteriorated in the last few decades. Fat is rather soft and causes fairly significant problems in the technology of producing sausages **(Pipek et al., 2012)**. Fat consistency depends on the content of saturated and unsaturated fatty acids. With the growing content of unsaturated fatty acids, the consistency of the fat changes as well, causing the fat to become softer. Softer fat is suitable for the production of minced meat products and unsuitable for the production of sausages, where solid fat is required **(Feiner, 2006)**. The influence of technology used on the standard nature of the finished product was also confirmed by **Čáslavková et al., (2014)**.

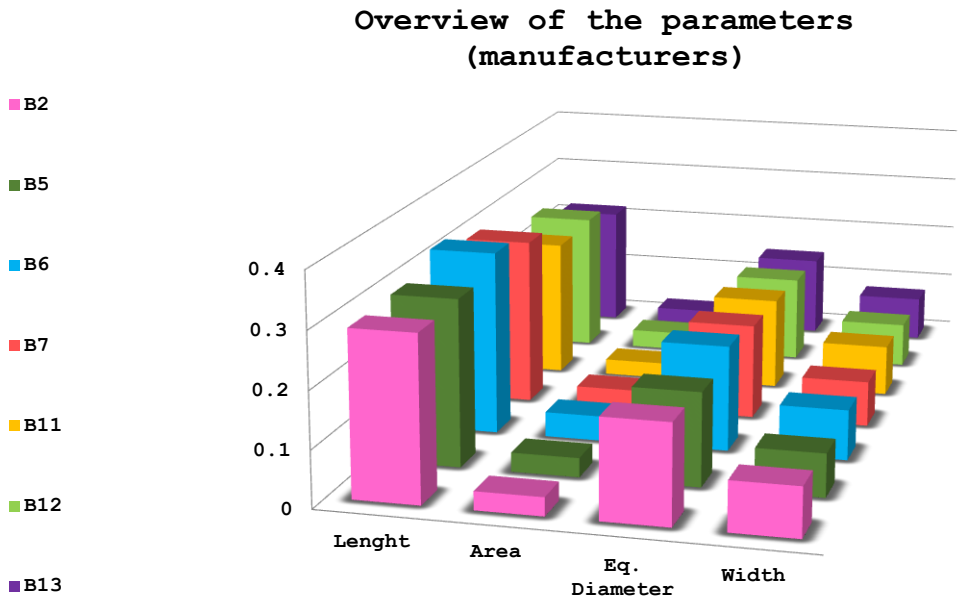

Figure 3 3D chart shows a summary of the parameters for the size and shape of contained fat grains for each manufacturer.

The subject of the examination was the dry fermented sausage Poličan. Image analysis was used as the evaluation method. The results showed a statistically significant difference (p <0.05) between manufacturers using different technologies (preparation of the product only in a bowl cutter, or in a combination of a bowl cutter and a vaccum filler with a sausage grinder). The results indicate that the desirable grain size of 1.5 – 3 mm is typical for technology using a vaccum filler with a sausage grinder.

CONCLUSION

The studied method of image analysis appears to be a suitable method for the detection of fat grains in soft sausages. Using a properly programmed visualization system, it is possible to obtain information regarding the size, shape and many other geometric parameters of the fat particles contained in the product in a very short span of time. The data obtained in the image analysis are then very easy to be processed statistically. By our results, we determined that the present study has a high representation of fat grains in size classes 0 – 0.25 and 0.26 – 0.5 mm. Based on gained data, we derived an assumption that grains within these categories formed due to the smearing of the raw material during technological processing. In comparison, only a very small number representation was detected for fat grains in size classes of 2.6 – 5.0, 5.1 – 8.0 and above 8 mm. From this point of view, it is worth pondering why there is a fat grain size limit of 8 mm for the Gothaj sausage set by legislation.

The comparison of the results obtained also showed that the structure of the cut product differs between individual manufacturers. In addition, there is a noticeable difference between individual lots by the same manufacturer.

REFERENCES

Brosnan, T., Da-Wen, S. 2004. Improving quality inspection of food products by computer vision - a review. *Journal of Food Engineering*. vol. 61, no. 1, p. 3-16. http://dx.doi.org/10.1016/S0260-8774(03)00183-3

Čáslavková, P., Pospiech, M., Štarha, P., Kameník, J., Saláková, A., Tremlová, B., Řezáčová Lukášková, Z. 2014. Použití analýzy obrazu k hodnocení nákroje trvanlivého fermentovaného salámu Poličan (The use of digital image analysis for the evaluation of slices of dry fermented sausage type Poličan). *Maso*. vol. 14, no. 6, p. 20-27.

Chmiel, M., Słowiński, M., Dasiewicz, K. 2011. Application of computer vision systems for estimation of fat content in poultry meat. *Food Control*. vol. 22, no. 8, p. 1424-1427. http://dx.doi.org/10.1016/j.foodcont.2011.03.002

Czech decree No. 326/2001 Coll. that implements Section 18, letters a), d), g), h), i) and j) of Act No. 110/1997 Coll., on foodstuffs and tobacco products and on the amendment and additions to some related acts, as amended, for meat, meat products, fish, other water animals and products of them, eggs and products of them, as amended.

Elmasry, G. M., Nakauchi, S. 2016. Image analysis operations applied to hyperspectral images for non-invasive sensing of food quality – A comprehensive review. *Biosystems Engineering*. vol. 142, p. 53-82. http://dx.doi.org/10.1016/j.biosystemseng.2015.11.009

Feiner, G. 2006. *Meat products handbook: Practical science and technology*. Elsevier, 672 p. ISBN 9781845690502

Girolami, A., Napolitano, F., Faraone, D., Di Bello, G., Braghieri, A. 2014. Image analysis with the computer vision system and the consumer test in evaluating the appearance of Lucanian dry sausage. *Meat science*. vol. 96, no. 1, p. 610-616. http://dx.doi.org/10.1016/j.meatsci.2013.08.006

Pipek, P. 1998. *Technologie masa II*. (*Meat technology II*.) 1st ed. Praha : Karmelitánské nakladatelství. 360 p. ISBN 80-7182-283-8

Pipek, P., Rohlík, B. A., Potůček, T., Šimoniová, A. 2012. The composition of pork lard as a raw material in meat production. *Maso International*, vol. 02, p. 115-119.
Available at: http://www.maso-international.cz/the-composition-of-pork-lard-as-a-raw-material-in-meat-production/

Pospiech, M., Tremlová, B., Randulová, Z., Řezáčová Lukášková, Z. 2009. Microscopic methods in analysis of foodstuff - review. *Potravinarstvo*, vol. 1, Special Issue, p. 38-41.
http://www.potravinarstvo.com/dokumenty/potravinarstvo_no1_2009.pdf

Pospiech, M., Horák, R., Čáslavková, P., Tremlová, B., Saláková, A., Kameník, J. 2013. Srovnání obrazové analýzy barvených a nebarvených nákrojů masných výrobků (Comparison of colored section and uncoloured section of the meat products using image analysis). *Potravinarstvo*. vol. 7, Special issue, p. 111-115. http://www.potravinarstvo.com/dokumenty/mc_march_2013/bezpecnost_potravin_zivocisneho_povodu/pospiech.pdf

Saláková, A., Pavlík, Z., Kameník, J., Steinhauserová, I., 2013. Gothajský salám (*Gothaj sausage*). *Maso*, vol. 13, no. 4, p. 18-23.

Šedivý, V. 1998. *Spotřební normy pro masné výrobky (Consumer standards for meat products)*. 3rd edition. Tábor, Czech Republic : OSSIS, 319 p., ISBN 80-902391-0-2

Steinhauser, L., 1995. *Hygiena a technologie masa* (*Hygiene and Meat Technology*). Tišnov, Czeh Republic : Last, 664 p. ISBN 80-900260-4-4

Zapotoczny, P., Szczypiński, P. M., Daszkiewicz, T. 2016. Evaluation of the quality of cold meats by computer-assisted image analysis. *LWT-Food Science and Technology*. vol. 67, p. 37-49.

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COMPARISON OF THE INFLUENCE OF DEFINED STORING CONDITIONS ON ASIAN AND EUROPEAN PEAR CULTIVARS

Petr Šnurkovič, Josef Balík, Jan Goliáš, Miroslav Horák, Anna Němcová

ABSTRACT

The study observed changes in the material composition of European (Conference) and Asian (Yali) pears during the storage period at different temperatures. Three different temperatures were selected for storing, i.e. 1 °C, 5 °C and 20 °C. Assessed for each of the pieces of fruit were flesh firmness, titratable acidity, soluble solids content, content of organic acids, and production of ethylene and carbon dioxide. Fruit stored at 1 °C and 5 °C was analysed before the moment of putting to the store and then after 25, 55 and 70 days of storing. Fruit stored at 20 °C was analysed before the moment of putting to the store and then after 14, 22 and 30 days of storing. The respiration intensity observed through carbon dioxide production at refrigeration conditions was approximately of the same progress for both of the varieties. For the Yali variety, the intensity of respiration of the fruit at the start of the storing period strongly decreased.The same progress was recorded for the Conference variety. Storing at 20 °C increased the respiration intensity. The varieties Yali and Conference which were stored under the temperature of 5 °C had the highest CO_2 production after 70 days of storage. For both varieties the lowest ethylene production during storage was observed at 1 °C. Ethylene production was higher in Yali pear fruits. The Yali variety stored at 20 °C from the beginning produced up to 10 times higher concentration of ethylene than the fruit of the Conference variety. The highest amount of ethylen by the Conference variety was produced by the fruits which were stored under the temperature of 5 °C. At the beginning of the storage period the Conference pears had two-fold higher flesh firmness than the Yali fruit. Fruits of the varieties Yali and Conference which were stored 70 days under the temperature of 1 °C had the highest flesh firmness. For soluble solids and titratable acidity no clear progress was recorded. Malic acid was predominant in both varieties. The Yali fruit contained more citric acid than the Conference fruit. In both varieties soluble solids gradually increased in the early days of storage at all of the monitored temperatures.

Keywords: pear fruit; storage; ethylene; carbon dioxide; acid

INTRODUCTION

The pear tree is included in the Rosaceae family and is an economically important tree species grown in temperate zones **(Bao et al., 2008)**. Consumers evaluate it very positively, mainly because of its distinctive taste properties. Pears fruit are the major source of simple sugars – glucose and fructose etc., organic acids (malic acid and citric acid), minerals, and, last but not least, it also contains a significant amount of ascorbic acid. Sugar and organic acid content have a significant effect on the sensory quality of the fruit, which is also assessed through the physical properties, particularly the size, colour, texture and aromatic properties **(Arzani et al., 2008)**. While stored, the fruit reduces its metabolic activity due to the changes in the concentration of oxygen and carbon dioxide as well as a result of temperature. In addition to keeping good storage conditions, it is necessary to eliminate all the adverse conditions during the growth and ripening of the fruit. **(Chen et al., 2006)**. In recent years, the fruit of Asian pears has been receiving increasing awareness of consumers in Europe. Linked to this is a growing range of cultivars in retail chains, but also in the production practice. To achieve the least possible qualitative and quantitative losses during storage, it is necessary to set optimum storing conditions for individual species or cultivars. Compared to Europe's pears Asian pears are more juicy, and are therefore more susceptible to damaging caused by bruising during handling, which can lead to excessive softening of the flesh and rotting during storage **(Mahajan and Dhatt, 2004)**. To initiate ripening, European pears fruit require the action of rather low storage temperatures, or treatment with exogenous ethylene **(Elgar et al., 1997)**. During storage, climacteric fruits exhibit an increased intensity of respiration and the fruit produces ethylene. Non-climacteric species do not show increased respiration when ripening and the fruit produces ethylene. Cultivars of European pears of *Pyrus comunnis* L. rank among climacteric fruits and feature the formation of butyrous consistency. Cultivars of *Pyrus pyrifolia* Nakai pears are among both climacteric and non-climacteric species; they remain firm and juicy when stored for a long time, but lack the butyrous consistency. **(Jackson, 2003; Itai et al., 2003; Itai and Fujita, 2008)**.

MATERIAL AND METHODOLOGY

Fruit material

The experiment studied the cultivars of European Conference (Figure 2) pears and Asian Yali (Figure 1) pears. The fruit came from orchards of the Horticultural

Figure 1 *Pyrus communis* Yali variety.

Figure 2 *Pyrus communis* Conference variety.

Faculty, Mendel University Brno, Czech Republic. Once harvested, the fruit of the Yali and Conference varieties were immediately stored under three different storage conditions, at the temperature of 1 °C, 5 °C and 20 °C, normal atmosphere. During storage, the fruit was sampled to determine the physico-chemical parameters, i.e. production of ethylene, production of carbon dioxide, flesh firmness, titratable acidity, soluble solids, and organic acids. For both varieties and for storage temperatures of 1 °C and 5 °C, taking samples from the refrigeration room occurred after specified days of storage: the beginning, i.e. day 0, then day 25, day 55 and day 70. For the storage temperature of 20 °C sampling took place on day 0, day 14, day 22 and day 30. Analysis was carried out each time for three samples per variety to calculate the mean and standard deviation of the parameter observed.

Parameters

Flesh firmness was determined by a manual penetrometer with the punch diameter of 11 mm and expressed in MPa. Total acids were assessed by a pH meter with a combined electrode by alcalimetric titration using the 0.1 $mol.l^{-1}$ NaOH (up to pH 8.1). Contents of acids were expressed as content of malic acid (%). Soluble solids content (SSC) was expressed by the index of refraction (°Bx) measured by Abbe refractometer. Contents of CO_2 and ethylene were determined using the GC Agilent 4890C apparatus. Ethylene was estimated by capillary gas chromatography (CGC) using a flame ionisation detector FID. The content of CO_2 was estimated concurrently using a thermal conducting detector TCD. Organic acids (malic and citric) were assessed by HPLC. Assessment conditions: Column: Prevail 5µm Organic Acid 110A HPLC Column 250×4.6 mm, flow rate of mobile phase 25 mM KH_2PO_4 $1mL.min^{-1}$, wavelength 210 nm, temperature +30 °C. Contents of organic acids were expressed in $mg.kg^{-1}$.

RESULTS AND DISCUSSION

For the Yali variety, the intensity of respiration of the fruit at the start of the storing period strongly decreased as measured by the production of carbon dioxide (Figure 3). The same progress was recorded for the Conference variety, which was associated with a decrease in metabolic processes due to cooling the fruit to 5 °C or as low as 1 °C. Conversely, storing at 20 °C increased the respiration intensity which was particularly true for the Conference variety (Figure 4), where the course was typical of the climacteric fruit type (carbon dioxide production over 40 $mg.kg^{-1}.h^{-1}$ on day 14 of storage).

Under refrigerated conditions (1 °C and 5 °C) the respiration intensity did not change significantly and production of carbon dioxide reached a maximum of 10 $mg.kg^{-1}.h^{-1}$ within the initial 55 days of storage. At the end of storage (day 70) both varieties responded to the temperature of 5 °C by similarly high increase in carbon dioxide production (more than 30 $mg.kg^{-1}.h^{-1}$).

For both varieties, the lowest ethylene production during storage was observed at 1 °C (Figures 5 and 6). The ethylene production slightly grew during storage at both

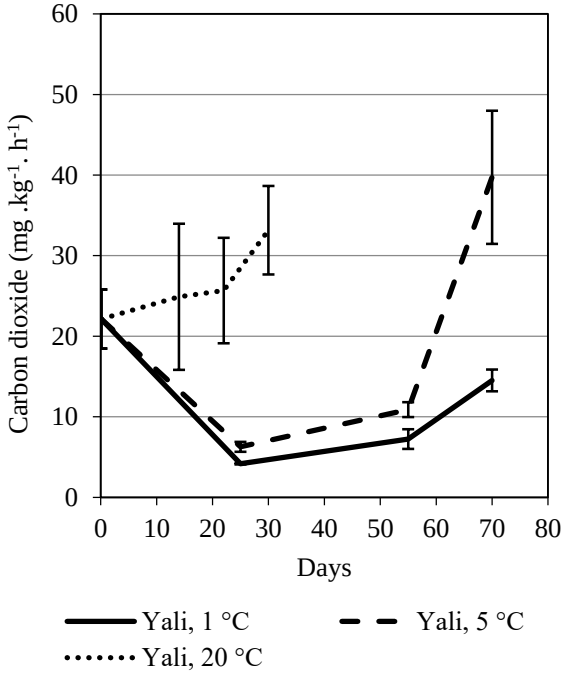

Figure 3 Carbon dioxide generated during the period of storing pears of the Yali variety at different teperatures.

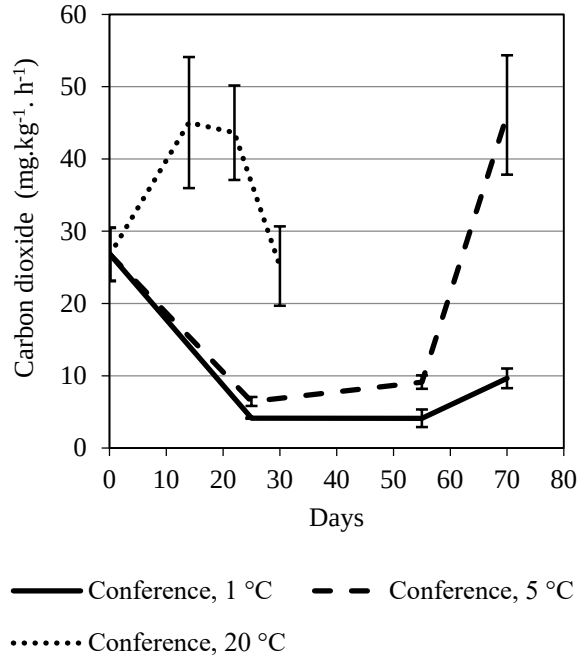

Figure 4 Carbon dioxide generated during the period of storing pears of the Conference variety at different teperature.

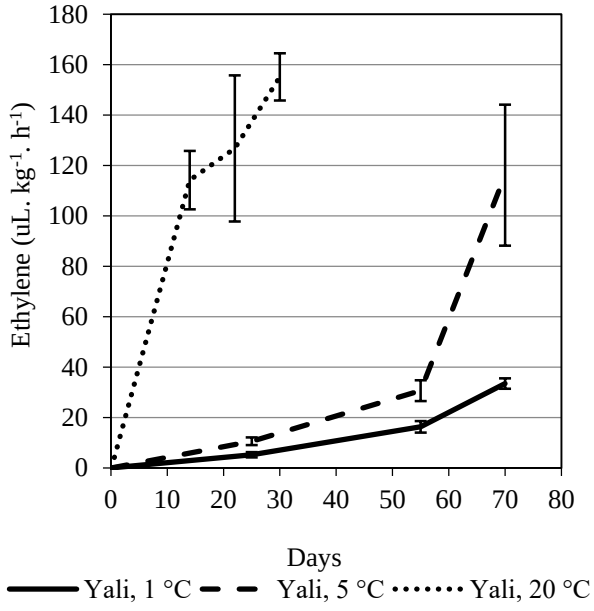

Figure 5 Ethylene generated during the period of storing pears of the Yali variety at different temperatures.

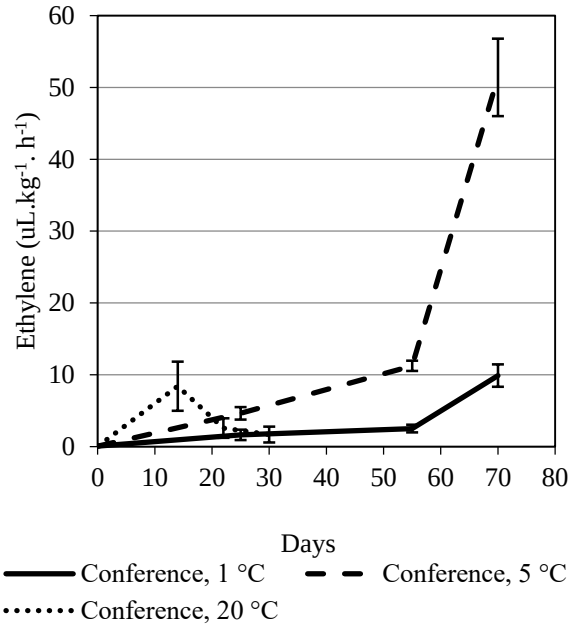

Figure 6 Ethylene generated during the period of storing pears of the Conference variety at different temperatures.

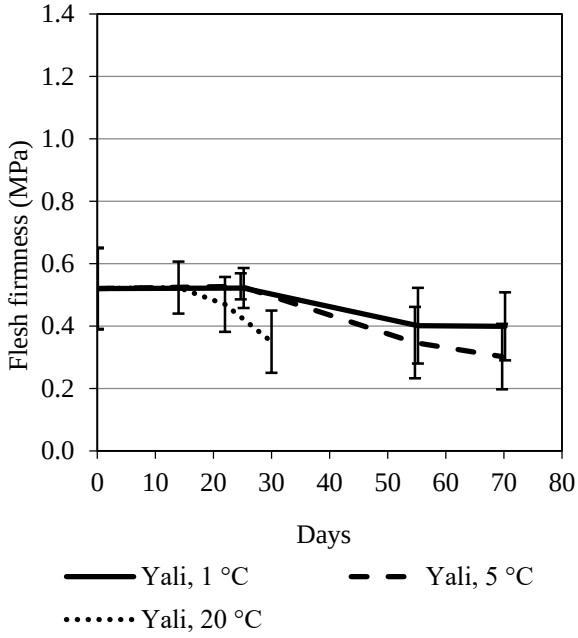

Figure 7 Changed flesh firmness during the period of storing pears of the Yali variety at different temperatures.

refrigerator temperatures for the studied varieties. After 55 days of storage, ethylene production was observed to undergo a rather significant increase which particularly applied to the fruit stored at 5 °C. The Yali variety stored at 20 °C from the beginning produced up to 10 times higher concentration of ethylene than the fruit of the Conference variety. The similar results were achieved by **Arzani et al., (2008)** who monitored the difference during storage of European and Asian pear varieties when the fruit was stored for five months and analysis was conducted after storage month 1, 3 and 5. The Asian fruit was observed to contain 5 times less ethylene than that of European origin. A slight increase in ethylene content occurred during storage in both of the species

At the beginning of the storage period the Conference pears (Figure 8) had two-fold higher flesh firmness than the Yali fruit (Figure 7). The documented changes in the fruit firmness during the storage period make it apparent that the ripening process is greatly influenced by the temperature of storage of pears and was greatly slowed at 1 °C. Far more sensitiveness towards the temperature of post-harvest storage was seen in the Conference pears

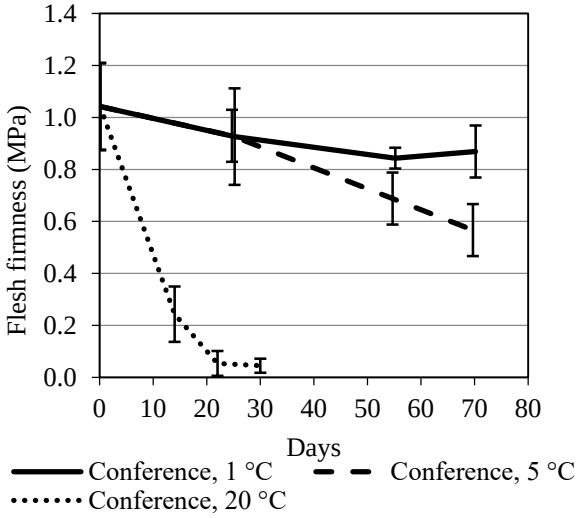

Figure 8 Changed flesh firmness during the period of storing pears of the Conference variety at different temperatures.

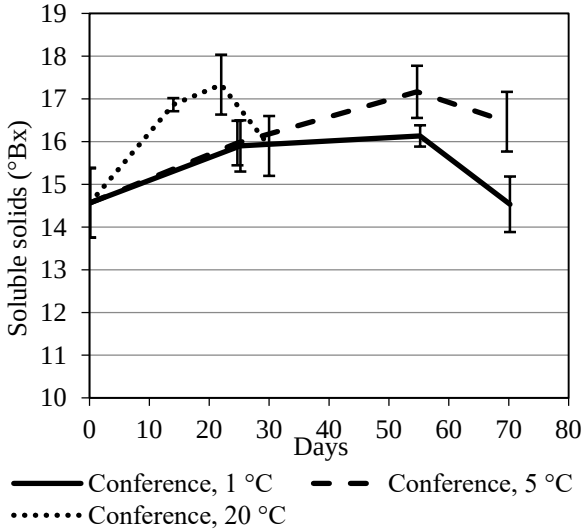

Figure 9 Changed soluble solids during the period of storing pears of the Conference variety at different temperatures.

when it achieved a completely soft structure at the storing temperature of 20 °C after 22 days.

In both varieties soluble solids gradually increased in the early days of storage at all of the monitored temperatures. The Conference pears featured soluble solids of 14.8% immediately after putting into the store (Figure 9). A rapid rise in the content of soluble solids occurred for the pears that were stored at 20 °C; for the temperature of 1 and 5 °C, the increase was gradual until the storage day 50. The Yali pears featured soluble solids of 11% immediately after putting into the store (Figure 10). A gradual rise in the content of soluble solids (55 days) occurred only in the fruit stored at 1 °C. At the end of the storage period there was a decrease of 0.5% approximately.

Chen et al., (2006) noted a similar pattern as regards soluble solids content. The fruit contained 11% soluble solids after storage; over the next two months, the content gradually increased up to 12%; between month 3 and month 4 there was stagnation in terms of content and the

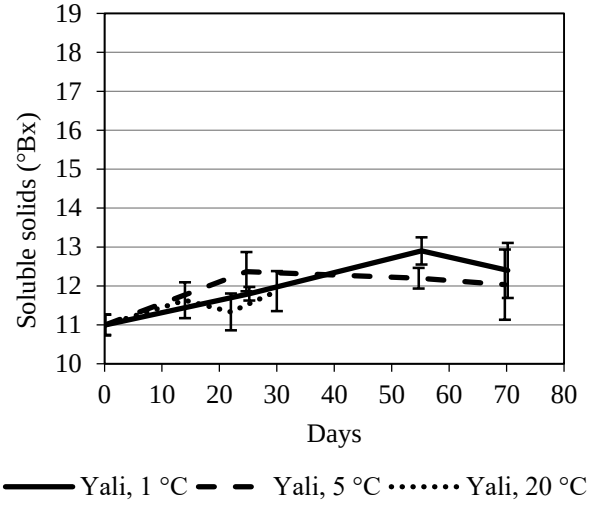

Figure 10 Changed soluble solids during the period of storing pears of the Yali variety at different temperatures.

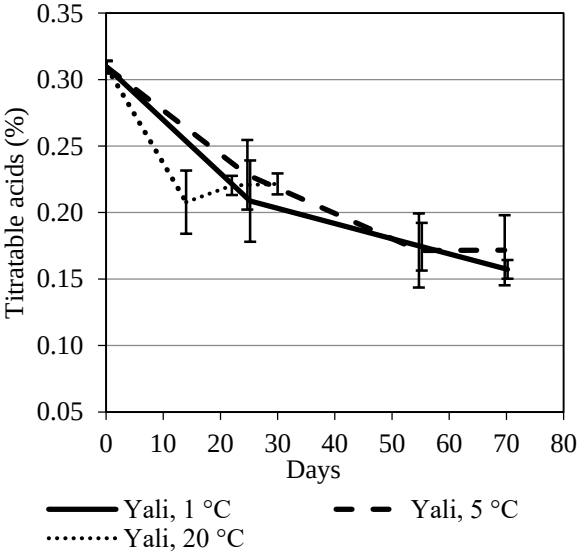

Figure 11 Changed titratable acidity during the period of storing pears of the Yali variety at different temperatures.

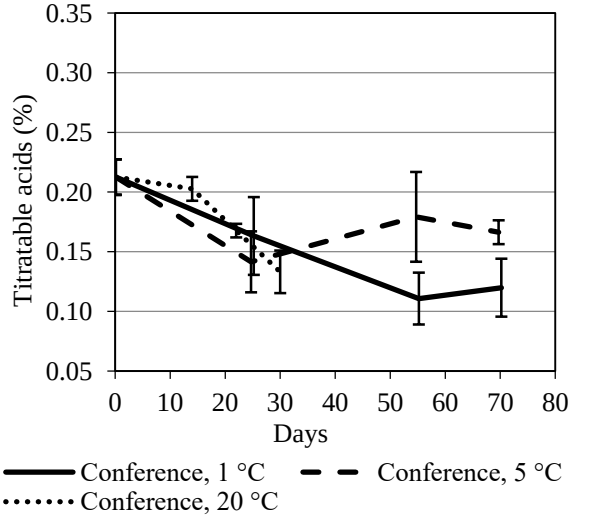

Figure 12 Changed titratable acidity during the period of storing pears of the Conference variety at different temperatures.

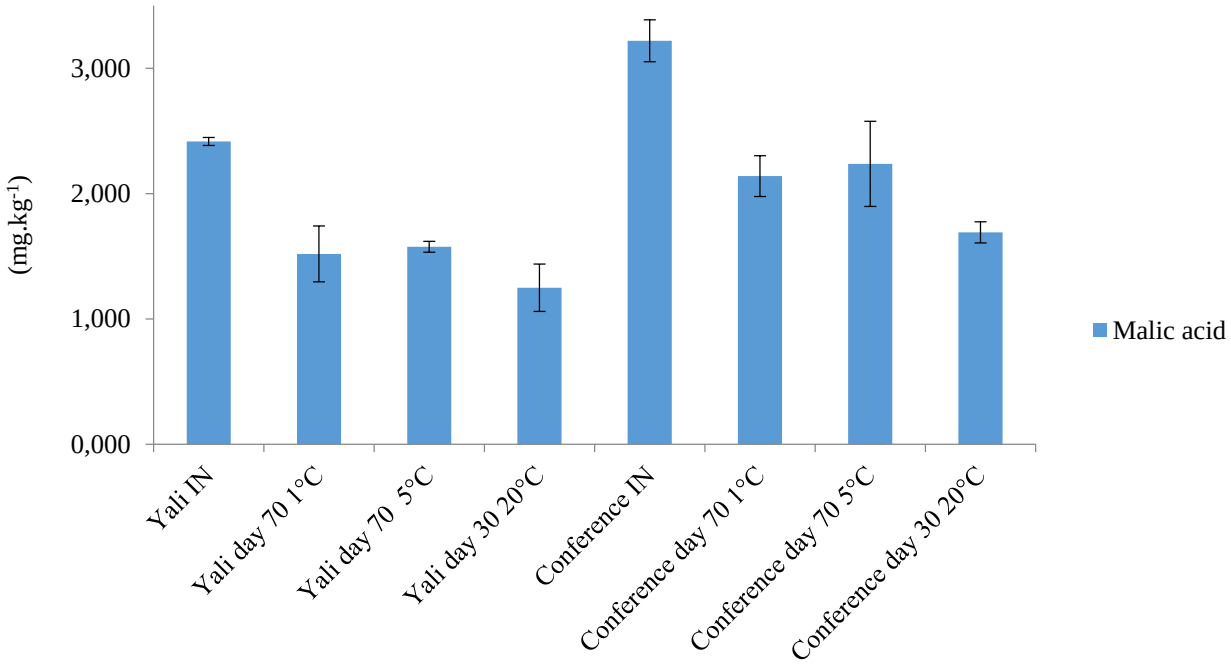

Figure 13 Changes in the content of malic acid at the beginning and the end of the storage period of the pear varieties.

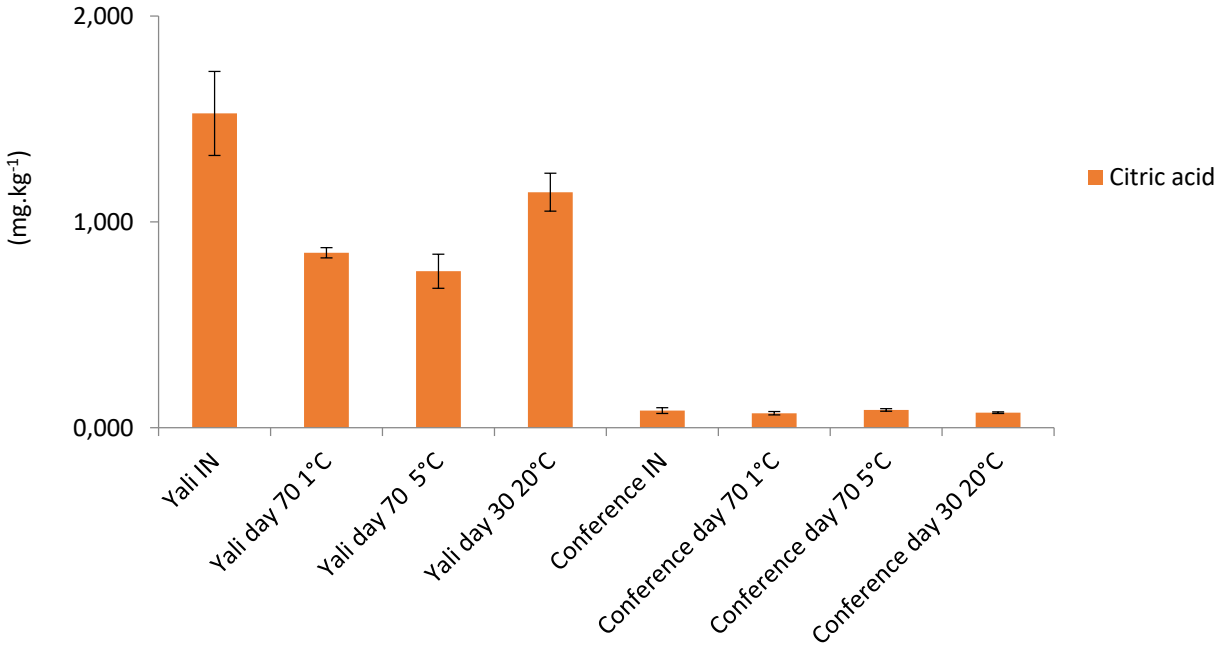

Figure 14 Changes in the content of citric acid at the beginning and the end of the storage period of the pear varieties**.**

last month of storage there was a drop to 11.5%. Immediately after the harvest the fruit flesh firmness was 0.8 MPa while a reduction was seen over the five-month period, the value being approximately identical every month (0.3 MPa). After 5 months of storage, the fruit was measured to show flesh firmness of 0.65 MPa. **Crisosto et al., (1994)** surveyed changes in the content of soluble solids in Asian pears during the storage period of one month. With the figure being around 10% all the time, almost no changes were noted.

A certain increase in soluble solids during storage could be linked to weight loss of fruit while stored. Rather, reducing titratable acidity of the fruit of the pear varieties observed is documented through the trend of ventilating acids during the fruit post-harvest storage period, which particularly applies to the Yali cultivar. The Conference variety contained 0.31% titratable acidity at the beginning of storage period; after 70 days of storage at 1 and 5 °C the content halved (Figure 12). The Yali variety contained 0.20% titratable acidity at the beginning of storing period. A significant reduction of content was seen particularly for fruit stored at 20 °C and 1 °C (Figure 11). A very similar trend as regards the acid content was also reported in the publication of **Arzani et al., (2008)** who monitored titratable acidity in European and Asian pears. At the beginning of storage the Asian pears contained 0.25%

while the European fruit had 0.30%; after month 5 the acidity decreased by almost a half. The decrease in titratable acidity was also seen by **Mahajan and Dhatt (2004)** who monitored titratable acidity of Asian pear varieties during the period of cold storage of 75 days. After putting into the store the acid content was 0.36%; after 75 days of storage it reached 0.15%.

For acidity, malic acid is represented the most in the fruit of both of the studied varieties (Figure 13). Compared with the initial concentration of 3,220 $mg.kg^{-1}$, a half-drop occurred for the Conference variety after 30 days of storage at 20 °C when only 1,691 $mg.kg^{-1}$ was measured. In terms of malic acid concentration, both of the varieties were found to show statistically significant differences between the beginning (IN) and the end of the storage period on all of the three dates (Table 1).

The Yali pears were noticed to show statistically significant differences between the beginning (IN) and the end of the storage period on all of the three dates, in terms of citric acid concentration. The Yali pears contained over ten-fold amount of citric acid than the Conference pears (Figure 14). For the Conference variety no statistically significant differences were found in terms of citric acid content.

The average content of malic acid in the Conference pears was 2,300 $mg.kg^{-1}$. For Williams Pears, i.e. another pear variety, malic acid concentration was monitored by Colaric et al., (2006). The average content was around 2,200 $mg.kg^{-1}$.

CONCLUSION

The study observed changes in the material composition of European (Conference) and Asian (Yali) pears during the storage period at different temperatures. Ethylene, carbon dioxide, flesh firmness, titratable acids, soluble solids and organic acids formed the main monitored parameters. Under refrigerating conditions, the intensity of respiration did not change significantly in the initial 55 days of storage. At the end of the storage period there was a significant increase in carbon dioxide production in both of the two varieties. A similar carbon dioxide content (30 $mg.kg^{-1}.h^{-1}$) was found after 30 days of storing the fruit of both varieties at 20 °C. Generation of ethylene slightly grew during the storage period for the varieties studied. The Yali pears produced higher levels of ethylene than the Conference pears in all of the monitored settings. Increasing of the metabolism through the production of ethylene, fundamentally ranges in values of Q_{10} not very different from the production of CO_2. Temperature quotients Q_{10} for respiration are higher for the cultivar Conference, while the Q_{10} for the production of the ethylene are higher for the cultivar Yali. The fruit of the Conference variety had a stronger flesh; at 20 °C, however, there was a very rapid softening and after 20 days of storage, the structure of these pieces of fruit was entirely soft. The Conference pears featured a higher soluble solids content under all of the storage conditions; the figure was steadily increasing in both of the varieties and a slight decline occurred toward the end of the storage period. The Yali pears had a higher titratable acidity at the beginning of the storage period, which in both varieties dropped below the value found when putting the fruit into the store; this occurred during the storage period under all of the studied conditions. For organic acids, malic acid was represented at the highest concentration in both of the varieties. After 30 days of storing the Conference pears at 20 °C, the content of malic acid decreased by 50% compared with the original concentration. Over a ten-fold amount of citric acid was determined for Yali pears compared with the Conference pears.

REFERENCES

Arzani, K., Khoshghalb, H., Malakouti, J. M., Barzegar, M. 2008. Postharvest Fruit Physicochemical Changes and Properties of Asian (*Pyrus serotina* Rehd.) and European (*Pyrus communis* L.) Pear Cultivars. *Horticulture, Enviroment and Biotechnology*, vol. 49, no. 4, p. 244-252.

Bao, L., Chen, K., Lu, B., Zhang, D., He, Y., Li, X., Teng, Y. 2008. An assessment of genetic variability relationships within Asian pears based on AFLP markers. *Scientia Horticulturae*, vol. 116, no. 4, p. 374-380. https://doi.org/10.1016/j.scienta.2008.02.008

Colaric, M., Stampar, F., Solar, A., Hudina, M. 2006. Influence of branch bending on sugar, organic acid and phenolic content in fruits on Williams pears. *Jurnal of the science of food and agriculture*, vol. 86, no. 14, p. 2463-2467.

Crisosto, H. C., Garner, D., Crisosto, M. G. 1994. Late Harvest and Delayed Cooling Induce Internal Browning of 'Ya Li'and 'Seuri' Chinese Pear. *Horticultural Science*, vol. 29, no. 6, p. 667-670.

Chen, L. J., Wu, H. J., Wang, Q., Deng, H., Hu, S. X. 2006. Changes in the Volatile Compounds and Chemical and Physical Properties of Kuerle Fragrant Pear (*Pyrus serotina* Reld) during Storage. *Journal of Agricultural and Food Chemistry*, vol. 54, no. 2, p. 8842-8847. https://doi.org/10.1021/jf061089g PMid:1709013

Elgar, J. H., Watkins, B. C., Murray, H. S., Gunson, A.F. 1997. Quality of 'Buerre Bosc' and 'Doyenne du Comice' pears in relation to harvest date and storage period. *Postharvest Biology and Technology*, vol. 10, no. 1, p. 29-37. https://doi.org/10.1016/S0925-5214(96)00058-0

Itai, A., Fujita, N. 2008. Identification of Climacteric and Nonclimacteric Phenotypes of Asian Pear Cultivars by CAPS Analysis of 1-Aminocyclopropane-1-Carboxylate Synthase Genes. *Horticultural Science*, vol. 43, no. 1, p. 119-121.

Itai, A., Kotaki, T., Tanabe, K., Tamura, F., Kawaguchi, D., Fukuda, M. 2003. Rapid identification of 1-aminocyclopropane-1-carboxylate (ACC) synthase genotypes in cultivars of Japanese pear (*Pyrus pyrifolia* Nakai) using CAPS markers. *Theoretical and Applied Genetics*, vol. 106, no. 7, p. 1266-1272. https://doi.org/10.1007/s00122-002-1186-8 PMid:12748778

Jackson, J. E. 2003. Biology of apples and pears. Cambridge, UK : Cambridge University Press. ISBN-13 978-0-511-05900-1 https://doi.org/10.1017/CBO9780511542657

Mahajan, B. V. C, Dhatt, A. S. 2004. Studies on postharvest calcium chloride application on storage behaviour and quality of Asian pear during cold storage. *Journal of Food, Agriculture & Environment*, vol. 2, no. 3-4, p. 157-159.

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DISTRIBUTION OF INVASIVE PLANTS IN THE NITRA RIVER BASIN: THREATS AND BENEFITS FOR FOOD PRODUCTION

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ABSTRACT

Invasive plants are introduced multicellular organisms of the kingdom Plantae, which produce their food by photosynthesis. An invasive plant has the ability to thrive and spread aggressively outside its native range. A naturally aggressive plant may be especially invasive when it is introduced to a new habitat. The basic literature emphasizes mainly the ecological and environmental effects of invasive plants. Impacts of these plants on the food production have never been studied in details. The direct and indirect or potential effects of occurrence of invasive plants on food production have been analysed on basis of published data according to eight selected criteria: food, fodder for animals, food and drink additives, indirect support for food production, weeds on arable lands, meadow weeds, allergenic plants in food and toxic plants. The principal components analysis of habitat preferences of invasive plants in the Nitra river basin showed that the majority of invasive plants growing along rivers is edible (*Fallopia* spp., *Helianthus tuberosus*, *Impatiens glandulifera*) and invasive plants preferring drier agricultural fields or grasslands are toxic and/or allergenic with low or zero level of edibility (*Ambrosia artemisiifolia*, *Heracleum mantegazzianum*). The plants living in drier conditions may produce more toxins to protect the sources (eg. water) in their tissues than plants near water flows where there is abundance of sources.

Keywords: allergenic plant; edible plant; fodder; invasive plant; toxic plant

INTRODUCTION

Biological invasions are mostly understood as the dissemination of non-native plant species in new areas. Plant invasiveness is neither a life form nor a taxonomic issue, but a set of species properties enabling growth in certain habitats. We have only a few generalisations on the invasiveness of plants or on their attributes (if they do exist) and usually we cannot predict biological invasions **(Fehér et al., 2012)**. According to the European strategy on invasive alien species **(Genovesi and Shine, 2004)**, an alien species is a species, subspecies or lower taxon introduced outside its natural past or present distribution; this includes any part of such species that might survive and subsequently reproduce. An invasive alien species is an alien species whose introduction and/or spread threaten biological diversity. In this paper, we consider 'invasive' plants alien species in accordance with the Slovak legislation valid in time of our study (the Proclamation of the Ministry of Environment of the Slovak Republic No. 173/2011).

The basic literature emphasizes mainly the ecological and environmental effects of invasive plants **(Genovesi, Shine, 2004)** but their impacts on food production have never been studied in details (no summary exists). Our goal was to monitor, in the studied area (Nitra river basin, SW Slovakia), the number of localities of selected invasive plant species and to evaluate the effect of different invasive species on food production, including their positive and negative externalities.

MATERIAL AND METHODOLOGY

The area of the Nitra river basin is 5144 km^2, the length of the main flow is 196.7 km. The catchment area belongs to the European continental climate area of the temperate zone. During the research period, we surveyed 302 localities of invasive plants between 1999 and 2009. Invasiveness of plants was classified according to the Proclamation of the Ministry of Environment of the Slovak Republic No. 173/2011 (*Ambrosia artemisiifolia*, non-native *Fallopia* spp., *Helianthus tuberosus*, *Heracleum mantegazzianum*, *Impatiens glandulifera*, *Solidago canadensis*, *Solidago gigantea*, Figure 1 – 7). We used ordination (multivariate gradient analysis) for comparison of species relations to selected habitats (principal components analysis, PCA in Canoco 4.5 and CanoDraw for Windows). The direct and indirect or potential effects of occurance of observed invasive plants on food production were analysed on basis of published data according to 8 selected criteria: food (edible plants or edible parts of plants), fodder for animals (forage), food and drink additives (spicy plants, therapeutic plants, tea herbs), indirect support for food production (e.g. melliferous plants), weeds on arable lands (competition with food plants), meadow weeds (competition with fodder plants), allergenic plants in food and toxic plants.

RESULTS AND DISCUSSION

We found that all identified invasive plants influence food production (Figure 1 – 7 and Table 1).

Figure 1 *Ambrosia artemisiifolia.*

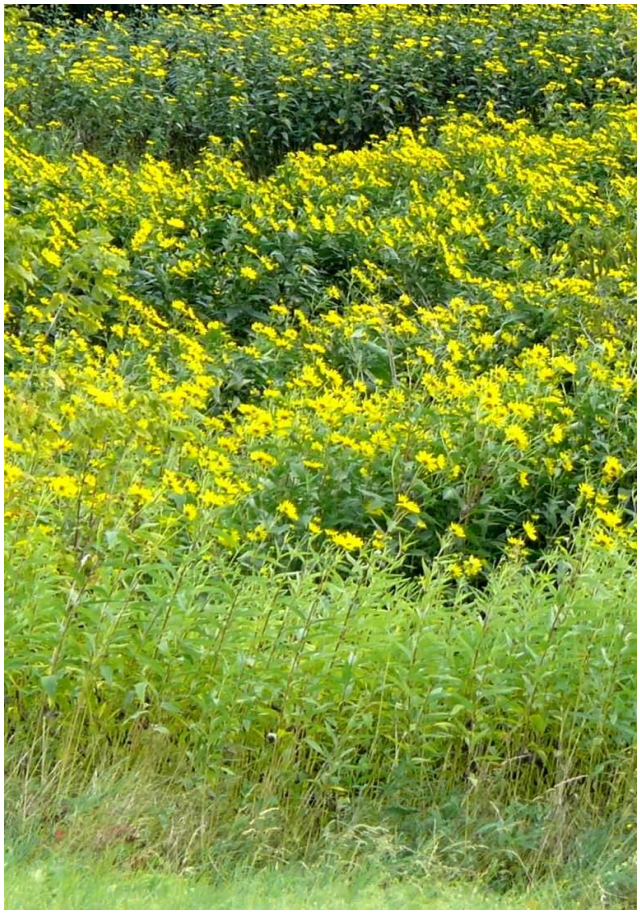

Figure 3 *Helianthus tuberosus.*

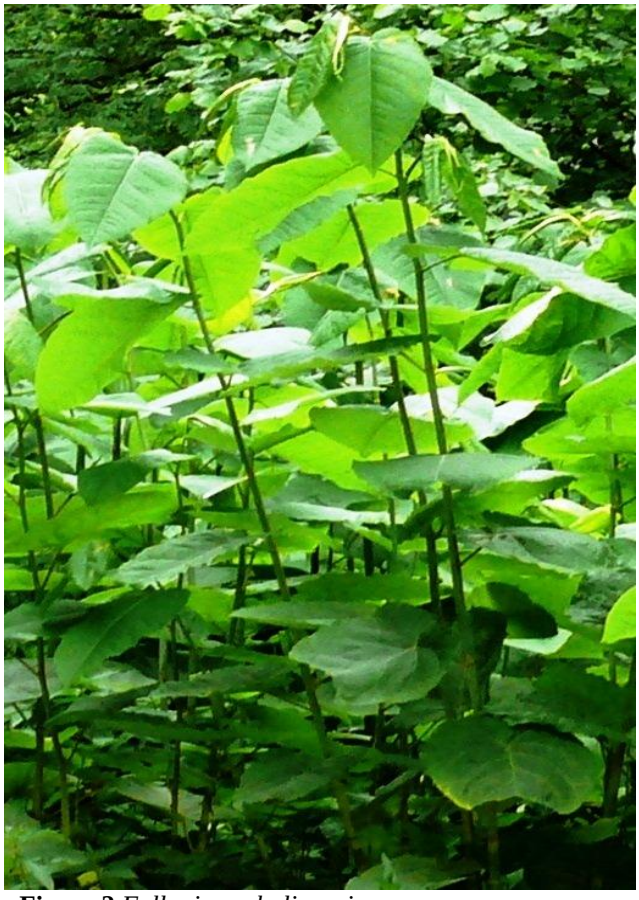

Figure 2 *Fallopia sachalinensis.*

Figure 4 *Heracleum mantegazzianum.*

Figure 5 *Impatiens glandulifera.*

Figure 6 *Solidago Canadensis.*

Figure 7 *Solidago gigantean.*

Ambrosia artemisiifolia is a common field weed in the southern part of the Nitra region, competing with food crops and it is also allergenic. *A. artemisiifolia* is a low quality fodder for animals and can be used as a therapeutic plant. All three identified non-native invasive species of *Fallopia* genus (*F. japonica*, *F. sachalinensis*, *F. ×bohemica*) are of great importance: they can be eaten by humans (e.g. in jam) or animals (fodder), they contain resveratrol usable in healing cancer and they also support food production by their melliferous potential. Negative effects of *Fallopia* species are based on their weedy character (e.g. competition for sources, decrease of biodiversity). *Helianthus tuberosus* has a similar utilization as the *Fallopia* species (food, fodder, therapeutic and melliferous potential) but its importance is higher in food and feed production (its edible tubers contain inuline important for peoples suffering from diabetes). It is a weed as well. We could not identified positive impacts of occurrence of *Heracleum mantegazzianum*, which is a toxic meadow and rarely field weed causing allergenic symptoms (blisters) when touching it. *Impatiens glandulifera* is an edible and melliferous plant growing as a weed on alluvial meadows and forest margins. *Solidago canadensis* is a medical and tea herb plant with importance in feeding animals and maintain bee keeping. Its negative impact is based on its expansion on meadows and possible toxicity. *Solidago gigantea* is also a melliferous medical plant growing as a weed on fields and meadows.

Table 1 Benefits and losses generated by invasive plants, quantified by number of scientific papers dealing with effects of invasive plants on food production.

Effects	Positive effects				Negative effects			
Plant species	**Food (edible plants or edible parts of plants)**	**Fodder for animals (forage)**	**Food and drink additives (spicy plants, therapeutic plants, tea herbs)**	**Indirect support for food production (e.g. melliferous plants)**	**Weeds on arable lands (competition with food crops)**	**Meadow weeds (competition with fodder plants)**	**Allergenic plants in food**	**Toxic plants**
Ambrosia artemisiifolia	-	1[a]	1[b]	-	4[c,d,e,f]	1[g]	4[c,d,f, h,]	-
Fallopia japonica, F. sachalinensis, F. ×bohemica	1[g]	1[i]	4[j,k,l,m]	2[i,l]	1[g]	1[n]	-	-
Helianthus tuberosus	3[o,p,q]	3[r,o,s]	1[t]	1[o]	1[o]	1[o]	-	-
Heracleum mantegazzianum	-	1[g]	-	-	2[u,v]	2[w,x]	1[g]	3[y,z,aa]
Impatiens glandulifera	1[bb]	-	-	2[cc,dd]	-	4[ee,n,bb,ff]	-	-
Solidago canadensis	-	1[gg]	5[hh,ii,jj,kk,ll]	1[mm]	-	2[nn,mm]	-	1[oo]
Solidago gigantea	-	1[g]	2[pp,qq]	1[mm]	1[rr]	1[ss]	-	-

Note: a – Feleafel, Mirdad, 2013, b – Chen et al., 2013, c – Sauliene et al., 2011, d – Smith et al., 2013, e – Fumanal et al., 2008, f – Dechamp, 2013, g – Fehér, 2000-2014 unpublished, h – Richter et al., 2013, i – Bailey, Conolly, 2000, j – Frantik et al., 2013, k – Alberternst, Böhmer, 2011, l – Fan et al., 2010, m – Strašil, 2006, n – Schnitzler et al., 2011, o – Swanton et al., 1992, p – Takeuchi, Nagashima, 2011, q – Erdal et al., 2011, r – Seiler, Campbell, 2006, s – Gleich et al., 1998, t – Gedrovica et al., 2011, u – Pergl et al., 2012, v – Mullerova et al., 2005, w – Pyšek, Pyšek, 1995, x – Tiley et al., 1996, y – Jakubska-Busse et al., 2013, z – Schib et al., 1996, aa – Drever, Hunter, 1970, bb – Zybartaite et al., 2011, cc – Chittka, Schurkens, 2001, dd – Bartomeus et al., 2010, ee – Love et al., 2013, ff – Clements et al., 2008, gg – Mysterud, Austrheim, 2008, hh – Schilcher et al., 1989, ii – Bornschein, 1987, jj – Hiller, Bader, 1996, kk – Sutovska et al., 2013, ll – McCune, Johns, 2002, mm – Amtmann, 2010, nn – Skorka et al., 2010, oo – Chizzola, Brandstaetter, 2006, pp – Choi et al., 2010, qq – Webster et al., 2008, rr – Weber, 2001, ss – Botta-Dukát, Dancza, 2001.

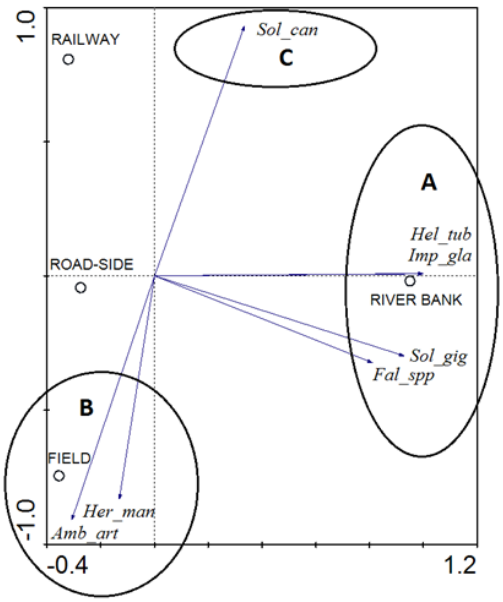

Figure 8. Principal componants analysis of habitat preferences of invasive plants.
Note: A – edible plants (food or feed), B – toxic and/or allergenic plants, C – cannot be classified. *Amb_art Ambrosia artemisiifolia, Fal_spp Fallopia japonica or F. sachalinensis or F. ×bohemica, Hel_tub Helianthus tuberosus, Her_man Heracleum mantegazzianum, Imp_gla Impatiens glandulifera, Sol_can Solidago canadensis, Sol_gig Solidago gigantea.*

The ordination of habitat preferences of invasive plants shows that the majority of invasive plants growing along rivers is edible (*H. tuberosus, F.* spp., *I. glandulifera)* and invasive plants preferring (usually drier) agricultural fields or meadows (including pastures) are toxic or allergenic with very low level of edibility (*A. artemisiifolia, H. mantegazzianum*) (Figure 8).

The negative impact of biological invasions is well known (decrease of biodiversity, toxic aliens, e.g. *Asclepias syriaca, Lupinus polyphyllus, Robinia pseudoacacia, Datura stramonium, Lycium barbarum*) but there are only few papers focused on possible positive effect of biological invasions. **Willerding (1988)** listed edible weeds in crops (*Bromus secalinus, Chenopodium album, Fallopia convolvulus, Echinochloa crus-galli*), medical weeds (*Chenopodium album, Polygonum aviculare*) and color production from weeds (*Polygonum aviculare, Fallopia convolvulus, Chenopodium album*). We confirmed four positive and four negative groups of potential influences or impacts of invasive plants in the Nitra river basin. The most important fact we identified by PCA was edibility of palnts near the river flow and toxicity of plants in drier areas. The majority of plants secondary metabolites (terpenoids, nitrogen-containing compounds and phenolics) are produced for benefit of plants, e.g. chemical defence to protect plants from herbivory or microbial infections (toxins, crystalline exudates on the leaf surface, malodorous smell from trichomes, bitter taste of plant tissue etc.). Environmental stress (e.g. drought) increases toxin production (in some cases palatable species become unpalatable to the herbivores, c.f. **Louda, Ferris, Blaa 1987; Harborne 1997)**. The plants living in drier conditions (individuals of the same species or representatives of different species) may produce more toxins to protect the sources in their tissues than plants near water flows where there is abundance of sources (water, nutrients etc.).

CONCLUSION

The principal components analysis of habitat preferences of invasive plants in the Nitra river basin shows that the majority of invasive plants growing along rivers is edible (*F.* spp., *H. tuberosus, I. glandulifera*) and invasive plants preferring drier agricultural fields or grasslands are toxic and/or allergenic with low or zero level of edibility (*A. artemisiifolia, H. mantegazzianum*).

REFERENCES

Alberternst, B., Böhmer, H. J. 2011. NOBANIS – Invasive Alien Species Fact Sheet –*Fallopia japonica*. Database of the European Network on Invasive Alien Species – NOBANIS [online] s.a. [cit. 2014-04-02] Available at: http://www.nobanis.org.

Amtmann, M. 2010. The chemical relationship between the scent features of goldenrod (*Solidago canadensis L.*) flower and its unifloral honey. *Journal of Food Composition and Analysis*, vol. 23, no. 1, p. 122-129. https://doi.org/10.1016/j.jfca.2009.10.001

Bailey, J. P., Conolly A. P. 2000. Prize-winners to pariahs - A history of Japanese Knotweed s.l. (*Polygonaceae*) in the British Isles. *Watsonia*, vol. 23, no. 1, p. 93-110.

Bartomeus, I., Vila, M., Steffan-Dewenter, I. Combined effects of *Impatiens glandulifera* invasion and landscape structure on native plant pollination. 2010. *Journal of Ecology*, vol. 98, no. 2, p. 440-450. https://doi.org/10.1111/j.1365-2745.2009.01629.x

Bornschein U. 1987. Pharmakognostische, phytochemische und biosyntheti sche untersuchungen über *Solidago virgaurea, Solidago gigantea* und *Solidago canadensis*. PhD Thesis, Humboldt University Berlin 1987.

Botta-Dukat, Z., Dancza, I. 2001. *Effect of weather conditions on the growth of Solidago gigantea.* In Brundu, G., Brock, J., Camarda, I., Child, L., Wade, M., Plant Invasions: Species Ecology and Ecosystem Management. Kerkwerve : Backhuys Publishers. ISBN: 90-5782-080-3

Chen, H. G., Tuck, T., Ji, X. H., Zhou, X., Kelly, G., Cuerrier, A., Zhang, JZ. 2013**.** Quality Assessment of Japanese Knotweed (*Fallopia japonica*) grown on Prince Edward Island as a source of resveratrol. *Journal of Agricultural and Food Chemistry*, vol. 61, no. 26, p. 6383-6392. https://doi.org/10.1021/jf4019239

Chittka, L. Schurkens, S. 2001. Successful invasion of a floralmarket – an exotic Asian plant has moved in on Europe's riverbanks. *Nature*, vol. 411, no. 6838, p 653-653. https://doi.org/10.1038/35079676

Chizzola, R., Brandstaetter, M. 2006. Case report: possible causality between ingested Canadian golden rod and colic signs and successive mortality in horses. *Wiener Tierarztliche Monatsschrift*, vol. 93, no. 7-8, p. 166-169.

Choi, I. Y., Song, Y. J., Lee, W. H. 2010. DPPH radical scavenging effect and antimicrobial activities of some herbal extracts. *Korean Journal of Horticultural Science and Technology*, vol. 28, no. 5, p. 871-876.

Clements, D. R., Feenstra, K. R., Jones, K., Staniforth, R. 2008. The biology of invasive alien plants in Canada. 9. *Impatiens glandulifera* Royle. *Canadian Journal of Plant Science*, vol. 88, no. 2, p. 403-417. https://doi.org/10.4141/cjps2011-280

Dechamp, C. 2013. *Ambrosia pollinosis*. *Revue des Maladies Respiratoires*, vol. 30, no. 4, p. 316-327. https://doi.org/10.1016/j.rmr.2012.10.632

Drever, J. C., Hunter, J. A. A. 1970. Giant Hogweed Dermatitis. *Scottish Medical Journal*, vol. 15, no. 9, p. 315-319.

Erdal, S., Canli, O., Algur, O. F. 2011. Inulinase production by *Geotrichum candidum* using Jerusalem artichoke as sole carbon source. *Romanian Biotechnological Letters*, vol. 16, no. 4, p. 6378-6383.

Fan, E. G., Zhang, K., Zhu, M. Z., Wang, Q. A. 2010. Obtaining resveratrol: from chemical synthesis to biotechnological production. *Mini-Reviews in Organic Chemistry*, vol. 7, no. 4, p. 272-281. https://doi.org/10.2174/157019310792246454

Fehér, A., Končeková, L., Glemnitz, M., Berger, G., Pfeffer, H., Herzon I. 2012. *Maintaining and promoting biodiversity*. In Jakobson, Ch. ed., *Sustainable Agriculture.* Uppsala : Baltic University Press, p. 371-387. ISBN 978-91-86189-10-5

Feleafel, M. N., Mirdad, Z. M. 2013. Hazard and effects of pollution by lead on vegetable crops. *Journal Of Agricultural and Environmental Ethics*, vol. 26, no. 3, p. 547-567. https://doi.org/10.1007/s10806-012-9403-1

Frantik, T., Kovarova, M., Koblihova, H., Bartunkova, K., Nyvltova, Z., Vosatka, M. 2013. Production of medically valuable stilbenes and emodin in knotweed. *Industrial Crops and Products*, vol. 50, p. 237-243. https://doi.org/10.1016/j.indcrop.2013.07.017

Fumanal, B., Girod, C., Fried, G., Bretagnolle, F., Chauvel, B. 2008. Can the large ecological amplitude of *Ambrosia*

artemisiifolia explain its invasive success in France? *Weed Research*, vol. 48, no. 4, p. 349-359. https://doi.org/10.1111/j.1365-3180.2008.00627.x

Gedrovica, I., Karklina, D. Fras, A., Jablonka, O. Boros, D. 2011. The non-starch polysaccharides quantity changes in pastry products where Jerusalem artichoke (*Helianthus tuberosus* L.) added. *Procedia Food Science*, vol. 1, p. 1638-1644. https://doi.org/10.1016/j.profoo.2011.09.242

Genovesi, P. Shine, C. 2004. *European strategy on invasive alien species*. Nature and Environment no. 37, Strasbourg : Council of Europe Publishing. ISBN 92-871-5487-2

Gleich, V. E., Katzel, R., Reichelt, L. 1998. Investigations on the food preferences of fallow deer for Jerusalem artichoke in a research preserve. *Zeitschrift fur Jagdwissenschaft*, vol. 44, no. 2, p. 57-65. https://doi.org/10.1007/BF02308850

Harborne, J. B. 1997: *Plant secondary metabolism*. 2nd ed. In Crawley, M. J. ed., *Plant ecology*, Oxford : Blackwell Science, p. 132-155. ISBN 0-632-03639-7

Hiller K., Bader G. 1996. Goldruten-kraut. Die Gattung Solidago-eine pharmazeutische Bewertung. Portrait einer Arzneipflanze. *Zeitschrift für Phytotherapie*, vol. 17, p. 123-130.

Jakubska-Busse, A., Sliwinski, M., Kobylka, M. 2013. Identification of bioactive components of essential oils in *Heracleum sosnowskyi* and *Heracleum mantegazzianum* (Apiaceae). *Archives of Biological Sciences*, vol. 65, no. 3, p. 877-883. https://doi.org/10.2298/ABS1303877J

Schib, R. J., Theus, L., Oeschger, M. G., Gossweiler, Meier P. J. B. 1996. Acute poisonings with toxic giants in Switzerland between 1966 and 1994. *Schweizerische Medizinische Wochenschrift*, vol. 126, no. 25, p. 1085-1098.

Louda, S. M., Ferris, M. A., Blaa, M. J., 1987: Variation in methylglucosinolate and insect damage to *Cleome serrulata* along a natural soil moisture gradient. *Journal of Chemical Ecology*, vol. 13, no. 3, p. 569-582. https://doi.org/10.1007/BF01880100

Love, H. M., Maggs, Ch. A., Murray, T., Provan, J. 2013. Genetic evidence for predominantly hydrochoric gene flow in the invasive riparian plant *Impatiens glandulifera* (Himalayan balsam). *Annals of Botany*, vol. 112, no. 9, p. 1743-1750. https://doi.org/10.1093/aob/mct227

McCune, L. M., Johns, T. 2002. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the Indigenous Peoples of the North American boreal forest. *Journal of Ethnopharmacology*, vol. 82, no. 2-3, p. 197-205. https://doi.org/10.1016/S0378-8741(02)00180-0

Mullerova, J, Pysek, P., Jarosik, V., Pergl, J. 2005. Aerial photographs as a tool for assessing the regional dynamics of the invasive plant species *Heracleum mantegazzianum*. *Journal of Applied Ecology*, vol. 42, no. 6, p. 1042-1053. https://doi.org/10.1111/j.1365-2664.2005.01092.x

Mysterud, A., Austrheim, G. 2008. The effect of domestic sheep on forage plants of wild reindeer; a landscape scale experiment. *European Journal of Wildlife Research*, vol. 54, no. 3, p. 461-468. https://doi.org/10.1007/s10344-008-0171-1

Pergl, J., Pysek, P., Perglova, I., Jarosik, V. 2012. Low persistence of a monocarpic invasive plant in historical sites biases our perception of its actual distribution. *Journal of Biogeography*, vol. 39, no. 7, p. 1293-1302. https://doi.org/10.1111/j.1365-2699.2011.02677.x

Pyšek, P., Pyšek, A. 1995. Invasion by *Heracleum mantegazzianum* in different habitats in the Czech Republic. *Journal of Vegetation Science*, vol. 6, no. 5, p. 711-718. https://doi.org/10.2307/3236442

Richter, R., Berger, U. E., Dullinger, S., Essl, F., Leitner, M., Smith, M., Vogl, G. 2013. Spread of invasive ragweed: climate change, management and how to reduce allergy costs. *Journal of Applied Ecology*, vol. 50, no. 6, p. 1422-1430. https://doi.org/10.1111/1365-2664.12156

Sauliene, I., Gudzinskas, Z., Veriankaite, L., Malciute, A., Lesciauskiene, V. 2011. Distribution of Ambrosia plants and airborne pollen in Lithuania. *Journal of Food, Agriculture and Environment*, vol. 9, no. 2, p. 547-550.

Schilcher, H., Boesel, R., Effenberger, S., Segebrecht, S. 1989. Neuere Untersuchungsergebnisse mit aquaretisch, antibakteriell und prostarop wirksamen Arzneipflanzen. *Zeitschrift für Phytotherapie*, vol. 10, no. 1, p. 77-82.

Schnitzler, A., Aumaitre, D.,Schnitzler, Ch. 2011. From rurality to ferality: a case study from the upper Moselle valley, France. *Revue d`Ecologie – La Terre et la vie*, vol. 66, no. 2, p. 117-133.

Seiler, G. J., Campbell, L. G. 2006. Genetic variability for mineral concentration in the forage of Jerusalem artichoke cultivars. *Euphytica*, vol. 150, no. 1-2, p. 281-288. https://doi.org/10.1007/s10681-006-9119-2

Skorka, P., Lenda, M., Tryjanowski, P. 2010. Invasive alien goldenrods negatively affect grassland bird communities in Eastern Europe. *Biological Conservation*, vol. 143, no. 4, p. 856-861. https://doi.org/10.1016/j.biocon.2009.12.030

Smith, M., Cecchi, L., Skjøth, C. A., Karrer, G., Šikoparija, B., 2013. Common ragweed: a threat to environmental health in Europe. *Environment International*, vol. 61, no. 1, p. 115-26. https://doi.org/10.1016/j.envint.2013.08.005

Strašil Z. 2006. *Křídlatky (Reynoutria)*. In Petříková, V. ed. *Energetické plodiny* (*Energetic plants*). Praha : Profi Press. p. 33-39. ISBN 80-86726-13-4

Sutovska, M., Capek, P., Kocmalova, M., Franova, S., Pawlaczyk, I., Gancarz, R. 2013. Characterization and biological activity of *Solidago canadensis* complex. *International Journal of Biological Macromolecules*, vol. 52, no. 1, p. 192-197. https://doi.org/10.1016/j.ijbiomac.2012.09.021

Swanton, C. J., Cavers, P. B.,Clements, D. R., Moore, M., J. 1992. The biology of Canadian weeds. 101. *Helianthus-tuberosus* L. *Canadian Journal of Plant Science*, vol. 72, no. 4, p. 1367-1382. https://doi.org/10.4141/cjps92-169

Takeuchi, J., Nagashima, T. 2011. Preparation of dried chips from Jerusalem artichoke (*Helianthus tuberosus*) tubers and analysis of their functional properties. *Food Chemistry*, vol. 126, no. 3, p. 922-926. https://doi.org/10.1016/j.foodchem.2010.11.080

Tiley, G. E. D., Dodd, F. S., Wade, P. M, 1996. Biological flora of the British Isles: *Heracleum mantegazzianum* Sommier and Levier. *Journal of Ecology*, vol. 84, no. 2, p. 297-319. https://doi.org/10.2307/2261365

Weber, E. 2001. Current and potential ranges of three exotic goldenrods (Solidago) in Europe. *Conservation Biology*. vol. 15, no. 1, p. 122-128. https://doi.org/10.1111/j.1523-1739.2001.99424.x

Webster, D., Taschereau, P., Belland, R., Sand, C., Rennie, R. 2008. Antifungal activity of medicinal plant extracts; preliminary screening studies. *Journal of Ethnopharmacology*, vol. 115, no. 1, p. 140-146. https://doi.org/10.1016/j.jep.2007.09.014

Willerding, U. 1988. Zur Entwicklung von Ackerunkrautgesellschaften im Zeitraum vom Neolithikum bis in die Neuzeit. In Küster, H. ed., *Der prähistorische Mensch und seine Umwelt*. Stuttgart, Geramny : Festschrift für Udelgard Körber-Grohne , p. 31-41. ISBN 3806207992

Zybartaite, L., Zukauskiene, J., Jodinskiene, M., Janssens, S. B., Paulauskas, A., Kupcinskiene, E. 2011. RAPD analysis of genetic diversity among Lithuanian populations of *Impatiens glandulifera*. *Zemdirbyste-Agriculture*, vol. 98, no. 4, p. 391-398.

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VERIFICATION FOR THE PRESENCE OF INHIBITORY SUBSTANCES IN POULTRY MEAT AFTER THE CONSUMPTION OF THE FEED MIXTURE SUPPLEMENTED WITH FERMENTED FEED

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ABSTRACT

The European Union has an obligation to ensure that feed produced in the European Union is safe for animals and also humans by ensuring food of animal origin is safe and wholesome. An increasing demand for safe, wholesome and nutritious animal products has led to the search for alternative substances in animal feed. Fermented feed has gained a lot of popularity in many animal diets today. They meet the demand for animal nutrition due to the formation of target substances with the desired properties. As some of them are attracting attention as potential antimicrobial agents that inhibit the growth of certain microorganisms, and the products of animal origin are controlled for the presence of residues of inhibitory (antimicrobial) substances, the aim of this work was to verify the presence of inhibitory substances in poultry meat (muscle, heart, liver, kidneys of broiler chickens) after the consumption of the feed mixture with addition of fermented feed (wheat bran fermented with the strain *Umbelopsis isabellina* CCF 2412) in a dose of 10 % of the total amount of the feed. The detection of residues was performed by two approved microbiological screening methods, the screening test for the detection of antibiotic residues (STAR) and the Premi®Test. Both methods detected the positive results and pre-identified the presence of residues of the inhibitory substances not only in the meat of broiler chickens but also in the investigated fermented feed. Due to the antimicrobial potential of the fermented feed and the possible presence of the false positive results, each positive result must be confirmed by a confirmatory analysis.

Keywords: meat; feed; residues; screening

INTRODUCTION

Animal production occupies a very important place in farming in the European Union (EU) and satisfactory results in terms of public and animal health depend to a large extent on the use of appropriate good quality feedingstuffs **(Directive 2002/32/EC)**. Feed shall not be placed on the market or fed to any food-producing animal if it has an adverse effect on human or animal health and makes the food derived from food-producing animals unsafe for human consumption **(Regulation (EC) No 178/2002)**.

Action by the EU relating to public and animal health are based on the precautionary principle. One of these actions is to ban the use of antibiotics as growth promoters in food-producing animals according to **Regulation (EC) No 1831/2003** with effect from 1 January 2006. The ban of in-feed use of antibiotics has brought unintended impacts on animal production industries in the EU, such as the increase of infections in animals and the decrease of animal production **(Cheng et al., 2014)**. On the other hand, this ban has opened up a space for the development of alternative substances and alternative feeding methods to decrease animal diseases and increase the production of safe, wholesome and nutritious animal products intended for human consumption. The subject of active search for alternatives have become probiotics, prebiotics, organic acids, minerals, enzymes, herbs (plant extracts), propolis extract, phytogenic feed additives, aromatic phenolic components, or fermented feed **(Niba et al., 2009; Bobko et al. 2016; Haščík et al. 2016)**.

Fermented feed has gained a lot of popularity in animal production today for enhancing the nutrient accessibility in some feed and developing and/or improving the flavour and texture of many fermented products **(Ivey et al., 2013; Čertík et al., 2013a)**. The extensive rise of biotechnology over the past years has opened several strategies that could be applied for the formation of functional food/feed. Biotechnological approach based on solid state fermentations (SSF) is one of the most perspective techniques to enrich cereals (rice bran, wheat bran, oat flakes, peeled barley, etc.) used in animal nutrition with desired metabolites. During this process, useful microorganisms (lower filamentous fungi) grow on various cereal substrates by utilization of nutrients from these substrates. As a result, a cereal-based functional biomaterial with the demanded properties is formed. An advantage of the SSF process is that fermented materials

can be directly used for food/feed applications without any downstream process **(Čertík and Klempová, 2016)**.

One of the most valuable fungus able to grow on various cereal substrates is *Umbelopsis isabellina*. This fungal strain belongs to a group of oleaginous microorganisms able to accumulate large amount of lipids in their cells and is characterized by the simultaneous formation of both polyunsaturated fatty acids and a mixture of carotenoid pigments **(Klempová et al., 2013)**. Polyunsaturated fatty acids and carotenoid pigments are essential compounds for animal and human health. They are important modulators of a number of vital physiological processes, they have a high antioxidant activity and beneficial (protective) effects in the prevention and widespread of the modern civilization diseases **(Klempová et al., 2013; Fiedor and Burda 2014)**. However, the recent studies have shown that polyunsaturated fatty acids are attracting attention as potential antimicrobial agents due to their safety, high efficiency, wide spectrum of activity and the lack of resistance mechanisms **(Huang et al., 2010; Desbois and Lawlor 2013; Sayegh et al., 2016)**.

Due to the fact that the animal products and animal feed are carefully controlled and monitored for the presence of the residues of antimicrobial substances and their derivatives in accordance with **Council Directive 96/23/EC** and due to the formation of natural bioactive agents with potential antimicrobial activity in fermented cereals, the aim of this work was to verify the presence of inhibitory substances in poultry meat after the consumption of the commercial feed mixtures with addition of wheat bran fermented with the strain *Umbelopsis isabellina* in a dose of 10% of the total amount of the feed.

MATERIALS AND METHODS

Sample material

A total of 8 tissue samples and 3 feed samples were used for residues analysis. Tissue samples (muscle /2/, heart /2/, liver /2/, kidneys /2/) were obtained from broiler chickens (ROSS 308) from two (experimental /1/ and control /1/) groups slaughtered at the age of 37th days after elapse of the withdrawal period by legally permitted method. Feed samples were obtained from the feed supplied to broiler chickens (medicated feed mixtures BR2 containing a polyether ionophoric antibiotic salinomycin authorised according to **Commission Regulation (EC) No 167/2008** for the prevention of coccidiosis in chickens for fattening at a minimum/maximum content of active substance in complete feed 60 – 70 mg.kg^{-1} /1/ and unmedicated feed mixture BR3 /1/ both commercially produced by De Heus a.s., Czech Republic, and fermented feed /wheat bran fermented with the fungal strain *Umbelopsis isabellina* CCF 2412 produced according to **Čertík et al. (2013b)**.The strain *Umbelopsis isabellina* CCF 2412 was obtained from Culture Collection of Fungi (Charles University, Czech Republic).

Experimental animals

The feeding regime for the control group consisted of the administration of BR1, BR2 and BR3 according to the age of broiler chickens and the feeding regime for the experimental group consisted of the administration of BR1, BR2 and BR3 with the addition of fermented wheat bran in a dose of 10% of the total amount of the feed. The animals were housed in accredited premise of the Clinic for birds, exotic and free-living animals at the University of Veterinary Medicine and Pharmacy in Košice fulfilling the conditions for the preservation of the health and welfare of the animals. All broiler chickens had free access to feed and water. Tissue and feed samples were individually packed and stored in a freezer at -20 °C until the analysis.

Sample analysis

For the verification of the presence of inhibitory substances in the tissues of broiler chickens after the consumption of the feed mixture supplemented with fermented wheat bran, the screening test for the detection of antibiotic residues (STAR) **(R–25, 2006)** as the plate method and the Premi®Test **(R–26, 2006)** as the tube method both developed on the principle of inhibition of growth of the test organism by an antimicrobial substance presents in the sample were used. Both methods are officially approved for screening food-producing animals and their products for residues of antimicrobial substances in many EU member states, including Slovakia.

STAR protocol

Culture media, test strains (bacteria) and standards were purchased from Merck (Germany), Oxoid (UK), Difco (USA), Sigma-Aldrich (USA) and American Type Culture Collection (USA). Test plates (*Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 /addition of trimethoprim at a concentration of 0.005 μg.mL^{-1} agar medium/, *Bacillus subtilis* BGA, *Escherichia coli* ATCC 11303, *Kocuria rhizophila* ATCC 9341, *Bacillus cereus* ATCC 11778) were prepared according to the procedure set by the method. A cylindrical core obtained from each frozen tissue sample (muscle, heart, liver, kidneys) using a sterile cork borer (diameter 9 mm) was cut into slices of 2 mm in thickness with a sterile lancet and placed in parallel in each of five test plates. For analysis of feed samples, a clear supernatant obtained by centrifugation of 10 g of feed sample dissolved in 30 mL of sterile demineralized water was used. The clear supernatant was applied on the surface of the test plates using a paper disc (diameter 9 mm, 30 μL, Albet Lab Science, Germany). The test plates were incubated under the conditions set by the method.

Premi®Test protocol

100 μL of the juice obtained by thawing the tissue sample in a microwave oven on defrost setting and 100 μL of clear supernatant obtained by preparation of feed samples as described above in Section STAR were pipetted onto the agar in the ampoule inoculated with *Bacillus stearothermophilus* var. *calidolactis.* The ampoules were subjected to pre-incubation and further incubation according to the manufacturer's instructions (R-Biopharm AG, Germany*).*

Reading the method results

Diffusion of any antimicrobial substance from the sample into an agar medium seeded with a sensitive test organism present results in the formation of an inhibition

zone around the sample, in which the growth of the test organism is inhibited (STAR) or from a delayed or absent colour change of the agar medium due to impaired growth of the test organism (Premi®Test). The size of the inhibition zone and the colour change of the agar medium is directly proportional to the concentration of the antimicrobial substance in the sample.

After incubation of the STAR test plates, the diameters of clear inhibition zones around the tissue samples or the filter paper discs moistened with clear supernatant were measured in mm from the edge of the sample (disc) to the outer limit of the inhibition zone using a digital caliper with a precision of 0.01 mm (Mitutoyo, Japan). The samples were considered positive, if they gave the inhibition zone equal or superior to 2 mm in width on the *Bacillus subtilis* BGA, *Escherichia coli* ATCC 11303, *Kocuria rhizophila* ATCC 9341 and *Bacillus cereus* ATCC 11778 test plates, and equal or superior to 4 mm in width on the *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 test plates. To verify that the operating conditions were systematically respected, a quality control on each test plate was performed.

After incubation of the Premi®Test ampoules with *Bacillus stearothermophilus* var. *calidolactis* test strain, a colour from the bottom 2/3 part of the ampoule was read. A clear colour change from purple to yellow indicated that a sample was considered negative (the absence of antimicrobial substance above the limit of detection). No clear colour change from purple to yellow indicated that a sample was considered positive (the presence of antimicrobials above the limit of detection). The samples with the light shades of purple colour were considered dubious (the presence of antimicrobials at the limit of detection).

RESULTS AND DISCUSSION

The results of screening for the presence of inhibitory substances in the examined animal and feed matrices are presented in Table 1 and Table 2. The photos documenting the positive, dubious and negative results detected by the STAR and the Premi®Test are presented in Figure 1 and Figure 2.

As shown in Table 1 and Table 2, both microbiological screening methods detected the presence of inhibitory substances in the animal and feed matrices. Evaluating the results after the screening of tissues samples of broiler chickens from the experimental and control group by the STAR, comparable results were detected. The inhibition of the growth of the test strain was observed only in two of five test strains, namely *Kocuria rhizophila* ATCC 9341 and *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149. No inhibition zones were observed on the *Bacillus cereus* ATCC 11778, *Bacillus subtilis* BGA and *Escherichia coli* ATCC 11303 test plates. The only inhibition zone observed around the kidney sample from the experimental group was not considered positive. Muscle yielded a positive result on the *Kocuria rhizophila* ATCC 9341 test plates, heart on the *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 test plates, and liver and kidneys on both the *Kocuria rhizophila* ATCC 9341 and *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 test plates.

Evaluating the results after the screening of samples of commercially produced feed mixtures BR2 and BR3 and fermented wheat bran by the STAR, all feed samples showed inhibition on the several test plates. Medicated BR2 yielded a positive result on four *(Bacillus cereus* ATCC 11778, *Bacillus subtilis* BGA, *Escherichia coli* ATCC 11303, *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149) of the five test plates. unmedicated BR3 yielded a positive result on two (*Bacillus subtilis* BGA, *Escherichia coli* ATCC 11303) of the five test plates, and fermented wheat bran yielded a positive result on the test plates identical with examined animal matrices (the *Kocuria rhizophila* ATCC 9341 and *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 test plates).

Table 1 The results of screening for the presence of inhibitory substances in the examined matrices by using the STAR.

		STAR				
Group of broiler chickens	**Matrix / No**	***B. cereus*** IZ (mm ±SD)	***B. subtilis*** IZ (mm ±SD)	***E. coli*** IZ (mm ±SD)	***K. rhizophila*** IZ (mm ±SD)	***B. stearothermophilus*** IZ (mm ±SD)
Control	**Muscle /9/**	**-**	**-**	**-**	**2.13 ±0.42**	**-**
	Heart /10/	**-**	**-**	**-**	1.56 ±0.58	**7.89 ±0.77**
	Liver /11/	**-**	**-**	**-**	**5.00 ±0.43**	**11.57 ±0.67**
	Kidneys /12/	**-**	**-**	**-**	**2.35 ±0.31**	**7.93 ±0.44**
Experimental	**Muscle /5/**	**-**	**-**	**-**	**2.43 ±0.17**	**-**
	Heart /6/	**-**	**-**	**-**	1.00 ±0.10	**6.60 ±0.39**
	Liver /7/	**-**	**-**	**-**	**4.34 ±0.33**	**10.69 ±1.01**
	Kidneys /8/	**-**	1.53 ±0.20	**-**	**2.26 ±0.61**	**9.17 ±1.30**
Feed	**BR 2 /13/**	**3.30 ±0.70**	**6.33 ±2.24**	**3.05 ±0,78**	0.87 ±0.07	**6.70 ±0.77**
	BR 3 /14/	**-**	**3.79 ±1.52**	**2.65 ±0,44**	**-**	**-**
	FWB /15/	**-**	**-**	**-**	**2.40 ±0.76**	**5.98 ±0.82**

Note**:** IZ – inhibition zone**,** bold numerals indicate positive results, SD – standard deviation, FWB – fermented wheat bran.

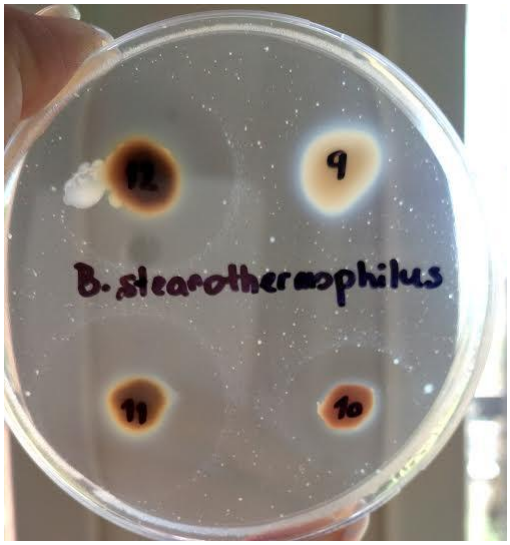

Figure 1 A visual demonstration of the results detected by the STAR.

Table 2 The results of screening for the presence of inhibitory substances in the examined matrices by using the Premi®Test.

Group of broiler chickens	Premi®Test Matrix / No	Result
Control	**Muscle /9/**	±
	Heart /10/	-
	Liver /11/	±
	Kidneys /12/	-
Experimental	**Muscle /5/**	±
	Heart /6/	±
	Liver /7/	±
	Kidneys /8/	-
Feed	**BR 2 /13/**	+
	BR 3 /14/	-
	FWB /15/	+

Note: IZ – inhibition zone, + positive results, - negative results, ± dubious results. FF – fermented wheat bran.

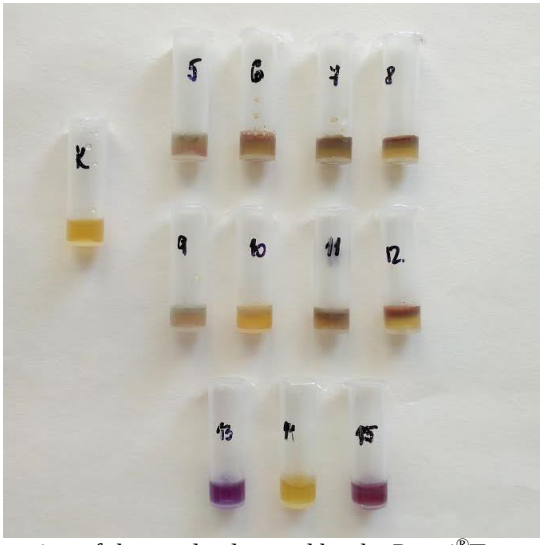

Figure 2 A visual demonstration of the results detected by the Premi®Test.

Evaluating the results after the screening of animal tissue and feed samples by the Premi®Test with *Bacillus stearothermophilus* var. *calidolactis* test strain, only medicated BR2 and fermented wheat bran yielded the positive results.

The muscle and liver from the control animals and the muscle, heart and liver from the experimental animals yielded a dubious result.

No positive result was observed in kidneys obtained from both animal groups and unmedicated BR3 feed mixture.

Summarizing the results of screening for the presence of inhibitory substances in the tissues of broiler chickens after the consumption of the feed mixture without addition of fermented wheat bran (control group) and with addition of fermented wheat bran (experimental group) we can state that both microbiological screening methods detected the positive results and pre-identified the presence of residues of the inhibitory substances not only in the meat of broiler chickens from the control group but also in the meat of broiler chickens from the experimental group. Both microbiological screening methods also revealed the presence of the inhibitory substances in wheat bran fermented with the strain *Umbelopsis isabellina*.

Microbiological methods based on the inhibition of bacterial growth by antimicrobial residues are an interesting screening step because they are capable of detecting a wide range of antimicrobial residues with one single method. They generally give results in less than 4 h (tube methods) or in 18-24 h (plate methods) **(Gaudin et al. 2008)**. The STAR which was developed at the European Union Reference Laboratory in *Fougères* (France) and the Premi®Test which was developed by DSM Gist B.V. (The Netherlands) have been most widely used in residue screening **(Gaudin et al. 2004; Stead et al., 2004; Stead et al., 2005; Gaudin et al. 2008; Kožárová et al. 2009; Pikkemaat et al. 2009; Gaudin et al. 2010; Kožárová et al., 2011; Magalhães et al., 2012, Gondová et al., 2014)**. The Premi®Test allows for the detection of broad spectrum of most relevant antimicrobial compounds simultaneously while the STAR allows for distinguishing (pre-identify) the families of antimicrobials, i.e. each test plate is sensitive to one or two families of antimicrobials. *Bacillus cereus* ATCC 11778 test plates are specific for tetracyclines, *Bacillus subtilis* BGA test plates for aminoglycosides, *Escherichia coli* ATCC 11303 test plates for quinolones, *Kocuria rhizophila* ATCC 9341 test plates for macrolides, and *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 test plates for b-lactams and sulphonamides.

According to the declared sensitivity of the respective test strain of the STAR to the respective family of antimicrobials we can assume that the positive animal samples were suspected for the presence of ß-lactams, sulphonamides and macrolides, and the positive feed samples, except medicated BR2, for the presence of all antimicrobial families. If the sample exhibits the positive result on more than one test plates, the confirmation should be directed onto specific family/families of antimicrobials detected.

Due to the fact that all screening methods have defined a false compliant rate of <5 % (β-error) by the **Commission Decision 2002/657/EC**, the false compliant (positive) results may be affected by inhibitory substances other than antimicrobials. These inhibitors (naturally occurring inhibiting substances presented in the food or feed matrices) can influence the test result and inhibit the growth of the test strain/strains. To prevent these inhibitors interfering with the test result, a special sample-procedure required by the Premi®Test for kidneys and feed involving the pre-incubation (heat pre-treatment of the sample at 80 °C for 10 min.) step was applied for all investigated matrices.

Currently, there are no published studies dealing with the screening of residues of inhibitory substances in meat of broiler chickens fed with fermented feed or feed supplemented with various bioactive substances. To reach a final conclusion regarding the presence of residues of antimicrobial substances such as antibiotics, sulphonamides, and quinolones or the presence of bioactive agents possessing antimicrobial activity in fermented cereals in meat of broiler chickens, further research is needed.

CONCLUSION

Screening methods have established performance criteria. They must detect the presence of a substance or class of substances at the level of interest (in compliance with legislation) with a false compliant rate of <5 %. Considering the results obtained in our work and growth inhibition of two of the five test strains after the screening of meat of broiler chickens fed with feed mixture with addition of fermented wheat bran, it is necessary for reaching true scientific conclusion and confirmation or exclusion of false positive and false negative results to perform a confirmatory analysis providing information on a substance/substances that cause/causes the inhibition of growth of the respective test strain.

REFERENCES

Bobko, M., Haščík, P., Mellen, M., Bobková, A., Tkáčová, J., Czako, P., Pavelková, A., Trembecká, L. 2016. Effect of different phytogenic additives on oxidation stability of chicken meat. *Potravinarstvo*, vol. 10, no. 1, p. 164-169. https://doi.org/10.5219/567

Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. OJ L 221, 17.8.2002, p. 8-36.

Commission Regulation (EC) No. 167/2008 of 22 February 2008 concerning a new authorization of coccidiostats as feed additive for ten years. OJ L50, 23.2.2008, p. 1-3.

Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. OJ L 125, 23.5.1996, p. 10-32.

Čertík, M., Klempová, T. 2016. Functional cereal-derived biomaterials enriched with lipophilic compounds prepared by fungal solid-state fermentations. *Materials Science Forum*, vol. 851, p. 8-13. https://doi.org/10.4028/www.scientific.net/MSF.851.8

Čertík, M., Adamechová, Z., Guothová, L. 2013a. Simultaneous enrichment of cereals with polyunsaturated fatty acids and pigments by fungal solid state fermentations. *Journal of Biotechnology*, vol. 168, p. 130-134. https://doi.org/10.1016/j.jbiotec.2013.03.016 PMid:23583333

Čertík, M., Klempová, T., Guothová, L., Mihálik, D., Kraic, J. 2013b. Biotechnology for the functional improvement of cereal-based materials enriched with polyunsaturated fatty acids and pigments. *European Journal of Lipid Science and Technology*, vol. 115, no. 11, p. 1247-1256. https://doi.org/10.1002/ejlt.201300092

Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L., Yuan, Z. 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Frontiers in Microbiology*, vol. 5, p. 69-83. https://doi.org/10.3389/fmicb.2014.00217 PMid:24860564

Desbois, A. P., Lawlor, K. C. 2013. Antibacterial activity of long-chain polyunsaturated fatty acids against *Propionibacterium acnes* and *Staphylococcus aureus*. *Marine drugs*, vol. 11, no. 11, p. 4544-4557. https://doi.org/10.3390/md11114544 PMid:24232668

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10-22.

Fiedor, J., Burda, K. 2014. Potential role of carotenoids as antioxidants in human health and disease. *Nutrients*, vol. 6, no. 2, p. 466-488. https://doi.org/10.3390/nu6020466 PMid:24473231

Gaudin, V., Hedou, C., Rault, A., Verdon, E., 2010. Validation of a Five Plate Test, the STAR protocol, for the screening of antibiotic residues in muscle from different animal species according to European Decision 2002/657/EC. *Food Additives and Contaminants A*, vol. 27, no. 7, p. 935-952. https://doi.org/10.1080/19440041003677483 PMid:20432095

Gaudin, V., Juhel-Gaugain, M., Morétain, J. P., Sanders, P. 2008. AFNOR validation of a Premi®Test, a microbiological-based screening tube-test for the detection of antimicrobial residues in animal muscle tissue. *Food Additives and Contaminants A*, vol. 25, no. 12, p. 1451-1464. https://doi.org/10.1080/02652030802429088 PMid:19680855

Gaudin, V., Maris, P., Fuselier, R., Ribouchon, J. L., Cadieu, N., Rault, A. 2004. Validation of a microbiological method: the STAR protocol, a five-plate test, for the screening of antibiotic residues in milk. *Food Additives and Contaminants*, vol. 21, no. 5, p. 422-433. https://doi.org/10.1080/02652030410001667575 PMid:15204543

Gondová, Z., Kožárová, I., Poláková, Z., Maďarová, M. 2014: Comparison of four microbiological inhibition tests for the screening of antimicrobial residues in the tissues of food-producing animals. *Italian Journal of Animal Science*, vol. 13, no. 4, p. 728-734. https://doi.org/10.4081/ijas.2014.3521

Haščík, P., Trenbecká, L., Bobko, M., Kačániová, M., Čuboň, J., Kunová, S., Bučko, O. 2016. Effect of diet supplemented with propolis extract and probiotic additives on performance, carcass characteristics and meat composition of broiler chickens. *Potravinarstvo*, vol. 10, no. 1, p. 223-231. https://doi.org/10.5219/581

Huang, C. B., George, B., Ebersole, J. L. 2010. Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. *Archives of Oral Biology*, vol. 55, no. 8, p. 555-560. https://doi.org/10.1016/j.archoralbio.2010.05.009 PMid:20541177

Ivey, M., Massel, M., Phister, T. G. 2013. Microbial interactions in food fermentations. *Annual Reviews in Food Sciences and Technology*, vol. 4, p. 141-162. https://doi.org/10.1146/annurev-food-022811-101219 PMid:23190140

Klempová, T., Basil, E., Kubatová, A., Čertik, M. 2013. Biosynthesis of gamma-linolenic acid and betacarotene by *Zygomycetes* fungi. *Biotechnoly Journal*, vol. 8, p. 794-800. https://doi.org/10.1002/biot.201200099 PMid:23625863

Kožárová, I., Janošová, J., Máté, D., Tkáčiková, S. 2009. Evaluation of different microbial inhibition tests for the Food Additives and Contaminants 617 detection of sulphamethazine residues in the edible tissues of rabbits. *Food Additives and Contaminants A*, vol. 26, no. 7, p. 978-987. https://doi.org/10.1080/02652030902839715 PMid:19680973

Kožárová, I., Mačanga, J., Goldová, M., Major, P., Tkáčiková, S. 2011. Detection of maduramycin residues of chickens and pheasants by the screening test for antibiotic residues (STAR). *Food Additives and Contaminants A*, vol. 28, no. 5, p. 608-618. https://doi.org/10.1080/19440049.2011.561879 PMid:21442501

Magalhães, C. G., De Paiva, C. R., Botelho, B. G., De Oliviera, A. M. G., De Souza, L. F., Nonaka, C. V., Santos, K. V., Farias, L. M., Carvalho, M. A. R. 2012. In-house validation of Premi®Test, a microbiological screening test with solvent extraction, for the detection of antimicrobial residues in poultry muscles. *Food Additives and Contaminants A*, vol. 29, no. 4, p. 535-540. https://doi.org/10.1080/19440049.2011.627571 PMid:22059477

Niba, A. T., Beal, J. D., Kudi, A. C., Brooks, P. H. 2009. Potential of bacterial fermentation as a biosafe method of improving feeds for pigs and poultry. *African Journal of Biotechnology*, vol. 8, no. 9, p. 1758-1767.

Pikkemaat, M. G., Rapallini, M. L. B. A., Oostra-Van Dijk, S., Elferink, J. W. A. 2009. Comparison of three microbial screening methods for antibiotics using routine monitoring samples. *Analytica Chimica Acta*, vol. 637, no. 1-2, p. 298-304. https://doi.org/10.1016/j.aca.2008.08.023 PMid:19286043

R 25. 2006. Screening test for determination of antibiotic residues using bacterial strain (STAR). Available at: http://www.svssr.sk/dokumenty/zakladne_info/Diagnostika/R%2025%20STAR%20test.pdf

R 26. 2006. Detection of inhibitory substances in meat to Premi®Test. Available at: http://www.svssr.sk/dokumenty/zakladne_info/Diagnostika/R%2026%20PREMi%20test.pdf

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 031, 1.2.2002, p. 1-24.

Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29-43.

Sayegh, F., Elazzazy, A., Bellou, S., Moustogianni, A., Elkady, A. I., Baeshen, M. N., Aggelis, G. 2016. Production of polyunsaturated single cell oils possessing antimicrobial and anticancer properties. *Annals of Microbiology*, vol. 66, no. 3, p. 937-948. https://doi.org/10.1007/s13213-015-1176-0

Stead, S., Richmond, S., Sharman, M., Stark, J., Geijp, E., 2005. A new approach for detection of antimicrobial drugs in food: Premi®Test coupled to scanner technology. *Analytica Chimica Acta*, vol. 529, no. 1-2, p. 83-88. https://doi.org/10.1016/j.aca.2004.09.071

Stead, S., Sharman, J. A., Tarbin, J. A., Gibson, E., Richmond, S., Stark, J., Geijp, E. 2004. Meeting maximum

residue limits: an improved screening technique for rapid detection of antimicrobial residues in animal food products. *Food Additives and Contaminants*, vol. 21, no. 3, p. 216-221. https://doi.org/10.1080/02652030310001647280 PMid:15195469

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ANTIBIOTIC RESISTANCE IN BACTERIA *STAPHYLOCOCCUS* SPP. ISOLATED FROM SAMPLES OF RAW SHEEP'S MILK

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ABSTRACT

From samples of raw sheep's milk were determined results of bacteriological examination from two herds in region of Eastern Slovakia in three years lasting study. The occurrence of *Staphylococcus spp.* 41.6% (124) was determined from 298 samples. The seven species of staphylococci were on a regular basis isolated: *S. epidermidis* (34), *S. chromogenes* (26), *S. aureus* (16). Alternately have been recorded *S. warneri* (16), *S. schleiferi* (15), *S. haemolyticus* (9) and S. *xylosus* (8). All isolated pathogens were tested by in vitro test on Mueller-Hinton agar by disc methods on resistance to 10 types of antibiotics. Highest value of resistance was determined to Penicilin 21.0%, Neomycin 10.5% and Novobiocin 9.7%. Lower resistance was in to Oxacilin 7.2% and Amoxicilin 6.5%. Minimal resistance was founded to Cefoxitin 0.8%, Linkomycin 2.4%, Erytromycin, and Streptomycin 3.2%. Was founded total resistance (21.0%) to all antibiotics in *S. epidermidis* (34) during the three years, *S. chromogenes* (26) showed resistance to 8 types of antibiotics (12.9%), *S. aureus* (16) to 6 antibiotics (10.5%) and *S. warneri* (16) to 4 antibiotics (5.6%). It was confirmed that sheep's milk remains a major source of staphylococci. Bacteria in comparison with isolates from cows' raw milk, showed lower values of resistance, but were resistant to more than two antibiotics. Recorded occurrence of resistance in staphylococci may be connected with a minimum use of antibiotics in the treatment of mastitis and other diseases in sheep herds. Reported resistance to the tested antibiotics became the basis for the recommendation to use preparations to treat mastitis in sheep principally by the detection of resistance to antibiotics contained.

Keywords: sheep; raw milk; antibiotic; resistance; *Staphylococcus* spp.

INTRODUCTION

The bacteria *Staphylococcus* are important pathogens in human and animals medicine. Specific properties above 50 species of *Staphylococcus* spp. caused differences in pathogenesis of many inflammatory diseases as evidenced by the extensive studies of *S. aureus*, which produces the greatest amount of substances called virulence factors **(Spergser et al., 2003)**.

Staphylococcus spp. is the main causative agent of ovine mastitis, with higher prevalence in cases of clinical and subclinical manifestations **(Bergonier et al., 2003; Fagundes et al., 2010)**.

In the environmental mastitis *Staphylococcus* spp. and *Escherichia coli* are the main pathogens responsible for the inflammation **(Baskaran et al., 2009)** and, together with coagulase negative staphylococci (CoNS) strains, are the most frequent pathogens, particularly such as *S. epidermidis, S. simulans, S. hyicus, S. warneri, S. sciuri* and *S. xylosus* in ovine mastitis **(Hariharan et al.,2004)**.

In sheep, intramammary antibiotic therapy using a combination of penicillin with many drugs has been found to be effective in reducing the load of mastitis pathogens after lambing **(Chaffer et al., 2003)**. Mastitis can be cured by treatment with antibiotics after identification of the causative agents. Antibiotic sensitivity tests can be performed to ensure adequate treatment **(Bergonier and Berthelot, 2003)**.

Owing to the important role of antimicrobials in mastitis control programs, the determination of antimicrobial susceptibilities of mastitis pathogens is necessary for therapy and monitoring the spread of resistant strains among populations **(Gentilini et al., 2002; Bengtsson et al., 2009)**. Also, antimicrobial resistant bacteria of animal origin can be transferred to humans via food chain and can pose public health problems **(Skovgaard, 2007)**.

Antibiotic resistance is one of the important problems encountered in the treatment and control of mastitis. Mastitis caused by resistant bacteria is difficult to cure and has severe consequences. Thus, determination of the antibiotic susceptibilities of pathogens causing mastitis is of crucial importance for the treatment and control of mastitis in dairy ewes **(Tel et al., 2012)**.

Increasing the consumption of antibiotics in veterinary and human medicine is in the last period, accompanied by the phenomenon of an increase in bacterial resistance. The use of antimicrobials in animal nutrition, in the production of plants, feed and food prices can have a negative impact on public health through the increase in resistant bacteria or bacteria producing resistant genes that pass into the organism of people directly or indirectly **(Bíreš et al., 2009)**.

Hleba et al. (2010) was focused on monitoring the resistance of bacteria in the work of the family *Enterobacteriaceae*, which are considered a reservoir of genes resistant to antibiotics in animal husbandry. About

bacteria *Staphylococcus*, which are frequently isolated from milk of ruminants, it is generally stated that they can download the following "mediated" resistance genes from another environment **(Pyörälä and Taponen, 2009)**.

Infections caused by resistant species of microorganisms cause costly treatment of animals as well as humans. These infections lengthen a pathological condition and, if not treated with antibiotics are administrative (according to current observed sensitivity of bacteria) can lead to increased mortality **(Witte, 2006)**.

It is therefore the identification of these reservoirs and a mechanism to transfer the key to reduction and the reduction of resistant bacteria in the commercial sphere of the food chain man **(Nováková et al., 2009)**.

Vasil' et al. (2009) noticed on increasing occurrence of subclinical and latent mastitis caused by CoNS resistant to antibiotics. For this reason, control of antibiotic resistance bacterial pathogens of mastitis should be the starting point for ensure the effectiveness of control methods applied **(Virdis et al., 2010; Bennedsgaard et al., 2006)**.

Recently, it has been recognised that antimicrobial susceptibility of CoNS, which represent the majority of organisms isolated from ovine milk, is important for the early recognition of newly emerging resistant milk-borne bacterial agents **(Onni et al., 2011)**.

The aim of our study was to determine the occurrence of *Staphylococcus* spp. in samples of raw sheep's milk and their resistance to 10 antibiotics by disc method.

MATERIAL AND METHODOLOGY

Characteristic of experiment and samples collection

During three years, in the frequency of two times a month were bacteriological examined samples of raw and sheep's milk, with a focus on the isolation of the bacteria *Staphylococcus* spp. Samples were collected from the tanks as the total pool after milking from different two sheep holdings on Eastern Slovakia.

The experiment was carried out in two herds - herd A of 450 sheep of Lacaune breed and herd B 400 sheep breed of Improved valaska. During summer season (from April to September) sheep were machine milked two times per day in milk parlous: herd A - 2x14 Miele Melktechnik, (Hochreiter Landtechnik, Germany) and Herd B - 2x20, Alfa-Laval, Tumba, Sweden).

Level on both farms with tradition of machine milking sheep can be characterized as a standard. Sheep have secured adequate food during all year, and grazing on adjacent pastures. It is ensured selection of sheep according health of the mammary gland; decommissioning ewes with chronic form of mastitis from herds of sheep; milking max. 180 seconds and with individual post-milking; observance of hygiene program during milking with an emphasis on teat disinfection after milking; Treatment of clinical mastitis cases on the base of antibiogram results.

Microbiology analyses

Bacteriology analyses were centred on the isolation of *Staphylococcus* spp. Cultivation and identification of bacteria was performed on the 5 % blood agar, Medium No. 110, Baird Parker agar. Colouring by Gram, catalase activity, coagulation of the rabbit's plasma, haemolysis, and the pigments production were carried out, too. Isolated bacteria were examined by the commercial set Staphytest24 (Erba-Lachema, Brno, Czech Republic), and results were evaluated using the identifying programme TNW, version 7.0 (Erba-Lachema, Brno, Czech Republic).

Resistance to antibiotics

We tested all isolated pathogens by *in vitro* test on Mueller-Hinton agar by zone disc methods (EUCAST, 2015) after 24h incubation at 37 °C on resistance to 10 antibiotics: Ampicilin 10 μg, Amoxicilin 25 μg, Oxacilin 5 μg, Erythomycin 10 μg, Linkomycin 15 μg, Neomycin 10 μg, Novobiocin 5 μg, Penicilin 10 μg, Streptomycin 10 μg, Cefoxitin 30 μg (OXOID Ltd. Basingstoke, Hants, UK). Resistance or sensitivity of the bacteria tested was interpreted by reference zones in accordance with the instructions EUCAST (2015). In tests were used according to recommendations following control strains: *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* CCM 4418, *Staphylococcus haemolyticus* CCM 2737. Occurrence of resistance to the tested antibiotics for each species of staphylococci has been registered continuously numerically.

RESULTS AND DISCUSSION

Several authors in their studies from France and Spain recorded, that the species of *Staphylococcus* spp. belongs to general aetiological agents of intramammary infections in small ruminants (*S. aureus* in clinical and CoNS in subclinical cases). From the CoNS is more frequently *S. epidermidis* and *S. chromogenes* what are also determined in our study **(Bergonier et al., 2003; Berthelot et al., 2006)**.

The frequency of occurrence of staphylococci in raw sheep´s milk collected twice a month in two different herds of sheep in Eastern Slovakia during the three milking seasons are described in Table 1.

From all isolated species of staphylococci were regular isolated *S. epidermidis* (34), *S. chromogenes* (26), and *S. aureus* (16). Alternately were isolated *S. warneri* (16), *S. schleiferi* (15), *S. xylosus* (8), and *S. haemolyticus* (7).

In general, microbial contamination of raw milk occurs from three main sources: from within the udder, from the exterior of the udder, and from the surface of milk handling and storage equipment **(McKinnon et al., 1990)**.

From the total three years valuation the occurrence of resistance to 10 types of antibiotics, resulted to occurrence of resistance predominantly in two species of staphylococci during year (*S. intermedius* and *S. haemolyticus*). Maximal annual finds had species *S. epidermidis* (16), *S. warneri* (12) and *S. schleiferi* (11).

Continuous valuation of the occurrence of resistance in staphylococci are characterised by minimum values. An objective assessment in the context of individual species and year on year was complicated due to the small number of groups.

In the total group of *Staphylococcus spp.* (n = 124) were determined the resistance to all ten types of antibiotics. Highest value of resistance was determined to Penicilin 21.0%, Neomycin 10.5% and Novobiocin 9.7%. Lower resistance was in to Oxacilin 7.2% and Amoxicilin 6.5%.

Table 1 Resistance to 10 antibiotics in bacteria *Staphylococcus* spp. (n = 124) isolated from 298 samples of raw sheep's milk.

Staphylococcus spp.	year	n	Resistance (%)									
			1	2	3	4	5	6	7	8	9	10
S. aureus (n = 16)	I.	8		1	1				1	2		
	II.	5						1	1	1	2	
	III.	3			1			1		1		
S. epidermidis (n = 34)	I.	16	1		3			2	1	5	1	
	II.	8	1	1		1	1		3	2		1
	III.	10			1			1		1		
S. chromogenes (n = 26)	I.	10	1	2	1			3	1			
	II.	9		1		2	1		1	2		
	III.	7					1			1		
S. warneri (n = 16)	I.	4							1	1		
	II.											
	III.	12			1			2		2		
S. schleiferi (n = 15)	I.	11		1	1				1	2	1	
	II.											
	III.	4						1		1		
S. haemolyticus (n = 9)	I.	7		1				2	1	1		
	II.	2							1	1		
	III.											
S. xylosus (n = 8)	I.											
	II.	3	1			1				1		
	III.	5		1						1		
Resistance (∑n)		124	4	8	9	4	3	13	12	25	4	1
Resistance (%)*		100	3,2	6.5	7.2	3.2	2.4	10.5	9.7	21.0	3.2	0.8

Note: (1) Ampicilin 10 μg; (2) Amoxicilin 25 μg; (3) Oxacilin 5 μg; (4) Erytromycin 10 μg; (5) Linkomycin 15 μg; (6) Neomycin 10 μg; (7) Novobiocin 5 μg; (8) Penicilin 10 μg; (9) Streptomycin 10 μg; (10) Cefoxitin 30 μg; * – resistance of all 124 testedbacterium on antibiotics.

Minimal resistance was founded to Cefoxitin 0.8%, Linkomycin 2.4%, Erytromycin, and Streptomycin 3.2%.

The resistance in most numbered species (groups with n ≥15) are described in Table 2. Was founded total resistance (21.0%) to all antibiotics in *S. epidermidis* (34) during the three years. *S. chromogenes* (26) showed resistance to 8 types of antibiotics (12.9%), *S. aureus* (16) to 6 antibiotics (10.5%) and *S. warneri* (16) to 4 antibiotics (5.6%).

Our results are consistent with the work **Kirkan et al. (2005)**, where 300 cases of mastitis were isolated 60 bacteria of CoNS (20.0%), which showed resistance to penicillin (90.0%) and oxacillin, (75.0%). For comparison **Virdis et al. (2010)** report increased resistance of CoNS on ampicillin and kanamycin and of *S. aureus* on the oxytetracycline.

The importance of CoNS in the aetiology of the subclinical mastitis in sheep is the undisputed. However, the frequency of their occurrence is species-specific and is different depending on the technological level of the holding and the geographical conditions of the site **(Gentilini et al., 2002)**. While our work we describe six types of CoNS, **Gelasakis et al. (2015)** under the transparent processing of the twelve works published over the last ten years in this context, it has registered a total of twenty species have been isolated in the mastitis of sheep. Despite the finding that the CoNS are pathogens of lower virulence, the severity of their occurrence lies in the progressive trend of increasing the share of the prevalence of clinical forms of mastitis. In particular, these are the bacteria *S. epidermidis*, *S. chromogenes*, *S. simulans* and *S. xylosus*, in which was recorded the occurrence of antibiotic resistance **(Onni et al., 2010)**. And for that reason we

Table 2 Resistance in species of the genus *Staphylococcus spp.*(only in groups with n ≥15), which were isolated from raw sheep's milk.

Antibiotics	µg	*S. aureus* (n = 16)	*S. epidermidis* (n = 34)	*S. chromogenes* (n = 26)	*S. warneri* (n = 16)
Penicilin	10 U	4	8	2	3
Ampicilin	10	–	2	1	–
Amoxicilin	25	1	1	3	–
Oxacilin	5	2	4	1	1
Erythromycin	10	–	1	2	–
Lincomycin	15	–	1	2	–
Streptomycin	10	2	1	–	–
Neomycin	10	2	3	2	2
Novobicin	5	2	4	3	1
Cefoxitin	30	–	1	–	–
Resistance (∑n)		13	26	16	7
***Resistance (%)**		**10.5**	**21.0**	**12.9**	**5.6**

Note: * - resistance from all 124 tested bacteria *Staphylococcus spp.*

prefer to take the view that in the coming period will need to be monitored the antibiotic resistance in CoNS bacteria, in order to ensure the effective clinical therapies of mastitis and, secondly, that it will be important for the early detection of resistance in this important group of bacteria isolated from ewe's milk.

Information about the occurrence of sporadic resistance to antibiotics, are usually a reflection of the range of intramammary drugs the use of veterinary practice in the country. For the acceptance of data in the published studies, is important information about the frequency of resistance isolates of CoNS to tested drugs, as well as the period during which the bacteria have been isolated. **Ebrahimi et al. (2007)** from nine antibiotics recorded, increased resistance of the CoNS to amikacin, and to a lesser extent resistance to ampicillin, penicillin, tetracyklin, and oxytetracyklin. Good sensitivity was detected to chloramphenicol, ciprofloxacin, streptomycin and gentamicin, it is important for potential use in the treatment of clinical mastitis.

The objectivity of the results about resistance to antibiotics of the bacteria can be achieved by using a phenotypical method, in parallel with molecular identification of markers of resistance. This is a PCR detection of number of specific genes responsible for individual properties, which determine the resistance **(Franco et al., 2012)**. Record the occurrence of two or more genes may be associated with the multiresistance to antibiotics in bacteria of *Staphylococcus spp*. Increasing incidence of multi-drug resistant strains of CoNS, compared with CoPS in general supports the hypothesis, that the CoNS can play an important role as a source of resistance genes for *S. aureus* **(Taponen & Pyorälä, 2009)**.

The results obtained a three-year monitoring of resistance in staphylococci isolated from samples of raw sheep's milk must be considered as continuous in the light of the ongoing two-year continuation of the experiment yet. It was confirmed that sheep's milk remains a major source of staphylococci; however, the current level of treatment effectively eliminates the potential threat to the health of consumers of dairy. In comparison with numerous types of staphylococci isolates from cow's raw milk, tested strains had lower values of resistance, but were resistant to more than two antibiotics.

CONCLUSION

Using bacteriological examination of 298 samples of raw sheep milk were obtained data on frequency of bacteria *Staphylococcus* spp., 41.6% (n = 124). Recorded the incidence of resistance in staphylococci, which were isolated from raw sheep's milk was linked to minimal use of antibiotics in the treatment of mastitis and other diseases in sheep.

REFERENCES

Baskaran, S. A., Kazmer, G. W., Hinckley, L., Andrew, S. M., Venkitanarayanan, K. 2009. Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens in vitro. *Journal of Dairy Science*, vol. 92, no. 4, p. 1423-1429. https://doi.org/10.3168/jds.2008-1384

Bengtsson, B., Unnerstad, H. E., Ekman, T., Artursson, K., Nilsson-Ost, M., Waller, K. P. 2009. Antimicrobial susceptibility of udder pathogens from cases of acute clinical mastitis in dairy cows. *Veterinary Microbiology*, vol. 136, no. 1-2, p. 142-149. https://doi.org/10.1016/j.vetmic.2008.10.024 PMid:19058930

Bennedsgaard, T. W., Thamsborg, S. M., Aarestrup, F. M., Enevoldsen, C., Vaarst, M., Christoffersen, A. B. 2006. Resistance to penicillin of *Staphylococcus aureus* isolated from cows with high somatic cell counts in organic and conventional dairy herds in Denmark. *Acta Veterinaria Scandinavica*, vol. 48, p. 24.

Bergonier, D., Berthelot, X. 2003. New advances in epizootiology and control of ewe mastitis. *Livestock Production Science*, vol. 79, no. 1, p. 1-16. https://doi.org/10.1016/S0301-6226(02)00145-8

Bergonier, D., Crémoux, R., Rupp, R., Lagriffoul, G. Erthelot, X. 2003. Mastitis of dairy small ruminants. *Veterinary Research*, vol. 34, no. 5, p. 689-716. https://doi.org/10.1051/vetres:2003030

Berthelot, X., Lagriffoul, G., Concordet, D., Barillet, F., Bergonier, D. 2006. Physiological and pathological thresholds of somatic cell counts in ewe milk. *Small Ruminant Research*. vol. 62, no. 1-2, p. 27-31. https://doi.org/10.1016/j.smallrumres.2005.07.047

Bíreš, J., Húska, M., Vasiľ, M. 2009. Antimikrobiálna rezistencia–aktuálny problém vo veterinárnej medicíne a vo verejnom zdraví, (Antimicrobial resistance – current problem in veterinary medicine and public health). *Potravinarstvo*, vol. 3, no. 2, p. 1-3. Available online: http://www.potravinarstvo.com/dokumenty/potravinarstvo_no2_2009.pdf

Ebrahimi, A., Lotfalian, Sh., Karimi, S. 2007. Drug resistance in isolated bacteria from milk of sheep and goats with subclinical mastitis in Shahrekord district. *Iranian Journal of Veterinary Research*, vol. 8, no. 1, ser. no. 18, p. 76-79.

EUCAST: European Society of Clinical Microbiology and Infectious Diseases. 2015. Disk Diffusion Method for Antimicrobial Susceptibility Testing - Version 5.0 (January 2015). Available online: http://www.eucast.org

Fagundes, H., Barches, L., Filho N. A., Menezes Ferreira, M. L., Fernandes Oliveira C. A. 2010. Occurrence of *Staphylococcus aureus* in raw milk produced in dairy farms in Sao Paulo Stat, Brazil. *Brazilian Journal of Microbiology*, vol. 41, no. 2, p. 376-380. https://doi.org/10.1590/S1517-83822010000200018

Franca, C. A., Peixoto, R. M., Cavalcante, M. B., Melo, N. F., Oliveira, C. J. B., Veschi, J. L. A., Mota, R. A., Costa M. M. 2012. Antimicrobial resistance of *Staphylococcus spp.* from small ruminant mastitis in Brazil. *Pesquisa Veterinária Brasileira*, vol. 32, no. 8, p. 747-753. https://doi.org/10.1590/S0100-736X2012000800012

Gelasakis, A. I., Mavrogianni, V. S., Petridis, I. G., Vasileiou, N. G. C., Fthenakis, G. C. 2015. Mastitis in sheep - The last 10 years and the future of research. *Veterinary Microbiology*, vol. 101, p. 136-146. https://doi.org/10.1016/j.vetmic.2015.07.009 PMid:26216457

Gentilini, E., Denamiel, G., Betancor, A., Revuelto, M., Rodriguez, F. M., De Torrest, R. A. 2002. Antimicrobial susceptibility of coagulase negative *staphylococci* isolated from bovine mastitis in Argentina. *Journal of Dairy. Science*, vol. 85, no. 8, p. 1913-1917. https://doi.org/10.3168/jds.S0022-0302(02)74267-7

Hariharan, H., Donachie, W., Macaldowie, C., Keefe, G. 2004. Bacteriology and somatic cell counts in milk samples from ewes on a Scottish farm. *Canadian Journal of Veterinary Research*, vol. 68, no. 3, p. 188-92. PMid:15352543

Hleba, L., Kačniová, M., Čuboň, J., Haščik, P., Pochop, J., Kunová, S., Mihok, M. 2010. Antimikrobiálna rezistencia – aktuálny problem vo veterinárnej medicíne a vo verejnom zdraví, (Antibiotic resistance of enterobacteriaceae genera isolated from sheep milk products). *Potravinarstvo*, vol. 4, special no., p. 158-165. Available online: http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/2/Hleba.pdf

Chaffer, M., Leitner, G., Zamir, S., Winkler, M., Glickman, A., Ziv, N. Saran, A. 2003. Efficacy of dry-off treatment in sheep. *Small Ruminant Research*, vol. 47, no. 1, p. 11-16. https://doi.org/10.1016/S0921-4488(02)00194-3

Kirkan, S., Goksoy, E. O., Kaya, O. 2005. Identification and antimicrobial susceptibility of *Staphylococcus aureus* and coagulase negative staphylococci from bovine mastitis in the Aydin region of Turkey. *Turkish Journal of Veterinary and Animal Science*, vol. 29, p. 791-796.

McKinnon, C. H., Rowlands, G. J., Bramley, A. J. 1990. The effect of udder preparation before milking and contamination from the milking plant on bacterial numbers in bulk milk of eight dairy herds. *Journal of Dairy Research*, vol. 57, no. 3, p. 307-318.

Nováková, I., Kačániová, M., Haščík, P., Pavličová, S., Hleba, L. 2009. The resistant to antibiotics in strains *E. coli* and *Enterococcus* sp. isolated from rectal swabs of lambs and calves, *Lucraristiintifice Zootehniesi Biotehnologii*, vol. 42, no. 2, p. 322-326.

Onni, T., Sanna, G., Larsen, J., Tola, S. 2011. Antimicrobial susceptibilities and population structure of *Staphylococcus epidermidis* associated with ovine mastitis. *Veterinary Microbiology*, vol. 148, no. 1, p. 45-50. https://doi.org/10.1016/j.vetmic.2010.07.024

Pyörälä, S., Taponen, S. 2009. Coagulase-negative staphylococci - Emerging mastitis pathogens. *Veterinary Microbiology*, vol. 134, no. 1-2, p. 3-8.

Skovgaard, N. 2007. New trends in emerging pathogens. *International Journal of Food Microbiology*, vol. 120, no. 3, p. 216-219. https://doi.org/10.1016/j.ijfoodmicro.2007.07.046

Tel, O. Y., Aslantaş, O., Keskin, O., Yilmaz. E. S. Demir, C. 2012. Investigation of the antibiotic resistance and biofilm formation of *Staphylococcus aureus* strains isolated from gangrenous mastitis of ewes. *Acta Veterinaria Hungarica*, vol. 60, no. 2, p. 189-197. https://doi.org/10.1556/AVet.2012.016 PMid:22609990

Taponen, S., Pyorälä, S. 2009. Coagulase-negative staphylococci as cause of bovine mastitis-Not so different from *Staphylococcus aureus*? *Veterinary Microbiology*, vol. 134, no. 1-2, p. 29-36. https://doi.org/10.1016/j.vetmic.2008.09.011 PMid:18977615

Vasiľ, M., Elečko, J., Farkašová, Z., Bíreš, J. 2009. The reduction on the occurrence of mastitis in dairy herd using the innovation of housing conditions, sanitary of milk storage and applying the therapy of mastitis during the lactation. *Folia Veterinaria*, vol. 53, suppl. II, p. 186-189.

Virdis, S., Scarano, Ch., Cossu, F., Spanu, V., Spanu, C., De Santis, E. P. L. 2010. Antibiotic resistance in *Staphylococcus aureus* and coagulase negative Staphylococci isolated from goats with subclinical mastitis. *Veterinary Medicine International*, p. 1-6.

Witte, W. 2000. Selective pressure by antibiotic use in livestock. *Internet Journal of Antimicrobial Agents*, vol. 16, suppl. 1, p. 19-24. https://doi.org/10.1016/S0924-8579(00)00301-0

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RISK ELEMENTS IN SELECTED TYPES OF VEGETABLES

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ABSTRACT

Vegetable has an important role in human nutrition. Various parts of the plants have been part of the human diet since the beginning. Vegetables have a number of properties that make its consumption very healthful. It not only is a good source of vitamins, minerals and fiber but also contains protective components so called phytonutrients, has an antioxidant and antimicrobial effects. Daily intake of vegetables offers many health benefits, helps to improve health for example the function of digestive and immune system, reduces the risk of various diseases and so we should take care to its regular consumption. It is widely used, except that it is the basic raw material for the preparation of foods and is also an important raw material for the processing industry. Nowadays has become environmental pollution by heavy metals as a big problem. The contamination of water, soil as well as air pollution by heavy metals negatively affects agricultural production and production of non-harmful to health, safe and quality food, which may be adverse effects on human health. Therefore, it is important that we devote this issue more attention. The aim of this work was to identify and determine content of heavy metals in selected vegetables. Defined objectives have been achieved by analyzing of selected species samples of root from brassica vegetables: carrot (*Daucus carota* L. ssp. *sativus*), parsley (*Petroselinum hortesne HOFFM* conv. *radicosum*), kohlrabi (*Brassica oleracea* L. var. *gongylodes*), celery (*Apium graveolen*s L. var. *rapaceum*) and beetroot (*Beta vulgaris* L. var. *conditiva* ssp. *vulgaris*). The crops were bought in local market. The obtained results were compared with the results obtained from analyzes of vegetables that were grown in home conditions respectively from markets of local growers. All crops were grown in Slovak Republic. By using Varian AA 240FS and AAS method were analyzed the contents of risk metals in selected vegetables. It was confirmed that in selected types of vegetables analyzed element was not exceeded the limit values established by *Codex Alimentarius* of Slovak Republic. From the results, also can be concluded that higher contents of heavy metals (Cu, Mn, Ni, Cd, Pb) were mostly in samples from home gardeners than in samples came from local market.

Keywords: heavy metals; vegetables; contamination; local market

INTRODUCTION

Vegetable is a fundamental part of the food chain and a typical component of a wide range of the dishes. Vegetable has an important place in a rational human nutrition for its high biological and low-energy value **(Uher, 2011).**

In the view of the attractiveness to consumers is significant diversity of aromas, flavors, species and varieties with the possibility of its culinary use. On the other hand, the nutritional value it is important to its good and quick digestibility **(Kopec, 2010)**.

Vegetables, as an important source of chemo protective substances, have irreplaceable position within the food of plant character **(Hegedűsová et al., 2015)**.

Vegetable is very important in human nutrition because it ensures adequate intake of most vitamins, minerals, fiber and phytochemical substances. The consumption of vegetables in the daily diet is nearly related with a number of positive health effects, good health, a lot of positive effects to gastrointestinal system, reduces the risk of heart disease, stroke, anemia and chronic diseases such as diabetes and some forms of cancer **(Dias, 2012)**.

Carrot is a huge source of pro-vitamin A and also a good source of fiber and trace elements as potassium, sodium, magnesium, calcium, and molybdenum. Carotenoids, flavonoids, phenolic compounds, and vitamins are contained in carrots and have an effect as antioxidants **(Dias, 2014)**.

Parsley has been used as carminative, gastro tonic, diuretic, antiseptic of urinary tract, anti-urolithiasis, anti-dote and anti-inflammatory and for the treatment of amenorrhea, dysmenorrhea, gastrointestinal disorder, hypertension, cardiac disease, urinary disease, otitis, sniffle, diabetes and also various dermal disease in traditional and folklore medicines **(Farzaei et al., 2013)**.

Celery contains vitamin C and other materials that are health enhancing substances such as phalides, which lowers cholesterol and coumarins that helps prevent cancer. Celery seeds of anti-rheumatism, sedative, antiseptic urinary tract, increased excretion of uric acid, blood pressure lowering, to some extent against fungal diseases, diuretic, analgesic, anti-inflammatory, detoxification **(Modaresia et al., 2012)**.

Kohlrabi is watery, easily digestible tuber of distinctive taste. The mean of water concentration is 91.7%. It has 3.7% of sugars, 1.6% of protein, 0.2% of fat and 2.2% of fiber. Kohlrabi is rich of glucosinolates that give it the characteristic odor and taste and have anticancer effects **(Uher, 2011).**

Beetroot (*Beta vulgaris* L.) is a member of the *Chenopodiaceae* family, cultivated for its large roots, although leaves are also utilizable. Seeds, roots and leaves of the plant are rich of polyphenols and a water-soluble nitrogen pigments group named betalains. The betalains family represents the principal pigment in beetroot and the characteristic red-violet color is regarded as major attribute

for beetroot quality and acceptability **(Ninfali and Angelino, 2013)**.

Environmental contamination with heavy metals is increasingly coming to the fore and it is one of the most serious problems of modern society nowadays. Their riskiness arises from the substantial persistence, toxicity and ability to bio accumulate into environmental components and consequently into the food chain **(Burges et al., 2015; Douay et al., 2013; Roman and Popiela, 2011)**.

Copper is an essential element for many organisms, but in high concentrations it becomes toxic. It has wide application in industry and agriculture **(Bui et al., 2016)**.

Nickel level is low in most foods and vegetables except for a few cases, such as tea plants and *Camillia sinensis* L., which have the ability to accumulate nickel. Nickel is a moderately toxic element compared to other transition metals that can lead to serious illness, including malignant tumours and nasopharynx, lung, and dermatological diseases. However, nickel is an essential element for humans, other animals, and plants **(Jiang et al., 2006; Spears, 1984)**.

According to **Beneš (1994)** many plants tolerate higher cadmium content in the soil (tomatoes, potatoes). Sensitive plants react negatively to a Cd concentration in soil 4 – 13 $mg.kg^{-1}$ (spinach, soybean, tobacco), which is not very high by hygienically point of view. Higher concentrations of cadmium in soil inhibit the uptake of Ca^{2+} and Mg^{2+} by plants.

The plants can be resist to cadmium retention of excess ions in the roots or on the borders of metabolically important organs, reduce activity of excess ions and their transfer to a physiologically inert form, or create alternative exchange reaction that is less sensitive to cadmium. Cadmium accumulation and distribution in plant organs is clearly acropetal character: roots >stems >leaves >fruits (seeds). The highest concentration of cadmium is in root and greenhouse vegetables. Cadmium concentration increases as follows: oat <wheat <bean <pea <sunflower <corn <radish <tomato <carrot <salad **(Kočík, 1995)**.

Short-term exposure to high levels of Pb can cause brain and kidney damage, and gastrointestinal distress, while long-term exposure may affect blood, liver, and the central nervous and reproductive systems. Chronic low level exposure causes serious damage, in particular to the central nervous system, the vasculature and the kidneys **(Gupta et al., 2013)**.

MATERIAL AND METHODOLOGY

The aim of this work was to identify participation of individual heavy metals in selected vegetables from local market (vegetables from home conditions and vegetables from local growers both from Slovak Republic). In this work were analyzed root from brassica vegetables:

1. carrot (*Daucus carota* L. ssp. *sativus*)
2. parsley (*Petroselinum hortesne* HOFFM conv. r*adicosum*)
3. kohlrabi (*Brassica oleracea* L. var. *gongylodes*)
4. celery (*Apium graveolens* L. var. *rapaceum*)
5. beetroot (*Beta vulgaris* L.var. *conditiva* ssp. *vulgaris)*

Sample preparation

Samples of vegetables were washed, dried, then grated. We transfer them to dry in a Petri dishes in the oven Venticel 111 (Czech Republic) at 80 °C to constant weight. Part of the fresh sample was used for the determination of the dry matter by Ultra X (Germany).

Determination of heavy metals (by AAS)

Mineralization of samples (1 – 2 g of dried vegetables) was in a mixture of distilled water with concentrated nitric acid in a ratio 1:1. The weighed samples were put into teflon vessels with 5 cm^3 of distilled water with 5 cm^3 of concentrated nitric acid. Closed vessels with samples were mineralized by microwave digestion unit MARS X-press (USA).

After mineralization were analyzed samples filtered through quantitative filter paper MUNKTELL (Germany) grade 390.84 $g.m^{-2}$ (green) to volumetric flasks (50 cm^3).

Flasks were refilled with distilled water to the mark and after that was the determination of heavy metals by VARIAN AA 240FS (Australia) under the conditions:

Cd – detection limit – 0.001 $mg.L^{-1}$, sensitivity 0.01 $mg.L^{-1}$
Pb – detection limit – 0.02 $mg.L^{-1}$, sensitivity 0.1 $mg.L^{-1}$
Cu – detection limit – 0.002 $mg.L^{-1}$, sensitivity 0.03 $mg.L^{-1}$
Zn – detection limit – 0.006 $mg.L^{-1}$, sensitivity 0.008 $mg.L^{-1}$
Co – detection limit – 0.005 $mg.L^{-1}$, sensitivity 0.05 $mg.L^{-1}$
Cr – detection limit – 0.003 $mg.L^{-1}$, sensitivity 0.04 $mg.L^{-1}$
Ni – detection limit – 0.008 $mg.L^{-1}$, sensitivity 0.06 $mg.L^{-1}$
Mn – detection limit – 0.003 $mg.L^{-1}$, sensitivity 0.02 $mg.L^{-1}$
Fe – detection limit – 0.005 $mg.L^{-1}$, sensitivity 0.04 $mg.L^{-1}$

Analysis determination has not a deviation more than 3%, the gas flow: air: 13.5 $L.min^{-1}$, acetylene 2.0 $L.min^{-1}$.

For statistical evaluation of results was used a statistical program STATISTICA 6.0 Cz. The results tested on the level of descriptive statistical evaluation, and overall visual indication of the level factor, variability and deviations were expressed as text. We used T-test at the confidence level $p \leq 0.05$.

RESULTS AND DISCUSSION

The figures showed a comparison of the content of risk metals in selected types of vegetables that were bought in local market and the commercial local shops network and markets of local growers.

Table 1 The process of mineralization – time, temperature (total time 55 minutes).

Stage	Power (W)	Power (%)	Initialization Time (min)	Temp. (°C)	Duration time (min)
Initialization	800	90	15	160	0
Mineralization	800	90	0	160	20
Cooling	–	–	–	–	20

The contents of risk metals were compared with the maximum limit values set by *Codex Alimentarius* of Slovak Republic. The contents of zinc, manganese, cobalt and iron do not have defined limit values in *Codex Alimentarius of SR*.

According to obtained results of copper content can be defined in the order of vegetable species from markets of local growers: parsley >celery >carrot >beetroot >kohlrabi.

According to obtained results of copper content can be defined in the order of vegetable species from commercial local market: parsley >celery >beetroot >carrot >kohlrabi.

The highest copper content was detected in parsley from market with value 1.85 ±0.02 mg.kg^{-1} and the lowest in kohlrabi (0.21 ±0.01 mg.kg^{-1}). The most significant difference between samples of obtained results of copper content in vegetable from market and from local shop network was found in celery.

Mahmood and Malik (2014) identified a high copper content in carrot (5.34 mg.kg^{-1}). The copper content in all types of vegetable was not exceeded the highest permissible limit set by *Codex Alimentarius* of SR.

According to obtained results of zinc content can be defined in the order of vegetable species from markets of local growers: parsley >celery >beetroot >carrot >kohlrabi.

According to obtained results of zinc content can be defined in the order of vegetable species from commercial local market: celery >beetroot >parsley >kohlrabi >carrot.

The lowest zinc content was detected in carrot from local market with value 1.76 ±0.03 mg.kg^{-1}.

Shaheen et al., (2016) obtained in carrot 0.07 mg.kg^{-1} of zinc content. The highest zinc content from both sources was in celery (4.46 ±0.03 mg.kg^{-1}).

According to obtained results of manganese content can be defined in the order of vegetable species from markets of local growers: kohlrabi >parsley >celery >beetroot >carrot.

According to obtained results of manganese content can be defined in the order of vegetable species from commercial local market: kohlrabi > celery > parsley > beetroot > carrot.

The highest manganese content was detected in kohlrabi from market with value 2.66 ±0.03 mg.kg^{-1}and the lowest in carrot (0.55 ±0.02 mg.kg^{-1}). **Shaheen et al., (2016)** identified in carrot 6.98 mg.kg^{-1} of manganese content, which is about 12 times more than the value measured by us.

According to obtained results of iron content can be defined in the order of vegetable species from markets of local growers: parsley >celery >kohlrabi >beetroot >carrot.

According to obtained results of iron content can be defined in the order of vegetable species from commercial local market: parsley >kohlrabi >beetroot >celery >carrot.

The highest iron content compared to all analyzed vegetables, was detected in parsley from local market as well as from market (25.8 ±0.53 mg.kg^{-1}).

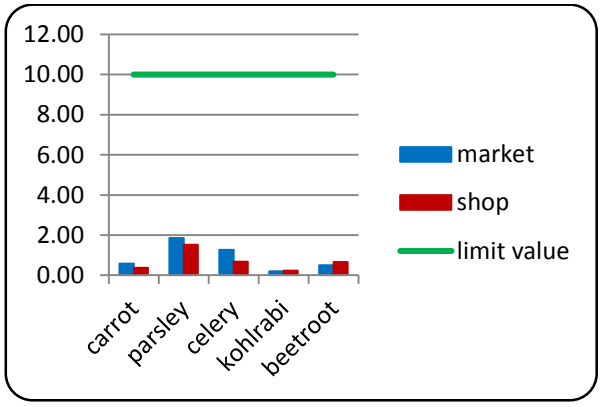

Figure 1 Evaluation of copper content in selected types of vegetables (mg.kg^{-1}), median, n = 6 and comparison with the maximum permissible limits set by *Codex Alimentarius* of SR.

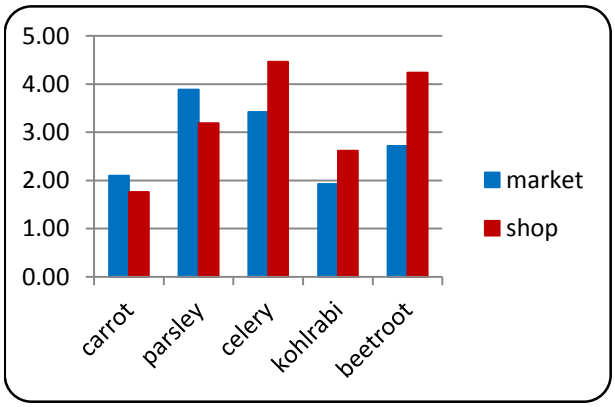

Figure 2 Evaluation of zinc content in selected types of vegetables (mg.kg^{-1}), median, n = 6.

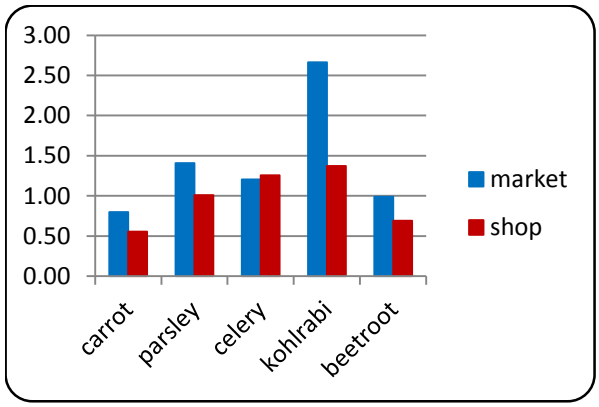

Figure 3 Evaluation of manganese content in selected types of vegetables (mg.kg^{-1}), median, n = 6.

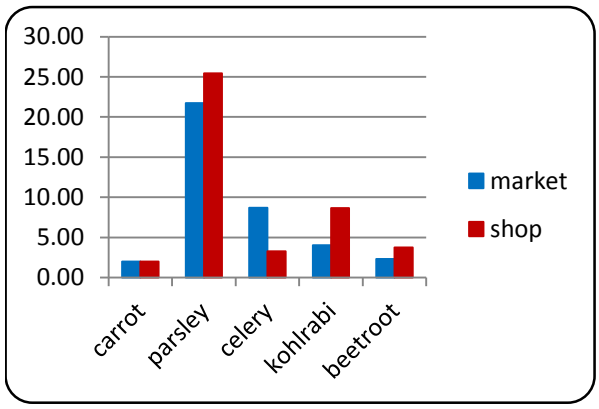

Figure 4 Evaluation of iron content in selected types of vegetables (mg.kg^{-1}), median, n = 6.

In vegetable samples bought in market, except celery, was iron content smaller than in vegetables bought in local market.

According to obtained results of nickel content can be defined in the order of vegetable species from markets of local growers: parsley >celery >kohlrabi >beetroot >carrot.

According to obtained results of nickel content can be defined in the order of vegetable species from commercial local market: celery >parsley >beetroot >kohlrabi >carrot.

The highest nickel content was detected in parsley from market with value 0.419 ±0.02 mg.kg^{-1}. **Guerra et al., (2012)** identified even higher nickel content of parsley (0.70 mg.kg^{-1}). The lowest nickel content was in carrot (0.05 ±0.02 mg.kg^{-1}).

The nickel content in all types of vegetable was not exceeded the highest permissible limit set by *Codex Alimentarius* of SR.

According to obtained results of cobalt content can be defined in the order of vegetable species from markets of local growers: celery >beetroot >kohlrabi >carrot >parsley.

According to obtained results of cobalt content can be defined in the order of vegetable species from commercial local market: kohlrabi >beetroot >parsley >celery >carrot.

The lowest cobalt content was in parsley from market (0.03 ±0.01 mg.kg^{-1}). The highest cobalt content was detected in celery from local market (0.20 ±0.03 mg.kg^{-1}).

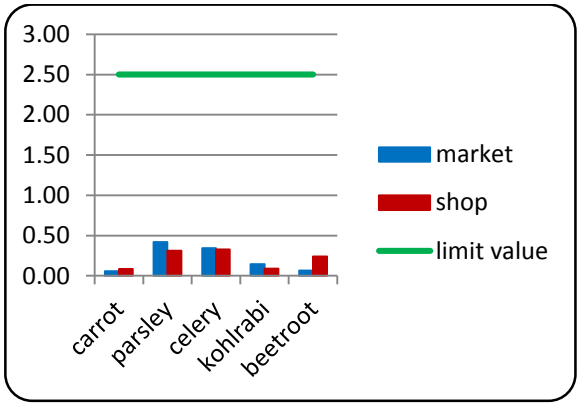

Figure 5 Evaluation of nickel content in selected types of vegetables (mg.kg^{-1}), median, n = 6 and comparison with the maximum permissible limits set by *Codex Alimentarius* of SR.

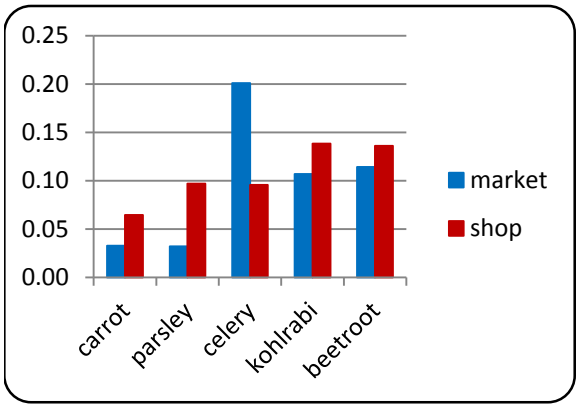

Figure 6 Evaluation of cobalt content in selected types of vegetables (mg.kg^{-1}), median, n = 6.

The most significant difference between samples of obtained results of cobalt content in vegetable from market and from local market was found in celery and parsley and the lowest significance of difference was in beetroot. **Guerra et al., (2012)** obtained in parsley 0.47 mg.kg^{-1} of cobalt content.

According to obtained results of cadmium content can be defined in the order of vegetable species from markets of local growers: celery >parsley >beetroot >kohlrabi >carrot.

According to obtained results of cadmium content can be defined in the order of vegetable species from commercial local market: beetroot >celery >kohlrabi >carrot >parsley.

The highest cadmium content in selected types of vegetables was detected in beetroot from local market (0.05 ±0.02 mg.kg^{-1}) and the lowest in parsley (0.003 ±0.01 mg.kg^{-1}). **Guerra et al., (2012)** identified high cadmium content in beetroot (0.09 mg. kg^{-1}). The cadmium content in all types of vegetable was not exceeded the highest permissible limit set by *Codex Alimentarius* of SR.

According to obtained results of lead content can be defined in the order of vegetable species from markets of local growers: celery >carrot >beetroot >parsley >kohlrabi.

According to obtained results of lead content can be defined in the order of vegetable species from commercial local market: celery >beetroot >parsley >kohlrabi >carrot.

The lowest lead content was in carrot from local shop network (0.002 ±0.01 mg.kg^{-1}). **Mahmood and Malik**

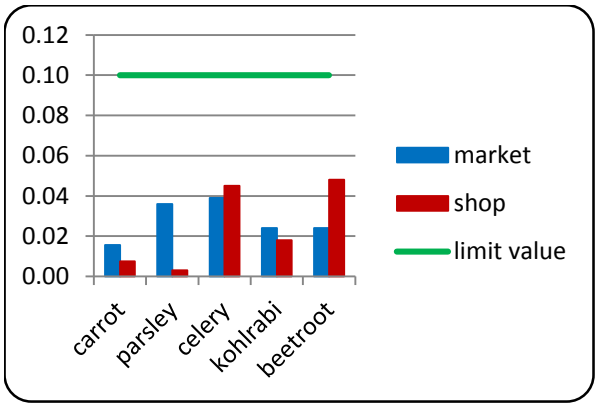

Figure 7 Evaluation of cadmium content in selected types of vegetables (mg.kg^{-1}), median, n = 6 and comparison with the maximum permissible limits set by *Codex Alimentarius* of SR.

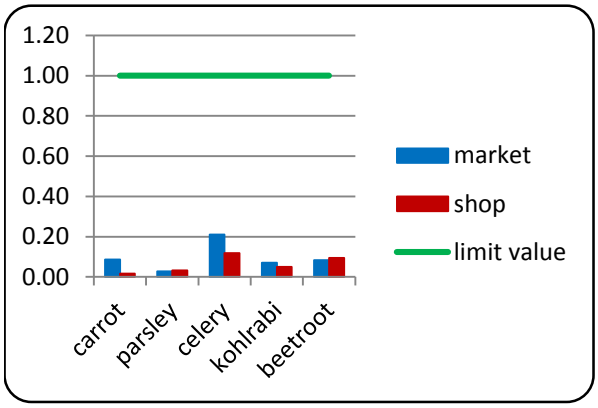

Figure 8 Evaluation of lead content in selected types of vegetables (mg.kg^{-1}), median, n = 6 and comparison with the maximum permissible limits set by *Codex Alimentarius* of SR.

(2014) identified in carrot 0.29 mg.kg^{-1} of lead content. The highest lead content was detected in celery from market (0.21 ±0.02 mg.kg^{-1}).

By evaluation of lead content in all types of vegetable from local shop network can be concluded that highest lead content was detected in celery.

Between samples of obtained results of heavy metals content in vegetable from market and from local shop network was not found significant difference.

CONCLUSION

This work focuses to the contents of risk metals in selected types of vegetable. Vegetable accumulates these elements mainly by roots from polluted water and soil as well as above-ground parts that are exposed to air pollution. A lot of heavy metals are accumulated mostly in the root system of plants.

We have analyzed samples of a root of brassica vegetable, in which it has been measured the content of these risk metals – copper, iron, cadmium, lead, manganese, chromium, zinc, cobalt and nickel.

Samples of carrot from markets of local growers contained the most of zinc content (2.098 ±0.03 mg.kg^{-1}) compared with the other elements in this vegetable, and obtained results shows that the highest zinc content from local market was in celery (4.24 ±0.03 mg.kg^{-1}). From all these evaluated risk elements was detected the highest iron content in parsley (25.45 ±0.53 mg.kg^{-1}), and also in celery (8.69 ±0.02 mg.kg^{-1}) and kohlrabi (8.65 ±0.03 mg.kg^{-1}).

The contents of risk metals were compared with the maximum limit values set by *Codex Alimentarius* of Slovak Republic. By evaluation of obtained results can be concluded that the content no one of analyzed elements does not exceed maximum permissible limits. The obtained results indicated that consumption of these types of vegetable do not pose any risk of heavy metal danger to consumer.

The findings of obtained and evaluated results shows that mostly higher contents of risk metals were in samples of vegetable bought from markets of local growers than in samples from local market.

REFERENCES

Beneš, S. 1994. *Obsahy a bilance prvků ve sférach životního prostředí* (*Content and balance of elements in the spheres of environment*). I. II. Část. Praha : Ministerstvo zemědelství České Republiky (I. II. Part. Prague Ministry of agriculture of Czech Republic).

Bui, T. L., Do-Hong, L. CH., Dao, T., Hoang, T. C. 2016. Cooper toxicity and the influence of water quality of Dongnai River and Mekong River waters on copper bioavailability and toxicity to three tropical species. *Chemosphere*, vol. 144. p. 872-878. https://doi.org/10.1016/j.chemosphere.2015.09.058 PMid:26421627

Burges, A., Epelde, L., Garbisu, C. 2015. Impact of repeated single-metal and multimetal pollution events on soil quality. *Chemosphere*, vol. 120, p. 8-15. https://doi.org/10.1016/j.chemosphere.2014.05.037 PMid:25462295

Codex Alimentarius of Slovak Republic 2006 Decree no. 18558/2006-SL (*Potravinový kódex SR 2006 Výnoxs č.18558/2006-SL*) Available at: http://www.svps.sk/legislativa/legislativa_kodex.asp.

Dias, J. C. S. 2012. Nutritional Quality and Health Benefits of Vegetables: A Review. *Food and Nutrition Sciences*, vol. 3, no. 10, p. 1354-1374. https://doi.org/10.4236/fns.2012.310179

Dias, J. C. S. 2014. Nutritional and Health Benefits of Carrots and Their Seed Extracts. *Food and Nutrition Sciences*, vol. 5, no. 22, p. 2147-2156. https://doi.org/10.4236/fns.2014.522227

Douay, F., Pelfrêne, A., Planque, J., Fourrier, H., Richard, A., Roussel, H., Girondelot, B. 2013. Assessment of potential health risk for inhabitants living near a former lead smelter. Part 1: metal concentrations in soils, agricultural crops, and home grown vegetables. *Environmental Monitoring and Assessment*, 2013. vol. 185, no. 5, p. 3665-3680. https://doi.org/10.1007/s10661-012-2818-3 PMid:22886627

Farzaei, M. H., Abbasabadi, Z., Ardekani, M. R. S., Rahimi, R. 2013. Parsley: a review of ethnopharmacology, phytochemistry and biological activities. *Journal of Traditional Chinese Medicine*, vol. 33, no. 6, 2013, p. 815-826. https://doi.org/10.1016/S0254-6272(14)60018-2

Guerra, F., Trevizam, A. R., Muraoka, T., Marcante, N. C., Brazaca, S. G. C. 2012. Heavy metals in vegetables and potential risk for human health. *Scientia Agricola*, vol. 69, no. 1, p. 54-60. https://doi.org/10.1590/s0103-90162012000100008

Gupta, D. K., Huang, H. G., Corpas, F. J. 2013. Lead tolerance in plants: strategies for phytoremediation. *Environmental Science and Pollution Research*, vol. 20, no. 4, p. 2150-2161. https://doi.org/10.1007/s11356-013-1485-4 PMid:23338995

Hegedűsová, A., Mezeyová, I., Hegedűs, O., Musilová, J., Paulen, O. 2015. Selenium content increasing in the seeds of garden pea after foliar biofortification. *Potravinarstvo*, vol. 9, no 1, p. 435-441. https://doi.org/10.5219/559

Jiang, N., Chang, X., Zheng, H., He, Q., Hu, Z. 2006. Selective solid-phase extraction of nickel(II) using a surface-imprinted silica gel sorbent. *Analytica Chimica Acta*, vol. 577, no. 2, p. 225-231. https://doi.org/10.1016/j.aca.2006.06.049 PMid:17723676

Kočík, K, Ducsay, L. 1995. *Active biomonitoring of risk elements in the soil - plant. Contaminants in agriculture*. Nitra : SAU, 1997, p. 29-31.

Kopec, K. 2010. *Zelenina ve výživě člověka*. Praha : Grada Publishing, 168 s. ISBN 978-80-247-2845-2

Mahmood, A., Malik, R. N. 2014. Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian Journal of Chemistry*, vol. 7, no. 1, p. 91-99. https://doi.org/10.1016/j.arabjc.2013.07.002

Modaresi, M., Ghalamkari, G., Jalalizand A. 2012. The Effect of Celery (*Apium graveolens*) Extract on the Reproductive Hormones in Male Mice. *APCBEE Procedia*, vol. 4, p. 99-104. https://doi.org/10.1016/j.apcbee.2012.11.017

Ninfali P., Angelino D. 2013. Nutritional and functional potential of Beta vulgaris cicla and rubra. *Fitoterapia*, vol. 89, p. 188-199. https://doi.org/10.1016/j.fitote.2013.06.004 PMid:23751216

Roman, A., Popiela, E. 2011. Studies of chosen toxic elements concentration in multiflower bee honey. *Potravinarstvo*, vol. 5, no. 2, p. 67-69. https://doi.org/10.5219/134

Shaheen, N., Irfan, N. M., Khan, I. N., Islam, M. S., Ahmed, M. K. 2016. Presence of heavy metals in fruits and vegetables: Health risk implications in Bangladesh. *Chemosphere*, vol. 152, p. 431-438. https://doi.org/10.1016/j.chemosphere.2016.02.060 PMid:27003365

Spears, J. W. 1984. Nickel as a “newer trace element” in the nutrition of domestic animals. *Journal of Animal Sciences*, vol. 59, no. 3, p. 823-835. https://doi.org/10.2527/jas1984.593823x PMid:6386782

Uher, A. 2011. *Zelenina vo výžive a jej rizikové factory (Vegetable in nutrition and their risk factors)*. 2. ed. Nitra : Slovenská poľnohospodárska univerzita, 65 p. ISBN 978-80-552-0518-2.

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IMPACT OF THE SYMBIVIT PREPARATION ON QUANTITATIVE AND QUALITATIVE INDICATORS OF TOMATO (*Lycopersicon esculentum* Mill.)

Alena Andrejiová, Ivana Mezeyová, Alžbeta Hegedűsová

ABSTRACT

The aim of the work was the verification of Symbivit preparation containing mycorrhiza fungi from the genus *Glomus* in the cultivation of tomato (varieties Uno Rosso F1 and Brixol F1). The impact of mycorrhiza on the growth parameters of seedlings (overground mass, the mass of the root system, stem diameter, plant height), total fruits yield and quality by the spectrophotometric determination of the total carotenoids in fresh fruits was evaluated. According to the statistical evaluation by the method of multifactorial analysis of variance there was found significant effect of the preparation on all evaluated growth parameters. The increase of the overground part in case of variety Uno Rosso F1 was about 62.43%, in Brixol F1 it was about 75.55% in comparison with control variant. Similarly, the increase in the weight of the root system was found for variety Uno Rosso F1 about 31.38% and for Brixol F1 about 35.98%, as well as in plants height of variety Uno Rosso F1 about 14.06% and of Brixol F1 about 31.84 % when compared to control. Application of Symbivit preparation in tomato cultivation had positive effect on total yields of tomato fruits of both selected varieties. Effect of application of Symbivit preparation on the carotenoids content in tomato fresh fruits was not prove to be statistically significant, as well as it was not found significant difference in the content of total carotenoids when evaluating the influence of the variety.

Keywords: tomato; mycorrhiza; growth parameters; yield; carotenoids

INTRODUCTION

Global production of tomato reaches at the present historically the highest value exceeding 163 million tons per year, which makes tomato (*Lycopersicon esculentum* Mill.) the most grown and industrially processed vegetable species in the world. In Slovakia in terms of production it is the second most cultivated vegetables **(Valšíková et al., 2015)**. Besides the taste this species is important for its valuable compounds, particularly carotenoids, concretely lycopene and β-carotene, which are important protective substances with antioxidant effects **(Petříková et al., 2012; García-Valverde et al., 2013; Perveen et al., 2015)**. The most important carotenoid in tomatoes is lycopene **(Yoshida et al., 2011; Mendelová et al., 2012; Mendelová et al., 2013)**.

To increase the production of vegetables in field conditions and for the ecologisation of the production the research is carried out by the use of preparations on the base of mycorrhizal fungi. Arbuscular mycorrhiza fungi have a role in horticulture as a sustainable, biological protection against pathogens, stress from salinity of the soil and heavy metals. For the host plant by the mycelium, which has bigger digging depth than the root system of the plant, they further improve the supply of water and nutrients, especially of phosphorus, which is immovable and often in hard acceptable form to plants, and of nitrogen. For it the plant supplies of 20% of bounded carbon to fungus. Exchange of nutrients is made through the symbiotic structures inside the root cells of the plant called arbusculers **(Parniske, 2008, Gutjahr and Parniske, 2013)**. The main reason for the use of preparations based on mycorrhizal fungi as it was shown in many researches is promoting of the seedlings growth, increasing of the yields, increasing of the resistance to environmental stress factors, the uniformity the crop, earlier and richer setting of inflorescence. Impact of the arbuscular mycorrhiza was also tested on qualitative parameters of tomato plants by **(Nedorost and Pokluda, 2012)**, where they found the positive effect of the inoculation that resulted in the increased content of the vitamin C. One of the preparations is Symbivit that is suitable for tomato, pepper and other fruit vegetables, beans, peas, allium vegetables, fruit bushes and trees. It can be used for field conditions growing as well as in greenhouses and plastic greenhouses. It is not suitable for brassica plants and for *chenopodiaceae* family. Composition of granule formulation Symbivit consists from clay carriers, from the natural ingredient that supports mycorrhiza - extracts of marine organisms, keratin, and milled minerals and from six species of mycorrhizal fungi. They are: *Glomus intraradices* BEG14 *Glomus mosseae* BEG95, *Glomus. etunicatum* BEG92, *Glomus claroideum* BEG96, *Glomus microaggregatum* BEG56, *Glomus geosporum* BEG199 **(Vojtíšková et al., 2011)**.

The aim of the work was to verify the Symbivit product for cultivation of tomato, namely the impact of mycorrhiza on growth, yield and quality of selected tomato varieties. For this purpose, the experiment was conducted, which was consisted from the evaluation of plants under laboratory conditions at the stage of pre-cultivated seedlings and at the same time the small plot field trial was established. In the experimental part of the work the effect of the used preparation on growth parameters of seedlings

was evaluated, on the achieved yield and fruit quality by the determination of the total carotenoids.

MATERIAL AND METHODOLOGY

The experiment was established in a Botanical garden of Slovak University of Agriculture (further BZ SUA) in 2015. Sowing was realized in the term of 17th March, 2015 in to seeding trays followed by spacing out of the seedlings on 31st March, 2015 at the growth phase of the first true leaf in containers with volume of 0.2 L by the use of a complete growing medium on the base of peat. Control variant i.e. 30 pieces of seedlings of Brixol F1 variety and 30 pieces of seedlings of Uno Rosso F1 varieties was used without Symbivit application. The same number of the plants was grown with the using of growing medium enriched by Symbivit preparation at a dose of 175 g of product per 14 L of substrate. The effect of used preparation on growth parameters of the seedlings was evaluated in a growth phase of 10 true leaves compared to control (pre-cultivated seedlings without the use of the preparation).

The experiment continued in field conditions. Territory of interest belongs to the warm climate area which is suitable for growing of fruit vegetables. On the basis of agrochemical soil analysis and recommended standards for the production of tomatoes there was two weeks before planting applied 206 kg of potassium sulphate (K_2SO_4).ha^{-1} and LAD (60% of N normative), what amounted 294 kg LAD.ha^{-1}. The remaining 40% of N (197 kg LAD.ha^{-1}) was applied in the early July in the phase of

Figure 1 Brixol F1 **(Andrejiová, 2015)**.

Figure 2 Uno Rosso F1 **(Andrejiová, 2015)**.

full ripening of the plant. Planting of the plants in field trial was conducted on 19th May, 2015 in single spacing 0.7 m x 0.3 m, with two monitored variants: control (planting of the seedlings pre-cultivated without the use of the Symbivit preparation) and variant with Symbivit (application of the Symbivit preparation to the substrate for seedling cultivation), each in three replications. Fruit harvest was carried out in full botanical maturity gradually in five terms from 1st July to 5th October, 2015.

Characteristics of Tomato Varieties

Brixol F1 – mid-early determinate variety suitable for combined harvesting. It has a compact moderate growth. The fruits are elongated, resistant to cracking in more frequent rainfall and irrigation. Due to the high content of lycopene they are quickly coloured in full red colour. The variety is very fertile when grown under irrigation and in warm climate (Figure 1).

Uno Rosso F1 – mid-early determinate variety, suitable for mechanized harvesting. It has strong growth with high fertility. The fruits are small, slightly elongated, they weighs 60 – 70 grams and are resistant to cracking in more frequent rainfall and irrigation. The storage time is at least 20 days (Figure 2).

Determination of the growth parameters of the seedlings

In evaluation of the seedlings the plant height, stem diameter, weight of overground part and root system in fresh mass were evaluated. 15 plants within each variant and variety were taken in account. Plant height was measured from the root collar to growth top of the plant. Plant diameter was measured 10 mm above the root collar. The weight of the overground part and root system in fresh matter was evaluated individually by the abolition of the root in the place of root collar.

Determination of total carotenoids

Carotenoids were estimated by spectrophotometric measurement of substances absorbance in petroleum ether extract on spectrophotometer PHARO 100 at 445 nm wavelengths. As a dissolution reagent, there was used acetone **(Hegedűsová et al., 2007)**.

Statistical analysis

The analysis of variance (ANOVA), the multifactor analysis of variance and the multiple Range test were done using the Statgraphics XVII (StatPoint Inc. USA). Significant differences among means were tested (Tukey HSD test, $p < 0.05$).

RESULTS AND DISCUSSION

A. Evaluation of the seedling growth parameters

The weight of the overground parts

Application of the Symbivit preparation had a positive effect on the weight of the plants, which led to weight increasing in the variety Uno Rosso F1 about 62.43% and in Brixol F1 variety about 75.55% compared to the control treatment (Table 1, Figure 3) according to evaluating of the weight of overground part of the seedlings. **Hernádi et al., (2012)** studded the effect of the Symbivit on peppers and similar as in our research they found that after application of Symbivit the significant increasing in weight of overground parts was occurred (19.32 g) comparing to control (13.22 g), which means an increase about 46.14%. **Başak et al., (2011)** examined the effect of endomycorrhizal preparation on tomato seedlings with the similar composition as Symbivit. They noticed a significant impact of the preparation on the weight of overground part, which was in variety Aspendos after preparation using 2.22 g in comparison to control 0.81 g, which represents an increase about 174%. The weight of overground mass was in case of variety Donna after preparation use 1.95 g versus 0.69 g in control. This represents an increase about 182.61%.

The weight of the root system

Considering the effects of application of a Symbivit preparation on the weight of the root system of pre-cultivated tomato seedlings it can be said that the increase in weight was noticed compared to the control treatment in case of Uno Rosso F1 variety about 1.61 g, what represent an increase about 31.38% and in Brixol F1 variety about 1.54 g, what represent an increase about 35.98%. **Oseni et al., (2010)** also found a statistically significant increase in the weight of the root system of tomato seedlings 14, 28 and 42 days after application of mycorrhizal product Biocult containing mycorrhiza fungus *Glomus etunicatum* a *Glomus intraradices.* **Salvioli et al., (2008)** examined the effect of mycorrhizal fungi *Glomus mosseae* BEG12 on tomato variety Pearson. For tomatoes with application of *Glomus mosseae* there was demonstrated a statistically significant increase in weight of the overground parts as well as in weight of the root system.

Diameter of the stem

Diameter of the stem in case of monitored tomato seedling plants of a variety Uno Rosso F1 after application of Symbivit preparation reached in average 4.67 mm (Table1). In comparison to control treatment (4.08 mm) an increase about 12.63% was observed. In the variety Brixol F1 application of Symbivit preparation had no statistically significant effect on stem diameter compared to the control treatment.

Plant height

Application of Symbivit preparation had significant effect on the plant high of tomato seedlings (Figure 3). It was noticed an increase in average of 34.16 mm for variety Uno Rosso F1, what means rising about 14.26% and in variety Brixol F1 about 67.92 mm, consequently an increase about 31.84% compared to control. Similar research conducted on pepper seedlings by **Vojtíšková et al., (2011)** showed a positive impact of Symbivit preparation on plant height in variety Slávy F1.

B. Yield

When evaluating the quantitative parameter, obtained yield of fresh fruit, it can be concluded that the applied preparation had a positive impact on achieving yields (Figure 4). In variety Uno Rosso F1 the yield was after application of Symbivit an average 216.87 $t.ha^{-1}$. Compared to the control variant an increase about 19.07% was observed.

Table 1: Evaluation of seedlings growth parameters after application of Symbivit in the frame of each variety*.

Variety/variant	Uno Rosso F1		Brixol F1	
Estimated parameter	**Control**	**Symbivit**	**Control**	**Symbivit**
Weight of overground part (g)	8.73 ±1.28 a	14.18 ±2.11 b	6.79 ±0.74 A	11.92 ±1.79 B
Weight of root system (g)	5.13 ±0.73 a	6.74 ±1.29 b	4.28 ±0.35 A	5.82 ±0.53 B
Diameter of the stem (mm)	4.08 ±0.19 a	4.67 ±0.42 b	4.46 ±0.48 A	4.96 ±0.59 A
Plant height (mm)	242.92 ±25.29 a	277.08 ±30.17 b	213.33 ±19.72 A	281.25 ±28.95 B

Note: *Means ± standard deviation. Different letters in rows denote significantly different, n = 15, p <0.05.

Figure 3 A – Symbivit, B – control.

Brixol F1 variety is characterized by high fertility. In a variant Symbivit the yield reached 234.52 t.ha^{-1}, which meant an increase compared to control only about 3.54% (8.33 t.ha^{-1}). Following the statistical analysis by the methods of analysis of variance, the impact of variant as well as of variety on crop yields was not statistically significant (Figure 4). **Hernádi and Sasvári (2012)** studied the effect of the Symbivit preparation in the cultivation of pepper where they found a significant increase in fruit yield after application of Symbivit from 3189.56 g/100 plants (control) to 5251.27 g/100 plants, what represented an increase about 39.26%.

Helyes et al., (2015) in his work shows a positive effect of mycorrhiza on yield of tomato fruit in Uno Rosso F1 variety which was recorded in 2013. However, in previous experimental year in 2012 the decline has been noticed, except of Triple Red variety, in case of other varieties Heinz, Uno Rosso and Strombolino. **Damaiyanti et al., (2015)** also found a positive effect of mycorrhizal fungi on fertility of tomato variety Betavila F1. In the variants with mycorrhizal fungi in a dose of 5 g, 10 g, 20 g, the yield (kg/plant) was increased about 21.60%, 24.07% and 35.80% comparing to the control.

C. Total carotenoids content in fresh fruit

Total carotenoids content in fresh tomato fruits after application of Symbivit in variety Uno Rosso F1 was 9.69 mg.100 g^{-1} of fresh matter. The control sample reached 7.94 mg.100 g^{-1} fresh matter. The content of carotenoids after application of Symbivit preparation was increased in Uno Rosso F1 about 1.75 mg.100 g^{-1}, what means an increase about 22.04% compared the control. In contrary for Brixol F1 variety the content of carotenoids in the fresh matter after Symbivit application was 10.36 mg.100 g^{-1}, and for the control the value reached 11.10 mg.100 g^{-1}. That means decline about 6.67% comparing to control. The effect of application of a Symbivit preparation on the content of carotenoids in tomato fresh fruits was not proved to be statistically significant, as well as between the varieties was not found any significant difference in the content of total carotenoids (Figure 5). **Helyes et al., (2015)** also found miscellaneous results of mycorrhiza impact on carotenoid content depending on the variety.

In his work reports that under the mycorrhiza influence the content of lycopene, which is a significant part of carotenoids in tomato fruit, was increased in case of Heinz and Strombolino varieties, which contained

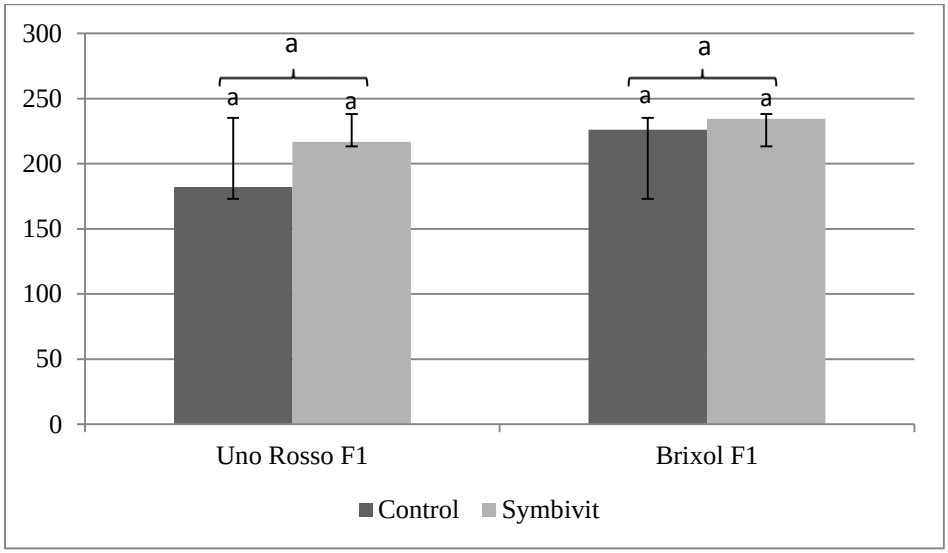

Figure 4: Effect of application of Symbivit preparation on the yield of selected tomato varieties (t.ha^{-1}) ± standard deviation. Note: Columns marked with different letters are significantly different at the level of p <0.05. The same letters above the varieties mean that between them is not a significant difference.

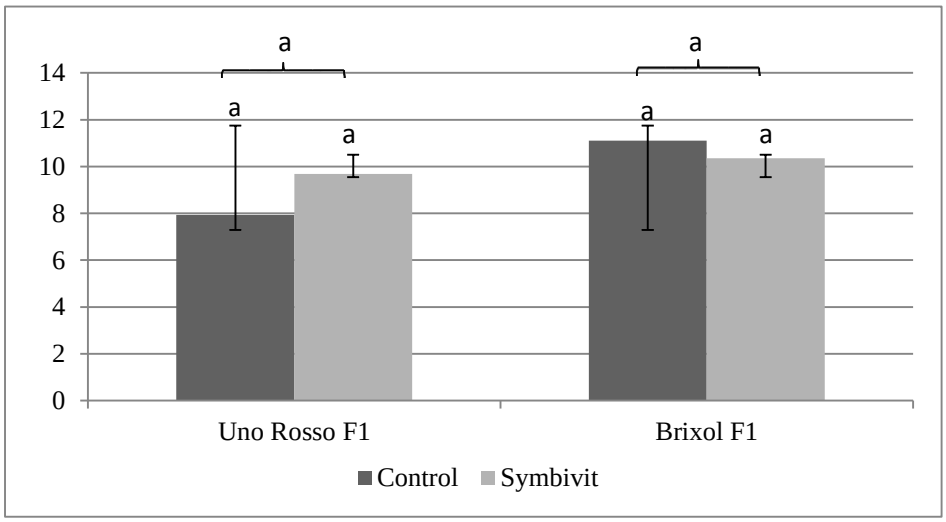

Figure 5 Effect of application of Symbivit preparation on the total carotenoid content in fruits of selected tomato varieties (mg.100 g^{-1} fresh matter) ± standard deviation. Note: Columns marked with same letters are not significantly different at the level of p <0.05. The same letter above the varieties mean that between them is not a significant difference.

9.97 mg.100 g^{-1} and Heinz and 12.22 mg.100 g^{-1} of lycopene compared to control 8.56 mg.100 g^{-1} and 10.86 mg.100 g^{-1}, and it decreased in case of Triple Red and Uno Rosso F1 varieties, containing 10.89 mg.100 g^{-1} and 9.21 mg.100 g^{-1} vs. control 12.46 mg.100 g^{-1} and 9.96 mg.100 g^{-1}.

Hart et al., (2014) found a statistically significant increase in the total carotenoids in the tomato fruits after application of mycorrhizal fungi *Glomus etunicatum* a *Glomus intraradices*.

CONCLUSION

The effect of mycorrhizal granular Symbivit preparation on observed growth parameters in the phase of seedling in both varieties Uno Rosso F1 and Brixol F1 was positive. Based on the statistical evaluation by the method of multifactorial analysis of variance the effect of the preparation on all evaluated parameters was shown to be very significant. Furthermore, it can be concluded that application of the Symbivit preparation in tomato cultivation had positive impact on achieved yields of both observed varieties. However, in assessing of the fruit quality by determining of the total carotenoids it can be said that the choosing of the variety has proved to be a limiting factor in the use of estimated preparation. Based on the statistical evaluation the effect of the Symbivit preparation was not prove to be statistically significant factor of increasing the fertility and total carotenoids in tomato fruit. For more complex results, there is recommendation to verify Symbivit preparation in future in cultivation of a wider range of tomato varieties as well as its use in the production of other types of fruit vegetables.

REFERENCES

Başak, H., Demđr, K., Kasim, R., Okay, F. Y. 2011. The effect of endo-mycorrhiza (VAM) treatment on growth of tomato seedling grown under saline conditions. *African Journal of Agricultural Research*, vol. 6, no. 11, p. 2532-2538.

Damaiyanti, D. R. R. 2015. Effects of arbuscular mycorrhiza inoculation on growth and yield of tomato (*Lycopersicum esculentum* Mill.) under salinity stress. *Journal of degraded and mining lands managemenet*, vol. 3, no 1, p. 447-452.

García-Valverde, V., Navarro-González, I., García-Alonso, J., Periago, M. J. 2013. Antioxidant Bioactive Compounds in Selected Industrial Processing and Fresh Consumption Tomato Cultivars. *Food and Bioprocess Technology*, vol. 6, no. 2, p 391-402. https://doi.org/10.1007/s11947-011-0687-3

Gutjahr, C., Parniske, M. 2013. Cell and Developmental Biology of Arbuscular Mycorrhiza Symbiosis. *Annual reviews*, vol. 29, p.593-617.

Hart, M., Ehret, D. L., Krumbein, A., Leung, C., Murch, S., Turi, C., Franken, P. 2014. Inoculation with arbuscular mycorrhizal fungi improves the nutritional value of tomatoes. *Mycorrhiza*, vol. 25, no. 5, p. 359-376. https://doi.org/10.1007/s00572-014-0617-0 PMid:25391485

Hegedüsová, A., Musilová, J., Jomová, K., Hegedüs, O., Bystrická, J. 2007. *Laboratórne experimenty z organickej chémie a biochémie pre špecializáciu Chémia životného prostredia* (Laboratory experiments of organic chemistry and biochemistry for study specialization Chemistry of environment). UKF : Nitra, 2007, pp.103. ISBN 978-80-8094-211-3.

Helyes, L., Pék, Z., Daood, H. G., Posta, K. 2015. Effect of Mycorrhizae on Main Antioxidant Content of Processing Tomato. *ISHS Acta Horticulturae 1081: XIII International Symposium on Processing Tomato*, p. 105-110. https://doi.org/10.17660/actahortic.2015.1081.10

Hernádi, I., Sasvári, Z. 2012. Arbuscular Mycorrhizal Inoculant Increases Yield of Spice Pepper and Affects the Indigenous Fungal Community in the Field. *Hort. Science*, vol. 47, no. 5, p. 603-606.

Mendelová, A., Andrejiová, A., Líšková, M., Kozelová, D., Mareček, J. 2012. Analysis of carotenoids and lycopene in tomato (*Lycopersicon esculentum* Mill.) and their retention in tomato juice. *Potravinarstvo*, vol. 6, no. 2, p. 36-38. https://doi.org/10.5219/195

Mendelová, A., Mendel, Ľ., Fikselová, M., Czako, P. 2013. Effect of drying temperature on lycopene content of processed tomatoes. *Potravinarstvo*, vol. 7, no. 1, p. 141-145. https://doi.org/10.5219/300

Nedorost, L., Pokluda, R. 2012. Effects of Arbuscular Mycorrhizal Fungi on Tomato Yield and Nutrient Uptake under Differentfertilization Levels. *Acta universitatis agriculturae et silviculturae mendelianae brunensis*, vol. 60, no. 8, p. 181-186. https://doi.org/10.11118/actaun201260080181

Oseni, T., Shongwe, N. S., Masarirambi, M. T. 2010. Effect of Arbuscular Mycorrhiza (AM) Inoculation on the Performance of Tomato Nursery Seedlings in Vermiculite. *International Journal of Agriculture and Biology*, vol. 12, no. 5.

Parniske, M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology*, vol. 6, no. 10, p. 763-775. https://doi.org/10.1038/nrmicro1987 PMid:18794914

Perveen, A. R., Suleria, H. A. R., Anjum, F. M., Butt, M. S., Pasha, I., Ahmad, S. 2015. Tomato (*Solanum lycopersicum*) carotenoids & lycopenes chemistry; Metabolism. Absorption. Nutrition and Allied health claims - A comprehensive review. *Critical Reviews in Food Science and Nutrition*, vol. 55, no. 7, p. 919-929. https://doi.org/10.1080/10408398.2012.657809 PMid:24915375

Petříková, K., Hlušek, J. et al. 2012. *Zelenina (Vegetable)*. Praha : Profi Press, 191 p. ISBN 978-80-86726-50-2.

Salvioli, A., Novero, M., Lacourt, I., Bonfante, P. 2008. The impact of mycorrhizal symbiosis on tomato fruit quality. [online] 2016-20-19 [2016-20-19] Available at: http://orgprints.org/12844/1/12844.pdf.

Valšíková, M., Bellan, Ľ., Rehuš, M. 2015. Produkcia rajčiakov v Slovenskej republike (Lycopersicum production in Slovak republic), *Zahradnictví*, vol. 12, p. 28-29.

Vojtíšková, J., Nedorast, Ľ. Pokluda, R. 2011. Využití mykorhizní symbiózy při předpěstování papriky (Use of mycorrhiza in pre-cultivated peppers). In *Salaš,* P. (ed.) *Rostliny v podmínkách měnícího se klimatu, (Plants in a changing climate)*, Lednice 20.-21. October 2011, Czech Republic. *Úroda* (scientific appendix) p. 662-667. ISSN 0139-6013. Available at: http://www.cbks.cz/Rostliny2011/prispevky/VojtiskovaNedorostPokluda.pdf

Yoshida, K., Yokoyama, H., Oteki, T., Matsumoto, G., Aizawa, K., Inakuma, T. 2011. Evaluation of the effect of dietary lycopene, the main carotenoid in tomato (*Lycopersicon esculentum*), on the in vivo renal reducing ability by a radiofrequency electron paramagnetic resonance method. *Journal of Agricultural and Food Chemistry*, vol. 59, no. 7, p. 2966-2971. https://doi.org/10.1021/jf1041883 PMid:21381743

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CHANGES OF VITAMIN C CONTENT IN CELERY AND PARSLEY HERB AFTER PROCESSING

Magdaléna Valšíková, Ivana Mezeyová, Marián Rehuš, Mirolav Šlosár

ABSTRACT

Humans and other primates have lost the ability to synthesize vitamin C and therefore the only source is diet. Vitamin C or ascorbic acid has labile nature, it is removed or destroyed in specific degree immediately after harvest, but storage and post – harvest processing also contribute to its degradation. The aim of work was to determine the vitamin C content in the herb of selected celery and parsley varieties in dependence on chosen postharvest processing and to compare it with fresh herb. There were chosen five bulb forms varieties of celery (*Apium graveolens*) – Makara, Ilonaa, Hegy Köi, Talar and Diamant. In case of parsley (*Petroselinum crispum*) there were evaluated one variety of curly parsley, one variety of herb parsley – Petra, and five varieties of root parsley – Lenka, Eagle, Ginate D´Italia, Titana and Arat. Every variety was harvested in three terms, followed by vitamin C content estimation in fresh herb, after drying and after freezing. The content of vitamin C was estimated by HPLC method by the help of liquid chromatograph with UV detector. There was found the significant difference in content of vitamin C in parsley as well as in celery when comparing the fresh herb with herbs after post – harvest processes – drying (by air circulation in laboratory hall) and freezing. After processing of herbs in both observed species the vitamin C content decreased, in case of freezing it was about 65% (celery) and 61% (parsley), after drying about 86% (celery) and 82% (parsley) in comparison with fresh herb. The effect of processing played more important role in influencing of vitamin C content than variety in case of both selected species. For using of celery and parsley not only as culinary herb, but as a notable source of ascorbic acid it is the most important fresh herb intake.

Keywords: parsley; celery; ascorbic acid; freezing; drying

INTRODUCTION

Vitamin C, also known as ascorbic acid, L-ascorbic acid or L-ascorbate, is the most important vitamin for human nutrition that is supplied by fruits and vegetables. Actually, vitamin C is almost a generic name for all compounds that exhibit the same biologic activity as ascorbic acid **Stan et al. (2014)**. Although there are many functions of vitamin C, his role in health is discussed mostly in relation to its role as an antioxidant and its effects on cancer, blood pressure, immunity, drug metabolism and urinary excretion of hydroxyproline **Barrita et al. (2013)**. Vitamin C reinforced the immune system, supports digestion and stimulates appetite. It stimulates liver function and helps with the gout. It neutralizes harmful substances from cigarette smoke **Juríková et al. (2013)**. It can act as an anti-carcinogen and reduces the risk of cardiovascular diseases **Šlosár et al. (2008)**. Due to hypovitaminosis the fatigue starts and the resistance to infection decreases. Critical periods are particularly in the end of winter and spring period when the intake of ascorbic acid from natural sources is small **Keresteš (2011)**.

Humans and other primates have lost the ability to synthesize vitamin C as a result of a mutation in the gene coding for L-gulonolactone oxidase, thus vitamin C must be obtained through the diet **Carr et al. (1999)**. In addition, it is well known that AA content declines during storage, especially in thermally treated foods, with the consequent formation of non-enzymatic browning compounds, which serve as indicators of organoleptic quality loss of the product **Uddin et al. (2002)**. Amounts of nutrients are removed or destroyed by storage of raw materials or technological processing (drying, grinding) **Leahu et al. (2013)**. The lowest ascorbic acid value was found after convective drying, followed by vacuum drying and microwave drying. With convective drying, the appearance values of the dried parsley decreased when the drying period was extended **Akbudak et al. (2011)**. Vitamin losses are also caused by sunlight. Vegetables loses during a short staying in the sun even 50% of vitamin C. In the shadow, it loses only 15% during the same period **Jedlička (2012)**. Due to the labile nature of vitamin C, extraction procedures are designed to avoid the loss of vitamin **Odriozola et al. (2007)**.

Efficient sources of ascorbic acid are parsley (*Petroselinum crispum*) and celery (*Apium graveolens*), both from family Apiaceae, which are ranged between vegetables as well as culinary herbs, in dependence on variety, with wide spectrum of using. Besides vitamin C they are rich in other antioxidants, essential vitamins, elements like iron, calcium, potassium, phosphorus, magnesium, sulfur, crystal and also in volatile and fixid oils (lipids). In Apiaceae petroselinic acid is the major fatty acid **Shams et al. (2015)**. For their specific taste and smell, mainly due to volatile oils they are worldwide used as culinary herbs as well. Celery plays a role in prevention of cardiovascular disease, lowering blood glucose and serum lipid, decrease blood pressure and strengthener the heart. This herb has anti- bacterial, anti-fungal and anti-inflammatory effects. Also, a powerful antioxidant property has been attributed to compounds such asapigenin, apiein, vitamins A and C **Kooti et al. (2014)**.

Parsley with high iron content protects against to anaemia. Parsley has been reported to have possible medicinal attributes as an antioxidative, antimicrobial, anticoagulant, antihyperlipidemic and antihepatotoxic **Yanardag et al. (2003)**. It is a useful source of calcium. It has a diuretic effect; it is used in diseases like the kidneys, bladder infections, urinary and kidney stones, inflammation of the prostate and intestinal colic **Juríková et al. (2012)**. In Slovakia and Central Europe, it is primarily grown as root parsley, in lower extent as leaf parsley. Especially in case of leaf type there was bred several forms with variously divided and curly leafs **(Kóňa, 2006; Uher et al., 2009)**.

The aim of the work was to determine the vitamin C content in the herb of selected celery and parsley varieties in dependence on postharvest processing.

MATERIAL AND METHODOLOGY

The project was led on the land of the Slovak Agricultural University in the Department of Vegetables-Production. Celery was sowing in greenhouse, because of its requirements to warm conditions. Sowing was realised on 14th of February, 2013 and it was replanted in to rooting boxes on 21st of March, 2013, followed by planting out in to prepared soil on 7th of May, 2013. The plant spacing was 40 x 30 cm, 9 pieces in row, three rows for each observed variety. Parsley was sowing directly into the soil of 18th of April, 2013, in two rows for each variety.

There were used five bulb forms varieties of celery (Apium graveolens) – Makara, Ilonaa, Hegy Köi, Talar and Diamant. In case of parsley (Petroselinum crispum) there were evaluated one variety of curly parsley, one variety of herb parsley – Petra, and five varieties of root parsley – Lenkab, Eagle, Ginate D´Italia, Titana and Arat.
The trail consists from three variants:

- Analyses in fresh herb,
- Analyses in dried herb
- Analyses in frozen herb

In every variant, there were done three terms of harvest - a first one, second and third harvest. After harvesting of the plants vitamin C was determined in the laboratory of the Department of Vegetables - Production (Horticulture and Landscape Engineering Faculty). In case of fresh herb the analysis were done immediately after harvest, in dried herbs the sample was analysed one month after harvest in average (after drying by air circulation in laboratory hall of department) and in case of frozen herbs after three months of freezing. Terms of harvesting and terms of evaluations in laboratory after processing are figured in (Table 1). Both species were analysed at the same time.

The content of vitamin C estimation

HPLC method of vitamin C content estimation **Stan et al. (2014)** was used by the help of liquid chromatograph with UV detector, for separation was used RP C18 column, mobile phase was methanol : water (5:95, v/v), UV detection was adjusted to 258 nm (HPLC fy. VARIAN).

Statistical analyses

The obtained data were processed into tables in Microsoft Office Excel 2007. Then analysis of variance (ANOVA) were used by the help of the LSD test (significance level $\alpha = 0.05$) for statistical analyses in the program Statgraphic Centurion XVII (StatPointInc. USA).

RESULTS AND DISCUSSION

The value of vitamin C content in celery ranged in interval from 45.73 ±5.47 mg.100 g^{-1} (variety Diamant) to 56.79 ±8.72 mg.100 g^{-1} (variety Hegy Köi) in case of fresh herb, from 17.38 ±1.47 mg.100 g^{-1} (Talar) to 20.34 ±3.51 mg.100 g^{-1} (Hegy Köi) in case of frozen herb and from 5.82 ±1.46 mg·100 g^{-1} (Talar) to 9.06 ±1.85 mg.100 g^{-1} (Hegy Köi). Variety Hegy Köi reached the highest values in case of all evaluated thermal processes, but from the point of view of variety impact on vitamin C content, the varieties created almost homogenous group expect of statistical significant differences between couples of varieties Diamant - Hegy Köi and Hegy Köi – Talar according to used statistical analyses (Table 2).

Jedlička (2012) features the average values of vitamin C content in fresh herb of celery equal to 3.1 mg.100 g^{-1}. The values in case of all our varieties are higher. On the other hand, in comparison with the results of **Kóňa (2006)** there were found lower values, as he features average values of vitamin C in celery herbs 88.96 mg.100 g^{-1}, whereby the maximal values reached 142.00 mg.100 g^{-1}, minimal values 27.92 mg.100 g^{-1}. It corresponds with the results of **Kopec (2010)** with the average values for celery equal to 89.00 mg.100 g^{-1}.

Table 1 Dates of vitamin C content analyses in chosen varieties of celery and parsley.

Date of herb harvest	4th July 2013	19th August 2013	20th September 2013
Drying of herb until	26th July 2013	9th September 2013	14th October 2013
Freezing of herb until	24th October 2013	12th December 2013	8th January 2014

Figure 1 Parsley tops ready for drying.

Figure 2 Celery tops ready for drying.

When evaluating impact of thermal process on vitamin C content (Figure 3), there were high significant differences in case of all observed variants. In fresh herb the values were the highest, and then there was rapid decreasing in frozen herb variant, the lowest values were found in third variant – dried herb. It is in accordance with the results of **Roslon et al. (2010)**, where they tested leaves from two cultivar varieties of celery: 'Safir' – a leaf variety and 'Jabłkowy' – a celeriac variety. Fresh leaves contained on average 104.90 mg.100 g^{-1} of vitamin C. Freezing and drying caused decreasing content of vitamin C in investigated raw material.

The content of vitamin C in fresh herb of parsley reached values from 197.14 ±25.55 mg.100 g^{-1} (Eagle) to 170.43 ±19.05 mg.100 g^{-1} (Ginate D´Italia) as it is mentioned in Table 3. When comparing to **Kóňa (2006)**, our results are similar to average values of vitamin C content in parsley fresh herb, as he mentions 179.33 mg.100 g^{-1} in average, 340.00 mg.100 g^{-1} for maximal values and 150.00 mg.100 g^{-1} for minimal values. According to **Kopec (2010)** average vitamin C content was 136.90 mg.100 g^{-1}, which is lower than our results. In generally parsley herb belongs to sources with the highest content of vitamin C in vegetables. It is obvious as well from our results (Figure 3) in comparison of average values both observed crops, in all three variants. In **Matějková et al. (2010)** the vitamin C content was the highest by parsley leaves (1692 mg.kg^{-1}), parsley contained high vitamin C amounts also in root (515 mg.kg^{-1}). Chrysanthemum, mustard and mizuna also showed high ascorbic acid content according to **Kudrnáčová et al. (2015)** where they proved them to be another important source of vitamin C. Ascorbic acid content detected in their spring varieties ranged from 1890 to almost 3000 mg.kg^{-1}. The influence of variety on vitamin C content is figured in table 3, whereby there was found significant differences between varieties according to used statistical analyzes. Varieties Eagle and Arat reached significantly the highest values. Similarly, then in case of celery, there was found the significant difference in content of vitamin C in fresh herb and then in herbs after following post – harvest processes – drying and freezing. As it is represented in Figure 3, the highest values were found in case of fresh herb variant, following by frozen variant and the drying looks like the worse choice for vitamin C content preservation. It corresponds with results of **Leahu et al. (2013)** where the greatest values of acid ascorbic concentration were registered in the fresh parsley 347.60 ±6.2 mg.$100g^{-1}$. The greatest reduction in the content of ascorbic acid was in the dried dill samples (89.33%), followed by parsley (75.41%). Freezing of plants was decreasing the vitamin C content, but drying and higher temperatures have higher influence on the degradation of vitamin C in comparison to control. According to **Garba et al. (2014)** it was found that total vitamin C, was higher at lower drying temperature of 40 ˚C as expected. As the drying air temperature increases from 40 – 60 ˚C, decreased in ascorbic acid was observed. Similarly, there was observed the effect of temperature and storage period on the preservation of vitamin C, thiamine and riboflavin in leaves and whole plants (leaves with petioles and stems) of dill by **Lisiewska et al. (2003)**. In dill, the treatment of blanching affected a decrease in the level of vitamin C by 35 – 48%. The losses affected by blanching in the content of vitamin C in leafy vegetables ranged from 47% even to 80%. The content of vitamin C depends as well on the term of storage, even in refrigerator. **Howard et al. (1999)** showed a linear decrease in vitamin C content during refrigerated storage of vegetables.

After processing of herbs in both observed species the vitamin C content decreased in comparison with fresh herb, in case of freezing it was about 65% (celery) and 61% (parsley), after drying about 86% (celery) and 82% (parsley). Influence of the processing on AA content was observed in study of **Mareček et al. (2016)**, where they determined primary and secondary metabolites (including AA) in selected varieties of potatoes. The highest content was in the fresh tubers, whilst heat treatment reduces AA amount. Among the assessed cultivars the highest content showed tubers of variety Red Anna with purple skin (73.72 mg.kg^{-1}). Conversely, variety Picasso reached its lowest value (35.02 mg.kg^{-1}). The amount of vitamin C decreases due to storage conditions. From results presented

Table 2 Vitamin C content in celery (*Apium graveolens*) in dependence on variety and thermal processing*.

celery (*Apium graveolens*)			
Variety	**fresh herb**	**frozen herb**	**dried herb**
	mg.100 g^{-1}	mg.100 g^{-1}	mg.100 g^{-1}
Makar[ab]	54.24 ±8.30	18.39 ±3.04	7.11 ±1.68
Ilona[ab]	49.48 ±5.47	17.87 ±4.37	8.20 ±1.85
Hegy Köi[b]	56.79 ±8.72	20.34 ±3.51	9.06 ±1.85
Talar[a]	47.67 ±3.28	17.38 ±1.47	5.82 ±1.46
Diamant[a]	45.73±5.47	16.06 ±3.36	6.51 ±1.36

Note: *Means ± standard deviation. Different lowercase letters in column with names of varieties marks significant differences at p <0.05 by LSD in ANOVA (Statgraphic).

Table 3 Vitamin C content in parsley (*Petroselinum crispum*) in dependence on variety and thermal processing*.

parsley *(Petroselinum crispum)*			
Variety	**fresh herb**	**frozen herb**	**dried herb**
	mg.100 g^{-1}	mg.100 g^{-1}	mg.100 g^{-1}
Curly Parsley / CP[ab]	171.90 ±23.26	63.98 ±15.25	31.50 ±6.50
Petra[ab]	172.63 ±8.52	62.23 ±8.568	30.09 ±5.98
Lenka[bc]	183.84 ±16.31	76.63 ±12.87	34.18 ±8.03
Eagle[c]	197.14 ±25.55	80.82 ±15.69	36.63 ±9.04
Ginate D´Italia[a]	170.43 ±19.05	59.56 ±9.27	28.42 ±6.43
Titan[ab]	171.50 ±13.30	66.42 ±12.09	28.42 ±5.74
Arat[c]	194.91 ±16.06	81.94 ±13.36	36.54 ±7.79

Note: *Means± standard deviation. Different lowercase letters in column with names of varieties marks significant differences at p <0.05 by LSD in ANOVA (Statgraphic).

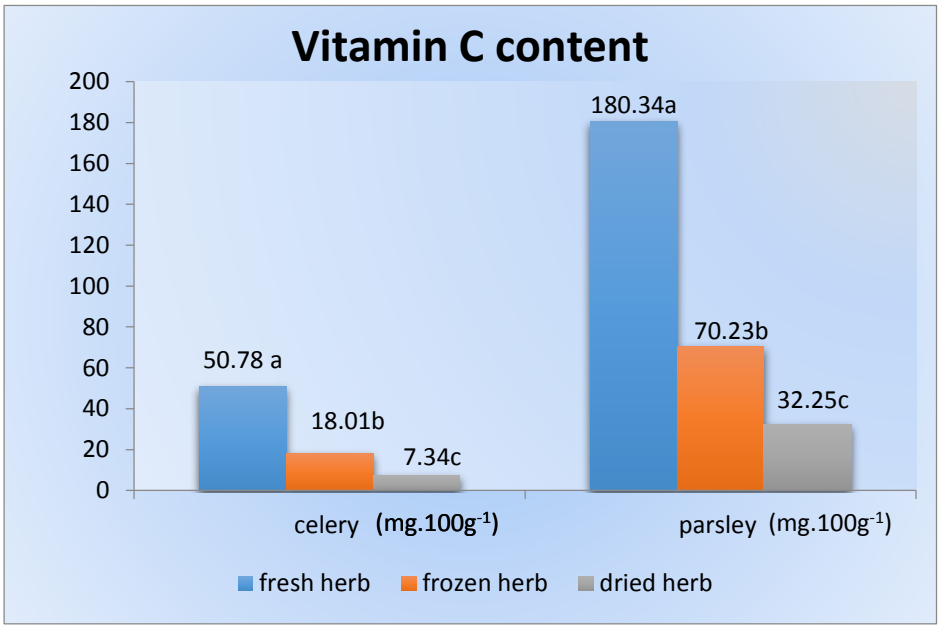

Figure 3 Graphical representation of statistical analysis of vitamin C content in celery and parsley herb depending on the observed variant. Note: *Different lowercase letters in graphs marks significant differences at p <0.05 by LSD in ANOVA (Statgraphic).

by **Matějková et al. (2014)** where they analysed variety, growing site, year and storage influence on the ascorbic acid content by selected vegetables there was noted statistically significant decrease of vitamin C after 30-days storage. The losses of vitamin C were highest in carrot (45%), followed by parsley (25%), garlic (24%) and onion (22%).

CONCLUSION

The submitted work was oriented to determination of vitamin C content in herb of selected celery and parsley varieties in dependence on postharvest processing. There were chosen 5 celery and 7 parsley varieties to evaluation. Every variety was harvested in three terms, followed by vitamin C content estimation in fresh herb, after drying and after freezing. Freezing of plants was decreasing the vitamin C content, but higher influence on the degradation of vitamin C has drying, which was confirmed by statistical analyses. In fresh herb the values were the highest 180.30 mg.100g^{-1} for parsley and 50.78 mg.100g^{-1} for celery, and then there was rapid decreasing in frozen herb variant to 70.23 mg.100g^{-1} (parsley) and to 18.01 mg.100g^{-1} (celery), followed by the lowest values in third variant – dried herb with the values 32.25 mg.100g^{-1} (parsley) and 7.34 mg.100g^{-1} (celery). The influence of variety on vitamin C content was confirmed only in some cases, the effect of processing plays significantly more important role in quantity of ascorbic acid in both selected species. Their fresh herb is notable source of tested antioxidant, very popular as culinary herb for its aromatic profile, but with additional value of medicinal effects.

REFERENCES

Akbudak, N., Akbudak, B. 2011. Effect of Vacuum, Microwave and Convective Drying on Selected Parsley Quality. *International Journal of Food Properties*, vol. 16, no. 1, p. 205-215. https://doi.org/10.1080/10942912.2010.535400

Barrita, J. L. S., Sánchez, M. S. S. 2013. Antioxidant Role of Ascorbic Acid and His Protective Effects on Chronic Diseases. In Morales-González, J. A. (ed.) *Biochemistry, Genetics and Molecular Biology*. Chapter 18 Antioxidant Role of Ascorbic Acid and His Protective Effects on Chronic Diseases. 512 p. ISBN 978-953-51-1123-8. https://doi.org/10.5772/52181

Carr, A. C., Frei, B. 1999. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *The American Journal of Clinical Nutrition*, vol. 69, no. 6, p. 1086-107. PMid:10357726

Garba, U., Kaur, S. 2014. Effect of drying and pretreatment on anthocyanins, flavenoids and ascorbic acid content of black carrot (*Daucus carrota* L.). *Journal of Global Biosciences*, vol. 3, no. 4, p. 772-777.

Howard, L. A., Wong, A. D., Perry, A. K., Klein, B. P. 1999. Beta-carotene and ascorbic acid retention in fresh and processed vegetables. *Journal of Food Science*, vol. 64, no. 5, p. 929-936. https://doi.org/10.1111/j.1365-2621.1999.tb15943.x

Jedlička, J. 2012. *Ovocie a zelenina pri prevencii a liečbe ochorení ľudí (Fruit and vegetables in the prevention and treatment of human diseases)*. Nitra, Slovak Sepublic : SPU, 190 p. ISBN 978-80-552-0859-6.

Juríková T., Balla Š. 2012. *Zdraviu prospešné účinky potravín pri bezlepkovej diéte (Health benefits of foods with gluten-free diet)*. Nitra, Slovak Republic, SPU. ISBN 978-80-358-0194-0.

Keresteš, J. 2011. *Zdravie a výživa ľudí (Health and human nutrition)*. CAD Press, 1040 p. ISBN 978-80-88969-57-0.

Kóňa J. 2006. *Koreňové zeleniny (Root vegetables)*. Nitra, Slovakia, Garmond. 77 p. ISBN 978-80-89148-31-8.

Kooti, W., Ali-Akbari, S., Asadi-Samani, M., Ghadery, H., Ashtary-Larky, D. 2014. A review on medicinal plant of Apium graveolens. *Advanced Herbal Medicine*, vol. 1, no. 1, p. 48-59.

Kopec. K. 2010. *Zelenina ve výživě člověka (Vegetables in human nutrition)*. Praha, Czech Republic : Grada Publishing, a.s., 159 p. ISBN 978-80-247-2845-2.

Kudrnáčová, E., Kouřimská, L. 2015. Qualitative parameters of non-traditional types of vegetables - determination of nitrates and ascorbic acid content, *Potravinarstvo*, vol. 9, 2015, no. 1, p. 237-241. https://doi.org/10.5219/466

Leahu, A. Damian, C., Oroian, M., Miclescu, V., Ropciuc, S. 2013. Variation in content of antioxidant and free radical scavenging activity of basil (*Ocimum basilicum*), dill (*Anethum graveolens*) and parsley (*Petroselinum sativum*). *Food and Environment Safety - Journal of Faculty of Food Engineering*, vol. 12, no. 4, p. 347-353.

Liseiwska, Z., Słupski, J., Zuchowicz, E. 2003. Influence of temperature and storage period on the preservation of vitamin C, thiamine and riboflavin in frozen dill (*Anethum graveolens*), *Food Science and Technology*, vol. 6, no. 2.

Mareček, J., Ivanišová, E., Frančáková, H., Musilová, J., Krajčovič, T., Mendelová, A. 2016. Evaluation of primary and secondary metabolites in selected varieties of potatoes, *Potravinarstvo*, vol. 10, no. 1, p. 145-151. https://doi.org/10.5219/562

Matějková, J., Petříková, K. 2010. Ascorbic acid contents in selected vegetables in relation to variety, growing site, year and storage. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 58, no. 1, p. 95-100. https://doi.org/10.11118/actaun201058010095

Odriozola, S. I., Hernándezjover, T., Martínbelloso, O. 2007. Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry*, vol. 105, no. 3, p. 1151-1159. https://doi.org/10.1016/j.foodchem.2007.02.037

Roslon, W., Osinska, E., Gajc-Wolska, J. 2010. The influence of raw material stabilization on the quality of celery (*Apium graveolens* L.) leaves. *Acta horticulturae*, vol. 877, no. 877, p. 201-208. https://doi.org/10.17660/ActaHortic.2010.877.20

Shams, K. A., Abdel-Azim, N. S., Tawfik, W. A., Hassanein, H. D., Saleh, M. A., Hammouda, F. M. 2015. Green extraction techniques: Effect of extraction method on lipid contents of three medicinal plants of Apiaceae. *Journal of Chemical and Pharmaceutical Reserch*, vol. 7, no. 4, p. 1080-1088.

Stan, M., Soran, M. L., Marutoiu, C. 2014. Extraction and HPLC Determination of the Ascorbic Acid Content of Three Indigenous Spice Plants. *Journal of Analytical Chemistry*, vol. 69, no. 10, p. 998-1002. https://doi.org/10.1134/S106193481410013X

Šlosár, M., Čekey, N. 2008. Hlúbová zelenina – významná súčasť výživy človeka. *Zahradnictví*, vol. 12, no. 8, p. 16-17.

Uddin, M. S., Hawlader, M. N. A, Ding, L., Mujumdar A. S. 2002. Degradation of ascorbic acid in dried guava during storage. *Journal of Food Engineering*, vol. 51, p. 21-26, https://doi.org/10.1016/S0260-8774(01)00031-0

Uher A., Kóňa J., Valšíková M., Andrejiová A., 2009. *Zeleninárstvo poľné pestovanie (Vegetable growing field)*. Nitra, Slovak Republic : SPU, 212 p. ISBN 978-80-552-0199-3.

Yanardag, R., Bolkent, S., Tabakoglu-Oguz, A., Ozsoy-Sacan, O. 2003. Effects of Petroselinum crispum extract on pancreatic B cells and blood glucose of streptozotocin-induced diabetic rats. *Biological and Pharmaceutical Bulletin*, vol. 26, no. 8, p. 1206-1210.

https://doi.org/10.1248/bpb.26.1206
PMid:12913280

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THE ESTIMATED POSSIBILITIES OF PROCESS MONITORING IN MILK PRODUCTION BY THE SIMPLE THERMODYNAMIC SENSORS

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ABSTRACT

The characterization and monitoring of thermal processes in thermodynamic systems can be performed using the thermodynamic sensors (TDS). The basic idea of thermodynamic sensor is possible to use in many various applications (eq. monitoring of frictional heat, thermal radiation, pollution of cleaning fluid, etc.). One of application areas, where the thermodynamic sensor can find the new area for a using, is a production of milk products - cheese, yogurt, kefir, etc. This paper describes the estimated possibilities, advantages and disadvantages of the use of thermodynamic sensors in diary productions and simple experiments for characterization and monitoring of basic operations in milk production process by thermodynamic sensors. The milk products are often realized by fermenting or renneting process. Final stages of fermentation and renneting processes are often determined on the base of sensory evaluation, pH measurement or by analytical method. The exact time of the fermentation process completion is dependent on various parameters and is often the company know-how. The fast, clean and simple non-analytical non-contact method for monitoring and for the determination of process final stages does not exist in this time. Tests of fermentation process, renneting process and yoghurt process by thermodynamic sensors were characterized and measured in this work. Measurement of activity yeasts was tested in first series of experiments. In second series of experiments, measurement of processes in milk production was tested. First results of simple experiments show that the thermodynamic sensors might be used for determination of time behaviour of these processes. Therefore, the milk products (cheese, yogurt, kefir, etc.) is opened as a one of new application areas, where the thermodynamic sensor can be used.

Keywords: thermodynamic sensor; fermentation process; yeast

INTRODUCTION

The principle of thermodynamic sensor

The characterization and monitoring of thermal processes in various thermodynamic systems can be performed using the thermodynamic sensors (TDS) that are based on the principle of balance equilibrium. These sensor systems are one of the newer group of sensors for sensing the thermal quantities. The basic idea, basic model and theory of ideal thermodynamic sensor integration as an ideal element in large models of thermodynamic system were presented in patent **(Industrial Property Office of the Czech Republic, 2006)**. The original theory of ideal thermodynamic sensor as a process and media energy activity monitoring device was presented in **Reznicek et al., 2005, Reznicek et al., 2006, Reznicek et al., 2008 and Reznicek, 2014**.

The principle of these thermodynamic sensors is based on measurement of energy, which is supplied to circuit to temperature setting and equilibration of temperature element with ambient. The sensor element is very often integrated with an amplifier and a converter to defined electrical signal (U, I, f), which is very easily connected to other measuring systems.

Three groups of influences have effect on thermodynamic sensors (Figure 1). First group of influences is presented by influences I1. This group have effect only on a temperature of sensitive element T2. Various physical quantities (temperature, radiant heat, humidity, flow of liquid), which are possibly transformed to temperature energy, are theoretically measured in this group.

Second group of influences is presented by influences I2. This group change the temperature properties between the sensitive elements T1 and T2 (difference energy E1 and E2). Volume, density, flow of liquid, pressure and many other quantities can be theoretically measured measure in this group.

Last group of influences is presented by influences I3, which have effect on a temperature of both sense elements T1 and T2. If both sense elements have the same sensitivity, this group does not have effect on output of voltage signal of thermodynamic sensor.

Advantages and disadvantages of TDS

A common feature of thermodynamic measurement system is the monitoring of major events. However, technological windows of production processes are decreasing due to increasing the demands on the reliability and quality of the target product. Accordingly, the demands on the controlling and monitoring increase up to the possibility limit when using current technologies.

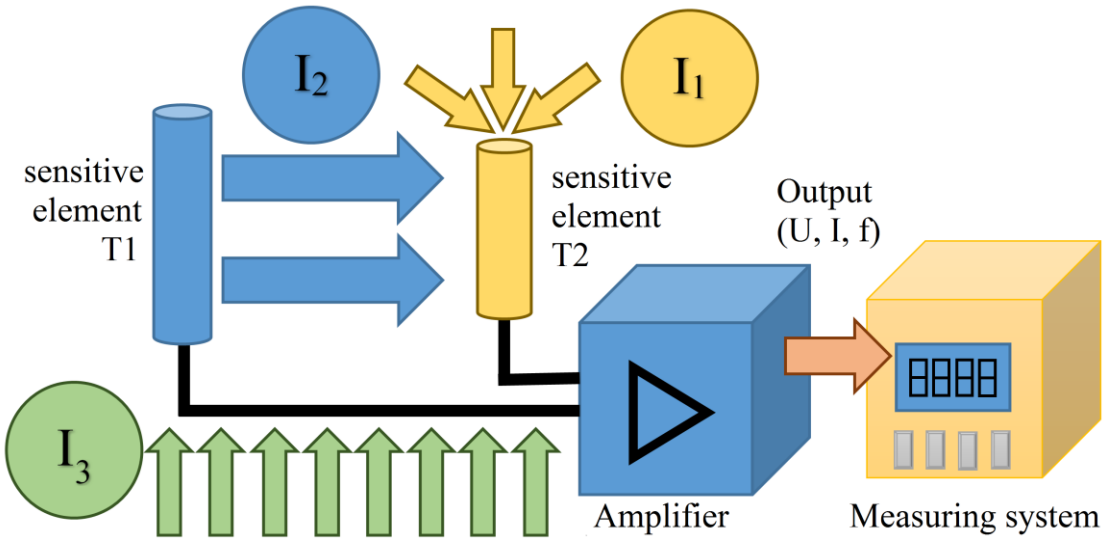

Figure 1 The group of influences I_1, I_2 and I_3, which have effect on thermodynamic sensor.

Therefore, the need for monitoring in area of zero temperature differences and identification of minor events (eg. an unexpected of unwanted event with a small temperature difference) by measurement system increases **(Reznicek, 2014)**.

The using of thermodynamic sensors that are based on the principle of balance equilibrium is one of the ways of monitoring these events on the background of major events (eg. temperature stabilization). Therefore, advantages of TDS are:

- High speed and high sensitivity over other temperature sensors' types, for example thermocouples.
- Possibility of heat flow measuring.
- Directionality of measurements.
- Possibility to process monitoring in zero temperature difference
- Possibility of monitoring and identification of minor events.
- Possibility of easy connect to the control and regulation circuits.

However, TDS also has these disadvantages:

- The system only gives information about the relative temperature changes.
- The measurement is highly dependent on the construction of the measuring system in a specific application.

Therefore, the basic idea of thermodynamic sensor is possible to use in various applications - monitoring of the breakage of glass fibers during their production, monitoring of frictional heat in engine, monitoring reception signal at the antenna, temperature stabilization of the detector nanoparticles, etc. **(Reznicek, 2014)**.

The estimated possibilities of process monitoring in food production by the simple TDS

Many thermal processes exist in food production – cooking, frying, baking, cooling, freezing, sterilization, pasteurization, drying, etc. The TDS can be used to monitor various parameters in many of these processes, which must be very often controlled and recorded for HACCP and customers' certification. It is assumed eg. the possibility to monitor the purity of the oil which is used in frying or monitoring the purity of cleaning emulsion similarly as was tested in project TAČR GAMA – TA01011754 "New methods for the cleaning of assembled substrates with higher effectivity and lower ecological and energy comsumption impact". Because the thermodynamic sensor is very sensitive and fast, it is possible to measure very small temperature changes that may be produced for example by groups of yeast. Therefore, it is possible use TDS in the production:

- dairy products – fermentation processes, yogurt processes, renneting processes,
- breweries – fermentation processes,
- distilleries – fermentation processes,
- control unwanted development of yeast and other microflora (sterilization, canning),
- bakeries –controls vitability yeast,
- pickled cabbage – fermentation processes, etc.

A production of dairy products (cheeses, yogurts, kefirs, etc.) can be one of new application areas, where the thermodynamic sensors can be used. Milk and products from milk are one of important ingredients of food in people´s lives, especially for children. Milk and dairy products are sources of vitamins, proteins, fat, minerals, lactose, etc., which are not fungible in people´s sustenance. The dairy production is composed of complicated and sophisticated processes **(Semjan, 1994)**. These processes are exacting to precision, temperature stability and hygiene. This is a reason for close quality checking.

In the dairy productions, the thermodynamic sensors can continuously monitor, control and regulate the main process parameters – required temperatures and deviations from it (sterilization and pasteurization temperature of milk, dairy products, storage temperature etc.). However, the sensors can also monitor other production parameters, which can be converted to a change of temperature flow – purity and quantity of basic raw materials (milk, marmalade, ...), purity and the amount of auxiliary media (rinsing water, auxiliary gas, etc.), monitoring and control unwanted of unwanted

event – sanitizing inspection of manufacturing devices and space, wear down control of production machines etc.

The production of dairy products is often realized by fermenting or renneting processes. The simple clean non-contaction non-analytical method for determination of final process does not exist in this time. Final stages of fermenting or renneting processes are often determined on the base of sensory evaluation or analytical measurements. The different ways to solving these problems are looking for now. One of the possible ways for solution of this problem can be characterisation and measurement of these processes by thermodynamic sensors.

MATERIAL AND METHODOLOGY

Chemicals

Measurement of activity yeasts was tested in first series of experiments. Dried yeast "Instantní Droždí", S. I. Lesaffre, caster sugar "Cukr bílý krystal", Cukrovary a lihovary TTD, a.s., Dobrovice, and distilled water were used as the material for first measurement.

The basic material used for second measurements of milk products was raw milk from farm Farma Hole s.r.o., Velké Přílepy. The yoghurt "White country yoghurt with probiotic BiFi culture", Hollandia Karlovy Vary a.s., Toužim, was used as start culture for production of yoghurt and the rennet proces was made by the reneet Laktochym (1 : 5.000), MILCOM, a.s., Praha.

Experiment

All the measurements were done by using the TDS sensors, which was fabricated in HIT, s.r.o., Nedachlebice. The sensors, a simple measuring circuit, power source and multimeter Metex 3270 D or Almemo 2390-5 (Ahlborn Mess- und Regelungstechnik GmbH, Germany) as voltmeter, which was controlled by computer, were main parts of workplace. The workplace for experiments with thermodynamic sensors is shown on Figure 2.

Temperature was 25 °C in case of first experiments with water and 35 °C in temperature-controlled box in case of second experiments with milk products.

Statistical analysis

The data were analysed using software Excel 2013 (Microsoft Corporation, USA) and the results were expressed by graph. Each experiment was measured at least three times and the resulting curve was calculated as the mean value of these measurements.

RESULTS AND DISCUSSION

Measurement of activity of yeasts was tested in first series of experiments. Experiments were made with water in room temperature (25 °C). Volume of water was 25 mL. First experiment **(Figure 3)** was focused on fermenting process, where the yeasts weight was changed. The test weight was 0.1 g, 0.5 g, 1 g and 1.25 g. Weight of sugar was 1 g. Results show a dependence of yeasts activity on yeasts weight in first fermentation phase and stabilization of yeasts activity on constant value in second phase of process. The initial weight of the yeast affects the increase rate of the total yeast's activity. It is assumed that increase of the yeasts number affects heat flow between elements T1 and T2 (influence I2). Next, increase of the number of active yeasts increases a heat, whitch coming to the element T2 (the effect I1). After reaching a maximum, the number of active yeasts decreases, therefore the heat coming to the element T2 is also reduced. The weight of sugar was changed (0.5 g; 1 g; 1.25 g) in next experiment (Figure 4). Weight of yeasts was 0.3 g. The yeasts activity is increased with weight of sugar in first fermentation phase and is stabilized on constant value in second phase of process again.

Viable yeast can be used as an inoculum for many fermentations processes in the food industries. The fermentation process increases the total value of the fermented foods, eq. improving the quantity and quality of proteins and their accessibility, increase the digestibility of starch, increasing the content of vitamins, especially group B (riboflavin, thiamine, niacin, folic acid), increase the availability of mineral elements, etc. **(Arora, Jood and Khetarpaul, 2010; Charalampopoulos et al., 2002; Rivera-Espinoza and Gallardo-Navarro, 2010; Kocková and Valík, 2011)**.

Traditionally, yeast viability has been measured in colony-forming units after plating of cells on growth medium or by direct microscopic counting using dye exclusion methods **(Jones, 1987a, b; Fung, 1994; Lloyd and Hayes, 1995; Haugland, 1996)**. Next measurement of yeast viability can be made a flow cytometry **(Deere et al., 1998; Attfield et al., 2000)** or monitoring CO_2 as product of yeast activity. For yeast identification, PCR-RAPD and RFLP-PCR methods **(Drozdz et al., 2015)** can be used. These methods can be used for determination of yeast grow curve. The grow curve of yeast **(Halasz and Lasztity, 1990; Walker, 1998; Neal, 2004)** and measured curves for fermenting process of yeasts in water with a change of yeasts weight have a very similar character. In accordance with the weight of the inoculum, the measured voltage measured voltage is increased and stabilized after some time. Similarly, the character of the measured curves for fermenting process of yeasts in water with a change of sugar weight is also in accordance with the present character of growth curves.

In second series of experiments, measurement of processes in milk production was tested. Experiments were made with raw milk in temperature-controlled box (35 °C).

Figure 5 shows a characterisation of yogurt process. Start and final stage of this proces is demonstrated. In the yogurt production, a mixed base startet yoghurt culture with the bacterial strain *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* added to the milk. Both living organisms must be in a product in the optimal ratio. *L. delbrueckii* ssp. *bulgaricus* partially degrades the casein. This releases valine, histidine, methionine, glutamic acid and leucine, which are stimulating the growth of *Streptococcus salivarius* subsp. thermophilus. Streptococci forms lactic acid and creates favorable conditions for the growth of lactobacilli **(Courtin and Rul, 2004; Walstra, Wouters and Geurts, 2006)**. It is possible to find a hint of two peaks on the curve of yogurt process on Figure 5. These peaks can be assigned activity lactococci and streptococci in the starter culture. End of measurement can also be influenced by a change the viscosity in the final stage of the process. The example of rennet process measurement is shown on Figure 6.

Figure 2 The workplace for experiments.

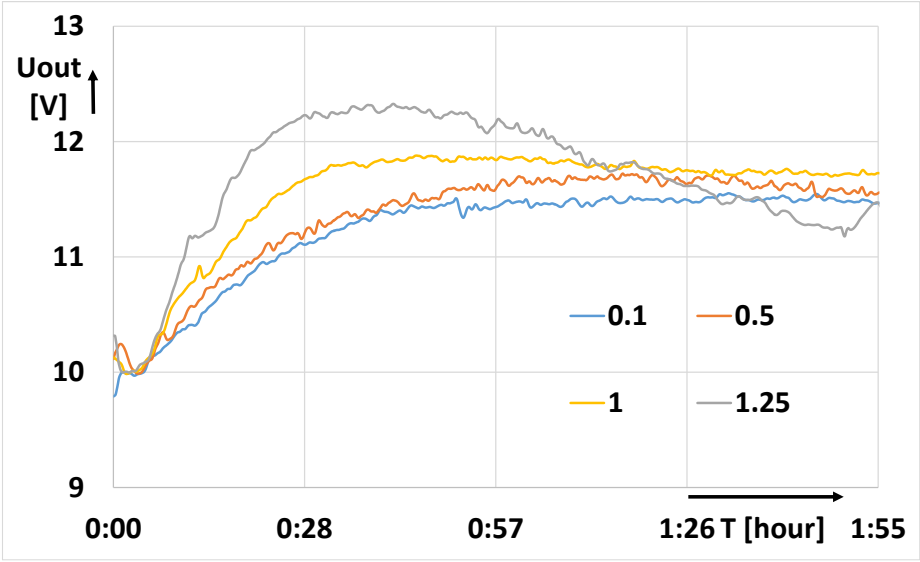

Figure 3 The fermenting process of yeasts in water - a change of yeasts weight.

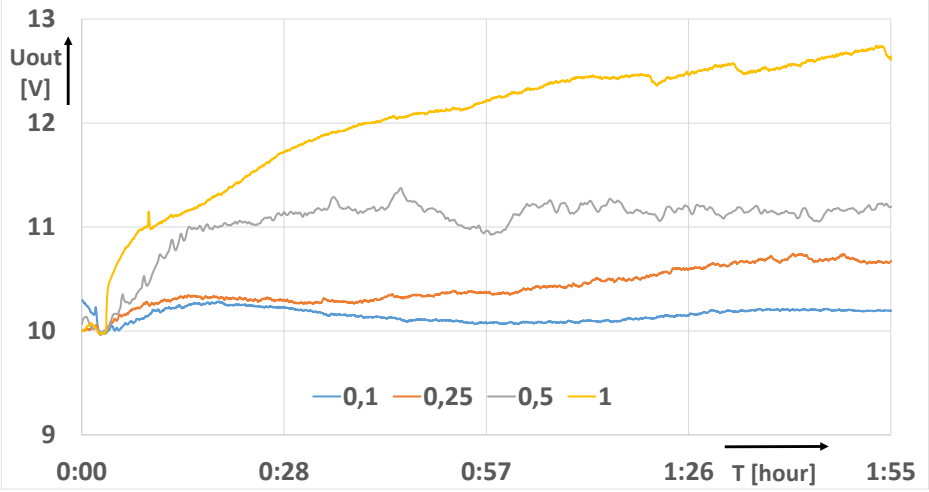

Figure 4 The fermenting process of yeasts in water.

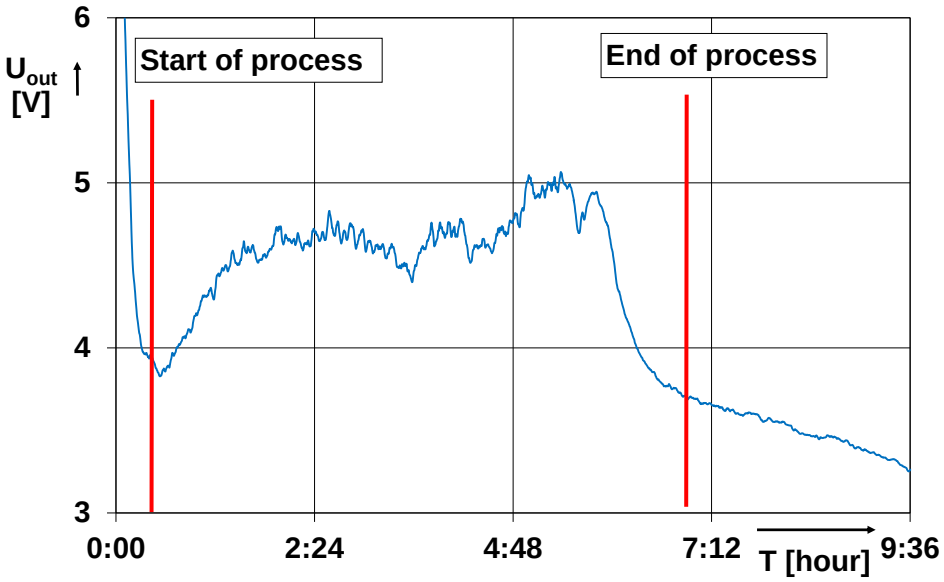

Figure 5 The yogurt process.

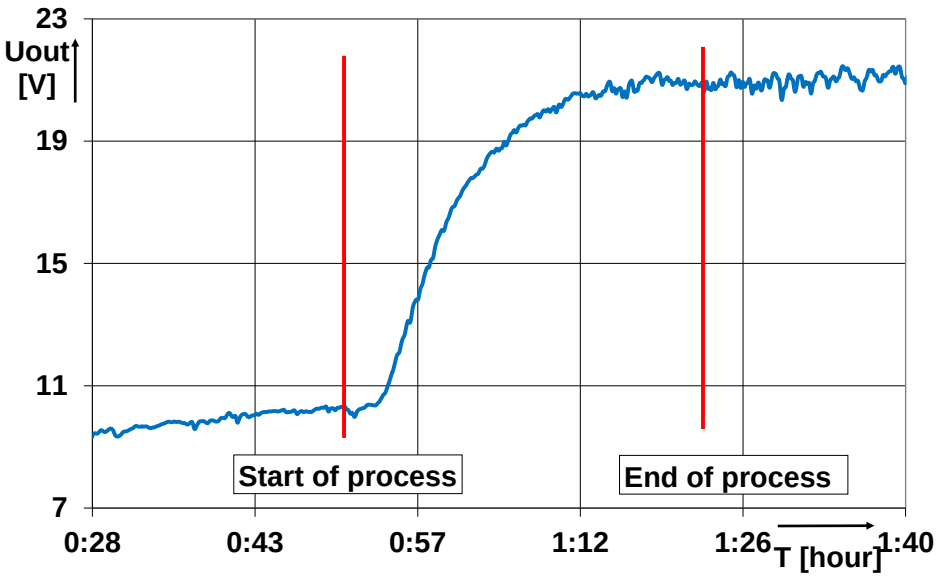

Figure 6 The rennet process.

The final stage of this proces is demonstrated again. The measurement is influenced by a change the viscosity, which have impact to termal properties of yogurt. After adding rennet (very often with chymosin enzyme) to milk, the casein micelles start to aggregate and forming a gel **(Walstra, Wouters and Geurts, 2006)**.

Because this is a new area of application of TDS and new method of measurement, it can not yet compare the results with other authors. Results can only be estimated in comparison with standard methods, which are less flexible and time and material consuming.

Although the number of measurement repetition was low, the first results show a possibilities TDS in monitoring of yeast activity in fermentation processes and determination of final stage in yogurt and rennet processes.

CONCLUSION

The thermodynamic sensor was tested in basic operations in milk production. Tests of rennet process, yogurt process and fermentation process were characterized and measured with thermodynamic sensor, which was borrowed from HIT, s.r.o. First results of simple experiments show that the thermodynamic sensors can be used for time behavior and end determination of this processes.

REFERENCES

Arora, S., Jood, S., Khetarpaul, N. 2010. Effect of germination and probiotic fermentation on nutrient composition of barley based food mixtures. *Food Chemistry*, vol. 119, no. 2, p. 779-784. https://doi.org/10.1016/j.foodchem.2009.07.035

Attfield, P. V., Kletsas, S., Veal, D. A., Van Rooijen, R., Bell, P. J. L. 2000. Use of flow cytometry to monitor cell damage

and predict fermentation activity of dried yeasts. *Journal of Applied Microbiology*, vol. 89, no. 2, p. 207-214. http://dx.doi.org/10.1046/j.1365-2672.2000.01100.x

Charalampopoulos, D., Wang, R., Pandiella, S. S., Webb, C. 2002. Application of cereals and cereal components in functional foods: a review. *International Journal of Food Microbiology*, vol. 79, no. 1-2, p. 131-141. https://doi.org/10.1016/S0168-1605(02)00187-3

Courtin, P., Rul, F. 2004. Interactions between microorganisms in a simple ecosystem: yogurt bacteria as a study model. *Le Lait*, vol. 84, no. 1-2, p. 125-134. http://dx.doi.org/10.1051/lait:2003031

Deere, D., Shen, J., Vesey, G., Bell, P., Bissinger, P., Veal, D. 1998. Flow cytometry and cell sorting for yeast viability assessment and selection. *Yeast*, vol. 14, p. 147-160. https://doi.org/10.1002/(SICI)1097-0061(19980130)14:2<147::AID-YEA207>3.0.CO;2-L

Drozdz, I., Makarewicz, M., Sroka, P., Satora, P., Jankowski, P. 2015. Comparison of the yeast microbiota of different varieties of cool-climate grapes by PCR-RAPD. *Potravinarstvo*, vol. 9, no. 1, p. 293-298. http://dx.doi.org/10.5219/484

Fung, D. Y. C. 1994. Rapid methods and automation in food microbiology: a review. *Food Reviews International*. vol. 10, p. 357-375. https://doi.org/10.1080/87559129409541006

Halasz, A., Lasztity, R. 1990. *Use of Yeast Biomass in Food Production*. CRC Press. 352 p. ISBN 9780849358661.

Haugland, R. 1996. *Handbook of Fluorescent Probes and Research Chemicals*. Oregon, USA : Molecular Probes Inc.

Industrial Property Office of the Czech Republic. *Technique of referential temperature and temperature difference measurement, asymetric temperature sensor and asymetric referential unit to technique application*. Patent owner: Reznicek, Z. Czech Republic. Patent no. CZ-297066. 2006-07-19.

Jones, R. P. 1987a. Measures of cell death and deactivation and their meaning: part I. *Process Biochemistry*, vol. 22, p. 118-128.

Jones, R. P. 1987b. Measures of cell death and deactivation and their meaning: part II. *Process Biochemistry*, vol. 23, 130-134.

Kocková, M., Valík, Ľ. 2011. Potential of cereals and pseudocereals for lactic acid fermentations. *Potravinarstvo*, vol. 5, no. 2, p. 27-40. https://dx.doi.org/10.5219/127

Lloyd, D., Hayes, A. J. 1995. Vigour, vitality and viability of microorganisms. *FEMS Microbiology Letters*, vol. 133, p. 1-7. https://doi.org/10.1111/j.1574-6968.1995.tb07852.x

Neal, D. 2004. *Introduction to Population Biology*. Cambridge, UK : Cambridge University Press. 394 p. ISBN 978-0521532235

Semjan, Š. 1994. *Mliekárstvo (Dairy technology)*. Nitra, Slovak Republic : SPU, 204 p. ISBN 80-7137-157-2.

Reznicek, Z., Tvarozek, Y., Reznicek, M., Szendiuch, I. 2005. Temperature balanced process media energy activity monitoring, *International Conference EDS-IMAPS CS 2005 proceeding*. Brno, Czech Republic. p. 94-100. ISBN 80-214-2990-9.

Reznicek, Z., Tvarozek, Y., Reznicek, M., Szendiuch, I. 2006. Hybrid Constant Temperature Regulator. *International Conference EuroSimE It 2006 proceeding*, Como, Italy, p. 502-505. ISBN 1-4244-0275-1.

Reznicek, Z. S., Szendiuch, I., Reznicek, M., Reznicek Z. Jr. 2008. Thick Film Double Thermodynamic Sensor System. In *2nd Electronics System integration Technology Conference proceedings*. Greenwich, UK, 1-4 September 2008. p. 1301 - 1304. ISBN 978-1-4244-2813-7. https://doi.org/10.1109/estc.2008.4684542

Reznicek, M. 2014. *Thermodynamic sensors based on the principle of balance equilibrium*: dissertation theses. Brno, Czech Republic : FEKT VUT Brno, 93 p.

Rivera-Espinoza, Y., Gallardo-Navaro, Y. 2010. Non-dairy probiotic products. *Food Microbiology*, vol. 27, no. 1, p. 1-11. https://doi.org/10.1016/j.fm.2008.06.008 PMid:19913684

Walker, G. M. 1998. *Yeast Physiology and Biotechnology*. Wiley. 362 p. ISBN 9780471964469.

Walstra, P., Wouters, J. T., Geurts, T. J. 2006. *Dairy science and technology*. London, UK : CRC press, Taylor & Francis Group. 808 p. ISBN 978-0-8247-2763-5.

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Thermodynamic sensors that were borrowed from HIT, s.r.o., were used for the measurement of experiments in the area of monitoring food production that are presented in this paper.

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THE POSSIBILITIES OF INCREASING LIGNAN CONTENT IN FOOD

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ABSTRACT

Lignans are bioactive substances which belong to polyphenols. This compounds can be found in plants including coniferous trees. Lignans are secondary plant metabolites with wide range of biological effects, such as antimicrobial, antivirus or anticancer. They also serve as antioxidants and are naturally occurring compounds which are found in food rich in fibre. There are more than 200 lignans that originate from more than 70 plant families. They can be found in all parts of the plant, mainly in seeds. Almost 37% of total lignan intake in human diet comes from drinking tea and coffee. Fruit and vegetable contain only about 1% of lignans, but they are also significant source of lignans because they are consumed in higher amounts than seeds. 7-hydroxymatairesinol is the main representative of lignans. It is white powder with great health benefits and it is present in the knots of coniferous trees, especially in knots of spruce. Lignans were extracted from the knots and used for fortifying fruit and vegetable spreads. Subsequently, the fortified products became subject to sensory analysis, their antioxidant capacity was measured by the FRAP method, total polyphenols content was found and lignan content determined using the HPLC method. The aim was enriching commonly consumed foods by healthy lignans to avoid negative effects on the sensory quality of these products by the bitter taste of the lignan extract. Of the tested foods, plum jam and red pepper paste are the best options as they best block the bitter taste of lignans. There was a positive increase in antioxidant capacity in food products fortified by the lignan extract. For plum jam, strawberry jam, strawberry spread and red pepper paste, the more lignans were added to the products, the greater was the level of antioxidant capacity. The highest antioxidant capacity was reached in samples with the added amount of 340 mg of lignan per kg of product. As with the antioxidant capacity, total polyphenols content is dependent on the quantity of added lignans. Plum jam is the only exception, for which there was no statistically evident difference between the doses of 170 mg and 340 mg of lignans per kg. The values of lignans measured for samples with added 340 mg of lignans per kg range from 313 mg to 339 mg. For samples with addition of 170 mg of lignans per kg the measured values range from 129 to 164 mg per kg. Although lignans are beneficial for health, they are not acceptable to deteriorate taste of the product. The samples containing the highest dose of lignans, i.e. 340 mg of lignans per kg, were rated as the least acceptable by consumer. Evaluated as the most suitable in this regard was plum jam with a dose of 170 mg of lignans per kg of product where lignans were not found to possess a sensory effect on the acceptability of the product.

Keywords: lignan; polyphenol; antioxidant capacity; soft fruit products

INTRODUCTION

Lignans are bioactive substances and members of the group of phenolic compounds referred to as phenylpropanoids. Found in the plant kingdom, in higher vascular plants, including conifers, they are involved in the protection of plants from infesting by microorganisms, or from exposure to insects and belong to the most common secondary metabolites of plants. They are dimers produced through oxidative dimerisation of two phenylpropanoid units linked by central carbons of their side propane chains. Linking additional bonds under the involvement of propane segments of the molecule in various oxidation states gives rise to all the possible structural types of lignans. Subsequent transformation produces norlignans, or conioids and neolignans. There are currently more than a thousand of known species and more are constantly being discovered **(Peterson et al., 2010)**. Lignans act against several types of cancer, such as breast, uterus, prostate, and colon cancer. **Dinkova-Kostova et al. (1996)** report that lignans are applied as anticancer agents or phytoestrogens, have antioxidant, antiviral, antibacterial and insecticidal effects and, finally, they protect against cardiovascular diseases. Lignans can penetrate cell membranes, thus influencing the various cell processes. **Peterson et al. (2010)** considers some sources of lignans functional food with their protective function. This study investigates weather addition of lignan extract to chosen foods increased the value of total polyphenols and antioxidant activity without negative influence on consumer acceptability of the enhanced food. Lignans belong to polyphenols which are related to sensorial qualities such as colour, bitterness, astringency, etc. in foods and beverages.

Lignans are naturally occurring compounds that are found particularly in foods rich in fibre. As reported by **Slanina (2000)**, there are more than 200 lignans, originating from more than 70 families of plants. They were found in all parts of plants; of these, typical are primarily the bark and wood of trees and the resin **(MacRae, Towers 1984)**. They occur in diverse seeds, legumes, nuts, fruits and vegetables **(Slanina, 2000)**.

Humans receive most of lignans from beverages, particularly from coffee and tea (up to 37% of total income). In fruits, lignans are present in very low rates

ranging from about 1% of total solids **(Johnsson et al., 2002)**. Despite this, however, fruits and vegetables are ranked among the significant sources as they are consumed in greater quantities than seeds **(Landete, 2012; Haramatha, 2005)**. **Hussain et al. (2006)** were adding flour of linseed into biscuits to increase their quality parameters, e.g. the content of lignans and fibre.

Lignans also occur in heartwood of trees, particularly in the woody species that feature soft wood. For species of trees of hardwood type, they contain mostly flavonoids. Pine wood is rich in stilbenes. **Holmbom et al. (2003)** found that knots of trees contain 5% – 10% lignans. Levels of lignans reached in knots of the Norway spruce (*Picea abies*) ranged from 6% to 29%; of these, 7-hydroxymatairesinol (HMR) was the most represented member, which accounted for 85% of total lignans. Supplements based on this substance (i.e. HMR) and available on the market include HMRlignan™ and others. According to **Taskinen et al.,(2004)** and **Brusentsev and Eklund (2015)** HMR has significant biological effects and is generally an anticancer and antioxidant agent. It also acts against hormone-dependent diseases. **Willför et al. (2005)** suggest that there are significant differences in terms of lignan content in knots obtained from a single tree.

Due to the beneficial and demonstrable health effects and the low content in foods the aim of this study was to extract lignans and add them to food. The research investigating the lignan levels in knots of conifers formed the basis for producing an alcoholic extract from such knots; the lignans generally contained in the substance include 7-hydroxymatiresinol (HMR) and α-conidendrin. The extract was added into selected types of intermediate products made of fruits and vegetables in order to increase the content of lignans and heighten the antioxidant capacity of the above.

MATERIAL AND METHODOLOGY

Chemicals for extraction: ethanol. for analysis: 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox); 2,4,6-tris(2-pyridyl)-s-triazine; hydrochloric acid; acetic acid; iron trichloride; sodium acetate trihydrate. Folin-Ciocalteu reagent; gallic acid monohydrate; sodium carbonate.
For HPLC analysis: methanol; acetonitrile; ortho-phosphoric acid; and formic acid.

Preparation of food products – the procedure: Knots of the Norway spruce (Picea abies) were milled using Cutting Mill SM 100 (Retsch, Haan, Germany). Lignans were extracted from the obtained chips by water and ethanol under the conditions as described in the design of the application for national patent No. 2014-870 **(Híc et al., 2014)**.

The extract contained mainly 7-hydroxymatairesinol (HMR) and a low level of α-conidendrin (CONI) and was used to fortify plum jam, strawberry jam, and strawberry/blueberry spreads intended for adding to yoghurt and bakery products. The test also involved a red pepper paste, which is added to milk-based spreads. Three lignan doses were selected, i.e. 0; 170; and 340 mg and expressed as HMR per kg of the product. Five kinds of spreads and 3 lignan doses were used. Measurement of antioxidant capacity, total polyphenols content and lignan content was done 3 times. After fortifying, the fruit and vegetable products were pasteurised at 85 °C for 20 minutes and stored for 1 month once modified as above. Subsequently, sensory analysis of samples was carried out and the antioxidant capacity, the total content of polyphenols and lignan levels determined.

Modification of food products prior to the chemical analysis: First of all, extracts were made from the samples to determine the antioxidant capacity, the total polyphenols content and the lignan level taking the steps according to **Wicklund et al. (2005)**. Five grams of the sample were homogenised with a small amount of methanol (100%) using a grinding mortar. The process took place 1-2 minutes. The sample was then quantitatively transferred into a volumetric flask of 50 mL, which was filled with methanol up to the mark. The flask with the sample was placed into an ultrasound device for 15 minutes. The sample was transferred into a centrifuge tube and centrifuged 5 minutes at 3,000 rpm.

Antioxidant capacity: Antioxidant capacity was determined by the FRAP method, which is based on the reduction of ferric complex TPTZ using potassium ferricyanide or possibly ferric chloride; almost colourless substances, they produce colour complexes of iron and after the reduction that can be measured using a spectrophotometer. The determination made use of 23 mM sodium acetate in the solution of 34 mM acetic acid. The reaction mixture contained 12 mM $FeCl_3$, 10 mM 2,4,6-tri (2-pyridyl)-s-triazine in the solution of 40 mL HCl and a buffer, the ratio of 1 : 1 : 10. Two mL of the reaction mixture were mixed with 25 µL of a sample diluted with distilled water using a plastic cuvette. The obtained solution was measured after ten minutes using Helios β spectrophotometer at a wavelength of 593 nm. Antioxidant capacity was calculated from the calibration curve for Trolox.

Total polyphenols: Total polyphenols (TP) content was determined by the method using the Folin-Ciocalteau reagent. Using a 50 mL volumetric flask, 0.5 mL of the sample was mixed with 20 mL of distilled water and 1 mL of the Folin-Ciocalteau reagent. The flask was shaken and after 3 minutes 5 ml of 20% Na_2CO_3 were added. After mixing, the flask was filled to the mark with distilled water. After 30 minutes the sample was measured at a wavelength of 700 nm using Helios β spectrophotometer. Total polyphenols content was calculated from a calibration curve for gallic acid.

Lignans: Concentrations of lignans, i.e. 7-hydroxymatairesinol (HMR) and α-conidendrin (CONI), were assessed by HPLC using an HP apparatus (Hewlett Packard 1050) with a diode array detector (DAD Agilent G1315B), and the Phenomenex Luna C18 column (2) (3 µm, 2 x 150 mm). The mobile phase consisted of water - acetonitrile - o-phosphoric acid. Mobile phase A consisted of 5% acetonitrile +0.1% o-phosphoric acid; mobile phase B consisted of 80% acetonitrile +0.1% o-phosphoric acid. Used for separation was the gradient from 20% B to 80% B within 20 minutes, the flow rate was 0.25 mL/min.

Column temperature was 25 °C. HMR and CONI were detected at 220 nm.

Sensory analysis: Nine trained assessors took part in the sensory analysis within the scope of ISO 8586 requirements; the procedure was underway in the sensory laboratory of the Faculty of Horticulture Lednice that meets the ČSN EN ISO 8589 Standard. The method used for assessment was one according to the graphic scale (ISO 4121). The results were recorded on an unstructured graphic scale, the length of 100 mm. The assessors were asked to taste the samples and indicate their perceived intensity of a bitter and an astringent taste, and the consumer acceptability, where applicable, using the graphic scale. When evaluating, a distance was measured between the marks assigned to the sample by the assessor and the beginning of the scale, with 1 mm = 1 score, meaning that the person was able to give at least 0 scores and a maximum of 100 scores.

Statistical evaluation: The results were processed by the statistical program Statistica 10.0. Test of homogeneity was done followed by parametric analysis of variance (ANOVA). Tukey´s LSD test with level of significance 0,05 was done from post hoc test (Table 1).

RESULTS AND DISCUSSION

The values of antioxidant capacity measured by FRAP were dependent on the content of lignans in samples of products (Figure 1). For plum jam, strawberry jam, strawberry spread and red pepper paste, the more lignans were added to the products, the greater was the level of antioxidant capacity. The highest antioxidant capacity was reached in samples with the added amount of 340 mg of lignan per kg of product. Conversely, the lowest antioxidant capacity was found in samples to which no lignans were added. All the differences are statistically significant (p = 0.05), except the blueberry spread. Different results were probably due to the relatively high antioxidant capacity of the spread alone. Similar findings were reported by **Balík et al. (2014)**, where the antioxidant values of grape juices were under a major impact by the manufacturing technology rather than by the addition of lignans.

As with the antioxidant capacity, total polyphenols content is dependent on the quantity of added lignans. Plum jam is the only exception, for which there was no statistically evident difference between the doses of 170 mg and 340 mg of lignans per kg (Figure 2). Since lignans are among polyphenols and feature antioxidant capacity, the conclusions presented are in accordance with assumptions. Samples that contained most of polyphenols were those fortified with 340 mg of lignans per kg of the product while the lowest values of total polyphenols were measured for samples to which lignans were not added at all. The graph shows that the addition of lignans increases the total polyphenols content in the samples. The lignan content in the products was tested by HPLC. It is apparent that the clean samples contain no lignans (Figure 3). The values measured for samples with added 340 mg of lignans per kg range from 313 mg to 339 mg. For samples with addition of 170 mg of lignans per kg the measured values range from 129 to 164 mg per kg.

The addition of the lignans extract was assumed to bring in food adverse secondary organoleptic characteristics such as bitterness or astringency. Although lignans are beneficial for health, they are not acceptable to deteriorate taste of the product. Due to the fact above, some products were assessed as unsuitable for fortifying using a lignan extract (Figure 4). When conducting sensory assessment of consumer acceptability, samples containing the highest dose, i.e. 340 mg of lignans per kg, were rated as the least acceptable by consumer. Evaluated as the most suitable in this regard was plum jam with a dose of 170 mg of lignans per kg of product where lignans were not found to possess a sensory effect on the acceptability of the product while

Table 1 Evaluation of antioxidant capacity, total polyphenols and sensory assesment of foods enriched with lignans.

Sample	Antioxidant capacity(FRAP) (mmol Troloxu.kg^{-1})	Total polyphenols (mg.kg^{-1})	Sensory assesment (points)
Plum jam 340	8.48 g	1963.86 h	60.33 b c
Plum jam 170	7.41 f	1893.07 h	72.33 f
Plum jam 0	6.05 d	1661.23 g	72 f
Strawberry jam 340	8.21 g	1465.22 f	71.67 f e d
Strawberry jam 170	6.94 e	1313.38 ed	74.78 f
Strawberry jam 0	5.7 dc	1170.65 c	75.33 f
Strawbery spreads 340	7.01 e	1345.18 e	46.78 a
Strawbery spreads 170	5.33 c	1099.13 b	63 d c
Strawbery spreads 0	4.31 b	881.33 a	71 e f
Blueberry spreads 340	14.15 i	2628.88 k	49.44 a b
Blueberry spreads 170	12.74 h	2446.68 j	62.44 d c
Blueberry spreads 0	13 h	2308.88 i	72.95 f
Red pepper paste 340	5.98 d	1293.75 d	61.33 e d c
Red pepper paste 170	4.42 b	1108.84 b	61.22 e d c b
Red pepper paste 0	2.97 a	871.79 a	68 f e d c

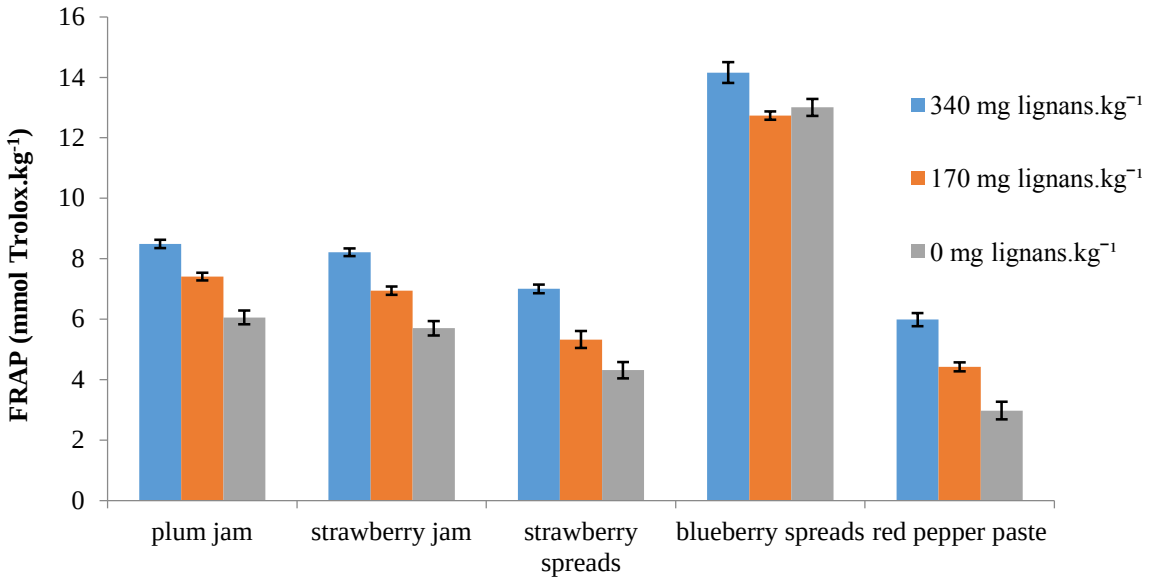

Figure 1 The values of antioxidant capacity for lignan-fortified fruit and vegetable spreads (FRAP method).

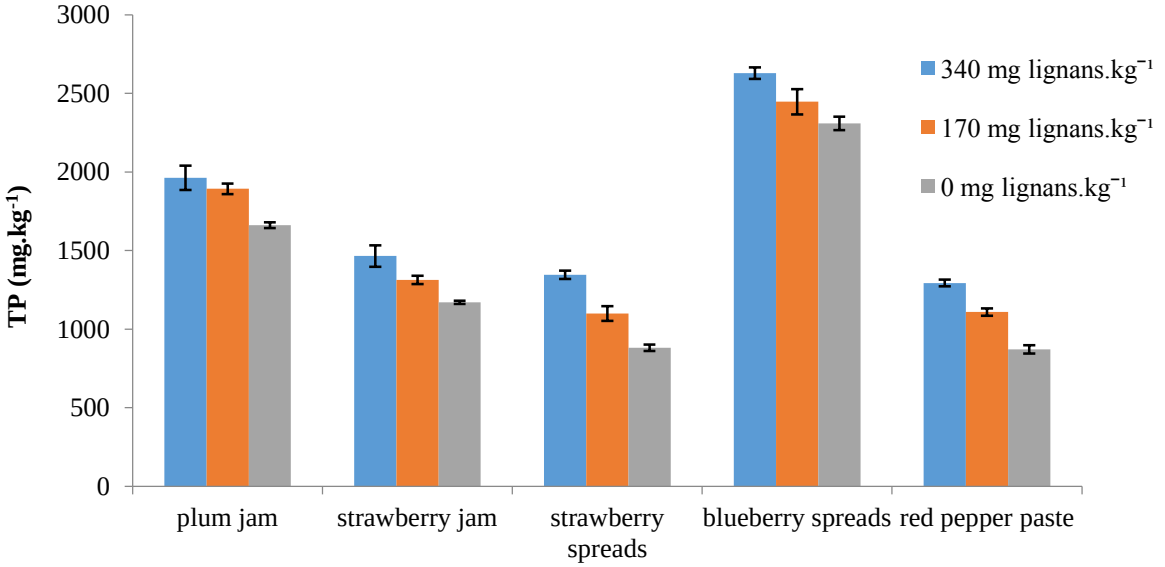

Figure 2 The values of total polyphenols for lignan-fortified fruit and vegetable spreads.

any higher dose caused a decline in consumer acceptability. A similar trend was recorded for strawberry jam, where no statistically significant difference was detected between samples (p = 0.05). For strawberry spread, a statistically significant difference was found between the sample containing 340 mg of lignans per kg and the sample containing 170 mg of lignans per kg and the one with no added lignans. When evaluating the red pepper paste, no statistically significant difference was found between the samples. Possible reason is that tasters might not be able to recognize difference among variants due to high pungency. The suitability also applies to the red pepper paste which, given its pungent taste, covered up the taste changes (Figure 4). Similar experiment was done

The study states that lignans HMR and CONI are the most present lignans in wooden chips of spruce tree. Several doses of wooden chips were added to white and red grape musts which were then pasteurised and evaluated after some time of storing. The results showed that in the year 2008 by **Perlman et al. (2008)** who extracted grape pomace. They proposed the fortification of grape juice with the grape pomace polyphenol extract. The new beverage was tested at different extract concentrations for sensorial acceptance and antioxidant activity. An unacceptable astringency was noticed at 4% with antioxidant activity increasing three times. **Draijer et al. (2009)** added a mixture of red wine and grape polyphenols to a soy drink. Subsequently the enhanced beverage was given daily to 35 males with positive impact on the blood pressure. Similar research was done by **Novotná et al. (2016)** who increased the content of lignans 7-hydroxymatairesinol (HMR) and α-conidendrin (CONI) in grape musts by adding of spruce chips. enrichment by spruce chips increased the antioxidant capacity and also the amount of total polyphenols in the samples. The woody aroma and woody taste of the samples were increased simultaneously as sensory assessment showed. **Balík et al. (2016)** performed an

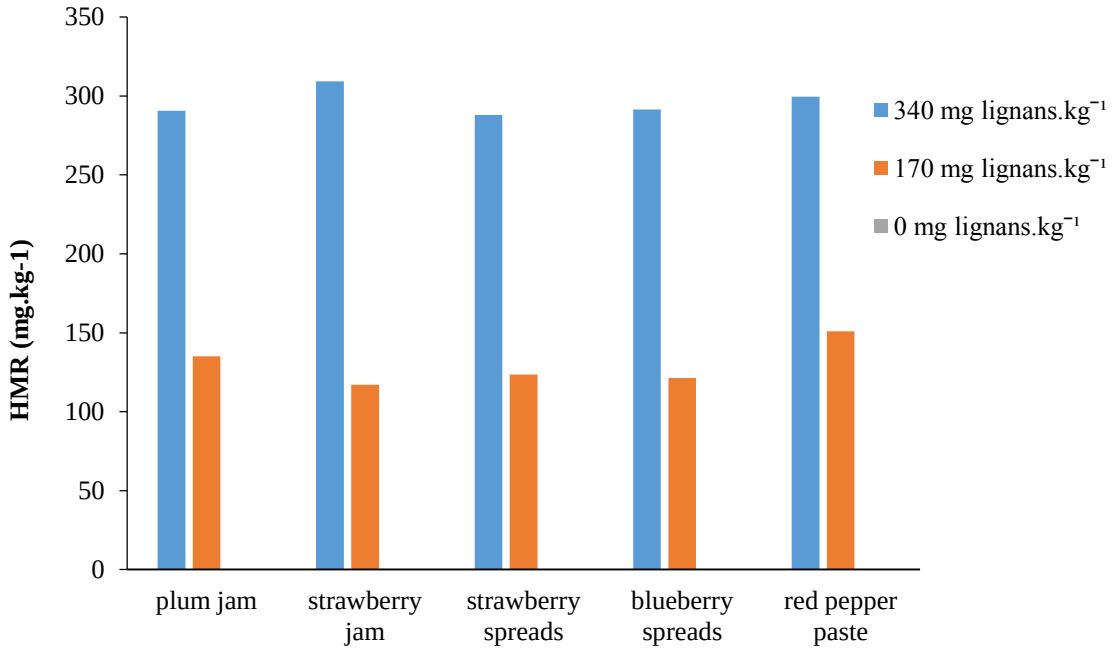

Figure 3 The lignan content in fruit and vegetable spreads.

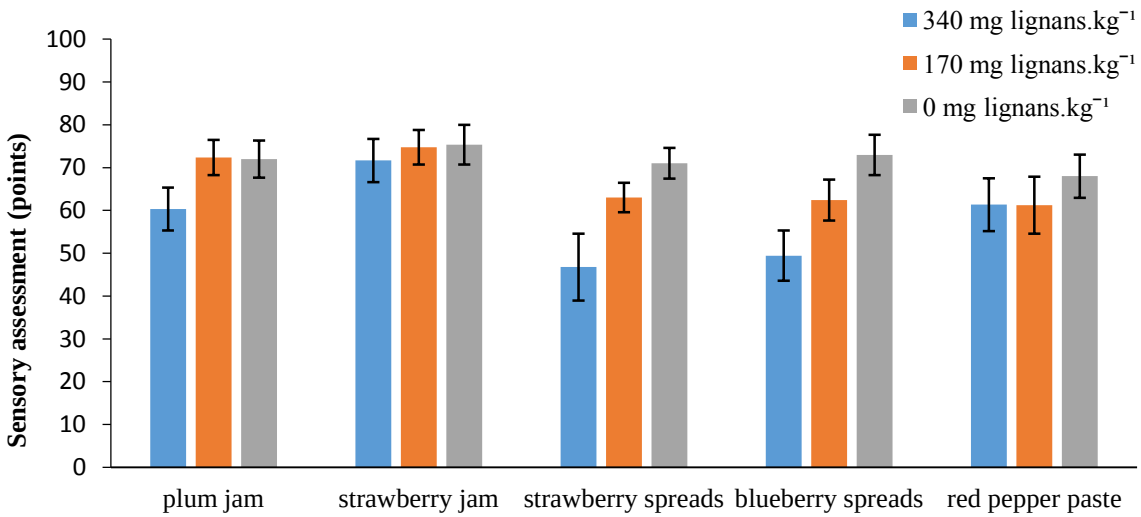

Figure 4 Sensory assessment of the consumer acceptability of food products depending on the addition of lignans.

experiment when lignan extract from wooden chips was added to white and red wines. Antioxidant capacity and total polyphenols content were measured after different times of storing. Increase of lignan content was observed in all samples after adding of the extract. The lignan content was stable even after 13 months of storing. Antioxidant capacity and total polyphenols content were not significantly influenced by extract enrichment. **Kapoor and Ranote (2016)** added the extract from jamun fruit of *Syzygium cumini* into pear juice in order to increase the antioxidant capacity. The products were sensoricaly evaluated and the results showed that juice with 4% of jamun phenolic powder reached the highest sensory evaluation. Enriched juiced showed about 9.24% higher content of total polyphneols than juices without powder enrichment. Antioxidant capacity was higher about 18.13%.

Storage period of 6 months caused significant decrease of bioactive compounds and antioxidant activity in supplemented pear juice. **Liu et al., (2016)** investigated the effect of oak chips on evolution of phenolic of bog bilberry syrup wine during bottle-aging. Results showed that the oak chips treatment significantly increased the content of phenolic compounds. The content of total polyphenols and antioxidant capacity decreased during 6 months of aging. **Massini et al., (2016)** increased the antioxidant capacity and total polyphenols of vegetable juices. They made an extract of apple peels which was rich especially in flavan-3-ols, flavonol glycosides and dihydrochalcones. Results showed that the addition of apple peel phenolic extract led to significantly higher radical scavenging capacity and to an increased protection against lipid peroxidation compared to control.

CONCLUSION

Lignans, as health-promoting substances, possess a great potential; their consumption is, however, low within the population since their levels are not high in common foodstuffs. For this reason, the exploitation of unconventional sources of lignans presents an attractive way of sourcing and further use. Extraction methods ensure that the substances can be sourced from potential waste materials such as wood chips. Application into foods, however, is often associated with changes in the sensory quality of the foodstuff as a result of the bitter flavour of the extract. It is therefore appropriate to add lignans into foods that are distinctive in terms of taste.

The results of evaluating the consumer acceptability show that the addition of 340 mg of lignans per kg of product is the least acceptable from a sensory aspect. In such samples, the assessment ranged from 46.7 to 71.6 scores out of a total of 100. In terms of consumer experience, the samples containing 170 mg of lignans per kg were almost as acceptable for the assessors as were the control samples with no addition of lignans. In samples containing 170 mg of lignans per kg the scoring ranged from 61.2 to 74.7 out of a total of 100. In terms of added lignans, strawberry spread intended for adding to yoghurt was the least suitable while in strawberry jam both of the lignan doses were rated positively. Addition of lignans into plum jam is the best when the amount is 170 mg per kg, as with the blueberry spread. For the red pepper paste, both lignan doses were rated almost equally and reached over 60 scores out of 100. In food products fortified by the lignan extract there was a positive increase in antioxidant capacity. Of the tested types of fruit and vegetable spreads, plum jam, strawberry jam and red pepper paste are the most suitable materials, their naturally distinctive flavour overlapping the taste of lignan extract being the very reason. $mg.kg^{-1}$

REFERENCES

Balík, J., Soural, I., Híc, P., Tříska, J., Vrchotová, N., Moos, M., Houška, M., Landfeld, A., Strohalm, J., Novotná, P. 2014. Grape juices with an increased content of lignans. *Proceedings 37th World Congress of Vine and Wine*. Mendoza, Argentina, Nov. 9-14, Book of Abstracts, p. 784-785.

Balík, J., Híc, P., Kulichová, J., Novotná, P., Tříska, J., Vrchotová, N., Strohalm, J., Houška, M. 2016. Wines with increased lignan content by the addition of lignan extracts. *Czech Journal of Food Science*, vol. 34, p. 439-444. https://doi.org/10.17221/575/2015-CJFS

Brusentsev, Y., Eklund, P. 2015. Synthesis and applications of diphoshine ligands derived from the lignan hydroxymatairesinol. *Catalysis Today*, vol. 241, p. 260-263. https://doi.org/10.1016/j.cattod.2014.02.032

Dinkova-Kostova, A., Gang, T., Davin, R., Bedgar, D., Chu, A., Lewis, N. 1996. (+)-Pinoresinol/(+)-lariciresino reductase from Forsythia intermedia. *The Journal of Biological Chemistry*, vol. 271, no. 47, p. 29473-29482. PMid:8910615

Draijer, R., den Hartog, S., Mulder, T. P. J. *Food composition. Patent US20090186118, 2009.*

Haramatha, J. 2005. Strukturní bohatství a biologický význam lignanů a jim příbuzných rostlinných fenylpropanoidů. *Chemické Listy*, vol. 99, no. 9, p. 622-632.

Holmbom, B. Eckerman, Ch., Eklund, P., Hemming, J., Nisula, L., Reunanen, M., Sjöholm, R., Sundberg, A., Sundberg, K., Willför, S. 2003. Knots in trees – a new rich source of lignans. *Phytochemistry Reviews*, vol. 2, no. 3, p. 331-340. https://doi.org/10.1023/B:PHYT.0000045493.95074.a8

Híc, P., Balík, J., Kulichová, J., Tříska, J., Vrtochová, N., Strohalm, J., Houška, M. 2014. *Extraction method of lignans from conifer knots and usage of extract for food purposes. Application for National Patent No. 2014-870, Industrial Property Office, Czech Republic.*

Hussain, S., Muhammad, A., Butt, M., Khan, M., Asghar, A. 2006. Physical and Sensoric Attributes of Flaxseed Flour Supplemented Cookies. *Turkish Journal of Biology*, vol. 30, p. 87-92.

Johnsson, P., Peerlkamp, N., Kamal-Eldin, A., Andersson, R. E., Andersson, R., Lundgren, L. N., Åman, P. 2002. Polymeric fractions containing phenol glucosides in flaxseed. *Food Chemistry*, vol. 76, no. 2, p. 207-212. https://doi.org/10.1016/S0308-8146(01)00269-2

Kapoor, S., Ranote, P. S. 2016. Antioxidant components and physico-chemical characteristics of jamun powder supplemented pear juice. *Journal of Food Science and Technology*, vol. 53, no. 5, p. 2307-2316. https://doi.org/10.1007/s13197-016-2196-x PMid:2740719

Landete, J. 2012. Plant and mammalian lignans: A Review of source, intake, metabolism, intestinal bacteria and health. *Food research international*, vol. 46, no. 1, p. 410-424. https://doi.org/10.1016/j.foodres.2011.12.023

Liu, S., Wang, S., Yuan, G., Ouyang, X., Liu, Y., Zhu, B., Zhang, B. Effect of Oak Chips on evolution of phenolic compounds and color attributes of bog bilberry syrup wine during bottle-aging. *Journal of Food Science*, vol. 81, p. 2697-2707. https://doi.org/10.1111/1750-3841.13532 PMid:27755647

MacRae, W. D., Towers, G. H. N. 1984. Biological activities of lignans. *Phytochemistry*, vol. 23, no. 6, p. 1207-1220. https://doi.org/10.1016/S0031-9422(00)80428-8

Massini, L., Rico, D., Martin-Diana, A. B., Barry-Ryan, C. 2016. Apple peel flavonoids as natural antioxidant for vegetable juice applications. *European Food Research and Technology*, vol. 242, no. 9, p. 1459-1469. https://doi.org/10.1007/s00217-016-2646-8

Novotná, P., Tříska, J., Híc, P., Balík, J., Vrchotová, N., Strohalm, J., Houška, M. 2016. Musts with and increased content of lignans from added spruce knot chips. *Czech Journal of Food Science*, vol. 34, no. 4, p. 318-324.

Perlman, D., Ramonas, L. 2008. *Astringency-compensated polyphenolic antioxidant-containing comestible composition. Patent US 2008/0044539.*

Peterson, J., Dwyer, J., Adlercreutz, H., Scalbert, A., Jacques, P., McCullough, M. 2010. Dietary lignans: physiology and potential for cardiovascular disease risk reduction. *Nutrition Reviews*, vol. 68, no. 10, p. 571-603. https://doi.org/10.1111/j.1753-4887.2010.00319.x PMid:20883417

Slanina, J. 2000. Biologická a farmakologická aktivita lignanů. *Chemické listy*, vol. 94, no. 2, p. 111-116.

Taskinen, A., Eklund, P., Sjohölm, R., Hotokka, M. 2004. The molecular structure and some properties of hydroxymatairesinol. *Journal of Molecular Structure (Theochem)*, vol. 677, no. 1-3, p. 113-124. https://doi.org/10.1016/j.theochem.2004.01.022

Wicklund, T., Rosenfeld, H. J., Martinsen, B. K., Sundfør, M. W., Lea, P., Bruun, T., Blomhoff, R., Haffner, K. 2005.

Antioxidant capacity and colour of strawberry jam as influenced by cultivar and storage conditions. *LWT - Food Science and Technology*, vol. 38, no. 4, p. 387-391. https://doi.org/10.1016/j.lwt.2004.06.017

WillföR, S., Nisula, L., Hemming, J., Reunanen, M., Holmbom, B. 2004. Bioactive phenolic substances in industrially important tree speciec. Part 2: Knots and stemwood of fir species. *Holzforschung*, vol. 58, no. 6, p. 650-659.

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THE ECONOMIC IMPACT OF REDUCED VALUE ADDED TAX RATES FOR GROCERIES

Slavomíra Martinková, Anna Bánociová

ABSTRACT

The value added tax represents one of the most important sources of state budget revenues of EU Member States. The basic value added tax rate is in the EU currently between 15% in Luxembourg to 27% applied in Hungary. The revenues from this tax represent an average of 17.5% of all tax revenues of EU countries and create an average GDP of 7.0% (year 2016, EU 28). As revenues from value added tax represent a stable income of state budget, the legislative changes in the system of value added tax, mainly its reductions as well as its imposition on groceries, can significantly influence further macroeconomic development. In the last year, the government of the Slovak Republic implemented changes in universal indirect taxing in such way that in addition to the standard value added tax rate of 20%, the Act No. 268/2015 on Value added tax adopted in 2016 a decreased value added tax rate of 10% on selected groceries, in order to support domestic producers and reduce the tax burden of low-income and middle-income groups. According to the European Commission (2007), the reduced rate of value added tax in selected cases has its justification and importance in the country's economy. The aim of this paper is to analyse the economic impact of the applied reduced value added tax on food in the Slovak Republic in the context of household expenditures and revenues of the state budget.

Keywords: reduced VAT rate on groceries; VAT rates; tax revenues; expenditure of household

INTRODUCTION

A modern system of value added tax (VAT) creation within the European Union should be in line with the goal of increasing the economic activity and ensuring the functioning of the internal market. The uniform VAT system is a part of **Council Directive 2006/112/ES** on common system of VAT, with the view to a neutral competition and harmonization of VAT legislation, including tax rates harmonization. The Member States committed with the adoption of this Directive to apply the standard VAT rate, fixed at a percentage of a taxable amount, which would be equal for both provided goods and services and not lower than 15%. At the same time, they committed to apply a maximum of two reduced rates on selected categories listed in the annex to that Directive, and these rates must be at the percentage of at least 5%, with the exception of lower rates only if these lower rates existed before 1993. The VAT system is currently used by almost all of the most advanced economies of the world, which were joined also by Slovakia when the Act No. 222/1992 on Value Added Tax was adopted **(European Commission, 2016; Bánociová, 2009; Šoltés and Jakubíková, 2008; Mihóková et al., 2016)**. This Act has been amended several times and the last amendment, effective since the 1st of January 2016, added a reduced VAT rate on selected foods at 10%, while these foods account for about 5% of the consumer basket.

Foods have a special position in household's consumption basket, which has been confirmed by several analyses, as they represent significantlly higher share of the total household costs compared to the share of other goods and services **(Syrovátka, 2011)** and they represent significantlly higher share of the total household costs compared to the share of gainful employment **(Kozelová et al., 2011)** and to the share of environmental education **(Kozelová et al., 2013; Kozelová et al., 2010)**. **The World Bank (2016)** states that the purchase of goods, beverages and tobacco represent more than 20% of total households' costs in the countries with high level of incomes, 30% of total households' costs in the countries with the middle-incomes and low-income countries account for 50% of total households' costs. Within the EU, most of the economies are considered to be economies with high level of incomes, which supports the assumption that the share of households' costs on the total purchases of goods represents estimated 20%. Despite this, the relatively high level of income distribution disparities in the countries, encourages the economic authorities to introduce reduced VAT rates in particular on food, in order to decrease tax burden of low-income and middle-income groups of households. The structure of household's expenditures distribution results from the functional demand system **(Deaton, 1986)**, the effects of indirect taxes on the public economies **(Myles, 1996)** and from consumer decisions influenced by economic factors **(Koutsoyiannis, 1979)**, healt factors **(Čapla et al., 2008; Zajác et al., 2012; Šoltés and Gavurová, 2015)** and social factors **(Nicholson, 1992; Šoltés and Gavurová, 2016)**.

The aim of this paper is to analyse the economic impact of reduced VAT on foods in the Slovak Republic (SR). Categorically we can state that implementation of reduced

VAT on selected types of groceries creates a space for broader economic consequences associated with the changes in household's consumption, as well as the changes in efficiency of occurred costs, income distribution in the country and with the change in pricing process **(Oosterhius, et al., 2008)**.

The paper is divided into three parts. The first part presents the theoretical implications of reduced VAT rate on food. The second part is devoted to the methodology and the possible options of VAT examination at international level. The third part is focused on the analyses and assessment of consequences of reduced VAT on food in conditions of Slovakia.

Economic significance of implementing reduced VAT on selected food

Knowledge of the impacts of tax changes contributes to the understanding of distribution effects of tax systems. **Aasness et al., (2002)** analyse the mentioned effects of indirect taxes, out of which the major tax reform is the impact of the reduced VAT on all goods and specifically on food.

Tax reduction or transfer increase lead to a partial increase of household's living standard because it allows an increase of private consumption. On the other side, within the distribution effects of indirect taxes, **Holman (2002)** points out the growth of goods' prices and restrictions in private consumption. The rice in prices does not suppress incentives for tax evasion.

From the macroeconomic point of view, the consumption taxation is the most often analysed by examining the estimated impact of hypothetical VAT unification on households **(Crawfood et al., 2010)**, by examining the distributional impact of previous VAT changes on households **(Klazar et al., 2007; Dušek and Janský, 2012)**, respectively by microsimulation of VAT reforms effects **(Åvitsland and Aasness, 2004)**. The results confirm that the impact of reduced VAT on food affects the growth of equality levels between economic entities. The distribution effects of tax changes and the impact of reduced VAT on food are the most commonly measured by aggregate indicators of living standards to the consumption of households **(Aasness, Benedictow and Hussein, 2002)**. **Dráb and Mihóková (2013)** expect that the economic growth will fluctuate in the same direction as the aggregate consumption, as consumption is one of the components of GDP. Determining the impact of VAT rate changes on country's economy represents a very complex issue and **Andrejovská and Martinková (2016)**. Consider also GDP being the most important classifier of changes. Subsequent development of changes in VAT rate on food significantly influences also the trends in agricultural market **(Harčariková, 2015)**.

The standard research of VAT usually analyses a horizontal supply curve, where the tax burden is passed on the consumer **(Stiglitz, 2000)**. The reduced VAT rate leads to different theoretical assumptions. Introduction to optimal indirect taxation at a reduced VAT was studied by **Ramsey (1927)** and later applied by **Boiteux (1956)**. Also **Ahmad and Stern (1984)** analysed the optimal indirect taxation. The conclusions of the authors show that VAT decrease leads to lower prices of goods and to increased demanded quantity in such manner that tax revenues are partially returned to their hypothetical previous level. The precondition to such situation is a sufficiently high demand elasticity.

Decrease in VAT rate in the context of pricing process changes was studied by **Crum et al. (1942)**. Reduced indirect universal tax categorically does not preclude maintaining a price of selected commodities at the same level and that is for two reasons. First, market conditions may be adjusted in a way that the total price paid by consumer is not affected by any tax change. Reduced VAT rate represents a profit for seller. Secondly, the conditions controlling the market price of commodity function in such way that a positive VAT rate change actually reduces the value of commodity in its full amount. Reduced tax rate represents a decrease of seller's revenues **(Mura, Buleca, Hajduová and Andrejkovič, 2015)**. **Carbonnier's paradox (2005)** reflects the changes in VAT rates into the prices of goods by analysing the competitive environment. The increase of VAT rate is transferred to customer in a higher extent than it is in case of the adequate VAT rate decrease. Similar conclusions were reached by **Benkovskis and Fadajeva (2013)**.

Within the economic significance of implementing the reduced VAT rate on food, it is desirable to further investigate the impact of reduced VAT on food in the context of food prices, short-term household spending, income equality of individual population groups and in the context of country's economic growth.

MATERIAL AND METHODOLOGY

Possibilities of international comparison of value added tax allow to track the selected indicator based on the analysis of economic activity of individual taxes or based on tax entities in the economic environment of a given state. Database of **Organisation for Economic Co-operation and Development (2016), European Commission (2016) and Eurostat database (Eurostat, 2016)** follow the VAT rates through standard and reduced VAT rates, as revenues of the state budget in its absolute amount, expressed in national currency, respectively in its relative amount in relation to total tax revenues or as % of GDP.

In order to fulfil the aim of this paper, the analytical part is divided into three parts. The first part presents a qualitative analysis, which compares standard and reduced VAT rates on food in selected EU countries, in order to capture the specifics of tax systems in 2016. The second and third part represent a quantitate analysis. The second part follows the development of household's expenditures on non-durable and semi-durable goods, as previous studies **(EU Commission, 2007)** argues that lower VAT rates should improve the overall fairness. This implies the creation of more equal income distribution and promotion of good consumption with the intention to map the position of individual EU countries in the context of reduced tax on selected food. The third part uses non-hierarchical clustering methods with the aim to identify the unique categories of tax characteristics of selected clusters in order to capture the economic position of Slovakia in a particular cluster.

Selected variables are categorized for individual countries in two groups:

- *tax variables*, such as VAT rate in its standard and reduced form in the context of its application to food, the share of VAT revenues as % of GDP whereas the changes and adjustments of VAT rates impact the revenues of state budget,
- *macroeconomic variables*, such as household consumption expressed as a share of household expenditures on non-durable, semi-durable goods and services as percentage of GDP, the equality of living standard determined by indicator *1 – Gini coefficient*, which is a key measure of inequality stated in the economic literature **(Aaberge, 2001)**.

The data are obtained from the OECD database **(OECD, 2016)**, Eurostat database **(Eurostat, 2016)** and from the report of European Commission. The analyses were processed through software R.

Analysis of implementation of reduced VAT rates on food in selected EU countries

The analytical part of the paper is divided into three parts and those are the qualitative analysis of reduced VAT rates on food, the quantitative analysis of impact of reduced VAT rates on food in the context of household expenditures on consumption of non-durable goods and the third part presents the position of SR in international comparison of reduced VAT rate on food.

Qualitative analysis of reduced VAT rate on food

The first part of the analysis studies the currently implemented reduced VAT rates on food in individual EU Member States and their comparison with the standard VAT rates. Figure 1 below illustrates the aforementioned VAT rates as published by the **European Commission (2016)** to January 2016, expressed as a percentage.

When analysing the reduced VAT rates on selected food, we study several differences in EU28 countries. Currently, the lowest VAT rate on food is applied in the United Kingdom (UK) at 0%. On the other side, the highest rate is currently used in Hungary (HU) at 22%. The categories of reduced VAT rate on food by subject of taxation is shown below in Table 1.

In most countries, the most commonly applied reduced VAT rate is the rate for particular individual group of foods, divided into categories of basic groceries, selected foods (bread, butter, milk and other) and food for infants.

Most countries use at least one reduced VAT rate. Out of the five clusters, the cluster with the highest number of countries with the reduced VAT rate is the cluster with selected foods, further divided in several categories.

CZ applies the reduced VAT rate on food classified as essential baby food (10%) and on food in general VAT rate of 15%. HU uses the reduced VAT rate mainly on wild pigs, cattle and sheep (4%) and other food is taxed by VAT rate of 18%. Similarly, in IE, where the reduced rate is applied on livestock used for food preparation (4.8%) and further 9% and 13.5% VAT. HR introduced 5% on selected foods (bread, milk and baby food) and on food in general VAT of 13%. IT also applies two rates on food of 4% and 10%, the same like in PL 5% and 8%, in PT 6% and 13%, on FR 5.5% and 10% and in ES 4% and 10%.

The reduced rate on all basic groceries is used in CY and EL. All groceries are taxed by the reduced rate in LU, NL and SI. BE includes in selected foods also „takeaway" food. In contrary, some countries restrict the usage of reduced VAT rates only on narrow scale of food, e.g. in LV i tis only baby food. DK is the only EU country which applies only one rate, without the existence of reduced rates.

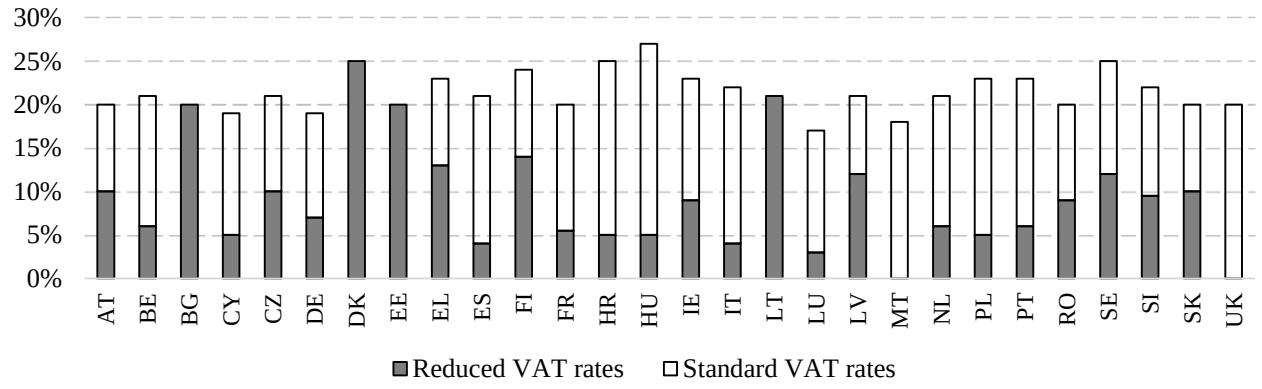

Figure 1 Standard VAT Rates And Reduced VAT Rates on Food by the EU Member States (European Commission, 2016).

Table 1 Basic categories of foods based on the implementation of reduced VAT rates on food in the EU Member States.

Without reduced VAT rate	BG, DK, EE, LT
Selected foods	BE, CZ, DE, ES, FR, HR, HU, IE, IT, LV , PL, PT, RO, SE, SK
Foods supporting agro-food producers	FI,
Food	LU, NL, SI
Basic groceries	AT, CY, EL, MT, UK

Source: Authors' elaboration.

Slovakia has joined other European countries by introducing the reduced VAT rate of 10% and this rate is applied only on selected category of food. The average reduced rate represents 9.14%, which is slightly less than rate in SR.

The impact of reduced VAT rate in the context of short-term household expenditures and equalities of income distribution in country

The second part of the analysis tracks the impact of reduced VAT rate in the context of short-term household expenditures spent on non-durable or semi-durable goods and services, for the period of second quarters of 2015 and 2016. The previous studies **(EU Commission, 2007)** argue that lower VAT rates should improve the overall fairness, which implies the creation of a more equal income distribution and should increase the consumption of goods. The first argument generates more equal income distribution on consumption, especially of cheaper goods. Expenditure on such goods shall include expenditures of household's day to day needs. Decrease in VAT on food affects households in changing their consumption in a positive way.

The second argument is based on the observation that the reduced VAT rates on selected goods could promote consumption of other goods in case of excess consumption value. In the context of effectiveness of reduced VAT rate, its impact is influenced by the level of income distribution.

The standard of living in the country is monitored by the indicator *1 – Gini coefficient*. The more the value is closer to 1, the income distribution is more equal **(Aaberge, 2001)**. Data on the Gini coefficient are published annually by Eurostat **(Eurostat, 2016)** and they are tracked in this paper for two latest available periods 2014 and 2015. The assumption is a shift of income distribution impact on household expenditures by one period backwards. The results of comparison of two indicators are shown on graphs below (Figure 1 and Figure 2).

OECD indicates the average amount of total expenditures at 60% of GDP, while the expenditures on non-durable consumption represent on average 52.47% (2015) and 52.42% (2016) in EU28. The average value of living standard reached 69.48%. Thus the countries represent the economies with a greater income equality in a long term. The increase of the Gini coefficient values occurred in countries with different socio-political profiles, but certain characteristic differences remained unchanged. The highest equality is reported in SI, FI, SE, DK and NL. In countries with a low share of household expenditures and lower VAT rate on food, a reduced VAT rate has only a limited impact on income distribution and the associated compliance costs are relatively low, e.g. SE, LU, NL. In countries with a high share of household expenditures on non-durable consumption, the reduced rate should have a greater impact on income distribution, e.g. CY, EL, RO.

In the case of Slovakia, the development of household expenditures on non-durable consumption did not change significantly as it slightly decreased by 0.6% to 50.0%. The data show that the household expenditures on non-durable consumption represent stable and easily estimable expenses, which did not change significantly during two monitored periods. VAT decrease had no significant impact on changes in household consumption of non-durable and semi-durable goods. The coefficient of equality of disposable incomes increased in SR to 76.3%, which represents an increase of 2.4%. Compared internationally, Slovakia is among the countries with the lowest income differences. Thus, it ranks to the group of countries such as SI, SE, CZ.

Position of SR in international comparison with EU countries in the context of reduced VAT rate on food

The third part of the analysis is based on non-hierarchical clustering procedures, k-means method of non-hierarchical clustering and on the assumption of optimal number of 4 clusters. The non-hierarchical clustering input variables include data on the level of tax rate on food in the country in 2016, data on final short-term household expenditures in

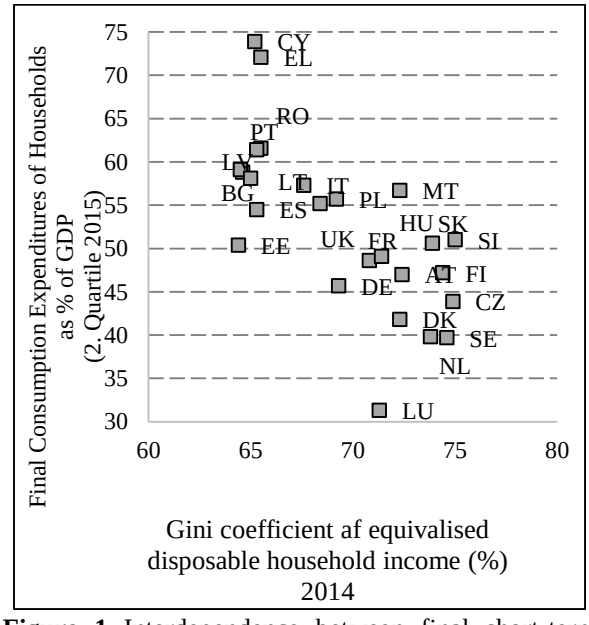

Figure 1 Interdependence between final short-term expenditures of households and living standards in country in 2015.

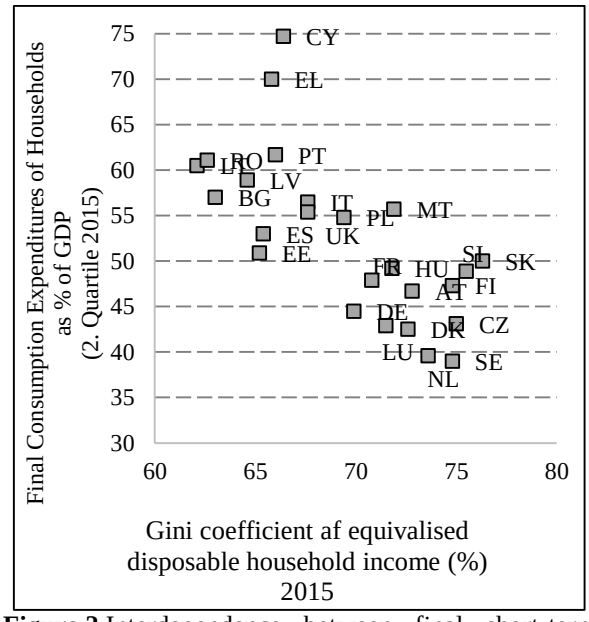

Figure 2 Interdependence between final short-term expenditures of households and living standards in country in 2016.

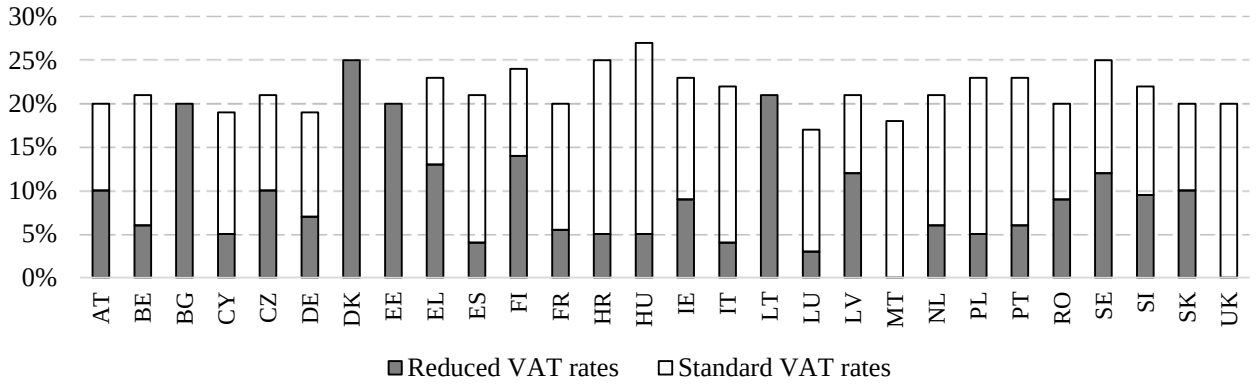

Figure 1 Standard VAT Rates And Reduced VAT Rates on Food by the EU Member States (European Commission, 2016).

Table 2 Basic categories of foods based on the implementation of reduced VAT rates on food in the EU Member States.

Without reduced VAT rate	BG, DK, EE, LT
Selected foods	BE, CZ, DE, ES, FR, HR, HU, IE, IT, LV , PL, PT, RO, SE, SK
Foods supporting agro-food producers	FI,
Food	LU, NL, SI
Basic groceries	AT, CY, EL, MT, UK

Source: Authors‘ elaboration.

the second quarter of 2016, the expected revenues from VAT in 2016 determined as the average of previous 8 years. The result is shown as clusters of two, four, eight and eleven countries (Figure 4). Input variables are shown below in (Figure 5).

The first cluster is characterized by significantly lower average VAT rate on food (5%) than the other clusters and includes also countries with zero or low VAT rate. The average amount of VAT revenues represents 6.90% of GDP and household expenditures on non-durable consumption are at 57.13% of GDP. The second cluster contains the highest number of countries and the VAT rate ranges from 3% (LU) to 14% (FR). What matters is the amount of VAT revenues, which is at 7.50% of GDP and represents an average value of countries in this cluster. The household expenditures in this cluster are reported at below average level (EU average is 52.06% of GDP). The third cluster represents the countries with the above average level of VAT revenues and also the above average value of household expenditures on non-durable consumption. The last cluster is characterized primarily by the countries that do not apply the reduced VAT rate on food. The amount of VAT revenues represents on average 8.7% of GDP and the household expenditures on non-durable consumption are at 53.22% of GDP. Within the 28EU Member States were identified differences in reduced VAT rates, as well as in tax revenues and household expenditures. These differences are in the macroeconomic indicators of countries, but also in the economic policies of individual governments. **Remeta (2015)** notes that changes in VAT will affect in SR mainly the wealthier households and that to support the tax progressivity should be used fiscal Instruments, since VAT decrease is reflected in consumer prices only partially. Transfer of selected food in the group of commodities with the reduced VAT rate has no significant negative impact on state budget, as it is mainly the compensation of VAT rate change from 19% to 20%.

CONCLUSION

The improving macroeconomic situation encourages the countries to implement an expansive fiscal policy, which uses the reduced VAT rate on selected food as one of its instruments. In order to analyse the economic impact of introducing reduced VAT in SR, the paper concludes that the reduced VAT on food has been implemented by majority of EU countries benefiting from the reduced Vat rate on selected food. The country created opportunities for wider economic consequences associated with the change in household consumption. By implementing the reduced VAT rate on selected food, Slovakia has been ranked among the countries with the lowest income differences and the average household expenditures on non-durable goods, while the introduction of reduced VAT rate has no significant impact on the development of tax revenues.

The interim results in the area of VAT revenues indicate that the introduction of reduced VAT on food can be considered as a positive measure of the government.

REFERENCES

Aasness, J., Benedictow, A., Hussein, M. F. 2002. Distributional efficiency of direct and indirect taxes. *Economic Research Programme on Taxation Report*, no. 69, p. 50.

Act No. 222/1992 Coll. on value added tax. The National Council of the Slovak Republic.

Ahmad E., Stern, N. 1984. The Theory of Reform and Indian Indirect Taxes. *Journal of Public Economics*, vol.

25, no. 3, p. 259-298. https://doi.org/10.1016/0047-2727(84)90057-4

Andrejovská, A., Martinková, S. 2016. The Impact of Value Added Taxes Rates on the Economy of the European Union Countries using Data Mining Approach. *Journal of Applied Economic Sciences*, vol. 6, no. 44, p. 1084-1096.

Åvitsland, T., Aasness, J. 2004. *Combining CGE and microsimulation models: Effects on equality of VAT reforms.* Discussion Papers No. 392, October 2004. Statistics Norway, Research Department. 42 p.

Bánociová, A. 2009. Analýza vývoja dane z pridanej hodnoty v Slovenskej republike (Analysis of the development of value added tax in the Slovak Republic). *E&M Ekonomie a management*, no. 4, p. 104-115.

Benkovskis, K., Fadejeva, L. 2013. The *Effect of VAT Rate on Price Setting Behaviour in Latvia*, Evidence from CPI Micro Data, Working Paper, no. 01. Riga : Latvijas Banka, 48 p.

Boiteux, M. 1956. Sur la gestion des Monopoles Publics astreints a l'equilibre budgetaire (The management of public monopolies subject to budgetary equilibrium). *Econometrica*, vol. 24, no. 1, p. 22-40. https://doi.org/10.2307/1905256

Čapla, J., Zajác, P., Golian, J. 2008. Nový návrh nariadenia Európskeho parlamentu a rady o poskytovaní informácií o potravinách spotrebiteľom. *Potravinarstvo*, vol. 2, no. 2, p. 5-8. http://www.potravinarstvo.com/dokumenty/potravinarstvo_no2_2008.pdf

Carbonnier, C. 2005. *Is tax shifting asymmetric? Evidence from French VAT reforms, 1995-2000. Working Papers, no. 34.* Paris : Paris-Jourdan Sciences Economiq, 24 p.

Council directive 2006/112/EC of 28 November 2006 on the common system of value added tax. OJ L 347, 11.12.2006, p. 1–118

Crawford, I., Keen, M., Smith S. 2010. Value added tax and excises. In Mirrlees, J. Adam, S., Besley, T., Blundell, R., Bond, S., Chote, R., Gammie, M., Johnson, P., Myles, G., Poterba, J. (eds.) *Dimensions of tax design: the Mirrlees review*, p. 275-365. ISBN 978-0-19-955375-4.

Crum, W. L., Fennelly, J. F., Seltzer, L. H. 1942. *Fiscal Planning for Total War*. Cambridge : NBER, 358 p.

Deaton A. 1986. Demand Analysis. In Griliches Z., Intriligator, M. D. (eds.)*: Handbook of Econometrics*, vol. 3, no. 30, p. 1767-1839.

Dráb, R., Mihóková, L. 2013. Determinants of Fiscal Consolidation Success in V4 Countries, In Mirdala, J. (Ed.) *Financial Aspects of Recent Trends in the Global Economy*, vol. 2, Craiova, Romania : ASERS Publishing, p 220-243.

Dušek, L., Janský, P. 2012. Dopady změn daně z přidané hodnoty na reálné příjmy domácností (Impact of changes in the value added tax on real incomes of households). *Politická ekonomie*, vol. 60, no. 3, p. 309-329. https://doi.org/10.18267/j.polek.844

EU Commision. 2016. *VAT Rates Applied in the Member States of the European Union, Situation at 1st January 2016.* Bruxells: Final report.

EU Commission. 2007. *Taxation papers. Study on reduced VAT applied to goods and services in the Member States of the European Union*, EU Commission: Working paper No. 13-2007. 108 p.

Eurostat, 2016. Eurostat© Database [online] s.a. [cit. 2016-10-15] Available at: http://ec.europa.eu/eurostat/data/database.

Harčariková, M. 2015. Proposal of new outperformance certificates in agricultural market. *Agricultural Economics (Zemědělská Ekonomika)*, vol. 61, no. 9, p. 400-409.

Holman, R. 2002. *Ekonomie.* 3rd ed. Praha, Czech Republic : Nakladatelství C. H. Beck, 726 p. ISBN 80-7179-681-6. 726 p.

Klazar, S., Slintáková, B., Svátková, S., Zelený, M. 2007. Dopad harmonizace sazeb DPH v ČR. *Acta Oeconomica Pragensia*, vol. 15, no. 1, p. 45-55.

Koutsoyiannis A. 1979. *Modern Microeconomics*. 2nd ed. London : Macmillan. 581 p. ISBN 0-333-25349-3. https://doi.org/10.1007/9781349160778

Kozelová, D., Mura, L., Matejková, E., Lopašovský, Ľ., Vietoris, V., Mendelová, A., Bezáková, M., Chreneková, M. 2011. Organic products, consumer behaviour on market and european organic product market situation. *Potravinarstvo*, vol. 5, no. 3, p. 20-25. https://doi.org/10.5219/96

Kozelová, D., Vietoris, V., Fikselová, M. 2013. Quality and Availability of Organic Foods by Slovak Consumers. *Potravinarstvo*, vol. 7, no. 1, p. 146-150. https://doi.org/10.5219/306

Mihóková, L., Andrejovská, A., Buleca J. 2016. Estimation of vat gap in Slovak Republic. *Actual Problems of Economics*, vol. 180, no. 6, p. 327-336.

Mura, L., Buleca, J., Hajduova, Z., Andrejkovic, M. 2015. Quantitative Financial Analysis of small and medium food enterprises in a developing country. *Transformation in Business & Economics*, vol. 14, no. 1, p. 212-224.

Myles, G. 1996. Imperfect Competition and the Combination of Ad Valorem and Specific Taxation. *International Tax and Public Finance*, vol. 3, no. 1, p. 29-44. https://doi.org/10.1007/BF00400145

Nicholson, W. 1992. *Microeconomic Theory, Basic Principles and Extensio*ns. 5th ed. Fort Worth : Dryden Press, 762 p. ISBN 0-03055043-2.

OECD, 2016. Organisation for Economic Co-operation and Development© Database [online] s.a. [cit. 2016-10-15] Available at: https://data.oecd.org/.

Oosterhius, F., Rayment, M., Varma, A, Jantzen, J, van der Woerd, H, Mudgal, S, Tinetti, B., Gerdes, H., Neubauer, A, Stocker, A, Dodoková, A, Greño, P. 2008. *The use of differential VAT rates to promote changes in consumption and innovation.* Final report. Amsterdam, Netherland : Institute for Environmental Studies. 150 p.

Ramsey, F. 1927. A Contribution to the Theory of Taxation. *Economic Journal*, vol. 37, no. 145, p. 47-61. https://doi.org/10.2307/2222721

Remeta, J., Perret, S., Jareš, M., Brys, B. 2015. Moving Beyond the Flat Tax – Tax Policy Reform in the Slovak Republic, *OECD Taxation Working Papers*, no. 22. p. 80. https://doi.org/10.1787/5js4rtzr3ws2-en

Šoltés, M., Gavurová, B. 2016. Economic aspects of social services development in Slovakia in the context of demographic changes. *Scientific papers of the University of Pardubice. Series D, Faculty of Economics and Administration*, no. 37, p. 14-25.

Šoltés, V., Gavurová, B. 2015. Modification of Performance Measurement System in the intentions of Globalization Trends. *Polish Journal of Management Studies*, vol. 11, no. 2, p. 160-170.

Šoltés, V., Jakubíková, E. 2008. Taxation and Public Finance in the Slovak Republic. *Taxation and Public Finance in Transition and Development Economies*, p. 603-308. https://doi.org/10.1007/978-0-387-25712-9_32

Stiglitz, J. E. 2000. *Economics of the Public Sector*, 3rd Ed., New York : W. W. Norton and Company. 823p. ISBN 0393966518.

Syrovátka, P. 2011. Effects of changes in the tax environment on the consumer demand for food. *Agricultural Economics*, vol. 57, no. 7, p. 350-355.

World Bank, 2016. World Bank Group© Database [online] s.a. [cit. 2016-10-15] Available at: http://data.worldbank.org/.

Zajác, P., Tomáška, M., Murárová, A., Čapla, J. and Čurlej, J. 2012. Quality and Safety of Raw Cow's Milk in Slovakia in 2011. *Potravinarstvo*, vol. 6, no. 2, p. 64-73. https://doi.org/10.5219/189

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NUTRITIONAL VALUES OF EDIBLE *COLEOPTERA* (*TENEBRIO MOLITOR*, *ZOPHOBAS MORIO* AND *ALPHITOBIUS DIAPERINUS)* REARED IN THE CZECH REPUBLIC

Anna Adámková, Lenka Kouřimská, Marie Borkovcová, Martin Kulma, Jiří Mlček

ABSTRACT

Edible insects have gained the status of highly nutritious food with high protein and fat content. However, nutritional value of insects is not constant. It could be affected by species, developmental stage, rearing technology, nutrition or sex. This study's goal is to determine the protein and fat contents of three edible beetle species (giant mealworm – larvae of *Zophobas morio*, mealworm – larvae of *Tenebrio molitor* and, lesser mealworm – larvae of *Alphitobius diaperinus*) bred in the Czech Republic. Based on the obtained results, all investigated species could be considered as a reasonable source of lipids and two of them (mealworm and lesser mealworm) are also an excellent source of protein. Crude protein content of mealworm (630 g. kg^{-1} DM) was found to be higher than in other studies. The investigated species of lesser mealworm contained 600 g of crude protein/kg DM, which was equal to the results of other authors. Most authors report a higher content of nitrogen in the giant mealworm than were the values measured by this experiment (390 $g.kg^{-1}$ DM). The lipid content in the tested samples was found in a range of 170 – 390 $g.kg^{-1}$ DM. The highest lipid content was found in the larvae of giant mealworm and the lowest lipid content was found in the larvae of mealworm. The determined fat content of lesser mealworms was 290 $g.kg^{-1}$. The fatty acid profiles of all samples were also determined.

Keywords: edible insects; nutrition; protein, fat; fatty acid; *Coleoptera*

INTRODUCTION

Edible insects form a common part of the human diet in many parts of the world **(van Huis et al., 2013; Vantomme et al., 2012)**. They are also being considered an extra food source in countries where people have limited access to sufficient, safe and nutritious food to maintain a healthy and active life **(Kampmeier and Irwin, 2009; van Huis et al., 2013; Vantomme et al., 2012)**.

Edible insects are seen as an interesting alternative source of proteins and lipids **(Zielińska et al., 2015)**. They are also believed to be an ideal option for the space agriculture **(Katayama et al., 2008)**. In the developing countries, edible insects may serve as a potential animal protein source because of its better digestibility and utilization than vegetable protein **(Hoffman and Falvo, 2004)**. They could also help the children suffering from malnutrition **(Brázdová, 2011)**.

Some species of insects could serve as an important source of lipids. Fatty profile of insects varies among different species as well as among the developmental stages within one species **(Finke, 2004)**. It may also be easily affected by the feed composition **(Schaefer, 1968; Bukkens, 1997; Mariod, Abdel-Whab and Ain, 2011).** Fatty acid composition of insect is reported to be similar to that of poultry or fish **(DeFoliart, 1992)**.

Entomophagy is not very common in Europe. Insects are usually considered a delicacy or a means to diversify one's diet. Although the amount of information about the insects' nutritional composition and the potential risks has been recently increased, insects are still not considered a standard human food. A list of edible insects (including mealworm, giant mealworm, and lesser mealworm) was published by **EFSA (2015)** together with the risk related to production and consumption of insects as food and feed. Although entomophagy is considered to be safe due to its long history, manipulation with and consumption of edible insect may involve some risks **(EFSA, 2015)**. These risks are usually represented by collecting the insects in dangerous areas without protective equipment, consuming inappropriate developmental stages or inadequate culinary treatment **(Ramos-Elorduy, 2005; Belluco et al., 2013; Mlček et al., 2014)**. The toxic substances content or allergic reactions (mostly to chitin) are among other potential risks of edible insect consumption **(Park, Kim and Yang, 2009)**.

Available data about nutritional values of insect species bred in Europe are not sufficient. The mealworm is probably the most-studied species **(Bernard, Allen and Ullrey, 1997; Oonincx and Dierenfeld, 2012; Bednářová et al., 2013** and **van Broekhoven et al., 2015)**. It could be considered as a good source of protein and lipids, although the nutritional composition varies among individual developmental stages. The highest protein content (637.0 – 676.5 $g.kg^{-1}$ DM) and the lowest fat content (148.8 – 184.0 $g.kg^{-1}$ DM) were found in adult beetles. However, adult beetles are not very suitable for human consumption because of the high anti-nutritional substances content (wings, exoskeleton, legs etc.).

From the nutritional point of view the larvae (protein: 477.6 – 527.0 $.kg^{-1}$ DM, fat 189.0 – 382.9 $.kg^{-1}$ DM) and pupae (protein: 531.3 – 546.0 $.kg^{-1}$ DM, fat

308.0 –366.5 g.kg^{-1} DM) seem to be more interesting. The nutrient content of hormonally modified mealworm form ("super mealworm") is known as well. These mealworms with artificially delayed pupation have the protein content comparable to other mealworms (471.8 g.kg^{-1} DM) but the fat content differs significantly (430.8 g.kg^{-1} DM) (calculated from **Finke, 2002**).

Giant mealworm, whose larvae could reach 55 mm **(Friedrich and Volland, 2004)**, is also considered to be a good source of quality protein and lipids. The nutritional composition of this species was determined by various authors **(Barker, Fitzpatrick and Dierenfeld, 1998; Finke, 2002; Bednářová et al., 2013; Yi et al., 2013; Bosch et al., 2014; van Broekhoven et al., 2015)**. The protein content of giant mealworm larvae was 431.3 – 516.2 g.kg^{-1} DM, the fat content was 328.0 – 435.4 g.kg^{-1} DM. **Oonincx and Dierenfeld (2012)** evaluated the nutrient content of giant mealworms adults and determined the protein level to be 680.5 g.kg^{-1} DM and lipid content 142.5 g.kg^{-1} DM.

The nutrient content of lesser mealworm is only available for larvae stages. **Bosch et al. (2014)** reported 648 g.kg^{-1} DM of protein and 222 g.kg^{-1} DM of fat. **Yi et al. (2013)** determined 580.3 g.kg^{-1} DM of protein and 239.5 g.kg^{-1} DM of fat. **Van Broekhoven et al. (2015)** found protein content to be 617 – 650 g.kg^{-1} DM and fat content 134 – 243 g.kg^{-1} DM.

Besides the factor of the above-mentioned development stage, the nutrient content of insects is also affected by feed composition, microclimate, environment, sex and other factors **(Oonincx and van der Poel, 2011)**. **Van Broekhoven et al. (2015)** reported that the feeding mixture change caused differences in content of both fat and protein (by 8 % and 11 % respectively). This research is therefore focused on the determination of basic nutrient contents of three edible insect species reared under defined farming conditions in the Czech Republic and the comparison of the obtained data with results from other countries and wild species.

MATERIAL AND METHODOLOGY

Material

The insect samples tested for the purposes of this study were larvae of darkling beetles (*Zophobas morio*, Fabricius, 1776), which are known by the common name superworm or giant mealworm, mealworm (larvae of *Tenebrio molitor*, Linnaeus, 1758) and lesser mealworm (larvae of *Alphitobius diaperinus*, Panzer, 1797). All of them are common warehouse pests and can be easily kept and bred in the European climate conditions. The samples were purchased in the ultimate or penultimate instars (most suitable to culinary purposes) from a private company Radek Frýželka, Brno. The insect species were fed by a mixture of plant material (carrots, cabbage, Chinese cabbage, tomatoes, and potatoes). Prior to the analysis, the insects were fasted for 48 hours to minimize the effects of food retained in the gut, then killed in boiling water (100 °C) and finally dried at 105 °C for 12 h. The obtained samples were then homogenized for 1 minute by the coffee grinder Scarlett Silver Line SL-1545 (ARIMA, UK) and stored at 4 – 7 °C. All sample analyses were done at least in triplicate. The used chemicals were of the p.a. grade and were purchased from the Sigma Aldrich company.

Methods

Nitrogen and crude protein content determination

The nitrogen and crude protein were analysed using the Kjeldahl's method **(ISO 1871:2009)**. The samples (1 g) and blank runs were mineralised at 420 °C for 105 min. The distillation was performed on Kjeltec™ 2200 (FOSS, Denmark) for 4 minutes. The protein content was calculated using nitrogen-to-protein conversion factor of 6.25.

Fat content determination

The fat content determination was performed by extraction using Soxhlet method **(Soxhlet, 1879)** on the Gerhardt Soxtherm SOX414 (C. Gerhardt GmbH & Co. KG, Germany). Approximately 5 g of dried and homogenized samples (with the accuracy of 0.0001 g) were put into extraction thimbles and extracted by 150 ml of petroleum ether via cold water extraction (program: 70 °C for 120 minutes). The extraction flask was then dried at 103 °C and weighed until a constant sample weight was attained.

Fatty acid profile determination

The esterification of lipids extracted form samples of insects via the Soxhlet extraction was performed according to the **ISO 12966-2:2011** standard using 0.25 M methanolic KOH (test weight of fat for esterification was 0.5 g). Methyl esters of fatty acids were analysed by GC Agilent 7890 (Agilent Technologies, USA) with a flame ionization detector (detector temperature: 250 °C) equipped with a RestekRt®-2560 column (100 m × 0.25 mm ID × 0.2 μm film) from Restek Corporation. Hexane was used as a solvent and the sample volume of 1 μL was injected in split mode (ratio 20:1) into the injector heated to 225 °C. The initial oven temperature was 70 °C (hold 2 min), ramp1 to 225 °C at 5 °C/min (hold 9 min), ramp2 to 240 °C at 5 °C/min (hold 15 min). Helium was used as carrier gas with the flow rate of 1.2 mL/min. The methylated fatty acids were identified using a Restek Food Industry FAME mix (cat#35077). Real chromatogram of Restek Food Industry FAME mix is shown in Figure 1. The proportions of fatty acids were calculated using the area normalisation method.

Statistical analysis

The data were analysed using Excel 2013 (Microsoft Corporation, USA) and the results were expressed by means ±standard deviations.

RESULTS AND DISCUSSION

Crude protein and fat contents of the three investigated edible insect species are shown in Table 1. The obtained values of crude protein in tested insects ranged from 390 to 630 g.kg^{-1} DM. The protein content of *Tenebrio molitor* was found to be higher than in the studies of **Bernard, Allen and Ullrey (1997); Finke (2002); Ramos-Elorduy (2006); Oonincx and Dierenfeld (2012); Yi et al. (2013)** or **van Broekhoven et al. (2015)**. It was also higher than the levels reported by **Bednářová et al. (2013)** who measured the nutrient content of insects bought from a local Czech supplier. The protein content of *Alphitobius diaperinus*is found in this study were consistent with the results reported by **Yi et al. (2013); Bosch et al. (2014)** and **van Broekhoven et al. (2015)**.

Table 1 Lipid and crude protein contents of three edible insect species.

species	crude protein	lipids
	g.kg^{-1} DM ±SD	
Giant mealworm *(Zophobas morio)*	390 ±1	390 ±4
Mealworm *(Tenebrio molitor)*	630 ±4	170 ±1
Lesser mealworm *(Alphitobius diaperinus)*	600 ±5	290 ±3

Table 2 Fatty acid profile of analysed samples.

Fatty acid composition	TM (%)	ZM (%)	AD (%)
SFA			
C8:0	<0.1	1.8	<0.1
C10:0	<0.1	0.4	<0.1
C12:0	0.2	0.1	0.1
C13:0	0.1	<0.1	<0.1
C14:0	3.5	1.7	1.4
C15:0	0.2	0.4	0.3
C16:0	18.4	30.2	26.4
C17:0	0.3	0.7	0.7
C18:0	6.6	8.8	10.9
C19:0	0.1	0.1	0.2
C20:0	0.3	0.2	0.6
C22:0	0.1	0.1	<0.1
Sum of SFA	29.7	44.6	40.6
MUFA			
C14:1, cis - 11	0.1	<0.1	<0.1
C16:1, trans - 11	0.1	<0.1	<0.1
C16:1, cis - 9	1.4	0.7	1.1
C17:1, cis - 10	0.1	0.2	0.2
C18:1, trans - 9	<0.1	<0.1	0.2
C18:1, cis - 9	36.5	31.1	35.9
C20:1, cis - 11	0.1	0.1	0.4
Sum of MUFA	38.4	32.1	37.8
PUFA			
C16:2, trans - 7,10	0.3	1.1	0.3
C 18:2, trans - 9,12	<0.1	<0.1	0.2
C18:2, cis -9,12	30.5	21.2	20.2
C 20:2, cis - 11,14	<0.1	<0.1	0.2
C18:3, cis - 9,12,15	1.1	0.9	0.4
C 20:4, cis - 5,8,11,14	<0.1	<0.1	0.4
Sum of PUFA	31.8	23.2	21.6

Note: TM - larvae of *Tenebrio molitor*, ZM - larvae of *Zophobas morio*, AD - larvae of *Alphitobius diaperinus.*

Figure 5 Giant mealworm *(Zophobas morio)* **(Karwath, 2005)**.

Figure 6 Mealworm *(Tenebrio molitor)* **(Halasz, 2008)**.

Figure 7 Lesser mealworm *(Alphitobius diaperinus)* **(USDA-ARS-GMPRC, 2016)**.

Information about this species reared in the Czech Republic is not available. Compared to traditional protein sources in human nutrition, the content of proteins of both above mentioned species *T. molitor* and *A. diaperinus* are comparable to beef loin (640 $g.kg^{-1}$ DM) or beef flank (640 $g.kg^{-1}$ DM) **(Pipek, 1995; Steinhauser, 1995)**. Crude protein content of the giant mealworm (390 $g.kg^{-1}$ DM) was lower than the contents reported by **Barker, Fitzpatrick and Dierenfeld (1998); Finke (2002); Yi et al. (2013); Bosch et al. (2014)** and also the only known Czech author dealing with this issue **Bednářová et al. (2013)**. On the other hand, the protein content was similar to that determined by **van Broekhoven et al. (2015)**. In comparison with the conventional food, this species could be considered similar to roast pork (410 $g.kg^{-1}$ DM) **(Pipek, 1995; Steinhauser, 1995)**. The differences between obtained results and other studies could probably be caused by using different feeding mixtures or analysing different developmental stages of the sampled larvae.

While the protein contents of the investigated insects varied significantly, the lipid content (Table 1) was found in a range of 170 – 390 $g.kg^{-1}$ DM. The highest lipid content was found in the larvae of the giant mealworm. The analysed lipid content of the giant mealworm was similar to the findings of other papers **(Finke, 2004; Bednářová et al., 2013; van Broekhoven et al., 2015)**. The fat content of *T. molitor* was lower than previously published works suggested. Higher values were reported by **Finke (2004); Bednářová et al. (2013); Yi et al. (2013)** and **van Broekhoven et al. (2015)**. The samples of lesser mealworm contained about fifty grams more lipids than **van Broekhoven et al. (2015)** reported. They discovered the possibility of changes in fat content (up to 10 %) to be caused by feed mixture changes. Therefore, the differences between results of this study and other reported values could be caused by the variety of used feed. In terms of lipid content, all tested species are comparable to a number of traditional foods such as eel meat (300 $g.kg^{-1}$ DM), pork rump (320 $g.kg^{-1}$ DM) or young goose meat (360 $g.kg^{-1}$ DM) **(Pipek, 1995; Steinhauser, 1995)**.

From a nutritional point of view, fatty acid content is very important. Our results in Table 2 show the fatty acid profiles of the fat extracted from the giant mealworm – larvae of *Zophobas morio*, mealworm - larvae of *Tenebrio molitor* and lesser mealworm - larvae of *Alphitobius diaperinus*. Real chromatogram samples of fatty acids composition for all selected insect species are shown in Figure 2, Figure 3 and Figure 4. The recommended ratio of fatty acids for human nutrition is SFA : MUFA : PUFA 1.25 : 1.5 : 1, but the ratio found in *Zophobas morio* is 1.9 : 1.4 : 1. The determined MUFA : PUFA ratio meets the requirements for human consumption (1.4 : 1), but the amount of SFA is significantly higher. Similar ratio is reported by **Bednářová (2013) and Jabir (2012)** – 2.2: 1.9 : 1 and 2.1 : 1.1 : 1. However, **Barroso (2014**) described a lower ratio. Higher SFA content was also determined in the case of lesser mealworms. On the other hand, **Tzompa-Sosa (2014)** presents a lower ratio (1.4 : 1.6 : 1) in case of these species.

In contrast to these species, the amount of SFA in mealworm was significantly lower. Similar results were published by **Zielinska (2015) and Barroso (2014)**. **Tzompa-Sosa (2014)** reported a significant content of MUFA (1.1 : 2.3 : 1), but on the contrary **Bednářová (2013)** measured a greater amount of PUFA (0.7 : 0.8 : 1).

These differences can be caused by a different type of feed and breeding conditions, which were not fully specified by the authors.

Professional and general public pays considerable attention to the ratio of fatty acids n-6 : n-3, which WHO recommends to be 5 : 1 for human nutrition **(Dostálová, Dlouhý and Tláskal, 2012)**. This ratio has a protective effect against non-infectious civilization diseases. The content of n-6 fatty acids in all species, that we analysed, was significantly higher (the ratios of n-6 : n-3 were 26 : 1 for TM, 22 : 1 for ZM, and 53 : 1 for AD). The giant mealworms were the closest to this requirement. On the other hand, lesser mealworms had the highest ratio from the analysed samples. Therefore, the lesser mealworm does not seem to be a perfect primary nutritional source for a long-term human consumption. However, the fatty acids proportions could be affected by changing the insects' feed composition. Balanced diet of people eating insects is also important.

A higher content of unsaturated (n-9) oleic acid was measured in all samples. The amount of this acid is comparable to the traditional sources such as beef tallow (26 – 50 %), and sheep tallow (30 – 42 %), but **DeFoliart (1992)** reported that composition of fatty acids is similar to poultry and fish. The second most represented fatty acid in giant mealworm and lesser mealworm was palmitic acid (31.1 % and 26.4 %). A similar content of this acid is to be found in rabbit lard (32 %) **(Velíšek, 2002)**. Linoleic acid was the third most abundant fatty acid in these samples (21.2 % and 20.2 %). However, in case of mealworm, the second most represented acid was the essential linoleic acid (30.5 %) and the third was palmitic acid (18.4 %). The highest content of essential α-linolenic acid was also measured in mealworm. Therefore, mealworm could be the most suitable insect of the analysed species for human consumption.

The descending order of the first four minor fatty acids for giant mealworm is the same as reported by **Bednářová et al. (2013)**. However, the ratios found differed slightly. Also, some fatty acids not detected by **Bednářová et al. (2013)** were determined. An example of such is the α-linolenic acid, whose content was measured to be twice the amount of the arachidic acid. Unfortunately, **Bednářová (2013)** did not mention the composition of the feed mixture. Our results are also in line with the data reported by **van Broekhoven et al. (2015)**. The order of the first four acids is identical, while the mutual ratio varied in dependence on feed (i.e. when insects were fed by feed with high starch and low protein content, the ratio between linoleic and oleic acid was 0.22 : 1; when they were fed by high-protein and low-starch feed this ratio changed to 0.94 : 1).

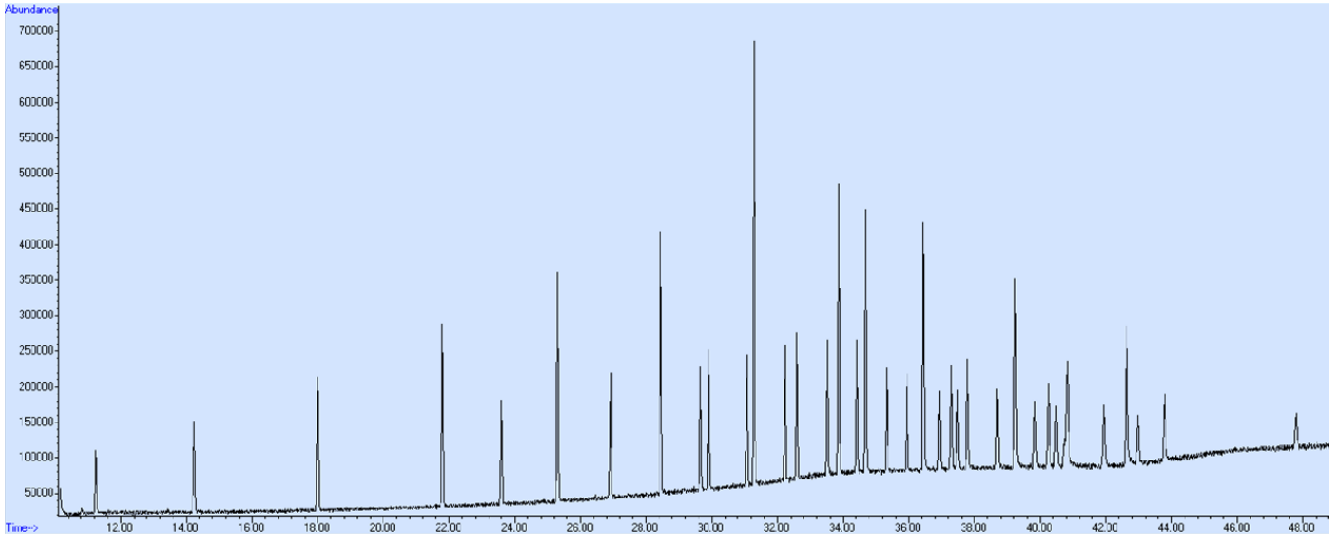

Figure 1 Chromatogram - fatty acids composition of Restek Food Industry FAME mix (cat#35077).

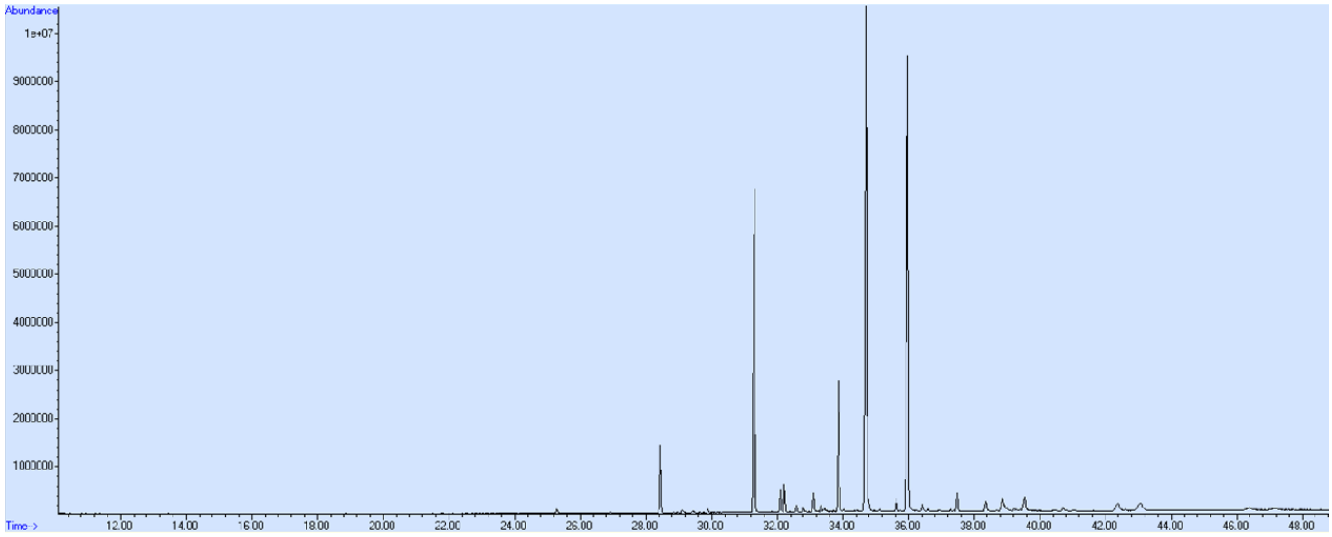

Figure 2 Chromatogram – fatty acids composition of mealworm – larvae (*Tenebrio molitor*) reared in the Czech Republic.

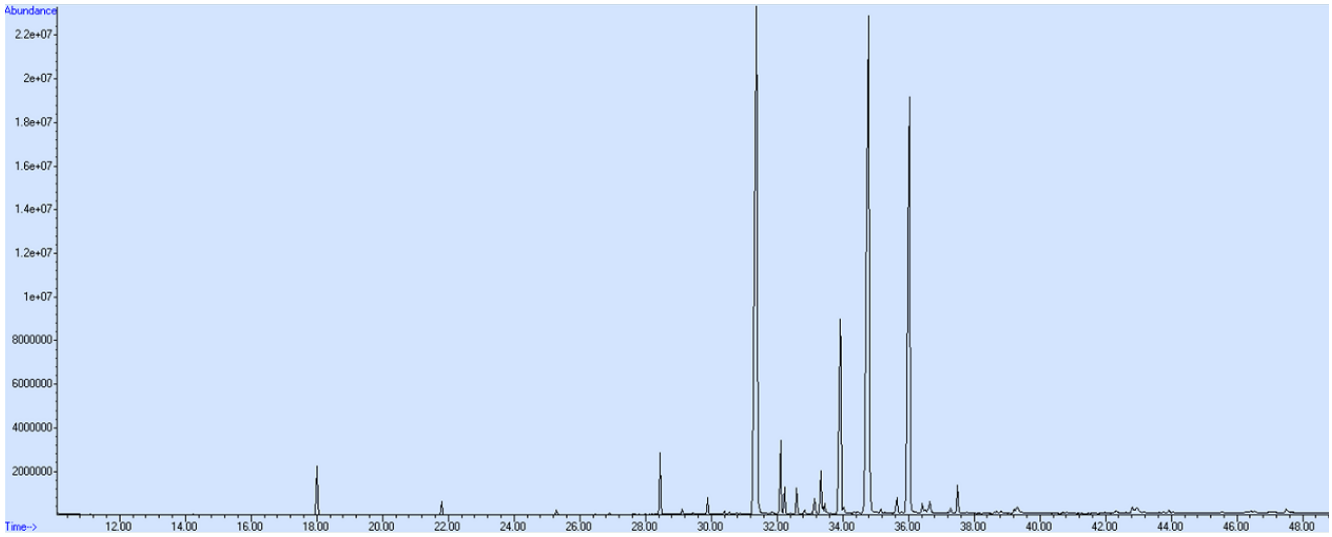

Figure 3 Chromatogram – fatty acids composition of giant mealworm – larvae (*Zophobas morio*) reared in the Czech Republic.

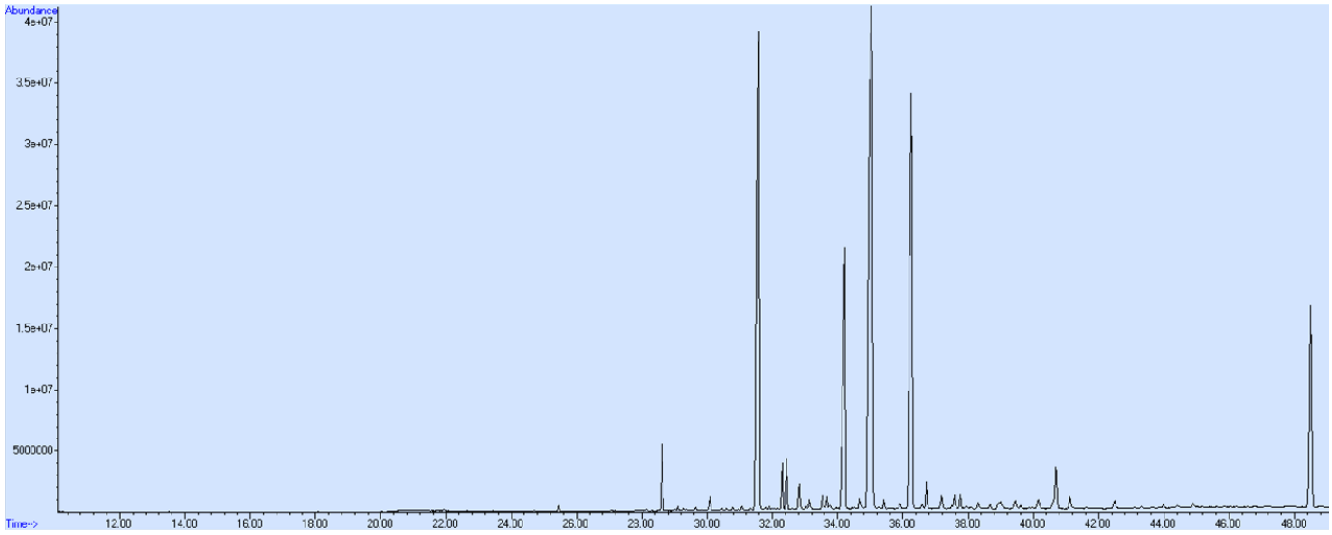

Figure 4 Chromatogram – fatty acids composition of lesser mealworm – larvae (*Alphitobius diaperinus*) reared in the Czech Republic.

CONCLUSION

This work was focused on the nutritional composition determination of three edible insect species reared in the Czech Republic. Based on the obtained results, all investigated species (*Zophobas morio*, *Tenebrio molitor* and *Alphitobius diaperinus*) could be considered as a reasonable source of lipids and two of them (mealworm and lesser meal worm) are also an excellent source of proteins. The results of fatty acids profile of the giant mealworm and lesser mealworm showed that they are not very suitable as the main food ingredient due to a high SFA content and an inappropriate n-6 and n-3 ratio. Out of all measured samples, mealworm has the highest content of linoleic and α-linolenic acid, which are among essential components of human nutrition.

REFERENCES

Barker, D., Fitzpatrick, M. P., Dierenfeld, E. S. 1998. Nutrient composition of selected whole invertebrates. *Zoo Biology*. vol. 17, no. 2, p. 123-134. https:/doi.org/10.1002/(SICI)1098-2361(1998)17:2<123::AID-ZOO7>3.0.CO;2-B

Barroso, F. G., de Haro, C., Sánchez-Muros, M-J., Venegas, E., Martínez-Sánchez, A., Pérez-Bañón, C., 2014. The potential of various insect species for use as food for fish. *Aquaculture*., vol. 422-423, p. 193-301. https://dx.doi.org/10.1016/j.aquaculture.2013.12.024

Bednářová, M., Borkovcová, M., Mlček, J., Rop, O., Zeman, L. 2013. Edible insects - species suitable for entomophagy under condition of Czech Republic. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 61, no. 3, p. 587-593. https://dx.doi.org/10.11118/actaun201361030587

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C. C., Paoletti, M. G., Ricci, A. 2013. Edible Insects in a Food Safety and Nutritional Perspective: A Critical Review. *Comprehensive Reviews in Food Science and Food Safety*, vol. 12, no. 3, p. 296-313. https://dx.doi.org/10.1111/1541-4337.12014

Bernard, J. B., Allen, M. E., Ullrey, D. E. 1997. Feeding captive insectivorous animals: Nutritional aspects of insects as food. In *Nutrition Advisory Group Handbook, Fact Sheet* 3: p. 1-7. Retrieved from the web: http://nagonline.net/801/feeding-captive-insectivorous-animals-nutritional-aspects-insects-food/

Borkovcová, M., Bednářová, M., Fišer, V., Ocknecht, P. 2009. *Kuchyně hmyzem zpestřená* 1 (The food variegation by insects). Brno, Czech Republic : Lynx, 135 p. ISBN 978-80-86787-37-4.

Bosch, G., Zhang, S., Oonincx, D. G. A. B., Hendriks, W. H. 2014. Protein quality of insects as potential ingredients for dog and cat foods. *Journal of Nutritional Science*, vol. 3, e29, p. 1-4. https//dx.doi.org/10.1017/jns.2014.23

Brázdová, Z. 2011. Výživa člověka. Výživa dítěte. (Human Nutrition. Child nutrition.). [online]. Brno: Ústav preventivního lékařství LF MU. [cit. 2014-04-07]. Available at: http://www.med.muni.cz/index.php?id=237

Bukkens, S. G. F. 1997. The nutritional value of edible insects. *Ecology of Food and Nutrition*, vol. 36, no. 2-4, p. 287-319. https://dx.doi.org/10.1080/03670244.1997.9991521

ČSN 57 0153:1986. *Metody zkoušení výrobků z masa a sterilovaných pokrmů v konzervách. Stanovení obsahu bílkovin podle Kjeldahla*. (Methods for testing products from meat and sterilized meals in cans. Determination of protein content by the Kjeldahl method.)

DeFoliart, G. R. 1992. Insects as human food: Gene DeFoliart discusses some nutritional and economic aspects. *Crop Protection*, vol. 11, no. 5, p. 395-399. https://dx.doi.org/10.1016/0261-2194(92)90020-6

Dostálová, J., Dlouhý, P., Tláskal, P. 2012. Výživová doporučení pro obyvatelstvo České republiky. *Výživa a potraviny*, (Nutrition recommendations for Czech Republic. Nutrition and Food). Společnost pro výživu, vol. 2012, no. 3. [cit.2016-02-02]. Available at: http://www.vyzivaspol.cz/vyzivova-doporuceni-pro-obyvatelstvo-ceske-republiky

EFSA, 2015. Risk pro Available at: file related to production and consumption of insects as food and feed. [online], [cit.2016-02-02]. Available at: http://www.efsa.europa.eu/ /de/efsajournal/pub/4257

Finke, M. D. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biology*, vol. 21, no. 3, p. 269-285. https://dx.doi.org/10.1002/zoo.10031

Finke, M. D. 2004. Nutrient content of insects. In *Encyclopedia of Entomology*, USA: Springer, p. 1563-1575. ISBN-978-0-306-48380-6. https://dx.doi.org/10.1007/0-306-48380-7_2920

Friederich, U., Volland, W. 2004. *Breeding Food Animals: Live Food for Vivarium Animals*. Malabar, Florida: Krieger publishing company. 178 p. ISBN 1575240459.

Halasz, P. 2008. *Tenebrio molitor*. Available at: https://en.wikipedia.org/wiki/Mealworm#/media/File:Mealworm_01_Pengo.jpg

Hoffman, J. R., Falvo, M. J. 2004. Protein-Which is best. *Journal of Sports Science and Medicine*. vol. 3, no. 3, p. 118-130. PMid:24482589

Horniaková, E., Bíro, D., Pajtáš, M., Gálik, B., Šimko, M., Juráček M., Rolinec M. 2010. *Základ vyýživy*. (Basics of nutrition.) 1st ed. Nitra: Slovenská poľnohospodárska univerzita, 137 p. ISBN-978-80-552-0446-8.

ISO 12966-2:2011. *Animal and vegetable fats and oils -- Gas chromatography of fatty acid methyl esters*. Geneva, Switzerland: ISO, 15 p.

ISO 1871:2009. *Food and feed products -- General guidelines for the determination of nitrogen by the Kjeldahl method.* Geneva, Switzerland: ISO, 7 p.

Jabir, M. D. A. R., Razak, S. A., Vikineswary, S., 2012. Chemical Composition and Nutrient Digestibility of Super Worm Meal in Red Tilapia Juvenile. *Pakistan Veterinary Journal*, vol. 32, no. 4, p. 489-493.

Kampmeier, G. E., Irwin, M. E. 2009. Commercialization of insects and their products. In Resh, V. H., Carde, R. (eds.) *Encyclopedia of Insects*, San Diego: Academic Press, p. 220-227. ISBN: 978-0-12-374144-8. https://doi.org/10.1016/B978-0-12-374144-8.00068-0

Karwath, A. 2005. *Zophobas morio.* Available at: https://en.wikipedia.org/wiki/Zophobas_morio#/media/File:Zophobas_morio_larva_-_top_(aka).jpg

Katayama, N., Ishikawa, Y., Takaoki, M., Yamashita, M., Nakayama, S., Kiguchi, K., Kok, R., Wada, H., Mitsuhashi, J. 2008. Entomophagy: A key to space agriculture. *Advances in Space Research*. vol. 41, no. 5, p. 701-705. ISSN 0273-1177. https://dx.doi.org/10.1016/j.asr.2007.01.027

Komprda, T., Zorníková, G., Rozíková, V., Borkovcová, M., Przywarová, A. 2013. The effect of dietary Salvia hispanica seed on the content of n-3 long-chain polyunsaturated fatty acids in tissues of selected animal species, including edible insects. *Journal of Food Composition and Analysis*, vol. 32, no. 1, p. 36-43. https://dx.doi.org/10.1016/j.jfca.2013.06.010

Mariod, A. A., Abdel-Wahab, S. I., Ain, N. M., 2011. Proximate amino acid, fatty acid and mineral composition of two Sudanese edible pentatomid insects. *International Journal of Tropical Insect Science*, vol. 31, no. 3, p. 145-153. https://dx.doi.org/10.1017/S1742758411000282

Mlcek, J., Rop, O., Borkovcova, M., Bednářová, M. 2014. A comprehensive look at the possibilities of edible insects as food in Europe - A Review. *Polish Journal of Food and Nutrition Sciences*, vol. 64, no. 3, p. 147-157. https://dx.doi.org/10.2478/v10222-012-0099-8

Oonincx, D. G. A. B., Dierenfeld, E. S., 2012. An investigation into the chemical composition of alternative invertebrate prey. *Zoo Biology*, vol. 31, no. 1, p. 40-54. https://dx.doi.org/10.1002/zoo.20382

Oonincx, D. G. A. B., van der Poel, A. F. B. 2011. Effects of diet on the chemical composition of migratory locusts (Locustamigratoria). *Zoo Biology*, vol. 30, no. 1, p. 9-16. https://dx.doi.org/10.1002/zoo.20308

Pánek, J., Pokorný, J., Dostálová, J. 2002. *Základy výživy a výživová politika*. (Basics of of nutrition and nutrition policy) 1st ed. Praha, Czech Republic : Vysoká škola chemicko-technologická, 219 p. ISBN: 80-7080-468-8.

Park, S. K., Kim, H. I., Yang, Y. I. 2009. Roles of vascular endothelial growth factor, Angiopoietin 1, and Angiopoietin 2 in nasal polyp. *Laryngoscope*, vol. 119, no. 2, p 409-413. https://dx.doi.org/10.1002/lary.20085

Pipek, P. 1995. *Technologie masa* I. (Meat Technology I.) Praha, Czech Republic : VŠCHT, 334 p. ISBN 80-7080-174-3.

Ramos-Elorduy, J. 2005. Insects a hopeful food. In *Ecological implications of Minilivestock for Sustainable Development*. USA : Science Publishers Inc., p. 263-291. ISBN 9781578083398.

Ramos-Elorduy, J. 2006. Threatened edible insects in Hidalgo, Mexico and some measures to preserve them. *Journal of Ethnobiology and Ethnomedicine*, vol. 2, no. 1, p. 1-10. https://dx.doi.org/10.1186/1746-4269-2-51

Rumpold, B. A., Schlüter, O. K. 2013. Nutritional composition and safety aspects of edible insects. Molecular Nutrition and Food Research, vol. 57, no. 5, p. 802-823. https://dx.doi.org/10.1002/mnfr.201200735

Schaefer C. H., 1968. The relationship of the fatty acid composition of Heliothiszea larvae to that of its diet. *Journal of Insect Physiology*, vol. 14, no. 2, p. 171-178. https://dx.doi.org/10.1016/0022-1910(68)90029-2

Soxhlet, F. 1879. Die gewichts analytische Bestimmung des Milch fettes. *Dingler's Polytechnisches Journal*, vol. 232, p. 461-465. Available at: http://dingler.culture.hu-berlin.de/article/pj232/ar232136

Steinhauser, L. 1995. *Hygiena a technologie masa*. (Hygiene and technology of meat.). 1st ed. Brno: LAST. ISBN 80-900260-4-4.

Tzompa-Sosa, D. A., Yi, L. Y., van Valenberg, H. J. F., van Boekel, M. A. J., Lakemond, C. M. M. 2014. Insect lipid profile: aqueous versus organic solvent-based extraction methods, *Food Res. Int.*, vol. 62, p. 1087-1094. https://doi.org/10.1016/j.foodres.2014.05.052

USDA-ARS-GMPRC, 2016. *Alphitobius diaperinus*. Available at: http://www.ozanimals.com/Insect/Lesser-Mealworm-Beetle/Alphitobius/diaperinus.html

Van Broekhoven, S., Oonincx, D. G. A. B., van Huis, A., van Loon, J. J. A. 2015. Growth performance and feed conversion efficiency of three edible mealworm species (*Coleoptera: Tenebrionidae*) on diets composed of organic by-products. *Journal of Insect Physiology*, vol. 73, no. 0, p. 1-10. https://dx.doi.org/10.1016/j.jinsphys.2014.12.005

van Huis, A., van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., Vantomme, P. 2013. *Edible insects: Future prospects for food and feed security*. Rome, Italy: FAO UN, Forestry Department, 201 p. Retrieved from the web: http://www.fao.org/docrep/018/i3253e/i3253e.pdf

Vantomme, P., Mertens, E., van Huis, A., Klunder, H. 2012. *Assessing the Potential of Insects as Food and Feed in assuring Food Security*, Summary report of Technical Consultation Meeting 23-25 January 2012. Rome, Italy: FAO UN, Forestry Department, 27 p.

Velíšek, J. 2002. *Chemiepotravin*. (Food Chemistry.) 2nd ed. Tábor: OSSIS, 303 p. ISBN 80-86659-01-1.

WHO, 2003. Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expertconsultation. WHO technical report series; 916. Geneva, Switzerland: WHO. ISBN 92 4 120916.

Yi, L., Lakemond, C. M. M., Sagis, L. M. C., Eisner-Schadler, V., van Huis, A., van Boekel, M. A. J. S. 2013. Extraction and characterisation of protein fractions from five insect species. *Food Chemistry*, vol. 141, no. 4, p. 3341-3348. https://dx.doi.org/10.1016/j.foodchem.2013.05.115

Zielińska, E., Baraniak, B., Karaś, M., Rybczyńska, K., Jakubczyk, A., 2015. Selected species of edible insects as a source of nutrient composition. *Food Research International*, vol. 77, no. 3, p. 460-466. https://dx.doi.org/10.1016/j.foodres.2015.09.008

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PLACING OF AROMA COMPOUNDS BY FOOD SALES PROMOTION IN CHOSEN SERVICES BUSINESS

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ABSTRACT

There are several ways to get higher sale involving human senses too. One of the options is a security of stimulating atmosphere of sale/ business environment. In addition to equipment, design and staff, the lighting, sound (noise), and last ones but not least smell, respectively air quality significantly take part on that. Aroma is the element that inherently belongs to the visual merchandising tools. It can influence not only emotions, memory, but total customers´ satisfaction and preferences as well as spending time in that place. In this context, it is important to find right compromise when choosing the aromatic compounds for various products, in the process of their application (intensity, process of aromatization) and security of sufficient air quality, because everybody perceives the odours with different sensitivity. Properly chosen smell and factors of air quality bring for business operators (services trades) many advantages; from staff, who more relax and friendly behave to the guests until making of various associations and stimulations of customers, who spend time inside of service. Fragrance or air quality looks like based on of present researches as the most important factor directly on point-of-sale, while aroma acquires the importance in case of memory too. On the one side, the thinks, which are seen or heard by customers, could be memorized by them few days or weeks, so on the second side, the thinks which are smelled, could be memorized by guests many decades. Except this, the Scientifics studies show, that over 75 % of all emotions are generated based on scent´ perception. The main aim of this paper is a research, how aroma influences customer purchasing decision (preferences) in chosen service provider through the tracking of daily sales of baked baguettes (Paninis) with using of aroma equipment; Aroma Dispenser.

Keywords: food sales promotion; aroma compounds; services business; consumer behaviour

INTRODUCTION

The consumer perceives the store environment with all of his five emotional organs. This perception was more described by **Solomon et al. (2006)**, who present that perception is a process in which the people choose, organize and interpret the information from outside **(Benda Prokeinová and Hanová, 2016)**.

Such as taste, a smell is a chemical sense too. Specialized receptors in the nasal cavity detect incoming molecules which enter to the nose with air flow and they bind to receptor cells. Olfactory receptors located too high in the nasal cavity send electrical impulses for processing into olfactory bulb *(bulbus olfactorium)* in the brain of limbic system. It is nervous structures of forebrain by vertebrates which involves for the smell/ odours feeling and in fact, it is the gateway to the brain **(Carter et al., 2014)**.

In according to **Neumann (2011)** smell is considered to the strong emotional trigger. It´s second most important sense directly behind sight. The research of this sense is confirmed by the study done in Paderborn (Germany). It was focused on that, the smell in 85% influences customer´ meaning about offered goods. But the willingness to shopping increases thanks to smell in 14.8% and approximately in 15% increase the time, which the customer spends in a shop. Growth by 19% was described **Michon, 2003)**. Scent marketing is not merely „a child" of the 1990s and was already used in ancient day. For instance with general interest for product and willingness to communicate. **Sikela (2014)** presents, that aroma or odour are associated from history and are partially related to memories.

Vysekalová (2014) publishes, that smell is a sense, which significantly can influence customer at point-of-sale. This is not, of course unpleasant odours, but attention is given mainly to specific smells like the smell of fresh bread or sausages. In choice of fragrance, the goal that traders want to achieve with using of aroma is too important. Suitable intensity of scents might arouse positive emotions among customers and even increase the relic of purchase.

Guéguen and Petr (2006) present, scents appear to be relevant to two forms of consumption: a) product evaluation (scent products) and b) sale´s environment (ambient aroma). Scents have an influence on restaurant customers' behaviour **(Paluchová, et al., 2015)**. The study found that both length of time and the amount of money spent were positively affected by lavender. However, the lemon aroma was found to have no effect on either the above two variables. The positive effect that the lavender scent had on the length of time spent in the restaurant was caused by its relaxing effect. One of the first study's drawing attention to the significance of scent within the marketing industry occurred in 1932 **(Chebat and**

Alain Corbin speaks of an „olfactory revolution" in the eighteenth century, which was influenced by the French

Revolution and a new standing of hygiene. Today scent marketing is intensifying the relevance of olfactory perception and is compelling us to learn about the perception of odours once more **(Müller, Alt and Michelis, 2011)**. Scent marketing relies on the neuropsychological processing of olfactory stimuli in the human brain **(Emsenhuber, 2009; Tarczydło, 2013)**. Within this category we define two types of ambient scents. The first is objective ambient scent, which we define as the application of ambient scent technology with the application of ambient scent technology with the intention of affecting the attitudes and behaviour of consumers for the benefit of the retailer **(Bradford and Desrochers, 2009)**.

From the day, we are born we experience food odours during anticipation and consumption of food. The smell of freshly baked bread entices people to buy and eventually eat a loaf **(Boesveldt et al., 2016)**. Olfactory food cues presented in the anticipatory phase of eating are found to increase the appetite for congruent products and decrease the appetite for incongruent products. This phenomenon is referred to as sensory-specific appetite **(Rybanská et al., 2014)**. Besides product-specific effects found that savoury odours) increased the appetite for (other) savoury foods and decreased the appetite for sweet foods, and vice versa **(Ramaekers et al., 2014)**. In earlier research, exposure to food odours (pizza, cookies) increased appetite, liking and craving for the food that was smelled in restrained eaters **(Fedoroff, Polivy and Herman, 2003)**. Moreover, **Gaillet et al. (2014)** found that non-attentively perceived odours in the environment affected food choice. Participants placed in a waiting room with pear odour, chose fruity desserts more often compared to participants that had been waiting in an unscented room. Brief exposure to the smell and sight of pizza increased prospective intake for pizza and other savoury foods, but not for sweet foods **(Ferriday and Brunstrom, 2011)**.

In this time, in the world exist many studies which use neuroimaging and biometric methods in order to show the influence of odours on brain activity **(Lorig, 2000; Pinto et al., 2014; Berčík, et al., 2015)**, as well as the research about influence of fragrances on emotions from the perspective of mood and physiology **(Warrenburg, 2005)**. This research of emotions was expanded for physical peace and performance the presence of mock ambient odour **(Knasko et. al., 1990)**.

Facts and myths about aromatherapy, analysis of odour effects on mood, physiology and on behaviour were also examined **(Herz, 2009)**. Detailed knowledge about brain processes influenced by aroma was presented by one study thanks functional magnetic resonance fMRI, which was realized in real conditions **(McGlone, Österbauer, Demattè and Spence, 2013; Kleinová et al., 2015)**. It is needed to emphasize, that almost none of the studies don´t take into account the quality of the air in the environment and changes of preferences, for example with influence of weather, wherein almost all they are limited to the laboratory conditions.

Alankin (2016) published, the aroma marketing services may include: a) creation of a scent logo/corporate aroma; b) distributing bioactive fragrance compositions indoors (the method of scent space), considering the given industry specificity, and consequently special composition for; c) influencing the customer behaviour in the point of sale, reaching out to the customer´s consciousness and sub-consciousness, creating emotions; d) atomising fragrance compositions related to the brand of the product during events influencing the customer´s sense of wellbeing, creating atmosphere and therefore facilitating friendly communication, e) using scent in an advertising campaign (scent advertisement).

MATERIAL AND METHODOLOGY

The object of research is an influence of aroma compounds on consumers 'purchase behaviour in chosen pub restaurant „SPORTPUB BREZNO". On base of implementation of aroma equipment - Aroma Dispenser, this is made on the principle of micro-particles atomization in real conditions in pub restaurant. We tried to research the influence of aroma on consumer preferences by selling of baked baguettes (Panini). In primary research, we monitored daily sale (amount) of baked baguettes without using of aroma stimulus and then we placed two kinds of fragrances inside of pub restaurant "SPORTPUB BREZNO" (Table 1).

Table 1 Used Aroma Compounds.

Aroma filling	Aroma type	Producer	Volume
Crunchy bread	Aerosl spray	Reimarom	250 mL
Chicken soup	Aerosl spray	Reimarom	250 mL

Table 2 Determination of dummy variables.

Without aroma	0
With aroma	1

Both aroma fillings are mostly made from natural ingredients and they are produced under strictly view of IFRA – International Fragrance Association (www.ifraorg.org). The observation was realized on March and April 2016. First month (March, 2016), there were used any fragrances and then in second month (April, 2016), aroma of *„Crunchy bread"* was exercised in first two weeks and after this, last two weeks, fragrance of *„Chicken soup"* was tested. The research, how sale and purchase behaviour were changed, was realized in pub restaurant SPORTPUB BREZNO. In this service provider is non-smoking space. Interior is designed in higher visual standard in the comparison with other pub restaurants within mentioned region (see Figure 1). Opening time in this SPORTPUB BREZNO is daily from 1pm until 11pm (weekly) and from 1pm until 2am (at weekend).

The aroma dosage in second phase of research (second month, April 2016) was done daily in 20 second intervals in time from 3pm until 11pm. Aroma compounds were placed in the middle of pub restaurant SPORTPUB BREZNO (Figure 2).

Primary data processing was done through descriptive statistics (frequencies, averages and standard errors), as well as through inductive statistics (regression statistics). This test verifies a dependence of quantitative symbol on one quantitative symbol or on more quantitative symbols **(Ostertagová, 2012)**. Because of qualitative variables presence, we choose dummy variables (Table 2).

Figure 1 Interior design in SPORTPUB Brezno.

Figure 2 Setting and positioning of diffuser.

Subsequently, we tested an influence of qualitative variable (dummy variables) on quantitative one. This was done through regression, while we tested, if quantitative variable (quantity sale of baked baguettes) depends on qualitative variable (each aroma).

We determined these hypotheses:

- H0: Quantity sale (nzmber of baked baguettes) without placing of specific aroma = quantity sale (number of baked baguettes) with placing of specific aroma; it means that aroma doesn´t influence the quantity sale (number of baked baguettes)
- H1: Aromatic compounds have an impact on the quantity sale (number of baked baguettes).

The test we realized through the statistical supplement "Data analysis" in Microsoft Excel, we had chosen Regression. We focused on the Significance F value (Figure 7) and based on a comparison with a significance level $\alpha = 0.05$, the hypothesis was accepted. The obtained values of the average number of sold baguettes have been grouped into clearly arranged figures that allow us to compare the effect of odours on changes in shopping preferences.

RESULTS AND DISCUSSION

The most important fact whether aroma application supporting an appetite in a hospitality services oriented to restaurant influences the sale of baked banquettes. Figure 3 illustrates the average of two-month observation of baked banquettes´ sale. Total comparison of period in research realization (first month without aromatization and second month with aromatization) is possible to state, that in case of aroma compounds application was achieved the total higher sale by 2.6% in April 2016.

As can be seen from the Figure 3, higher sale of Paninis was including the influence of aroma only in certain days within the observation period. The biggest change in baguettes selling on the basis of average values occurred on Sunday, where sale increased by 200% during the application of aerosol sprays. The same situation can be observed on Wednesday, where was an increase by 100% and also on Fridays (an increase of almost 17%). The opposite effect is based on an average of eight weeks demonstrated in the case of Saturdays, where sale decreased by 60%, then on Monday a decrease by 33% and also on Tuesday, a decline of 50%.

Any change occurred during the research time only on Thursdays, which could be due to the fact that this was the last day before the supply of which is related to the smallest selection (most preferred flavours/ types of baguettes, they were no longer in offer). The average sold quantities (Figure 3) can be greatly distorted by seasonal factors and because in the month of March was the Easter time, when we could expect some changes in customer habits (a lent), and also the fact that during the Easter Monday, SPORTPUB was closed.

Except tracking of the number of sold pieces of baguettes, object of research interest were customer preferences by choosing of different types of baguettes too. On Figure 4 can be seen the average that in case of non-application of odour stimulus, guests prefer these kinds of Paninis.

In first month, customers mainly preferred a flavour of bacon and ham. Other flavours within offered portfolio of semi-finished products were consumed at relatively the same level in 15%.

After implementation of aromatic diffuser, that first two weeks an aroma of *„Crunchy bread"* was activated and then last two weeks of the month, on April 2016, an smell of *„Chicken soup"* was placed. On Figure 5 are seen preferential changes by choosing of baked baguettes.

During aroma dosage, guests greatly preferred the flavours of Paninis: bacon and egg (35%), and ham and cheese (25%) in comparison with other flavours. This 15% growth in case of bacon flavour could largely relate with Eastern holiday (for example, in lent time at the end of March 2016, customers preferred tuna baguettes). On the contrary, lower interest was in sale of tuna, chicken kebab flavours (decrease by 5%) and totally lowest with prosciutto (decrease by 10%).

In comparison of period (second month) within which different fragrances were applicate (two weeks aroma of *„Crunchy bread „and* two weeks smell of *„Chicken soup"*). We determined on based of average values of sold quantities some differences too (Figure 6). Although the general comparisons, in the case of smell of *"Crunchy bread"* were sold by 5% baguettes more than in the case of aroma of *"Chicken soup"*. Figure 6 shows that up to three days in week were sold more baguettes during implementation of *"Chicken soup"* fragrance (on Saturdays, Tuesdays and Thursdays) while in case of *"Crunchy bread"* smell just two days (on Fridays and Sundays).

Part of this paper focuses on the gaining of knowledge about whether the sold quantities of baguettes dependent on aroma stimulus and subsequently to provide recommendations for pubs and restaurant providers. The nature of this research requires the statistical evidence about the effects of aromas on total sale of baguettes. Based on aggregate data, we realized regression (Figure 7) and we obtained following results: $\alpha >$significance F, $0.05 > 0.028$. The value of Significance F is less than the Significance level α, therefore null hypothesis was rejected and we accepted the alternative hypothesis that the effect of specific odour has an influence on total sale of Paninis. There is a dependency between variables; the model is statistically significant and useful for demonstrating the impact of one variable on another. The effect of smells on the sale of snacks (baguettes) is statistically verifiable.

Except seasonal effects impact, the research results could be different because of fact, that in SPORTPUB did not ensure adequate air exchange, because over-limit values of individual factors of air quality (e.g. an amount of CO_2 particles, temperature, humidity) can significantly eliminate stimulating effects of aromatic compounds circulation in this pub restaurant. Finally, the results also reflected the fact that an area of 120 m^2 was due to the financial performance used only one diffuser, which is determined based on the recommendations of the manufacturer to the spaces with an area of 30 m^2.

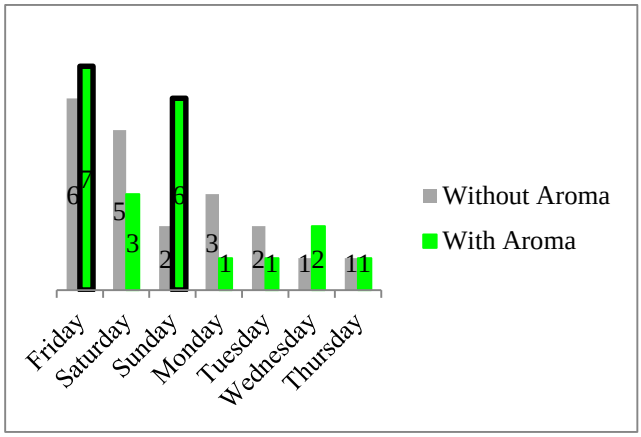

Figure 3 Average daily sales of baked baguettes during first month.

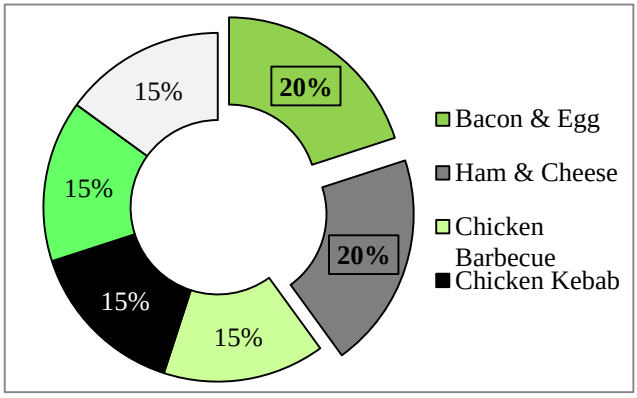

Figure 4 Average consumer preferences before implementation of aromatic diffuser.

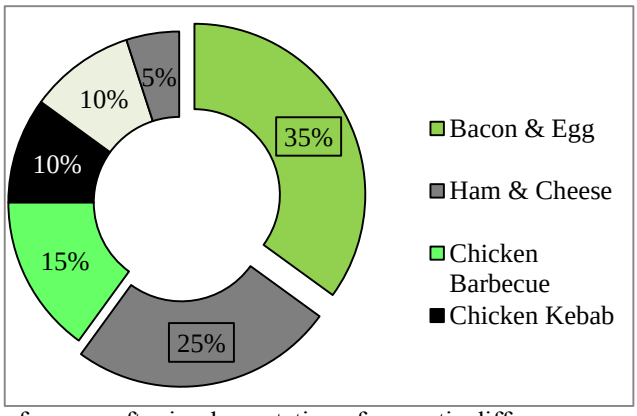

Figure 5 Average consumer preferences after implementation of aromatic diffuser

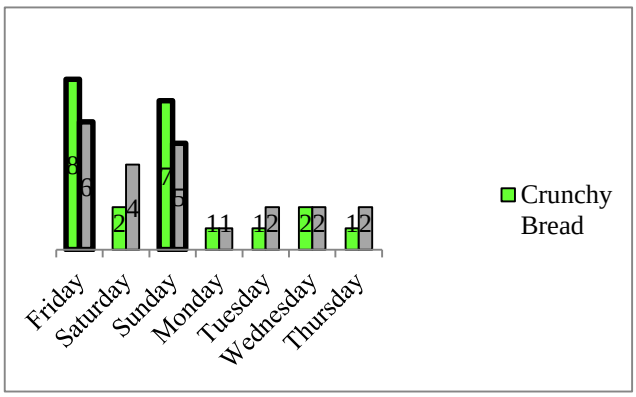

Figure 6 Average daily sales of baguettes with placing of aromatic compounds consumer preferences after implementation of aromatic diffuser.

Regression Statistics	
Multiple R	0.582239583
R Square	0.339002932
Adjusted R Square	0.283919843
Standard Error	1.860743290
Observations	14

ANOVA

	df	SS	MS	F	Significance F
Regression	1	21.3087557603687	21.3087557603	6.15439219165928	0.0289154083805951
Residual	12	41.5483870967742	3.46236559139		
Total	13	62.8571428571429			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95,0%	Upper 95,0%
Intercept	1.225806451	0.779301028905 03	1.57295628537	0.1417108300011	-0.47214	2.92375753156406	-0.47214	2.92375753156 40
X Variable 1	0.548387096	0.221052099116 38	2.48080474678	0.0289154083805952	0.0667559471945426	1.03001824635384	0.06675594719	1.03001824635 38

Figure 7 Output from regression through dummy variables.

CONCLUSION

The acquired values about realized sale of baked baguettes in chosen period including aromatic stimulus, as a forms of sales promotion provide only a minimal effect. Nevertheless, we have shown through statistical test the effect of specific odour on total sale of Paninis, while we would like to state, that in this case only a small increase, which cannot be considered as economically efficient. The higher baguettes´ sale by 2.6% not covers the costs for the acquisition of aroma equipment and of the aromatic compounds. In this case, it is necessary to highlight certain facts that it could significantly affect the overall results of the experiment.

Firstly, it was used only one diffuser (aromatic compound designed for space with an area of 30 m^2) in pub restaurant SPORTPUB in real condition of 120 m^2. There is most modern method based on the principle of nebulization (aroma particles in 1 000 times smaller in comparison to conventional aerosol freshener). During all research time, in SPORTPUB was not constant quality of the air (e.g. the amount of CO_2 particles, temperature, and humidity), as well as the measurement of various parameters too. It can be assumed that an over-limit or under-limit values can significantly eliminate the stimulating effect of the aromatic compounds spread in this pub restaurant. Within the period, seasonal effect reflected and related to the period of Easter holiday (e.g. lent time and changes in preferences).

The favourable atmosphere supporting sale through olfactory stimulation within the sale/business service assumes the choice of optimal aroma compound that is consistent with offered product, ensure a certain level of air quality and, last but not least, the rational method of application (intensity, dose, timing). In the world with a lot of visual stimulus, it can be expected that over time will become the olfactory stimulation as a form of sales promotion common part of each business unit, which returns back not only in the form of satisfied customers, but also in the form of higher profits of sales.

In the future, we plan to realize a similar research with three aroma compounds (smell streamer), which operate on the principle of nebulization. Research will be conducted six months, while we will respect air quality in the environment (because ensuring the constant conditions would be difficult in external environment). In addition to tracking sale also we aim to test the impact of aromatics compounds on consumer emotions, current research methods are in today modern world of technology deficient. We are insufficient focus on the use of biometrics and neuroscience methods. The most commonly used research methods in studying of smell effects on emotions humans used electroencephalography, which will be with olfactometry a part of a continuing research.

REFERENCES

Alankin, A. 2016. "Multisensory Experiential Marketing". Experiential Marketing Blog. Eventige Media Group. Available at: https://eventige.com/multisensory-experiential-marketing/

Barborová, P., Jančovičová, J., Zajác, P., Čapla, J., Vietoris, V. 2013. Perception of basis tastes and threshold sensitivity during testing of selected judges. *Potravinarstvo*, vol. 7, no 1, p. 12-17. https://dx.doi.org/10.5219/259

Berčík, J., Horská, E., Wang, W. Y. R., Chen, Y. Ch. 2015. How can food retailing benefit from neuromarketing research: a case of various parameters of store illumination and consumer response? In *EAAE. Seminar of the European association of agricultural economics*. Hague, Netherlands : European association of agricultural economists, p. 22.

Bradford, K. D., Desrochers, D. M. 2009. The Use of Scents to Influence Consumers: The Sense of Using Scents to Make Cents. *Journal of Business Ethics*, vol. 90, no. 2, p. 141-153. https://doi.org/10.1007/s10551-010-0377-5

Carter, R., Aldridge, S., Page, M., Parker, S. 2014. *The Human Brain Book*. New York, USA : DK Publishing, p. 264. ISBN 978-1465416025.

Chebat, J. Ch., Michon, R. 2003. Impact of ambient odours on mall shoppers' emotions, cognition, and spending. A test of competitive causal theories. *Journal of Business Research*, vol. 56, p. 529-539. https://doi.org/10.1016/S0148-2963(01)00247-8

Emsenhuber, B. 2009. Scent Marketing: Subliminal Advertising Messages. *Conjunction with Informatik*. Lübeck, Germany. Available at: https://www.pervasive.jku.at/Research/Publications/_Documents/Scent%20Marketing%202009.pdf

Fedoroff, I., Polivy, J., Herman, C. P. 2003. The specificity of restrained versus unrestrained eaters' responses to food cues: General desire to eat, or craving for the cued food? *Appetite*, vol. 41, no. 1, p. 7-13. https://doi.org/10.1016/S0195-6663(03)00026-6

Ferriday, D., Brunstrom, J. M. 2011. „I just can't help myself": Effects of food-cue exposure in overweight and lean individuals. *International journal of obesity*, vol. 35, p. 142-149. https://doi.org/10.1038/ijo.2010.117 PMid:20548302

Gaillet-Torrent, M., Sulmont-Rossé, C., Issanchou, S., Chabanet, C., Chambaron, S. 2014. Impact of a non-attentively perceived odour on subsequent food choices. *Appetite*, vol. 76, p. 17-22. https://doi.org/10.1016/j.appet.2014.01.009 PMid:24462492

Guéguen, N., Petr, Ch. 2006. Odors and consumer behavior in a restaurant. *International Journal of Hospitality Management*, vol. 25, no 2, p. 335-339. https://doi.org/10.1016/j.ijhm.2005.04.007

Herz, R. S. 2009. Aromatherapy facts and fictions: a scientific analysis of olfactory effects on mood, physiology and behavior. *International Journal of Neuroscience*, vol. 119, no. 2, p. 263-290. https://doi.org/10.1080/00207450802333953 PMid:19125379

Kleinová, K., Paluchová, J., Berčík, J., Horská, E. 2015. Visual merchandising and its marketing components in the chosen restaurants in Slovakia. *Procedia Economics and Finance*, vol. 34, p. 3-10. https://doi.org/10.1016/S2212-5671(15)01594-4

Knasko, S. C., Gilbert, A. N., Sabini, J. 1990. Emotional State, Physical Well-Being, and Performance in the Presence of Feigned Ambient Odor. *Journal of Applied Social Psychology*, vol. 20, no. 16, p. 1345-1357. https://doi.org/10.1111/j.1559-1816.1990.tb01476.x

Kozelová, D., Matejková, E., Fikselová, M., Dékányová, J. 2014. Analysis of consumer behavior at chocolate purchase. *Potravinarstvo*, vol. 8, no 1, p. 62-66. https://doi.org/10.5219/325

Lorig, T. S. 2000. The application of electroencephalographic techniques to the study of human olfaction: a review and tutorial. *International Journal Psychophysiology*, vol. 36, no. 2, p. 91-104. https://doi.org/10.1016/S0167-8760(99)00104-X

McGlone, F., Österbauer, R. A., Demattè, L. M., Spence, S. 2016. The Crossmodal Influence of Odour Hedonics on Facial Attractiveness: Behavioural and fMRI Measures. Chapter 11. In Signorelli, F., Chirchiglia, D. (eds.) *Neuroscience*. Rijeka, Croatia, InTech. ISBN 978-953-51-1160-3 https://dx.doi.org/10.5772/56504

Müller, J., Alt, F., Michelis, D. 2011. Pervasive Advertising. London, UK : Springer Dordrecht Heidelberg, p. 361. ISBN: 978-0-85729-351-0. https://doi.org/10.1007/978-0-85729-352-7 PMid:21412843

Neumann, P. 2011. Navoďte v predajni pocity pohody (Do in a store feeling of peace). *Tovar & Predaj*, vol. 1, no. 1, p. 20-21.

Ostertagová, E. 2012. *Aplikovaná štatistika v počítačovom prostredí MATLABU (Applicated statistics in computer enviroment)*. Košice, Slovak Republic : Equilibria, s.r.o., p. 192. ISBN 9788081430060.

Paluchová, J., Kleinová, K., Berčík, J., Horská, E. 2015. The perception of merchandising visual components in the fast food restaurants of chosen shopping mall. In *ICABR X. International Conference on Applied Business Research*, 14.9.-18.9. 2015. Brno, Czech Republic : Mendel University, p. 426-437. ISBN 978-80-7509-379-0

Prokeinová, R., Hanová, M. 2016. Modelling consumer´s behaviour of the meat consumption in Slovakia. *Agricultural economics*, vol. 62, no. 5, p. 235-245.

Ramaekers, M. G., Boesveldt, S., Gort, G., Lakemond, C. M. M., van Boekel, M. A. J. S., Luning, P. A. 2014. Sensory-specific appetite is affected by actively smelled food odors and remains stable over time in normal-weight women. *Journal of Nutrition*, vol. 144, no. 8, p. 1314-1319. https://doi.org/10.3945/jn.114.192567 PMid:24966408

Ramaekers, M. G., Boesveldt, S., Lakemond, C. M. M., van Boekel, M. A. J. S., Luning, P. A. 2013. Odours: Appetizing or satiating? Development of appetite during odor exposure over time. *International journal of obesity*, vol. 38, no. 5, p. 650-656. https://doi.org/10.1038/ijo.2013.143 PMid:23917805

Rita J. C., Pinto, I. P. P. P., Xavier, M. do R. A., Calado, S. J., Mariano. 2014. Analysis of the Human Reaction to Odors Using Electroencephalography Responses. In *Proceedings of the World Congress on Engineering*. London, UK : WCE, p. 1-5.

Rybanská, J., Nagyová, Ľ., Košičiarová, I. 2014. Sensory marketing: sensory perceptions and emotions as the key factors influencing consumer behaviour and decision-making. In *Marketing identity*. Trnava, Slovak Republic : University of Ss. Cyril and Methodius. p. 198-215. ISBN 978-80-8105-666-6.

Sikela, H. 2014. Vyzkoušejte aróma marketing, váš úspěch je ve vzduchu (Try aroma marketing, your success is in an air) Available at: http://www.podnikatel.cz/clanky/vyzkousejte-aroma-marketing-vas-uspech-je-ve-vzduchu/

Solomon, M. R., Marshall, G. W., Stuart, E. W. 2006. *Marketing: Očima světových marketing manažerů (Eyes global marketing managers)*. Brno, Czech Republic : Computer Press, p. 572. ISBN 80-251-1273-X.

Sýkora, V., Šulcerová, H., Mihok, M., Pytel, R. 2016. Influence of type and shelf-life on two brands complementary food in color, vitamins, and sensory evaluation. *Potravinarstvo*, vol. 10, no. 1, p. 265-271. https://doi.org/10.5219/618

Tarczydło, B. 2013. Scents and Elements of Aroma Marketing in Building of an Appropriate Brand Image. Chap.10. In Jaki, A., Mikuła, B. (eds.) Knowledge economy society managing organizations: concepts and their applications. Kraków, Poland : Publisher AGH, p. 98-107. Available at: https://cfm.uek.krakow.pl/media/files/0b/25/CFM_2014_ksiazka_2.pdf#page=98

Vysekalová, J. 2014. *Emoce v marketingu (Emotion in marketing)*. Praha, Czech Republic : Grada Publishing, p. 296. ISBN 978-80-247-4843-6.

Warrenburg, S. 2005. Effects of fragrance on emotions: moods and physiology. *Journal of Chemical Senses*, vol. 30, no. 1, p. 248-249. https://doi.org/10.1093/chemse/bjh208 PMid:15738139

Zoon, H. F. A., de Graaf, C., Boesveldt, S. 2016. Food Odours Direct Specific Appetite. *Foods*, vol. 5, no. 1, p. 2-10. https://doi.org/10.3390/foods5010012

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COMPARISON OF PHYTOESTROGENS DIETARY INTAKE FROM VEGETABLES AND FRUIT IN SELECTED POPULATION IN SLOVAKIA

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ABSTRACT

Phytoestrogens are compounds that are naturally present in almost all plant foods to a varying degree. They include several different classes of chemical compounds known as isoflavones, coumestans and lignans. In our work we analyzed intake of phytoestrogens is based upon our answer questionaries' in different ages. Evaluating health effects of phytoestrogens is difficult and depends on numerous factors, including the kind and dose (amount) of phytoestrogens eaten and the age, gender, and health of the person. We are exposed daily to highly variable amounts of phytoestrogens. While adults are eating a vegetarian diet or those taking dietary supplements containing phytoestrogens have high levels of exposure, infants drinking soy-based formula have the highest exposure levels by far. Accurate information about dietary phytoestrogens is therefore important but there is very limited data concerning food contents. In this study, we analyzed the phytoestrogen content in fresh and processed fruits and vegetables. The comprehensive database of phytoestrogen content determined simultaneously in vegetables and fruits foods has been developed. The important source of phytoestrogens in Slovak men and women is garlic. Phytoestrogen intake of fruits in men as an in woman is very low. Slovak populations consume a lot of fruits but the total intake is low due to the lower content of phytoestrogens.

Keywords: phytoestrogens; daily intake; vegetables; fruits

INTRODUCTION

Phytoestrogens are classified into groups according to their chemical structure. The greatest estrogenic activity is found in flavones, flavonols, flavanones, lignans, chalcones and isoflavones. The most common of these are the isoflavones and lignans, which are found mainly in fruit, vegetables and whole grains **(Leathaby et al., 2007)**. The intake of 400 – 600 $g.d^{-1}$ of fruits and vegetables is associated with reduced incidence of many common forms of cancer, and diets rich in plant foods are also associated with a reduced risk of heart disease and many chronic diseases of ageing. These foods contain phytochemicals that have anti-cancer and anti-inflammatory properties which confer many health benefits. Many phytochemicals are colourful, and recommending a wide array of colourful fruits and vegetables is an easy way to communicate increased diversity of intake to the consumer **(Herber, 2004)**.

Estrogens play a major role in the control of energy homeostasis and glucose metabolism. They act on hypothalamic nuclei controlling food intake, energy expenditure, and body fat distribution **(Mauvais-Jarvis et al., 2013)**. Phytoestrogens are naturally occurring estrogen-like compounds commonly found in various foods, especially soybeans and soy products, flaxseed, and sesame seed **(Miller and Snyder, 2012)**. Phytoestrogens are probably the most bioactive components of soy and interest in these compounds stems from their structural similarity to 17-estradiol (the most abundant circulating estrogen). Consequently phytoestrogens can interact with estrogen receptors (ER) and mediate estrogenic responses. For example, where endogenous levels of circulating estrogen are high (e.g. during the ovulatory menstrual cycle phase in pre-menopausal women), phytoestrogens can act as ER antagonists by competing with activity of natural estrogens, but with a lower potency. When circulating estrogen is low (e.g. in men or post-menopausal women), phytoestrogens can operate as ER agonists **(Pilsáková et al. (2010)**.

Phytoestrogens are plant-derived compounds that, because of their structural similarity with mammalian estrogens, may display both estrogenic and anti-estrogenic effects. There are three major classes of phytoestrogens: the isoflavones, lignans and coumestans. Isoflavones are found in high concentration in soybean, soybean products (e.g. tofu) and red clover. Lignans are mainly found in flaxseed. The amount of phytoestrogen in any given plant varies considerably based on location of crop, time of harvest and crop conditions, processing, and preparation. The metabolism of phytoestrogens in humans is complex: once ingested, lignans are transformed by the intestinal microflora and converted to hormone-like compounds, while isoflavones (which are present in soy as glycosides) are initially hydrolyzed by glucosidases of the intestinal bacteria and then metabolized to glucuronide conjugates in the intestine and liver. Thus the bioavailability of phytoestrogens depends on the intestinal microflora **(Woodside et al., 2006)**.

According to a number of epidemiological and clinical studies in this area, phytoestrogens have been generally accepted to have a beneficial, rather than a deleterious effect in humans. Potential health benefits of

phytoestrogens may be attributable to metabolic properties that do not involve estrogen receptors, such as influence on enzymes, protein synthesis, cell proliferation, angiogenesis, calcium transport, Na+/K+ adenosine triphosphatase, growth factor action, vascular smooth muscle cells, lipid oxidation, and cell differentiation **(Adlercreutz and Mazur, 1997; Knight and Eden, 1996)**.

A phytoestrogen-rich diet is known to increase urinary excretion and circulating plasma levels of phytoestrogen metabolites. However, it is not known how a phytoestrogen rich diet may affect intake of other nutrients. Phytoestrogens are found in soy products, legumes, fruit and vegetables, foods that have been proposed to have health-promoting effects. It is important, however, that the effect of inclusion of phytoestrogen-rich foods in the diet on the intake of other micro- and macronutrients is determined to assess the impact that public health nutrition messages to encourage an increase in soy consumption may have on the nutritional balance of the diet **(Borelli and Enrst, 2010)**.

The objective of our study was to estimate daily intake of the phytoestrogens from selected vegetables and fruit sources in selected Slovak population.

MATERIAL AND METHODOLOGY

A package of 60 questions has been carefully prepared and developed on the basis of literature previously published in scientific journal. The major questions covers: age; sex; dietary habits (focused to selected foods which are marked as the most common phytoestrogens sources) With the help of several options, respondents reported total intake of phytoestrogens from 13 kinds of vegetables including: broccoli, cabbage, carrots, corn, potatoes, garlic, salad, olives, onion, pumpkin, spinach, tomatoes and zucchini and 12 kinds of fruits as: apples, bananas, currants, blackberries, blueberries, dried apricots, dried dates, dried pears, dried grapes, grapefruit, grapes, orange, and peach.

We analyzed dietary intake of 9 phytoestrogens: formononetin (FOR), daidzein (DAI), gensteín (GEN), glycitein (GLY), matairesinol (MAT), larinesinol (LAR), pinolaricinesinol (PINO), secoiresinol (SECO) and coumestrol (COU). From total collected answers we subsequently recalculated number of each of the nine received phytoestrogens, depending on the specified frequency, portion or unit dose to each respondent separately. These data were calculated to determine accurately the individual phytoestrogens in vegetables, fruits (which was presented by **Thompson et al., (2006)**. After a recount of 293 respondents (217 women and 76 men) individually, we counted the total number of nine phytoestrogens, and we received a total amount of phytoestrogens for each respondent. This content was analyzed and recalculated to a daily intake of mg.100g^{-1}, depending on the gender and age of respondents. The number of men from 21 to 26 is shown separately because the number of these respondents was less than 10; the resulting average could thus adversely affect the correct information when comparing the intake of phytoestrogens. Men aged from 30 to 39 was recorded in our survey as one single respondent, while for women aged from 30 to 39 is not recorded any respondent. Statistics were calculated to summarize the eating occasions by kind of fruit and vegetables and age of respondents. For the calculation we used Microsoft Excel.

RESULTS AND DISCUSSION

Daily intake of phytoestrogen had been calculated according to data collected from dietary questionnaire, realized on 293 (divided into Males – covered by 76 individuals and Females – 217 individuals) respondents coming from Slovakia. Questionnaire was designed to determine intake frequency as well as amount of selected food fruits and vegetables. Respondents reported a total intake of 13 kinds of vegetables (Table 1 – Table 4) As is evident from the results of an important source of phytoestrogens in Slovak men and women is a garlic. Resulting values were compared with a study **Kuhnle et al., (2009**), which reported a single daily intake of phytoestrogens for the English population living in the UK. **Kuhnle et al., (2009)** reported a study of the data for the daily intake of broccoli: 0.07 mg.100 g^{-1}, cabbage 0.06 mg.100 g^{-1}, carrots 1.25 mg. 100 g^{-1}, garlic 0.09 mg.100 g^{-1}, 0.33 mg.100g^{-1}, olives 0.33 mg.100g^{-1}, onion 0.31 mg.100 g^{-1}, pumpkin 0.72 mg.100g^{-1} potatoes 0.40 mg.100 g^{-1}, corn 0.09 mg.100 g^{-1} tomatoes 0.07 mg.100 g^{-1}, due to the higher levels of phytoestrogens.

Table 1 Daily intake of phytoestrogens for men in selected vegetables mg.100 g^{-1}.

Age	Broccoli	Cabbage	Carrots	Corn	Potatoes	Garlic	Salad
21	0.009	0.008	0.000	0.000	0.022	0.147	0.000
22	0.010	0.015	0.000	0.001	0.032	0.252	0.003
23	0.011	0.008	0.000	0.000	0.023	0.067	0.002
24	0.003	0.016	0.002	0.002	0.022	0.294	0.006
25	0.004	0.003	0.001	0.000	0.022	0.012	0.000
26	0.003	0.008	0.000	0.000	0.005	0.012	0.000
30 – 39	0.057	0.016	0.002	0.001	0.000	0.098	0.011
40 – 49	0.003	0.008	0.001	0.000	0.020	0.148	0.004
50 – 59	0.006	0.024	0.001	0.000	0.053	0.234	0.001
60 – 69	0.006	0.011	0.000	0.000	0.027	0.141	0.001
70 – 79	0.004	0.018	0.000	0.000	0.016	0.151	0.001
80 – 89	0.015	0.012	0.000	0.000	0.025	0.116	0.000

Table 2 Daily intake of phytoestrogens for men in selected vegetables mg.100 g^{-1}.

Age	Olives	Onion	Pumpkin	Spinach	Tomatoes	Zucchini
21	0.004	0.003	0.000	0.000	0.003	0.001
22	0.008	0.014	5.388	0.000	0.003	0.000
23	0.001	0.004	0.000	0.000	0.003	0.000
24	0.001	0.077	0.000	0.000	0.011	0.000
25	0.000	0.005	0.000	0.000	0.000	0.000
26	0.000	0.001	0.000	0.000	0.000	0.000
30 – 39	0.006	0.019	0.000	0.004	0.011	0.009
40 – 49	0.000	0.019	0.000	2.084	0.015	0.003
50 – 59	0.000	0.024	0.000	0.000	0.006	0.000
60 – 69	0.000	0.017	0.000	0.000	0.004	0.000
70 – 79	0.000	0.010	0.000	0.000	0.002	3.820
80 – 89	0.000	0.017	0.000	0.000	0.002	0.000

Table 3 Daily intake of phytoestrogens for women in selected vegetables mg.100 g^{-1}.

Age	Broccoli	Cabbage	Carrots	Corn	Potatoes	Garlic	Salad
20 – 29	0.008	0.010	0.000	0.000	0.019	0.115	0.002
30 – 39	0.030	0.017	0.001	0.000	0.022	0.478	0.007
40 – 49	0.013	0.019	0.001	0.000	0.035	0.108	0.007
50 – 59	0.013	0.012	0.001	0.000	0.017	0.204	0.003
60 – 69	0.008	0.013	0.001	0.000	0.024	0.184	0.002
70 – 79	0.006	0.010	0.001	0.000	0.018	0.193	0.003
80 – 89	0.003	0.007	0.001	0.000	0.014	0.143	0.000

Table 4 Daily intake of phytoestrogens for women in selected vegetables mg.100 g^{-1}.

Age	Olives	Onion	Pumpkin	Spinach	Tomatoes	Zucchini
20 – 29	0.004	0.009	0.000	0.001	0.000	0.005
30 – 39	0.000	0.031	0.000	0.004	8.336	0.002
40 – 49	0.001	0.017	0.000	0.002	0.000	0.011
50 – 59	0.000	0.012	0.000	0.002	0.000	0.006
60 – 69	0.001	0.015	0.000	0.001	0.000	0.004
70 – 79	0.000	0.012	0.000	0.001	0.000	0.005
80 – 89	0.000	0.008	0.000	0.001	2.778	0.002

Table 5 Daily intake of phytoestrogens for men in selected fruits mg.100 g^{-1}.

Age	Apples	Bananas	Currants	Blueberries	Blackberries	Dried Apricots
21	0.001	0.000	0.006	0.000	0.002	0.000
22	0.001	0.000	0.000	0.001	0.000	0.004
23	0.002	0.000	0.000	0.000	8.946	0.002
24	0.012	0.001	0.002	0.000	0.001	0.000
25	0.001	0.000	0.000	0.000	0.000	0.000
26	0.004	0.000	0.000	0.000	0.000	0.000
30 – 39	0.000	0.000	0.000	0.000	0.000	0.000
40 – 49	0.001	0.000	0.001	0.000	0.000	0.216
50 – 59	0.003	0.000	0.005	0.013	0.002	0.000
60 – 69	0.003	0.000	0.000	0.000	0.000	0.006
70 – 79	0.002	0.000	0.001	0.003	0.000	0.004
80 – 89	0.007	0.001	0.000	0.000	0.000	0.000

In the next part of the questionnaire, respondents answer to the frequency of intakefor 12 kinds of fruits as: apples, bananas, currants, blackberries, blueberries, dried apricots, dried dates, dried raisins, grapes, grapefruit, orange and peach.

The result showed that Slovak populations consume a lot of fruits but the total intake is low due to the lower content

of phytoestrogens. That corresponding with study of free-living adults in the UK **(Day et al., 1999)**, such as bananas raw tomatoes apples and cucumbers also contained only small amounts of phytoestrogens. The results of men's daily intake (Table 5 and 6) were compared with a study **Curlej et al. (2015)** total phytoestrogens content of all fruits were for men aged 50 – 59 years 0.067 mg.100g^{-1} for men aged 60 – 69 years 0.017 mg.100g^{-1} and for men aged 70 – 79 years, the total sum received phytoestrogens 0.021 mg.100g^{-1}. When comparing our work with the presented study can be seen increases of phytoestrogens intake in men aged 51 – 59 years and another rise was recorded in the age group of men from 70 to 79 years. When men aged 60 – 69 years, were lower daily intake of phytoestrogens from fruits.

Results in the second gender determination, which women are (Table 7 and Table 8) were also compared with the study of **Curlej et al., (2015)** the total intake of phytoestrogens from fruits determined as follows: women aged 50 – 60 years are taken daily 0.033 mg.100 g^{-1}, aged 61 to 70 years was value 0.035 mg.100g^{-1}, women in the age group 71 – 80 years are taken daily 0.036 mg.100g^{-1}. Phytoestrogens from fruits in the age group 81– 90 years women take daily 0. 019 mg.100 g^{-1}. In our work we noted an increase in the daily intake of women aged 50 – 59 years (0.067 mg.100 g^{-1}) and a further increase in women aged 80 – 89 years (0.032 mg.100g^{-1}). For the remaining two age groups has been an increase in dietary intake of phytoestrogens on average 0.015 mg.100 g $^{-1}$. Average intake (summary for male and female) of isoflavones at retirees of selected Slovakia region is represented by following values: 0.0226 (50 – 60 age intervals); 0.1485 (61 – 70 age intervals); 0.2599 (71 – 80 age intervals) and 0.005 mg.day^{-1} (over 81).

Presented values are in accordance to conclusion identified in our work and apparently lower than those found in Japanese population (50 mg.day^{-1}) presented by **Messina (1995)**; or population of Asia (a range between 25 to 45 mg.day^{-1}) **(Coward et al., 1961)**.

Table 6 Daily intake of phytoestrogens for men in selected fruits mg.100 g^{-1}.

Age	Dried dates	Dried raisins	Grapefruit	Grapes	Orange	Peach
21	0.000	0.0000	0.000	0.000	0.000	0.003
22	0.000	0.000	0.000	0.000	0.004	0.002
23	0.002	0.001	0.000	0.000	0.004	0.002
24	0.009	0.004	0.002	0.011	0.037	0.020
25	0.000	0.000	0.000	0.000	0.000	0.000
26	0.000	0.000	0.000	0.000	0.005	0.001
30 – 39	0.000	0.000	0.000	0.000	0.001	0.003
40 – 49	0.216	0.014	6.303	0.000	0.001	0.002
50 – 59	0.021	0.001	0.000	0.004	0.017	0.004
60 – 69	0.000	0.000	0.000	0.001	0.006	0.004
70 – 79	0.007	0.000	0.000	0.001	0.002	0.001
80 – 89	0.000	0.001	0.000	0.012	0.011	0.000

Table 7 Daily intake of phytoestrogens for women in selected fruits mg.100 g^{-1}.

Age	Apples	Bananas	Currants	Blueberries	Blackberries	Dried Apricots
20 – 29	0.002	0.000	0.000	0.001	0.000	0.003
30 – 39	0.002	0.000	0.000	0.000	0.000	0.000
40 – 49	0.006	0.001	0.000	0.000	0.000	0.016
50 – 59	0.005	0.000	0.002	0.001	0.001	0.002
60 – 69	0.004	0.000	0.001	0.001	0.000	0.007
70 – 79	0.004	0.000	0.001	0.001	0.000	0.011
80 – 89	0.003	0.000	0.000	0.000	5.964	0.036

Table 8 Daily intake of phytoestrogens for women in selected fruits mg.100 g^{-1}.

Age	Dried dates	Dried raisins	Grapefruit	Grapes	Orange	Peach
20 – 29	0.002	0.000	0.000	0.001	0.004	0.007
30 – 39	0.000	0.000	0.000	0.000	0.000	0.000
40 – 49	0.003	0.000	0.000	0.001	0.002	0.008
50 – 59	0.015	0.000	0.000	0.003	0.005	0.007
60 – 69	0.001	0.000	0.000	0.001	0.005	0.004
70 – 79	0.002	0.000	0.000	0.001	0.003	0.003
80 – 89	0.000	0.000	0.000	0.000	0.002	0.002

CONCLUSION

Phytoestrogens are estrogen hormone-like chemicals found in plants. They include a group of chemicals such as isoflavones, flavones, coumestans and lignans. Phytoestrogens are available in medically formulated pills. However, dietary phytoestrogen can also be found naturally in wide variety of plant and fruit based foods, with the amount varying depending on the plant and fruit type. The study demonstrated that phytoestrogen sources are diverse. Food is also a part of traditions and culture. This can mean that eating has an emotional component as well. Sources of phytoestrogens from fruits and vegetables are different depending on dietary habits and traditions of the Slovak population.

REFERENCES

Adlercreutz, H., Mazur, W. 1997. Phyto-estrogens and western diseases. *Annals of Medicine*, vol. 29, no. 2, p. 95-120. https://doi.org/10.3109/07853899709113696 PMid:9187225

Borrelli, F., Ernst, E. 2010. Alternative and complementary therapies for the menopause. *Maturitas*, vol. 66, no. 4, p. 333-343. https://doi.org/10.1016/j.maturitas.2010.05.010 PMid:20580501

Coward, L., Barnes, N. C., Setchell, K. D. R., Barnes, S. 1961. The isoflavone genestein diadzein soyabean foods from American and Asian diets. *Journal of Agricultural and Food Chemistry*, vol. 41, no. 11, p. 1961-1967. https://doi.org/10.1021/jf00035a027

Čurlej, J., Zidek, R., Belej, Ľ. , Zajác, P., Čapla, J. 2015. Phytoestrogens dietary intake and health status of retiree from middle-notrh Slovakia region. *Potravinarstvo*, vol. 9, no. 1, p. 573-579. https://doi.org/10.5219/572

Day, N., Oakes, S., Luben, R., Khaw, K. T., Bingham, S., Welch, A. 1999. EPIC-Norfolk: Study design and characteristics of the cohort. European ProspectiveInvestigation of Cancer. *British Journal of Cancer*, vol. 80, suppl. no. 1, p. 95-103. PMid:10466767

Herber, D. 2004. Vegetables, fruits and phytoestrogens in the prevention of diseases. *Journal of Postgraduate Medicine*, vol. 50, no. 2, p. 145-149.

Knight, D. C., Eden, J. A. 1996. A review of the clinical effects of phytoestrogens. *Obstetrics and Gynecology*, vol. 87, no. 5, p. 897-904. PMid:8677131

Kunhle, G. C., Dell´aquila, C., AspinalL, S. M., Runswick, S. A., Joosen, A. M. C. P., Mulligan, A. A., Bingham, S. A. 2009. Phytoestrogen content of fruits and vegetables commonly consumed in the UK based on LC–MS and 13C-labelled standards. *Food Chemistry*, vol. 116, no. 2, p. 542-554. https://doi.org/10.1016/j.foodchem.2009.03.002

Leathaby, A. E., Brown, J., Marjoribanks, J., Kronenberg, F., Roberts, H., Eden, J. 2007. Phytoestrogens for vasomotor menopausal symptoms. *Cochrane Database of Systematic Reviews*, vol. 4. https://doi.org/10.1002/14651858.CD001395.pub3 PMid:17943751

Mauvais-Jarvis, F., Clegg, D. J., Hevener, A. L. The role of estrogens in control of energy balance and glucose homeostasis. *Endocrine Reviews*, vol. 34, no. 3, p. 309-338. https://doi.org/10.1210/er.2012-1055 PMid:23460719

Messina, M. 1995. Isoflavone intake by Japanese were overestimated (letter to the editor). *The American Journal of Clinical Nutrition*, vol. 62, no. 3, p. 645. PMid:7661128

Miller, P. E., Snyder, D. C. 2012. Phytochemicals and cancer risk: a review of the epidemiological evidence. *Nutrition in Clinical Practice*, vol. 27, no. 5, p. 599-612. https://doi.org/10.1177/0884533612456043 PMid:22878362

Pilsáková, L., Riecansky, I., Jagla, F. 2010. The physiological actions of isoflavone phytoestrogens. *Physiology Research*, vol. 59, no. 5, p. 651-654. PMid:20406033

Thompson, L. U., Boucher, B. A., Liu, Z., Cotterchio, M., Kreiger, N. 2006. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. *Nutrition and Cancer*, vol. 54, no. 2, p. 184-201. https://doi.org/10.1207/s15327914nc5402_5 PMid:16898863

Woodside, J. V., Morton M. S., Cooper A., Leathem, A. J. C. 2006. Short-term consumption of phytoestrogen-rich foods in humans alters dietary macro- and micronutrient intake. *Nutrition Research*, vol. 26, no. 1, p. 7-10. https://doi.org/10.1016/j.nutres.2005.11.007

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Table of Contents

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