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THE EFFECT OF UV-C IRRADIATION ON GRAPE JUICE TURBIDITY, SENSORIC PROPERTIES AND MICROBIAL COUNT

Peter Czako, Peter Zajác, Jozef Čapla, Vladimír Vietoris, Lenka Maršálková, Jozef Čurlej, Ľubomír Belej, Jozef Golian, Lucia Benešová, Patrícia Martišová

ABSTRACT
In this work, we investigated the effect of UV-C radiation (254 nm) on turbidity, microbial count and sensoric properties of the grape juices treated or not treated with sulphur dioxide. The UV-C radiation is considered to be germicidal against microorganisms. This technology is routinely used to treat drinking water. Application of this method for the purpose of treating the wine was tested in few studies. These studies have shown a positive effect on the inactivation of microorganisms, but they not dealt in detail with the sensory properties of grape juice after the treatment. The main idea of using this method appears to eliminate the sulphur dioxide from wine making technology. There are people who have a genuine allergy to sulfites, and these allergies are often linked with asthma. These people have an rapid onset of symptoms when drinking liquids like wine treated with sulphur dioxide. In our work we have found that the application of this method of treating the grape juice is problematic. Intensity of UV-C radiation increases the turbidity of grape juices. This effect was observed in all grape juices with or without addition of sulphur dioxide and also in clarified or not clarified grape juices. We found that UV-C radiation negatively affect the sensory properties of grape juices. This effect was more pronounced in grape juices treated with SO_2. The smell and taste were significantly negatively changed. Exposure of grape juice treated with sulphur dioxide to UV-C radiation can probably lead to arising the sulphur compounds, which affects the smell and taste of grape juices. Also, it is very likely that the negative change in taste and smell may affect the quality of produced wines. For this purpose, we do not recommend to use UV-C treatment for the grape juice treatment. It will be interesting to conduct a detailed analysis of the grape juices composition before and after UV-C radiation treatment.

Keywords: grape wine; UV-C; irradiation; treatment; grape juic turbidity

INTRODUCTION
The aim of this work was to investigate the effect of the UV-C radiation on grape juice turbidity, sensoric properties and microbial count.

Ultraviolet C (UV-C) irradiation is one of the emerging techniques for the inactivation of microorganisms in liquid food products, and it holds considerable promise also for treatment of wine. This application can be of particular interest to reduce or even eliminate the use of sulphur dioxide as a preservative in winemaking, given its potential health risks **(Rizzotti et al., 2011)**.

Ultraviolet radiation involves the use of radiation from the electromagnetic spectrum from 100 to 400 nm and is categorized as UV-A (320 – 400 nm), UV-B (280 – 320 nm) and UV-C (200 – 280 nm) **(Guerrero-Beltrán and Barbosa-Cánovas, 2004)**.

UV-C radiation is considered to be germicidal against microorganisms such as bacteria, viruses, protozoa, yeasts, moulds and algae, where the highest germicidal effect is obtained between 250 and 270 nm **(Bintsis, Litopoulou-Tzanetaki, and Robinson, 2000)**.

Microbial inactivation caused by UV-C (254 nm) radiation is based on the rearrangement of the microorganism's nucleic acid which directly interferes with the ability of microorganisms to reproduce **(Bintsis et al., 2000; Thompson, 2003; Tran and Farid, 2004; Gabriel and Nakano, 2009)**.

The UV-C rays damage the structure of DNA, rendering microorganisms incapable of reproduction. Each type of microorganism requires a specific dose for destruction. In general, bacteria die at doses greater than 20 mj/cm^2, while molds can tolerate up to 300 mj/cm^2. Germicidal ultraviolet lamps gradually loose power with use. In order to stabilize the quantity of rays emitted in terms of time-surface units, the input power is increased as a function of the length of time in use (around a total of 10,000 hours) **(Delfini and Formica, 2001)**.

Cleaning and disinfection are important operations in food processing because of the significant contributions to product hygiene and food safety. The UV-C treatment is a physical method, which can be applied for this purpose **(Otto et al., 2011)**.

Ultraviolet rays fall perpendicular to the surface being irradiated but can not pass through any type of material, including glass. The surfaces to be treated must be smooth to avoid casting shadows in which microorganisms could be protected. In oenology UVC irradiation could be used to keep the mouth of bottles clean during transport from the sterilizer to the bottling line, and to the corker. UV irradiation could provide partial sanitation of metallic capsules of corks, as well the air around the bottler and corker **(Delfini and Formica, 2001)**.

UV-C radiation has offered a wide spectrum of effective inactivation of wine-associated microorganisms such as *Brettanomyces, Saccharomyces, Acetobacter, Lactobacillus, Pediococcus* and *Oenococcus* and therefore may hold promise as an alternative technology to inactivate spoilage microorganisms at different stages of vinification in conjunction with reduced SO_2 levels. From practical point of view, further studies pertaining to the long and short term effect of UV-C radiation on the sensorial and chemical properties of wine are imperative **(Fredericks, du Toit and Krügel, 2011)**.

The application of the UV-C treatment to different grape juice and wine samples in five diverse wineries assured the desired decrease of viable microorganisms **(Lorenzini et al., 2010)**.

Microbial reduction is in correlation with the UV-C dosage (J L^{-1}): higher microbial reductions were obtained with exposure to higher UV-C dosages (J L^{-1}). As a result, the highest microbial reduction was obtained after 3672 J L^{-1} **(Fredericks, du Toit and Krügel, 2011)**.

Ultraviolet treatment is performed at low temperatures and is classified as a non-thermal disinfection method **(Tran and Farid, 2004)**.

The penetration effect of UV-C radiation depends on the type of liquid, its UV-C absorptivity, soluble solids and suspended matter in the liquid. The greater the amount of soluble solids, the lower the intensity of penetration of the UV-C light in the liquid **(Guerrero-Beltrán & Barbosa-Cánovas, 2005)**.

The UV-C treatment did not to alter the color and chemical parameters of grape juice and wine, even in the case of prolonged treatments **(Lorenzini et al., 2010)**.

The advantages associated with UV-C radiation used as a non-thermal method is that no known toxic or significant nontoxic byproducts are formed during the treatment, certain organic contaminants can be removed, no off taste or odor is formed when treating water, and the treatment requires very little energy when compared to thermal pasteurization processes. Fruit juice that undergo thermal pasteurization or sterilization tend to change color and lose some of its aromas and vitamins during the process of heating **(Choi and Nielsen, 2005)**.

The usage of UV-C treatment needs aware staff and requires clean equipments and facilities to keep must and wine stable during storage in barrels and in bottles. Indeed, after the treatment, the samples could be newly contaminated by spoilage microorganisms in poorly sanitized containers. The promising results reported here demonstrated that physical methods, such as UV-C, an incoming technology already commercially available, could exert a highly positive impact in the winemaking industry by eliminating or reducing the use of SO_2 in the near future **(Lorenzini et al., 2010)**.

Unluturk and Atilgan (2015) were investigated the effects of UV-C irradiation on the inactivation of *Escherichia coli* and on the shelf life of freshly squeezed turbid white grape juice. UV exposure was not found to alter pH, total soluble solid, and titratable acidity of juice. There was a significant effect on turbidity, absorbance coefficient, color, and ascorbic acid content. The microbial shelf life was doubled after UV-C treatment, whereas the quality of juice was adversely affected similarly observed in the control samples.

Scientific hypothesis

The UV-C treatment has an effect on the turbidity, sensory properties and total viable count of grape juice.

MATERIAL AND METHODOLOGY

Grape juice samples

Grape juices (*Chardonnay*) samples were taken from Chateau Malanta Vinery Company (Slovakia). The juices were taken 24 and 96 hours after sedimentation. The sterile 5 L glass bottles were used for sampling.

Sample A: Grape juice taken after 24 hours of sedimentation without addition of SO_2,

Sample B: Grape juice taken after 24 hours of sedimentation with adidion of 32 mg L^{-1} SO_2,

Sample C: Grape juice taken after 96 hours of sedimentation without addition of SO_2,

Sample D: Grape juice taken after 96 hours of sedimentation with addition of 32 mg L^{-1} SO_2.

Instruments and equipment

The UV-C reactor that consisted of a 1 L glass graduated measuring cylinder, at which the 45 cm UV-C lamp 254 nm, VanGraven 40 W (light output, 15 Watt/m of lamp) was vertically located. The UV-C lamp has been anchored to a long rod and was fixed in the center of the measuring cylinder. The lamp was shielded by quartz so the grape juice would not be in direct contact with the lamp. The reactor was placed on a magnetic stirrer.

Grape juice UV-C treatment

1 L of grape juices sample was treated: 0, 1, 10, 20 and 30 minutes with UV-C light.

Microbiological analyisis

Pour plate technique was applied on PCA to enumerate total aerobic count, then plates were incubated at 35 °C for 48 h.

UV-C Dose and energy used for treratment

UV-C Dose was calculated following these formulas.

The surface area of the whole lamp (A) and the surface area of the working part of the lamp (B) were calculated according to the same formula:

$$\text{Surface A and B } (m^2) = 2\ r\ \pi\ d$$

Where:

r – is radius of the lamp (m),

d – is length of the lamp (m).

Percentage of the surface of the lamp immersed in grape juice (working part of the lamp):

$$C\ (\%) = (B / A)\ 100$$

Where:
A – is surface area of the whole lamp (m^2),
B – is surface area of working part of the lamp (m^2),
C – is the percentage of the surface of working part of the lamp from the whole surface area (%)

Intensity (I) of the radiation:

$$I\ (W) = (D / 100) \times C$$

Where:
D – is the total UV-C output of the lamp (W) (according to the manufacturer 15 Watt),
C – is the percentage of the surface of working part of the lamp from the whole surface area (%).

UV-C dose was calculated according this formula:

$$\text{UV-C Dose}\ (J\ L^{-1}) = I / V\ t$$

Where:
I – is the intensity of the radiation (Watt),
V – is the volume of the grape juice in reactor (L),
t – is the time of the treatment (s).

Data used for calculation:
π: 3.14
r : 0.0125 m
d : 0,58 m (length of the whole lamp)
d : 0,185 m (length of the working part of the lamp)
A: 0.04553 m^2
B: 0.01452 m^2
C: 31,8965%
D:15 W
I: 4.7845 W
V: 0.42 L
t: 60 s, 120 s, 600 s, 1200 s, 1800 s.

Calculated UV-C doses: 1 min (683 J L^{-1}), 2 min (1366 J L^{-1}), 10 min (68349 J L^{-1}), 20 min (13670 J L^{-1})

Turbidity measurement

The intensity of turbidity was measured with spectrophotometer Neogen 4700 (Advanced Instruments, USA.

Sensoric analysis

The grape juice smell and taste was analysed after the UV-C radiation treatment. We have used scale of the intensity of foreign smell (0 – odorless, 10 – strong odor) and scale of the intensity of foreign taste (0 – without foreign taste, 10 – strong foreign taste). The number of certified assessors was 7.

Reactor description

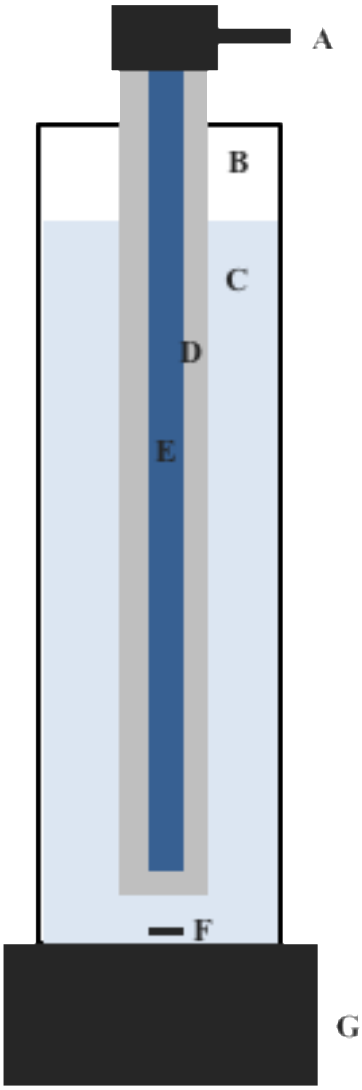

Figure 1 Description of the UV-C (254 nm) reactor used in this study.

Note:
A – Power supply
B – Measuring cylinder
C – Wine
D – Quartz sleave
E – UV-C lamp (15 W)
F – Magnet
G – Magnetic stirrer

Statistical analysis

We used the statistical program Tanagra 1.4 (Lumière University, Lyon, France) according to **Rakotomalala (2005)** for evaluation of turbidity results in relation to UV-C treatment time. The Shapiro-Wilks test was used to test the normality of data. Statistical analysis of turbidity results in relation to different UV-C treatment times was performed with one-way ANOVA for each grape juice (A, B, C and D). We were testing the null hyphotesis $H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_4$ for main effect of factor A (grape juice turbidity) within four groups (0, 1, 10 and 20 min UV-C treatment). Consequently, we have used the paired Student's t-test for evaluation of differences among

obtained results between groups. We have also used the same procedure for evaluation of the differences between grape juices (A – B and C – D). Differences between samples were considered as statistically significant at p <0.05. We have also performed the Principal Components Analysis (PCA) to reducing the original data and show position of grape juices according to the different UV-C treatment times.

The sensoric analysis data was analysed in statistical program Tanagra 1.4 (Lumière University, Lyon, France). Multiple comparison of samples was performed by nonparametric Friedman's test.

RESULTS AND DISCUSSION

The effect of the UV-C radiation treatment on the grape juice microbial counts is presented in the Table 1 and Figure 2. The effect of UV-C radiation treatment on the grape juice turbidity is presented in the Table 2 and Figure 3. The effect of UV-C radiation treatment on the grape juice sensory properties is presented in Table 3, Table 4, Figure 4 and Figure 5.

The effect of UV-C radiation (254 nm) was studied to reduce the microbial count in different grape juices with or without addition of SO_2. UV-C radiation is considered to be germicidal against microorganisms such as bacteria **(Bintsis, Litopoulou-Tzanetaki, and Robinson, 2000)**. We agree, the application of UV-C radiation reduced the microbial count in grape juices (Table 1). In grape juice A, an aplicaiton of 0.68 kJ L^{-1} UV-C reduced the log of microbial count from initial log 7.37 to log 4.53, dose 6.83 kJ L^{-1} to log 3.86 and dose 13.67 kJ L^{-1} to log 2.83. In grape juice B, an aplicaiton of 0.68 kJ L^{-1} UV-C reduced the log of microbial count from initial log 7.21 to log 3.93, dose 6.83 kJ L^{-1} to log 3.55 and dose 13.67 kJ L^{-1} to log 1.33. In grape juice C, an aplicaiton of 0.68 kJ L^{-1} UV-C reduced the log of microbial count from initial log 7.47 to log 6.53, dose 6.83 kJ L^{-1} to log 6.00 and dose 13.67 kJ L^{-1} to log 4.13. The reduction of log microbial counts with addition of SO_2 was more effective (Figure 2). The microbial counts in grape juice D was reduced to log 0, because this grape juice was sampled 96 hours after sedimentation and was effectively treated with SO_2.

Application of UV-C treatment in order to reduce microorganism counts in clarified grape juices in small demijohns, which are used by small producers is questionable according to our research. Some microbial counts can survive the treatment. The growth and spoiling capacity of the surviving microbes should be investigated in further research.

Lorenzini et al., (2010) found more effective method of treatment. These authors used reactor with 40 UV-C (254 nm) germicidal lamps in series, and exploits an advanced turbulent flow system to optimize the penetration of UV-C and improve microbial inactivation. In their study, grape juice and wine batches were circulated at a constant flow rate of 4000 $L.h^{-1}$ by means of an eccentric screw pump and the dosage was 1000 J L^{-1}. Also, the fluid systems described in **(Fredericks, du Toit and Krügel, 2011)** was effective. **Rizzotti et al., 2015** was applied UV-C light treatment to ten different white and red wines during winemaking, for the first time at industrial scale, using a commercial turbulent flow system. The effect of 1.0 kJ L^{-1} dosage treatment on the viability of the natural microbial population, (total yeasts, lactic acid bacteria and acetic acid bacteria), was investigated. Data indicated that the UV-C irradiation was effective in reducing microbial counts for up to five log CFU mL^{-1}, depending on the wine.

The main aim of our study was to determine whether the UV-C radiation (254 nm) affects the sensory properties of grape juices.

According to **Fredericks, du Toit and Krügel (2011)** further studies pertaining to the long and short term effect of UV-C radiation on the sensorial and chemical properties of wine are imperative.

Lorenzini et al. (2010) were investigated the effect of UV-C treatment on chemical composition of grape juice and wine. Authors of this study found, UV-C treatment did not to alter the color and chemical parameters of grape juice and wine, even in the case of prolonged treatments. In contrast to our study, these authors didn't use the SO_2 for grape juice treatment. Authors reported, after UV-C irradiation no changes were observed for alcohol, reducing sugars, glycerine content, total acidity, pH and color in all the samples, including the red wines, which were subjected to higher UV-C dosage. These authors didn't study the sensory properties of treated grape juice.

In our study, we have used lower doses (0.68 kJ L^{-1} and 1.37 kJ L^{-1}) and higher doses (6.83 kJ L^{-1} and 13.67 kJ L^{-1}) to study the effect of UV-C treatment on the sensory properties of grape juice.

Unfortunatelly, we have found that UV-C treatment of grape juice has affected the sensory properties both in lower and higher doses.

The effect of UV-C radiation on grape juice turbidity is presented in Figure 3. We have observed incrase in turbidity in all UV-C treated grape juices with or without addition of SO_2. In average, an application of 0.68 kJ L^{-1} UV-C increased the turbidity 1.1 times, dose 6.83 kJ L^{-1} 1.2 times and dose 13.67 kJ L^{-1} 1.3 times.

The sensory properties, the smell (Table 3) and taste (Table 4) were changed immediately when grape juices were treated with SO_2. The sensory properties of grape juices sampled 24 and 96 hours after sedimentation without addition of SO_2 was changed slightly after 1 min of treatment (UV-C dose 0.68 kJ L^{-}1) and changed markedly (p-value <0.001) after 2 min of treatment (UV-C dose 1.37 kJ L^{-1}). We can describe the smell and taste after the treatment like baked and strongly simmilar to hydrogen sulphide. This smell and taste was very unpleasant and nasty.

The Principal Components Analysis of four grape juices with and without addition of sulphur dioxide according to the different UV-C treatment times is presented in Figure 6. This figure contains four groups of data that are separated by a distance, which indicates clearly that the UV-C radiation treatment affected the turbidity of grape juices. The turbidity of grape juices with and without addition of SO_2 was increased in relation to the length of UV-C treatment (p <0.05). This findig is in agreement with **Hartly (2008)**. All the wines absorb strongly upper end of the UV spectrum. Wavelengths of 375 and 440 nm have been identified as critical in promoting harmful reactions.

Table 1 The effect of UV-C radiation treatment (254 nm) on the grape juice microbial counts.

	A	B	C	D
UV-C light (254 nm) treatment (min) and Dose (kJ L^{-1})	**Grape juice sampled 24 hours after sedimentation without addition of SO_2**	**Grape juice sampled 24 hours after sedimentation with addition of SO_2**	**Grape juice sampled 96 hours after sedimentation without addition of SO_2**	**Grape juice sampled 96 hours after sedimentation with addition of SO_2**
	CFU mL^{-1}			
0 min	22 x 10^6	17 x 10^6	27 x 10^6	0
0 (kJ L^{-1})	252 x 10^5	152 x 10^5	321 x 10^5	0
1 min	58 x 10^3	9 x 10^3	32 x 10^5	0
0.68 (kJ L^{-1})	2 x 10^4	80 x 10^2	360 x 10^4	0
10 min	26 x 10^2	16 x 10^2	28 x 10^4	0
6.83 (kJ L^{-1})	219 x 10^1	103 x 10^1	190 x 10^3	0
20 min	9 x 10^2	2 x 10^1	14 x 10^3	0
13.67 (kJ L^{-1})	50 x 10^1	23 x 10^0	130 x 10^2	0

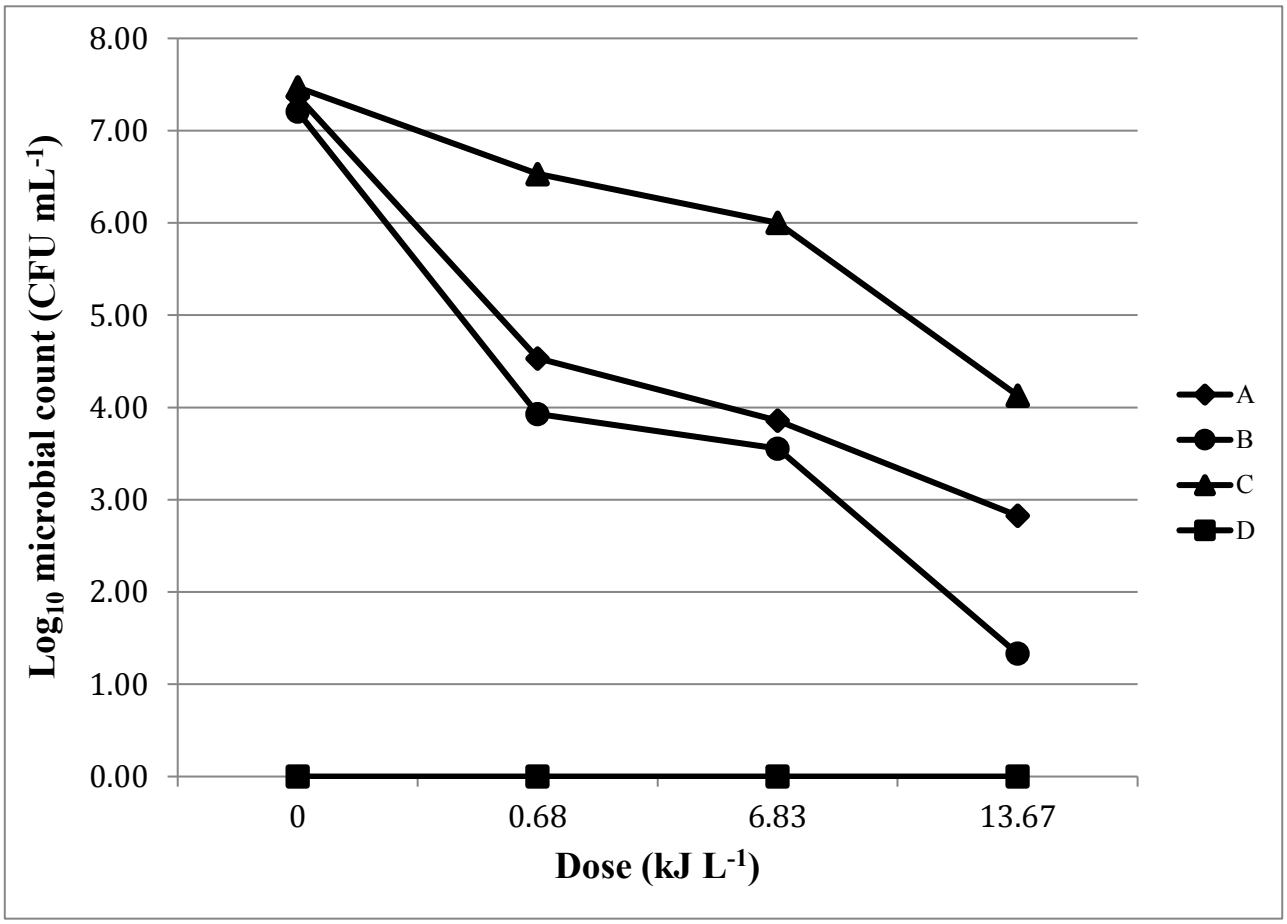

Figure 2 The effect of UV-C (254 nm) radiation treatment on the grape juice microbial counts.

Table 2 The effect of UV-C radiation (254 nm) treatment on the grape juice turbidity.

	A	B	C	D
UV-C light (254 nm) treatment (min) and Dose (kJ L^{-1})	**Grape juice sampled 24 hours after sedimentation without addition of SO_2**	**Grape juice sampled 24 hours after sedimentation with addition of SO_2**	**Grape juice sampled 96 hours after sedimentation without addition of SO_2**	**Grape juice sampled 96 hours after sedimentation with addition of SO_2**
	Average values of absorbance (405 nm)			
0 min	0.541	0.589	1,623	0.375
0 (kJ L^{-1})	cv = 3.5 x 10^{-6}	cv = 2.1 x 10^{-6}	cv = 4.0 x 10^{-6}	cv = 6.0 x 10^{-7}
	sd = 0.002	sd = 0.001	sd = 0.002	sd = 0.001
1 min	0.565	0.656	1.673	0.393
0.68 (kJ L^{-1})	cv = 2.1 x 10^{-5}	cv = 8.2 x 10^{-6}	cv = 3.7 x 10^{-6}	cv = 3.4 x 10^{-6}
	sd = 0.005	sd = 0.003	sd = 0.002	sd = 0.002
10 min	0.654	0.749	1.808	0.415
6.83 (kJ L^{-1})	cv = 1.9 x 10^{-6}	cv = 3.5 x 10^{-6}	cv = 5.8 x 10^{-6}	cv = 2.0 x 10^{-6}
	sd = 0.001	sd = 0.002	sd = 0.002	sd = 0.001
20 min	0.674	0.804	1.868	0.471
13.67 (kJ L^{-1})	cv = 1.3 x 10^{-6}	cv = 3.0 x 10^{-6}	cv = 7.0 x 10^{-6}	cv = 4.4 x 10^{-6}
	sd = 0.001	sd = 0.002	sd = 0.003	sd = 0.002

Note: each measurement (n = 30).

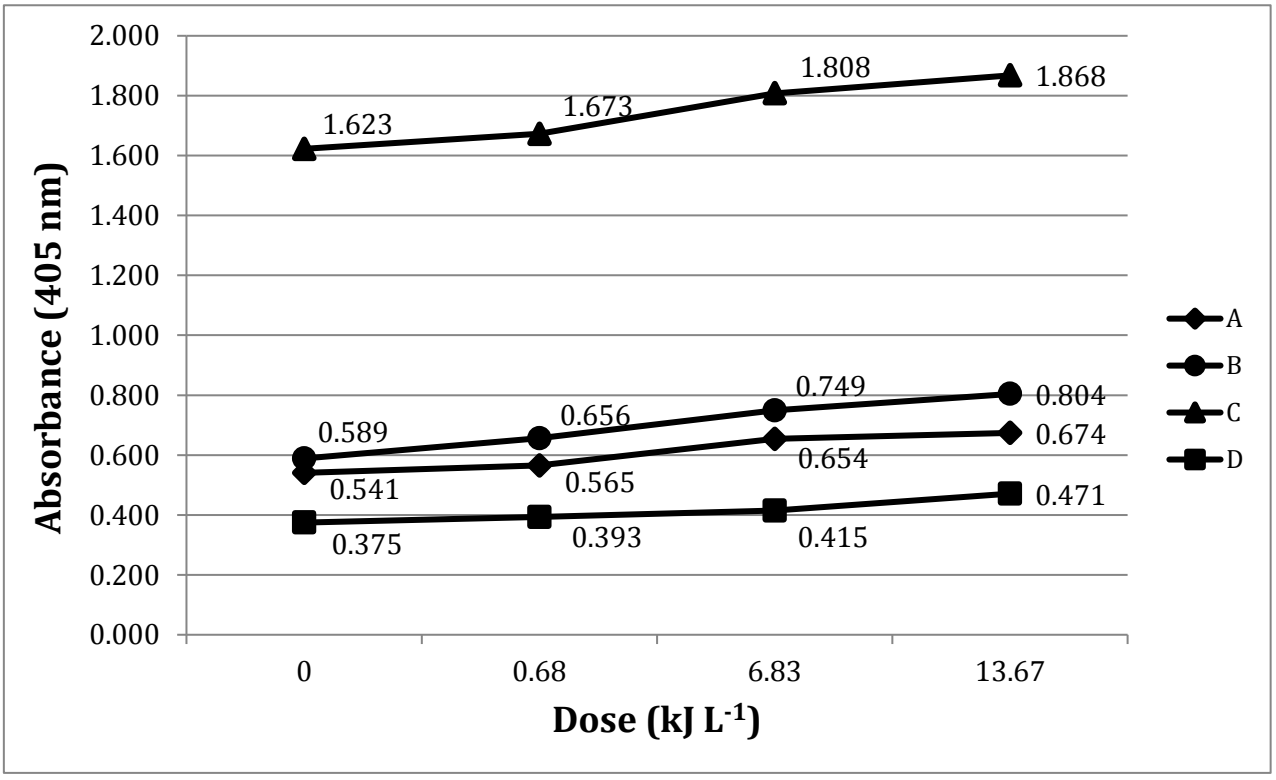

Figure 3 The effect of UV-C radiation (254 nm) on grape juice turbidity.

Table 3 The effect of UV-C radiation (254 nm) treatment on the grape juice smell.

	A	B	C	D
UV-C light (254 nm) treatment (min) and Dose (kJ L^{-1})	**Grape juice sampled 24 hours after sedimentation without addition of SO_2**	**Grape juice sampled 24 hours after sedimentation with addition of SO_2**	**Grape juice sampled 96 hours after sedimentation without addition of SO_2**	**Grape juice sampled 96 hours after sedimentation with addition of SO_2**
	Intensity of foreign smell in the grape juice (0 – 9)			
0 min 0 (kJ L^{-1})	0, 0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0, 0
1 min 0.68 (kJ L^{-1})	2, 3, 3, 2, 3, 2, 5	9, 9, 9, 9, 9, 9, 9	4, 3, 2, 2, 3, 3, 3	9, 9, 9, 9, 9, 9, 9
2 min 1.37 (kJ L^{-1})	8, 9, 7, 8, 8, 8, 7	9, 9, 9, 9, 9, 9, 9	9, 8, 8, 8, 7, 8, 9	9, 9, 9, 9, 9, 9, 9
10 min 6.83 (kJ L^{-1})	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9
20 min 13.67 (kJ L^{-1})	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9

Note: scale of the intensity of foreign smell (0 – odorless, 10 – strong odor). The number of certified assessors: 7.

Multiple comparison of samples by nonparametric Friedman's test revealed there is no significant difference in 0 mins, 10 mins and 20 mins treatments among samples A,B,C and D. In 1 minute and 2 minutes treatments are significant differences (***p* <0.001**). This hypothesis is supported by Figure 4 and Figure 5.

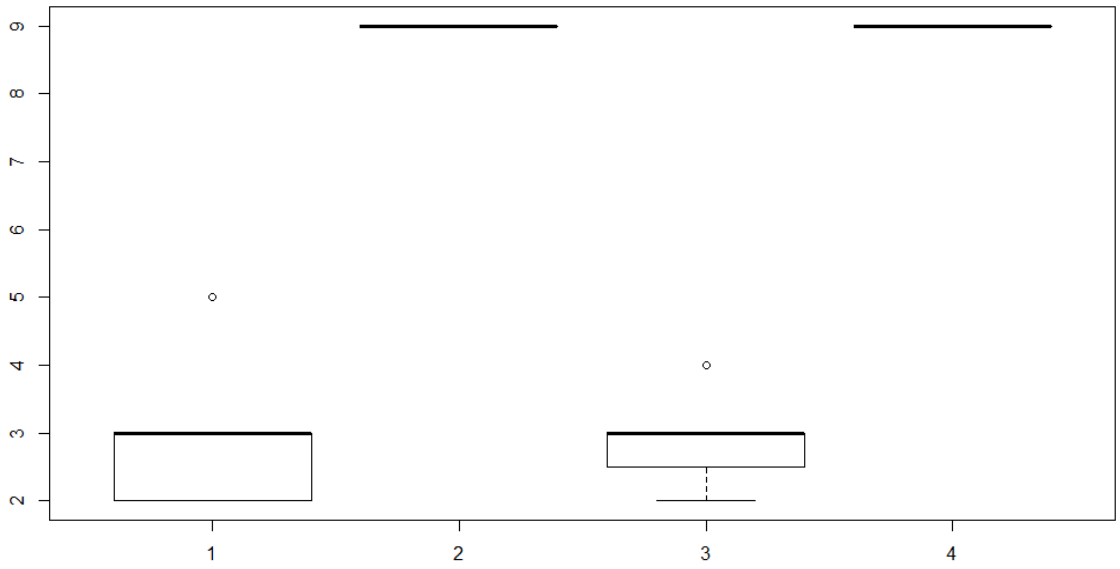

Figure 4. Boxplot of sensory smell values of samples UV-C radiated by (254 nm) 1 minute treatment.

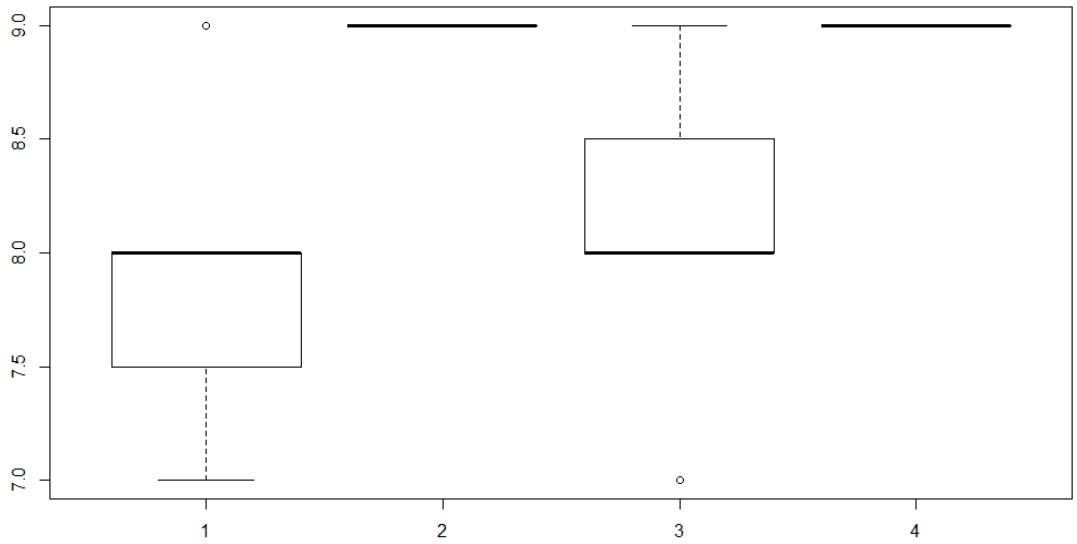

Figure 5. Boxplot of sensory smell values of samples UV-C radiated by (254 nm) 2 minutes treatment.

Table 4 The effect of UV-C radiation (254 nm) treatment on the grape juice taste.

	A	B	C	D
UV-C light (254 nm) treatment (min) and Dose (kJ L^{-1})	**Grape juice sampled 24 hours after sedimentation without addition of SO_2**	**Grape juice sampled 24 hours after sedimentation with addition of SO_2**	**Grape juice sampled 96 hours after sedimentation without addition of SO_2**	**Grape juice sampled 96 hours after sedimentation with addition of SO_2**
	Intensity of foreign smell in the grape juice (0 – 9)			
0 min 0 (kJ L^{-1})	0, 0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0, 0
1 min 0.68 (kJ L^{-1})	2, 3, 3, 2, 3, 2, 3	9, 9, 9, 9, 9, 9, 9	2, 2, 2, 3, 4, 3, 5	9, 9, 9, 9, 9, 9, 9
2 min 1.37 (kJ L^{-1})	7, 8, 8, 8, 7, 8, 8	9, 9, 9, 9, 9, 9, 9	8, 8, 7, 8, 7, 8, 9	9, 9, 9, 9, 9, 9, 9
10 min 6.83 (kJ L^{-1})	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9
20 min 13.67 (kJ L^{-1})	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9	9, 9, 9, 9, 9, 9, 9

Note: scale of the intensity of foreign taste (0 – without foreign taste, 10 – strong foreign taste). The number of certified assessors: 7.

Multiple comparison of samples by nonparametric Friedman's test for taste attributes revealed there is no significant difference in 0 mins, 10 mins and 20 mins treatments among samples A,B,C and D. In 1 minute and 2 minutes treatments is significant difference (p <0.001). Visualization of effect is similar to Figure 4 and Figure 5.

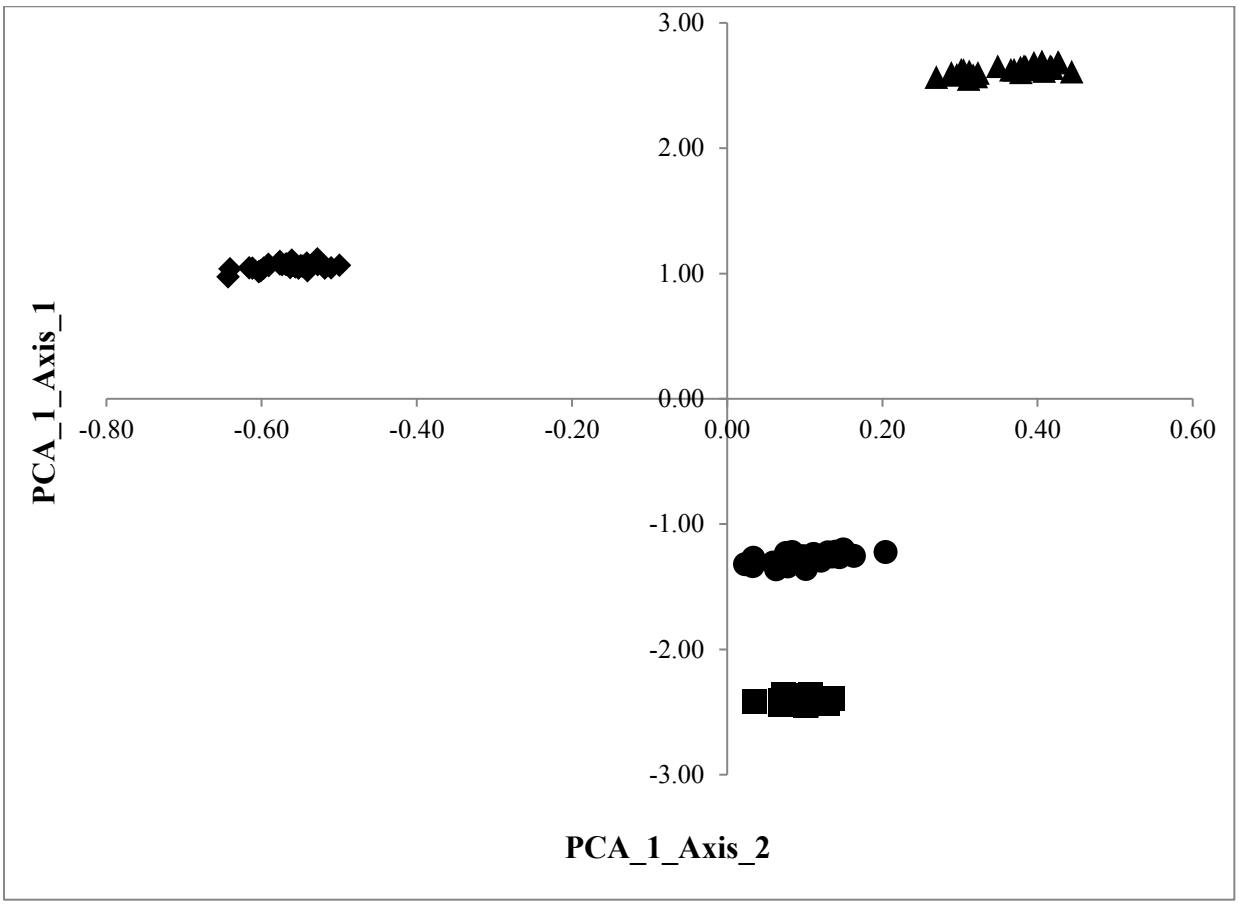

Figure 6 The PCA analysis of four grape juices with and without addition of sulphur dioxide according to the different UV-C treatment times. The PCA_1_Axis_1 and PCA_1_Axis_2 represents the turbidity data of four grape juices after 1 min (■), 2 min (●), 10 min (◆) and 20 min (▲) treatment with UV-C. The turbidity of grape juices with and without addition of sulphur dioxide was increased in relation to the length of UV-C treatment.

TExposure of wine to light results in what is known as light-struck flavours and aromas. These are produced by the initiation of chemical reactions in the wines, resulting in the formation of sulphurous compounds with an unpleasant smell and taste. The reactions can occur within minutes of exposure to light and a tiny amount of the sulphurous compounds can impart a noticeable (bad) taste and aroma to a wine.

The subject of further investigation may be the effect of fermentation on grape juices treated with UV-C radiation. It would be useful to found how the changed sensory properties of grape juice affect the final product.

CONCLUSION

The UV-C (254 nm) radiation increases the turbidity of grape juices (p <0.001). This effect was observed in all grape juices with or without addition of sulphur dioxide and also in clarified or not clarified grape juices.

We found UV-C radiation negatively affect the sensory properties of grape juices (p <0.001). This effect was more pronounced in grape juices treated with sulphur dioxide. The smell and taste were significantly negatively changed. Exposure of grape juice treated with sulphur dioxide to UV-C radiation can probably lead to arising the sulphur compounds, which affects the smell and taste of grape juices. Also, it is very likely that the negative change in taste and smell may negatively affect the quality of produced wines. For this reason, we do not recommend to use UV-C light for the grape juice treatment. It will be interesting to conduct a detail analysis of the grape juices composition before and after UV-C radiation treatment in next work.

REFERENCES

Bachman, K. C., Wilcox, C. J. 1990. Effect of rapid cooling on bovine milk fat hydrolysis. *Journal of Dairy Science.* vol. 73, p. 617-620. http://dx.doi.org/10.3168/jds.S0022-0302(90)78711-5

Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R. 2000. Existing and potential applications of ultraviolet light in the food industry. A critical review. *Journal of the Science of Food and Agriculture*, vol. 80, p. 637-645. http://dx.doi.org/10.1002/(SICI)1097-0010(20000501)80:6<637::AID-JSFA603>3.0.CO;2-1

Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R. K. 2000. Existing and potential applications of ultraviolet light in the food industry- a critical review. *J. Sci. Food Agric.* vol. 80, p. 637-645. http://dx.doi.org/10.1002/(SICI)1097-0010(20000501)80:6<637::AID-JSFA603>3.0.CO;2-1

Choi, L. H., Nielsen, S. S. 2005. The effect of thermal and non-thermal processing methods on apple cider quality and consumer acceptability. *Journal of Food Quality*, vol. 28, p. 13-29. http://dx.doi.org/10.1111/j.1745-4557.2005.00002.x

Delfini, C., Formica, J. V. 2001. *Wine Microbiology: Science and Technology.* NEW YORK, USA : CRC Press, Marcel Dekker, Inc., 499 p. ISBN: 0-8247-0590-4.

Fredericks, I. N., du Toit, M, Krügel, M. 2011. Efficacy of ultraviolet radiation as an alternative technology to inactivate microorganisms in grape juices and wines. *Food Microbiology,* vol. 28, no. 3, p. 510-517. http://dx.doi.org/10.1016/j.fm.2010.10.018

Gabriel, A. A., Nakano, H. 2009. Inactivation of Salmonella, E. coli, and Listeria monocytogenes in phosphate-buffered saline and apple juice by ultraviolet and heat treatments. *Food Control*, vol. 20, p. 443-446. http://dx.doi.org/10.1016/j.foodcont.2008.08.008

Guerrero-Beltrán, J. A., Barbosa-Cánovas, G. V. 2004. Review: Advantages and limitations on processing foods by UV light. *Food Science and Technology International*, vol. 10, p. 137-148. http://dx.doi.org/10.1177/1082013204044359

Guerrero-Beltrán, J. A., Barbosa-Cánovas, G. V. 2005. Reduction of Saccharomyces cerevisiae, Escherichia coli and Listeria innocua in apple juice by ultraviolet light. *Journal of Food Process Engineering*, vol. 28, p. 437-452. http://dx.doi.org/10.1111/j.1745-4530.2005.00040.x

Hartly, A. 2008. The effect of ultraviolet light on wine quality. Banbury, Oxon, UK : WRAP, 24 p. ISBN: 1-84405-386-5

Koutchma, T. N., Forney, L. J., Moraru, C. I. 2009. *Ultraviolet Light in Food Technology.* CRC Press, Boca Raton, p. 13-20 and 75-81. http://dx.doi.org/10.1201/9781420059519

López-Malo, A., Palou, E. 2005. Ultraviolet light and food preservation. In: Barbosa-Cánovas, G. V., Tapia, M. S., Cano, M. P. (Eds.), *Novel Food Processing Technologies*. CRC Press, Boca Raton, p. 405-419.

Lorenzini, M., Fracchetti, F., Bolla, V., Stefanelli, E., Rossi, F., Torriani, S. Ultraviolet light (UV-C) irradiation as an alternative technology for the control of microorganisms in grape juice and wine. In Conference proceeding: International Organization of Vine and Wine - 33rd World Congress of Vine and Wine, 8th General Assembly of the OIV, Volume: V-2010_n° 1240 OR.II.19. Available at: http://www.uiv.it/wp-content/uploads/2013/08/bibiliografia%20-Lorenzini_et_al._OIV-2010_n%C2%B0124.pdf

Otto, C., Zahn, S., Rost, F., Zahn, P., Jaros, D., Rohm, H. 2011. Physical methods for cleaning and disinfection of surfaces. *Food Eng. Rev.,* vol. 3, p. 171-188.

Rakotomalala, R. (2005). "TANAGRA: a free software for research and academic purposes", in Proceedings of EGC'2005, RNTI-E-3, vol. 2, pp.697–702.

Rizzotti, L., Levav, N., Fracchetti, F., Felis, G. E., Torriani, S. 2015. Effect of UV-C treatment on th microbial population of white and red wines, as revealed by conventional plating and PMA-qPCR methods. *Food Control,* vol. 47, p. 407-412. http://dx.doi.org/10.1016/j.foodcont.2014.07.052

Thompson, F., 2003. Ultraviolet light. In: Trugo, L., Finglas, P. M. (Eds.), *Encyclopedia of Food Sciences and Nutrition*. Elsevier Science Ltd, London, p. 5885-5889. http://dx.doi.org/10.1016/B0-12-227055-X/01218-9

Tran, M. T. T., Farid, M. 2004. Ultraviolet treatment of orange juice. *Innovative Food Science and Emerging Technologies*, vol. 5, p. 495-502. http://dx.doi.org/10.1016/j.ifset.2004.08.002

Unluturk, S., Atilgan, M. R. 2015. Microbial safety and shelf life of UV-C treated freshly squeezed white grape juice. *J. Food Sci.*, vol 80, no. 8, p. 1831-1841. http://dx.doi.org/10.1111/1750-3841

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ANTIOXIDANT PROPERTIES OF CUMIN (*BUNIUM PERSICUM* BOISS.) EXTRACT AND ITS PROTECTIVE ROLE AGAINST ULTRASOUND-INDUCED OXIDATIVE STRESS TESTED BY MICRORNA BASED MARKERS

Katarína Ražná, Nishonoy Khasanova, Eva Ivanišová, Davranov Qahramon, Miroslav Habán

ABSTRACT

Bunium persicum Boiss. seeds have been used for medicinal and nutritional properties such as antioxidant, antihelmetic and antimicrobial activity. The aim of this study was to to tested protective role of cumin extract against abiotic stress by microRNA markers. Secondary also was to evaluate antioxidant activity as well as total polyphenol, flavonoid and phenolic acid content of cumin extract. We observed that cumin DNA itself has not been damaged by sonication teratment. This protective impact indicates that cumin antioxidant properties can efficiently quench free radicals induced by sonication. On the other side, ultrasound-mediated formation of reactive oxygen species did induce the DNA polymorphism of lettuce samples which was detected by miRNAs-based markers. The range of sonication impact was time-dependent. Markers based of miRNA-DNA sequences has proven to be an effective tool. We have confirmed statistically significant differences ($p \leq 0.01$) in miRNAs markers ability to detect the polymorphism due to sonication treatment. The antioxidant activity was determined by a method using DPPH radical and phosphomolybdenum method, total polyphenol content with Folin – Ciocalteu reagent, total flavonoid with aluminium-chloride mehod and total phenolic acid with Arnova reagent. Results showed that cumin is rich for biologically active substances and can be used more in different kind of industry as a cheap source of these substances. Antioxidant activity with DPPH method was 1.18 mg TEAC.g^{-1} (TEAC – Trolox equivalent antioxidant capacity per g of sample) and by phosphomolybdenum method 45.23 mg TEAC.g^{-1}. Total polyphenol content achieved value 4.22 mg GAE.g^{-1} (GAE – gallic acid equivalent per g of sample), total flavonoid content value 10.91 mg QE.g^{-1} (QE – quercetin equivalent per g of sample) and total phenolic acid content value 5.07 mg CAE.g^{-1} (CAE – caffeic acid equivalent per g of sample).

Keywords: cumin; antioxidant; ultrasound; microRNA marker

INTRODUCTION

Changing environmental conditions are giving rise to a variety of free radicals, which plants have to deal with them in order to survive. Besides that, free radicals and other reactive oxygen species (ROS) are produced in our body as byproducts of various biological processes of metabolism **(Dua et al., 2012)**. Reactive oxygen species, such as single oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide, are highly reactive, toxic molecules, which are generated normally in cells during metabolism **(Saeed et al., 2012)**. However, overproduction of ROS is also harmful to the body because they cause severe oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation, with subsequent tissue injury **(Dua et al., 2012)**. In addition to those aspects, the technological methods of food processing and preservation include such approaches that themselves represent the source of free radicals and reactive oxygen species which can ultimately cause damage of the products. The different applications of ultrasound in the food industry include ultrasound and microbial inactivation, ultrasound in filtration, ultrasound-assisted extraction and ultrasound in enhancing fermentation **(Bhattacharya, 2015)**.

Ultrasound can result in cavitation and acoustic microstreaming, which modifies cellular ultrastructure, enzyme stability and cell growth. It can also cause breaks in extracellular polymers, release DNA from the nucleus, decrease cell stability, alter cell membrane permeablity and modify charges on the surfaces of cells **(Rokhina et al., 2009)**. In biological applications, sonication may be sufficient to disrupt or deactivate a biological material. Cells being sonicated are subjected to various stimulation including mechanical, thermal, and oxidative stress **(Riesz and Kondo, 1992)**. A cell may sense this stimulation by chemical changes and special movements of molecules

within the cell, and then convey the information to the other parts of the cell through certain signal transduction networks. Receiving such information, the cell may undergo fundamental changes to respond and adapt itself to the stimulations. By doing so, the cell may be able to protect itself from the stimulation, repair any damage and so on **(Suslick, 1990)**. Ultrasound irradiation may lead to various kinds of interactions with the living organism depending upon the sonication conditions. Under certain sonication conditions, it was reported that ultrasound induced gene expression changes in cells **(Abdollahi et al., 2004; Wei et al., 2012)**. Ultrasound (10 – 60 kHz) have been used to modify growth and developmental processes in plants **(Rokhina et al., 2009)**. Sonication also affects the endogenous hormonal balance of treated plant tissue or cells and thereby promotes growth and development **(da Silva and Dobranszki, 2014)**.

Plants contain a wide variety of antioxidant phytochemicals or bioactive molecules, which can neutralize the free radicals and thus retard the progress of many chronic diseases associated with oxidative stress **(Ani et al., 2006)**. Natural antioxidant agents have attracted much interest because of their ability to scavenge free radicals **(Saeed et al., 2012)**. Antioxidants are the compounds that can delay, inhibit or prevent the oxidation of biomolecules like lipids, proteins or nucleic acids. Antioxidants may scavenge the free radicals or break the chain reaction due to their redox properties **(Schafer et al., 2003)**. In general, there are two categories of antioxidants-natural and synthetic. These days, interest has increased in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are restricted due to their carcinogenicity and toxicity **(Barlow, 1990; Klimešová et al., 2015)**. Naturally occurring antioxidants in leafy vegetables, fruits and seeds such as ascorbic acid, vitamin E and phenolic compounds have abiliy to reduce oxidative damage associated with many diseases including cancer, cardiovascular diseases, cataract, arthiritis, diabetes **(Saphira et al., 1989)**.

Cumin (*Bunium persicum* L.) is a flowering and annual herb from *Apiaceae* family which might have originated in the area between Central Asia and Northern India. Cumin is a high value herbaceous spice widely used for culinary, flowering, perfumery and carminative purposes. One of the commonly used spices for their special aromatic effect in food preparations that are prevalent in Central Asia, Caucasus, Crimea. It is also used in herbal medicine as a stimulant, carminative and astringent. Cumin seeds have been reportedly used for traditional treatment of toothache, dyspepsia, diarrhoea, epilepsy and jaundice **(Sultana et al., 2010)**. The plant type of *Bunium persicum* L. varies from dwarf (30 cm) to tall (80 cm) compact or spreading, moderately to highly branched, tuberous and perennial herb **(Panwar, 2000)**. The study of antioxidant properties and antioxidant compounds of cumin **(Dua et al., 2012)** indicate that polyphenol rich methanolic extract of cumin had efficient free radical scavenging and metal chelating activity to protect biomolecules like proteins, lipids and DNA against oxidative stress. A significant protection role of bitter cumin (*Cuminum nigrum* L.) extract againts DNA damage induced by hydroxyl radicals recorded **Ani et al. (2005)**. They also tested the antibacterial activity of cumin extract finding out that the growth of *Bacillus subtilis*, *Bacilus cereus* and *Staphylococcus aureus* was significantly inhibited. Due to contamination of cumin seeds by microorganisms, **Fatemi et al. (2011)** tested the effect of decontamination treatment by gamma-irradiation on the chemical composition and antioxidant properties of cumin extracts. The research showed that total percentage of ten essential oil components was not affected and the changes in flavonoid content were nonsignificant. The antioxidant activities of cumin extract were not altered by gamma-irradiation treatment.

Emerging evidence has suggested that a class of small regulatory RNAs, called microRNAs (miRNAs) play a critical role in regulation of DNA damage response **(Wan et al., 2011)** as a response to various biotic and abiotic stress. Certain miRNAs are either under or over-expressed or new miRNAs are synthesized under stress. Mostly miRNA target genes which encodes various transcriptional factors or functional enzymes having important roles in abiotic stress response **(Bej and Basak, 2014; Kantar et al., 2010)**. Emerging evidence has suggested that miRNAs molecules regulate the DNA damage response by a mechanism based on the nature and intensity of damage **(Simon et al., 2009)**. Although several DNA damage responsive miRNAs and their targets have been identified, there is a need to establish the complex interconnections between miRNAs and their DNA damage response targets **(Wan et al., 2011)**. **Zhang et al. (2011)** found that DNA damage led to increased levels of some pre-miRNAs and mature miRNAs suggesting functional connection between DNA damage response and miRNA processing and maturation.

The aim of this work was to study the protective role of seed cumin extracts and its antioxidant properties on plant DNA from lettuce as a model plant under conditions of sonication induced oxidative stress. An oxidative stress has been recorded on molecular level by microRNA-based markers. Secondary also was to evaluate antioxidant activity as well as total polyphenol, flavonoid and phenolic acid content of cumin extract.

Scientific hypothesis

We have tested the protective role of antioxidant properties of seed cumin extract in conbination with abiotic oxidative stress, on DNA polymorphism. We hypothesized that the effect of sonication-induced oxidative stress can be recorded on molecular level by microRNAs-based markers.

MATERIAL AND METHODOLOGY

Biological material

Cumin (*Bunium persicum* L.*)* seeds were grown in the mountain area of Uzbekistan. They were procured from the local market, identified and authenticated at Department of Botany, National University of Uzbekistan.

Lettuce (*Lactuca sativa* L.) grown in climatic chamber with phytotron system (Pol-Eko Aparatura KK350 Top +Fit) of the AgroBioTech, Research Centre of the Slovak University of Agriculture in Nitra. The plants were grown under photoperiod 16/8 hours (day/night), temperature 21/18 °C, humidity 50% and light intensity 15,000 lx.

Chemicals

All chemicals for antioxidant analyses were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA). Chemicals for molecular analyses were analytical grade and were purchased from ThermoFisher Scientific (USA), Promega (USA), Biotium (Canada) and Microsynth (Switzerland).

Sample preparation

An amount of 0.2 g of cumin sample was extracted with 20 mL of 80% ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids, phenolic acids). Extraction as well as measurements were carried out in triplicates.

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.

Phosphomolybdenum method

Phosphomolybdenum method was realized according to method **Prieto et al. (1999)** with slight modifications. The mixture of sample (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, 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 extracts was measured by the method of **Singleton and Rossi (1965)** using Folin-Ciocalteu reagent. 0.1 mL of each sample 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 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25 – 300 $mg.L^{-1}$; $R^2 = 0.998$) 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)**. 0.5 mL of sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M potassium acetate and 4.3 mL of distilled water. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.5 – 20 $mg.L^{-1}$; $R^2 = 0.989$) was used as the standard and the results were expressed in $\mu g.g^{-1}$ quercetin equivalents.

Total phenolic acid 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 $mg.L^{-1}$, $R^2 = 0.999$) was used as a standard and the results were expressed in $mg.g^{-1}$ caffeic acid equivalents.

Genomic DNA extraction and sonication treatment

The genomic DNA (3 independent samples) was isolated from leaves of model plant *Lactuca sativa* L. based on method by **Padmalatha a Prasad (2007)**. The methodology of this experiment was based on **Dua et al. (2012)**. Genomic DNA extracted from cumin seeds (3 independent samples) was isolated by **Padmalatha a Prasad (2007)** and used as a control. DNA was quantified by NanoPhotometer™ (IMPLEN). The reaction mixture (9 µL) contained 2.70 µg of DNA in 20.0 $mmol.dm^{-3}$ phosphate buffer saline (PBS) (pH 7.4) and different concentrations of cumin extract (0.5%; 1.0% and 1.5%). The samples were pre-incubated for 15 min at ambient temperature. The oxidation stress was induced by sonication of DNAs during different time periods (5, 10 and 15 minutes) in laboratory ultrasonic bath (type K-5LE, KRAINTEK CZECH, s.r.o., Czech Republic) with the ultrasound frequency 38 KHz and power 150 W. Each treatment was performed in duplicates. The reaction was terminated by the addition of loading buffer (Blue/Orange 6x, Promega) and the mixture was subjected to gel electrophoresis in 1.5% agarose/TBE buffer run at 60V. DNA was visualized and photographed by UV-transilluminator (G-Box, Syngene, United Kingdom) system to assess the DNA damage.

In the second type of experiments, the fresh lettuce leaves (100 mg) of were incubated in 1.5 mL of 1×PBS and diferent concentrations (0.5%; 1% and 1.5%) of ethanolic cumin extract, for 15 minutes and consequently the samples were affected by sonication in duration 1, 5, 10 and 15 minutes. Each treatment was performed in duplicates. DNA was extracted using the modified method according to **Padmalantha and Prasad (2006)**. In total 30 samples od DNA were extracted from leaf tissues.The DNA concentration was quantified by the ImplenNano Photometer® (Germany), measuring the absorbance at 260nm. The purity and integrity was assessed by the absorbance 260/280nm ratio. And diluted then into 50 $ng.\mu L^{-1}$ with nuclease-free water for PCR amplification.

microRNA molecular markers assay

Genomic DNAs isolated from lettuce leaves were analyzed by molecular markers based on conservative families of microRNA (miR156 and miR168). The miRNA based markers PCR amplified in 20 µl reaction

mixture that contained 70 ng of genomic DNA, 1x DreamTaq Buffer (KCl, $(NH_4)_2SO_4$, 20mmol.dm^{-3} $MgCl_2$), 2 units DreamTaq DNA polymerase, 0.8 mmol.dm^{-3} dNTPs, 10 mmol.dm^{-3} of each primer and nuclease-free water for PCR amplification. The PCR amplification program used '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 a final extension at 72 °C for 10 min.

The primers for the miRNA-based markers were designed according to the mature miRNA sequences, originated from the miRNA database (http:www.mirbase.org/). Two miRNA-based forward primers, miRNA156 (5`-GTGCAGGGTCCGAGGT-3`) and miR168 (5`-CACGCATCGCTTGGTGCAGGT-3`) and one universal miRNA reverse primer (5`-CCAGT GCAGGGTCCGAGGTA-3`) were used and combined to perform a marker assay.

PCR product were separated on 3 % agarose and 10% TBE-Urea gels (miRNA assay) in 1 x TBE Running Buffer at a constant power of 90 V , 120mA for 60min, respectively at 180 V, 15 mA for 90 min. The polyacrylamide gels were stained with GelRed^{TM} (0.5 μg.mL^{-1}) and were visualized under UV by the G-Box electrophoresis documentation system. The DNA fragment size was compared to 100bp Gene Ruler and in the case of polyacrylamide gels by 10bp DNA ladder.

The DNA amplification profile by both markers was analyzed by GeneTool software (Syngene, Germany)**.** Each fragment is characterized by quantity and volume of profile in pixels. Profiles are recorded on the basis of the set threshold value in which the analysis is carried out.

Statisic analysis

The statistical analyses were performed with the statistical program Statgraphics (version 5.0) and the SAS program (the SAS system v 9.2.). Results of microRNA-based markers assay were evaluated by One-Way ANOVA. Differences were considered significant at $p \leq 0.01$. To test differences was used Tuckey test. Results of antioxidant activity as well as total polyphenols, flavonoids and phenolic acids are presented as mean value with standard deviation.

RESULTS AND DISCUSSION

Antioxidant activity

Antioxidant activity (Table 1) of tested cumin extract was 1.18 mg TEAC.g^{-1} evaluated with DPPH method and 45.23 mg TEAC.g^{-1} by phosphomolybdenum method. **Sharififar et al. (2010)** tested methanolic extract of cumin and reported strong activity with DPPH method – 45.7 μg.mL^{-1}. This authors also reported that in general, the cumin essential oil showed higher activity than solvent extracts from the cumin **(Sharififar et al., 2010)**. Similarly **Chizzola et al. (2014)** tested antioxidant activity of methanolic extract of cumin and their value varied from 9.2 to 14.4 mg Trolox equivalents in the DPPH test and from 6.1 to 10.9 mg.g^{-1} in the Fe-reduction test. In our study we tested activity of ethanolic extract, but many researchers also detected strong antioxidant activity of black cumin essential oil. **Shahsauari et al. (2008)** reported strong activity evaluated with DPPH methos of this oil with value 0.88 mg.mL^{-1} (EC50). These authors also showed that *Bunium persicum* essential oil is able to reduce the oxidation rate of the soybean oil in the accelerated condition at 60 degrees C (oven test). Study of **Kareshk et al. (2015)** showed that *B. persicum* essential oil as a natural source can be used for production of new prophylactic agent for use in toxoplasmosis. *Bunium persicum* Boiss. is an economically important medicinal plant growing wild in the dry temperature regions in Iran, Kazakhstan and Uzbekistan with the strong potential of widely using in different kind of industry.

Total polyphenol, flavonoid and phenolic acid content

Total polyphenol content in evaluated cumin extract achieved value 4.22 mg GAE.g^{-1}, total flavonoid value 10.91 mg QE.g^{-1} and total phenolic acid value 5.07 mg CAE.g^{-1} (Table 1). Our results are comparable with findings of **Chizzola et al. (2014)** which tested these compounds in methanolic extract of cumin and total polyphenol content in their study was between 8.7 – 14.7 mg GAE.g^{-1}, total flavonoid content between 4.3 – 9.5 mg CE.g^{-1} (catechin equivalent). **Nickavar and Abolhasani (2009)** reported a total flavonoid content of cumin extract in value 20.2 mg.g^{-1} as rutin-equivalents. **Seri et al. (2017)** measured total polyphenol conten in hydroalcoholic extract of cumin and found value 122.41 mg GAE.g^{-1}. These authors also published that this hydroalcoholic extract might have therapeutic potentials in the prevention of glycation-mediated diabetic complications. **Souri et al. (2008)** published total polyphenol content in cumin in amount 214.03 ±4.10 mg GAE.100 g^{-1}.

MicroRNA-based markers assay

The ultrasound is widely used tool not only in medicine,

Table 1 Antioxidant activity, total polyphenol, flavonoid and phenolic acid content in cumin (*Bunium persicum* Boiss.) ethanolic extract.

Sample	DPPH (mg TEAC.g^{-1})	PM (mg TEAC.g^{-1})	TPC (mg GAE.g^{-1})	TFC (mg QE.g^{-1})	TPA (mg CAE.g^{-1})
Cumin (*Bunium persicum* Boiss.)	1.18 ±0.11	45.23 ±2.38	4.22 ±0.78	10.91 ±1.63	5.07 ±0.42

Note: DPPH - radical scavenging activity; PM – phosphomolybdenum method; TPC – total polyphenol content; TFC – total flavonoid content; TPA – total phenolic acid content; TEAC – trolox equivalent antioxidant capacity; GAE – gallic acid equivalent; QE – quercetin equivalent; CAE – caffeic acid equivalent; ±standard deviation.

but its applications in food industry and agriculture are more than significant. In addition, the thermal and chemical effects of ultrasound could be considered as a kind of serious environmental abiotic stress factor equal to the impacts of the current advanced telecommunication technologies on human beings. Therefore the research on the impact of sonication treatment on biological subjects is necessary. Ultrasound induces thermal, mechanical and chemical changes on morphological, cytological and molecular level. The ultrasound bioeffects include formation of free radicals and reactive oxygen species **(Milowska and Gabryelak, 2007)**. The use of genomic DNA as a template for fragmentation by sonication is one of the necessary approaches prior to library construction or subcloning for DNA sequencing **(Sambrook and Russell, 2006; Lee and Abdullah, 2014)**. Antioxidant properties of spices and herbs are known since ancient times and their pharmaceutical and medicinal potential is inexhaustible and still brings new possibilities of their use.

The protective role of cumin seed extract has been tested both on isolated genomic DNA treated by ultrasound and on plant tissue material effected by ultrasound during various time points. Non-treated genomic DNA isolated from lettuce and cumin seeds represented the control samples. Additional control was the cumin DNA treated with ultrasound in duration of 5, 10 and 15 minutes as the rest of tested samples. From the Figure 1 can be seen the effect of sonication on DNA degradation in the presence of different concentration of cumin extract. In comparison to non-treated control samples (C0 and C1) and sonicated samples of cumin DNA (C25, C210 and C215), dagradation proces of DNA due to the ultrasound treatment is visible, having increasing tendency with the time of action. It seems that the pretreatment of DNA with the cumin extract of various concentrations (0.5%; 1.0% and 1.5%), does not have any impact on DNA protection againts the damage, probably due to unsifficient of cumin extract in these solutions. But on the other hand, it can be observed that the cumin DNA, itself (Figure 1; $C2_5$, $C2_{10}$ and $C2_{15}$) has not been disrupt by sonication at any time point. This protective impact of the extract indicates that antioxidant properties of cumin seeds can efficiently quench free radicals induced by sonication. This corresponds to the results of Dua et al. (2012). As the authors stated, the presence of extract equivalent to 0.5 µg and 1.0 µg cumin in the incubation mixture could prevent damage of DNA.

In order to analyze the impact of ultrasound-mediated ROS formation at the molecular level more closely, we applied the markers based on microRNA sequences, due to

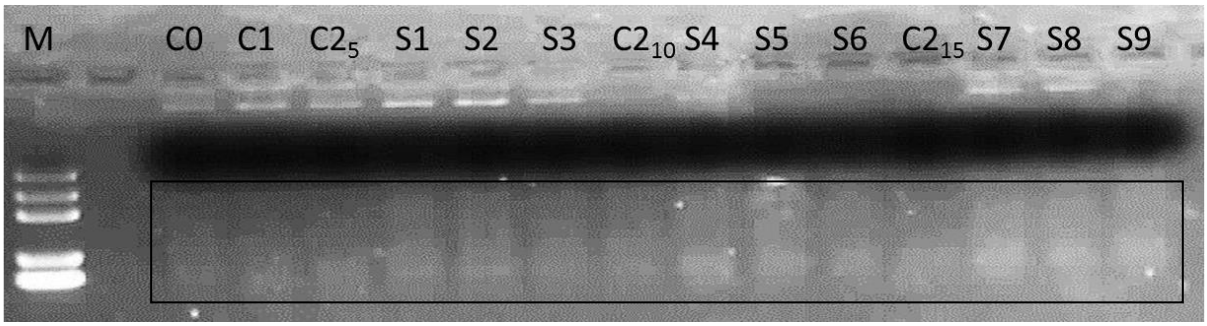

Figure 1 The effect of cumin seeds extract during the sonication treatment of lettuce DNA vizualized on 1.5% agarose gel. Note: M – DNA marker; control samples C0 – lettuce DNA non-treated, C1 – cumin DNA non-treated, C2 – cumin DNA treated with ultrasound in duration of 5, 10 and 15 minutes; samples S1, S2 and S3 - lettuce DNA in 0.5%, 1.0% and 1.5% cumin extract, treated by sonication 5 min; S4, S5 and S6 – lettuce DNA in 0.5%, 1.0% and 1.5% cumin extract, treated by sonication 10 min; S7, S8 and S9 – lettuce DNA in 0.5%, 1.0% and 1.5% cumin extract, treated by sonication 15 min. The highlighted area shows the degree of DNA degradation.

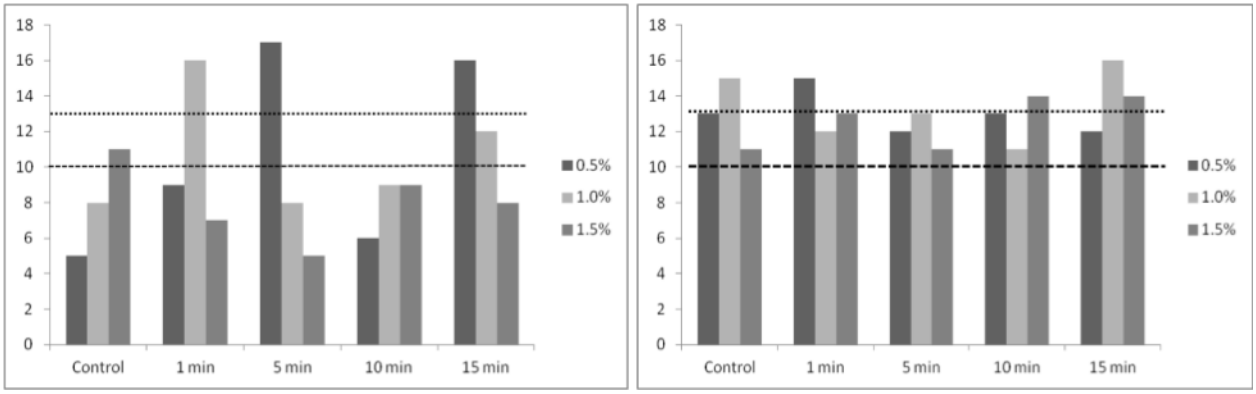

Figure 2 Number of DNA fragments of lettuce (*Lactuca sativa* L.) samples after ultrasound treatment, preceded by cumin extracts pre-incubation, detected by marker miRNA156 (left) and miR168 (right). Linear lines show numbers of DNA fragments in cotrol samples of cumin (interrupted line) and lettuce (starred line).

their role in regulation of DNA damage response **(Zhou et al., 2007; Kruszka et al., 2012)**. For this reason were pieces (approximately 100 mg) of fresh leaf tissue material taken and prior to ultrasound treatment, they were incubated 15 minutes in PBS buffer together with various concentrations of cumin seed extract.

Consequently, the total genomic DNA was extracted and loaded on 1.5% agarose gel (picture not shown). Along with this proccesure was DNA analyzed by miRNA-based markers in touchdown PCR method. For these purposes two types of miRNA families have been chosen, conservative miR156 and miR168. Both types of miRNAs are considered as biomarkers of abiotic stress **(Bej and Basak, 2014; Kruszka et al., 2012; Zhou et al., 2007)**. The family miR156 targets squamosa promoter binding protein (SBP), transcription factor which is involved in

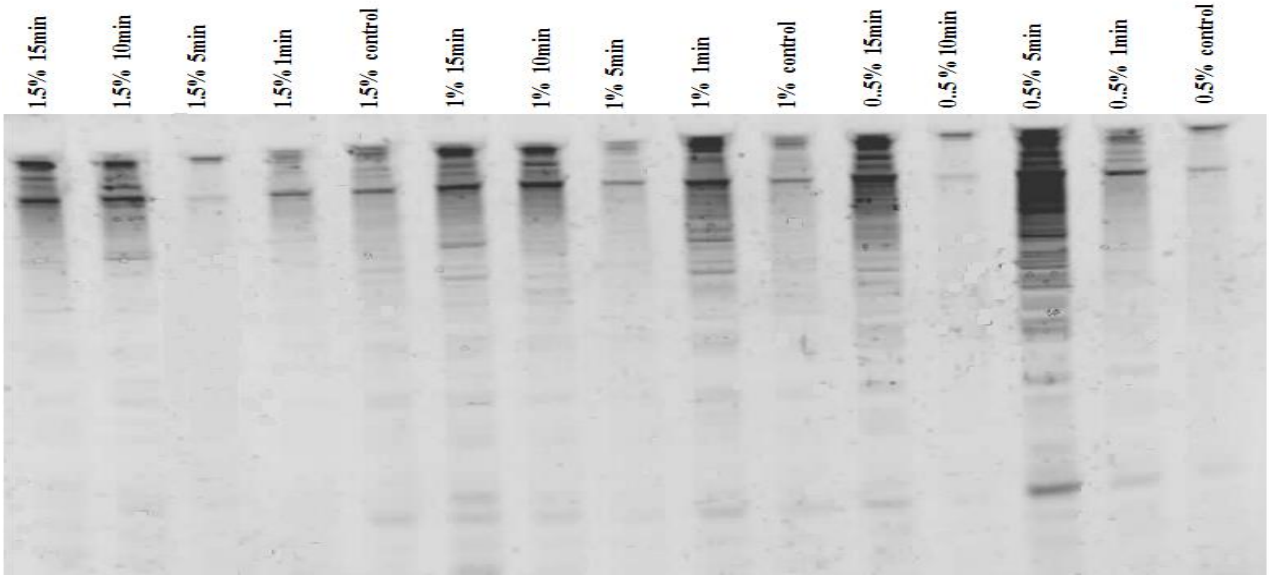

Figure 3 An example of the the gel with the PCR products of lettuce generated by miR156 marker.

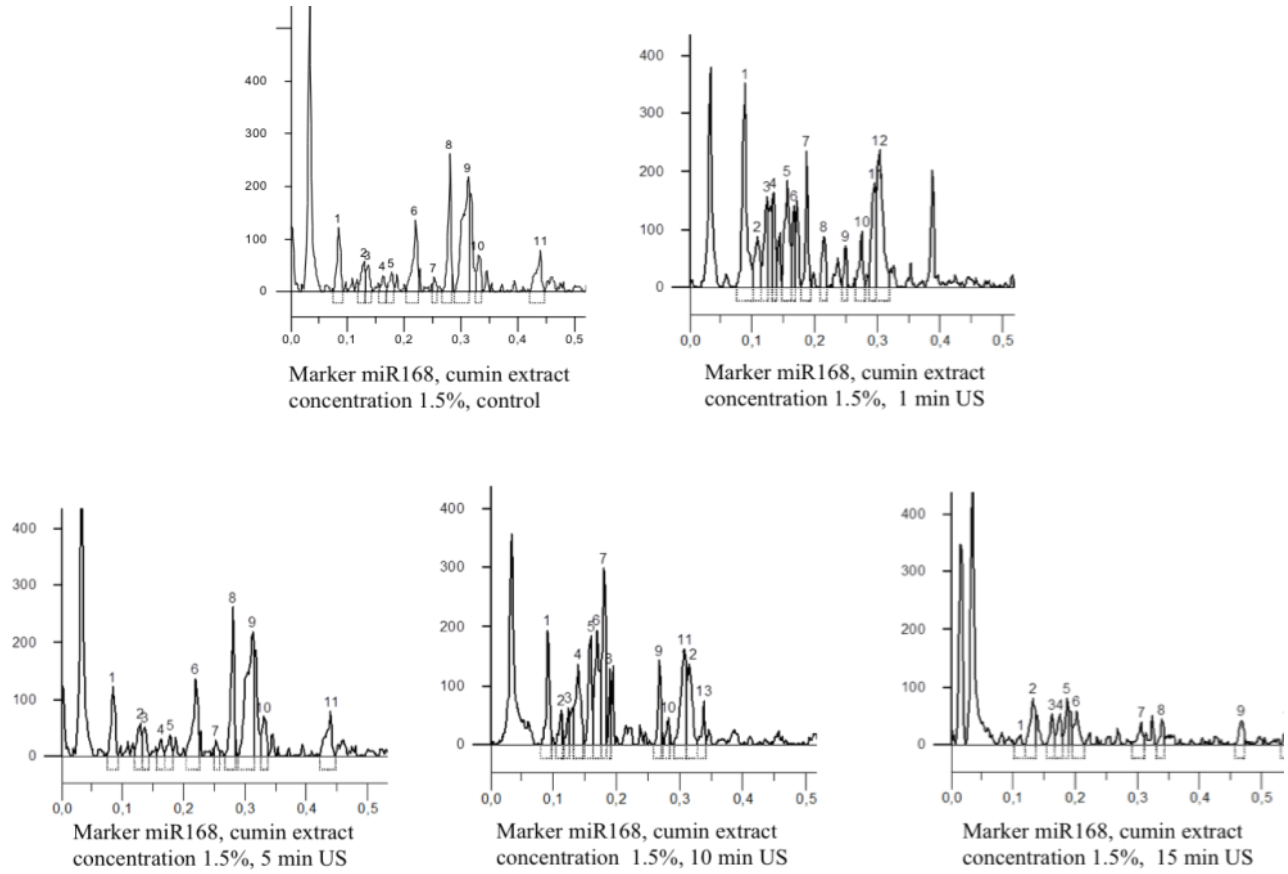

Figure 4 An example of the profiles of miRNA-based DNA fragments in control and treated samples by the ultrasound (US) generated by miR168 marker. Before treatment were samples incubated in solution of 1.5% cumin seed extract.

many biologically important processes **(Barvkar et al., 2013)**. One of the target sequences of miR168 family are sequences of cytochrome P450 which is involved in a wide range of biosynthetic reactions, which include ROS inactivation **(Kruszka et al., 2012)**. Based on the number and profile of individual peaks representing DNA fragments, we could observe more sensitive response to applied ultrasound treatment in miR168 marker in comparison to the marker miR156 (Figure 2) regardless to cumin extract pre-treatment of the samples. An example of the the gel with the PCR products of lettuce generated by miR156 marker shows figure 3.

The number of amplified fragments by miR168 was doubled in comparison to miR156. This difference was statistically significant (significance level 0.0056 at $p \leq 0.01$). More interestingly, the profile of peaks generated by marker miR168 has been distinguished (Figure 4) and the increasement of the marker activity has been recorded depending on the length of the ultrasound treatment. The extent of DNA damage may lead to activation of miRNAs which suggest that these molecules regulate the DNA damage response by a mechanism based on the intensity of DNA damage **(Simon et al., 2009)**.

In contrary, the profiles of DNA fragments generated by marker miR156 was quite balanced (Figure 3a, b and c). Our results correspond to observations of **Kruszka et al., (2012)**, **Xin et al. (2010)** and **Lu et al. (2005)**. As it has been recorded in our previous studies **(Ražná et al., 2015)** the polymorphism and expression of miR156 and miR168 markers was not only species- but also tissue- and developmentally-specific.

Molecular effects of ultrasound can be observed on the level physical, chemical and stress-induced changes which arise as a result of thermal, mechanical and acoustic axposure **(Rokhina et al., 2009)**. With the duration of treatment the temperature is increasing which may lead to denaturation of proteins and this consequently interferes with cellular machineries to repair proteins and membranes **(Bej and Basak, 2014)**. In response to heat stress the miR156 and miR168 in wheat were found to be up regulated **(Xin et al., 2010)**. Differential expression profile of miR156 and miR168 was studied in different plant species under various abiotic and biotic stress factors **(Kruszka et al., 2012)**. In most of tested abiotic stress conditions was marker miR168 up regulated.

However, in the study of *Populus trichocarpa* **(Lu et al., 2005)** was observed that miR156 was found to be down regulated during the mechanical stress and miR168 was up regulated in tension-stressed tissues. As **Bej and Basak (2014)** has stated in the context of response of UV-B radiation stress-responsive miRNAs families, that their reaction to the stress factor may be species-specific.

The results demonstrated that ultrasound at the used intensity and frequency is inducing the polymorphic variability at molecular level what was detected by the miRNA-based DNA markers. This effect of ultrasound was dependent on the duration of the treatment. Our results are in agreement with the observation of **Milowska and Gabryelak, (2007)**. **Juhaimi et al. (2017)** studied the effect of preultrasonic process on oil content and fatty acid composition of black cumin seeds and found out the major decrease in linoleic acid content after sonication for 30 minutes.

At the same time, we conducted the same type of experiments with the incense (*Boswellia sacra* FLUECKIGER) (results not shown). The antioxidant properties as well as the DNA degradation profile due to the ultrasound treatment have shown similar behavior. However, significant differences in the number and profile of individual peaks representing miRNA-based DNA fragments, with respect to tested concentrations of cumin extract has not been noticed. The reason for the observed phenomenon may be caused by low level of antioxidants in the particular sample of cumin seeds and subsequently with an insufficient concentration of the extracts in the solutions applied during the samples pre-treatment. The antioxidant properties of cumin seed extract are dependent of the total radical scavenging activity (DPPH) reflecting the total phenolic and flavonoid content. **Abdelhaliem and Al-Huqail, (2016)** observed distinct variations in DPPH and the content of phenols and flavonoids among and between 11 cumin accessions. Observed variations could be due to high variability in the substances with antioxidant characteristics which may be induced by diverse ecogeographical areas of cumin accessions cultivation.

CONCLUSION

Cumin (Bunium persicum Boiss.) is very interešting medicinal plant which contains various bioactive compounds. This plant is not typically for Slovak repuplic, but nowadays start be very popular mainly in gastronomy due to the specifical flavour. Results in this study showed that cumin had good antioxidant activity as well as polyphenol composition. Nevertheless, it is clear that reactive oxygen species, induced by sonication treatment did cause the polymorphism at the molecular level detected by miRNAs-stress markers, despite the co-operation of lettuce samples and the cumin extract. MiRNAs-based molecular markers effectively dettected the variability at the molecular level caused by abiotic stress factors. Specifically, the response of miR168 marker was statistically more sensitive in comparison to miR156 marker. The results, at the same time, pointed out the fact that the changes that took place within the molecules of biological objects exposed to ultrasonic waves, might be significant for food technological processes.

REFERENCES

Abdollahi, A., Domhan, S., Jenne, J. W., Hallaj, M., Dell´Aqua, G., Mueckenthaler, M., Richter, A., Martin, H., Debus, J., Ansorqe, W., Hynynen, K., Huber, P. E. 2004. Apoptosis signals in lymphoblasts induced by focused ultrasound. *The FASEB Journal*, vol. 18, no. 12, p. 1413-1414. https://doi.org/10.1096/fj.04-1601fje

Ani, V., Varadaraj, M. C., Naidu Akhilender, K. 2006. Antioxidant and antibacterial activiries of polyphenolic compounds from bitter cumin (*Cuminum nigrum* L.). *European Food Research and Technology*, vol. 224, p. 109-115. https://doi.org/10.1007/s00217-006-0295-z

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. 1149-161. https://doi.org/10.1007/s00425-012-1833-5

Barlow, S. M. 1990. Toxicological Aspects of Antioxidants Used as Food Additives. In: Hundson, B. J. *Food Antioxidants*. Amsterdam, Netherlands : Springer Netherlands, p. 253-307. ISBN 978-94-010-6824-6. https://doi.org/10.1007/978-94-009-0753-9_7

Bej, S., Basak, J. 2014. MicroRNAs: The potential biomarkers in plant stress response. American Journal of Plant Sciences, vol. 5, p. 748-759. https://doi.org/10.4236/ajps.2014.55089

Bhattacharya, S. 2015. Conventional and Advanced Food Processing Technologies. Chapter 21. In Jambrak, A. R. et al. *Application of Ultrasonics in Food Preservation and Processing*. Hoboken, New Jersey, USA : John Wiley and Sons, Ltd. p. 515-536. ISBN 9781118406281.

da Silva, J. A. T, Dobranszki, J. 2014. Sonication and ultrasound: impact on plant growth and development. *Plant Cell, Tissue and Organ Culture*, vol. 117, no. 2, p. 131-143. https://doi.org/10.1007/s11240-014-0429-0

Chizzola, R., Saeidnejad, A. H., Azizi, M., Oroojalian, F., Mardan, H. 2014. Bunium persicum: variability in essential oil and antioxidant acitivty of fruits different Iranian wild populations. *Genetic Resources and Crop Evolution*, vol. 61, no. 8, p. 1621-1631. https://doi.org/10.1007/s10722-014-0158-6

Dua, A., Gupta, S. K., Mittal, A., Mahajan, R. 2012. A study of antioxidant properties and antioxidant compounds of cumin. *International Journal of Pharmaceutical and Biological Archives*, vol. 3, no. 5, p. 1110-1116.

Farmakopea Polska. 1999. 5th ed. Warszawa, Poland : PTFarm. 664 p.

Fatemi, F., Dadkhah, A., Rezaei, M. B., Dini, S. 2013. Effect of ɣ-irradiation on the chemical composition and antioxidant properties of cumin extracts. *Journal of Food Biochemistry*, vol. 37, no. 4, p. 432-439. https://doi.org/10.1111/j.1745-4514.2011.00641.x

Juhaimi, F. A., Uslu, N., Özcan, M. M. 2017. The effect of preultrasonic process on oil content and fatty acid composition of hazelnut, peanut and black cumin seeds. *Journal of Food Processing and Preservation*, vol. 42, no. 1, p. 1-4.

Kantar, M., Unver, T., Budak, H. 2010. Regulation of Barley miRNAs upon dehydration stress correlated with target gene expression. *Functional and Integrative Genomics*, vol. 10, no. 4, p. 493-507. https://doi.org/10.1007/s10142-010-0181-4
PMid:20676715

Kareshk, A. T., Keyhani, A., Mahmouduand, H., Oliaei, R. T., Asadi, A., Andishmand, M., Azzizian, H., Babaei, Z., Ziaali, N. 2015. Efficacy of the Bunium persicum Boiss. essential oil against Toxoplasmosis in mice model. *Iran Journal of Parasitology*, vol. 10, no. 4, p. 625-631.

Klimešová, M., Horáček, J., Ondřej, M., Manga, I., Koláčková, L., Nejeschlebová, Ľ., Ponížil, A. 2015. Microbial contamination of spice sused in production of meat products. *Potravinárstvo*, vol. 9, no. 1, p. 154-159. https://doi.org/10.5219/440

Kruszka, K., Pieczynski, M., Windels, D., Bielewicz, D., Jarmolowski, A., Kulinska-Szweykowska, Z., Vazquez, F. 2012. Role of microRNAs and other sRNAs of plants in their changing environments. *Journal of Plant Physiology*, vol. 169, no. 16, p. 1664-1672. https://doi.org/10.1016/j.jplph.2012.03.009
PMid:22647959

Lee, L. J., Abdullah, M. 2014. Optimization of genomic DNA shearing by sonication for next-generation sequencing library preparation. *Asia-Pacific Journal of Molecular Biology and Biotechnology*, vol. 22, no. 3, p. 200-208.

Lu, S., Sun, Y., Shi, R., Clark, C., Li, L., Chiang, V. 2005. Novel and mechanical stress-responsive MicroRNAs in Populus trichocarpa that are absent from Arabidopsis. *Plant Cell*, vol. 17, no. 8, p. 2186-2203. https://doi.org/10.1105/tpc.105.033456
PMid:15994906

Milowska, K., Gabryelak, T. 2007. Reactive oxygen species and DNA damage after ultrasound exposure. *Biomolecular Engineering*, vol. 24, no. 2, p. 263-267. https://doi.org/10.1016/j.bioeng.2007.02.001
PMid:17353145

Padmalatha, K, Prasad, M. N. V. 2005. Optimization of DNA isolationand PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India. *African Journal Biotechnology*, vol. 5, no. 3, p. 230-234.

Panwar, K. 2000. Black caraway. In Arya P. S. *Spice crops of India*. New Delhi : Kalyani Publishers, p. 172-178.

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. https://doi.org/10.1006/abio.1999.4019
PMid:10222007

Riesz, P., Kondo, T. 1992. Free radical formation induced by ultrasound and its biological implications. *Free Radical Biology and Medicine*, vol. 13, no. 3, p. 247-270. https://doi.org/10.1016/0891-5849(92)90021-8

Rogers, S. O., Bendich, A. J. 1994. Extraction of total cellular DNA from plants, algae and fungi. *Plant molecular biology Manual*, p. 183-190. https://doi.org/10.1007/978-94-011-0511-8_12

Rokhina, E. V., Lens, P., Virkutyte, J. 2009. Low-frequency ultrasound in biotechnology: state of the art. *Trends in Biotechnology*, vol. 27, no. 5, p. 298-299. https://doi.org/10.1016/j.tibtech.2009.02.001
PMid:19324441

Saeed, N., Khan, M. R., Shabbir, M. 2012. Antioxidant activity, totalphenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine*, vol. 12, p. 221. https://doi.org/10.1186/1472-6882-12-221

Sambrook, J., Russell, D. W. 2006. Fragmentation of DNA by sonication. *CSH protocols*, vol. 2006, no. 4.

SAS 2009. Users guide version 9. 2. SAS/stat (r) SAS institute inc. cary, nc, USA.

Shapira, Z., Terkel, J., Egozi, Y., Nyska, A., Friedman, J. 1989. Abortifacient potential for the epigeal parts of Peganum harmala. *Journal of Ethnopharmacology*, vol. 27, no. 3, p. 319-325. https://doi.org/10.1016/0378-8741(89)90006-8

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. https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9

Seri, A., Khorsand, M., Rezaei, Z., Hamedi, A., Takhshid, M. A. 2017. Inhibitory effect of Bunium persicum hydroalcoholic extract on glucose-induced albumin glycation, oxidation and aggregation in vitro. *Iran Journal of Medical Sciences*, vol. 42, no. 4, p. 369-376. PMid:28761203

Shahsavari, N., Barzegar, M., Sahari, M. A., Naghdibadi, H. 2008. Antioxidant activity and chemical characterisation of essential oil of Bunium persicum. *Plant Foods for Human*

Nutrition, vol. 63, no. 4, p. 183-188. https://doi.org/10.1007/s11130-008-0091-y PMid:18810640

Sharififar, F., Yassa, N., Mozaffarian, V. 2010. Bioactivity of major components from the seeds of Bunium persicum Boiss. *Pakistan Journal of Pharmaceutical Sciences*, vol. 23, no. 3, p. 300-309. PMid:20566444

Schafer, F. Q., Kelly E. E., Buettner, G. R. 2003. Oxidative stress and antioxidant intervention. In: Cutler, R. G. et al. *Critical Reviews of Oxidative Stress and Aging: Advances in Basic Science, Diagnostics and Intervention*. New Jersey, London, Singapore, Hong Kong : World Scientific, p 849-869. ISBN 981-02-4636-6.

Simone, N. L., Soule, B. P, Ly, D., Saleh, A. D., Savage, J. E., Degraff, W., Cook, J., Harris, C. C., Gius, D., Mitchell, J. B. 2009. Ionizing radiation-induced oxidative stress alters miRNA expression. *PLoS One*, vol. 4, no. 7, p. e6377. https://doi.org/10.1371/journal.pone.0006377 PMid:19633716

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.

Souri, E., Amin, G., Farsam, H., Barazandeh, T. M. 2008. Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. *DARU Journal of Pharmaceutical Sciences*, vol. 16, no. 2, p. 83-87.

Sultana, S., Ripa, F. A., Hamid, K. 2010. Comparative antioxidant activity study of some commonly used spices in Bangladesh. *Pakistan Journal of Biological Sciences*, vol. 13, no. 7, p. 340-343. https://doi.org/10.3923/pjbs.2010.340.343 PMid:20836290

Suslick, K. S. 1990. Sonochemistry. *Science*, vol. 247, no. 4949, p. 1439-1445. https://doi.org/10.1126/science.247.4949.1439 PMid:17791211

Xin, M., Wang, Y., Yao, Y., Xie, C., Peng, H., Ni, Z., Sun, Q. 2010. Diverse set of MicroRNAs are responsive to powdery mildew infection and hest stress in wheat (*Triticum aestivum* L.). *BMC Plant Biology*, vol. 10, p. 123. https://doi.org/10.1186/1471-2229-10-123 PMid:20573268

Wan, G., Mathur, R., Hu, X., Zhang, X., Lu, X. 2011. MicroRNA response to DNA damage. *Trends in Biochemical Sciences*, vol. 36, no. 9, p. 478-484. https://doi.org/10.1016/j.tibs.2011.06.002 PMid:21741842

Wei, M., Yang, C. Y., Wei, S. H. 2012. Enhancement of the differentiation of protocorm-like bodies of Dendrobium officinale to shoots by ultrasound treatment. *Journal of Plant Physiology*, vol. 169, no. 8, p. 770-774. https://doi.org/10.1016/j.jplph.2012.01.018 PMid:22437146

Willett, W. C. 2002. Balancing life-style and genomics research for disease prevention. *Science*, vol. 292, no. 5568, p. 695-698. https://doi.org/10.1126/science.1071055 PMid:11976443

Zhang, X., Wan, G., Berger, F. G., He, X., Lu, X. 2011. The ATM kinase induces microRNA biogenesis in the DNA damage response. *Molecular Cell*, vol. 41, no. 4, p. 371-383. https://doi.org/10.1016/j.molcel.2011.01.020 PMid:21329876

Zhou, X., Wang, G., Zhang, W. 2007. UV-B responsive microRNA genes in Arabidopsis thaliana. *Molecular Systems Biology*, vol 3, no. 103. https://doi.org/10.1038/msb4100143

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CONSTITUENTS OF THE ESSENTIAL OIL IN *SOLIDAGO CANADENSIS* L. FROM EURASIA

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ABSTRACT

Hydro distilled essential oils in air-dry samples of aerial parts of *Solidago canadensis* L., (Asteraceae) from eight local invasive populations were investigated by GC-MS analysis. A comparative study on quantity and composition of the essential oils obtained from plants, growing in different ecological and climatic conditions, ontogenesis phase and different plant organs was carried out The major compounds detected in oil samples of *S. canadensis* were α-pinene (1.3 – 61.27%), limonene (0.5 – 22.5%), bornyl acetat (3.4 – 29.8%) and germacrene D (1.8 – 39.2%). Samples from inflorescences contained the maximal percentage of monoterpene hydrocarbons, while the leaves' samples showed the maximal cumulative percentage of sesquiterpene and monoterpene hydrocarbons. Data obtained from our studies confirm the availability of alien invasive species *Solidago canadensis* for medicine and many other purposes. The variability of the qualitative and quantitative composition of essential oils in different geographical locations will allow futher selection of form containing the maximum amount of active substances.

Keywords: *Solidago canadensis*; essential oil; terpenes

INTRODUCTION

Nowdays alien invasive plants spread in the secondary distribution range in mass and can displace native species especially in the antropogenically transformated sites. The resource potential of these «new species» didn't studyied in detail yet. The direct and indirect effects of occurrence of invasive plants on food production and other positive externalities of its appearence have been analysed only for a few species **(Fehér et al., 2016)**. In this regard not without interest is genus *Solidago* L. that comprises about 100 species, five of them occurring in the Old World. *Solidago virgaurea* L. is the only one native for Eurasia. The other four – *S. canadensis* L., *S. gigantea* Ait., *S. rupestris* Raf. and *S. graminifolia* (L.) Salisb. (=*Euthamia graminifolia* (L.) Nutt.) are of North American origin **(Vinogradova et al., 2010)**. *S. canadensis* and *S. gigantea* are the invasive plants, widely distributed in Russia and almost all European countries. The plant material of medicinal usage, known as *Herba Solidaginis,* includes *S. virgaurea, S. gigantea* and *S. canadensis* **(Kalemba and Thiem, 2004)**. This material, its extracts and derivatives serve as components in many medications, applicable while treatment of the urinary tract and prostate diseases.

S. canadensis is used in medicine for treatment of several diseases. The flowers contain analgesic, astringent, febrifuge compounds, infusion of the dried powdered herb is used as an antiseptic agent and root is applied as poultice to burns **(Moerman, 1998)**. The species has been used in European phytotherapy for over 200 years for treatment of chronic nephritis, cystitis, urolithiasis, rheumatism and as an antiphlogistic drug **(Apati et al., 2003)**. The chemical composition of the essential oil in *S. canadensis* is poorly known. The essential oils obtained from fresh green parts, dried aerial parts, inflorescences, leaves and roots of *S. canadensis* collected from Poland, India, Egypt and China were previously investigated **(Kalemba et al., 1990; Weyerstahl et al., 1993; Xia et al., 1999; Mishra et al., 2010, 2011; El-Sherei et al., 2014)**. Results of those investigations showed that inflorescences of *S. canadensis* yielded 1.7%, 0.9% (leaves), and 0.2% (steams) of the essential oil. This oil contains 85% hydrocarbons and 15% terpenoids. Earlier, α-pinene, limonene, bornyl acetate, germacrene D, myrecene and γ-cadinene had been identified.

It is known that essential oils have antibacterial, antifungal, antioxidant **(Baratta et al. 1998; Burt, 2004; Bounatirou et al., 2007)**, antimycotic **(Arras and Grella, 1992)**, antiparasitic **(Pandey et al., 2000)**, antitoxigenic **(Juglal et al., 2002)**, and insecticidal **(Konstantopoulou et al., 1992)** properties. Essential oils can be used as natural preservative in the food industry too **(Petrová et al., 2015; Foltinová et al., 2017)**. For example, essential oil as an potential sources of antimicrobial ingredients are applied for chicken thighs meat treatment **(Kačániová et al., 2016)**. Essential oils can be used as well for the control

of plant pathogens such as *Aspergillus niger* and *Aspergillus tubingensis* **(Císarová et al., 2016)**.

Scientific hypothesis

The major goal of this work was the determination chemical composition of the essential oils and the characterization essential oil chemical polymorphism of *S.canadensis* invasive populations from Eurasia.

MATERIAL AND METHODOLOGY

Locating plants and data collection

The specimens of *S. canadensis* (Figure 1) were collected in eight local invasive populations from Austria, Ukraine, Kazakhstan and Russia (Moscow, Penza, Tver and Tula regions) geographically distant from each other (Table 1). The specimens were collected both at vegetative and flowering buds' formation phases (June – August 2015). For the purpose of phytochemical analysis, one to three specimens (all the aerial shoots) were sampled within 0.2 m^2 – patches in all the examined local populations, simultaneously with collection of the herbarium specimens. The herbarium specimens are kept in the herbarium of Main Botanical Garden RAS (MHA).

Chemicals

Various reference chemicals used in this study were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals used in this study were purchased from Merck (Darmstadt, Germany).

Methods of the Essential Oil Composition Study

Essential oil was obtained from an average sample of aerial parts (leaves, inflorescences and a mixture of inflorescences and leaves) of plants. The oil was extracted from chopped air-dried material by hydrodistillation according to **Ginzberg (1932)**. Qualitative composition of the oil was determined by gas chromatography at the Center for Collective Use of the Federal Research Center "Fundamentals of Biotechnology", Russian Academy of Sciences, Moscow (RFMEFI62114X0002), using a Shimadzu GS 2010 gas chromatograph with a GCMS-QP 2010 mass detector and SPB-1 nonpolar column (solid-phase-bound methyl silicone) (Supelco, Sigma-Aldrich Corporation) with the length of 30 m and diameter of 0.25 mm. The samples were dissolved in benzene at a ratio of 1 : 150 and chromatographed with the temperature-programming mode under the following conditions: injector temperature, 180 °C; interface, 205 °C; detector, 200 °C. The carrier gas was helium. The flow through the column was 1 cm^3/min and flow division was 1 : 10. Mass detector settings: logging mode, TIC; mass range, 30 – 400 m/z. The temperature regime for the substances with the retention index of 1300 consisted of the thermostat at 60 °C for 3 min; then, 2 °C.min^{-1} until 230 °C; and isotherm for 2 min. The temperature regime for the substances with a retention index higher than 1300

Figure 1 *Solidago canadensis* L. in the Moscow district, photo by Yu.Vinogradova, September 2016.

Table 1 Examined invasive populations of *Solidago canadensis* L.

Specimen	Locality	Content of essential oil, %
M-1	Moscow, Main Botanical Garden RAS, phase of vegetation (leaves)	0.7
M-2	Moscow, Main Botanical Garden RAS, phase of blooming (leaves)	0.1
M-3	Moscow, Main Botanical Garden RAS, phase of blooming (inflorescens)	0.4
Ms	Moscow region, Krasnogorsk district (N 55°48'; E 37°18')	0.3
As	Austria, Urban district of Vienne (N 48°11'; E 16°18')	0.4
Tv	Tver' region, Kimru district (N 56°51'; E 35°54')	0.1
Pz	Penza region, Zemetrino district (N 53°50'; E 42°62')	0.4
Kz	Kazakhstan, Urban district of Alma-Ata (N 43°15'; E 76°54')	0.2
Bs	Altaj region, Bijsk district (N 51°38'; E 84°30')	0.3
Sh	Sakhalin region, Urban district Yuzhno-Sakhalinsk (N 46°57'; E 142°44')	0.3
Uk	Ukraine, Kiev region, Mironovka district (N 49°38'; E 30°57')	0.2
Tl	Tula region, Venevo district (N 54°51'; E 38°35')	0.4

consisted of the thermostat at 60 °C for 1 min; then, 4 °C.min^{-1} until 230 °C; and isotherm for 2 min. The percentage composition of the oils was computed by the normalization method from the GC peak areas, which were calculated as mean values of two injections of each oil sample, without using response factors. The identity of the components was assigned by comparison of their retention indices, relative to a C9 – C17 hydrocarbon standard mixture.

Statistical analyses

Results are expressed as mean of triplicate trials. Data were analyzed by one-way analysis of variance (ANOVA) on the means of values (p <0.05). The essential oils composition was used to determine the relationship between the different samples by cluster analysis using PAST 2.17.

RESULTS AND DISCUSSION

Essential oil contents of *S. canadensis* varied between 0.07 and 0.70%. Accumulation of essential oil of this species depends on the ecological and climatic conditions of growth, ontogenesis phase and testing plant's organs.

The maximum content of oil was found in the leaves collected at vegetative phase (0.7%). Inflorescences collected at flowering buds' formation phase yielded more oil than the leaves (Table 1).

The trace amount of essential oil (0.1%) was recorded in the plants at the northernmost collection point- Tver region, Kimry district; the maximal quantity (0.4%) – in the plants from three local invasive populations (As, Pz and Tl).

Qualitative and quantitative variations in the oils' composition in leaves, inflorescences and aerial parts for all the specimens collected were observed (Table 2, 3).

In the composition of essential oil 63 components were identified. Mono- and sesquiterpene hydrocarbons were dominant in all the oil samples. Among the monoterpenes dominated α- and β-pinene, β-myrcene, limonene and bornyl acetate; sesquiterpenes – germacrene D, α- and β-caryophyllene, β-elemene. The remaining components of these classes were encountered in small amounts.

Composition of the essential oil significantly varies within different phases of plants ontogenesis (Table 2). During the vegetative period the monoterpene and the sesquiterpenes contents in leaves decrease from 80 to 20% after the flowering stage; oxygenated monoterpenes increases from 7% to 30%, respectively. Within a vegetatitive phase (before flowering) the major components of essential oil of leaves are α-pinene – 28.1%, germacrene D – 39.2%, bornyl acetate – 7.3%, limonene – 7.0%, β-myrcene – 7.3%. At the flowering buds' formation phase amount of essential oil in the leaves decreased to 0.10% and the main components' proportion changed (α-pinene – 1.3%, germacrene D – 16.6%, bornyl acetate 29.8%, limonene – 0.5%, β-myrcene – 0.2%). The inflorescences contain 0.4% essential oil and the major component is α-pinene – 61.2%, limonene – 13.7% and bornyl acetate – 8.5%.

The composition of essential oil significantly varies in aerial parts of *S. canadensis*, depending on the geographical locality of the invasive population, too. The monoterpene hydrocarbons were significantly higher in all the samples compared to sesquiterpenes.

Mostly quantitative rather than qualitative variation was observed in all the essential oils analyzed. The percentage of monoterpene hydrocarbons was higher in samples Pz, Ms, As, Tv, Uk and Tl (64 – 85%) compared to those of Kz, Bs and Sh (46 – 54%). α-Pinene (12.6 – 55.4%), β-pinene (0.7 – 5.4%), β-myrecene (1.8 – 6.9%) and limonene (6.4 – 22.5%) were the major monoterpenes in all the samples. The sesquiterpenes: germacrene D (3.0 –

Table 2 Essential oil composition (%) of the leaves and inflorescens *Solidgo canadensis* L.within different phases of plant's ontogenesis.

Compounds	RI	Leaves, phase of vegetation (M-1)	Leaves, phase of blooming (M-2)	Inflorescens, phase of blooming (M-3)
		Monoterpene		
α-Pinene	928	28.1	1.25	61.2
Camphene	940	0.99	0.13	1.40
Sabinen	960	0.53	–	0.91
β-Pinene	965	2.75	0.17	3.25
β-Myrcene	976	7.28	0.20	2.28
α-Phellandrene	989	1.47	–	–
Limonene	1016	6.98	0.52	13.7
α-Campholenal	1105	0.33	1.60	1.06
Bornyl acetate	1270	7.34	29.8	8.49
		Sesquiterpene		
β-Elemene	1477	1.05	0.71	–
β-Caryophyllene	1483	0.46	0.45	–
α-Caryophyllene	1492	0.30	0.39	–
Germacrene D	1497	39.2	16.6	1.81
β-Eudesmene	1499	0.18	1.13	0.23
Aromadendrene oxide	1557	–	1.74	0.15
Caryophyllene oxide	1571	–	2.50	–
Spathulenol	1603	0.17	2.97	–

Legend: RI – retention index relative to C_9-C_{17} n-alkanes on SPB-1 column (Supelco); M-1, M-2, M-3 – Moscow, Main Botanical Garden RAS; «–» – component is not present.

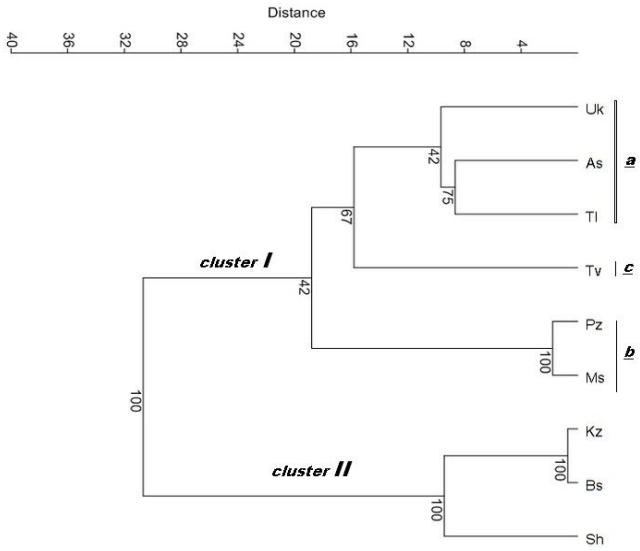

Figure 2 Dendrogram obtained by cluster analysis of the percentage composition of essential oils from the *Solidgo canadensis* L. samples examined. For abbreviations, see Table 1 and the text.

Table 3 Essential oil composition of *Solidgo canadensis* L. (%).

Compounds	RI	Ms	As	Tv	Pz	Kz	Uk	Tl	Bs	Sh
Monoterpene										
α-Pinene	928	41.6	43.6	25.1	52.4	12.6	30.0	24.3	14.1	13.8
Camphene	940	1.28	1.70	0.97	0.75	0.94	1.07	0.97	1.22	0.20
Sabinen	960	0.62	0.78	0.42	0.96	1.23	0.46	0.32	0.39	0.28
β-Pinene	965	3.52	5.36	2.48	4.73	3.91	4.20	2.42	3.62	0.71
β-Myrcene	976	6.38	1.82	4.10	1.12	4.88	6.90	2.60	4.88	2.29
Limonene	1016	22.5	10.7	6.42	20.3	19.7	15.4	17.3	18.1	17.5
α-Campholenal	1105	0.39	0.68	1.37	0.31	0.19	0.21	0.83	0.49	0.32
Bornyl acetate	1270	8.34	7.87	26.3	3.35	10.9	8.71	15.2	9.40	11.8
Sesquiterpene										
β-Elemene	1477	0.60	0.50	0.82	0.21	0.67	–	0.83	0.20	–
β-Caryophyllene	1483	0.56	1.09	0.66	0.61	0.93	1.41	0.77	018	0.64
α-Caryophyllene	1492	0.44	0.28	0.39	0.21	0.56	0.51	0.43	0.24	0.55
Germacrene D	1497	8.67	14.9	2.98	10.3	31.8	24.0	15.9	32.4	36.2
β-Eudesmene	1499	0.47	0.35	0.36	–	0.24	0.20	0.59	0.12	–
Aromadendrene oxide	1557	0.31	0.20	1.45	0.34	–	0.40	1.78	–	0.67
Caryophyllene oxide	1571	0.51	0.88	4.17	–	1.23	0.46	2.34	0.32	0.11
Spathulenol	1603	–	–	2.33	0.28	0.46	0.11	1.39	0.83	1.31

Note: RI – retention index relative to C_9-C_{17} n-alkanes on SPB-1 column (Supelco); «–» – component is not present.

36.2%), α- and β-caryophyllene (0.4 – 1.9%), β- and γ-elemene (0.2 – 1.8%) were present in all the oil samples. Oxygenated compounds, especially bornyl acetate (3.4 – 26.3%) showed their highest percentage in all the samples.

Cluster analysis (Figure 2), confirmed a high chemical correlation among all populations (S_{corr} >0.80%). Two clusters were defined in the bases of the amount of monoterpenes and germacrene D. In cluster 1, which included 6 out of the 9 samples, germacrene D ranged from 3 to 24%, whereas the three sample of cluster 2 showed a higher percentage (32 – 36%). Correlation was detected between the clusters and the geographical collection site. The most part of samples the essential oils studied belonged to the "European" chemotype. And only the three samples (Kz, Bs, Sh) belonged to the "Asian" chemotype. Sub-cluster a has a relative amount between 15.9 and 24.0%, sub-cluster b between 8.7 and 10.3% and sub-cluster c (Tv) has the lower relative amount (3%). No significant correlation was detected between the clusters and the geographical collection site (p = - 0.35, r= 0.49).

The presence of germacrene D as the major component in overwhelming majority oil samples is in agreement with the previous reports on its occurrence as a major constituent in the oils obtained from the fresh green parts (23.8%), leaves (28.4 and 64.06%) and fresh flowers (29.5%) of *S. canadensis* collected from Poland **(Kalemba et al., 1990; Weyerstahl et al., 1993; Kalemba and Thiem, 2004),** China **(Xia et al., 1999)**, India **(Mishra et al., 2010, 2011)** and Egypt **(El-Sherei et al., 2014)**, respectively. On the contrary, γ-cadinene previously identified as main constituents (27.1 and 12.7 – 20.4%) in the aerial parts and fresh flowers of *S. canadensis* collected Poland and Egypt during the flowering stage **(Kalemba et al., 1990; Weyerstahl et al., 1993; Kalemba and Thiem, 2004; El-Sherei et al., 2014)** was not detected in our oil samples. Moreover, all the samples collected in Poland, India and Egypt lacked bornyl acetate while the latter was

identified in considerable amounts (3.4 – 26.3%) in the oil of aerial parts of the plants, collected in Russia, Austria, Ukraine and Kazakhstan.

CONCLUSION

There is a great potential in finding new antimicrobial drugs from the wild. Natural crude drug extracts and biologically active compounds obtained from plant species used in traditional medicine can be prolific resources for new drugs. Moreover, according to literature, some monoterpenes and sesquiterpenes as α-pinene, myrcene, limonene and germacrene D have cytotoxic effects against different cell lines. We can support usage of such natural products as potent preservative agents, as well as possible candidates for new medical preparations. Data from our studies confirm the availability of *Solidago canadensis*: its aerial part containes from 0.1 to 0.7% of essential oil in the leaves and from 0.1 to 0.4% of essential oil in the inflorescens. The major compounds detected in oil samples of *S. canadensis* were α-pinene (1.3 – 61.27%), limonene (0.5 – 22.5%), bornyl acetat (3.4 – 29.8%) and germacrene D (1.8 – 39.2%). Samples from inflorescences contained the maximal percentage of monoterpene hydrocarbons, while the leaves' samples showed the maximal cumulative percentage of sesquiterpene and monoterpene hydrocarbons. The variability of the qualitative and quantitative composition of essential oils in different geographical locations will allow futher selection of form containing the maximum amount of active substances.

REFERENCES

Apati, P., Szentmihalyi, K., Kristo, S. T., Papp, I., Vinkeber, P., Szoke, E., Kery, A. 2003. Herbal remedies of Solidago – correlation of phytochemical characteristics and antioxidative properties. *Journal of Pharmaceutical and Biomedical Analysis*, vol. 32, no. 4-5, p. 1045-1053. https://doi.org/10.1016/S0731-7085(03)00207-3

Arras, G., Grella, G. E. 1992. Wild thyme, Thymus capitatus, essential oil seasonal changes and antimycotic activity. *Journal of Horticultural Science*, vol. 67, no. 2, p. 197-202. https://doi.org/10.1080/00221589.1992.11516237

Baratta, M. T., Dorman, H. J. D., Deans, S. G., Figueiredo, A. C., Barroso, J. G., Ruberto, G. 1998. Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour and Fragrance Journal*, vol. 13, no. 4, p. 235-244. https://doi.org/10.1002/(SICI)1099-1026(1998070)13:4<235::AID-FFJ733>3.0.CO;2-T

Bounatirou, S., Smiti, S., Miguel, M. G., Faleiro, L., Rejeb, M. N., Neffati, M., Costa, M. M., Figueiredo, A. C., Barroso, J. G., Pedro, L. G. 2007. Chemical composition, antioxidant and antimicrobial activities of the essential oils isolated from Tunisian Thymus capitus Hoff. et Link. *Food Chemistry*, vol. 105, no. 1, p. 146-155. https://doi.org/10.1016/j.foodchem.2007.03.059

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. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022 PMid:15246235

Císarová, M., Tančinová, D., Medo, J. 2016. Antifungal activity of lemon, eucalyptus, thyme, oregano, sage and lavender essential oils against Aspergillus niger and Aspergillus tubingensis isolated from grapes. *Potravinarstvo*, vol. 10, no. 1, p. 83-88. https://doi.org/10.5219/554

El-Sherei, M., Khaleel, A., Motaal, A. A., Abd-Elbaki, P. 2014. Effect of Seasonal Variation on the Composition of the Essential Oil of Solidago canadensis Cultivated in Egypt. *Journal of Essential Oil Bearing Plants*, vol. 17, no. 5, p. 891-898. https://doi.org/10.1080/0972060X.2014.901612

Fehér, A., Halmová, D., Fehér-Pindešová, I., Zajác, P., Čapla, J. 2016. Distribution of invasive plants in the Nitra river basin: threats and benefits for food production. *Potravinarstvo*, vol. 10, no. 1, p. 605-611. https://doi.org/10.5219/651

Foltinová, D., Tančinová, D., Císarová, M. 2017. Influence of essential oils on the growth of aspergillus flavus. *Potravinarstvo*, vol. 11, no. 1, p. 322-331. https://doi.org/10.5219/725

Ginzberg, A. S. 1932. Simplified method for determining essential oil in essential oil plants. *Khim.-Farm. Prom-st*., vol. 8, no. 9, p. 326-329.

Juglal, S., Govinden, R., Odhav, B. 2002. Spice oils for the control of co-occurring mycotoxin-producing fungi. *Journal of Food Protection*, vol. 65, no. 4, p. 683-687. https://doi.org/10.4315/0362-028X-65.4.683 PMid:11952220

Kačániová, M., Terentjeva, M., Puchalski, C., Petrová, J., Hutková, J., Kántor, A., Mellen, M., Čuboň, J., Haščík, P., Kluz, M., Kordiaka, R., Kunová, S. 2016. Microbiological quality of chicken thighs meat after four essential oils combination, EDTA and vaccum packing. *Potravinarstvo*, vol. 10, no. 1 , p. 107-113. https://doi.org/10.5219/548

Kalemba, D., Góra, J., Kurowska, A. 1990. Analysis of the essential oil of Solidago canadensis. *Planta Medica*, vol. 56, no. 2, p. 222-223. https://doi.org/10.1055/s-2006-960930 PMid:17221400

Kalemba, D., Thiem, B. 2004. Constituents of the essential oils of four micropropagated Solidago species. *Flavour and Fragrance Journal*, vol. 19, no. 1, p. 40-43. https://doi.org/10.1002/ffj.1271

Konstantopoulou, I., Vassilopoulou, L., Mavragani-Tsipidou, P., Scouras, Z. G. 1992. Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on Drosophila auraria. *Experientia*, vol. 48, no. 6, p. 616-619. https://doi.org/10.1007/BF01920251 PMid:1612141

Mishra, D., Joshi, S., Bisht, G., Pilkhwal, S. 2010. Chemical composition and antimicrobial activity of Solidago canadensis Linn. root essential oil. *Journal of Basic and Clinical Pharmacy*, vol. 1, no. 3, p. 187-190. PMid:24825986

Mishra, D., Joshi, S., Sah, S. P., Bisht, G. 2011. Chemical composition, analgesic and antimicrobial activity of Solidago canadensis essential oil from India. *Journal of Pharmacy Research*, vol. 4, no. 1, p. 63-66.

Moerman, D. 1998. *Native American Ethnobotany*. 1st ed. Oregon : Timber Press. 927 p. ISBN-10: 0881924539.

Pandey, R., Kalra, A., Tandon, S., Mehrotra, N., Singh, H. N., Kumar, S. 2000. Essential oil compounds as potent source of nematicidal compounds. *Journal of Phytopathology*, vol. 148, no. 7-8, p. 501-502. https://doi.org/10.1046/j.1439-0434.2000.00493.x

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. https://doi.org/10.5219/550

Vinogradova. Yu. K., Mayorov, S. R., Khorun, L. V. 2010. The Black book of the flora of the Middle Russia. Moscow, Russia : GEOS. 512 p. ISBN: 978-8-89119-487-9.

Weyerstahl, P., Marschall, H., Christiansen, C., Kalemba, D., Góra, J. 1993. Constituents of the essential oil of Solidago canadensis ("Goldenrod") from Poland. A correction. *Planta Medica*, vol. 59, no. 3, p. 281-282. https://doi.org/10.1055/s-2006-959673
PMid:17235976

Xia, W. X, He, W., Wen, G. Y. 1999. The constituents of essential oil from Solidago canadensis. *Chinese Bulletin of Botany*, vol. 16, no. 2, p. 178-180.

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ASSESSMENT OF THE PHYSICOCHEMICAL AND BACTERIOLOGICAL QUALITIES OF NONO – A FERMENTED COW MILK

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ABSTRACT

Nono is a spontaneously fermented yoghurt-like milk product consumed is a staple food commodity in parts of the Sub-Saharan West Africa. Nono is usually consumed along with 'Fura' as 'Fura da Nono' in Nigeria. Studies on physicochemical and bacteriological qualities were carried out on samples of Nono obtained from 5 different sources in Ado-Ekiti, Nigeria. The Nono samples were found to be nutritious, containing moderate levels of ash, crude fat, crude protein and carbohydrate. The pH of the Nono samples was relatively low (4.04 ±0.04), while the density and specific density were close to that of distilled water at room temperature. Total aerobic plate count of Nono samples was 1.8 ±0.02 × 106 CFU.mL^{-1}. A total of 15 bacteria species namely *Eubacterium nodatum*, *Bacillus subtilis*, *Chromobacterium violaceum*, *Propionibacterium acnes*, *Amycolatopsis benzotilytica*, *Tropheryma whipplei, Moraxella catarrhalis, Campylobacter gracilis, Neisseria sicca, Vibrio natiensis, Photobacterium damselae, Corynebacterium kutsceri, Corynebacterium xerosis, Lactobacillus fermentum* and *Lactobacillus casei* were isolated from the Nono samples. The gram-positive bacterial isolates were resistant to all antibiotics tested with the exception of Erythromycin where 40% susceptibility was obtained, while the gram-negative bacteria showed high resistance to the tested antibiotics, but with 80% susceptibility to Ofloxacin. The nono samples were observed to exhibit antibacterial activity against cultures of *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 29929 and *Staphylococcus aureus* ATCC 29293. Most of the bacteria isolated were of less public health importance, but the high prevalence of multi-drug resistance is of great concern.

Keywords: Nono; fermented milk; nutrition; antibiotics resistance

INTRODUCTION

Milk has been preserved since early times by fermentation techniques. Asia, Africa, the Middle East, Northern and Eastern Europe are known for the production of traditionally fermented milk products **(Savadogo et al., 2004)**. Nono is a spontaneously fermented yoghurt-like milk product consumed as a staple food commodity in parts of the Sub-Saharan West Africa. Its production and consumption gives economic benefits such as food security to the rural people in the region **(Akabanda et al., 2010)**.

Nono is produced by Fulani women in Nigeria. The Fulani is an ethnic group in northern Nigeria with the tradition of earning a living from cattle rearing and sales, but they are found all over the country. Cattle rearing is an integral part of the culture of this group of people. They produce their fermented milk, 'Nono', for sales by traditional methods. Fresh cow milk is collected in the morning in calabashes, sieved and left to ferment for a period of 24 to 48 hours under the ambient temperature (28 ±2 °C). Fermentation of milk is spontaneous (with its own natural bacteria). The curd separates from the whey, the curd is removed and used in the preparation of local cheese or butter, while the milk (whey) is left to ferment further for some few hours thereby converting it to yogurt-like **(Akabanda et al., 2010)**. Fermentation span varies from one producer to another resulting in products of variable quality and stability. The finished product – nono is sold in beautiful calabashes along with Fura (dough of boiled ground millet mixed with a host of other ingredients and spices) as 'Fura da Nono' (Figure 1).

The milking process, production and marketing of nono is not appealing to some people, especially the elites, because of the the quality of the water commonly used, the feeding methods of the cows, and above all the processing environment. Sanitary practices are essential in milk processing so as to minimize the risk of infection to people through consumption of milk products **(Chan et al., 2007)**.

Figure 1. Fulani woman displaying nono **(Dobby's Signature.com, 2014)**.

Common bacterial flora of fermented milk are the Lactic Acid Bacteria (LAB) which among others comprises of *Lactobacillus lactis*, *L. bulgarius*, *L. helvericus*, *Leuconostoc* species, *Streptococcus thermophilus*, *S. lactis* and *S. cremoris*; as well as the Propionic Acid Bacteria (PAB). Most coliforms inhabit the intestines of warm-blooded animals and are shed along with their faeces to living environments. Many other food borne pathogens equally originated from faecal contaminations **(Congan, 1995)**.

Pathogenic bacteria are not supposed to be present in any successfully fermented dairy product because of heat treatment and low acidity of the product. Their presence in these products is sign of re-contamination. The occurrence of *Enterococcus* species, coliforms, *Salmonella* species, *Clostridium* species *and Bacillus* species is a sign of re-contamination **(Ledenbach and Marshall, 2009)**.

Milk products have been frequently implicated in the transmission of diseases such as tuberculosis, brucellosis, diphtheria, scarlet fever and gastroenteritis. This is as a result of contaminations by the implicated human pathogens **(Bryan et al., 1988)**. The contamination could be attributed to three reasons. Firstly, the wide distribution of coliform in nature which predisposes products to contamination before and after pasteurization; secondly, conditions of storage and thirdly, some enteric pathogen may be disseminated by dairy products gotten from an infected animal **(Ledenbach and Marshall, 2009)**.

Quality and safety or raw cow's milk have to be regularly controlled **(Zajác et al., 2012)**.

Scientific hypothesis

There has been many contradicting reports on the safety of Nono consumption. But nono is believed to be safe for human consumption, hence the popular demand for this fermented milk product. The present study investigates the quality and safety of nono with the following objectives of the study:

i. Determining the physicochemical qualities of the nono
ii. Assessing for bacteria of public health importance in nono samples
iii. Evaluating the probiotic potential of nono.

MATERIAL AND METHODOLOGY

Collection of Samples

Samples of nono were purchased from 5 different locations in Ado-Ekiti, Nigeria, and brought in sterile bottles to the Microbiology Laboratory, Afe Babalola University, Ado-Ekiti, Nigeria, for analysis.

Physicochemical analyses of nono

Proximate nutrient composition and some other physico-chemical properties such as pH, density and specific density was carried out according to the **Association of Official Analytical Chemists (2000)**.

Isolation of aerobic organisms from nono

The Pour-plate technique was used for primary isolation of aerobic bacteria. Following serial dilution of the nono samples, 1 mL each of 10^{-4} and 10^{-5} dilutions were evenly spread on freshly prepared nutrient agar (Oxoid, England), in du regulatory microbial criteria for raw cow milk plicates and incubated at a temperature of 37 $^{\circ}$C for 24 hours. Bacterial load were estimated, and distinct colonies were picked and sub-cultured on nutrient agar plates.

Isolation of lactic acid bacteria from nono

This was carried out as described by **Ibrahim and Nural (2016)**, 1 mL each of 10^{-4} and 10^{-5} dilutions of Nono was evenly spread on freshly prepared deMan, Rogosa and Sharpe agar, MRS (Oxoid, England), distinct colonies were picked and sub-cultured on nutrient agar plates.

Characterization and identification of isolates

All isolates were characterized using standard morphological and biochemical tests as described by **Barrow and Feltham (1993)**. Bacterial isolates were identified using online bacterial identification application **(Gideon informatics, 1994-2017)**, with reference to Bergey's Manuals **(Brenner, et al., 2005a,b, Vos et al., 2009, and Krieg et al., 2010)**.

Antibiotic susceptibility test of bacterial isolates from nono

The susceptibility of the isolated aerobic bacteria to antibiotics was determined by Kirby-Bauer disc diffusion method on Mueller-Hinton agar **(CLSI, 2016)**. This test was carried out by making an even spread of the pure isolates on prepared Mueller-Hinton agar (Oxoid, England) using sterile swab sticks and aseptic placement of the antibiotic disks (Oxoid, England) using sterile forceps. The plates were incubated at 37 °C for 24 hours after which the zone of inhibition was measured and interpreted according to Clinical Laboratory Standards Instituite **(CLSI, 2016)**. Antibiotics used are; Augumentin, AUG (30 µg), Ofloxacin, OFL (5 µg), Ampicillin, AMP (30 µg), Ciprofloxacin, CIP (5 µg), Gentamicin, GEN (10 µg), Ceftazidime, CAZ (30 µg), Nitrofurantoin, NIT (300 µg), and Cefuroxime, CXM (30 µg), for gram negative isolates; and Streptomycin, STP (10 µg), Ampicillin (30 µg), Cloxacillin, CLX (5 µg), Tetracycline, TET (10 µg), Chloramphenicol, CHL (10 µg), Erythromycin, ERY (5 µg), Penicillin, PEN (10 µg), and Gentamicin (10 µg), for gram positive isolates.

In-vitro antibacterial activities of nono

The in-vitro antibacterial of nono was carried out by agar well diffusing assay with Mueller-Hinton agar (Oxoid, England) as described by **Clinical and Laboratory Standards (2016)**. Nono was centrifuged and the supernatant was tested against Salmonella typhimurium ATCC 14028, Escherichia coli ATCC 29929 and Staphylococcus aureus ATCC 29293. Each test was performed in triplicate.

Statisic analysis

All data were expressed as mean ± Standard deviation.

RESULTS AND DISCUSSION

Nutritional quality of nono

The Nono samples were found to be nutritious, containing moderate levels of ash, crude fat, crude protein and carbohydrate (Table 1). The pH of the nono products was relatively low (4.04 ±0.04), while the density and specific density were close to that of distilled water at room temperature (Table 1). The low pH value of 4.04 has the ability to inhibit spoilage microbes, as well as giving Nono its sharp taste. The pH values obtained in this study is in consistent with pH values of 4.1 – 4.4 reported earlier by **Bankole et al. (1992)** from Nono samples obtained from Zaria, Northern Nigeria.

Bacteriological quality of Nono

Aerobic plate count (bacterial load) of 1.8 ±0.02 x 10^6 CFU.mL^{-1} (6.26 $\log_{10}$ CFU.mL^{-1}) was recorded in Nono in this study. The bacterial load reported is slightly higher

Table 1 Physicochemical qualities of Nono.

Analyte	Value ±SD
pH	4.04 ±0.03
Density (g.mL^{-1})	0.96 ±0.01
Specific density (g.mL^{-1})	0.97 ±0.01
Moisture (%)	90.88 ±0.04
Ash (%)	3.81 ±3.67
Crude Fiber (%)	ND
Crude Fat (%)	1.35 ±0.07
Crude Protein (%)	2.88 ±0.78
Carbohydrate (%)	0.92 ±0.16
Energy (Kcal.kg^{-1})	230.25 ±3.04

SD – Standard Deviation.
ND – Not Detected.

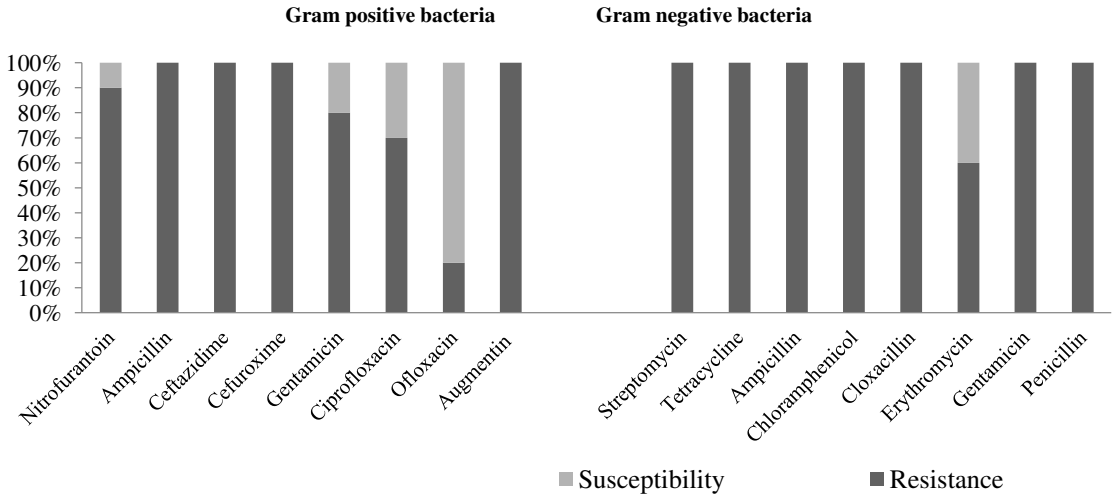

Figure 2 Antibiogram of bacteria isolated from Nono.

than the values of 4.1, 4.9 ±0.11 and 4.5 ±0.4 $\log_{10}$ CFU.mL^{-1} for raw whole milk reported by **Asaminew and Eyassu (2007)**, **Abebe et al. (2012)** and **Kunová et al (2017)**, respectively. The bacterial load of 6.26 $\log_{10}$ CFU.mL^{-1} compared favourable with the value of 6.68 ±1.19 $\log_{10}$ CFU.mL^{-1} and 5.59 ±0.69 $\log_{10}$ CFU.mL^{-1} of Nono produced in 24 and 48 hr respectively in Ghana **(Akabanda et al., 2010)**. However earlier studies by **Bankole et al. (1992)** reported an average aerobic plate count of 1.6 x 10^9 from Nono samples obtained from Zaria, Northern Nigeria.

Fifteen (15) different bacteria species were isolated. The aerobic bacteria isolated from Nono were *Eubacterium nodatum, Bacillus subtilis, Chromobacterium violaceum, Propionibacterium acnes, Amycolatopsis benzotilytica, Tropheryma whipplei, Moraxella catarrhalis, Campylobacter gracilis, Neisseria sicca, Vibrio natiensis, Photobacterium damselae, Corynebacterium kutsceri* and *Corynebacterium xerosis.* The isolated lactic acid bacteria were *Lactobacillus fermentum,* and *Lactobacillus casei.*

Most of thebacteria species isolated from nono in this study are not commonly associated with food related illnesses. However, other species of some of the genera, especially *Campylobacter, Bacillus,* and *Vibrio*, have been implicated in food related illnesses **(Donkor et al., 2007)**. *Campylobacter* species such as *C. jejuni, C. coli* and *C. fetus* are considered pathogenic while *B. cereus a*nd *B. anthrax* are of clinical importance and V. *cholerae* is the causative agent of cholera **(Shah et al., 1998; Logan, 1988 and Julian, 1997)**. Although these species were not reported in the study, the species reported are indicators of potential contamination by the organisms. This is an indication of inadequate hygienic practices during production **(Theodore et al., 2016 and Zajác et al., 2012)**.

According to **Harrigan (1998)**, raw milk drawn from a healthy udder normally will contain only a few hundreds to a few thousands of bacteria per milliliter, mostly from the genus *Micrococcus* and the udder diphtheriod, *Corynebacterium bovis*. Microbial contamination of milk often arises from the udder surface, bovine faeces, soil, bedding, feed, as well as milk-handling equipment. Lack of potable water and use of detergent was a major constraint to hygienic practices on the farm **(Theodore et al., 2016; Solomon et al., 2013 Galton et al., 1989)**.

The gram-positive bacteria isolated from Nono were resistant to all antibiotics tested with the exception of Erythromycin where 40% susceptibility was obtained, while the gram-negative bacteria showed high resistance to the tested antibiotics, but with 80% susceptibility to Ofloxacin (Figure 2).

All the bacteria were resistant to multiple drugs showing cluster of resistance to 5 – 8 antibacterial drugs (Table 2). High prevalence of multi-drug resistant bacteria among

Table 2 Antibiotics resistance cluster of bacteria isolated from Nono.

Bacteria	Drug cluster
Vibrio natiensis	AMP/CAZ/CXM/GEN/CIP/OFL/AUG
Photobacterium damselae	NIT/AMP/CAZ/CXM/GEN/CIP/OFL/AUG
Campylobacter gracilis	NIT/AMP/CAZ/CXM/GEN
Neisseria spp	NIT/AMP/CAZ/CXM
Moraxella catarrhalis	NIT/AMP/CAZ/CXM
Tropheryma whipplei	AMP/CAZ/CXM/GEN/CIP/OFL/AUG
Propionibacterium acnes	STR/TET/AMP/CHL/CLX/ERM/GEN/PEN
Amycolatopsis benzoatilytica	STR/TET/AMP/CHL/CLX/ERM/GEN/PEN
Eubacterium nodatum	STR/TET/AMP/CHL/CLX/ERM/GEN/PEN
Bacillus subtilis	STR/TET/AMP/CHL/CLX/ERM/GEN/PEN
Chromobacterium violaceum	STR/TET/AMP/CHL /CLX/ERM/GEN/PEN

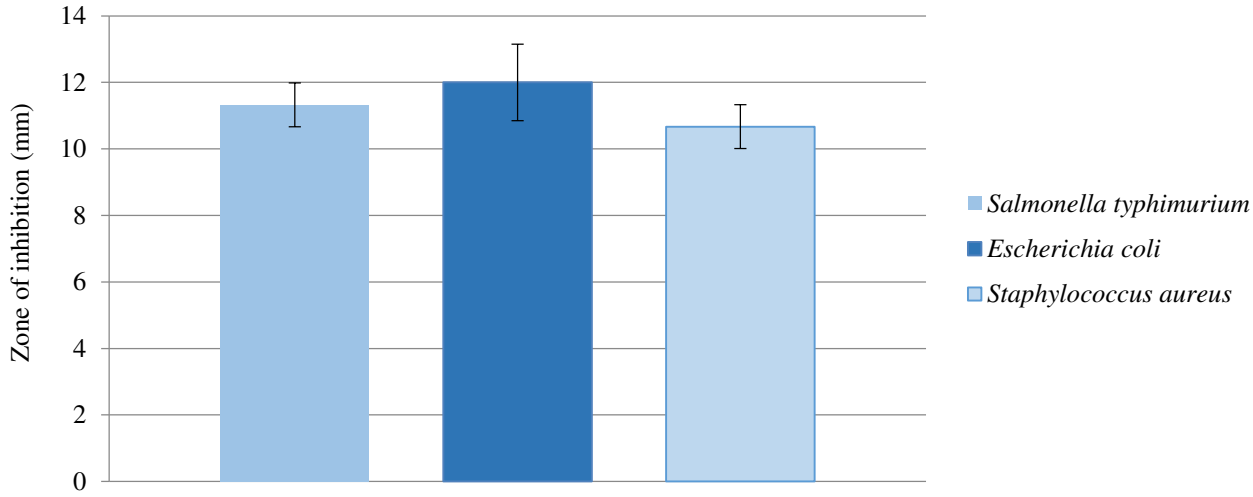

Figure 3 Antibacterial activity of nono against selected bacterial isolates.

food animals and meat processing equipment in Nigeria, has been well documented **(Osibote et al., 2014; Okiki et al., 2013)**. The high prevalence of bacteria that are resistant to multiple drugs in Nono is of great public health concern, because many people in Nigeria especially those from the Northern part of the country, have preference for nono to industrially produced yogurts.

Enterococci and members of the Enterobacteriaceae were not detected in all the samples (Nono) analyzed as against the report of **Fabianova (2010)** who reported the presence of Enterococci in raw cow milk. This may reflect the inhibitory effect of metabolites (acetic acid and lactic acid) and reduced pH produced by the mixed culture lactic acid bacteria thereby contributing to the quality and safety of the fermented milk product. Their presence, even at the initial fermentation period, suggested that they were probably introduced from the external environment, i.e. from the udder surface, bovine faeces, soil, beddings, human skin, and they survived because of the high pH **(Bezkorovainy, 2001; Harrigan, 1998)**.

Antimicrobial quality of Nono

The nono samples were also observed to exhibit antibacterial activity against cultures of *Salmonella typhimurium* ATCC 14028*, Escherichia coli* ATCC 29929 and *Staphylococcus aureus* ATCC 29293 (Figure 3).

According to the work of **Krishnendra et al. (2013)** selected lactic acid bacteria (*L. casei, L. fermentum* and *L. plantarum*) showed inhibitory properties against *Escherichia coli* and *Staphylococcus aureus*. The report of the author agrees with the findings of the study as the nono samples containing *L. casei* and *L. fermentum* showed inhibition of *S. aureus* and *E. coli.* The study is further established by the **Coconnier et al. (1997)** and **Bernet Camard (1997)** who reported the inhibition of *Salmonella typhymurium* by *L. fermentum* in their separate works.

Lactobacillus fermentum is a potent probiotic. Strains of *Lactobacillus fermentum* have been found to be pH and bile tolerant, strong enough to survive the stresses of the digestive system, the stomach pH between 1.5 and 3, and the upper intestine 3 – 5 gL^{-1} of bile **(Pan et al., 2011)**. *Lactobacillus fermentum* has the ability to reduce cholesterol levels. *L. fermentum* removes cholesterol *in vivo* by the absorption of cholesterol, which as a result accelerates cholesterol metabolism, as well as by the incorporation of cholesterol from the host into its cell membrane or walls **(Pan et al., 2011)**. **Marika and Zilmer (2009)** work on experimental introduction of the strain ME-3 of *Lactobacillus fermentum* into dairy products as a probiotic ingredient, revealed that the organism (*L. fermentum*) was able to suppress reputable contaminants of food such as pathogenic *Salmonella* species, *Shigella* species, and bacterial agents of urinary tract infections such as *E. coli* and *Staphylococcus* species.

Some *L. casei* strains are considered to be probiotic, and may be effective in alleviation of gastrointestinal pathogenic bacterial diseases **(WHO, 2002)**. According to **Ridwan et al (2008)** *Lactobacillus* species are safe and effective in treatment of acute and infectious diarrhoea. *L. casei* has been found to have a wide spectrum of coverage against pathogenic organisms that translocate from the gastrointestinal tract, thereby demonstrating therapeutic benefit in pancreatic necrosis **(Gratino and Alvarez, 2006)**. *L. casei* bacteria can also be used in the natural fermentation of beans to lower levels of the compounds causing flatulence upon digestion **(Gratino and Alvarez, 2006)**.

CONCLUSION

Since there are no standardized methods of processing nono, the products are of varying quality and stability, and if adequate hygienic practices are not ensured during processing, the quality of the final product may be compromised. Standard processing method that will ensure the production of Nono that meets International regulatory standards for safety should be employed. Thus microbial process technology can be transferred to the local producers. Education of producers on good manufacturing practices including basic hygienic principles will equally be crucial in achieving standard products.

In conclusion, Nono as a fermented milk product is of high nutritional value and a good probiotic food.

REFERENCES

Abebe, B., Zelalem, Y., Ajebu, N. 2012. Hygienic and microbial quality of raw whole cow's milk produced in Ezha district of the Gurage zone, South Ethiopia. *Wudpecker Journal of Agric Research*, vol. 1, no. 11, p. 459-465.

Akabanda, F., Glover, R. L. K., Owusu-Kwanteng, J., Tano-Debrah, K. 2010. Microbiological characteristics of fermented milk product, Nunu. *Nature and Science*, vol. 8, no. 9, p. 178-187.

Asaminew, T., Eyassu, S. 2007. Microbial quality of raw cow's milk collected from farmers and dairy cooperatives in Bahim Dar Zuria and Mecha district, Ethiopia. *Agriculture and Biology Journal of North America*, vol. 2, no. 1, p. 29-33.

Association of Official Analytical Chemists, AOAC:2000. *Official Methods of Analysis of AOAC International. 17th edition. Gaithersburg, MD.*

Bankole, M. O., Okagbue, R. N. 1992. Properties of Nono a Nigerian fermented milk food. *Ecology of Food and Nutrition.* vol. 27, no. 2, p. 145-149. https://doi.org/10.1080/03670244.1992.9991236

Barrow, G. I., Feltham, R. K. 1993. *Cowan and Steel's Manual for the identification of Medical Bacteria.* London. 3rd ed. UK : Cambridge University Press, 331 p. https://doi.org/10.1017/CBO9780511527104

Bezkorovainy, A. 2001. Probiotics: Determinants of survival and growth in the gut. *American Journal of Clinical Nutrition*, vol. 73, suppl. no. 2, p. 399-405.

Bernet-Camard, M. F., Lievin, V., Brassart, D., Neeser, J. R., Servin, A. L., Hudault, S. 1997. The human *Lactobacillus acidophilus* strain LA1 secretes a nonbacteriocin antibacterial substance(s) active in vitro and in vivo. *Applied Environmental Microbiology*, vol. 63, no. 7, p. 2747-2753. PMid:9212421

Brenner, D. J., Krieg, N. R., Staley, J. T. 2005a. The Gammaproteobacteria. In Garrity, G. M. *Bergey's Manual of Systematic Bacteriology*. New York, USA : Springer. 1108 p. ISBN 978-0-387-28022-6.

Brenner, D. J., Krieg, N. R., Staley, J. T. 2005b. The Proteobacteria. In Garrity, G. M. *Bergey's Manual of Systematic Bacteriology*. New York, USA : Springer. 1388 p. ISBN 978-0-387-29299-1.

Bryan, F. L., Michanie, S. C., Alvarez, P., Paniagua, A. 1988. Critical control points of street-vended foods in the Dominican Republic. *Journal of Food Protection* vol. 51, no. 5, p. 373-383. https://doi.org/10.4315/0362-028X-51.5.373

Chan, H., Kahan, Z., Hakkak, M., Ivany, K. 2007. Role of diet in prostrate cancer development and progression, *Journal of Oncology,* vol. 23, no. 32, p. 8152-8160. https://doi.org/10.1200/JCO.2005.03.1492 PMid:16278466

Clinical Laboratory Standards Institute, CLSI:2016. *Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI Supplement M100S. Clinical Laboratory Standards Institute.*

Coconnier, M. H., Lievin, V., Bernet-Camard, M. F., Hudault, S., Servin, A. L. 1997. Antibacterial effect of the adhering human *Lactobacillus* strain LB. *Antimicrobial Agents and Chemotherapy,* vol. 41, no. 5, p. 1046-1052.

Donkor, E. S., Aning, K. G., Quaye, J. 2007. Bacterial contaminations of informally marketed raw milk in Ghana. *Ghana Medical Journal,* vol. 200, no. 41, p. 2.

Dobby's Signature, 2014. Fura da nono drink recipe. [online] s.a. [2017-09-2] Available at: http://www.dobbyssignature.com/2014/06/fura-da-nono-drink-recipe.html.

Fabianová, J., Ducková, V., Čanigová, M., Kročko, M. 2011. Presence of Enterococci in cow milk and their antibiotic resistance. *Potravinarstvo*, vol. 4, no. 2, p. 17-21. https://doi.org/10.5219/45

Gideon Informatics, 1994-2017. Gideon-Microbiology-Identify Bacteria [online] s.a. [2017-09-2] Available at: www.gideononline.com.

Granito, M., Álvarez, G. 2006. Lactic acid fermentation of black beans (*Phaseolus vulgaris*): microbiological and chemical characterization. *Journal of the Science of Food and Agriculture*, vol. 86, no. 8, p. 1164-1171. https://doi.org/10.1002/jsfa.2490

Harrigan, W. F. 1998. *Laboratory Methods in Food Microbiology*. 3rd ed. San Diego : Academic Press. 532 p. ISBN 0-12-326043-4.

Ibrahim, K., Nural, A. 2016. Isolation, identification and characterization of Lactic Acid Bacteria from milk and yoghurts. *Journal of Food and Dairy Technology*, vol. 4, no. 3, p. 19.

Julian, M. K. 1997. Pathogenesis of enteric infection by Campylobacter. *Microbiology*, vol. 143, no. 1, p. 5-21.

Kunová, S., Golian, J., Zeleňáková, L., Lopašovský, Ľ., Čuboň, J., Haščík, P., Kačániová, M. 2017. Microbiological quality of fresh and heat treated cow's milk during storage. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 652-657. https://doi.org/10.5219/799

Krieg, N. R., Ludwig, W., Whitman, W. B., Hedlund, B. P., Paster, B. J., Staley, J. T., Ward, N., Brown, D., Parte, A. 2010. The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes. Bergey's Manual of Systematic Bacteriology. In Krieg, N. R. et al. *Bergey´s Manual of Systematic Bacteriology*. New York, USA : Springer. 908 p. ISBN 978-0-387-3-68572-4.

Krishnendra, S. N., Sakshi, D., Priyanka, P., Priyanka, S., Neelofar, S., Jitendra, N. 2013. Antagonistic activity of lactic acid bacteria from dairy products. *International Journal of Pure and Applied Bioscience*, vol. 1, no. 1, p. 28-32.

Ledenbach, L. H., Marshall, R. T. 2009. Microbiological Spoilage of Dairy Products. In Sperber, W. H. et al. *Compendium of the Microbiological Spoilage of Foods and Beverages*. Berlin, Germany : Springer Science+ Business Media, LLC, 369 p. ISBN 978-1-4419-0825-4.

Logan, N. A. 1988. *Bacillus* species of medical and veterinary importance. *Journal of Medical Microbiology*, vol. 25, no. 3, p. 157-165. https://doi.org/10.1099/00222615-25-3-157 PMid:3279213

Marika, M., Zilmer, M. 2009. *Lactobacillus fermentum* ME-3 An antimicrobial and antioxidative probiotic. *Microbial Ecology in Health and Disease*, vol. 21, no. 1, p. 1-27. https://doi.org/10.1080/08910600902815561 PMid:19381356

Okiki, P. A., Moro, D. D., Oguntayo, G., Ogbimi A. O. 2013. Association of plasmid mediated multi-drug resistant bacteria with high mortalities in Nigerian poultry. *Journal of Biology Agriculture and Health Care*, vol. 3, no. 15, p. 1-6.

Osibote, I. A., Okiki, P. A., Ekundayo, E. A., Adekunle, A. C. 2014. Prevalence of Multi-drug Resistant Bacterial Isolates from Meat Processing Equipment and Abattoir Environment in Ado Ekiti. *Advances in Biological Research*, vol. 8, no. 5, p. 207-211.

Pan, D. D., Zeng, X. Q., Yan, T. 2011. Characterization of *Lactobacillus fermentum* SM-7 isolated from koumiss, a potential probiotic bacterium with cholesterol-lowering effects. *Journal of the Science of Food and Agriculture*, vol. 91, no. 3, p. 512-518. https://doi.org/10.1002/jsfa.4214 PMid:21218486

Ridwan, B. U., Koning, C. J., Besselink, M. G., Timmerman, H. M., Brouwer, E. C., Verhoef, J., Gooszen, H. G. and Akkermans, L. M. 2008. Antimicrobial activity of a multispecies probiotic (Ecologic 641) against pathogens isolated from infected pancreatic necrosis. *Letters in Applied Microbiology*, vol. 46, no. 1, p. 61-67. PMid:17944834

Savadogo, A., Ouattara, A. T., Savadogo, W. Baro, N., Ouattara, S. A., Traore, S. A. 2004. Identification of exopolysaccharides producing lactic acid bacterianstrains from Burkina Faso fermented milk samples by specific primers. *African Journal of Biotechnology*, vol. 3, no. 3, p. 189-194.

Shah, M. F., John, A. M., John, J. M. 1998. Epidemiology, genetics and ecology of toxigenic vibrio cholerae. *Microbiology and Molecular Biology Reviews*, vol. 62, no. 4, p. 1301-1314.

Solomon, M., Mulisa, M., Yibeltal, M., Desalegn, G., Simenew, K. 2013. Bacteriological quality of bovine raw milk at selected dairy farms in Debre Zeit town, Ethiopia. *Journal of Food Sciences and Technology Research*, vol. 1, no. 1, p. 1-8.

Theodore, J. D. K., Bernard, M. H., Mwansa, M. S.,Yona, S., Delia, G. 2016. Microbial contamination and hygiene of fresh cow's Milk produced by smallholders in Western Zambia. *International Journal of Environmental Research and Public Health*, vol. 13, no. 7, p. 737. https://doi.org/10.3390/ijerph13070737 PMid:27455294

Vos, P., Garrity, G., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleifer, K. H., Whitman, W. B. 2009. *Bergey's Manual of Systematic Bacteriology. The Firmicutes*. 2nd ed. New York, USA : Springer. 1450 p. ISBN 978-0-387-68489-5.

World Health Organization (WHO), 2002. Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food [online] s.a. [2017-09-2] Available at: http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf.

Zajác, P., Tomáška, M., Murárová, A., Čapla, J., 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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INFLUENCE OF MILK THISTLE PRESSED PARTS ON RATS LIVER HISTOLOGY

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ABSTRACT
Milk thistle (*Silybum marianum*) is one of the best known and very often used herbs with positive effect on liver. The aim of this article was to study influence of addition of milk thistle pressed parts in feed ration on liver histology and weight increase of laboratory rats. The experiment was tested by 15 pieces of laboratory rats divided into 3 groups (A, B, C). The rats in first group (A) hade feed ration with addition of 10% milk thistle pressed parts, second group (B) hade feed ration with 20% and control group (C) hade feed ration without addition of milk thistle pressed parts. The silymarin content of pressed milk thistle seed was 26.2 mg/g. The aim of this article is to study influence of addition of milk thistle pressed parts in feed ration on liver histology and weight increase of laboratory rats. The hypothesis is that the feeding addition – milk thistle seed pressed parts has positive effect on weight performance and liver histology. Does the feeding addition have any effect on these health indicators? Does feeding of milk thistle seed pressed parts have any sense? In results, the groups with addition of milk thistle had significant bigger average gain increases than the control group. Histological results vary considerably among groups of rats. All rats in the control group had dystrophic liver with sinusoidal congestion. In most rats of the group A, the dystrophy was minimal without congestion. On the other hand, most of rats of the group B had liver dystrophy caused by large droplets steatosis with congestion. All rats in control group C were found to have significant dystrophy caused by steatosis. The results indicate rats receiving the addition of milk thistle pressed parts in their feed had a lower incidence of liver steatosis due to the hepatoprotective effects of silymarin.

Keywords: *Silybum marianum*; histology; growth performance; silymarin

INTRODUCTION
Effective agents of milk thistle stimulate regeneration of hepatocytes. Complex of milk thistle agents is called silymarin, this stabilises membranes of hepatocytes and thus silymarin increase hepatocytes resistance to toxins and oxidants. Effect of silymarin stimulates production of new hepatocytes. Extracts of milk thistle has been used in the treatment of acute and chronic liver disorders. Liver disorders are usually caused by toxins, drug, alcohol and hepatitis and gall bladder disorders **(Zhu et al., 2016)**. Silymarin appears to be safe and well tolerated **(Dhiman et al., 2005).** Pharmacological taking of milk thistle is known from history and has general dissemination.

Many studies have demonstrated that the active components of silymarin have many hepatoprotective properties **(Abenavoli et al., 2011)** and these studies are predominantly focused on human treatment however at present research get focused too on milk thistle (pressed milk thistle seed) like feed supplement for animals. Properties of production of *S. marianum* (such as oil, pressed seed or flour) markets too alternative uses **(Andrzejewska et al., 2015)**. **Jakubcova et al. (2015)** studied the antimicrobial and antioxidative effect of phytogenic additives, also phytobiotics have potential in animal feeding and it has effect on human nutrition in closing stage. Milk thistle constituents to the animal diet can promise for conventional methods of animal farming **(Kosina et al., 2017)** and seems to be a promising natural feed additive to improve the health condition **(Cullere et al., 2016)**. Results of different researches indicate positive effect of feeding *Silybum marianumon* growth performance but by way of contrast this exist some researches with different ambiguous results.

From point of influence of taking *S. marianum* on liver histology of view some studies state results, that using of *S. marianum* have not any significant effect on liver histology **(Blevins et al., 2010; Dhiman et al., 2005; Jacobs et al., 2002; Tedesco et al., 2004)** by contrast results **(Guo et al., 2016; Loguercio et al., 2012)** state association with improvement in liver histology. It should be noted that significant evaluation often is difficult and ambiguous, also next research is necessary and by reason this study can make a contribution to the issue of influence of *S. marianum* on liver histology.

Scientific hypothesis

The aim of this article is to study influence of addition of milk thistle pressed parts in feed ration on liver histology and weight increase of laboratory rats. The hypothesis is that the feeding addition – milk thistle seed pressed parts has positive effect on weight performance and liver histology. Does the feeding addition have any effect on these health indicators? Does feeding of milk thistle seed pressed parts have any sense?

MATERIAL AND METHODOLOGY

The experiment was established by 15 pieces of laboratory rats divided into 3 groups (A, B, C) in experimental facilities of Department of Animal Nutrition and Forage Production in Mendel University. The rats in first group (A) had feed ration with addition of 10% milk thistle pressed parts, second group (B) had feed ration with 20% and control group (C) had feed ration without addition of milk thistle pressed parts (only scraped barley). Served milk thistle pressed parts had flour-like structure and was mixed equally with scraped barley also the rats could not prefer any feeding component. Milk thistle variety MIREL was used. The feeding mixture and water were available *ad-libitum*. Water and feed ration were daily served and rests were removed.

The length of this experiment was 28 days. Starting average rat´s weight was 90 ±3 g. The rats were weighted in 0 – 7 – 14 – 21 – 28. The rats were sacrificed inhalational anaesthetic Isofuran way on 28th day and liver samples were taken to histological analyse. Histological analyse was performed at University of Veterinary and Pharmaceutical Sciences Brno.

Determination of silymarin

The content of silymarin was performed by HPLC method in Department of Chemistry and Biochemistry at Mendel University in Brno. Analysis of silymarin was performed on a HPLC-UV / VIS instrument (Dionex Ultimate 300). Chromatography column Hypersil GOLD Dim (150 x 4.6) was used for separation by temperature 30 °C. The sample (5 µL) was injected by autosampler. Flow rate was 1 mL.min^{-1}. Content of mobile phase was A: 0.1% formic acid, B: 100% methanol. The substances were leaved to infuse in an isocratic elution way (mobile phase A was 65% and mobile phase B was 35%). Detection of separated substances was in motion under circumstances of wavelength 288 nm.

Statisic analysis

The data were statistically processed using STATISTICA.CZ, version 10.0 (the Czech Republic). The results were expressed as average values (weight) with standard deviation (SD). Statistical significance was determined by the examining the basic differences between groups by ANOVA and Scheffé's test (one-way analysis). The differences with p <0.05 were considered to be significant.

RESULTS AND DISCUSSION

Growth Performance

The results show that the weight gain of the rats fed with mixtures of 10% the milk thistle was higher compared to rats of the control group. The highest average daily gains were found in rats fed by mixtures of 20% milk thistle. On the seventh day of the experiment, the difference between groups C and A was found to be statistically significant, same as the difference between groups C and B. Statistically significant difference was also observed between groups C and B on the 28th day. Rats receiving the addition of milk thistle in their feed dose grew more intensely than the control group. **Kosina et al. (2017)** describes the positive effect of the milk thistle on the growth potential of experimental animals (rabbits in this case). Therefore, the addition of the milk thistle in the feed dose could have a positive effect on the growth potential of animals. **Feng et al. (2016)** states that silymarin significantly affected weight gain in mice. This trend was also observed in our experiment.

In group C, all animals had wide-area liver dystrophy and congestive parenchyma according to histological images. Dilation of porto-biliary system and central veins were less common than in groups A, B.

In group A, liver parenchyma is showed like intact hardly any dystrophy with dilated porto-biliary system in the pictures LM 6, LM 7 and LM 8, whereas pictures LM 9 and LM 10 show distinct dystrophy caused by steatosis.

In group B, LM 11, LM 12, LM 14 and LM 15 show area-wide distinct liver dystrophy caused by steatosis.

In animals of all groups A, B, C, there was a more distinct dystrophy caused by steatosis. In groups B and C, the vast majority showed liver steatosis. In Group A, 40% of the animals were found with steatosis, 80% in group B, and 100% in group C. The results of **Guo et al. (2016)** suggest silymarin reduces inflammatory processes causing hepatic tissue damage. Also in our study, the incidence of hepatic dystrophy was lower in groups receiving the addition of milk thistle (especially group B). **Blevins et al. (2010)** and **Tedesco et al. (2004)** report histological examination of the liver found the occurrence of steatosis even in groups of animals fed by the addition of silymarin and did not confirm effect of silymarin reducing the incidence of steatosis.

Table 1 Average daily gain (g.day^{-1}) – Group C (Control group), Group A (rats fed with 10% part of milk thistle pressed parts), Group B (rats fed with 20% part of milk thistle pressed parts).

Groups	Average daily gains during the experiment				
	0.day (g.day^{-1} ±SD)	7th.day (g.day^{-1} ±SD)	14th.day (g.day^{-1} ±SD)	21th.day (g.day^{-1} ±SD)	28th.day (g.day^{-1} ±SD)
Group C	0.0	2.3 ±0,5^{a}	2.1 ±0.4^{a}	1.9 ±0.4^{a}	1.7 ±0.5^{a}
Group A	0.0	3.4 ±0.6^{b}	2.5 ±0.3^{a}	1.8 ±0.5^{a}	1.7 ±0.6^{b}
Group B	0.0	3.6 ±0.3^{b}	2.4 ±0.4^{a}	2.0 ±0.7^{a}	3.1 ±0.6^{a}

Note: Change in index ^{a},^{b} shows significant difference at the level (p <0.05)

Liver Histology

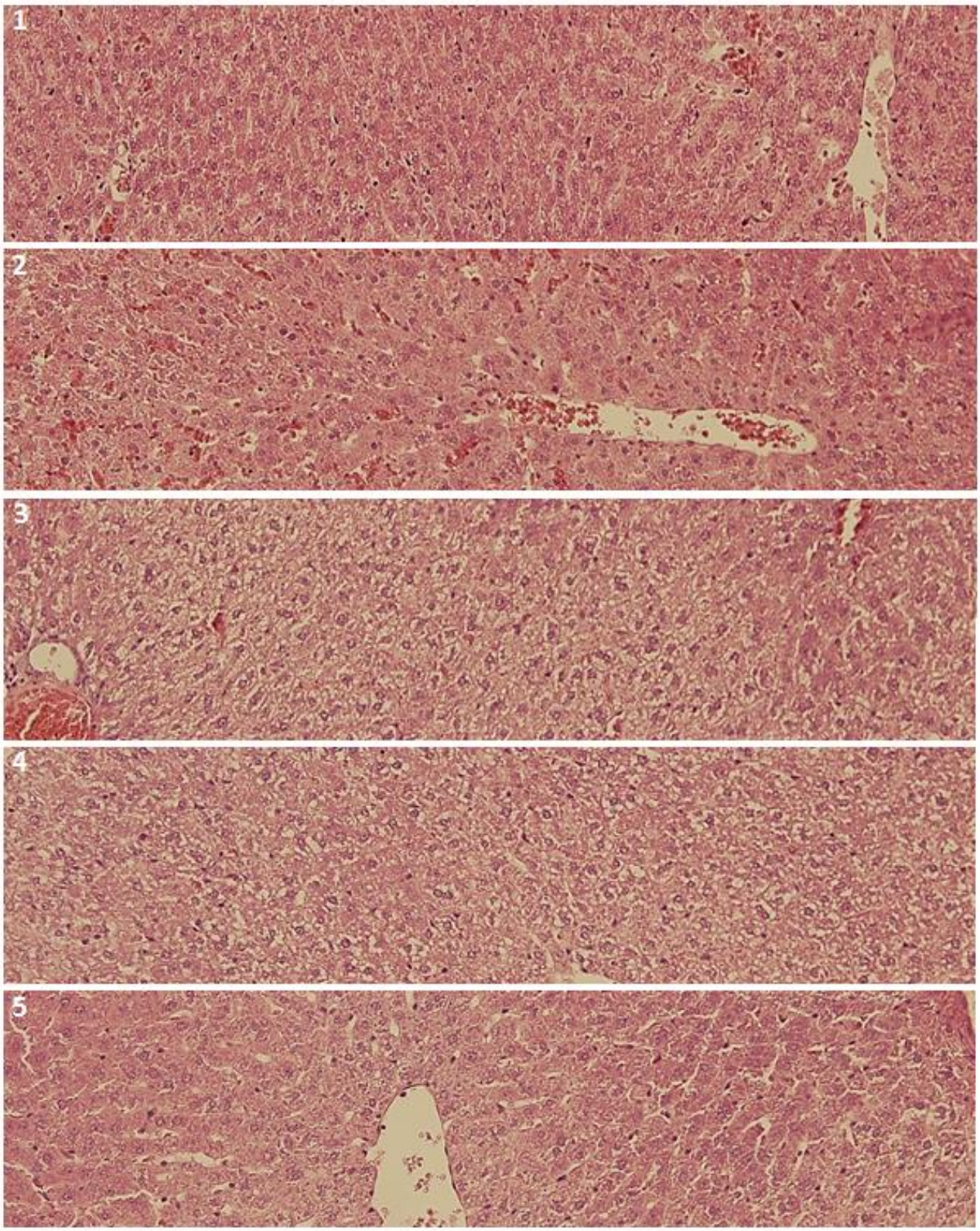

Figure 1 Liver Mash of rats in group C (control group). *Magnification 200x*.
Note: LM 1: Parenchyma is congestive, all-dystrophic, locally with trabecular system, without necrosis, with distinct dilation of porto-biliary system. LM 2: Locally distinct sinusoidal congestion, otherwise identical to LM 1. LM 3: Congestion, massive wide-area hepatodystrophy with structural destruction focuses. LM 4: Identical with LM 3. LM 5: Sinusoidal congestion, wide-area dystrophy but less distinct compared to the previous cases, trabecular structure in good condition.

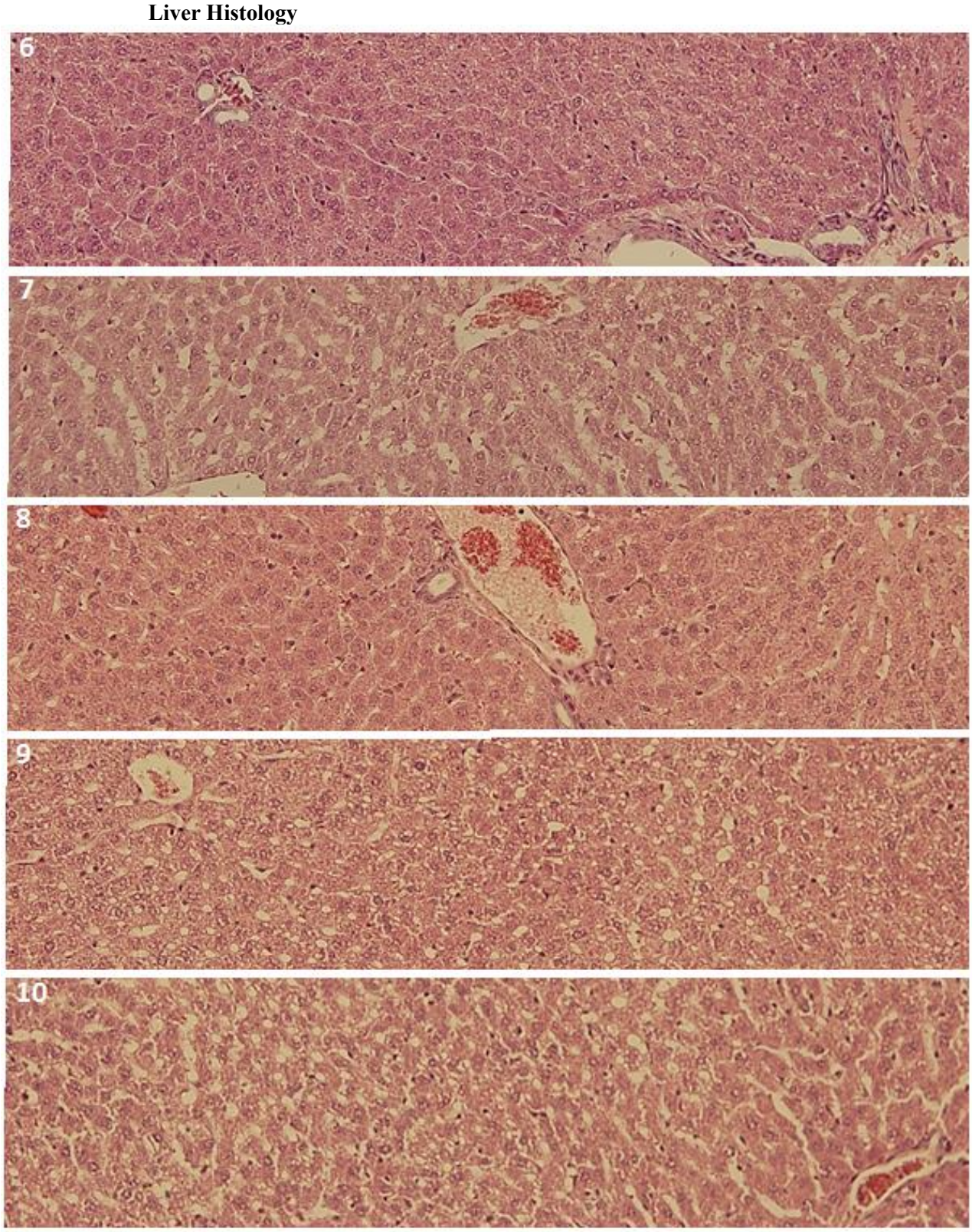

Figure 2 Liver Mash of rats in group A (10% part of milk thistle pressed parts). *Magnification 200x*.
Note: LM 6: Minimum dystrophy, dilatation of porto-biliary system, otherwise almost intact parenchyma.
LM 7: LM 7 is identical to LM 6. LM 8: LM 8 is identical to LM 6. LM 9: More distinct dystrophy caused by steatosis, trabeculary system in good condition, dilatation of central veins. LM 10: Distinct dystrophy caused by large droplets steatosis, moderate congestion, plates of hepatic cell with good structure.

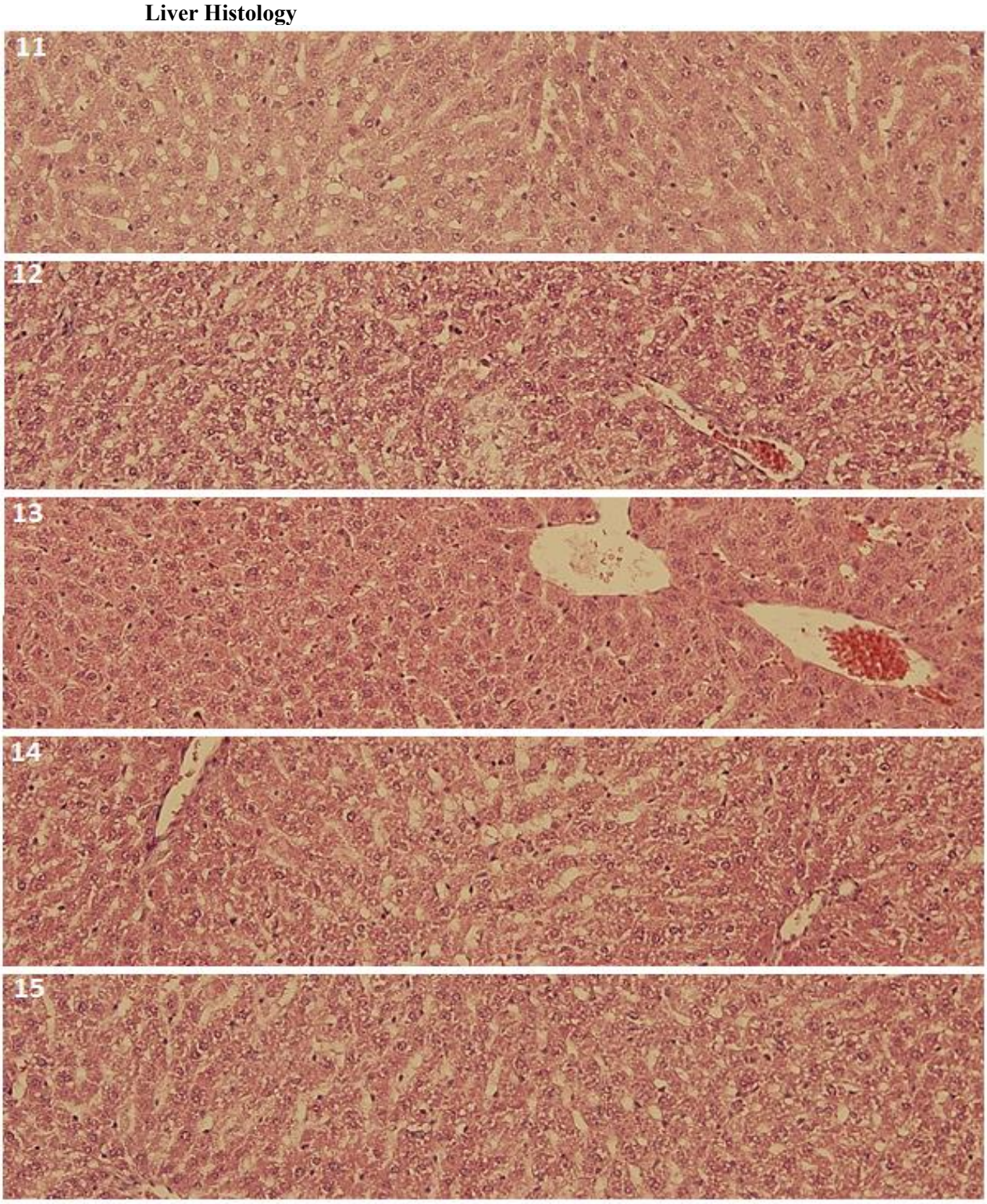

Figure 3 Liver Mash of rats in group B (20% part of milk thistle pressed parts). *Magnification 200x*.
Note: LM 11 is comparable with LM 10. LM 10: distinct dystrophy caused by large droplets steatosis, moderate congestion, plates of cells structure in good condition. LM 12: Wide-area massive dystrophy with persistence of trabeculary system, without necrosis, dilatation of porto-biliary system. LM 13: Compared to LM 12, significantly less dystrophic parenchyma, moderate congestion and dilatation of central veins, without alteration of plates of cells structure. LM 14: Congestion, wide-area dystrophy caused by large droplets steatosis. LM 15: Identical with LM 14. (Congestion, wide-area dystrophy caused by large droplets steatosis).

Dystrophy of the liver parenchyma caused by steatosis in rats of our experiment seems to be related to the barley monodiets – the animals received only barley and addition of milk thistle pressed parts in some groups. An unbalanced ratio of nutrients could be the cause of liver damage. Involvement of the health burden caused by inadequate nutrition was targeted in order to investigate hepatoprotective effects of silymarin as we do not anticipate the occurrence of histological findings in healthy animals with ideal nutrition. In animals receiving the addition of milk thistle pressed parts, the incidence of liver steatosis was lower which was probably related to the hepatoprotective effects of silymarin. From a histological point of view, the most convenient percentage of milk thistle pressed parts is 10% (group A) according to our results.

CONCLUSION

In the experiment, the effect of milk thistle was investigated on liver health in rats. In addition to liver histology, rats were also monitored for their continuous weight gains. Average daily weight gain of rats fed by mixtures with milk thistle compared to the control group increased, with the highest weight gain in the group B (20% addition of milk thistle). In animals in all groups A, B, C, there was more distinct dystrophy caused by steatosis. In groups B and C, the majority of rats were found with liver steatosis. According to histology findings, rats of group A were found with steatosis in 40%, 80% in Group B and 100% in group C. Dystrophy of liver parenchyma caused by steatosis in rats can be probably related to nutritional imbalance (barely monodiets). Induction of the health burden has been targeted in order to investigate the hepatoprotective effect of silymarin, as histologic findings are less probable in healthy animals with the ideal nutrition. In the animals fed with the addition of pressed parts of milk thistle, the incidence of liver steatosis was lower which may be related to hepatoprotective effect of silymarin. According to the results from the histological point of view, the most appropriate proportion of milk thistle pressed parts was 10% – group A. The content of silymarin in the pressed parts was 26.2 $mg.g^{-1}$.

REFERENCES

Abenavoli, L., Aviello, G., Capasso, R., Milic, N., Capasso, F. 2011. Milk thistle for treatment of nonalcoholic fatty liver disease. *Hepatitis Monthly*, vol. 11, no. 3, p. 173-177.

Andrzejewska, J., Martinelli, T., Sadowska, K. 2015. Silybum marianum: non-medical exploitation of the species. *Annals of Applied Biology*, vol. 167, no. 3, p. 285-297. https://doi.org/10.1111/aab.12232

Blevins, S., Siegel, P. B., Blodgett, D. J., Ehrich, M., Saunders, G. K., Lewis, R. M. 2010. Effects of silymarin on gossypol toxicosis in divergent lines of chickens. *Poultry Science*, vol. 89, no. 9, p. 1878-1886. https://doi.org/10.3382/ps.2010-00768 PMid:20709972

Dhiman, R. K., Chawla, Y. K. 2005. Herbal medicines for liver diseases. *Digestive Diseases and Sciences*, vol. 50, no. 10, p. 1807-1812. https://doi.org/10.1007/s10620-005-2942-9 PMid:16187178

Cullere, M., Zotte, A. D., Celia, C., Renteria-Monterrubio, A. L., Gerencser, Z., Szendro, Z., Kovacs, M., Kachlek, M. L., Matics, Z. 2016. Effect of Silybummarianum herb on the productive performance, carcass traits and meat quality of growing rabbits. *Livestock Science*, vol. 194, p. 31-36. https://doi.org/10.1016/j.livsci.2016.10.012

Feng, B., Meng, R., Huang, B., Shen, S., Bi, Y., Zhu, D. 2016. Silymarin alleviates hepatic oxidative stress and protects against metabolic disorders in high-fat diet-fed mice. *Free Radical Research*, vol. 50, no. 3, p. 314-327. https://doi.org/10.3109/10715762.2015.1116689 PMid:26758315

Guo, Y., Wang, S., Wang, Y., Zhu, T. 2016. Silymarin improved diet-induced liver damage and insulin resistance by decreasing inflammation in mice. *Pharmaceutical Biology*, vol. 54, no. 12, p. 2995-3000. https://doi.org/10.1080/13880209.2016.1199042 PMid:27387273

Jacobs, B. P., Dennehy, C., Ramirez, G., Sapp, J., Lawrence, V. A. 2002. Milk thistle for treatment of liver disease: A systematic review and meta-analysis. *American Journal of Medicine*, vol. 113, no. 6, p. 506-515. https://doi.org/10.1016/S0002-9343(02)01244-5

Jakubcova, Z., Horky P., Dostalova, L., Sochor, J., Tomaskova, L., Baron, M., Kalhotka, L., Zeman, L. 2015. Study of antioxidant and antimicrobial properties of grapevine seeds, grape and rosehip pressings. *Potravinarstvo*, vol. 9. no. 1, p. 382-387. https://doi.org/10.5219/503

Kosina, P., Dokoupilova, A., Janda, K., Sladkova, K., Siberova, P., Pivodova, V., Ulrichova, J. 2017. Effect of Silybummarianum fruit constituents on the health status of rabbits repeated 42-day fattening experiment. *Animal Feed Science and Technology*, vol. 223, p. 128-140. https://doi.org/10.1016/j.anifeedsci.2016.11.013

Loguercio, C., Andreone, P., Brisc, C., Brisc, M. C., Bugianesi, E., Chiaramonte, M., Cursaro, C., Danila, M., De Sio, I., Floreani, A. 2012. Silybin combined with phosphatidylcholine and vitamin E in patients with nonalcoholic fatty liver disease: A randomized controlled trial. *Free Radical Biology and Medicine*, vol. 52, no. 9, p. 1658-1665. https://doi.org/10.1016/j.freeradbiomed.2012.02.008 PMid:22343419

Tedesco, D., Domeneghini, C., Sciannimanico, D., Tameni, M., Steidler, S., Galletti, S. 2004. Silymarin, a possible hepatoprotector in dairy cows: Biochemical and histological observations. *Journal of Veterinary Medicine Series A-Physiology Pathology Clinical Medicine*, vol. 51, no. 2, p. 85-89. https://doi.org/10.1111/j.1439-0442.2004.00603.x PMid:15153078

Zhu, X. X., Ding, Y. H., Wu, Y., Qian, L. Y., Zhou, H., He, Q. 2016. Silibinin: a potential old drug for cancer therapy. *Expert Review of Clinical Pharmacology*, vol. 9, no. 10, p. 1323-1330. https://doi.org/10.1080/17512433.2016.1208563 PMid:27362364

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TIBIA MINERALIZATION OF CHICKENS DETERMINED TO MEAT PRODUCTION USING A MICROBIAL PHYTASE

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ABSTRACT

The target of the research was 6-phytase of microbial origin. It was used in feed mixtures for chickens determined to meat production. Its effect has been studied in relation to the tibia mineralization by calcium, phosphorus and magnesium. 6-phytase is a product of *Aspergillus oryzae*. That was obtained by means of biotechnological processes of production of commercially available enzymes. It was incorporated in the feed mixtures 0.1%. In a 38-day feeding trial, 300 one-day-old, as hatched, Cobb 500 chickens determined to meat production (100 birds per group) were fed on one concentrations of dietary non-phytate phosphorus (2.32, 2.31 $g.kg^{-1}$, respectively and supplemental microbial phytase (0 and 500 $FTU.kg^{-1}$ feed mixtures). Control group was used to compare the results and control feed mixtures contained 4.5 $g.kg^{-1}$ without microbial phytase. At days 40 it was selected 6 birds in every group, which were slaughter in accordance with the principles of welfare. Left tibias of every bird were used to determination of calcium, phosphorus and magnesium contents. According to *in vivo*, it was found that the addition of microbial phytase to reduced dietary non-phytate phosphorus increased concentrations of calcium (Ca), phosphorus (P) and magnesium (Mg) in tibia. The differences among groups were statistically significant ($p < 0.05$). It was concluded that reducing of dietary non-phytate phosphorus on the 2.32, 2.31 $g.kg^{-1}$, respectively, by monocalcium phosphate and microbial phytase supplementation in feed mixtures facilitated tibia mineralization at chicken determined to meat production.

Keywords: broiler; microbial phytase; additive; phosphorus; tibia; mineralization

INTRODUCTION

The availability of phosphorus in feedstuffs of plant origin is generally very low, ranging from 30 to 40% **(Nelson et al., 1968a)**. To increase phosphorus bioavailability, the most commonly used method is supplementing high dosage of inorganic phosphorus in feed, which leads to the excretion of large amounts of phosphorus in animal manure. Consequently, the cost of feed and the environmental adverse impact are increased. Moreover, phytate limits the availability of several other essential nutrients, such as minerals, protein and amino acids **(Biehl and Baker, 1996)**. Among the strategies designed to reduce excessive drainage of phosphorus but also calcium into the environment from the poultry industry, the two most important are (1) determining the exact phosphorus and calcium requirements for present modern chickens determined to meat production and formulating to meet those requirements exactly and (2) using additives such as microbial phytase to increase phytate phosphorus utilization and decrease excretion of these nutrients into environment **(Angel et al., 2005)**.

As phytase is increasingly used worldwide, science and technology related to the enzyme have evolved to a new exciting field at a fast pace. Clearly, supplemental phytases improve dietary phytate-phosphorus utilization by food-producing animals, and reduce environmental pollution of phosphorus from animal waste in areas of intensive animal production **(Kliment et al., 2010)**.

Phytases are a group of enzymes that catalyze the release of orthophosphate from inositol hexakisphosphate **(Mullaney and Ullah, 2003)**. The phytases are divided into four classes: histidine acid phosphatases, β-propeller phytases, cysteine phosphatases and purple acid phosphatases **(Puhl et al., 2007; Huang et al., 2011)**. These include the fungal phytases from Aspergillus ficuum **(Kostrewa et al., 1997)** A. fumigatus **(Xiang et al., 2004)** A. niger **(Oakley, 2010)** and Debaryomyces castellii **(Ragon et al., 2009)** and the bacterial phytases from Escherichia coli **(Lim et al., 2000)** and Klebsiella pneumoniae **(Böhm et al., 2010)**. The enzymes are additionally classified into 3-, 5- or 6-phytases (EC 3.1.3.8, EC 3.1.3.72 and EC 3.1.3.26, respectively) based on the carbon position on the inositol ring at which they initiate phosphate hydrolysis. Thus 3-phytases first remove the phosphate group at the C3 or C1 position (1L- vs. 1D-convention) while 6-phytases do so at the C6 position (or

C4 in the 1L convention) **(Lei and Porres, 2003)**. Very few 5-phytases have been identified (for example that from from Selenomonas ruminantium **(Chu et al., 2004)**.

The efficacy and safety of a microbial 6-phytase expressed via the use of synthetic genes in Aspergillus oryzae was investigated from days 8 to 22 of age using 480 Ross PM3 broiler chickens. Five treatments were tested. A diet containing 5.6 $g.kg^{-1}$ of phosphorus was fed to the control treatment. Another diet containing 4.1 $g.kg^{-1}$ phosphorus was fed to another treatment as negative control. This diet was fed in 3 other treatments with the addition of phytase (500, 1000, or 2000 $U.kg^{-1}$). Lower feed intake and higher weight gain was obtained with the treatment containing 2000 $U.kg^{-1}$ phytase compared to the two control treatments and the treatment containing 500 $U.kg^{-1}$ phytase, leading to a significant improvement in feed conversion ratio with the 2000 $U.kg^{-1}$ phytase. Tibia strength and ash were improved with the latter and were dose-dependent described by an exponential function. Safety test using a concentrated preparation of the novel 6-phytase enzyme did not reveal any toxicological significant findings. Tested 6-phytase improved broiler performance and reduced the need for phosphate **(Aureli et al., 2011)**.

Recently study demonstrated that diets containing non-phytate phosphorous closer to the requirements along with the use of supplemental phytase decreased the total phosphorus concentrations in litters without affecting phosphorus solubility in litters and amended soils **(Maguire et al., 2004)**.

Phytate phosphorus digestibility can be measured at either ileal or fecal levels. Total tract phosphorus digestibility can be used to determine phosphorus retention as it has fewer practical limitations. However, determination of phytate phosphorus release should be measured at the ileal level because in the hindgut phytate is degraded by the intestinal microflora, but the released phosphorus is not absorbed. Direct measurement of the hydrolysis of phytate phosphorus at the ileal level will give a good indication of the effectiveness of a phytase feed additive. In addition, tibia ash, bone strength and performance data are useful parameters for validation of matrix values (e.g. inorganic phosphorus, calcium, energy and digestible amino acids) for phytase **(Dersjant-Li et al., 2015)**.

Phosphorus supply may indeed play a controlling role for the total numbers of bacteria as suggested by the significant calcium/phosphorus and phytase effects which were independent of one another, and tended to be additive. Phytase seems to have an important role to play in modulating the gut flora but their effects are clearly framed by the background levels of phosphorus and calcium in the diet. The changes in the microbiome as a result of feeding a phytase at high inclusion levels 5000 FTU are novel, although it must be noted that although the changes were significant, the scale of the changes were not extraordinary. The fact that phytase, rather than calcium and phosphorus levels played a significant role in promoting better animal performance suggests that the linkage between microbiome structure and performance is not inextricable **(Ptak et al., 2015)**.

Benefits of phytase supplementation likely occur due to release of phosphorus and extraphosphoric effects simultaneously over a wide range of phytase concentrations. In contrast to the aforementioned knowledge **Watson et al. (2006)** noted that significant extra-phosphoric increases in body weight gain have been reported at concentrations ranging from phytase as low as 300 to 800 FT $U.kg^{-1}$.

Experimentally the low non-phytate phosphorus diet, medium and high non-phytate phosphorus feed mixtures were compared. They found the increasing ash percentage, phosphorus content and breaking strength of tibia on day 21 and 42 ($p < 0.001$). Supplementation of 6-phytase but also 3-phytase significantly improved the ash percentage on day 21 and phosphorus content of tibia at 21 and 42 days of age ($p < 0.001$). Dietary 6-phytase enhanced ash percentage (linear contrast, $P = 0.039$) and tended to increase breaking strength (linear contrast, $P = 0.094$) in tibia of chickens at 42 days of age compared to control diet. There was a significant interaction between non-phytate phosphorus levels and phytase sources on ash percentage at 42 days of age ($p < 0.01$). The ash percentage and phosphorus contents in ashes of bone are the main parameters for mineral deposition in animal bones **(Jiang et al., 2013)**.

A decrease in dietary phosphorus, especially in finishing chickens determined to meat production (21 to 38 days old) is a crucial issue in poultry production from an environmental and economic point of view. Nevertheless, phosphorus must be considered together with other dietary components such as calcium and microbial phytase. Phosphorus level is possible to decrease in finishing chickens, if the calcium content is appropriate. Nevertheless, decreasing the dietary phosphorus and calcium cannot allow a maximization of bone mineralization, but the optimal threshold remains to be determined **(Rousseau et al., 2012)**.

In chickens determined to meat production it has extensively been reported that phytase supplementation to maize-soybean meal feed mixtures permits total phosphorus concentration to be reduced without impairing bone mineralization **(Brož et al., 1994; Qian et al., 1996; Sebastian et al., 1996a; Yan et al., 2001**; **Viveros et al., 2002**; **Brenes et al., 2003**; **Dilger et al., 2004**; **Onyango et al., 2004, 2005; Payne et al., 2005**; **Catalá-Gregori et al., 2006)**.

Scientific hypothesis

The purpose of this study was to present the in vivo results of 6-phytase effect on tibia mineralization in chickens kept for meat production. In the experiment were used the feed mixtures of soy-cereal type with reduced of non-phytate phosphorus content.

MATERIAL AND METHODOLOGY

Carry out of experiment

In vivo experiment was realized on Zámostie Company poultry experimental station with deep litter breeding system. The experiment included 300 pcs of one-day-old hybrid chickens Cobb 500 divided into 3 groups (in each group n = 100) according to scheme in Table 1. Spaced deep litter box allowed chickens to an unlimited access to feed and water as well as to perform their natural activities. The bottom layer of the litter consisted of 8 cm

Table 1 Scheme of feed individual experiment.

Broiler chicks n = 6	Group	Experimental phase, feed mixture	Phosphorus, content per kg of feed mixture and phytase supplement
Cobb 500	Control (CG)	Starter (day 1 to 18)	non-phytate P 4.50 $g.kg^{-1}$
		Grower (day 19 to 32)	non-phytate P 4.50 $g.kg^{-1}$
		Finisher (day 33 to 38)	non-phytate P 4.50 $g.kg^{-1}$
	Experimental 1 (G-RP)	Starter (day 1 to 18)	non-phytate P 2.32 $g.kg^{-1}$
		Grower (day 19 to 32)	non-phytate P 2.32 $g.kg^{-1}$
		Finisher (day 33 to 38)	non-phytate P 2.32 $g.kg^{-1}$
	Experimental 2 (G-RP +MPH)	Starter (day 1 to 18)	non-phytate P 2.32 $g.kg^{-1}$ +0.1% (500 $FTU.kg^{-1}$) 6-phytase
		Grower (day 19 to 32)	non-phytate P 2.32 $g.kg^{-1}$ +0.1% (500 $FTU.kg^{-1}$) 6-phytase
		Finisher (day 33 to 38)	non-phytate P 2.31 $g.kg^{-1}$ +0.1% (500 $FTU.kg^{-1}$) 6-phytase

Note: n = multiplicity; P = phosphorus; CG = control group; G-RP = experimental group 1 with reduced non-phytate phosphorus content to 2.32 $g.kg^{-1}$ feed mixtures; G-RP +MPH experimental group 2 with reduced non-phytate phosphorus content to 2.32 $g.kg^{-1}$ and with addition 6-phytase microbial origin 0.1% (500 $FTU.kg^{-1}$) in feed mixtures.

Table 2. Nutrient and energy contents per kg of feed mixtures.

	Feed mixture					
	Starter		Grower		Finisher	
	experimental	control	experimental	control	experimental	control
ME_N, MJ	12.076	12.00	12.081	12.035	12.449	12.421
Crude protein, g	212.95	212.32	192.64	192.68	173.17	172.92
Lysine, g	11.60	11.58	9.97	10.00	10.07	10.00
Methionine, g	4.68	4.67	5.19	5.18	5.40	5.39
Methionine + cystine, g	7.85	7.83	8.04	8.03	7.96	7.94
Threonine, g	8.20	8.18	7.34	7.34	6.53	6.53
Calcium, g	8.18	8.14	7.22	7.19	7.20	7.24
P total, g	5.37	7.96	5.33	7.92	5.24	7.83
P non-phytate, g	2.32	4.50	2.32	4.50	2.31	4.50
6-phytase, FTU	0*, 500**	0*	0*, 500**	0*	0*, 500**	0*

Note: ME_N = metabolizable energy; P = phosphorus; * = Control group; experimental group 1 (reduced phosphorus content); ** = experimental group 2 (reduced phosphorus content +6-phytase).

of wood sawdust's and the top layer consisted of 5 cm of adjusted wheat straws. The chickens to the age of 14 days consumed a feed from plate feeders and water from the hat drinkers located on the floor. Older chickens consumed feed from the tube feeders and drank water from bucket drinkers till the end of the experiment. Microclimatic conditions were equal for all groups in accordance with the recommendations for the final type chickens Cobb 500. The temperature in the hall was ensured by the air conditioner as well as heating lamp to the age 18 days. Ventilation in the hall on the farm was ensured by the ventilation system and ventilation windows. Light regime was automatically adjusted according to the requirements for this type of chickens.

The experiment lasted 38 days and was divided into three phases:

- starter, from day 1 to day 18,
- grower, from day 19 to day 32,
- finisher, from day 33 to day 38 day

The feed mixtures of soybean-cereal type starter, grower and finisher were fed during the different stages. The experiment consisted of two experimental groups G-RP (reduced non-phytate phosphorus content to 2.32, 2.31 $g.kg^{-1}$ feed mixture, respectively without add-6-phytase) and G-RP + MP (non-phytate phosphorus content to 2.32, 2.31 $g.kg^{-1}$ feed mixture, respectively with the addition of compound 6-phytase 0.1% (500 $FTU.kg^{-1}$) in feed mixtures. The reduction of non-phytate phosphorus content was carried out by reducing of share feed ingredient monocalcium phosphate from 1.7% to 0.55% in the starter feed mixture, from 1.75% to 0.60% in the grower feed mixture and from 1.80 to 0.65% in the finisher feed mixture. The control group was used with commonly used feed mixtures to compare of results. In the control feed mixtures was the non-phytate phosphorus content 4.5 $g.kg^{-1}$ and without microbial phytase.

The procedure was chosen for the balance of phosphorus using feed phosphatase by **Angelovičová (1999)**, **Rada and Havlík (2010)**. They predict a phosphorus digestibility of plant feeds 30% in broiler chickens. A share of plant feeds is in our feed mixture starter 89.25%, in grower 93.00% and finisher 92.60%. In experimental feed mixtures was reduced phosphorus content through feed supplement *monocalcium phosphate* (MCP F Belgium) to 2.32 $g.kg^{-1}$ in feed mixtures starter and grower and to 2.31 $g.kg^{-1}$ in feed mixture finisher from need a standard for broiler chickens of non-phytate phosphorus 4.5 $g.kg^{-1}$ (control group). Nutrient and energy content per kg of feed mixtures is shown in Table 2.

In general, creating formulas of the feed mixtures for broiler chickens is predicted on average phosphorus digestibility 30% of plant feeds. The required amount of phosphorus is added in mineral form. The remaining 70% of phosphorus is therefore unavailable from plant feed, which is basically the inaccurate estimate. The percentage of digestible phytate is in the range from 0 to 60% **(Rada and Havlík, 2010)**.

The characteristics of the additive in the experimental feed mixtures

6-phytase of microbial origin used in experiment is commercial feed additive RONOZYME® P5000 (CT).

Gene encoding phytase in microscopic fungus *Peniophora lycii* was transferred to a host of microscopic fungus *Aspergillus oryzae* (DSM 14223) **(EFSA, 2010)**. *Aspergillus oryzae* vektora Phytase encoding cDNA inserts were introduced into *Aspergillus oryzae* vector pHD414. Plazmid DNA was isolated and transferred to *Aspergillus oryzae* with amdS$^+$ plasmid. Clones amd S$^+$ were detected in the medium, the phytase activity and isolated clones producing phytase. Phytases were then purified from Aspergillus oryzae supernetantov clones producing 6-phytase. cDNA nucleotide sequences *Peniophora lycii* phyA are deposited in the European Molecular Biology Laboratory database **(Classen et al., 1995)**.

6-phytáza is a product of *Aspergillus oryzae*. The safety of genetically modified *Aspergillus oryzae* was confirmed by the European Food Safety Authority. Authorization of product was characterized as a supplement improving a digestive physiology and as environmental enzyme.

Preparation of the sample for chemical analysis

At the end of the experiment the chickens were selected from each group of 6 pieces about the same body weight 1800 g. Left tibia of every chicken was removed from the carcasses immediately after the birds had been slaughtered via exsanguination of the *jugular veins* and *carotid arteries* on both sides. The tibiae were stripped of muscle and stored at +4 °C.

Calcium, phosphor and magnesium determine in tibia

The tibiae were boiled in distilled water for 5 min to facilitate removal of any remaining muscle and connective tissue, oven-dried at 105 °C for 24 h, cooled in a desiccator, weighed, and incinerated in a muffle furnace at 550 to 600 °C for 6 h in porcelain crucible. The ash was cooled in a desiccator, and weighed. The obtained ash was used to preparation of solution mineralized. In the solution of mineralized tibia was calcium and magnesium measured by atomic absorption spectrophotometer GBC Avanta at a wavelength of 422 and 285 nm and phosphorus content by JENWAY 6400 spectrophotometer at a wavelength of 666 nm.

Statistical analysis

The results are presented as average values, standard deviation and coefficient of variation. Scheffe's test was used at the significance level of $\alpha = 0.05$ to compare a difference between groups. SAS statistical package (5) was used to perform statistical analyses, verion 8.2.

RESULTS AND DISCUSSION

Approximately 50% of phytine phosphorus from feeds can be released by use of phytase **(Christensen et al., 1997)** but this value can be significantly lower since efficiency of the use of phytase depends on series of factors. In general, moderate reduction of mineral source of phosphorus, with addition of phytase in feed mixtures for broiler chickens is optimal, whereas greater reduction or complete exclusion of mineral phosphorus from diet even with increased concentrations of added enzyme phytase causes lower growth and lower consumption of food in broilers **(Lukić et al., 2005)**.

Calcium content in the tibia of chickens

Results of calcium content in the tibia of chickens are shown in Figure 1 and Table 3.

The highest calcium content in tibia of chickens was 84.86 g.kg^{-1}, which fed the feed mixtures with the reduced non-phytate phosphorus content and with the share of 6-phytase. It represents 43.46% of tibia ash. The lowest calcium content in tibia of chickens was 69.33 g.kg^{-1}, i.e. 33.09% of tibia ash, which were fed with the feed mixtures with the reduced non-phytate phosphorus content and without 6-phytase. In the control group was tibia calcium content of chickens 78.13 g.kg^{-1} (39.39%). The assessment of basic statistical characteristics shows that the lowest values of fluctuation of calcium content in tibia were at chickens fed by feed mixtures with the reduced non-phytate phosphorus content and with the share of 6-phytase. Maximum variation of calcium content in the tibia was in the control group. The differences of calcium content in tibia were statistically significant (p <0.05) between chickens fed by feed mixtures with reduced non-phytate phosphorus content and with the share 6-phytase and control group and also chickens fed by feed mixtures with reduced non-phytate phosphorus content without 6-phytase, between chickens of control group and chickens which fed by feed mixtures with reduced non-phytate phosphorus content and without 6-phytase. Based on the investigation *in vivo* effects of dietary phytase were clarified some relationships between dietary phytate and calcium. Our findings are consistent with the current literature knowledge **(Plumstead et al., 2008)**. A problem was the absence of any clear effect of dietary phytate concentration on calcium digestibility and retention. In contrast to earlier observations, in other experiment **(Nelson et al., 1968b)** the calcium requirement of 3-wk-old chicks was increased by at least 50% when dietary phytate levels were increased from 0.0 to 25.1%. The increased calcium requirement demonstrated in their study was hypothesized to be caused by increased binding of calcium in the intestine, because 1% phytate was found to be able to bind 0.36% calcium. It has been documented that microbial phytase improves the availability of calcium in corn and soybean feed mixture **(Schöner et al., 1991; Schöner et al., 1993; Kornegay et al., 1996). Sebastian et al. (1996a)** reported that phytase supplementation of a low-phosphorus feed mixture increased the relative retention of calcium by 12.2% in chickens. This conclusion was confirmed by their later work **(Sebastian et al., 1996b)** in which microbial phytase supplementation

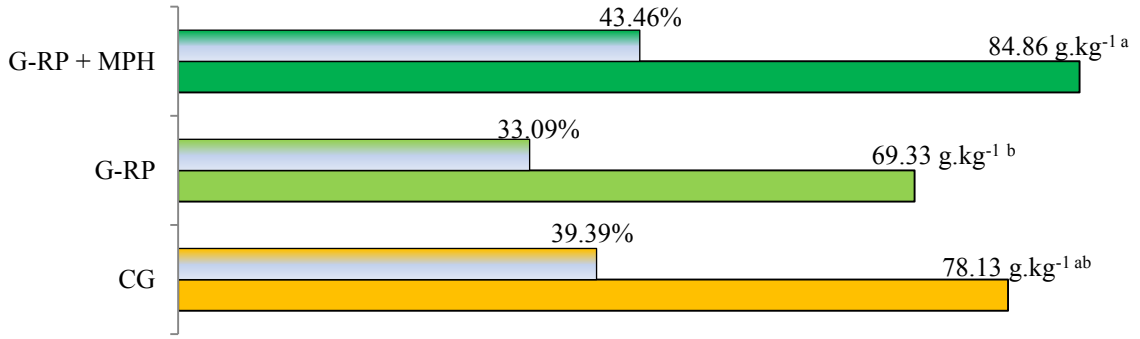

Figure 1. Mean calcium content in the tibia.
Note: CG = control group; G-RP = experimental group 1 with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP + MPH experimental group 2 with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU.kg^{-1}) in feed mixtures. Means bearing different superscripts differ statistically significant at (p <0.05).

Table 3 Basic statistical characteristics of calcium content in the tibia.

Group	n	SD (g.kg^{-1})	CV (%)
CG	6	3.16	4.05
G-RP	6	2.97	4.28
G-RP +MPH	6	1.51	1.79

Note: n = multiplicity; SD = standard deviation; CV = coefficient of variation; CG = control group; G-RP = experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP +MPH experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU kg^{-1}) in feed mixtures.

of a low-phosphorus feed mixture increased growth and relative retention of total phosphorus, calcium, and improved bone mineralization in chickens determined to meat production.

Phosphorus content in the tibia of chickens

Results of phosphorus content in the tibia of chickens are shown in Figure 2 and Table 4.

The highest phosphorus content in tibia of chickens was 24.09 g.kg^{-1}, which were fed by feed mixtures with the reduced non-phytate phosphorus content and with the share of 6-phytase, i.e. 12.55% of tibia ash. The lowest phosphorus content in tibia of chickens was 19.26 g.kg^{-1}, which fed the feed mixtures with the reduced non-phytate phosphorus content without 6-phytase, i.e. 9.21% of tibia ash. In the control group was tibia phosphorus content of chickens 21.88 g.kg^{-1} (11.02%).

The assessment of basic statistical characteristics shows that the lowest values of fluctuation of phosphorus content in tibia were at chickens, which were fed by feed mixtures with reduced non-phytate phosphorus content and with the share of 6-phytase. Maximum variation of phosphorus content in the tibia was in the control group. The differences of phosphorus content in tibia were statistically significant (p <0.05) between chickens fed by feed mixtures with reduced non-phytate phosphorus content and with the share 6-phytase and control group and between chickens, which were fed by feed mixtures with reduced non-phytate phosphorus content without 6-phytase and control group. Similar the results were confirmed by **Singh et al. (2013)** They investigated the influence of different levels (%) of dietary non-phytate phosphorus fed from 0 to 20 day (0.45, 0.40, 0.35, 0.30, 0.25, compared with feeding 0.20 non-phytate phosphorus with and without 500 FTU of phytase per kg of feed mixture) and from days 21 to 36 of age (0.414, 0.364, 0.314, 0.264, 0.214, compared with 0.164 non-phytate phosphorus with and without 500 FTU of phytase per kg of feed mixture) were evaluate using a total of 588 day-old commercial broiler chicks. Each treatment was replicated four times in a completely randomized design. In conclusion, the results showed that the combination of a lower level of non-phytate phosphorus and phytase may be used to increase dietary phosphorus utilization, without severe changes in performance and bone quality. The same conclusion also have found **Karimi et al. (2011)** where results experiment showed that the combination of a lower level of non-phytate phosphorus and phytase may be used to increase dietary phosphorus utilization, without severe changes in performance and bone quality. In fact, the ash content is closely related to phosphorus concentration. Enhancement of ash percentage and phosphorus content of tibia with application of either source of phytase suggests that phytase can increase mineral deposition in phosphorus deficient diet, which is in agreement with the results described in the scientific literature by **Viveros et al., 2002; Sebastian et al., 1996b**. **Ahmad et al. (2000)** observed that phytase in a low-phosphorus feed mixture increased the phosphorus retention by 20.4% and 12.73% as compared to a negative control and normal phosphorus diet supplemented with phytase, respectively.

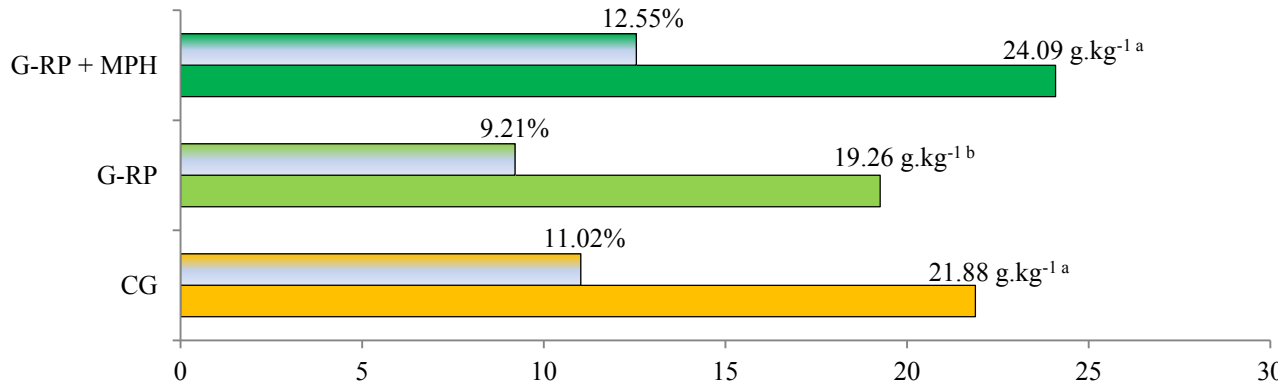

Figure 3. Mean phosphorus content in the tibia.
Note: CG = control group; G-RP = experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP +MPH experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU.kg^{-1}) in feed mixtures. Means bearing different superscripts differ statistically significant at p <0.05.

Table 4 Basic statistical characteristics of phosphorus content in the tibia.

Group	n	SD (g.kg^{-1})	CV (%)
CG	6	2.16	5.91
G-RP	6	1.14	4.05
G-RP +MPH	6	0.49	2.03

Note: n = multiplicity; SD = standard deviation; CV = coefficient of variation; CG = control group; G-RP = experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP +MPH experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU.kg^{-1}) in feed mixtures.

Magnesium content in the tibia of chickens

Results of calcium content in the tibia of chickens are shown in Figure 3 and Table 5.

The highest magnesium content in tibia of chickens was 1.27 g.kg^{-1}, which fed the feed mixtures with reduced non-phytate magnesium content and with a share of 6-phytase, i.e. 0,609% of tibia ash. The lowest magnesium content in tibia of chickens was 1.01 g.kg^{-1}, which fed the feed mixtures with reduced non-phytate magnesium content without 6-phytase, i.e. 0.47% of tibia ash. In the control group was tibia magnesium content of chickens 1.21 g.kg^{-1} (0.608%). The assessment of basic statistical characteristics shows that the lowest values of fluctuation of magnesium content in tibia were at chickens feeding the feed mixtures with reduced non-phytate magnesium content. Maximum variation of magnesium content in the tibia was at chickens feeding of feed mixtures with reduced non-phytate phosphorus and with a share 6-phytase. The differences of magnesium content in tibia were statistically significant (p *<0.05*) between chickens fed by feed mixtures with reduced non-phytate phosphorus content and with the share 6-phytase and control group and between chickens, which were fed by feed mixtures with reduced non-phytate phosphorus content without 6-phytase and control group.

Our findings are in accordance with the literature data that shows that supplemental phytase increases the mineral content in tibia **(Sohail and Roland, 1999)**.

However, we were unable to find any literature data on the phytase influence on magnesium content in broiler tibia. It is interesting to note that contents of magnesium was statistically higher in broilers fed by feed mixtures with reduced phosphorus content and 6-phytase addition in comparison with the feed mixtures without 6-phytase or with the commercial feed mixtures. In contribution to the application of phytase are results of the latest studies indicating the positive effect on digestibility and availability not only of calcium, phosphorus, but also the magnesium and other minerals and organic matters with the use of phytase.

Also, because of almost universal presence of phytate in grain cereals and oil meals, phytase represents enzyme with the highest potential among other enzymes which can be used in poultry production **(Lukić et al., 2009)**.

Research of the issues relating minerals and another substances and application of phytase in broiler production are equally important and are mutually complementary **(Huyghebaert et al., 2005)**.

These researches are especially important for the purpose of solving issues and problems which are very frequent and induced by rapid growth of broilers (bone deformations, predisposition to breaking and infections, etc.), as well as poor quality of the product, i.e. bone firmness, which is considered as serious disadvantage in the processing industry, because of frequent breaking of bones during handling, transportation and automatic broiler processing procedures.

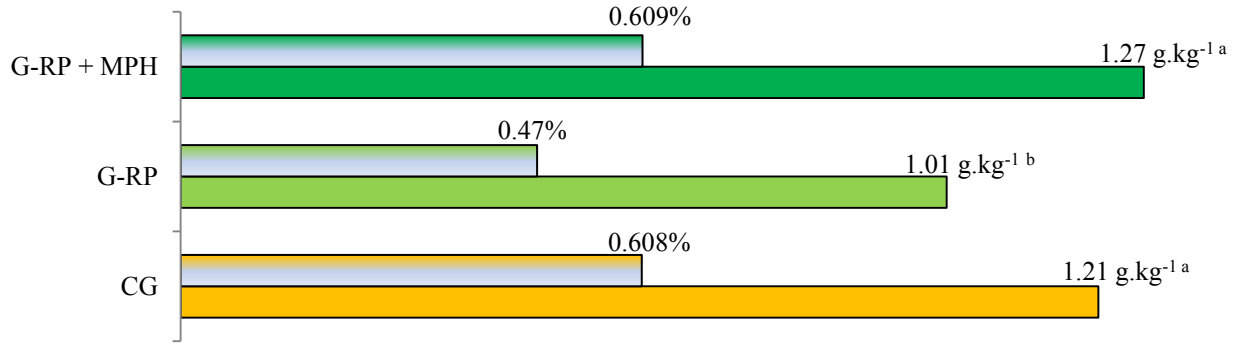

Figure 4. Mean magnesium content in the tibia.
Note: n = multiplicity; SD = standard deviation; CV= coefficient of variation; CG = control group; G-RP = experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP +MPH experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU.kg^{-1}) in feed mixture; Means bearing different superscripts differ statistically significant at p <0.05.

Table 5 Basic statistical characteristics of the magnesium content in the tibia.

Group	n	SD (g.kg^{-1})	CV (%)
CG	6	0.06	4.57
G-RP	6	0.02	2.41
G-RP+MP	6	0.08	6.61

Note: n = multiplicity; SD = standard deviation; CV = coefficient of variation; CG = control group; G-RP = experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} feed mixtures; G-RP +MPH experimental group with reduced non-phytate phosphorus content to 2.32 g.kg^{-1} and with addition 6-phytase microbial origin 0.1% (500 FTU.kg^{-1}) in feed mixtures.

CONCLUSION

The target of our research was 6-phytase of microbial origin and its effectiveness in relation to the tibia mineralization of chickens kept for meat production. In the experiment, we used a 6-phytase of microbial origin, which is the product of a microscopic fungus *Aspergillus oryzae*. We used the final type of hybrid combination Cobb 500. The experimental feed mixtures had reduced non-phytate phosphorus content 2.32 g.kg^{-1} versus 4.50 g.kg^{-1} control feed mixtures. We used the feed mixtures of soybean-cereal type (starter, grower and finisher). Based on the experiment, we came to the following conclusions that statistically significant (p <0.05) the highest content of calcium, phosphorus and magnesium was in the tibia of chickens fed by feed mixtures with reduced non-phytate phosphorus content and with the 6-phytase. Based on the experimental results, we can conclude that it has been confirmed scientific hypothesis, which we have established. Application of 6-phytase of microbial origin had a positive effect on the utilization of phytate phosphorus in feed, which resulted tibia mineralization.

REFERENCES

Ahmad, T., Rassol, S., Sarwar, M., Haq, A., Hasan, Z. 2000. Effect of microbial phytase produced from a fungus *Aspergillus niger* on bioavailability of phosphorus and calcium in broiler chicken. *Animal Feed Science and Technology*, vol. 83, no. 2, p. 103-114. https://doi.org/10.1016/S0377-8401(99)00122-4

Angel, R., Saylor, W., Dhandu, A. S., Powers, W., Applegate, T. J. 2005. Effects of dietary phosphorus, phytase and 25-hydroxycholecalciferol on performance of broiler chickens grown on floor pens. *Poultry Science*, vol. 84, no. 7, p. 1031-1044. https://doi.org/10.1093/ps/84.7.1031 PMid:16050120

Angelovičová, M. 1999. *Nutrition and feeding of high-yield poultry (Výživa a kŕmenie vysokoúžitkovej hydiny)*. Nitra : Slovak university of agriculture. 85 p. ISBN 80-7137-60.

Aureli, R., Umar Faruk, M., Cechova, I., Pedersen, P. B., Elvig-Joergensen, S. G., Fru, F., Broz, J. 2011. The efficacy of a novel microbial 6-phytase expressed in *Aspergillus oryzae* on the performance and phosphorus utilization in broiler chickens. *International Journal of Poultry Science*, vol. 10, no. 2, p. 160-168. https://doi.org/10.3923/ijps.2011.160.168

Biehl, R. R., Baker, D. H. 1996. Efficacy of supplemental 1 alpha-hydroxycholesterol and microbial phytase for young pigs fed phosphorus- or amino acid-deficient cornsoybean meal diets. *Journal of Animal Science*, vol. 74, p. 2960-2966. https://doi.org/10.2527/1996.74122960x PMid:8994910

Böhm, K., Herter, T., Müller, J. J., Borriss, R., Heinemann, U. 2010. Crystal structure of *Klebsiella* sp. ASR1 phytase suggests substrate binding to a preformed active site that meets the requirements of a plant rhizosphere enzyme. The *FEBS Journal*, vol. 277, no. 5, p. 1284-1296. https://doi.org/10.1111/j.1742-4658.2010.07559.x PMid:20392204

Brenes, A., Viveros, A., Arija, I., Centeno, C., Pizarro, M., Bravo, C. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. *Animal Feed Science and*

Technology, vol. 110, no. 1-4, pp. 201-219. https://doi.org/10.1016/S0377-8401(03)00207-4

Brož, J., Oldale, P., Perrin-Voltz, A. H., Rychen, G., Schulze, J., Simoes Nunes, C. 1994. Effects of supplemental phytase on performance and phosphorus utilization in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. *British Poultry Science*, vol. 35, no. 2, p. 273-280. https://doi.org/10.1080/00071669408417691 PMid:8062110

Catalá-Gregori, P., García, V., Hernández, F., Madrid, J., Cerón, J. J. 2006. Response of broilers to feeding low-calcium and phosphorus diets plus phytase under different environmental conditions: body weight and tibiotarsus mineralization. *Poultry Science,* vol. 85, 11, p. 1923-1931. https://doi.org/10.1093/ps/85.11.1923 PMid:17032825

Christensen, L., Bech, L., Ohman, A., Pettersson, D. 1997. Development, roduction and performance of microbial phytases. In *Proceedings 11th European symposium on poultry nutrition*. Faaborg, Denmark, p. 203-210.

Chu, H. M., Guo, R. T., Lin, T. W., Chou, C. C., Shr, H. L., Lai, H. L., Tang, T. Y., Cheng, K. J., Selinger, B. L., Wang, A. H. 2004. Structures of Selenomonas ruminantium phytase in complex with persulfated phytate: DSP phytase fold and mechanism for sequential substrate hydrolysis. *Structure*, vol. 12, no. 11, p. 2015-2024. https://doi.org/10.1016/j.str.2004.08.010 PMid:15530366

Classen, H. I., Scott, T. A., Irish, G. G., Huck, P., Swift, P. Bedford, M. R. 1995. The relationship of chemical and physical measurements to the apparent metabolisable energy (AME) of wheat when fed to broiler chickens with and without a wheat enzyme source. In *Proceedings of the 2nd European Symposium on Feed Enzymes*. Antalya, Turkey, p. 65-69.

Dersjant-Li, Y., Awati, A., Schulze, H., Partridge, G. 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *Journal of the Sciience of Food and Agriculture*, vol. 95, no. 5, p. 878-896. https://doi.org/10.1002/jsfa.6998 PMid:25382707

Dilger, R. N., Onyango, E. M., Sands, J. S., Adeola, O. 2004. Evaluation of microbial phytase in broiler diets. *Poultry Science*, vol. 83, no. 6, p. 962-970. https://doi.org/10.1093/ps/83.6.962 PMid:15206623

EFSA, 2010. EFSA Panel on Additives and Poducts or Substances used in Animal Feed (FEEDAP)[online] s.a. [cit. 2017-02-15] Available at: http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1915/abstract.

Huang, H., Zhang, R., Fu, D., Luo, J., Li, Z., Luo, H., Shi, P., Yang, P., Diao, Q., Yao, B. 2011. Diversity, abundance and characterization of ruminal cysteine phytases suggest their important role in phytate degradation. *Environmental Microbiology*, vol. 13, no. 3, p. 747-757. https://doi.org/10.1111/j.1462-2920.2010.02379.x PMid:21105982

Huyghebaert, G., Lippens, M., Lescoat, P., Nys, Y. 2005. The interaction between the macrominerals calcium and phosphorus, vitamin D and phytase in broilers. In *Proceed. 15th European symposium on poultry nutrition*. Balatonfüred, Hungary, p. 146-160.

Jiang, X. R., Luo, F. H., Qu, M. R., Bontempo, V., Wu., S. G., Zhang, H. J., Yue, H. Y., Qi, G. H. 2013. Effects of non-phytate phosphorus levels and phytase sources on growth performance, serum biochemical and tibia parameters of broiler chickens. *Italian Journal of Animal Science*, vol. 12, no. e60, p. 375-380. https://doi.org/10.4081/ijas.2013.e60

Karimi, A., Bedford, M. R., Sadeghi, G., Ghobadi, Z. 2011. Influence of dietary non-phytate phosphorus levels and phytase supplementation on the performance and bone characteristics of broilers. *Brazilian Journal of Poultry Science*, vol. 13, no. 1, p. 43-51.

Kliment, M., Angelovičová, M., Nagy, S. 2010. Use of phytase in broiler chicken diets: a review. *Potravinarstvo*, vol. 4, suppl. no., p. 166-171. Available at: http://www.potravinarstvo.com/dokumenty/mc_februar_2010/pdf/2/Kliment.pdf

Kornegay, E. T., Radcliffe, Kornegay, E. T., Radcliffe, J. S., Denbow, D. M. 1996. Influence of Natuphos phytase on calcium bioavailability in plant ingredients and development of calcium equivalency values for swine and poultry. In Coelho, M. B., Kornegay, E. T. *Phytase in animal nutrition and waste management*. Mount Olive : BASF Corporation, NJ. p. 419-434.

Kostrewa, D., Gruninger-Leitch, F., D'Arcy, A., Broger, C., Mitchell, D., Van Loon, A. P. G. M. 1997. Crystal structure of phytase from *Aspergillus ficuum* at 2.5 A resolution. *Nature Structural and Molecular Biology*, vol. 4, no. 3, p. 185-190. https://doi.org/10.1038/nsb0397-185

Lei, X. G., Porres, J. M. 2003. Phytase enzymology, applications, and biotechnology. *Biotechnology Letters*, vol. 25, no. 21, p. 1787-1794. https://doi.org/10.1023/A:1026224101580 PMid:14677699

Lim, D., Golovan, S., Forsberg, C. W., Jia, Z. 2000. Crystal structures of Escherichia coli phytase and its complex with phytate. *Nature Structural and Molecular Biology*, vol. 7, no. 2, p. 108-113. https://doi.org/10.1038/72371 PMid:10655611

Lukić, M., Sinovec, Z., Pavlovski, Z., Škrbić, Z., Cmiljanić, R. 2005. The reduction of mineral phosphor us source in diets for broiler and addition of phytase in condition of temperature stress. In *Proceedings 15th European symp. on poultry nutrition*. Balatonfüred, Hungary, p. 201-204.

Lukić, M., Pavlovski, Z., Škrbić, Z. 2009. Mineral nutrition of modern poultry genotypes. *Biotechnology in animal husbandry*, vol. 25, no. 5-6, p. 399-409.

Maguire, R. O., Sims, J. T., Saylor, W. W., Turner, B. L., Angel, R., Applegate, T. J. 2004. Influence of phytase addition to poultry diets on phosphorus forms and solubility in litters and amended soils. *Journal of Environmental Quality*, vol. 33, no. 6, p. 2306-2316. https://doi.org/10.2134/jeq2004.2306 PMid:15537954

Mullaney, E. J., Ullah, A. H. 2003. The term phytase comprises several different classes of enzymes. *Biochemical and Biophysical Research Communications*, vol. 312, no. 1, p. 179-184. https://doi.org/10.1016/j.bbrc.2003.09.176 PMid:14630039

Nelson, T. S., Sheih, T. R., Wodzinski, R. J., Ware, J. H. 1968a. The availability of phytate phosphorus in soybean meal before and after treatment with a mold phytase. *Poultry Science*, vol. 47, no. 6, p. 1842-1848. https://doi.org/10.3382/ps.0471842 PMid:4304685

Nelson, T. J., McGillivray, J. J., Shieh, T. R., Wodzinski, R. J., Ware, J. H. 1968b. Effect of phytate on the calcium requirement of chicks. *Poultry Science*, vol. 47, no. 6, p. 1985-1989. https://doi.org/10.3382/ps.0471985 PMid:4304686

Oakley, A. J. 2010. The structure of *Aspergillus niger* phytase PhyA in complex with a phytate mimetic. *Biochemical and Biophysical Research Communications*, vol. 397, no. 4, p. 745-749. https://doi.org/10.1016/j.bbrc.2010.06.024 PMid:20541524

Onyango, E. M., Bedford, M. R., Adeola, O. 2004. The yeast production system in which Escherichia coli phytase is expressed may affect growth performance, bone ash, and nutrient use in broiler chicks. *Poultry Science*, vol. 83, no. 3, p. 421-427. https://doi.org/10.1093/ps/83.3.421 PMid:15049495

Onyango, E. M., Bedford, M. R., Adeola, O. 2005. Efficacy of an evolved *Escherichia coli* phytase in diets of broiler chicks. *Poultry Science*, vol. 84, no. 2, p. 248-255. https://doi.org/10.1093/ps/84.2.248 PMid:15742961

Payne, R. L., Lavergne, T. K., Southern, L. L. 2005. A comparison of two sources of phytase in liquid and dry forms in broilers. *Poultry Science*, vol. 84, no. 2, p. 265-272. https://doi.org/10.1093/ps/84.2.265 PMid:15742963

Plumstead, P. W., Leytem, A. B., Maguire, R. O., Spears, J. W., Kwanyuen, P., Brake, J. 2008. Interaction of calcium and phytate in broiler diets. 1. Effects on apparent prececal digestibility and retention of phosphorus. *Poultry Science*, vol. 87, no, 3, p. 449-458. PMid:18281570

Ptak, A., Bedford, M. R., Świątkiewicz, S., Żyła, K., Józefiak, D. 2015. Phytase modulates ileal microbiota and enhances growth performance of the broiler chickens. *PLoS One*, vol. 10, no. 3, p. e0119770. https://doi.org/10.1371/journal.pone.0119770 PMid:25781608

Puhl, A. A., Gruninger, R. J., Greiner, R., Janzen, T. W., Mosimann, S. C., Brend Selinger, L. 2007. Kinetic and structural analysis of a bacterial protein tyrosine phosphatase-like myo-inositol polyphosphatase. *Protein Science*, vol. 16, no. 7, p. 1368-1378. https://doi.org/10.1110/ps.062738307 PMid:17567745

Qian, H., Veit, H. P., Kornegay, E. T., Ravindran, V., Denbow, D. M. 1996. Effects of supplemental phytase and phosphorus on histological and other tibial bone characteristics and performances of broilers fed semi-purified diets. *Poultry Science*, vol. 75, no. 5, p. 618-626. https://doi.org/10.3382/ps.0750618 PMid:8722910

Rada, V., Havlík, J. 2010. *Enzymes in nutrition of farm animal*s (Enzýmy vo výživy hospodárskych zvierat.) 1st Ed.; Praha – Uhříneves : Research Institute of Animal Production. 38 p. ISBN 978-80-7403-065-9.

Ragon, M., Hoh, F., Aumelas, A., Chiche, L., Moulin, G., Boze, H. 2009. Structure of *Debaryomyces castellii* CBS 2923 phytase. *Acta Crystallographica Section F Structural Biology and Crystallization Communications*, vol. 65, no. Pt4, p. 321-326.

Rousseau, X., Létourneau-Montminy, M. P., Même, N., Magnin, M., Nys, Y., Narcy, A. 2012. Phosphorus utilization in finishing broiler chickens: effects of dietary calcium and microbial phytase. *Poultry Science*, vol. 91, no. 11, p. 2829-2837. https://doi.org/10.3382/ps.2012-02350 PMid:23091139

Schöner, F. J., Hoppe, P. P., Schwarz, G. 1991. Comparative effects of microbial phytase and inorganic P on performance and on retention of P, Ca and crude ash in broilers. *Journal of Animal Physiology and Animal Nutrition*, vol. 66, p. 248-255. https://doi.org/10.1111/j.1439-0396.1991.tb00294.x

Schöner, F. J., Hoppe, P. P., Schwarz, G., Wiesche, H. 1993. Effects of microbial phytase and inorganic phosphate in broiler chickens: performance and mineral retention at various calcium levels. *Journal of Animal Physiology and Animal Nutrition*, vol. 69, p. 235-244.

Sebastian, S., Touchburn, S. P., Chavez, E. R., Laguė, P. C. 1996a. Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. *Poultry Science*, vol. 75, no. 12, pp. 1516-1523. https://doi.org/10.3382/ps.0751516 PMid:9000277

Sebastian, S., Touchburn, S. P., Chavez, E. R., Lague, P. C. 1996b. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed corn-soybean diets. *Poultry Science*, vol. 75, no. 6, p. 729-736. https://doi.org/10.3382/ps.0750729 PMid:8737837

Singh, A., Walk, C. L., Ghosh, T. K., Bedford, M. R., Haldar, S. 2013. Effect of a novel microbial phytase on production performance and tibia mineral concentration in broiler chickens given low-calcium diets. *British Poultry Science*, vol. 54, no. 2, p. 206-215. https://doi.org/10.1080/00071668.2013.775403 PMid:23647184

Sohail, S. S., Roland, D. A. 1999. Influence of supplemental phytase on performance of broilers four to six weeks of age. *Poultry Science*, vol. 78, p. 550-556. https://doi.org/10.1093/ps/78.4.550 PMid:10230908

Viveros, A., Brenes, A., Arija, I., Centeno, C. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. *Poultry Science*, vol. 81, no. 8, p. 1172-1183. https://doi.org/10.1093/ps/81.8.1172 PMid:12211310

Watson, B. C., Mathews, J. O., Southern, L. L., Shelton, J. L. 2006. The effects of phytase on growth performance and intestinal transit time of broilers fed nutritionally adequate diets and diets deficient in calcium and phosphorus. *Poultry Science*, vol. 85, no. 3, p. 493-497. https://doi.org/10.1093/ps/85.3.493 PMid:16553281

Xiang, T., Liu, Q., Deacon, A. M., Koshy, M., Kriksunov, I. A., Lei, X. G., Hao, Q. Thiel, D. J. 2004. Crystal structure of a heat-resilient phytase from *Aspergillus fumigatus*, carrying a phosphorylated histidine. *Journal of Molecular Biology*, vol. 339, no. 2, p. 437-445. https://doi.org/10.1016/j.jmb.2004.03.057 PMid:15136045

Yan, F., Kersey, J. H., Waldroup, P. W. 2001. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. *Poultry Science*, vol. 80, no. 4, p. 455-459. https://doi.org/10.1093/ps/80.4.455 PMid:11297284

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EFFECT OF SORBITOL ON DOUGH RHEOLOGY AND QUALITY OF SUGAR REPLACED COOKIES

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ABSTRACT

A high amount of sugar is used in bakery products, which may cause diabetes, high blood glucose levels and obesity. Due to these reasons, sugar is being replaced with substitutes. There is different carbohydrate-based sugar substitutes (polyols) that can efficiently replace sugar. Among polyols, sorbitol is an efficient replacer that can mimic sugar with minimal effects on cookie quality. Effects of different sorbitol levels (0 to 12.5%) were seen on the dough rheology. Mixographic studies showed that peak height and mixing time reduced with the addition of sorbitol. Farinographic studies showed that water absorption and the mixing tolerance index of dough reduced with the supplementation of sorbitol, whereas dough development time, arrival time, dough stability time and softening of dough increased. Extensographic studies revealed that sorbitol substitution produced hard, cohesive, adhesive and elastic dough. Sugar in cookies formulations was reduced from 100 to 50% by replacing with sorbitol 0 to 50%. Physical analysis of sorbitol containing cookies showed that the diameter and spread factor of cookies decreased with higher levels of sorbitol, whereas thickness, color, hardness and water activity of cookies increased. The calorific value of cookies decreased with the increasing levels of sorbitol. At upto 20% replacement of sugar, other parameters of cookies were not affected. Sensory evaluation of the cookies showed that hedonic points for sensory evaluation parameters reduced with the increasing levels of sorbitol, T_2 (20% replacement) showed maximum overall acceptability.

Keywords: calorific value; cookies; dough rheology; polyols; sensory evaluation

INTRODUCTION

Polyols are carbohydrates that are hydrogenated, meaning that a hydroxyl group replaces the aldehyde or ketone group found in sugars. These are also different from dense energy sweeteners such as cyclamate, polydextrose and aspartame aspartame, which are utilized in very low quantities so that their contribution to the calorific value of foods is very negligible. These sweeteners are 40% to 100% sweet as compared to sucrose and provide 0.2 to 3 kcal.g^{-1} energy as compared to sugars and starch. These are partially digested and absorbed by the human body. Some polyols, such as erythritol, sorbitol, and mannitol, occur naturally in foods **(Fitch and Keim, 2012)**. Some polyols can help retain moisture in foods, lower water activity to help to protect against spoilage, impart smoothness and creaminess by inhibiting sugar crystallization, provide viscosity, and/or assist in retaining flavor at high temperatures. They also provide bulkiness to foods once in the body. Most polyols are considered GRAS, specifically sorbitol, erythritol, isomalt, maltitol, lactitol, and polyglycitols. Xylitol and mannitol (special dietary purposes) are regulated as food additives. Excessive polyol load, more than 50 grams per day of sorbitol and/or more than 20 grams per day of mannitol, can create gastrointestinal disorders **(Kroger et al., 2006)**. Sorbitol is related to the family of sugar alcohols. The molecular formula of sorbitol is C6H14O6, molar mass 182.17 g.mol^{-1}, and density is 1.489 g.cm^{-3}. It is a colorless, odorless, and sweet-tasting liquid that is 60% sweet as compared to sucrose, and it provides 2.6 kcal.g^{-1} energy **(Fitch and Keim, 2012)**.

Bakery products continue to improve as the user becomes more conscious about the health benefits of consuming less supplemented sugars. Low sucrose or low calorie is a top-level marketing approach in bakery products and baking sectors. They do, however, provide functional benefits to bakery goods when sugars used are replaced with polyols. When used in place of sugar, polyols can make almost all bakery products healthier while still giving a sweet taste similar to sucrose. Bakery products prepared with polyols cannot become asslimy on the surfaces as early as products prepared with other sweeteners do. Microorganisms also cannot flourish well on these sweetening agents as they do on sucrose, and

hence the shelf life of products is extended. However, unlike sugars, polyols do not normally develop crisp and colored surfaces on baked commodities. The non-coloring character can be beneficial where a disturbance in browning is not required. Along with the non-browning characteristic, polyols often affect the cooling rate of baked products. Isomalt is an exception; it does not provide any typical cooling effect in bakery foods **(Baek et al., 2004)**.

Isomalt has a very low hygroscopicity compared with polyols, such as sorbitol, xylitol and maltitol, and has a very low glycemic index. In many baking formulations; substituting sugar with isomalt remains the color, aroma, taste, texture, appearance and volume of sugar **(Grabitske and Slavin, 2008)**. Maltitol, that is 90% sweet as compared to sucrose, is utilized as a sugar substituent in frostings, chocolate bars, low calorie muffins, icing creams, biscuits, breads and cakes. Malitol syrup is utilized to substitute maize syrup in similar bakery utilizations **(Nadji et al., 2005)**. Glucitol is the other name of sorbitol. The human body utilizes it very slowly. It can be produced by reducing glucose i.e. altering the functional group of glucose from aldehyde to hydroxyl. It is present in apples, plums and other stone fruits in appreciable quantities. Sorbitol provides bulk and functionality to bakery products but not as much sweetness **(Yan et al., 2010)**.

Scientific hypothesis

Bakery products are the most widely produced and consumed food items, after dairy products. Among the bakery products, cookies are the most significant. Cookies are famous and abundantly liked by all ages of people, including children. These are important bakery commodities that are used as snack foods by children and adults in Pakistan. Biscuits hold an important position in snack foods due to a variety taste, crispness, and digestibility. This project was selected to examine the influence of different sorbitol substitution levels on dough rheology and physio-chemical and sensory characteristics of cookies to reduce the total calories provided by the cookies and to improve their nutritional value.

MATERIAL AND METHODOLOGY

Procurement of Raw Materials

Raw materials used in the present study were purchased from local market. Wheat flour was purchased from "Farrukh Flour Mills" Jhang road, Fa cookies preparation. Sorbitol was purchased from local market. Sorbitol available in the market is basically imported from "India" a treatments plan has depicted in Table 1.

Analytical Methods for Wheat Flour and Treatments

Proximate Composition of Wheat Flour

Wheat flour was analyzed for moisture, total ash, crude protein, crude fiber and crude fat by using methods described in AACC **(AACC, 2000)**. The nitrogen free extract (NFE) was calculated by difference method.

Moisture Content

The moisture content in the flour sample was estimated according to the method mentioned in AACC method No. 44-15A **(AACC, 2000)**.

Total Ash

The ash content in flour sample was estimated by following the procedure given in AACC method No. 08-01 **(AACC, 2000)**.

Crude Fat

Flour sample was analyzed for crude fat according to the method No. 30-10 in **AACC (2000)**.

Crude Fiber

The crude fiber was determined by following the method No. 32-10 outlined in **AACC (2000)**.

Crude Protein

Kjeldahl apparatus (Model: D-40599, Behrs Labor Technik, Gmbh-Germany) was used to determine nitrogen % in the flour sample according to AACC method No. 46-10 **(AACC, 2000)**.

Nitrogen Free Extracts (NFE)

The nitrogen free extract (NFE) of wheat flour sample was calculated according to the following expression:

NFE (%) = 100 - (% Moisture +%Ash +%Crude Fat +%Crude Protein +%Crude Fiber).

Rheological Analysis of Treatments

The wheat flour sample was supplemented with sorbitol according to treatments (Table 1) respectively and effects of supplementation on flour rheology were studied as described below.

Mixographic Analysis

The mixographic studies were carried out by using mixograph equipped with 10g capacity bowl (National Mfg. Co., Lincoln, Nebraska). All the treatments were run through mixograph by adding 60% water to each sample according to the instructions given in AACC method No. 54-40A **(AACC, 2000)**. Each mixogram was interpreted for mixing time and maximum peak height percentage.

Farinographic Studies

The treatments were run through Brabender Farinograph equipped with 50 grams bowl capacity to assess the physical dough behaviour of different flour treatments according to AACC Method No. 54-21 **(AACC, 2000)**.

The parameters such as water absorption, dough development time, softening of dough, arrival time, departure time, dough stability time and mixing tolerance index were calculated from the farinograms as described below.

Extensograhic Analysis

All the treatments were analyzed through Brabender Extensograph according to the methods outlined in **AACC (2000)** separately.

Table 1 Treatment Plan.

Treatment	Sorbitol %
T_0	0.0
T_1	2.5
T_2	5.0
T_3	7.5
T_4	10.0
T_5	12.5

Legend: T_0 acts as control

Table 2 Cookies Recipe (T_0).

Ingredients	Amount
White flour	500g
Sugar	250g
Shortening	250g
Baking powder	7-8g
Egg	1
Cardamom Flavor	1 – 2mL

Water Hydration Capacity of Treatments

The water binding capacity or the amount of water retained by the flour has been subjected to centrifugation and was measured according to the procedures given in AACC method number 56-30 **(AACC, 2000)**.

Preparation of Cookies

Cookies of different treatments were prepared from the wheat flour according to methods 10-50 D **(AACC, 2000)**. Table 2 depicts used recipe for the preparation of control cookies (T_0).

Preparation Method

The ingredients weighed accurately. Dry ingredients were mixed thoroughly by mixer (Mod. A-200, Hobart, USA).

Analysis of Cookies

Physical Analysis of Cookies

Cookies of different treatments were analyzed firstly for their physical parameters after preparation. Cookies diameter, thickness and spread factor was measured according to their respective methods described in AACC **(AACC, 2000)**. Cookies treatments color was determined by methods described by **(Rocha and Morais, 2003)**. Texture and water activity was determined according to the methods described by **(Piga et al., 2005)**.

Calorific Analysis of Cookies

The amount of heat measured in calories that is released when a substance is completely oxidized in a bomb calorimeter is called the gross energy of substance. Calorific Value of the cookies treatments was estimated by using Oxygen Bomb Calorimeter (Werke IKA C200). The method of Krishna and Ranjhan with slight modifications was used in determination **(Krishna and Ranjhan, 1981)**.

Proximate Analysis of Cookies

Cookies of different treatments were analyzed for moisture, total ash, crude protein, crude fibre and crude fat by using methods described in AACC **(AACC, 2000)**. The nitrogen free extract (NFE) was calculated by difference method.

Sensory Evaluation of Cookies

The cookies of different treatments were rated using 9-point hedonic score system (9 = like extremely; 1 = dislike extremely) by taste panel **(Meilgaard et al., 2004)**.

Statistical Analysis

The results obtained from different parameters of all the treatments were exposed to statistical analysis. Completely Randomized Design (CRD) was used, followed by the Analysis of Variance Technique (ANOVA) and the results were interpreted according to the Least Significant Difference Test (LSD) at 5% level of significance as described by **(Steel et al., 1997)**.

RESULTS AND DISCUSSION

Chemical Composition of Wheat Flour

The results regarding the chemical composition of wheat flour indicated that it contains 12.70% moisture, 10.8% crude protein, 0.86% crude fat, 0.24% crude fiber, 0.56% ash and 74.74% nitrogen free extract. The results are in close agreement with the findings of others in the literature **(Ahmad, 2011; Mushtaq et al., 2010)**.

Rheological Analysis of Treatments

Mixographic Analysis

The statistical data regarding the mixographic parameters showed that mixing time and peak height of treatments decreases with the increasing levels of sorbitol (Table 3). T_5 showed minimum mixing time 3.2 minutes and minimum peak height 33%, whereas T_2 showed maximum mixing time 7 minutes and maximum peak height 63%, showing an overall decreasing trend. The decrease in mixing time and peak height was due to fact that in present study flour blended with different levels of sorbitol was run through mixograph rather than plain flour. These results were in close agreement with the findings of others in literature **(Asghar, 2004; Farooq, 1996)**.

Table 3 Effect of different treatment on mixograph parameters of dough.

Treatments	Mixing Time (minutes)	Peak Height (%)
T_0	5.50^{b}	55^{b}
T_1	6.60^{a}	60^{a}
T_2	7.00^{a}	63^{a}
T_3	4.80^{c}	49^{c}
T_4	3.80^{d}	42^{d}
T_5	3.20^{e}	33^{e}

Table 4 effect of different treatment on farinograph parameters of the dough**.**

T	W.A (%)	D.D.T (min)	A.T (min)	D.T (min)	D.S.T (min)	S.O.D (B.U.)	M.T.I (B.U.)
T0	57.20^{a}	7.30^{f}	1.25^{d}	16.50^{a}	15.25^{a}	60.00^{d}	40.00^{a}
T_1	56.20^{ab}	8.50^{c}	2.75^{a}	16.75^{a}	14.00^{d}	72.50^{bc}	38.00^{ab}
T_2	54.60^{b}	7.75^{e}	2.25^{b}	16.90^{a}	14.65^{bc}	71.00^{bc}	28.50^{d}
T_3	52.80^{c}	8.00^{d}	2.70^{a}	17.00^{a}	14.30^{cd}	78.00^{a}	32.50^{c}
T4	49.60^{d}	9.50^{a}	1.30^{d}	16.00^{a}	14.70^{bc}	75.00^{ab}	36.50^{b}
T5	48.00^{d}	9.00^{b}	1.75^{c}	16.80^{a}	15.05^{ab}	70.00^{c}	27.50^{d}

Table 5 effect of different treatment on extensograph parameters of dough.

T	Energy (cm^2)	Resistance to Extension (B.U.)	Extensibility (mm)	Maximum (B.U.)	Ratio No (B.U./mm)	Ratio No Maximum (B.U./mm)
T_0	49^{c}	28^{f}	51^{d}	803^{b}	0.50^{f}	15.60^{a}
T_1	48^{c}	260^{d}	55^{d}	759^{c}	4.70^{b}	13.80^{b}
T_2	45^{d}	398^{a}	63^{c}	586^{d}	6.30^{a}	9.20^{c}
T_3	48^{c}	56^{e}	52^{d}	824^{a}	1.10^{e}	15.80^{a}
T_4	57^{a}	370^{b}	107^{b}	378^{e}	3.50^{c}	3.50^{d}
T_5	55^{b}	286^{c}	126^{a}	302^{f}	2.30^{d}	2.40^{e}

Farinographic Studies

The statistical analysis regarding the farinographic characteristics showed that water absorption, dough stability time and mixing tolerance index of treatments decreases with the increasing levels of sorbitol, whereas arrival time, dough development time and softening of dough increases with increasing levels of sorbitol (Table 4). T_5 showed minimum water absorption and mixing tolerance index 48% and 27.5 B.U. respectively, whereas T_0 showed maximum water absorption and mixing tolerance index 57.2% and 40 B.U. respectively.

T_0 showed minimum arrival time, dough development time and softening of dough 1.25 minutes, 7.30 minutes and 60 B.U. respectively, whereas T_1, T_4 and T_3 showed maximum arrival time, dough development time and softening of dough 2.75 minutes, 9.50 minutes and 78 B.U. respectively. T_1 showed minimum dough stability time 14 minutes, whereas T_0 showed maximum dough stability time 15.25 minutes.

The decrease in water absorption trend is due to reduction in protein and complex carbohydrates contents in treatments respectively. The increase in dough development time and arrival time trend is due to less mixing behaviour of sorbitol as compared to wheat flour. The decrease in dough stability time and increase in softening of dough trend is also due to the less resistance of sorbitol to over mixing as compared to the wheat flour. The results are in close agreement with the findings of others in literature **(Rashid, 2007; Rakha, 2006)**.

Extensographic Analysis

The statistical data regarding the extensographic analysis of the treatments showed that work needed to deform the dough, resistance to extension of dough, dough extensibility and ratio no of dough increases with the increasing concentration of sorbitol; whereas maximum force required breaking the gluten strands and the ratio no maximum reduces with the increasing sorbitol contents (Table 5).

T_0 showed minimum values for energy, resistance to extension, dough extensibility and ratio no 49 cm2, 28 B.U., 51 mm and 0.5 B.U. /mm whereas T_4, T_2, T_5 and T_2 showed maximum values 57 cm2, 358 B.U., 126 mm and 6.5 B.U. /mm respectively. T_5 showed minimum values for maximum force and ratio no maximum 302 B.U. and 2.40 B.U. /mm whereas T_3 showed maximum values 824 B.U. and 15.80 B.U. /mm respectively.

Sorbitol (polyol) lowers the water absorption, increases the sliminess and hardness of the dough and hence increases the energy required to deform the dough, dough resistance to extension and dough extensibility. Maximum force required for breaking the gluten strands and ratio no maximum shown decreasing trend because as the flour is replaced with sorbitol the gluten content of treatment lowers and hence the concentration of gluten strands lowers, overall needing less force for breakage. The results are in close agreement with the findings of **(Alava et al., 2007; Akthar, 2011)**.

Table 6 effect of sorbitol on the physical charachteristic of cookeis**.**

T	Diameter (mm)	Thickness (mm)	Spread Factor	Color (CTn)	Hardness (g)	Fracturability (mm)	Water Activity
T_0	132.00^{b}	33.00^{d}	40.00^{ab}	154.67^{a}	1735.0f	20.39^{e}	0.1800^{e}
T_1	150.00^{a}	36.00^{b}	41.67^{a}	147.67^{ab}	3250.0d	21.37^{c}	0.1940^{de}
T_2	134.67^{b}	34.01^{cd}	39.54^{b}	140.67^{bc}	3045.0e	20.97^{d}	0.2050^{cd}
T_3	125.3^{c}	35.40^{bc}	35.42^{c}	152.33^{a}	4966.7b	21.49^{b}	0.2200^{bc}
T_4	127.33^{c}	35.04^{bc}	36.36^{c}	152.67^{a}	5450.0a	22.64^{a}	0.2350^{ab}
T_5	122.00^{d}	37.80^{a}	32.28^{d}	134.67^{c}	4125.0c	21.40^{c}	0.2450^{a}

Table 7 effects of sorbitol on the chemical analysis of cookies.

T	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	NFE (%)
T_0	3.30^{bc}	0.49^{f}	9.20^{a}	21.50^{a}	0.24^{f}	65.27^{a}
T_1	3.38^{bc}	0.54^{e}	9.12^{a}	21.54^{a}	0.27^{e}	65.15^{a}
T_2	3.50^{b}	0.56^{d}	9.05^{a}	21.60^{a}	0.30^{d}	64.99^{a}
T_3	3.60^{ab}	0.60^{c}	9.26^{a}	21.40^{a}	0.33^{c}	64.81^{a}
T_4	3.68^{ab}	0.63^{b}	9.32^{a}	21.46^{a}	0.37^{b}	64.54^{a}
T_5	3.80^{a}	0.66^{a}	9.05^{a}	21.60^{a}	0.40^{a}	64.49^{a}

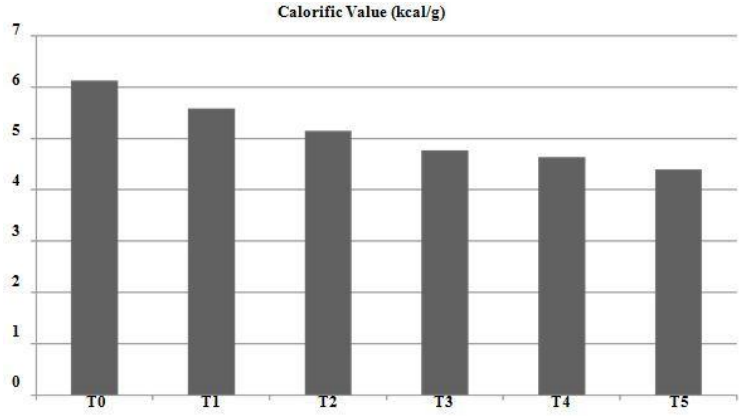

Figure 1 Effect of sorbitol on the calorific value of cookies.

Water Hydration capacity of Treatments

The statistical analysis revealed that water hydration capacity of treatments did not show significant effect of sorbitol replacement. T5 showed minimum (0.85 $g.g^{-1}$) water hydration capacity, whereas T0 showed maximum value (1.05 $g.g^{-1}$) respectively.

Proteins and fiber rich flours and meals show high water hydration capacity due to their more moisture retaining behavior. The results are in close agreement to the finding of others in literature **(Mahajan et al., 1999; Zoulias et al., 2002)**.

Analysis of cookies

Physical Analysis of cookies

The statistical analysis showed that diameter, spread factor and color of treatments decrease, whereas thickness, texture and water activity increases with the increasing concentration of sorbitol (Table 6).

T_5 showed minimum values of diameter, spread factor and color 122 mm, 32.28 and 134.67 CTn, whereas T_1 showed maximum values of diameter and spread factor 150 mm and 41.67 and T0 showed maximum value of color 154.67 CTn respectively. T0 showed minimum values of thickness, hardness, fracturability and water activity 33 mm, 1735 g, 20.39 mm and 0.18, whereas T_5 showed maximum values of thickness and water activity 37.80 mm and 0.24 and T4 showed maximum values for hardness and fracturability 5450 g and 22.64 mm respectively.

The decrease in diameter and spread factor and increase in the texture of cookies with the addition of sorbitol is due to the elastic shrinkage of gluten network. The increase in water activity of treatments is due to more hygroscopic nature of sorbitol as compared to the sucrose. Basically, polyols reduce the Millard and Caramel browning reactions as compared to sugars. The color of cookies reduces with the addition of sorbitol (colorimeter reading increases), but here trend is slightly different due to less controlled cookies preparation and baking (time and temperature) conditions and due to not proper calibration of colorimeter and human reading measuring errors. The results are in close agreement with the findings of **(Mushtaq et al., 2010; Pasha et al., 2002; Siddique, 1995)**.

Calorific Analysis of Cookies

The statistical data regarding the calorific analysis of treatments showed that calorific value of treatments decreases with the increasing sorbitol content (Figure 1). T_5 showed minimum calorific value 4.40 $kcal.g^{-1}$, whereas T_0 showed maximum value 6.13 $kcal.g^{-1}$ respectively. The decrease in the calorific value with the increasing sorbitol

levels was due to the less energy provided by the sorbitol as compared to the sugar. The results are in close agreement with the findings of **(Siddique, 1995; Bond and Dunning, 2006)**.

Chemical Analysis of cookies

Statistical data regarding the chemical analysis of cookie treatments revealed that moisture, ash, and crude fiber contents increase with the increasing sorbitol content, whereas increasing sorbitol levels have a non-significant effect on crude protein, crude fat, and nitrogen-free extract contents (Table 7).

T_0 showed minimum values of moisture, ash and crude fiber 3.30%, 0.49% and 0.27%, whereas T5 showed maximum values of moisture, ash and crude fiber 3.80%, 0.66% and 0.40% respectively. Crude protein ranged from 9.05 to 9.32%, crude fat ranged from 21.40 to 21.60% and nitrogen free extract varies from 64.49 to 65.27% between the treatments.

The moisture content of the treatments increases with the increasing sorbitol levels was due to more hygroscopic nature of sorbitol as compared to the sucrose. Ash contents of treatments increases with the increasing sorbitol levels are due to the higher ash content of sorbitol as compared to the sucrose. Crude fiber content of cookies increases with the higher concentration of sorbitol due to low digestion of sorbitol as compared to the sucrose. The results are in close agreement with the findings of others in the literature **(Mushtaq et al., 2010; Siddique, 1995; Winkelhausen et al., 2007)**.

Sensory Evaluation of Cookies

Statistical data showed that the hedonic points related to the sensory parameters of cookies decrease with the increasing levels of sorbitol in treatments (Table 8). T_5 showed minimum hedonic points related to taste and crispness at 6 and 6, respectively, whereas T_0 showed a maximum value of 6.75 and 6.90, respectively. T_4 showed minimum hedonic points related to mouth feel and overall acceptability of 6.20 and 5.75, whereas T0 and T2 showed maximum hedonic points of 7.05 and 7.32, respectively.

The hedonic points related to the treatments decrease with the increasing levels of sorbitol because sorbitol lowered the sweetness level, increased the hardness, and had an aftertaste in cookies as compared to sugar. The results are in close agreement with the findings of **(Mushtaq et al., 2010; Siddique, 1995; Rosell et al., 2001)**.

CONCLUSION

The present study showed that the dough rheology of wheat flour could not be significantly improved by sorbitol supplementation. Sorbitol lowered the mixographic and farinographic parameters values in the treatments. Sorbitol substitution also resulted in harder, cohesive, adhesive and elastic dough as compared to the control treatment. The physical properties of sorbitol-substituted cookies did not significantly improve, as replacement resulted in harder, more brittle, lighter in color, higher in water activity and lower in diameter and spread factor values. Calorific content of sorbitol-replaced treatments were lowered as compared to the control treatments. Chemical characteristics of sorbitol-containing treatments significantly improved with substitution, as moisture, ash, and crude fiber contents of sorbitol-containing cookies increased with the increasing levels of sorbitol. Crude protein, crude fat, and nitrogen-free extract did not significantly change with the sorbitol replacement in treatments. Sensory evaluation of treatments showed that sorbitol substitution lowered the hedonic points related to the sensory parameters with the increasing levels of sorbitol. T_2 (20% sugar replacement) showed hedonic points of all sensory parameters closest to the control (T_0). T_2 also showed maximum hedonic points related to the overall acceptability of all the cookie treatments, showing it as the best treatment of the research.

REFERENCES

AACC. 2000. Approved Methods of the American Association of Cereal Chemists. 11th Ed. American Association of Cereal Chemists, Inc. St. Paul. Minnesota, USA.

Ahmad, R. 2011. *Effect of fat replacement with polydextrose and maltodextrin on dough rheology and biscuit quality in soft dough biscuits* : dissertation theses. National Institute of Food Science and Technology, UAF : Pakistan.

Akthar, M. N. 2011. *Effect of sesame meal supplementation on physical, chemical and sensory properties of bread* : dissertation theses. National Institute of Food Science and Technology, UAF : Pakistan.

Alava, J. M., Sahi, S. S., Garcia-Alvarez, J., Turo, A., Chavez, J. A., Garcia, M. J. and Salazar, J. 2007. Use of ultrasound for the determination of flour quality. *Ultrasonics*, vol. 46, no. 3, p. 270-276. https://doi.org/10.1016/j.ultras.2007.03.002 PMid:17462688

Asghar, A. 2004. *Effect of polyols (sorbitol and mannitol) on quality and shelf life stability of frozen dough for the bakery products* : dissertation theses. National Institute of Food Science and Technology, UAF : Pakistan.

Baek, M. H., Yoo, B., Lim, S. T. 2004. Effect of sugars and sugar alcohols on thermal transition and cold stability of corn starch gel. *Food Hydrocolloids*, vol. 18, no. 1, p. 133-142. https://doi.org/10.1016/S0268-005X(03)00058-4

Bond, M., Dunning, N. 2006. Xylitol. In Mitchell, H. *Sweeteners and Sugar Alternatives in Food Technology*. Oxford, UK : Blackwell Publishing Ltd, p.295-324. ISBN 978-14051-3434-7.

Farooq, A. 1996. *Effect of polyols (Glycerol and Sorbitol) on the quality and shelf life of bread and chapatti prepared from local and imported wheats* : dissertation theses. Deptment of Food Technology, UAF : Pakistan.

Fitch, C., Keim, K. S. 2012. Position of the academy of nutrition and dietetics: Use of Nutritive and Nonnutritive Sweeteners. *Journal of Academic Nutrition and Diet*, vol. 112, no. 5, p. 739-758. https://doi.org/10.1016/j.jand.2012.03.009 PMid:22709780

Grabitske, H. A., Slavin, J. L. 2008. Low-digestable carbohydrates in practice. *Journal of American Diet Association*, vol. 108, no. 10, p. 1677-1678. https://doi.org/10.1016/j.jada.2008.07.010 PMid:18926133

Krishna, G., Ranjhan, S. K. 1981. Gross energy of herbage, urine, milk and silage. In Krishna G. *Laboratory Manual of Nutrition Research*. Delhi : Vikas Publishing House. ISBN 978-0706911237.

Kroger, M., Meister, K., Kava, R. 2006. Low-calorie sweeteners and other sugar substitutes: a review of safety issues. *Comprehensive Reviews in Food Science and Food Safety*, vol. 5, no. 2, p. 35-47. https://doi.org/10.1111/j.1541-4337.2006.tb00081.x

Mahajan, A., Bhardwaj, S., Dua, S. 1999. Traditional processing treatments as a promising approach to enhance the functional properties of rapeseed and sesame seed meals. *Journal of Agriculture and Food Chemistry*, vol. 47, no. 8, p. 3093-3098. https://doi.org/10.1021/jf981003m PMid:10552614

Meilgaard, D., Civille, G. V., Carr, B. T. 2004. *Sensory Evaluation Techniques*. 4th ed. New York, USA : CRC Press LLC. 600 p. ISBN 9781482216905.

Mushtaq, Z., Rehman, S. U. Zahoor, T., Jameel, A. 2010. Impact of Xylitol replacement on physiological, sensory and microbial quality of cookies. *Pakistan Journal of Nutrition*, vol. 9, no. 6, p. 605-610. https://doi.org/10.3923/pjn.2010.605.610

Nadji, H., Bruzzese, C., Belgacem, M. N., Benaboura, A., Gandini, A. 2005. Oxypropylation of lignins and preparation of rigid polyurethane foams from the ensuing polyols. Macromol. *Material Engineering*, vol. 290, no. 10, p. 1009-1016. https://doi.org/10.1002/mame.200500200

Pasha, I., Butt, M. S., Anjum, F. M., Shehazi, N. 2002. Effect of dietetic sweeteners on the quality of cookies. *International Journal of Agriculture and Biology*, vol. 4, no. 2, p. 245-248.

Piga, A., Catzeddu, P., Farris, S., Roggio, T., Scano, E. 2005. Texture evaluation of amaretti cookies during storage. *Food Research Technology*, vol. 221, no. 3-4, p. 387-439. https://doi.org/10.1007/s00217-005-1185-5

Rakha, A. 2006. *Nutritional Assessment of high protein cookies through biological assay* : dissertation theses. National Institute of Food Science and Technology, UAF : Pakistan.

Rashid, M. S. 2007. *Baking quality of wheat flour cookies supplemented with mungbean flour and its protein isolate* : dissertation theses. National Institute of Food Science and Technology, UAF : Pakistan.

Rocha, A. N., Morais, M. B. 2003. Shelf life of minimally processed food apple determined by color changes. *Food Control*, vol. 14, no. 1, p. 13-20. https://doi.org/10.1016/S0956-7135(02)00046-4

Rosell, C. M., Rojas, J. A., Benedito de Barber C. 2001. Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocolloids*, vol. 15, no. 1, p. 75-81. https://doi.org/10.1016/S0268-005X(00)00054-0

Siddique, M. A. 1995. *Effect of artificial sweeteners on the quality of cakes and Biscuits* : dissertation theses. Deptment of Food Technology, UAF : Pakistan.

Steel, R. G. D., Torrie, J. H., Dickey, D. 1997. Principles and Procedures of Statistics. A Biometrical Approach. 3rd ed. New York, USA : McGraw Hills Book Co. Inc. 672 p. ISBN 978-0070610286.

Winkelhausen, E., Malinovaka, R. J., Velickova, E., Kuzmanova, S. 2007. Sensory and microbiological quality of baked products containing xylitol as an alternative sweetener. *International Journal of Food Properties*, vol. 10, no. 3, p. 639-649. https://doi.org/10.1080/10942910601098031

Yan, Y., Mingming, H., Zhihua, W. 2010. Kinetic study on the liquefaction of cornstalk in polyhydric alcohols. *Industrial Crops and Products*, vol. 32, no. 3, vol. 349-352.

Zoulias, E. I., Oreopoulou, V., Kounalaki, E. 2002. Effect of fat and sugar replacement on cookie properties. *Journal of the Science of Food and Agriculture*, vol. 82, no. 14, p.1637-164. https://doi.org/10.1002/jsfa.1230

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TOTAL PHOSPHORUS, PHYTATE PHOSPHORUS CONTENTS AND THE CORRELATION OF PHYTATES WITH AMYLOSE IN SELECTED EDIBLE BEANS IN SRI LANKA

Keerthana Sivakumaran, Jagath Wansapala, Theja Herath

ABSTRACT

Phytate a major anti nutritional factors in legumes and it accounts for larger portion of the total phosphorus, while limiting the bioavailablity of certain divalent cations to the human body. Legumes of eleven varieties cultivated in Sri Lanka, Mung bean (MI5, MI6), Cowpea (Waruni, MICP1, Bombay, Dhawala, ANKCP1), Soybean (MISB1, Pb1) and Horse gram (ANKBlack, ANKBrown) were analyzed for phosphorus content and phytate content. Total phosphorus content was quantified by dry ashing followed by spectrophotometrical measurement of the blue colour intensity of acid soluble phosphate with sodium molybdate in the presence of ascorbic acid while phytate phosphorus using anion exchange chromatographic technique followed by spectrometrical measurement of the digested organic phosphorus and amylose content by Simple Iodine-Colourimetric method. Where the least value for phosphorus was observed 275.04 ±1.44 mg.100g^{-1} in ANKBlack (Horse gram) and the highest in MISB1 (Soyabean) with 654.94 ±0.05 mg.100g^{-1}. The phytate phosphorus content (which is a ratio of phyate to total phosphorus) was highest in Dhawala (Cowpea). The phytate phosphorus (which is a ratio of phyate to total phosphorus) was highest in Dhawala with 67.42% and least in Bombay (Cowpea) with 24.87%. The amylose content of the legumes was least in Pb1 with 8.71 ±0.13 mg.100mg^{-1} and the highest in MI6 22.58 ±0.71 mg.100mg^{-1}. The correlation between phyate and total phosphorus was significant ($p \leq 0.05$) and positive (r = 0.62). Similarly the correlation coefficient for phytate phosphorus and total phosphorus was significant ($p \leq 0.05$) and positive (r = 0.63). Amylose content of legumes was significantly correlated negatively ($p \leq 0.05$) with the total phytates content (r = -0.82).

Keywords: phytates; phosphorus; amylose; phytate phosphorus; legumes

INTRODUCTION

Phosphorus which is an essential mineral is important for human health and optimal livestock production. Phytic acid (phytate; myo-inositol 1,2,3,4,5,6, hexakisphosphate) is one of the anti-nutritional factors (ANFs) among naturally occurring constituent of plant seeds, roots, tubers, and some fruits and vegetables and it acts as a storage form of phosphate **(Reddy and Sathe, 2002)**. In seed and grains phytate is accumulated within subcellular single membrane particles, aleurone grains or protein bodies. Legumes are the richest source of macro nutrients such as protein, starch and micronutrients minerals and vitamins while they contribute to important health protective compounds such as phenolics, inositol phosphates and oligosaccharides. **Lolas and Markakis (1975)** stated that phytate accounts for 80% of the total phosphorus in most legumes. The recommended average daily intake of phytate for humans on vegetarian diets, is 2000 – 2600 mg, for inhabitants of rural areas in developing countries, on mixed diets, it is 150 – 1400 mg **(Greiner, 2006)**. Presence of phytates is of a major concern in the foods and animal feeds industries because the phosphorus in this form is unavailable to monogastric animals due to a lack of endogenous intestinal phytases; enzymes specific for the dephosphorylation of phytic acid **(Greiner, 2006)** .In poultry rearing where sufficient dietary intake of phosphorus is maintained for reducing phosphorus intake in poultry manure. In addition, the strong chelating characteristic of phytic acid which works on a broad pH range reduces the bioavailability of other essential dietary nutrients such as minerals (e.g. Ca^{2+} ,Zn^{2+},Mg^{2+}, Mn^{2+}, $Fe^{2+/3+}$, proteins and amino acids **(García-Estepa et al., 1999; Azeke, 2010)**. Phytate occurs primarily as Potassium-Magnesium salt in rice, beans, sesame seeds and as a Calcium- magnesium-Potassium salt in soyabeans. Phytic acid is hydrolysed enzymatically by phytases. Apart from the binding divalent cations dietary

phytic acid has beneficial effects by acting as antioxidant or anticancer agent **(Raboy, 2001)**.

Scientific hypothesis

Usually legume based food (cooked) items contain higher amounts phytate than the cereal-based food items. Few food items, such as sesame seeds (toasted), soy protein concentrate, rice (unpolished and cooked), maize bread (unleavened) and peanuts have exceptionally high amounts of phytate **(Dahiya, 2016)**. As such the aim of this study is to determine the correlation between Phosphorus content and the phytate contents in legumes, correlation between the phytate phosphorus and the total phosphorus content as well as the correlation between Amylose and total phytates in some commonly consumed legumes in Sri Lanka.

MATERIAL AND METHODOLOGY

Chemicals

Anion exchange resin (AG 1- X 4 Chloride form, 100-200 mesh) and the other reagents with analytical grade.

Materials

In this study, two varieties of mung bean (MI5 and MI6), five varieties from cowpea (ANKCP1, MICP1, Bombay, Wauni and Dhawala), two varieties from soybean (Pb01and MISB1) and two varieties from horse gram (ANKBlack, ANKBrown) recommended by the Department of Agriculture, Sri Lanka were selected. These eleven legume varieties were obtained by random sampling method under same field and similar environmental conditions from Angunakolapelessa, Grain Legumes and Oil Seed Crops Research and Development Centre, which is the main agriculture research centre located in Southern Dry Zone of Sri Lanka. Samples were stored in the cold room at 10 °C till further usage.

Sample preparation

Cleaned and dried whole legume seeds were ground with a RETSCH S/S CROSS BEATER Hammer Mill Sk1 to 0.5 mm (500 µm) sieve size and the flour was packed in an air tight polythene bag till further usage.

Determination of phytate phosphorus content

Anion exchange method described by **AOAC (2012)** in method 986.11 was used in determining the phytate content in the legumes.

A glass column about 0.7 mm x 30 mm with a valve with anion exchange resin AG 1 – X 4 Chloride form, 100 – 200 mesh was used in the determination of phytate phosphorus content.

Phytate extracted from duplicate test portions of dried legume flour using dilute HCl (1 mL), mixed with 1 mL Na_2EDTA-NaOH solution and placed on an ion exchange column, the elute was discarded. Then the column was eluted with 15 mL of distilled water followed by 0.1 M NaCl respectively. Both elutes were discarded. Finally the column was eluted with 15 mL of 0.7 M NaCl and the fraction was collected to a digestion tube. 0.5 mL of concentrated H_2SO_4 and 3 mL of concentrated HNO_3 were added to the tube and digested on a kjeldhal block at 250 °C until yellow fumes evolved. The boiling was continued until clear solution was obtained. When the flask was cooled 10ml of distilled water was added and heated for 10 minutes at low heat. After cooling the contents of the tube was transferred to a 50 mL volumetric flask followed by addition of 2ml of molybdate solution and 1ml sulfonic acid make up to the mark and mixed well. After 15 minutes absorbance was measured at 640nm.

The recovery of the column has been tested using standard Sodium phytate solution of concentration 2.8 µg.mL^{-1}. Triplicate samples were done with the standard phytate solution to test the recovery of the column. Standard curve plotted using Standard phosphate solution (Primary standard, 80 µg.mL^{-1} KH_2PO_4) was used in determining the phytate phosphorus content.

Determination of total phosphorus

Initially a standard curve for Phosphorus was plotted using 0.01 mg P.mL^{-1} standard phosphorus (KH_2PO_4) solution. Phosphorus (total) in foods method described in **AOAC (2012)** (method 995.11) was used to analyse the phosphorus content in eleven legume varieties. Flour (1.5 g) was weighed into a crucible and 0.5g of Zinc oxide was added and mixed. Then the samples were ashed in the muffle furnace at 550 °C for 4 hours. Then the crucibles were removed from the furnace and let to cool. To the cold crucibles, 5 mL of water, and 5 mL of concentrated HCl were added. The crucibles were covered with watch glass and boiled for 5 minutes in a water bath. The contents of the crucibles were filtered into a 100ml volumetric flask and rinsed the crucibles and watch glass with hot water through the filter into the flask. After cooling the flask to room temperature, 50% KOH was added until the solution was slightly opalescent. HCl was added until the opalescent disappears. The solution was cooled to room temperature and diluted to the volume with water. Then 10 mL of the solution was transferred into a 100 mL volumetric flask and diluted to the mark. Then 5 mL of the diluted solution was transferred to a 50 mL volumetric flask and 15 mL of deionized water was added. Then 20 mL of molybdate ascorbic acid solution prepared immediately before use, 25 mL of sodium molybdate solution and 10 mL of ascorbic acid solution was transferred to a 100 mL volumetric flask, the solution was mixed and diluted to markwas added and swirled. The flasks were loosely stoppered and placed in a metal basket. The metal basket was placed in vigorously boiling water bath for 15 minutes. Then the flasks were cooled under the tap water and diluted to the volume with deionized water. Absorbance was measured at 823nm.

Determination of amylose content

Initially a standard curve for Amylose was plotted using Standard potato amylose solution (0.40 mg.mL^{-1}). Powdered sample of the legume variety (particle size 0.5 mm, 100 mg) was precisely measured into an Erlenmeyer flask (100 mL). ethy alcohol (95%, 1 mL), NaOH (1 N, 9 mL) were added to the flask and boiled to gelatinize for 10 minutes in boiling water bath. The solution was cooled to room temperature and was transferred into a volumetric flask (100 mL) with two successive washings. An aliquot (5 mL) was transferred into a volumetric flask (100 mL).

Acetic acid (1 N, 1 mL) and Iodine/Potassium Iodide (2 mL) were added. The solution of each flask were diluted to 100 mL mark with distilled water. Meanwhile blank was prepared without sample with other same conditions. After stabilizing the samples at 30 °C, the absorption was measured 620 nm using UV-spectrophotometer.

Statistical analysis

All the data were analyzed using parametric tests. The data were statistically evaluated by one way ANOVA using Minitab 17 software. All test procedures were made at 5% significant level ($p \leq 0.05$).

Microsoft excel 2013 has been used for graphical illustration of data. The correlation between phosphorus and phytate contents were determined using Pearson's correlation test.

RESULTS AND DISCUSSION

Total phosphorus content in legumes

Legume sample is dry ashed to remove any organic compounds. Acid soluble phosphate forms a blue complex with Na_2MoO_4 in the presence of ascorbic acid as the reducing agent. Intensity of the blue colour is determined spectrophotometrically.

The phosphorus content of the legume varieties ranged from 275.04 ±1.44 mg.g^{-1} in ANKBrown to 654.94 ±0.05 in MISB1. There was a significant difference ($p \leq 0.05$) among the phosphorus content of the legume varieties (Refer to Tables). There was a significant difference ($p \leq 0.05$) in phosphorus contents between soya bean varietals of Pb1 and MISB1. There was no significant difference ($p > 0.05$) existing between Waruni and MICP1 varieties of Cowpea, while there was significant difference ($p \leq 0.05$) among Bombay, Dhawala and ANKCP1. According to **Ravindran et al. (1994)** it was reported that the phosphorus content of soyabeans, cowpea and green gram were 600 mg.100g^{-1}, 390 mg.100g^{-1} and 380 mg.100g^{-1} respectively which are in accordance to the results obtained. There was a significant difference ($p \leq 0.05$) between MI5 and MI6, and similarly ANKBlack and ANKBrown. According to **Vitorello et al. (2002)** grain phosphate content can vary depending the dose of fertilizer phosphorus and the difference in genotypes.

Phytate phosphorus contents of legumes

It was shown in the table the phytate phosphorus content of the legume varieties were significantly different ($p \leq 0.05$) from each other. The phytate phosphorus content ranged from 103.056 ±10.255 mg.g^{-1} in ANKBlack to 334.545 ±13.397 mg.g^{-1} in Pb1. There was no significant difference ($p > 0.05$) between the phytate phosphorus contents of Soya bean varieties Pb1 and MISB1 varieties. Meanwhile there was significant difference ($p \leq 0.05$) between the Cowpea varieties Dhawala and ANKCP1. There was no significant difference ($p > 0.05$) between Mung Bean varieties of MI5, MI6, and the cowpea varietals of Dhawala, Bombay and ANKBlack of Horse gram. The study of **Ologhobo and Fetuga (1982)** indicated that generally phytic acid phosphorus represented 31.3 – 59.4% of total phosphorus with an average of 47.2%. These results are partly consistent with a view that phytic acid is the principal form of phosphorus in many seeds and that about 40 – 80% of the total phosphorus contents of dry legume seeds are in the form of phytic acid phosphorus **(Lolas and Markakis, 1975)**.

Phytic acid is the principal form of phosphorus in many seeds and that about 40 – 80% of the total phosphorus contents of dry legume seeds are in the form of phytic acid phosphorus **(Lolas and Markakis, 1975)**. **Ologhobo and Fetuga (1982)** indicated that the soybean dry seeds were the richest source of phytate (1.47% dry weight basis) followed in descending order by cowpeas (1.37%). The ratio of phytate phosphorus as percentage of total phosphorus was highest in soybeans.

There was a significant, ($p \leq 0.05$) positive correlation between phosphorus and phytate contents with the correlation coefficient of 0.62 as shown in Figure 1. According to the findings of **Chitra (1994)**, there was a significant positive correlation (r = 0.99) between phytic acid and total phosphorus content in all the legumes.

Table 1 Phosphorus and phytate phosphorus.

Nameof Variety	Phosphorus mg.100g^{-1} ±SD	Phytate Phosphorus mg.100g^{-1} ±SD	Phytate P as a % of total phosphorus
Soya bean			
Pb1	573.70 ±3.37 ^{b}	334.55 ±13.40 ^{a}	58.31%
MISB1	654.94 ±0.05 ^{a}	286.23 ±0.92 ^{a}	43.70%
Cowpea			
Waruni	443.19 ±0.00 ^{d}	117.79 ±4.63 ^{d}	26.58%
MICP1	441.44 ±1.77 ^{d}	241.26 ±7.65 ^{b}	54.65%
Bombay	544.71 ±1.89 ^{c}	135.45 ±4.58 ^{d}	24.87%
Dhawala	377.70 ±0.31 ^{g}	254.64 ±23.27 ^{b}	67.42%
ANKCP1	427.45 ±0.00 ^{e}	192.14 ±0.95 ^{c}	44.95%
Mung bean			
MI5	373.09 ±0.64 ^{g}	131.83 ±28.48 ^{d}	35.33%
MI6	405.63 ±3.16 ^{f}	119.32 ±7.00 ^{d}	29.42%
Horse gram			
ANKBlack	284.49 ±4.66 ^{h}	103.06 ±10.26 ^{d}	36.23%
ANKBrown	275.04 ±1.44 ^{i}	159.12 ±19.10 ^{cd}	57.85%

Note: results are expressed as mean ±standard deviation of triplicates and Means that do not share a same letter are significantly different ($p \leq 0.05$).

Table 2 Amylose contents of legumes.

Name of variety	Amylose mg per 100mg sample ±SD
Soya bean	
Pb1	8.71 ±0.13 [e]
MISB1	8.99 ±0.18 [e]
Cowpea	
Waruni	20.85 ±0.16 [b]
MICP1	18.56 ±0.41 [c]
Bombay	21.02 ±0.19 [b]
Dhawala	20.60 ±0.22 [b]
ANKCP1	20.06 ±0.25 [b]
Mung bean	
MI5	22.24 ±0.91 [a]
MI6	22.58 ±0.71 [a]
Horse gram	
ANKBlack	20.10 ±0.04 [d]
ANKBrown	19.23 ±0.04 [d]

Note: results are expressed as mean ±standard deviation of triplicates and Means that do not share a same letter are significantly different ($p \leq 0.05$).

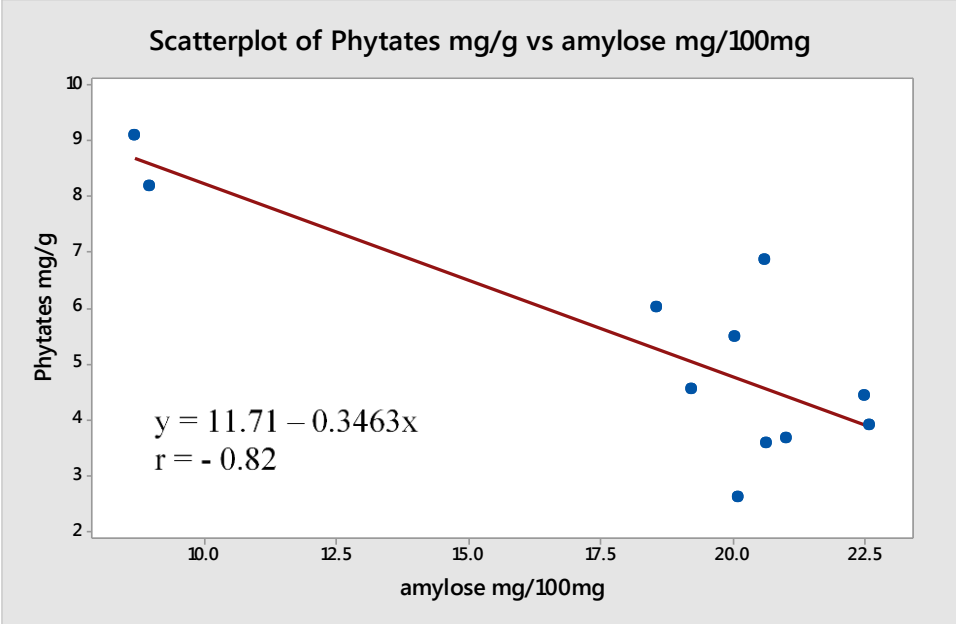

Figure 3 Correlation between total phytates – $mg.g^{-1}$ and amylose – $mg.100mg^{-1}$ in legumes.

According to the finding of **Raboy et al. (1984)** and **Mosenthin (2007)**, phytic acid and seed total phosphorus in soybean gradient stated were highly and positively correlated (r = 0.94)

The magnitude of correlation coefficient obtained in the analysis was low due to one of the reason of prolonged storage of legumes led to activation of phytase enzyme at high humidity and high temperature conditions which can lead to significant loss in phytates. According to **Chitra (1994)** the decrease in phytic acid was the lowest in soybean (29%) after 12 months of storage at 25 °C and 37 °C. The values obtained are in close agreement with the results reported by **Reddy and Sathe (2002)**.

An experiment conducted by **Cossa et al. (1999)** for maize samples phytate phosphorus and phosphorus contents were determined where the correlation coefficient was 0.70. Though some of the results of phytate phosphorus and total phosphorus obtained deviates from that reported in the literature, could be due to certain reasons such as variations in the environmental factors such as locations, irrigation conditions, type of soil, fertilizer applications, year of growing the cultivar etc.

The sample that has been used for the analysis was stored in the cold room till further usage which could had again led to loss in phytates with storage time **(Reddy and Sathe, 2002)**. The population in the developing countries consumes plant foods like legumes on a daily basis, there can be problems in meeting the daily dietary requirement for phosphorus, since it is clear from the experimental results that phytate phosphorus account for 29 to 67% of the total phosphorus which can adversely affect the mineral absorption **(Chitra, 1994)**. As such it is advisable to consume processed (fermented/cooked/germinated) legumes in order to reduce phytate contents **(Reddy and Sathe, 2002)**. There are studies stating that phytate containing foods are rich sources of dietary fiber which have great affinity for minerals at the same time, therefore

it is difficult to state that phyate availability solely affects mineral absorption **(Ravindran et al., 1994)**.

Amylose contents of legumes

The amylose content in legumes ranges from 8.705 ±0.129 mg.100g^{-1} in Pb1 to 22.580 ±0.714 mg.100g^{-1} in MI6. There is a significant difference ($p \leq 0.05$) existing among the amylose content of eleven legume varieties. The amylose content of MI5 and MI6 are significantly higher than the other varieties, which are not in the range of the values obtained by **Kaur et al. (2011)** where mung bean (*Vigna radiata* L) amylose contents were varied between 29.9 – 33.6 mg.100mg^{-1}. Similarly **Sandhu and Lim (2008)** who studied the digestibility of Indian legumes stated the amylose % of mung bean as 31.6 ±0.7 mg.100mg^{-1}. the amylose content of Soyabean are significantly lower ($p \leq 0.05$), which is lower that the values obtained by **Stevenson et al. (2006)**, where the apparent amylose content was 19 – 22 mg.100mg^{-1} and absolute amylose content was 11.8 – 16.2 mg.100mg^{-1} in *Glycine max (L.)Merr*. But according to **Gunathilake et al. (2016)** who observed that the carbohydrate contents in two varieties of Soyabean Pb1 and MISB1 as 18.0% and 15.0% respectively, it is evident that a lower amylose content can be as a result of lower total carbohydrate content. There is no significant difference ($p > 0.05$) between ANKBlack and ANKBrown varieties, the values obtained by **Marimuthu and Krishnamoorthi (2013)** for the amylose content of the South Indian horse gram was 32.14 ±0.10 mg.100mg^{-1}, similarly **Chavan et al. (2010)** stated the amylose content of black horsegram to be 36.30 ±1.40 mg.100mg^{-1} which are not in accordance to the values obtained in the experiment. The amylose content of MICP1 is significantly lower ($p \leq 0.05$) from the other cowpea varieties.

According to Pearsons correlation between phytates and amylose there is a significant negative ($p \leq 0.05$) correation existed between the phytate and amylose content of legumes (r = -0.82), according to **Dayakar et al. (2016)** there was a significant correlation between amylose and phytates contents in Sorghum was -0.26.

CONCLUSION

Soyabean contains the highest amount of Phosphorus of 654.94 ±0.05 mg.100g^{-1} in MISB 01 and least amount of 275.04 ±1.44 mg.100g^{-1} in ANK brown. Phytate phosphorus accounts for major portion of the total phosphorus ranging from 29.42% to 67.42% in legumes. There is a high positive correlation between phytate and Phosphorus as well as phytate phosphorus and phosphorus. While, there is a strong negative correlation between phytates and amylose content in legumes.

REFERENCES

AOAC International, 2012. Method 986.11 Phytate in foods. AOAC international, Suite 500, Maryland, USA.

AOAC International, 2012. Method 995.11 Phosphorus (total) in foods. AOAC international, Suite 500, Maryland, USA.

Azeke, M. A, Egielewa, S. J., Eigbogbo, M. U., Ihimire, I. G. 2010. Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). *Journal of food science and technology*, vol. 48, no. 6, p. 724-729. https://doi.org/10.1007/s13197-010-0186-y PMid:23572811

Cossa, J., Jeroch, H., Kerstin Oloffs, Kluge, H., Drauschke, W., Ackermann, R. 1999. Total Phosphorus and Phytate Phosphorus Content in Grain Maize (*Zea Mays*). *Journal of Agriculture in Tropics and Subtropics*, vol. 100, no. 2, p.181-188.

Dahiya, S. 2016. Role of phytate and phytases in human nutrition. *International Journal of Food Science and Nutriton*, vol. 1, no. 1, p. 39-42.

Dayakar Rao, B., Malleshi, N. G., Annor, G. A., Patil, J. V. 2016. *Millets Value Chain for Nutritional Security: A Replicable Success Model from India*. 1st ed. UK : CABI. 254 p. ISBN 978-1780648309.

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. https://doi.org/10.1016/S0963-9969(99)00092-7

Greiner, R., Konietzny, U, Jany, K. D. 2006. Phytate – an undesirable constituent of plant – based foods. *Journal fur Ernahrungsmedizin*, vol. 8, no. 3, p. 18-28.

Gunathilake, K. G. T., Wansapala, M. A. J., Herath, H. M. T. 2016. Comparison of nutritional and functional properties of mung bean (*Vigna radiate*) and cowpea (*Vignaun guiculata*) protein isolates processed by isoelectric precipitation. *International Journal of Innovative Research in Technology*, vol. 3, no. 2, p.139-148.

Chavan, U. D., Shinde, B. G., Kadam, S. S., Amarowicz, R. 2010. Isolation and Characterization of Starch from Horse Gram. *African Journal Food Science and Technology*, vol. 1, no. 3, p.64-67.

Chitra, U. 1994. *Effect of Storage and Processing on Phytic Acid Levels in Legumes and Its Interference With the Utilisation of Protein and Iron* : dissertation theses. Rajendranagar : Andhra Pradesh Agricultural University. 240 p.

Kaur, M., Sandhu, K. S., Singh, N., Lim, S. 2011. Amylose content, molecular structure, physicochemical properties and in vitro digestibility of starches from different mung bean (*Vigna radiata* L.) cultivars. *Starch – Stärke*, vol. 63, no. 11, p. 709-716. https://doi.org/10.1002/star.201100053

Lolas, G. M., Markakis, P. 1975. Phytic acid and other phosphorus compounds in beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry*, vol. 23, no. 1, p. 13-15. https://doi.org/10.1021/jf60197a016

Marimuthu, M., Krishnamoorthi, K. 2013. Nutrients and functional properties of horse gram (*Macrotyloma uniflorum*), an underutilized south Indian food legume. *Journal of Chemical and Pharmaceutical Research*, vol. 5, no. 5, p.390-394.

Ologhobo, D. A. 1989. Improving the nutritive value of soybean (*Glycine mx* (L.) Merr.) through processing: biochemical and protein quality studies. *Tropical Agriculture*, vol. 66, p. 290-296.

Ologhobo, D. A., Fetuga, B. L. 1982. Polyphenols, phytic acid and other phosphorus compounds of lima bean (*Phseolus lunntis*). *Nutrition Reports International*, vol. 26, p.605-611.

Raboy, V. 2001. Progress in Breeding Low Phytate Crops. In *Plant Breeding: A New Tool for Fighting Micronutrient Malnutrition*. National Small Grains Germplasm Research Facility, Aberdeen : National Small Grains Germplasm Research Facility, p. 503-505.

Raboy, V., Dickinson, D. B., and Below, F. E. 1984. Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium, and protein among lines of Glycine max and G. soja. *Crop Science*, vol. 24, no. 3, p. 431-434. https://doi.org/10.2135/cropsci1984.0011183X002400030001x

Ravindran, V., Ravindran, G., Sivalogan, S. 1994. Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. *Food chemistry*, vol. 50, no. 2, p. 133-136. https://doi.org/10.1016/0308-8146(94)90109-0

Reddy, N. R., Sathe, S. K. 2002. *Food phytates*. Boca Ranton, Florida : CRC press. 280 p. ISBN 9781566768672.

Sandhu, K. S., Lim, S. T. 2008. Digestibility of legume starches as influenced by their physical and structural properties. *Carbohydrate Polymers*, vol. 71, no. 2, p.245-252. https://doi.org/10.1016/j.carbpol.2007.05.036

Stevenson, D. G., Doorenbos, R. K., Jane, J., Inglet, G. E. 2006. Structures and functional properties of starch from seeds of three soybean (*Glycine max* (L.) Merr.) varieties. *Starch*, vol. 58, no. 10, p. 509-519. https://doi.org/10.1002/star.200600534

Vitorello, V. A., Coelho, C. M. M., Santos, J. C. P., Tsai, S. M. 2002. Seed phytate content and phosphorus uptake and distribution in dry bean genotypes. *Brazilian Journal of Plant Physiology*, vol. 14, no. 1, p. 51-58. https://doi.org/10.1590/S1677-04202002000100007

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COMPARISON OF RHEOLOGICAL PROPERTIES OF VARIETAL GRAPE SEED OILS

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ABSTRACT

The aim of this work was to determine the rheological properties of grape seed oils from different varieties selected at different temperatures. Measurement of the rheological properties of grape seed oils was performed on the instrument Anton Paar MCR 102. The rheological properties of the liquid have been performed at temperatures of 0 °C, 15 °C, 30 °C, 45 °C and 60 °C. The density of measured samples ranged from 0.905 ±0.002 to 0.948 ±0.002 g.mL^{-1}. Subsequently, there were determined viscosity values at a shear rate of 5 s^{-1}. As expected, the viscosity decreased with increasing temperature. Highest viscosity values reached grape seed oil of variety Pinot gris where at 0 °C the viscosity reached 0.206 ±0.037 Pa s. Samples measured at 0 °C showed non-Newtonian behavior, while at higher temperatures liquids behave as Newtonian substances. All grape seed oils samples exhibit non-Newtonian behavior at lower temperatures. In experiments carried out at 0 °C, it was observed that the tested samples tends to behave as shear-thinning system with thixotropic properties. At higher temperatures was, in line with other scientific works, observed that samples behave as Newtonian fluids. Knowledge of the rheological properties of oils are very important for their processing, storage, and may affect their quality.

Keywords: grape seeds; grape seed oil; rheology; viscosity

INTRODUCTION

Globally, there are produced more than 120 million tons of edible oils and fats while approximately 80% of them are derived from plant sources and thus they are referred to as vegetable oils. The development of natural oils is one of the alternatives how to protect the environment from hazardous materials **(Fasina and Colley, 2008)**. Oils and fats are the essential materials for margarine, shortening, salad oil and other specialty or tailored products, which have become significant ingredients in food preparation or processing in homes, restaurants, food manufactures and they are one of the main ingredients used to manufacture soaps, cosmetics and pharmaceutical products **(Rodenbush et al., 1999)**. The majority of edible oils and fats produced worldwide annually is derived from plant sources and these are referred as vegetable oils. Common commercially-available vegetable oils are colza, olive, sunflower and others **(Frančáková et al., 2015)**. New possibilities in terms of application are in larger scale offered by grape seed oil **(Hamm and Hamilton, 2000)**.

According to chemical composition, grape seed oil belongs to oils with a high proportion of unsaturated fatty acids (90%), 75% of which is represented by linoleic acid **(Baydar and Akkurt, 2001)**. Thus, it can be considered as very valuable in terms of nutrition. A high proportion of tocotrienols in wine oil, i.e. substances, which are together with tocopherols included in the group of vitamins E, makes this oil significantly different from the other described vegetable oils **(Hassanein and Abedel-Razek, 2009)**. Tocotrienols may have a much higher antioxidant capacity in comparison to tocopherols, which are often a single component representing vitamin E in other vegetable oils **(Choi and Lee, 2009; Hassanein and Abedel-Razek, 2009)**.

From the perspective of contained substances, grape seed oil has been studied rather in detail, however, information about its physical properties are insufficient **(Fasina and Colley, 2008)**. Oil viscosity has the greatest importance from the perspective of rheological properties. Oil viscosity is typically measured and defined in two ways, either based on its absolute (dynamic) viscosity or its kinematic viscosity. The absolute viscosity of oil is its resistance to flow and shear due to internal friction and it is measured with SI units of Pa s. In contrast, the kinematic viscosity of oil is its resistance to flow and shear due to gravity and it is measured with SI units of m^2.s^{-1}. The kinematic viscosity of oil can be obtained by dividing the absolute (dynamic) viscosity of oil with its corresponding density **(Diamante and Lan, 2014)**. In case of non-Newtonian fluids, we talk about the so-called apparent viscosity. This value is a variable depending especially on the shear rate value. Therefore, it is reasonable to state this value only when we know measuring conditions **(Singh and Heldman, 2001)**.

It has been well established that temperature has a strong influence on the viscosity of fluids with viscosity generally decreasing with increase in temperature **(Rao, 1999)**. The Power Law (Ostwald) is commonly used to describe the relationship of the temperature dependence on vegetable oil viscosity **(Fasina and Colley, 2008)**. Several researchers have reported the viscosity of vegetable oils at room temperature **(Lang et al., 1999; Diamante and Lan, 2014)**. The studies about temperature effect on viscosity of vegetable oils have been mostly carried out at temperatures above 30 °C. The absolute viscosity of fluids is an important property needed in fluid flow and heat transfer unit operations. This includes pumping, flow measurement, heat exchange, sterilization, freezing and many other operations. The aim of this study was to evaluate the rheological behavior of varietal grape seed oils at different temperatures.

Scientific hypothesis

Grape seed oils obtained through pressing at different temperatures behave as Newtonian fluids.

MATERIAL AND METHODOLOGY

Sample

For the purpose of this paper, grape seed oil of six grape species was used. The oil was pressed on the UNO FM 3F press. This press model is designed for pressing of all oily seeds. The drive is configured for the three-phase voltage with the possibility to change main drive speed by a frequency changer, which enables better optimization of press parameters. The press consists of an electric motor (power 1.5 kW), transmission, stamping system and motor starter including a frequency changer. The stamping system consist of a matrix, scroll, head, holder, nozzles, nozzles with diameter of 10 mm and heating cup.

Rheological measurement

The rheological evaluation of grape seed oil for this paper was performed on an Anton Paar MCR 102 Rheometer (Austria) with the measuring geometry cone-plate. The gap between the cone and the plate is set at the stable value of 0.103 mm. The diameter of the cone equaled to 50 mm with the angle of 1°. Rheological tests were performed at the temperatures 0 °C, 15 °C, 30 °C, 45 °C and 60 °C. Values of shear rates at individual rheological tests were following: Hysteresis loop test – the range of shear rate was set from 2 to 100 s^{-1}, Time dependent test – the constant value 50 s^{-1} of shear rate was set. The apparent viscosity was measured at the shear rate 5 s^{-1}.

The Power Law (Ostwald) model was used to evaluate dependence of the shear rate on the shear stress. This model is applied to fluids without initial yield stress and it was utilized for rheological description of foods. For example, authors **Al-Mahasneh et al. (2014)** state that the Power Law model is appropriate for description of rheological behavior of honey. This model is given by equation:

$$\tau = K \times \gamma^{n} \quad (1)$$

where: τ – shear stress (Pa), K – consistency coefficient, γ – shear rate (s^{-1}), n – flow behavior index.

The linear regression analysis of measured values was also carried out for the comparison.

Statisic analysis

Each measurement was repeated three times. Therefore, a standard deviation was determined for every value characterizing the sample of grape seed oil. The data were treated with a one-factor analysis of variance. Tukey HSD (honest significant difference) at the significance level of 0.05 was utilized for multiple comparisons of data. A statistical analysis was carried out using the software package ´Statistica 12.0´ (StatSoft Inc., USA).

RESULTS AND DISCUSSION

Sampling of wine marc from 6 species (Dornfelder–Dr, Blaufränkisch–BF, Pálava–Pa, Riesling–RR, Pinot gris–PG, Zweigelt–Zw) was carried out in order to separate seeds immediately after pressing on pneumatic presses in the processing season 2015. The compression pressure in the pneumatic presses was in the range of 1.8 bar. In order to separate seeds from wine marc, there was used a vibratory separator prototype, which applies the principle of mechanic vibrations transmitted to three plane sieves with different hole shape and size. In order to achieve successful pressing of seeds and for their potential storage, their initial humidity was decreased from 40 – 45% to 8 – 10%, because at higher humidity around 20% there occurs fast mould infection and subsequent spoilage. The temperature during drying in a chamber dryer did not exceed 40 °C. The varietal grape seed oil was pressed on the UNO FM 3F press. All oils were stored at room temperature (around 20 °C) and in a dark place before the analysis. Table 1 shows the species of tested oils according to varieties, place of their origin and volume weight.

The apparent viscosity was determined as the first rheological parameter of the analyzed samples. The values for apparent viscosities of the individual samples at various temperatures and at the shear rate of 5 s^{-1} are shown in Table 2. For comparison, the dynamic viscosity of wine ranged from 0.001479 to 0.001945 mPas in the

Table 1 Description of samples (n = 3).

Sample	Origin	Variety	Density at 20 °C ($g.mL^{-1}$ ±SD)
Dr	Velké Bílovice	Dornfelder	0.948 ±0.002
BF	Rakvice	Blaufränkisch	0.911 ±0.004
Pa	Rakvice	Pálava	0.943 ±0.001
RR	Velké Pavlovice	Riesling	0.941 ±0.003
PG	Velké Bílovice	Pinot gris	0.905 ±0.002
ZW	Rakvice	Zweigelt	0.933 ±0.001

Table 2 Apparent viscosity of samples at the shear rate 5 s^{-1} (n = 3).

Sample	Apparent viscosity (Pa s)				
	0 °C	15 °C	30 °C	45 °C	60 °C
Dr	0.195 ±0.005	0.075 ±0.002	0.042 ±0.000	0.027 ±0.000	0.019 ±0.001
BF	0.172 ±0.015	0.078 ±0.001	0.041 ±0.000	0.024 ±0.001	0.017 ±0.002
Pa	0.180 ±0.001	0.078 ±0.000	0.043 ±0.001	0.025 ±0.001	0.021 ±0.002
RR	0.163 ±0.020	0.079 ±0.000	0.044 ±0.001	0.028 ±0.001	0.018 ±0.001
PG	0.206 ±0.037	0.075 ±0.003	0.040 ±0.001	0.024 ±0.001	0.017 ±0.001
ZW	0.165 ±0.002	0.076 ±0.002	0.042 ±0.000	0.027 ±0.000	0.017 ±0.001

work presented by **Košmerl et al. (2000)**. The value of apparent viscosity in rendered fat at the temperature 10 °C and the shear rate 50 s^{-1} reached the approximate value 0.16 Pa s **(Trávníček et al., 2013)**. **Diamante and Lan (2014)** conducted determination of values of viscosity in vegetable oils of different origin. In case of grape seed oils, they determined the value 0.0227 Pa s at the temperature 50 °C, at the temperature 26 °C then viscosity reached the value 0.0466 Pa s. Authors of the publication state that in this range of temperatures grape seed oil behaved like Newtonian fluid. Also **Fasina and Colley (2008)** carried out determination of viscosity of vegetable oil at different temperatures. Results of their measuring in grape seed oil range from 0.0415 to 0.0169 Pa s (temperature 35 – 65 °C). As in the previous case, authors **Fasina and Colley (2008)** state that in the tested range of temperatures (35 – 180 °C) grape seed oil behaved like Newtonian fluid. It is then apparent from Table 2 that viscosity values are in correspondence with values measured by other authors **(Diamante and Lan, 2014; Fasina and Colley, 2008)**. It is also evident that according to expectations viscosity decreases with increasing temperature. The same effect of temperature on the absolute viscosities of vegetable oils was also observed by **Steffe (1992)**, **Abramovič and Klofutar (1998)** and **Santos et al. (2005)** for various vegetable oils at different temperatures.

In statistical evaluation of measured data by ANOVA, there was the hypothesis h0 accepted in all cases stating that there is no statistically significant difference in apparent viscosity among samples at individual temperatures at the level of importance $\alpha = 0.05$.

Afterwards, the so-called hysteresis loop test was performed. It consisted of gradual increasing of the shear rate to the set value. The sample was stressed at the constant shear rate value and then the shear rate was gradually decreased. If a loop was created, it was possible to describe the given sample as showing either thixotropic or anti-thixotropic (rheopectic) behavior. Thixotropic behavior is obtained from shear-thinning fluids in which no equilibrium is established between the structural breakdown and reformation process **(Pyle et al., 1997)** and can be described as a time dependent shear–thinning system. On the other hand, anti-thixotropy can be considered as a time dependent shear–thickening system.

Hysteresis loops of individual samples were observed at 0 °C. However, it was evident that hysteresis loops are not significant. This test was conducted also for further temperatures (15 °C, 30 °C, 45 °C and 60 °C). Significant hysteresis loops were not recorded at these temperatures.

It is possible to determine the so-called degree of thixotropy (in case of positive values) or antithixotropy (in case of negative values) when calculating the area of created loop. The software provided together with the equipment Anton Paar MCR 102 was used for the calculation. Calculation results are visible in Tab. 3. It is obvious from the table that values of the hysteresis area are very low and they decrease with increasing temperature. By the influence of high variability of measured data there were calculated high values of standard deviations (SD). Also according to the assumption (probably again by the influence of high variability of data) there were not recorded any statistically significant differences between individual samples and individual temperatures. For comparison, the value of the hysteresis area of heather honey at the temperature 10 °C is 25000 Pa s^{-1} **(Witczak et al., 2011)**, in case of wine lees this value moved around 1.25 Pa s^{-1} **(Lachman et al., 2015)**. However **Baudez (2006)** states that hysteresis area is simply a consequence of the shear localization rather than thixotropic behavior and its area is closely linked to the apparatus and the data sampling. This means that the loop test is only an approximate test for rheology evaluating of samples. Due to this reason, there is necessity of another types of rheological tests.

Thus, based on this type of test, it is not possible to decide if it is Newtonian or non-Newtonian fluid, especially due to high variability of data (Table 2). In case it was Newtonian fluid, dependence of the viscosity value on the shear rate value would be constant. If it was non-Newtonian fluid, viscosity would change with the increasing shear rate value. In Figure 1 there are shown

Table 3 Hysteresis areas of samples at various temperatures (n = 3).

Sample	Hysteresis area (Pa s^{-1})				
	0 °C	15 °C	30 °C	45 °C	60 °C
Dr	2.01 ±1.28	1.57 ±2.23	1.25 ±0.56	0.97 ±0.37	0.01 ±0.25
BF	2.91 ±1.07	2.46 ±1.40	1.17 ±0.73	0.34 ±0.28	0.13 ±0.47
Pa	5.20 ±3.42	4.40 ±1.37	1.29 ±0.11	0.20 ±0.20	0.12 ±1.13
RR	7.97 ±27.54	0.65 ±0.84	0.59 ±0.13	0.66 ±0.51	0.09 ±0.46
PG	3.04 ±15.80	1.95 ±1.00	0.46 ±0.04	0.23 ±0.35	0.11 ±0.20
ZW	4.61 ±0.42	1.74 ±0.75	0.53 ±0.44	0.16 ±0.05	0.06 ±0.05

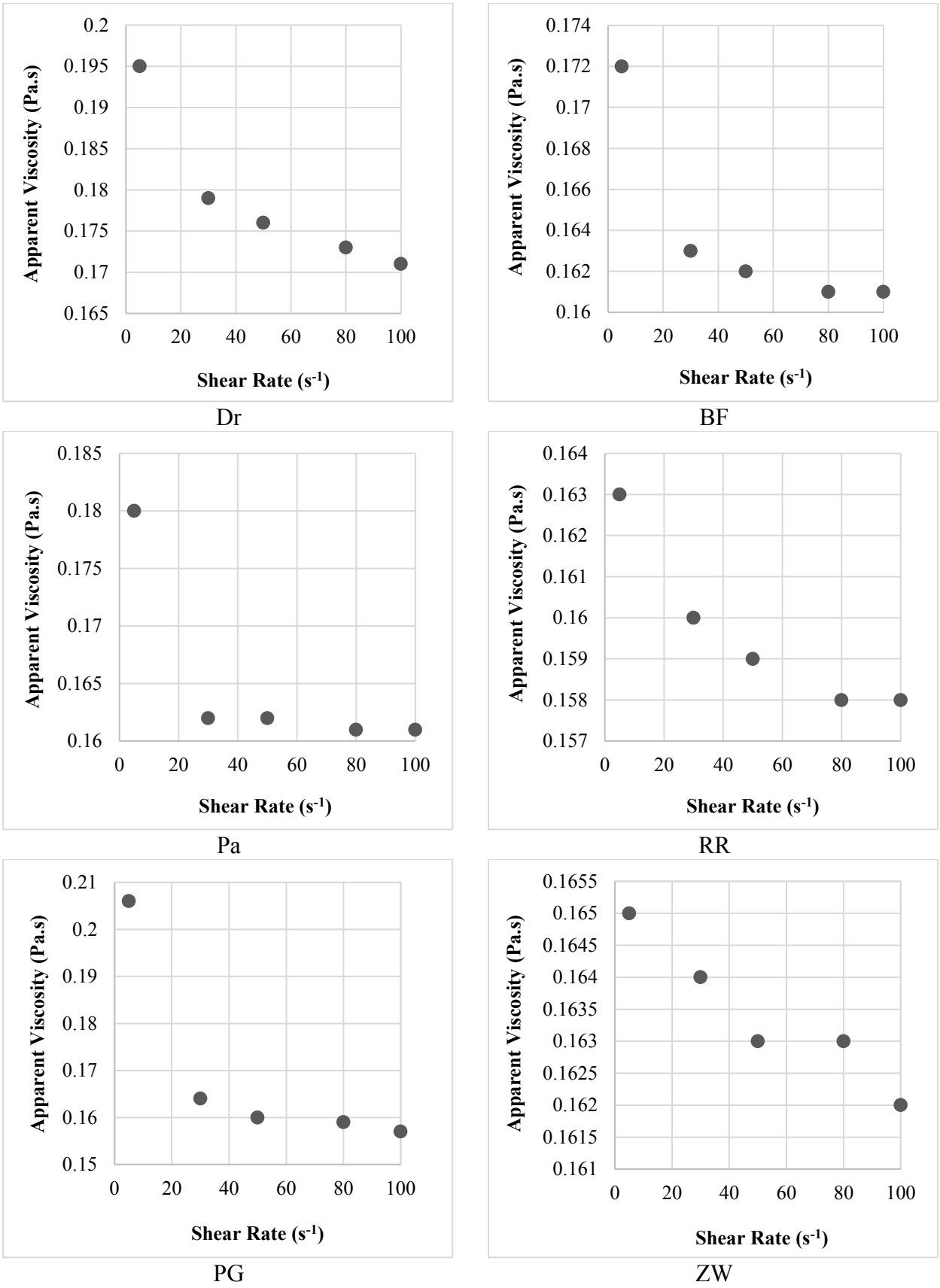

Figure 1 Dependence of the apparent viscosity on the shear rate at the temperature of 0 °C for samples.

dependences of the shear rate on the apparent viscosity in individual samples at the temperature 0 °C.

It is obvious from the picture, that there occurs decrease of viscosity with the increasing shear rate. This would indicate that it is the shear-thinning system, alternatively the shear-thinning system with thixotropic behavior. Nevertheless, it is necessary to mention that in some cases there are small changes of viscosity with the increasing shear rate. In case of increasing temperature, changes of viscosity are not almost noticeable in individual samples.

The next step utilized the Ostwald mathematical model to evaluate dependence of the shear rate on shear stress.

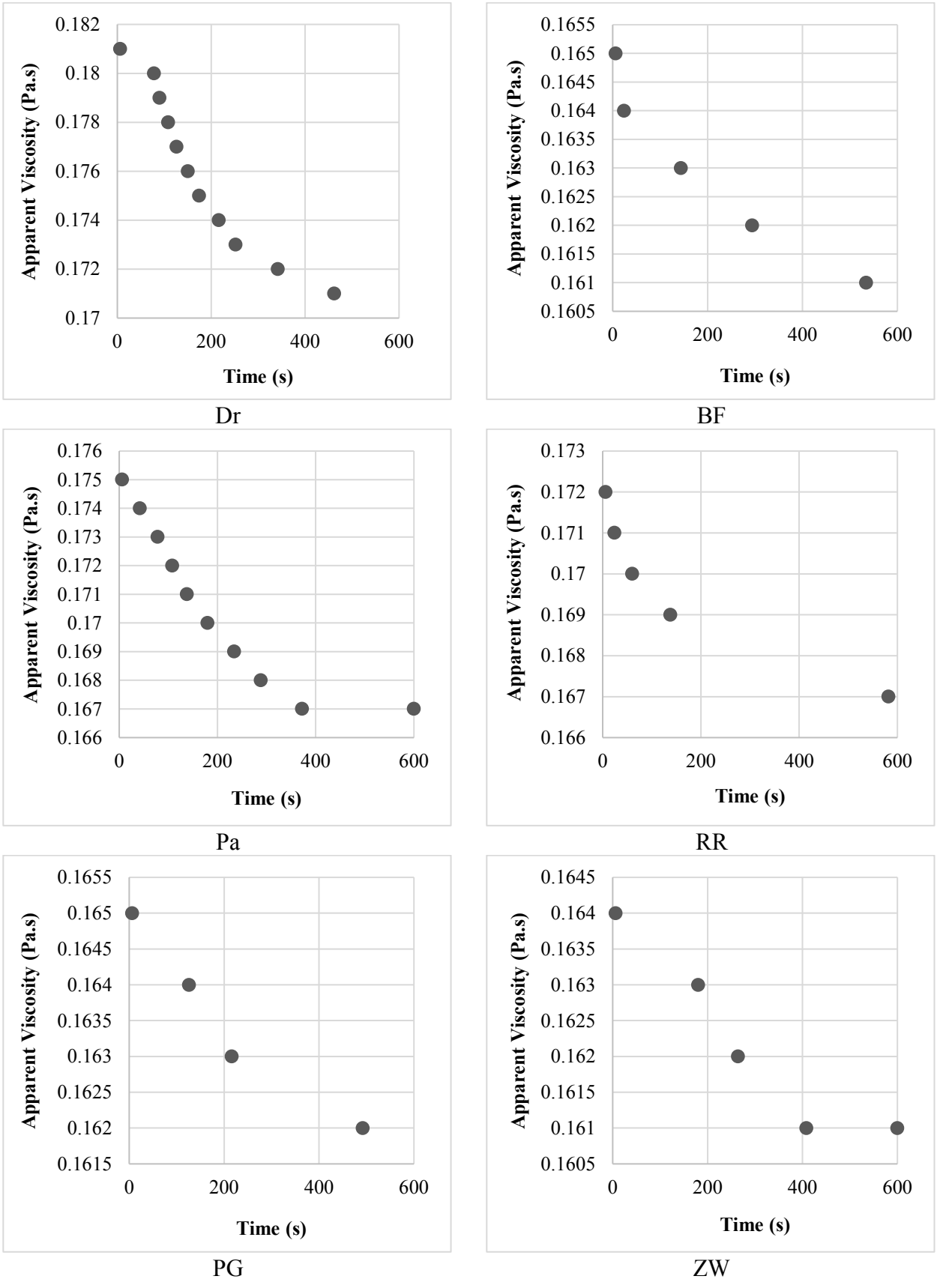

Figure 2 Change of the apparent viscosity over time at the temperature of 0 °C for samples.

This model is mostly used to describe flow curve material(s) with shear-thinning (pseudo plastic) or shear-thickening (dilatant) behavior without initial yield stress.

The results of calculation showed that determination coefficient of all temperatures and varieties is very high, ranging from R^2 = 0.9974 to R^2 = 0.9999. Authors

Diamante and Lan (2014) described dependence of the shear rate on shear stress by linear regression. The coefficient of determination in case of description of dependence of the shear rate on shear stress in grape seed oil ranged from $R^2 = 0.9995$ to $R^2 = 1.000$. In case of description of dependence of the shear rate on shear stress by linear regression, the coefficient of determination in individual samples ranged in the interval from $R^2 = 0.9996$ to $R^2 = 0.9999$.

Further important parameters of the Ostwald model are parameters *n* and *k*. The consistency index *k* indicates the extrapolated shear stress at the unit shear rate. The flow index is the rate of deviation from Newtonian behavior; when n <1, the apparent viscosity of the sample decreases (shear-thinning behavior), although the apparent viscosity of the sample increases when n >1 (shear-thickening behavior). If $n = 1$, it is Newtonian fluid. Values of coefficients *n* is always close to 1. Generally, it can be said, that coefficient *n* is the lowest at the temperature 0 °C. While the lowest value *n* was shown in case of the sample Dr at the temperature 0 °C ($n = 0.91 \pm 0.011$). The highest value of parameter *k* was calculated also for the sample Dr at the same temperature ($k = 0.246 \pm 0.013$). After completion of the analysis of variance there were found statistically significant differences in the coefficient *n* in samples Dr and Pa, RR, Zw at the temperature 0 °C and in samples PG and Pa, RR, Zw at the same temperature.

On the basis of past findings, it can be assumed that oil samples at low temperatures (0 °C) behave as shear-thinning fluids or as shear-thinning fluids with thixotropic behavior. Thixotropic fluids are time-dependent, or viscosity value changes over time at the constant press. It is necessary to conduct another type of test in order to confirm or rebut the presumption about thixotropic behavior of samples. The subsequent set of tests consisted of measuring of change of apparent viscosity depending on time at a constant value of shear rate.

Results of the experiment at the temperature 0 °C for individual samples of species are stated in Figure 2. It is apparent in pictures, that there occurs decrease of apparent viscosity over time in all samples. Nevertheless, it is necessary to remark that decrease is very small. In the Dr sample decrease of the apparent viscosity makes approximately 5% from the original value, in the BF sample approximately 2.5%, in the sample Pa 4.5%, in the sample RR 3%, PG 2%, and in the sample Zw also approximately 2%. After circa 10 minutes, apparent viscosity values stabilize and there does not occur further decrease.

From the perspective of previously stated information it is possible to consider that a system becomes a shear-thinning system with thixotropic properties at lower temperatures. At higher temperatures a system becomes Newtonian fluid. This phenomenon can be explained by the fact, that oil pressed from grape seeds contains micro particles, which are diffused within the fluid. In case of lower temperatures, intermolecular powers increase and probably help diffused particles to create aggregates in a stressed sample, which afterwards result in decrease of viscosity and so-called shear-thinning behavior with thixotropic properties. In case of longer pressure these aggregates disorganize and values of apparent viscosity stabilize. Another aspect, which can contribute to this behavior of fluid, is formation of ice crystals from water, which is in a small amount represented in oil. We also cannot forget the fact, that at lower temperatures there may occur crystallization of fats **(Bell et al., 2007)**. Generally, the crystal size, shape and alignment, degree of formation of mixed crystals and ability of crystals to flocculate into a network which increases firmness are important **(Opfer, 1978)**.

CONCLUSION

The professional publications cited in the text, which dealt with rheological properties of oils (including grape seed oils), imply that all monitored samples showed Newtonian behavior. Temperature 26 °C was the lowest temperature at which these fluids were tested **(Diamante and Lan, 2014)**. In the work, there were tested grape seed oil samples also at lower temperatures. Followings conclusions can be draw from this work:

1. Samples of grape seed oil behave like Newtonian fluid at higher temperature.
2. It was observed during the experiments that tested oils have the tendency to behave like the shear-thinning system with thixotropic properties at the temperature 0 °C.
3. This phenomenon may be caused for example by micro particles, which are due to higher values of intermolecular forces at lower temperatures attracted and create aggregates. Non-Newtonian behavior may be also caused by formation of crystals from water contained in oil or also by formation of crystals caused by fat crystallization at lower temperatures.

REFERENCES

Abramovič, H., Klofutar, C. 1998. The temperature dependence of dynamic viscosity for some vegetable oils. *Acta Chimica Slovenica*, vol. 45, no. 1, p. 69-77.

Al-Mahasneh, M., Rababah, T. M., Amer, M., Al-Omoush, M., 2014. Modeling physical and rheological behavior of minimally processed wild flowers honey. *Journal of Food Processing and Preservation*, vol. 38, no. 1, p. 21-30. https://doi.org/10.1111/j.1745-4549.2012.00734.x

Baudez, J. C. 2006. About peak and loop in sludge rheograms. *Journal of Environmental Management*, vol. 78, no. 3, p. 232-239. https://doi.org/10.1016/j.jenvman.2005.04.020 PMid:16112796

Baydar, N. G., Akkurt, M. 2001. Oil content and oil quality properties of some grape seeds. *Turkish Journal of Agriculture and Forestry*, vol. 25, p. 163-168.

Bell, A., Gordon, M. H., Jirasubkunakorn, W., Smith, K. W. 2007. Effect of composition on fat rheology and crystallisation. *Food Chemistry*, vol. 101, no. 2, p. 799-805. https://doi.org/10.1016/j.foodchem.2006.02.033

Diamante, L. M., Lan, T. 2014. Absolute Viscosities of Vegetable Oils at Different Temperatures and Shear Rate Range of 64.5 to 4835s^{-1}. *Journal of Food Processing and Preservation*, vol. 2014, 1- 6 p.

Fasina, O. O., Colley, Z. 2008. Viscosity and specific heat of vegetable oils as a function of temperature: 35 °C to 180 °C. *International Journal of Food Properties*, vol. 11, no. 4, p. 738-746. https://doi.org/10.1080/10942910701586273

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. https://doi.org/10.5219/556

Hamm, W., Hamilton, R. J. 2000. *Edible Oil Processing*. 2nd ed. New York, USA : CRCPress. 342 p. ISBN 978-1-444-33684-9.

Hassanein, M. M., Abedel-Razek, A. G. 2009. Chromatographic quantitation of some bioactive minor components in oils of wheat germ and grape seeds produced as by-products. *Journal of Oleo Science*, vol. 58, no. 5, p. 227-233. https://doi.org/10.5650/jos.58.227 PMid:19367078

Choi, Y., Lee, J. 2009. Antioxidant and antiproliferative properties of a tocotrienol-rich fraction from grape seeds. *Food Chemistry*, vol. 114, no 4, p. 1386-1390. https://doi.org/10.1016/j.foodchem.2008.11.018

Košmerl, T., Abramovič, H., Klofutar, C. 2000. The rheological properties of Slovenian wines. *Journal of Food Engineering*, vol. 46, no. 3, p. 165-171. https://doi.org/10.1016/S0260-8774(00)00079-0

Lachman, J., Rutkowski, K., Trávníček, P., Vítěz, T., Burg, P., Turan, J., Junga, P., Višacki, V. 2015. Determination of rheological behaviour of wine lees. *International Agrophysics*, vol. 29, no. 3, p. 307-311. https://doi.org/10.1515/intag-2015-0036

Lang, W., Sokhansanj, S., Sosulski, F. W. 1999. Modeling the Temperature Dependence of Kinematic Viscosity for Refined Canola Oil. *Journal of the American Oil Chemists´ Society*, vol. 69, no. 10, p. 1054-1055. https://doi.org/10.1007/BF02541080

Opfer, W. B. 1978. Margarine products: a review of modern manufacturing processes. *Chemistry and Industry*, vol. 18, p. 681-687.

Pyle, D. L., Fryer, P. J., Reilly, C. D. 1997. *Chemical Engineering for the Food Industry*. 1st ed., London , UK : Springer. 462 p. ISBN 978-1-4615-3864-6.

Rao, M. A. 1999. *Rheology of Fluid and Semifluid Foods: Principles and Applications*. 2nd ed. USA : Springer. 482 p. ISBN 978-0-387-70930-7.

Rodenbush, C. M. Hsieh, F. H., Viswanath D. D. 1999. Density and Viscosity of Vegetable Oils. *Journal of the American Oil Chemists´ Society*, vol. 76, no. 12, p. 1415-1419. https://doi.org/10.1007/s11746-999-0177-1

Santos, J. C. O., Santos, I. M. G., Souza A. G. 2005. Effect of heating and cooling on rheological parameters of edible vegetable oils. *Journal of Food Engineering*, vol. 67, no. 4, p. 401-405. https://doi.org/10.1016/j.jfoodeng.2004.05.007

Singh, R. P., Heldman, D. R. 2001. *Introduction to Food Engineering*. 4th ed. London, UK : Academic Press. 864 p. ISBN 9780080919621.

Steffe, J. F. 1992. *Rheological Methods in Food Process Engineering*. East Lansing, Mich, USA : Freeman Press. 418 p. ISBN 9780963203618.

Trávníček, P., Krčálová, E., Vítěz T. 2013. Rheopectic Behavior of Rendered Fat. *Polish Journal of Environmental Studies*, vol. 22, no. 5, p. 1499-1504.

Witczak, M., Juszczak, L., Gałkowska, D. 2011. Non-Newtonian behaviour of heather honey. *Journal of Food Engineering*, vol. 104, no. 4, p. 532-537. https://doi.org/10.1016/j.jfoodeng.2011.01.013

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EVALUATION OF ANTIOXIDANT POTENTIALS OF DIFFERENT SOLVENT-FRACTIONS OF *DIALIUM INDIUM* (AFRICAN BLACK VELVET TAMARIND) FRUIT PULP – *IN VITRO*

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ABSTRACT

Plant phytonutrients have been harnessed for their various curative properties both *in vitro* and *in vivo*. In this study African black velvet tamarind (ABVT) fruit pulp was evaluated for it antioxidant potentials using chloroform and hexane fractions through different antioxidant parameters. In the results; total phenolic contents quantified in mg GAE/dried sample in chloroform and hexane extracts were; 14.57 ±5.85 and 9.78 ±4.61, total flavonoid contents in chloroform and hexane extracts as; 48.58 ±0.00 and 27.35 ±0.00 while the FRAP (µg AAE.g^{-1} dried sample) was lower in chloroform (298.10 ±0.00) than hexane extracts (1029.81 ±0.00). More also, ability of varied concentrations of the extracts (with their IC50) to cause inhibition against Fe^{2+}-induced MDA that was determined by TBARS in rat's brain and liver tissue homogenates, Fe^{2+}-chelating ability and other antioxidant assays, showed an appreciable significant (p <0.05) difference. The various antioxidant properties showed by ABVT has indicated that, if the pulp is incorporated in diet, it could serve as an alternative in managing various ROS-induced degenerative ailments as it has been clearly demonstrated in the protection of brain and liver homogenates from Fe^{2+}-induced oxidative stress.

Keywords: Oxidative stress; cellular damage; antioxidants; *Dialium indium*; African Black velvet tamarind

INTRODUCTION

Oxidative stress induction through reactive oxygen species (ROS) has been studied explicitly and the roles play in promoting different diseases both in humans and animals directly or otherwise as a results of either a decrease in natural cellular antioxidant ability or an increased oxidants level cannot be over emphasized **(Afolabi and Oloyede, 2014a)**. Fe^{2+}, among series of metals has been implicated to play a crucial catalytic role in the generation/production of ROS which have the capacity to damage cellular lipids and other macromolecules resulting in wide range impairment in the cellular function and integrity through Fenton's reaction **(Afolabi, 2015; Britton et al., 2002)**. Several studies have shown that, when ROS are generated, several cellular protective pathways must have been disrupted by several mechanisms which could lead to cellular eruption and damage to enzymes localized in different cellular organelles **(Afolabi and Oloyede, 2014b; Akomolafe, 2013; Rani, 2013)**, which aftermath, would lead to the formation of several neurodegenerative, cardiovascular diseases and many other terminal ailments **(Oboh et al., 2013)**.

In recent times, attention has been shifted to exploiting plant-based products for the prevention and treatment of these different ROS linked diseases by various inhibition against its formation and different activities **(Raghuveer et al., 2011)**, also, in the body, several cellular defensive anti-oxidative mechanisms have been devised to fight against the initiation of ROS **(Britton et al., 2002)**. However, consumption of diets enriched in antioxidants may help in fighting the ROS-initiated diseases by strongly improving body's antioxidant status. *Dialium indium,* commonly known as African black velvet tamarind (ABVT) is a large tree found in many parts of Africa such as West Africa, Central African Republic and the Chad. The fruits are usually circular and frightened, usually has a sweet-Sour taste, black in colour with a stalk of about 6mm long, it belongs to the family *Fabaceae-caesalpiniodaea* **(Eziaku and James 2014; Okegbile and Taiwo, 1990)**. It is popularly called *Icheku* or *Nchichi* in Igbo, *Awin* among Yorubas and *Tsamiyar Kurm* in Hausa, Nigeria. The fruit is quite popular as a spice with belief

that fruit can bring down systemic cholesterol level. The velvet tamarind pulp is eaten in Southeastern Nigeria because of its refreshing properties and pleasant taste **(Nwaukwu and Ikechi, 2012)**. Black tamarind and its pulp has been found to be highly enriched in vitamin C and other vital essential nutrients needed in human diet which could also be used instead of synthetic drugs to treat stomach upset when soaked in water **(Nwaukwu and Ikechi, 2012; Dike, 2010)**.

Scientific hypothesis

It has been well elucidated in the current work that, when 0.1 mg.mL^{-1} of the various solvent extracts of ABVT was appraised, the trends that indicated various activities increasingly in concentration – dependent manner were observed. If 0.2 mg.mL^{-1} of the same extracts was subjected to the same assays as found in the current work, it could be scientifically assumed that same trend would be achieved, if and only if the same methods and procedures are engaged.

MATERIAL AND METHODOLOGY

Chemicals used

Chemicals and reagents used such as thiobarbituric acid (TBA), 1, 10-phenantroline, Folin-coicalteau's reagent, trichloroacetic acid (TCA), gallic acid with Diphenyl-2-picryl-hydrazyl (DPPH) from Sigma-Aldrich, Inc. (St Loius, MO), $FeCl_3$, Sodium carbonate, $AlCl_3$, Sodium dodecyl sulphate (SDS), $FeSO_4$ (Iron II Sulphate), potassium ferricyanide, ferric chloride acetic acid, hydrogen peroxide, methanol were procured BDH Chemicals Ltd., (Poole, England) and other chemicals used were of analytical grades and prepared in all-glass apparatus using distilled water.

Sample collection and preparation

African Black velvet tamarind (ABVT) fruits were obtained from a popular place in Ikare- Akoko in Ondo State, Nigeria. The voucher sample was taken to the herbarium of Plant Science Department of Ekiti State University, Ado-Ekiti, Ekiti State, where it was authenticated. Thereafter, the sample was treated and pulverized using a laboratory blender and the fine powders obtained stored at moderate temperature until further use.

Obtention of sample

Ethanolic extraction procedure

The blended ABVT pulp was air-dried to a constant weight in a ventilated place at ambient temperature of 30 ± 2 °C, pulverized using a laboratory blender and the fine powdery form obtained was stored at moderate temperature until further use. About 120 g of the sample was weighed and used for the extraction in 70% ethanol for 72 h.

Solvent Partitioning

About 5 g of the ethanolic extract of ABVT pulp was reconstituted in distilled water and then defatted with Pet-ether, the fat free aqueous residue was partitioned repeatedly with n-hexane in a separating funnel flask until exhaustion. The remaining marc was finally partitioned again with chloroform solvent until exhaustion. Thereafter, the aqueous residue was remove and the solvent fractions were concentrated in water bath at 50 °C.

Determination of total phenolic content

The total phenolic contents of the solvent extracts were determined by the method of **Singleton et al. (1999)**. 0.2 mL of the extract was mix with 2.5 mL of 10% folin-ciocalteau's reagent and 2 mL of 7.5% Sodium carbonate. The reaction mixture will be subsequently incubated at 45 ^{o}C for 40 min., and the absorbance was measured at 700 nm with garlic acid as standard and the result expressed in mg GAE.g^{-1} of the dried sample.

Determination of total flavonoid

The total flavonoid contents of the extracts were determined using a colorimeter assay developed by **Bao et al. (2005)**. 0.2 mL of each extract was added to 0.3 mL of 5% $NaNO_3$ at zero time. After 5 min, 0.6 mL of 10% $AlCl_3$ was added and after 6 min, 2 mL of NaOH was added to the mixture followed by the addition of 2.1 mL of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as µg quercetin equivalent/gram dry sample (µg QE.g^{-1} dry sample).

Determination of ferric reducing property (FRAP)

The reducing properties of the extracts were determined by the described method of **Pulido et al. (2000)**. 0.25 mL of the extract was mixed with 0.25 mL of 200 mmol.L^{-1} of Sodium phosphate buffer pH 6.6 and 0.25 mL of 1% KFC. The mixture was incubated at 50 ^{o}C for 20 min, thereafter 0.25 mL of 10% TCA was also added and centrifuge at 2000 rpm for 10 min, 1 ml of the supernatant was mixed with
1 mL of distilled water and 0.2 mL of 1% ferric chloride and the absorbance was measure at 700 nm and ascorbic acid was used as standard, with the result expressed in µg ascorbic acid equivalent/g dried sample (µg AAE.g^{-1} dried sample).

Fe^{2+} Chelating assay

The *in vitro* Fe^{2+} chelating ability of the ABVT pulp solvent extracts were assayed according to the method of **Puntel et al. (2005)**. Briefly, 0.9 mL of aqueous 0.5 mmol.L^{-1} $FeSO_4$ and 0.15 mL of leaf extract were incubated for 5 min at room temperature. Then, 78 µL of ethanolic solution of 1,10-phenanthroline was added. The absorbance of the orange colour solution was read at 510 nm. The *in vitro* Fe^{2+} chelating ability of the samples was calculated by using the following formula:

Chelating ability (%) = (Abs $_{control}$ – Abs s_{ample}) / Abs $_{control}$ x 100

Abs control = The absorbance of the control (reaction mixture in the absence of sample)
Abs sample = The absorbance of the reaction mixture (with the sample).

Estimation of DPPH Radical Scavenging Ability

The free radical scavenging ability of the ABVT pulp extracts against DPPH was determined using the described method of **Gyamfi et al. (1999)**. 1 mL of the extract was

mixed with 1mL of the 0.4 mmol.L^{-1} methanolic solution of the DPPH, the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

Determination of NO radical scavenging ability

The scavenging effects of the solvent extracts of ABVT pulp on nitric oxide (NO) radical were measured according to the method of **Mercocci et al. (1994)**. An amount of 100 – 400 µL of the aqueous extract was added in test tubes to 1 mL of SNP solution (25 mmol.L^{-1}) and the tubes were incubated at 37 °C for 2 h. An aliquot (0.5 mL) of the incubating mixture was removed and diluted with 0.3 mL of Griess reagent. The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank. Results were expressed as percentage Nitric oxide radical-scavenging ability.

(%) Nitric oxide radical scavenging activity = (Abs_{Ref} – Abs_{sample} / Abs_{Ref}) x 100

Degradation of deoxyribose (Fenton's reaction)

The ability of the solvent fractions of ABVT pulp to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the method of **Halliwell and Gutteridge (1981)**. Briefly, freshly prepared aqueous extract (0 – 100 µL) was added to a reaction mixture containing 120 µL 20 mmol.L^{-1} deoxyribose, 400 µL 0.1 M phosphate buffer (pH 7.4), 40 µL 20 mmol.L^{-1} hydrogen peroxide and 40 µL 500 µmol.L^{-1} FeSO4, and the volume for made to 800 µL with distilled water. The reaction mixture was incubated at 37 ^{o}C for 30 min, and the reaction was stop by the addition of 0.5 ml of 2.8% trichloroaceticacid (TCA), this was followed by the addition of 0.4 mL of 0.6% TBA solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at measured at 532 nm in spectrophotometer.

$$\text{Hydroxyl radical scavenging ability (\%)} = \frac{Abs_{control} - Abs_{Test\ sample}}{Abs_{control}} \times 100$$

where $Abs_{control}$ = absorbance of the control (reacting mixture without the test sample) and $Abs_{test\ sample}$ = absorbance of reacting mixture with the test sample.

Lipid peroxidation by thiobarbituric acid reactive species assay

Preparation of Tissue homogenates

The rats were decapitated under mild di-ethyl ether anesthesia while the cerebral and liver tissues were rapidly extracted and placed on ice and weighed. This tissues were subsequently homogenized in cold 0.1 mol.L^{-1} Tris-HCl buffer pH 7.4 (1:10 w/v). The tissue homogenates were centrifuged for 10 min at 3000 g to yield a pellet that was discarded and the supernatant was used for the assay.

Thiobarbituric acid reactive species assay

The lipid peroxidation assay was carried out using the modified method of **Ohkawa et al. (1984)**. Briefly, the reaction mixture consisting 100 µL of tissue, 30 µL of 0.1 mol.L^{-1} pH 7.4 Tris-HCl buffer, different concentrations of the extracts were incubated with 50 µL of the freshly prepared 250 µmol.L^{-1} $FeSO_4$ with distilled water at 37 °C for 1 h. The color reaction was carried out by adding 200, 500 and 500 µl each of the 8.1 % sodium dodecyl sulphate (SDS), 1.33 M acetic acid (pH 3.4) and 0.6% TBA respectively. The reaction mixture was incubated at 100 °C for 1 h. The absorbance was read after cooling at 532 nm in an ultraviolet visible-spectrophotometer. The results were expressed in percentage inhibition of Malondialdehyde (MDA) produced.

Statistical analysis

All experiments were carried out in duplicate. Results were expressed as mean values ±standard deviation (SD) of duplicated samples. Differences and levels of significance were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple test **(Zar, 1984)**. Significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Plants are naturally endowed with natural antioxidants that exhibit cell's defensive mechanisms through scavenging the reactive oxygen species (ROS), with protective effects against degenerative diseases in humans **(Shalaby and Shanab, 2013)**. They exhibit antioxidant activities through electron donation and thereby neutralizing the damaging effects of free radicals, whose formation is associated with aerobic cells normal natural metabolic processes **(Amic et al., 2003)**. This work reports the antioxidant properties of different solvent extracts of ABVT fruit pulp by considering various parameters. Most plants that are rich in phenolic and polyphenolic acids exhibit strong antioxidant and anti-radical activities **(Mary et al., 2003)**, capable of removing free radicals, activate antioxidant enzymes, chelate metal catalysts, reduce alpha-tocopherol radicals and inhibit oxidases **(Amic et al., 2003)**. This group of compounds constitute the main class of natural antioxidants present in plants **(Andrea et al., 2003)**. As represented in Figure 1, the phenolic contents in the different solvent extracts of ABVT pulp considered, were clearly revealed, chloroform extract demonstrated higher content of phenol groups than that of hexane extract, which means that, the potential to exhibit antioxidant activities will be more in the chloroform extract than that of hexane extract.

Also, the flavonoid contents of ABVT pulp solvent extract as shown in Figure 2, reveal that, chloroform extract of the ABVT pulp showed higher flavonoid content than that of hexane extract. Howbeit, the plant flavonoids have been unfolded in several works recently to have antioxidant activity both *in vitro* and *in vivo* **(Shimoi et al., 1996; Geetha et al., 2003)**, by suppressing the reactive oxygen formation, chelating trace elements involved in free radical production, scavenging reactive species and protecting antioxidant defensive mechanism in the cell **(Agati et al., 2012)**. Figure 3, shows the ferric reducing ability (FRAP) of the extracts expressed in μg ascorbic acid equivalent/g dried sample, the reducing power is associated with antioxidant activity and may serve as a significant indicator of the antioxidant activity **(Meir et al., 1995)**. The results in the Figure 3, indicated that, hexane extract of ABVT pulp has higher reductive

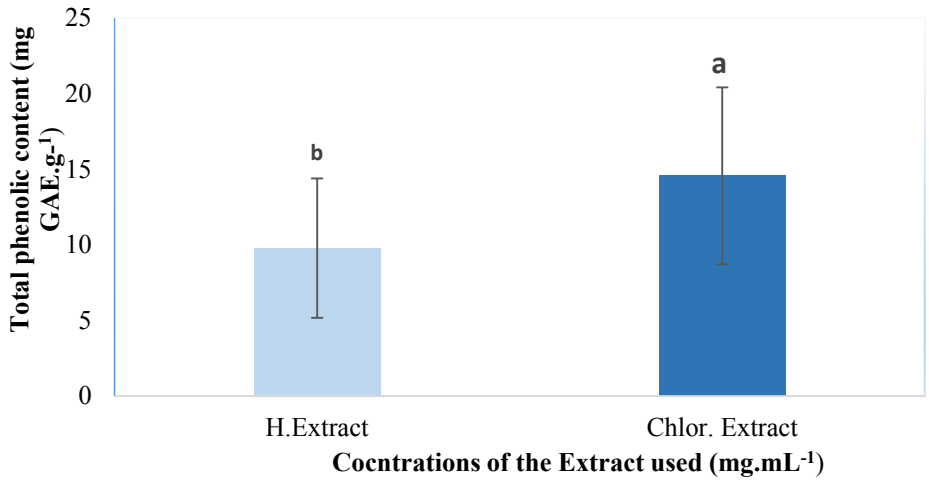

Figure 1 Total phenolic contents of hexane and chloroform fractions of ABVT pulp.
Key: H. Extract = Hexane Extract; Chlor. Extract = Chloroform Extract, GAE=Galic acid equivalent. The alphabets a and b show the levels of significant (p <0.05) difference between the extracts.

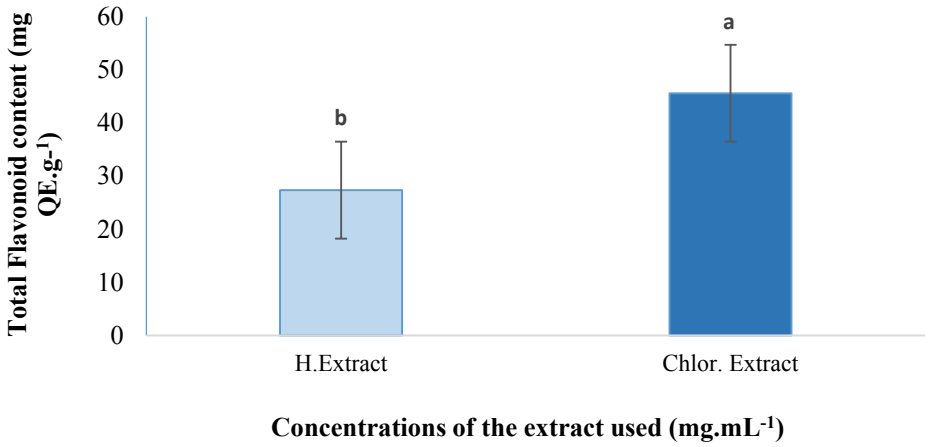

Figure 2. Total flavonoid contents of hexane and chloroform extracts of ABVT pulp.
Key: H. Extract = Hexane Extract; Chlor. Extract = Chloroform Extract, QE = Quercetin equivalent. The alphabets a&b show the levels of significant (p <0.05) difference between the extracts.

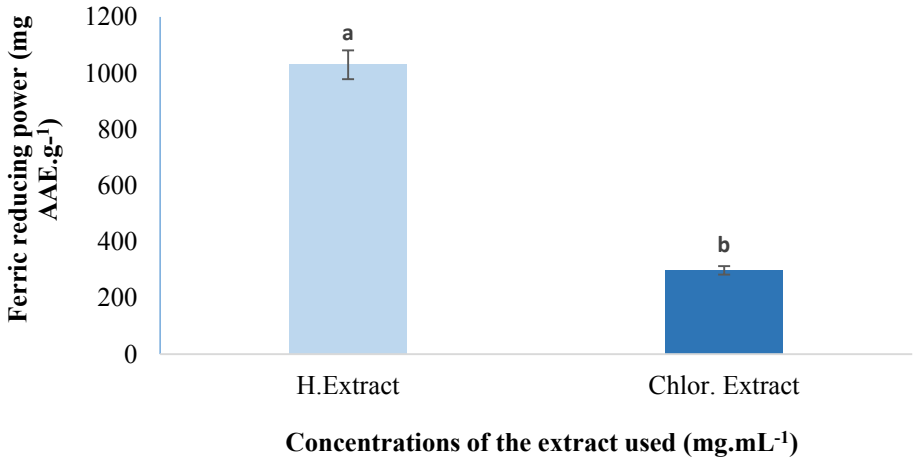

Figure 3. Ferric reducing abilities of hexane and chloroform extracts of ABVT pulp.
Key: H. Extract = Hexane Extract; Chlor. Extract = Chloroform Extract, AAE = Ascorbic acid equivalent. The alphabets a and b show the levels of significant (p <0.05) difference between the extracts.

capacity than that of chloroform extract. Whereas, in Figure 4, the disruption of *o*-phenanthroline-Fe^{2+} complex expressed as percentage inhibition in Figure 4, during incubation with both solvent extracts of ABVT fruit pulp, reveals that, H. Extract of ABVT pulp was also able to chelate Fe^{2+} considerably higher than Chlor. Extract. The ability of the H. Extract to chelate Fe^{2+} matched with the fact that, the H. Extract showed higher value for FRAP in

Figure 3. However, the chelating ability showed by these extracts could have been credited to the phenolic and flavonoid contents as reported by **Zhao et al. (2006)**. The possibility of an extracts/substances to chelate and deactivate transition metals by the antioxidant mechanism of action, has been said to prevent such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal-based catalytic reaction **(Oboh et al., 2007)**.

The Figure 5 relays the bleaching capacity of 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) chromogenic radical by antioxidant/reducing compounds present in ABVT pulp solvent extracts to its corresponding hydrazine **(Boligon et al., 2014)**. DPPH is a free radical donor that accepts an electron or hydrogen to become a stable diamagnetic molecule **(Afolabi and Oloyede, 2014)**. As shown in Table 1, there was no significant difference in the IC_{50} of the various concentrations of the solvent extracts of ABVT

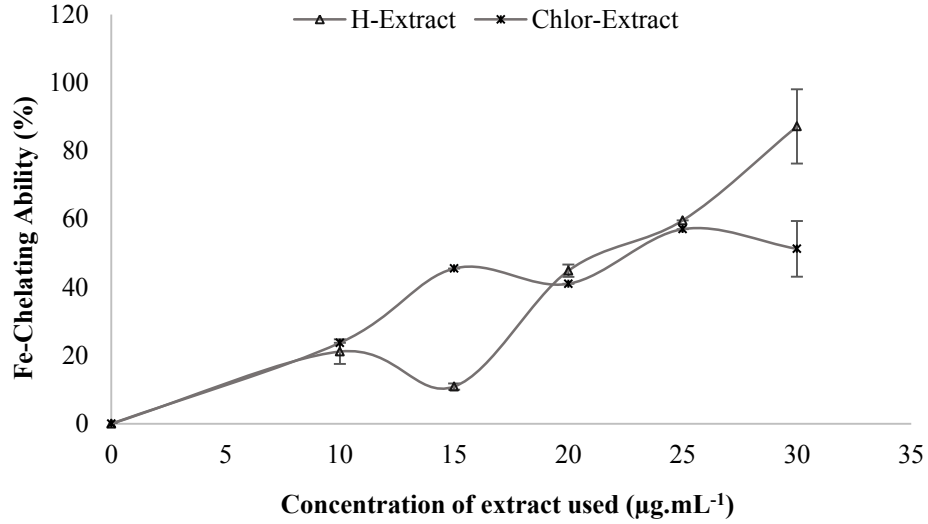

Figure 4 Fe^{2+} – chelating ability (%) of different solvent extracts of ABVT pulp.
Key: H. Extract = Hexane Extract; Chlor. Extract = Chloroform Extract.

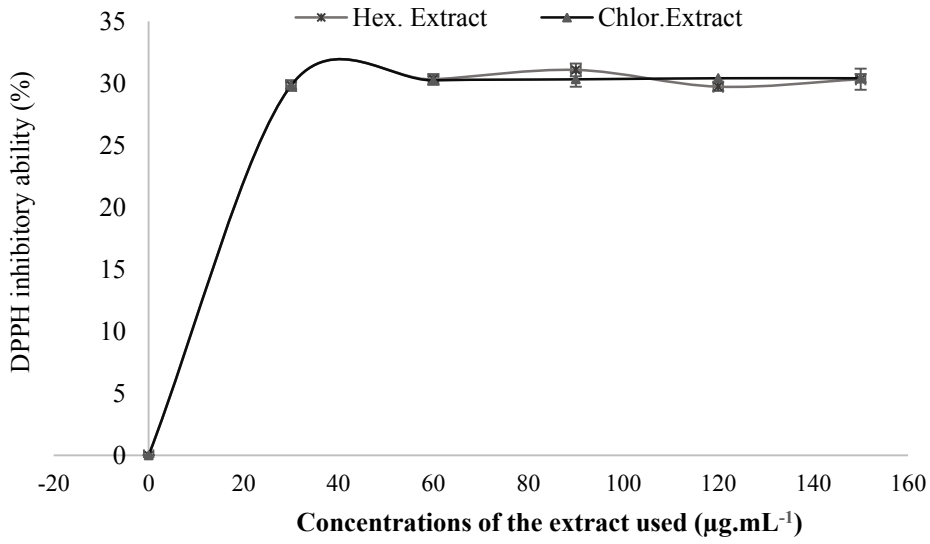

Figure 5 DPPH$^{\bullet}$ radical scavenging ability of different solvent extracts of ABVT pulp.
Note: Hex. Extract = Hexane Extract; Chlor. Extract = Chloroform Extract.

Table 1 The IC_{50} (µg.mL^{-1}) values (Concentration of the extracts that will cause 50 percent inhibition) of ABVT pulp solvent extracts calculated from a linear regression curve of the percentage (%) inhibitions against various concentrations of the extracts.

Assays(%)	Hexane extract	Chloroform extract
Fe^{2+}Chelation	30.16 ±2.16^{a}	23.94 ±1.35^{a}
DPPH Radical Scavenging	181.62 ±0.37^{a}	181.37 ±2.14^{a}
NO radical scavenging ability	107.20 ±2.79^{a}	73.24 ±0.63^{b}
Hydroxyl radical scavenging ability	131.61 ±13.55^{a}	131.61 ±0.49^{a}

Note: Results represent mean values (n = 2) ±SD. The values with the same superscript along the row are not significantly (p <0.05) different.

pulp examined. The inhibition of DPPH$^{\bullet}$ radical exhibited by both extracts, could be attributed to their various phenolic and flavonoid contents.

Nitric oxide (NO) is an important physiological messenger that participates in inflammatory processes which can directly/independently induce toxicity in the tissues resulting in vascular damage and other disorders at an increased level **(Moncada et al., 1991)**. The cellular damage is heightened when NO react with superoxide radical to form a non-radical peroxynitrite (ONOO-), which is a powerful oxidant **(Halliwell and Gutteridge 1999; Balavoine and Geletti, 1999)**. As presented in Figure 6, ABVT pulp solvent extracts showed considerable inhibitions against generation of NO induced radicals. The mechanisms of inhibition exhibited by the extracts are not known but could be ascribed to the presence of antioxidant contents, as ability of plants to demonstrate antioxidant properties against generation of oxidants have been linked to the presence of array of important phenolic and non-phenolic phytochemicals which includes phenolic acids, flavonoids and alkaloids **(Cheplick et al., 2007)**, however, this agrees with Figure 1 and 2.

The ability of the solvent fractions of ABVT pulp to prevent Fe^{2+}/H_2O_2 from causing decomposition of deoxyribose to generate OH radical through Fenton reaction was also carried out. H. Extract showed higher inhibitory effect against the production of hydroxyl radical at the very lower concentration than Chlor. Extract, the results are presented in Figure 7, with various IC_{50} in Table 1. This inhibition possibly could have been responsible for by the antioxidant parameters as shown in Figure 1 and 2 as well.

Likewise, incubation of both the liver as well as brain tissue homogenates with Fe^{2+} to test for the ability of

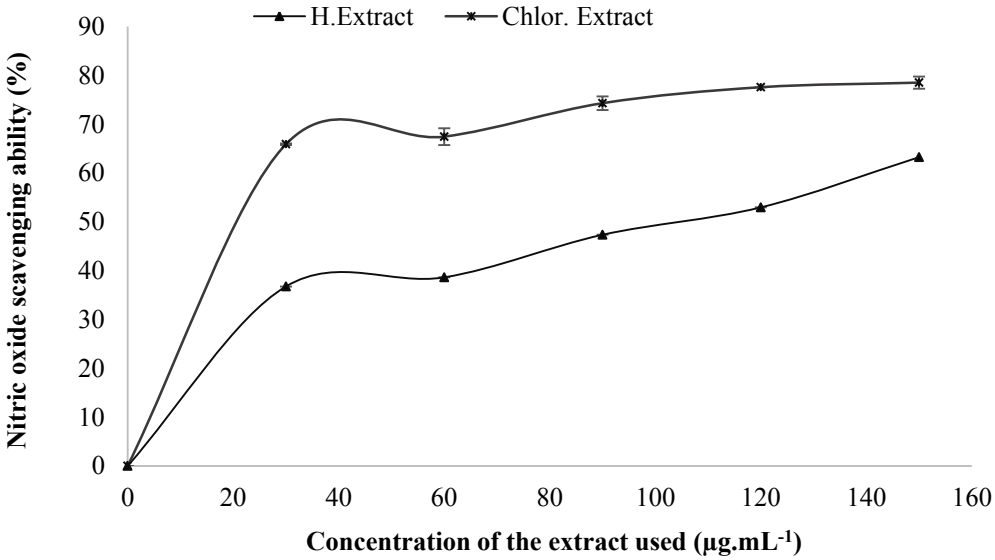

Figure 6 Nitric oxide inhibitory ability (%) of different solvent extracts of ABVT pulp.
Note: H. Extract = Hexane Extract; Chlor. Extract = Chloroform Extract.

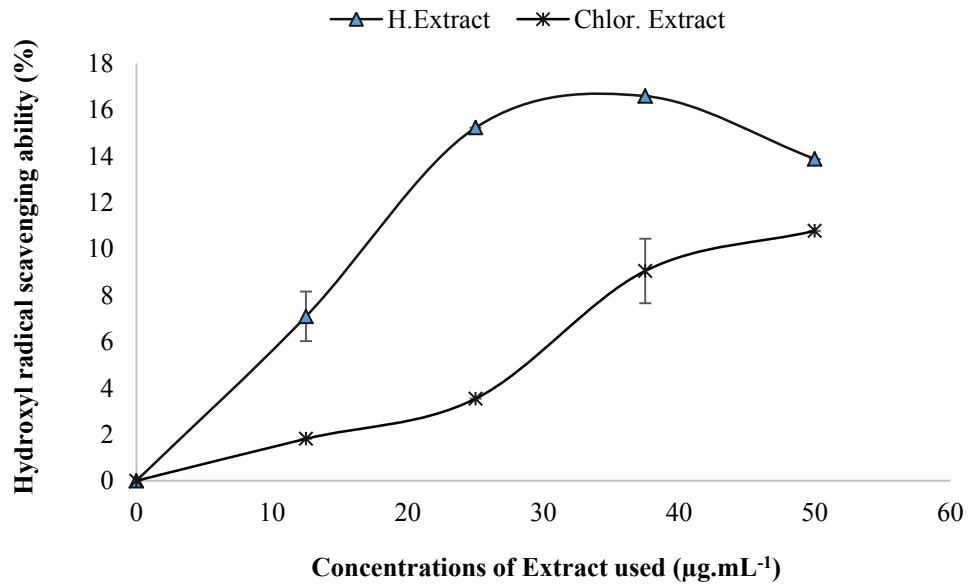

Figure 7 Hydroxyl radical scavenging ability (%) of different solvent extracts of ABVT pulp.
Note: H. Extra ct = Hexane Extract; Chlor. Extract = Chloroform Extract.

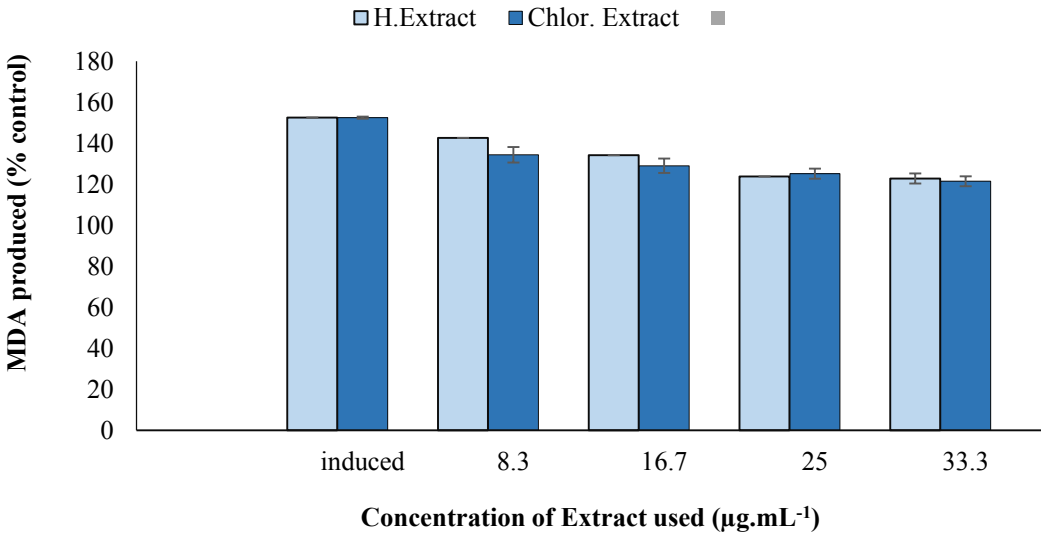

Figure 8a Inhibition of Fe^{2+} – induced lipid peroxidation in rat brain homogenate by different solvent extracts of ABVT pulp. **Key:** H. Extract = Hexane Extract; Chlor. Extract = Chloroform Extract.

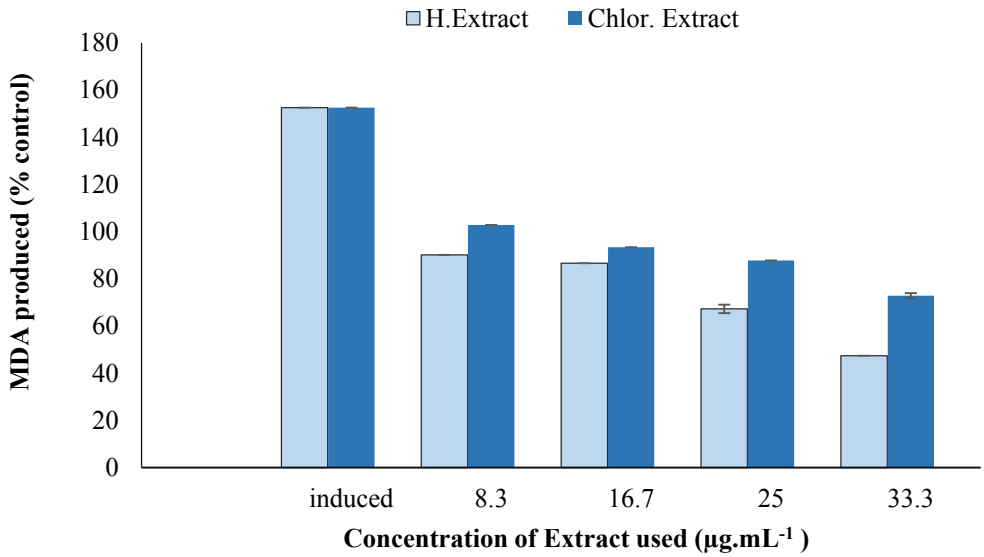

Figure 8b Inhibition of Fe^{2+} – induced lipid peroxidation in rat liver homogenate by different solvent extracts of ABVT pulp. Key: H. Extract = Hexane Extract; Chlor. Extract = Chloroform Extract.

ABVT pulp solvent extracts in inhibiting the formation of MDA was assayed for by lipid peroxidation reaction as shown in Figure 8 a and b. Lipid peroxidation is one of the major effects of ROS-mediated injury leading to the generation of a variety of relatively stable end products. ROS concentrations above the clearance capacity of the cell cause oxidative stress, mitochondrial dysfunction, cellular damage, in most cases, cell death **(Ferreiro et al., 2012)**. Several studies have implicated malondialdehyde (MDA), one of the byproducts as main indicator in monitoring the level of oxidative stress/damage caused by ROS in the tissues **(Aitken and Fisher, 1994)**. Incubation of both the liver and brain tissue homogenates with Fe^{2+} (iron II) induced oxidative stress, being perceptible by the increased MDA levels in homogenates in the absence of the extracts (Figure 8a and b). However, there were significant (p <0.05) reduction when the solvent extracts of ABVT pulp were introduced in the reaction with the various concentration considered in dose-dependent manner. The ability of the ABVT pulp solvent extract to inhibit formation of MDA could be traceable to the antioxidant properties of the extracts as being evidenced in Fe^{2+} chelation and/or scavenge free radicals produced by the Fe^{2+}-catalyzed production of reactive oxygen species (ROS), which is in agreement with Figure 4.

CONCLUSION

The potency of ABVT pulp solvent extracts having appraised, has indicated that the pulp, if incorporated in diet, could serve as an alternative in managing various

degenerative ailments as it has been clearly demonstrated in the protection of brain and liver from Fe^{2+}-induced oxidative stress. The mechanism for doing this has also been shown in Fe^{2+}-chelation, DPPH inhibition, nitric oxide inhibition and hydroxyl radical scavenging ability. All these could have been as a result of the antioxidant parameters in the form of phenolic/polyphenolic acid and flavonoids.

REFERENCES

Afolabi, O. B., Oloyede, O. I., Jaiyesimi, K. F., Obafemi, T. O., Awe, J. O., Fadaka, A. O. 2015. Antagonistic potentials of *Talinum triangulare* extracts against iron II – induced oxidative stress in tissue homogenates of wistar albino rat - in vitro. *World Journal of pharmacy and pharmaceutical sciences*, vol. 4, no. 6, p. 59-67.

Afolabi, O. B., Oloyede, O. I. 2014a. Antioxidant Properties of the Extracts of *Talinum Triangulare* and its Effect on Antioxidant enzymes in Tissue Homogenate of Swiss Albino Rat. *Toxicology International*, vol. 21, no. 3, p. 307-13. https://doi.org/10.4103/0971-6580.155377 PMid:25948971

Afolabi, O. B., Oloyede, O. I. 2014b. Comparative neuroprotective potentials of the extracts of *Talinum triangulare* against nitroprusside-induced damages in albino rats. *International Journal of Pharmacognosy*, vol. 1, no. 11, p. 705-708.

Agati, G., Azzarello, E., Pollastri, S., Tattini, M. 2012. Flavonoids as antioxidants in plants: location and functional significance. *Plant Science*, vol. 196, p. 67-76. https://doi.org/10.1016/j.plantsci.2012.07.014 PMid:23017900

Aitken, R., Fisher, H. 1994. Reactive oxygen species generation by human spermatozoa. *Bioessays*, vol. 16, no. 4, p. 259268. https://doi.org/10.1002/bies.950160409 PMid:8031303

Akomolafe, S. F, Oboh, G., Akindahunsi, A. A., Akinyemi, A. J., Oluwatosin, G. T. 2013. Inhibitory Effect of Aqueous Extract of Stem Bark of Cissus populnea on Ferrous Sulphate- and Sodium Nitroprusside-Induced Oxidative Stress in Rat's Testes *In Vitro*. *ISRN Pharmacology*, vol. 2013, p. 130989. https://doi.org/10.1155/2013/130989 PMid:23401792

Amic, D., Davidovic-Amic, D., Beslo, D., Trinajstic, N. 2003. Structure-radical scavenging activity relationship of flavonoids. *Croatica Chemica Acta*, vol. 76, no. 1, p. 55-61.

Andrea, L., Hóvári, J., Katalin, V. S., Lajos, B. 2003. The role of antioxidant phytonutrients in the prevention of diseases. *Acta biologica zegediensis*, vol. 47, no. 1-4, p. 119-125.

Balavoine, G. G., Geletti, Y. V. 1999. Peroxynitrite scavenging by different antioxidants. Part 1: convenient study. *Nitric Oxide*, vol. 3, no. 1, p. 40-54. https://doi.org/10.1006/niox.1999.0206 PMid:10355895

Bao, J., Cai, Y., Sun, M., Wang, G., Corke, H. 2005. Anthocyanins, flavonols and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. *Journal of Agricultural and Food Chemistry*, vol. 53, no. 6, p. 2327-2332. https://doi.org/10.1021/jf048312z PMid:15769176

Boligon, A. A., Machado, M. M., Athayde, M. L. 2014. Technical Evaluation of Antioxidant Activity. *Medicinal Chemestry*, vol. 4, p. 517-522. https://doi.org/10.4172/2161-0444.1000188

Britton, R. S., Leicester, K. L., Bacon, B. R. 2002. Iron toxicity and chelation therapy. *International Journal of Hematology*, vol. 76, no. 3, p. 219-28. https://doi.org/10.1007/BF02982791 PMid:12416732

Cheplick, S., Kwon, Y., Bhowmik, P., Shetty, K. 2007. Clonal variation in raspberry fruit phenolics and relevance for diabetes and hypertension management. *Journal of Food Biochemistry*, vol. 31, no. 5, p. 656-679. https://doi.org/10.1111/j.1745-4514.2007.00136.x

Dike, M. C. 2010. Proximate, phytochemical and nutrient compositions of some Fruits, Seeds and leaves of some Plant Species at Umudike, Nigeria. *ARPN Journal of Agricultural and Biological Science*, vol. 5, no. 1, p. 7-16.

Eziaku, O., James, A. O. 2014. Corrosion Inhibition of Copper Using African Black Velvet Tamarind (*Dialium indium*) Extract in Sulphuric Acid Environment. *Journal of Scientific Research & Reports*, vol. 3, no. 18, p. 2450-2458. https://doi.org/10.9734/JSRR/2014/9434

Ferreiro, E., Baldeiras, I., Ferreira, I. L., Costa, R. O., Rego, A. C., Pereira, C. F., Oliveira, C. R. 2012. Mitochondrial- and endoplasmic reticulum-associated oxidative stress in alzheimer's disease: From pathogenesis to biomarkers. *International Journal of Cell Biology*, vol. 2012, p. 735206. https://doi.org/10.1155/2012/735206 PMid:22701485

Geetha S., Sai-Ram, M., Mongia, S. S., Singh, V., Ilavazhagan, G., Sawhney, R. C. 2003. Evaluation of antioxidant activity of leaf extract of sea buckthorn (*Hippophae rhamnoides* L.) on chromium (VI) induced oxidative stress in albino rats. *Journal of Ethnopharmacology*, vol. 87, no. 2-3, p. 247-251. https://doi.org/10.1016/S0378-8741(03)00154-5

Gyamfi, M. A., Yonamine, M., Aniya, Y. 1999. Free radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally induced liver injuries. *General Pharmacology*, vol. 32, no. 6, p. 661-667. https://doi.org/10.1016/S0306-3623(98)00238-9

Halliwell, B., Gutteridge, J. M. 1981. Formation of thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS *Letters*, vol. 128, no. 2, p. 347-352. https://doi.org/10.1016/0014-5793(81)80114-7

Halliwell, B., Gutteridge, J. M. 1999. *Free radicals in biology and medicine.* 3rd ed. Oxford, UK : Oxford University Press, p. 936. ISBN 10: 0198552912.

Mary, N. K., Achuthan, C. R., Babu, B. H., Padikkala, J. 2003. *In vitro* antioxidant and antithrombotic activity of *Hemidesmus indicus* (L) R.Br. *Journal of Ethnopharmacology*, vol. 87, no 2-3. p. 187-191. https://doi.org/10.1016/S0378-8741(03)00119-3

Meir, S., Kanner, J., Akiri, B., Philosoph-Hadas, S. 1995. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *Journal of Agricultural and Food Chemistry*, vol. 43, no. 7, p. 1813-1819. https://doi.org/10.1021/jf00055a012

Mercocci, L., Marguire, J. J., Droy-Lefaiz, M. T., Parker, L. 1994. The nitric oxide-scavenging properties of Ginko biloba extract EGB 761. *Biochemical and Biophysical Research Communications*, vol. 201, no. 2, p. 748-55. https://doi.org/10.1006/bbrc.1994.1764

Moncada, S., Palmer, R. M., Higgs, E. A. 1991. Nitric oxide: physiology, pathophysiology and pharmacology.

Pharmacological Reviews, vol. 43, no. 2, p. 109-42. PMid:1852778

Nwaukwu, I. A., Ikechi, N. C. 2012. Biochemical Changes Induced by the Effect of Six Pathogenic Fungi on *Dialium guineense*: Black Velvet Edible Fruit. *IOSR Journal of Pharmacy and Biological Sciences (IOSRJPBS)*, vol. 2, no. 4, p. 20-24. https://doi.org/10.9790/3008-0242024

Oboh, G., Akinyemi, A. J., Ademiluyi, A. O. 2013. Inhibitory Effect of Phenolic Extract from Garlic on Angiotensin-1 Converting Enzyme and Cisplatin induced Lipid Peroxidation – *In Vitro*. *International Journal of Biological Sciences*, vol. 9, no. 2, p. 98-106.

Oboh, G., Puntel, R. L., Rocha, J. B. T. 2007. Hot pepper (*Capsicum annuum*, Tepin and *Capsicum chinese*, Habanero) prevents Fe2+-induced lipid peroxidation in brain – *in vitro*. *Food Chemistry*, vol. 102, no. 1, p. 178-185. https://doi.org/10.1016/j.foodchem.2006.05.048

Ohkawa, H., Ohishi, N., Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reactions. *Analytical Biochemistry*, vol. 95, no. 2, p. 351-8. https://doi.org/10.1016/0003-2697(79)90738-3

Okegbile, E. O., Taiwo, E. A. 1990. Nutritional Potentials of Velvet Tamarind (*Dialium guineense* Wild). *Nigerian Food Journal*, vol. 8, p. 115-121.

Pulido, R., Bravo, L., Saura-Calixto, F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, vol. 48, no. 8, p. 396-3402. https://doi.org/10.1021/jf9913458

Puntel, R. L., Nogueira, C. W., Rocha, J. B. T. 2005. Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in Rat Brain *In vitro*. *Neurochemical Research*, vol. 30, no. 2, p. 225-235. https://doi.org/10.1007/s11064-004-2445-7 PMid:15895826

Raghuveer, R., Sindhoori, T., Sanjeeva, K. A., Raju, C., Valya, N. 2011. Evaluation of *In-Vitro* Antioxidant and Free Radical Scavenging Activities of Punicagranatum Pericarp Extract. *International Journal of Pharmaceutical and Phytopharmacological Research*, vol. 1, no. 2, p. 49-55.

Rani, J. S. 2013. The efficacy of aqueous extract of Talinum triangulare on carbohydrate metabolic, non-enzymatic antioxidants and anti-oxidative enzymes in alloxan induced diabetic rats. *Archives of Applied Science Research*, vol. 5, no. 2, p.197-202.

Shalaby, E. A., Shanab, S. M. M. 2013. Antioxidant compounds, assays of determination and mode of action. *African Journal of Pharmacy and Pharmacology*, vol. 7, no. 10, p. 528-539. https://doi.org/10.5897/AJPP2013.3474

Shimoi, K., Masuda, S., Shen, B., Furugori, M., Kinze, N. 1996. Radioprotective effects of antioxidative plant flavonoids in mice. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, vol. 350, no. 1, p. 153-161. https://doi.org/10.1016/0027-5107(95)00116-6

Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin– Ciocalteu reagent. In Packer, L. *Methods in enzymology*. San Diego, California : Academic Press, p. 152-178.

Zar, J. H. 1984. *Biostatistical analysis*. New Jersey : Prentice-Hall, p. 620. ISBN 0-13-081542-X.

Zhao, H., Dong, J., Lu, J., Chen, J., Li, Y., Shan, L., Lin, Y., Fan, W., Gu, G. 2006. Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.). *Journal of Agricultural and Food Chemistry*, vol. 54, no. 19, p. 7277-86. https://doi.org/10.1021/jf061087w PMid:16968094

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HEATING AND DEHYDRATION OF GRAIN AND CEREALS AT A COMBINED ENERGY SUPPLY

Sergey Zverev, Otari Sesikashvili

ABSTRACT

The paper dwells on the development of experimental dependencies of heating and dehydration of grain and cereals when varying the irradiance, ambient temperature in the heat treatment zone and the initial moisture content of product, and the development of the mathematical models for heating and dehydration of some grains and cereals. The grain was heated on the laboratory equipment with quartz halogen linear infrared emitters. The irradiance on the working surface in the treatment zone was determined by calculation using a specially developed program. The ambient temperature was determined by a thermocouple thermometer placed in a ceramic tube. The grain temperature was estimated as average by weight by a thermocouple thermometer after its transfer into a thermally insulated container. The following dependencies have been obtained: 1 – Temperature dependence of the heating time for different heating modes and initial moisture content. 2 – Dependence of moisture content on the heating time under different conditions and initial moisture content. 3 – Dependence of moisture content on a temperature under different conditions and constant initial humidity. The models of the heat-moisture exchange and dehydration processes have been created, and the model parameters K_0 and K_T of the temperature dependence of some grains have been identified, as well as their dependence on moisture content and treatment modes has been evaluated. It has been established that this model describes adequately the process of dehydration to an extent limited by the upper temperature value of grain not much more than 100 ºC. Within not limited to the upper temperature value of grain not much more than 100 ºC. From the presented graphs (Figures 1.24 – 1.26) and earlier obtained results for barley and millet, it can be assumed that the model describes adequately experimental data on the small-sized (3 – 5 mm) objects.

Keywords: IR; radiation; grain; dehydration; heat

INTRODUCTION

Heat treatment, in particular, through the use of the radiative (infrared) energy supply, is an operation fairly common in the technological processes of processing food products, including grain. **(Pan and Atungalu, 2002; Zverev, 2009)**. Heat transfer is carried out in two ways: by convective method from the air medium in the processing zone, and by radiation method (infrared radiation). It could therefore be spoken of a combined heat supply. Industrial installations based on this principle of heating are used in small and medium-sized grain processing enterprises in the processes of production of instant cereals, cereal flakes, feed ingredients, including for decontamination and inactivation of anti-nutrients. The heating process, as a rule, is carried out at a high thermal head and is limited to the time of the beginning of darkening of the grain surface. The temperature of product varies continuously throughout the processing period, that is, the process is of a substantially non-isothermal nature.

The change in temperature during the heating process is paralleled by dehydration of product. The temperature and final grain-moisture content of the in the process of heating by infrared (IR) radiation is determined by a number of factors: heating time, initial grain-moisture content, and heat treatment modes (irradiance, ambient temperature within the exposure zone). As a rule, in industrial installations the modes are conditioned by the design, and they rarely change during the operation, even if such an option is available. The moisture content of source raw material depends on its conditions on delivery, storage conditions and can vary, sometimes within rather wide limits. The outlet temperature of product is controlled by its residence time within the exposure zone. The heating process, as a rule, is carried out at a high thermal head and is limited to the time of the beginning of darkening of the grain surface. The temperature of product varies continuously throughout the treatment period, that is, the process is of a substantially non-isothermal nature.

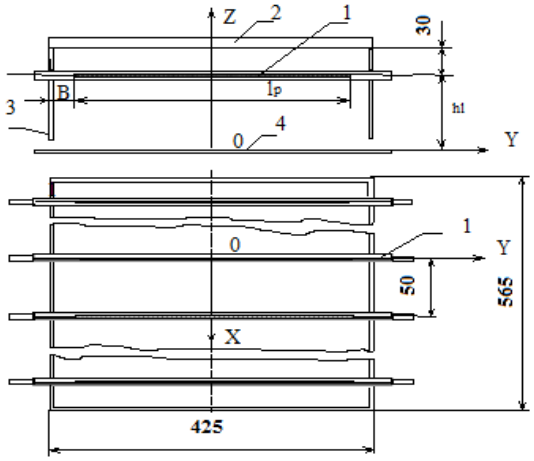

Figure 1 Diagram of the experimental-industrial block of the emitters: 1. The KGT-1000-220-type infrared source; 2 – Top reflector; 3 – Side reflector; 4 – A working surface of the processing zone.

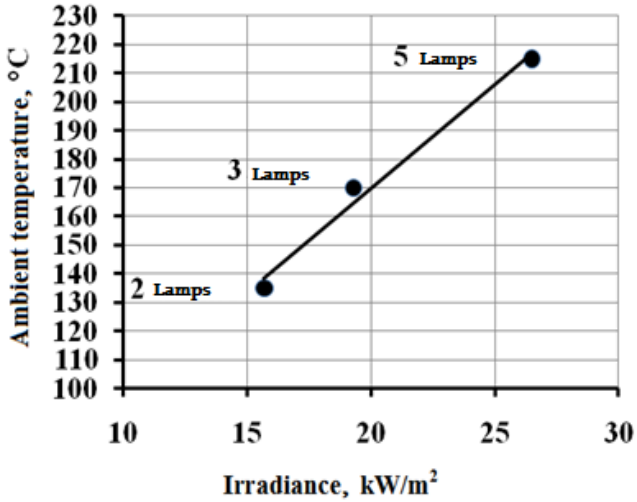

Figure 2 The dependency diagram of the ambient temperature in the treatment zone on the irradiance.

The work was aimed at obtaining the experimental dependencies and developing the models of heating and dehydration of grain (cereal) by varying irradiance, ambient temperature in the heat treatment zone of infrared heating of grain and cereals, and the initial moisture content of product.).

Scientific hypothesis

The temperature and final grain-moisture content of the in the process of heating by infrared (IR) radiation is determined by a number of factors: heating time, initial grain-moisture content, and heat treatment modes (irradiance, ambient temperature within the exposure zone). There is a correlation dependence of moisture content on the temperature of product, as well as influence of above listed factors on it. There is a probability of its invariable towards some of these factors.

MATERIAL AND METHODOLOGY

Heating of grain was carried out on the laboratory equipment with the KGT-1000-220-type quartz halogen linear infrared emitters.

Diagram of the equipment is shown in Figure 1.

The irradiance on the working surface in the processing zone was determined by calculation using a specially developed program. Variation by the number of the emitters, height of their installation above the monolayer of grain product and the type of pallet allowed changing independently, under a limited range, the irradiance and ambient temperature in the treatment zone. Change in the number of lamps, i.e. of the installed total power of the emitters in a fixed closed volume of the working area of the laboratory equipment leads to an increase in the ambient temperature. The dependency diagram of the irradiance and ambient temperature in the treatment zone when the lamps are installed at an altitude hl = 100 mm, is shown in Figure 2.

Grain was arranged in a monolayer on a pallet, which for a fixed time was placed in a heated treatment zone. Then it was poured into a thermally insulated container, where the average temperature of its mass was determined by using a thermocouple and an electronic thermometer.

As the subjects of research, there were used triticale (Triticosecale Wittmack) grain (the average thickness – 2.8 mm, the average weight of corn seed – 0.050 g), spelt (Triticum spelta) cereal (the average thickness – 2.1 mm, the average weight of corn seed – 0.031 g) and white

lupine (Lupinus albus) grain (the average thickness 4.4 mm, the average weight of corn seed – 0.23 g).

The significant factors influencing the rate of grain heating at a high-temperature micronization (HTM) are heat treatment modes (ambient temperature, irradiance on the surface of the monolayer) and moisture capacity of product. The experiment plans included the widest possible ranges of factors, however, for technical reasons, it had been impossible to cover the high irradiance region at a low ambient temperature.

The ambient temperature was determined by means of a thermocouple thermometer placed in a ceramic tube with a high reflection ratio in the infrared spectrum. The grain temperature was estimated as the average by weight by a thermocouple thermometer after its transfer into a thermally insulated container (reproducibility of the results was approximately ±3 °C), humidity - according to GOST (State Standard) 13586.5-2015 (Grain. Method for determination of moisture content).

Statisic analysis

Nonlinear modeling was carried out by using an application software package "STADIA-6", developed at the M.V. Lomonosov Moscow State University, **(Kulaychev, 1999)**.

Scoping the adequacy of the models is a complex procedure, requiring high computational costs, which are rapidly growing with dimensions of space of external parameters. By the volume, this task may greatly exceed the task of parametric optimization of a model itself (especially in the case of a nonlinear model), that's why for the newly-designed objects, it may not be resolved. Some indication of the adequacy of the models is provided by the Squared multiple correlation, R2, (Table.1 and 2). In addition, directly in the diagrams in Figure 21 – 26, we can see that the residual dispersion and the dispersion medium differ considerably.

RESULTS AND DISCUSSION

The relationships between a temperature and a heat time at different heat modes and initial moisture content

Figures 3 – 5 illustrate the empirical relationships between a temperature increment of some types of grain and cereals and the time ΔT(t) at different heat modes.

It is obvious that with the intensification of heat supply (by increasing the ambient temperature and/or the irradiance or the emitter power), the heating rate is increasing, that is, the heat time to a fixed temperature is reduced.

The effect of the initial moisture content on the nature of the products temperature change can be seen in Figures 6 – 8.

With increasing moisture content for fixed heating times and modes, the temperature slightly reduces.

The relationship between a moisture content and a heat time at different heat modes and initial moisture content

Similar relationships for the relative loss of moisture ΔU(t) / U_0 (relative to the initial moisture content) are shown in Figures 9 – 11. As can be seen, the rate of moisture loss depends heavily on the heat modes.

The relationship of a moisture content on a temperature at different heat modes and constant moisture content

Reconstruct the graphs of moisture loss into the functions of the temperature. The results are shown in Figures 12 – 14.

As can be seen, the relationships U(T) are practically invariant to heat modes, but they depend on the initial moisture content.

The morphology of the moisture content/temperature curves in the general case can be described as follows. At the initial stage of heating, a significant decrease in moisture content is observed, then the moisture content is decreasing, then it is diminishing by law close to linear, at

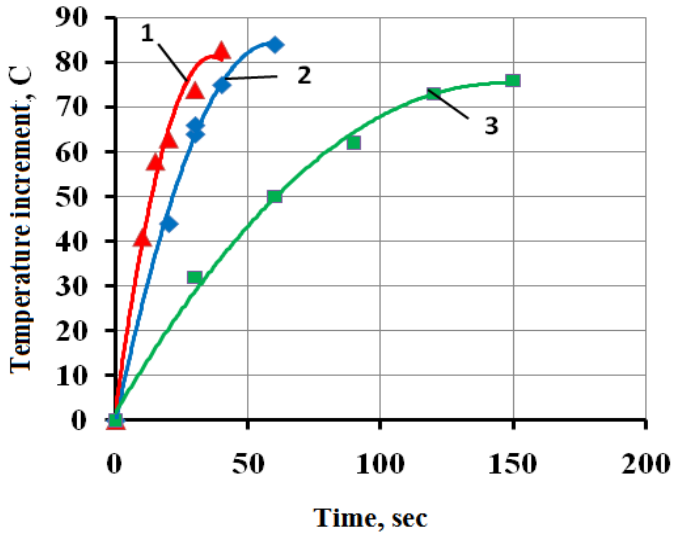

Figure 3 The relationship between a temperature increment of a RUNO-type spelt with initial moisture content of 18%, and a heat time at the different irradiance and ambient temperature T_c: 1 – E=23 kW.m^{-2}, T_c = 303 ºC; 2 – E = 11 kW.m^{-2}, T_c = 270 ºC; 3 – E = 0 kW.m^{-2}, T_c = 212 ºC.

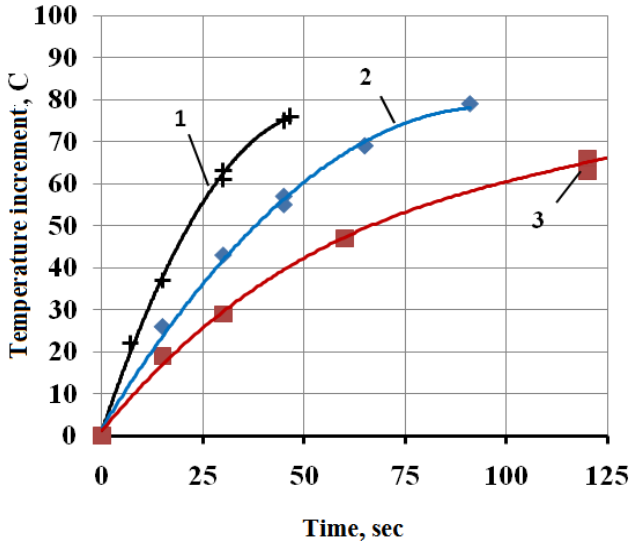

Figure 4 The relationship between a temperature increment of triticale and initial moisture content of 15%, with a heat time at the different irradiance and E ambient temperature T_c: 1 – E = 16 kW.m^{-2}, T_c = 271 ºC; 2 – E = 10 kW.m^{-2}, T_c = 204 ºC; 3 – E = 0 kW.m^{-2}, T_c = 148 ºC.

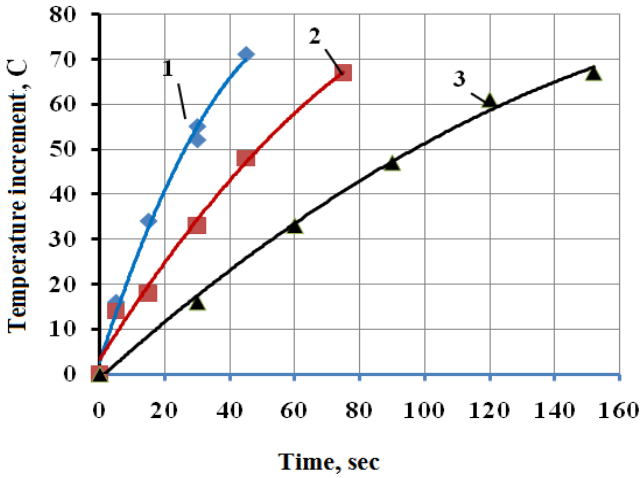

Figure 5 The relationship between a temperature increment of a DEGA-type white lupine with initial moisture content of 18%, and a heat time at the different irradiance and ambient temperature T_c: 1 – E = 22 kW.m^{-2}, T_c = 301 ºC; 2 – E = 13 kW.m^{-2}, T_c = 223 ºC; 3 – E = 0 kW.m^{-2}, T_c = 177 ºC.

the temperatures around 100 °C the rate of moisture loss increases again, and then decreases, falling to zero. The initial stage can be explained by the increased initial moisture content of the near-surface layers caused by the inadequate time of binning and by the non-aligned moisture content in the grain volume, or by the presence of a shell with different from kernel gyroscopic properties (for example, in lupine). The section near the temperature of 100 °C is associated with the intensification of evaporation near the critical point, and is due to the "burning out" of moisture. The nature of the dependence of a particular curve depends on product, initial moisture content, heat modes, the temperature range under consideration.

Modeling of the processes of heat-moisture exchange

There have been put forward many mathematical models. In general, the processes of heat and moisture exchange are interrelated and described by a system of corresponding nonlinear differential equations, which, as a rule, cannot be solved analytically. The application of numerical methods is also practically impossible, since the series of coefficients that are included in the equations are not defined. Therefore, we have to resort to various kinds of quite oversimplified assumptions and simplifications.

Let's use the solution obtained by A.V. Lykov in the form of exponential series **(Lykov and Mikhailov, 1963)**.

$$Y = 1 + a_1 \exp(-K_1 t) + a_2 \exp(-K_2 t) + a_i \exp(-K_i t), \quad (1)$$

where $Y = (T - T_0)/(T_\infty - T_0)$ – for the temperatures;
$Y = (U - U_0)/(U_\infty - U_0)$ – for moisture content;
T – temperature;
T_0 – wetting temperature;
T_∞ – temperature at $t \to \infty$;
U – moisture content;
U_0 – initial moisture content;
U_∞ –moisture content at $t \to \infty$;
t – time;

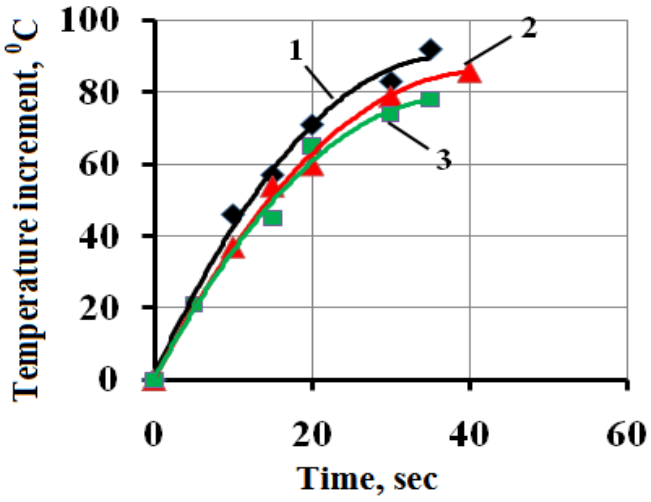

Figure 6 The relationship between a temperature increment of a RUNO-type spelt cereal and a heat time (the irradiance – 23 kW.m^{-2}, ambient temperature – 303 ºC) at moisture content, %: 1 – 12; 2 – 16; 3 – 22.

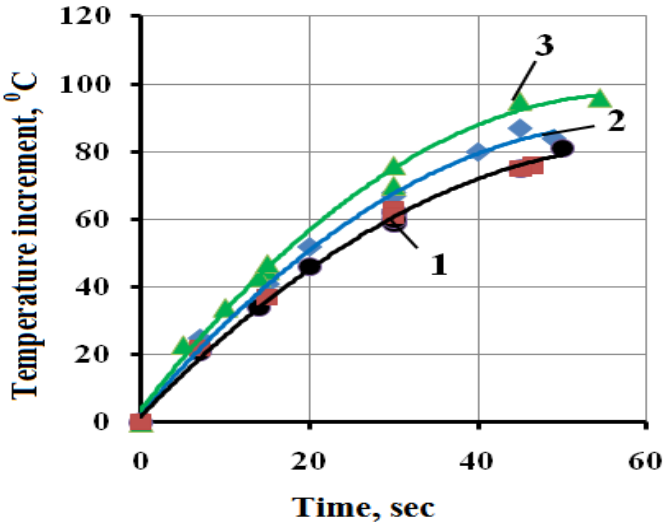

Figure 7 The relationship between a temperature increment of triticale grain and a heat time (the irradiance – 16 kW.m^{-2}, ambient temperature – 300 ºC) at moisture content, %: 1 – 18; 2 – 13; 3 – 10.

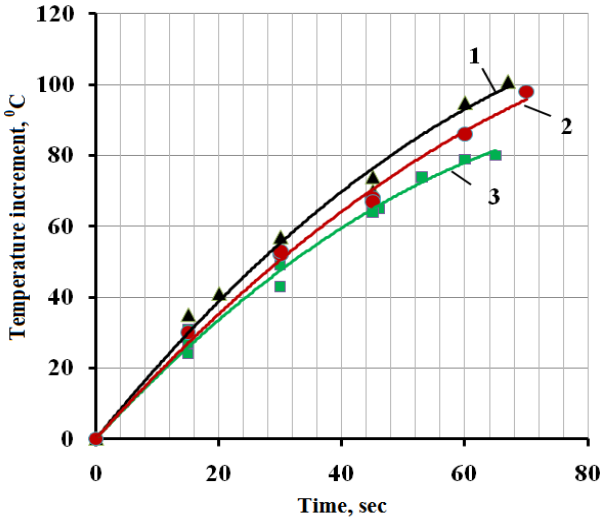

Figure 8 The relationship between a temperature increment of white lupine and a heat time (the irradiance – 15 kW.m^{-2}, ambient temperature – 263 ºC) at moisture content, %: 1– 7; 2 – 12; 3 – 17.

K – coefficients.

100 °C, and the rate of moisture loss increases sharply.

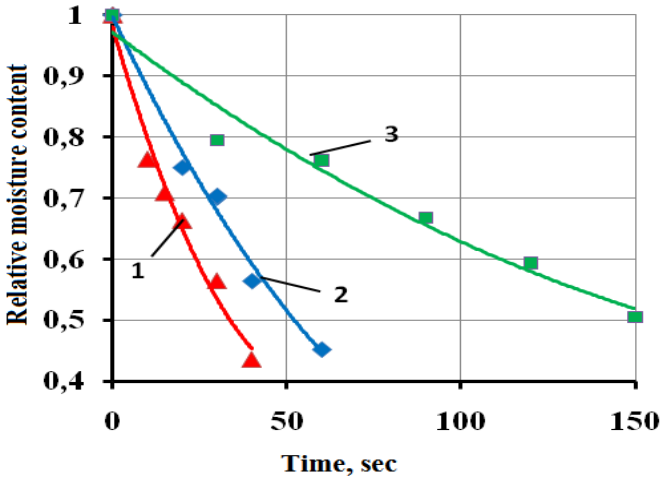

Figure 9 The relationship between a relative moisture content of a RUNO-type spelt cereal with initial moisture content of 18%, and a heat time at the different irradiance E and ambient temperature T_c: 1 – E = 23 kW.m^{-2}, T_c = 303 ºC; 2 – E = 11 kW.m^{-2}, T_c = 270 ºC; 3 – E = 0 kW.m^{-2}, T_c = 212 ºC.

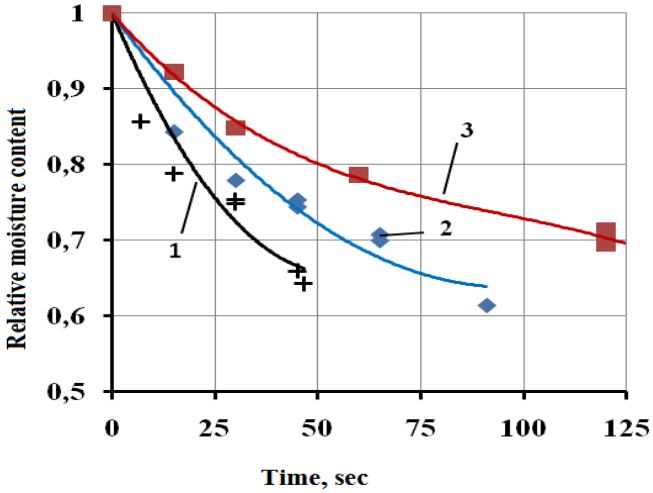

Figure 10 The relationship between a relative moisture content of triticale with initial moisture content of 18%, and a heat time at the different irradiance E and ambient temperature T_c: 1 – E = 16 кВт/м^2, T_c = 271 ºC; 2 – E = 10 кВт/м^2, T_c = 204 ºC; 3 –E = 0 кВт/м^2, T_c = 148 ºC.

A model of the convective-radiation heating

Taking advantage of using the first two terms of the series (1), and setting the coefficients to be constant, for the initial conditions ΔT(0) = 0, a model of heating can be represented in the form

$$\Delta T(t) = K_0[1 - \exp(-K_t t)]. \quad (2)$$

where $\Delta T = (T - T_0)$,

K_0, K_t – empirical coefficients.

The coefficients are assumed to be constant in time, but dependent on the heating conditions (initial humidity, irradiance and ambient temperature in the treatment zone). It is obvious that the condition of constancy (slight change) in the coefficients is not satisfied in the entire temperature range. It is known from the experiments that the intensive dehydration begins near the temperature of product at

Therefore, the proposed dependence can be regarded as an initial, rather rude approximation.

Analysis of the coefficients, including on a number of other grain crops as well, has revealed a correlation of K_0 with the initial moisture content, and of K_t with treatment modes.

As a rule, in industrial installations, the irradiance and temperature in the treatment zone are constant, i.e. K_t = const. From the batch to the batch of grain, its moisture content may change, which primarily affects the coefficient K_0.

Correlation between the model coefficients and moisture content and heat treatment modes

Based on the results of multi-factorial experiment, the parameters K_0 and K_t of the temperature dependence (4) for white lupine grain, triticale, and spelt cereal were identified, and their dependence on moisture content and treatment modes was evaluated. The results are shown in Figure 15 and Figure 16.

The effect of moisture content was evaluated at fixed heat treatment modes. From the graphs in Fig. 15, the correlation of the coefficient K_0 with moisture content is clearly visible, while for K_t such a correlation is not observed (Figure 16).

It is a little more difficult to evaluate the effect of heating

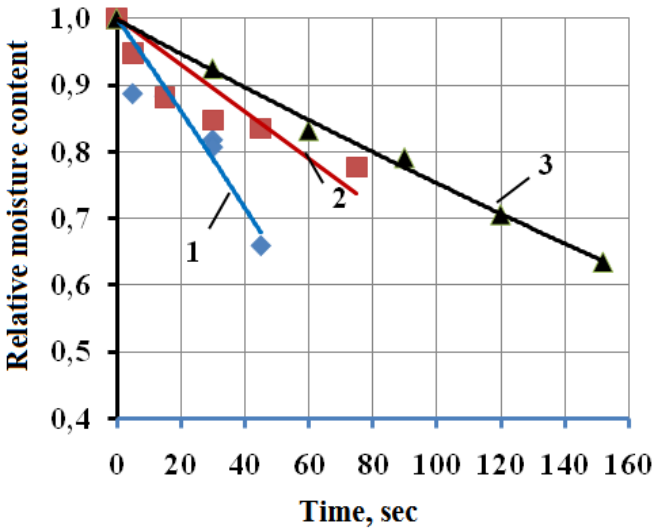

Figure 11 The relationship between a relative moisture content of a DEGA-type white lupine with initial moisture content of 15%, and a heat time at the different irradiance E and ambient temperature T_c: 1 – E=22 кВт/м^2, T_c=301 ºC; 2 – E=13 кВт/м^2, T_c=223 ºC; 3 – E=0 кВт/м^2, T_c=177 ºC.

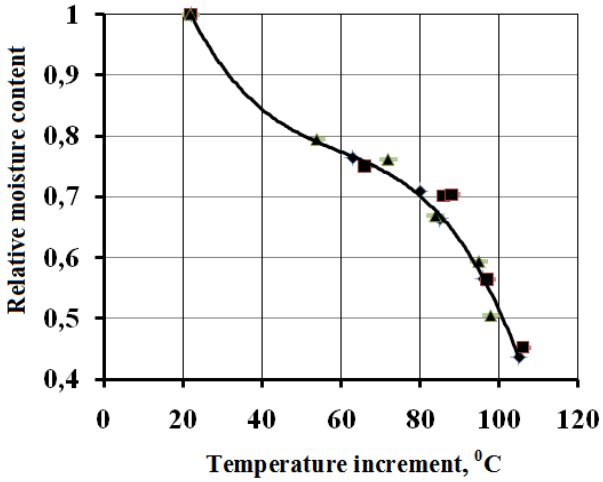

Figure 12 The relationship between a relative moisture content of a RUNO-type spelt cereal with initial moisture content of 18%, and a temperature at the different irradiance E = 0-23 kW/m^2 and ambient temperature T_c = 212-303 ºC.

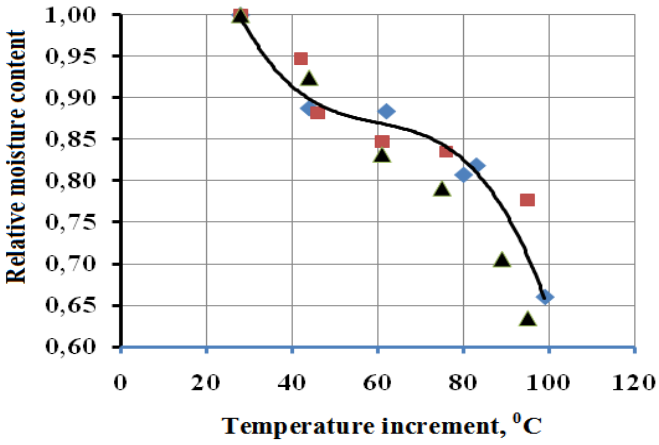

Figure 13 The relationship between a relative moisture content of triticale with initial moisture content of 18%, and a temperature at the different irradiance E = 0-16 kW.m^{-2} and ambient temperature T_c = 148-271 ºC.

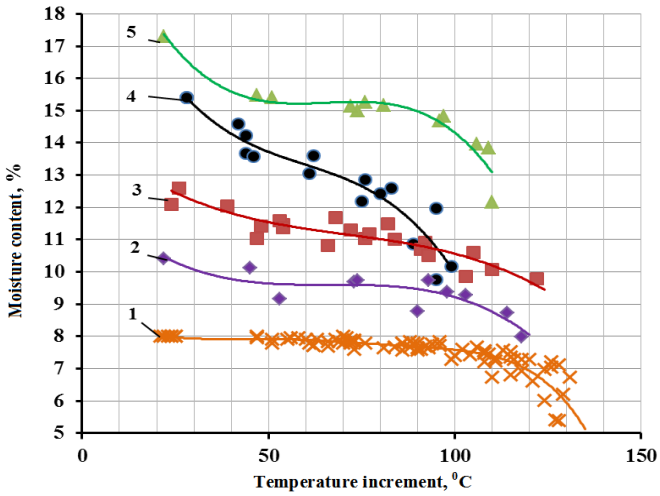

Figure 14 The empirical relationships between the change of moisture content of white lupine grain and a temperature at the different initial moisture contents and heat: 1 – initial moisture content – 8%, irradiance – 0 – 17 kW.m^{-2}, ambient temperature 157-308 ºC; 2 – initial moisture content – 10%, irradiance – 14 kW.m^{-2}, ambient temperature – 263 ºC; 3 – initial moisture content – 12%, irradiance – 0 – 22 kW.m^{-2}, ambient temperature – 183 – 301 ºC; 4 – initial moisture content – 15%, irradiance – 0 – 22 кВт/м^2, ambient temperature – 177 – 301 ºC; 5 – initial moisture content – 17%, irradiance – 14 кВт/м^2, ambient temperature – 263ºC.

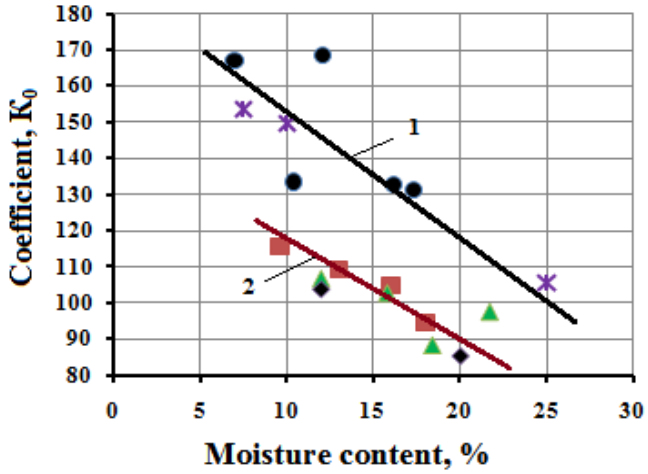

Figure 15 The dependence of coefficient K_0 on moisture content: 1 – lupine, the sunflower seed; 2 –triticale, spelt cereal and pearl barley.

modes, since a change in the irradiance leads to a change in the ambient temperature, and the evaluation of energy activity of the medium causes some difficulties.

A generalized model

Proceeding from the results of the analysis of the dependences of the model coefficients (2) on the initial moisture content of cereal and the ambient temperature in the treatment zone, a generalized model is proposed in the following form

$$\Delta T(t) = K0\,(1- KwW)\{\,1 - \exp[-Kt(E + KT\,\Delta Tc)\,t]\}. \quad (3)$$

Based on the results of a complete set of initial experimental data, the model parameters were identified taking into account the effect not only of moisture content, but also of heat treatment modes. The results of identification of model parameters (3) are given in Table 1.

A model of dehydration

We shall use the relationship (1). We shall confine ourselves to the first approximation, which, after substituting the model for the heating time (2) and the transformations, leads to a model for the relative current moisture content, as a function of a temperature increment

$$U/U_0 = (1 - \Delta T/ k_0)^C, \quad (4)$$

where ΔT – temperature increment, k_0 and C – empirical coefficients.

The value of the parameter k_0 can be taken from the results of the identification of the heating model

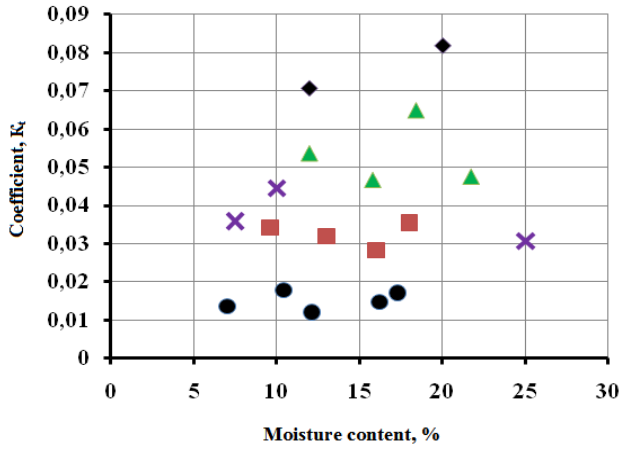

Figure 16 The dependence of coefficient K_t on moisture content: 1 – lupine, the sunflower seed; 2 –triticale, spelt cereal and pearl barley: ▲ – spelt; ■ – triticale; ● – lupine; ♦ – pearl barley; × – the sunflower seed.

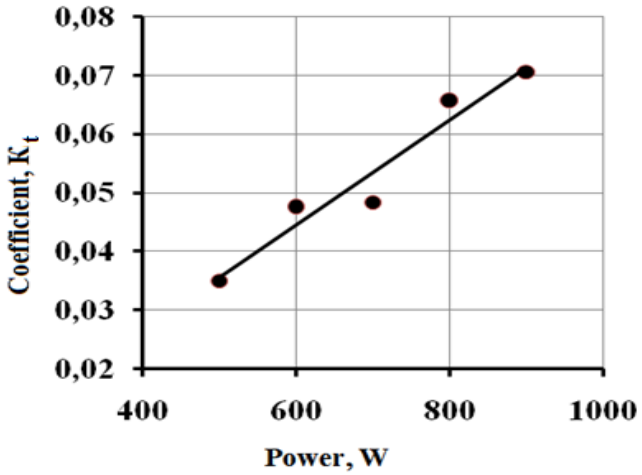

Figure 17 The dependence of coefficient K_t on the grossed installed capacity in the treatment zone of infrared emitters for pearl barley.

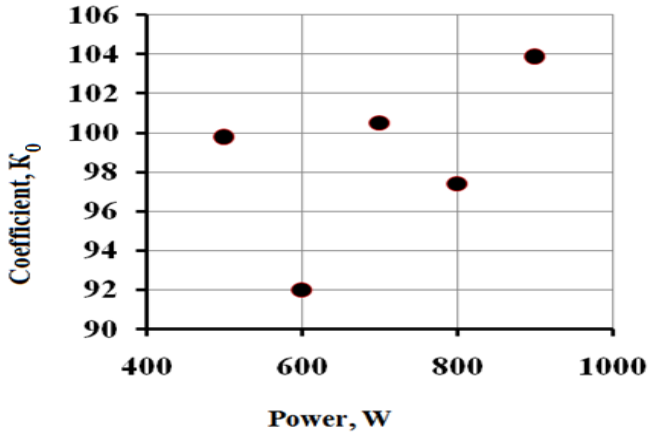

Figure 18 The dependence of coefficient K_0 on the grossed installed capacity in the treatment zone of infrared emitters

parameters or, just as the parameters B and C, may be identified by the results of dehydration experiments.

Identification of the parameter B and C by the results of the experiments with a wide variation in the initial moisture content, irradiance and temperature in the treatment zone, has shown a dependence on the initial moisture content and the absence of correlation with heating modes. As a result, a model is proposed

$$U/U_0 = (1 - \Delta T\, U_0/\, K_0)^C, \quad (5)$$

where U_0 – initial moisture content.

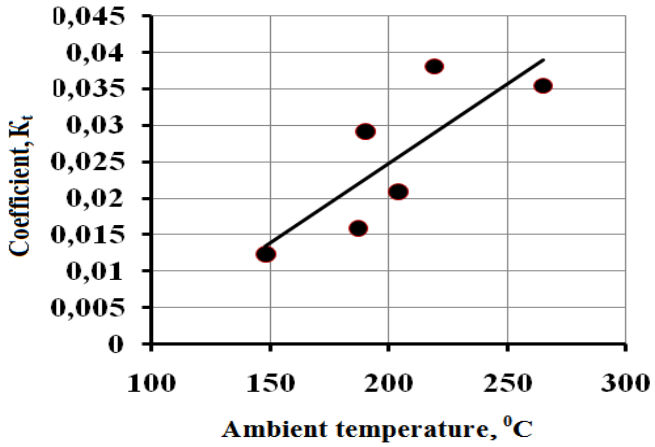

Figure 19 The dependence of coefficient K_t on the ambient temperature in the treatment zone for triticale grain.

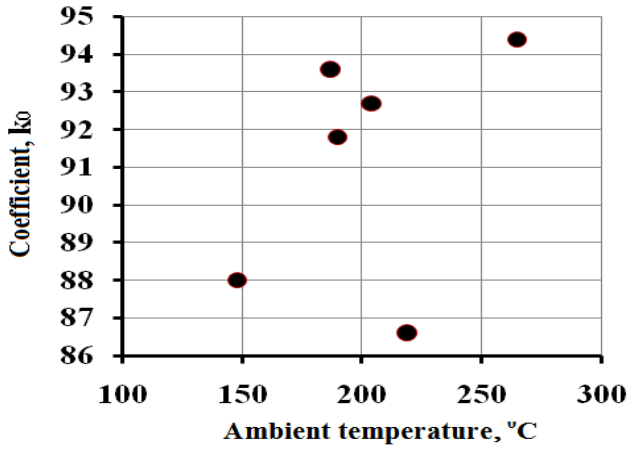

Figure 20 The dependence of coefficient K_0 on the ambient temperature in the treatment zone for triticale grain.

Table 1 Model parameters (3).

Type of product	K_0	K_T	K_t	K_w	Squared multiple correlation, R^2
Triticale grain	113.0	0.0714	0.00129	0.0125	0.993
Spelt cereal ("Runo")	121.1	0.0444	0.00149	0.0134	0.995
White lupine ("Dega")	128.3	0.2144	0.000298	0.00567	0.990

Calculated by a model (3) and experimental values of grain temperature are shown in Figures 21 – 23.

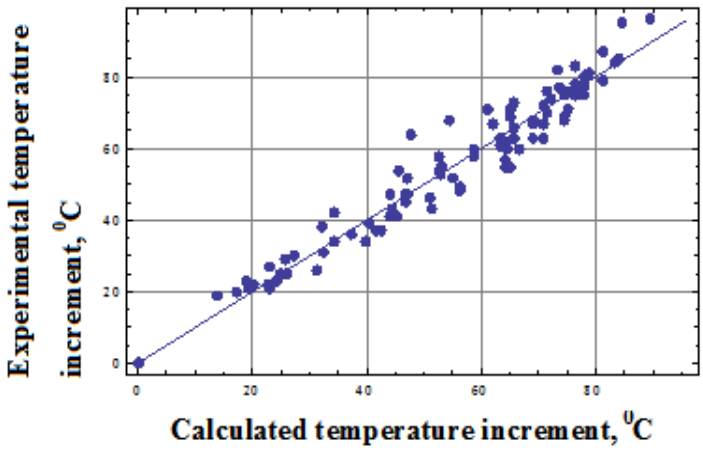

Figure 21 Experimental and calculated values of a temperature increment of triticale grain when varying by the irradiance of 0 – 22 kW.m^{-2}, ambient temperature 148 – 347 ºC and initial moisture content W= 10…21%.

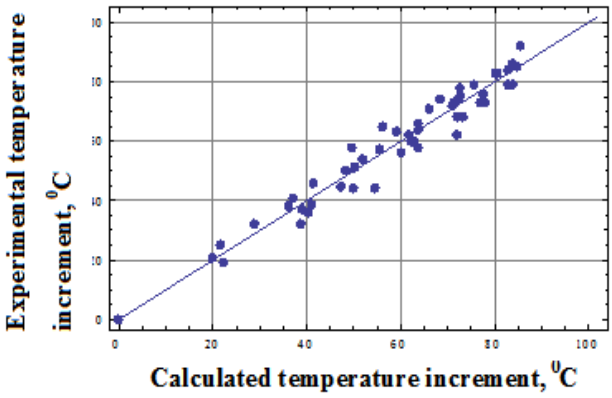

Figure 22 Experimental and calculated values of a temperature increment of spelt cereal when varying by the irradiance of 0 – 22 kW.m^{-2}, ambient temperature 132 – 313 ºC and initial moisture content W= 12…22%.

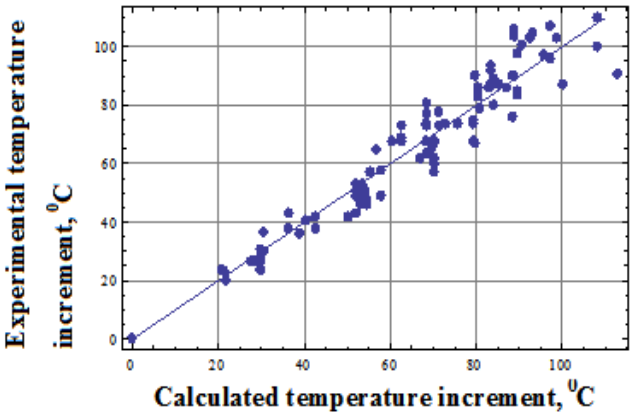

Figure 23 Experimental and calculated values of a temperature increment of white lupine when varying by the irradiance of 0 – 17 kW.m^{-2}, ambient temperature 142 – 275 ºC and initial moisture content W = 7…17%.

Table 2 Model parameters (4).

Type of product	K_0,	C	Squared multiple correlation, R^2
Triticale grain	27.3	0.44	
Spelt cereal ("Runo")	48.3	1.26	0.99
White lupine ("Dega")	30.4	0.32	

Calculated by a model (3) and experimental values of relative moisture content for triticale grain are shown in Figure 24.

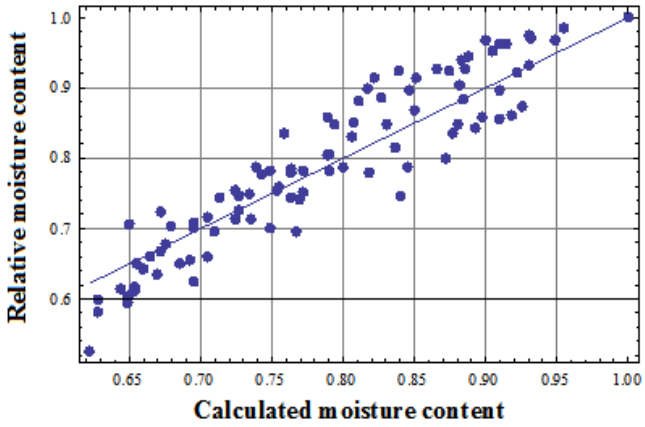

Figure 24 Calculated (K_0 = 27.3, C = 0.446, squared pair correlation R^2 = 0.998) and experimental values (initial moisture content U_0 = 0.11-0.27, irradiance E = 0-27 kW.m^{-2}, temperature in the treatment zone T_c=340 – 150 ºC) of relative moisture content for triticale grain.

However, it should be borne in mind that this model describes adequately the process of dehydration to an extent limited by the upper temperature value of grain not much more than 100 ºC.

However, it should be borne in mind that this model describes adequately the process of dehydration to an extent limited by the upper temperature value of grain not much more than 100 ºC. From the presented graphs (Figures 1.24 – 1.26) and earlier obtained results for barley and millet, it can be assumed that the model describes adequately experimental data on the small-sized (3 – 5 mm) objects.

CONCLUSION

Despite the assumptions made, the considered models describe adequately the grain temperature rise during infrared heating in the range of 10 – 100 °C and moisture content up to 15%. High correlation coefficients and a significant difference between the residual variance and the variance of the mean allow us to speak about the adequacy of models. We have to explain the physical reality of invariance of the dependence of moisture content on the temperature of infrared radiation.

Simplicity of models allows even in production conditions, having determined the coefficients of the model, to correct processing modes. At higher temperatures and initial humidity, more complex distributed models are needed.

An increase in the share of radiation heat supply, due to an increase in the absorption coefficient K_E, and due to the irradiance E, for example, as a result of more efficient design of the treatment zone, leads to a reduction in the heat time until a given temperature. In this case, we note that the nature of the change in the moisture content of the product does not change due to its invariance to the heating regimes.Ideally, all power of the emitter should be "pumped" into the product by the mechanism of radiation heating. This is understandable, because any excess temperature in the treatment zone above the ambient temperature leads to heat loss. Design activities should be aimed at minimizing heat losses, increasing the radiant efficiency of the emitter and the reflecting power of the screen system. However, the reduction of heat loss (due to the thermal insulation of the treatment zone) without increasing the reflecting power of screens leads to an increase in the ambient temperature to the value of infrared emitters or elements of design, which are unacceptable by the operating conditions.

REFERENCES

Kulaychev, A. 1999. The complete works in three volumes vol. 1. In Kulaychev, A. et al. *Methods and tools of data analysis in a Windows environment STADIA-6*. Information and computers, 344 p. ISBN 5-89-357-016-2.

Lykov, A. V., Mikhailov, Y. A. 1963. *Theory of heat and mass-transfer*. University of Michigan, USA : Israel Program for Scientific Translations publishers, 558 p.

Pan, Z., Atungalu, G. G. 2010. *Infrared Heating for Food and Agricultural processing.* Boca Raton, Florida, US : CRC Press. 300 p. ISBN-13: 9781420090970.

Zverev, S. V. 2009. *High-temperature micronization in production of cereal products*. Deli Print publishers. 222 p. ISBN 978-5-94343-202-6.

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QUANTITATIVE AND QUALITATIVE PARAMETERS IN ACORN SQUASH CULTIVAR IN THE CONDITIONS OF THE SLOVAK REPUBLIC

Miroslav Šlosár, Ivana Mezeyová, Alžbeta Hegedűsová, Ondrej Hegedűs

ABSTRACT

The species *Cucurbita pepo* includes several types of squashes; in Slovak Republic, well-known and oftenly grown squash types are patisson, zucchini, spaghetti squash, oil pumpkin etc. Several interesting squash types of *Cucurbita pepo* are grown abroad, including Acorn squash which is well-known mainly in USA. The aim of this study was to evaluate the important quantitative (yield per hectare, average fruit weight) and qualitative (total carotenoids, ascorbic acid, antioxidant activity, total soluble solids) yield parameters of Acorn squash fruits in comparison with patisson which is typical squash type of *Cucurbita pepo* in Slovak Republic. The field trial was realised in Košice-Šaca in 2016. Within experiment, four cultivars of Acorn type pumpkin were tested (Thelma Sanders; Jet Set; Table Gold; Cream of The Crop). The patisson 'Orfeus' was used as a comparative cultivar for evaluation of individual parameters of Acorn type pumpkin cultivars. Matured pumpkin fruits were harvested on the 7th September 2016. From aspect of yield quantity, Acorn cultivars are appeared as very interesting squash type with good yield potential for growing. The highest yield of squash fruits was found in the cultivar 'Cream of The Crop' (17.8 t.ha^{-1}). In mentioned Acorn cultivar, the yield was higher about 87.4% compared to the tested patisson cultivar 'Orfeus' (9.5 t.ha^{-1}). On the contrary, the average weight of squash fruits was reached in patisson cultivar 'Orfeus' (780.7 g). The qualitative parameters of fruits were expressively influenced by squash cultivar. The content of total carotenoids, ascorbic acid and total soluble solids was markedly higher in all Acorn cultivars, compared to the patisson cultivar 'Orfeus'. The highest content of total carotenoids (26.74 mg.kg^{-1} fresh weight) and ascorbic acid (238.79 mg.kg^{-1} f. w.) was found in the squash cultivar 'Table Gold'. The highest content of total soluble solids was determined in the cultivar 'Jet Set' (3.8 °Brix). On the contrary, the highest antioxidant activity (DPPH) was found in the patisson cultivar 'Orfeus' (10.80 %). On the basis of obtained results, it is possible to state that Acorn cultivars are very interesting squash type with promising yield potential for possible growing in conditions of Slovak Republic. In addition, Acorn squashes were expressed by higher content of several nutritional parameters compared to the typical squash type - patisson. Thus, these squashes could be an interesting vegetable for human nutrition.

Keywords: squash; Acorn; yield; quality

INTRODUCTION

The genus *Cucurbita* is a member of the *Cucurbitaceae* family which includes four major species, i. e. *Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita moschata* and *Cucurbita ficifolia*. A lot of cultivars of these species are grown around the world and belong to the major agricultural commodities **(Kim et al., 2012)**. According to **FAOSTAT (2017)**, total world production of pumpkins, squashes and gourds was more than 25 milions tones in 2014. The main production area was Asia (63.9%), followed by Europe (15.7%), American continents (11.3%), Africa (7.9%) and Oceania (1.1%). The main producers of these species were China, India, Russia, Ukraine, USA, Iran and Italy. The production of squashes in Slovak Republic was 1480 tones in 2014. In recent period, the squash production in Slovak Republic is characterized by increasing trend and its value was 3039 tones in 2015 **(Meravá, 2016)**. The most important squash species is *Cucurbita pepo* which includes several known types, e. g. pattypan squash, zucchini or oil squash. The Acorn squash is an iconic fall vegetable in the USA, known for its unique ribbed fruit shape and culinary properties. Of the diverse types of *Cucurbita pepo*, acorn squash is the longest-storing and it has the highest fruit quality **(Wyatt et al., 2015)**.

Carotenoids are known for several important biological activities. The most widely studied and well-understood nutritional role of carotenoids is their provitamin A activity **(Cazzonelli and Pogson, 2010)**. Carotenoids are known to be very efficient physical and chemical

quenchers of singlet oxygen ($^1O_2$), as well as potential scavengers of other reactive oxygen species (ROS). It is the special significance, because the uncontrolled generation and concomitant increase of ROS level in the body results in "oxidative stress", an essential contributor to the pathogenic processes of many diseases **(Fiedor and Burda, 2014)**. Carotenoids and some of their metabolites belong to the important antioxidants and they are suggested to play a protective role in a number of ROS-mediated disorders, i.e. cardiovascular diseases, several types of cancer or neurological, as well as photosensitive or eye-related disorders **(Tang, 2010)**.

One of the most important and effective antioxidant substances, abundant in edible parts of many vegetable species, is vitamin C, also known as ascorbic acid **(USDA, 2016)**. Though most animals are able to endogenously synthesize large quantities of vitamin C, humans do not have the capability to synthesize vitamin C due to a series of mutations of the gene encoding gulonolactone oxidase which catalyses the last enzymatic step in ascorbate synthesis. From this reason, it mus be ingested in natural food form, most suitably as a component of fresh vegetables or fruits **(Grosso et al., 2013)**. The vitamin C plays an important role in immune system, stimulation of leucocytes to the increased bacteria degradation or body resistance increase to the coldness **(Hacişevki, 2009)**. According to **Gonzalez and Miranda-Massari (2014)**, vitamin C is considered as a very strong reductant and radical scavenger. It reduces unstable oxygen, nitrogen, and sulfur radicals. In addition, vitamin C acts as primary defense against aqueous radicals in blood. **Pal, Sanal and Gopal (2011)** state that vitamin C can inactivate the urease enzyme, which allows the endurance of *Helicobacter pylori* and the colonization of the gastric mucosa at a low pH. Thus, it may inhibit the spread, growth, and colonization of *H. pylori* in the early periods of infection. This bacterium is considered as important risk factor in stomach cancer formation.

Scientific hypothesis

The squashes of Acorn type are not known and grown in the Middle European region. The impact of cultivar on the quantity and quality of Acorn squash fruits (*Cucurbita pepo*) was tested in conditions of Slovak republic. As a comparative sample, cultivar of patisson was used because it is squash species commonly grown in Slovak republic.

MATERIAL AND METHODOLOGY

The field trial with pumpkins was realised in Košice in 2016. The experimental locality is described as the slightly hot area. Within experimental period (May-September 2016), the average month air temperature was 18.9 °C. The total rainfall sum was 355 mm. According to the climatic normal 1961 – 2010 for Košice, average month temperature is 17.9 °C and total rainfall sum is 370 mm within period May-Spetember. Compared to the climatic normal, the experimental period can be evaluated as slightly cold and wetter.

Experiment organisation

Within experiment, four cultivars of Acorn type squash were tested ('Thelma Sanders', 'Jet Set', 'Table Gold', 'Cream of The Crop'). The patisson 'Orfeus' was used as a comparative cultivar for evaluation of individual parameters of Acorn type squash cultivars.

The total experimental area was 64 m^2 (8 m^2 for each cultivar). All cultivars were sowed in three replications. Each replication was presented by three planting holes with four seeds. The seeds were sowed on the 7th May 2016 and matured squash fruits were harvested on the 7th September 2016. The squash yield from all replications was sequentially calculated to the square unit of one hectare.

In all experiment area, same conditions were prepared for squash plants from aspect of fertilization or irrigation. According to agrochemical soil analyses, realised before experiment establishment, phosphorus and potassium could not have been applied because their sufficient content for squash growing. The calculated nitrogen dose was applied before squash seed sowing. The irrigation was realised in dependency on the weather and rainfalls. In the view of protection, plants were treated by several chemical preparations against mildew and silverleaf whiteflies.

Immediately after harvest, individual qualitative parameters of squash cultivars were analysed. The average sample from each replication was prepared from 3-4 fruits. All fruits were quartered and opposite quarters were used for qualitative analyses.

Total carotenoids (TC)

The extraction of samples was done at the Laboratory of Beverages, AgroBioTech Research Center, Slovak University of Agriculture (SUA) in Nitra. The estimation of total carotenoid content was realised in the laboratory of Department of Fruit Growing, Viticulture and Enology 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, Mezeyová, Andrejiová, 2015)**.

Ascorbic acid (AA)

The determination of ascorbic acid (vitamin C) content was realised in the Regional Institute of Public Health in Nitra. The modificated HPLC method of AA content estimation according to the **Stan et al. (2014)** was used by the help of liquid chromatograph with UV detector. For separation, Res Elut 5 C18 (150 x 4.6mm) was used (Varian, Palo Alto, California, USA). Mobile phase was acetonitrile – *phosphate buffer solution* (pH = 3.5) 5 : 95. UV detection was adjusted to 264 nm (Waters 2489 UV/VIS Detector).

Antioxidant activity (AOA)

The liophilisation of squash samples for AOA analysis was realised at the Department of Animal Nutrition, SUA in Nitra. The total antioxidant capacity was measured by the method of **Brand-Williams et al. (1995)** using DPPH (2.2-diphenyl-1-pikrylhydrazyl) scavenging activity calculated as inhibition of DPPH radicals in %. The absorbance was measured at 515.6 nm in the spectrophotometer Shimadzu UV/VIS-1240.

Figure 1 Field experiment with squashes.

Figure 2 Patisson 'Orfeus'.

Figure 3 Acorn 'Thelma Sanders'.

Figure 4 Acorn 'Jet Set'.

Figure 5 Acorn 'Table Gold'.

Figure 6 Acorn 'Cream of the Crop'.

Total soluble solids (TSS)

The total soluble solids content (°Brix) of individual squash cultivars were measured by a digital hand-held refractometer (Kern ORD 45BM, Balingen, Germany). The juice that exuded from the suash pulp was used as a sample. The average value of TSS for each cultivar was obtained by three measurements of sample **(Hegedüsová, Mezeyová, Andrejiová, 2015)**.

Statisic analysis

The obtained data were processed into tables in Microsoft Office Excel 2007. Then analysis of variance (ANOVA) was used by the help of the Tukey test (significance level $\alpha = 0.05$) for statistical analyses in the program StatgraphicCenturion XVII (StatPointInc. USA).

RESULTS AND DISCUSSION

Yield per hectare

All cultivars of Acorn squashes were characterized by markedly higher yield of fruits per hectare compared to the patisson cultivar 'Orfeus'. The difference of yield between Acorn squash cultivar 'Thelma Sanders' was showed as statistically non-significant. Differences among other

cultivars was evaluated as statistically significant (Table 3).

The yield of squash fruits was ranged from 9.5 t.ha^{-1} (patisson 'Orfeus') to 17.8 t.ha^{-1} (Acorn 'Cream of The Crop'). Differences among pattison cultivar and Acorn squash cultivars were varied in the range from 24.2 % (Acorn 'Thelma Sanders') to 87.4% (Acorn 'Cream of The Crop').

Kołota and Balbierz (2015) found similar yield of patisson fruits in cultivar 'Disco' (9.89 t.ha^{-1}) compared to the cultivar 'Orfeus', tested in realised experiment. Yield of other patisson cultivars, presented by authors, was markedly higher in comparison with cultivar 'Orfeus', e. g. 10.51 t.ha^{-1} ('Polo F1'), 11.60 t.ha^{-1} ('Gagat'), 13.27 t.ha^{-1} ('Okra'), 14.46 t.ha^{-1} ('Sunny Delight'). The fruit yield of Acorn squash type cultivars was the study subject of **Strang et al. (2001)**. Authors found significant yield variability in dependency on the tested Acorn cultivars. It was varied in the range from 6.17 t.ha^{-1} to 23.45 t.ha^{-1} (average = 13.58 t.ha^{-1}). In mentioned study, the lower yield was found in cultivars 'Table Gold' (10.43 t.ha^{-1}) in comparison with our study. On the contrary, the higher yield was reached in cultivar 'Cream of The Crop' (13.57 t.ha^{-1}), compared to our study. **Strang et al. (2006)** found relatively high yield of fruits in Acorn squash cultivar 'Autumn Delight' (18.29 t.ha^{-1}).

Average weight of fruits

In this study, statistically significant differences of average fruit weight among squash cultivars 'Orfeus' (patisson)↔'Jet Set'↔'Cream of The Crop' or 'Thelma Sanders'↔'Table Gold' were not showed. Other differences among cultivars were evaluated as statistically significant (Table 3).

The average fruit was ranged from 532.4 g to 780.7 g and it was increasing in following cultivar order: 'Table Gold' <'Thelma Sanders' <'Cream of The Crop' <'Jet Set' <'Orfeus' (patisson).

Barátová, Uher and Štefunko (2011) found the variability of average patisson fruit weight in the range from 757 g to 922 g. These results are realtively comparable to the cultivar 'Orfeus', used in this study. Compared to the tested Acorn squash cultivars, similar values of average fruit weight were presented in the studies of **Strang et al. (2011)** and **Strang et al. (2016)** with variability in the range from 453.6 g to 725.7 g.

Total carotenoid content (TC)

Carotenoids are natural compounds of many vegetable species and they predominantly define the yellow, orange or reddish colour of various intensity in the edible parts of many crops, e.g. carrot, sweet potato or some squash types. The most prominent carotenoid in the most of vegetable species, including *Cucurbita* species, is β-carotene **(Maiani et al., 2009; Šlosár et al., 2013)**.

The total carotenoid content in all Acorn squash cultivars was statistically significantly higher in comparison with patisson cultivar. Values of TC in tested squash cultivars were increasing in the following cultivar order: 'Orfeus' (1.10 mg.kg^{-1} fresh weight) <'Cream of The Crop' (7.31 mg.kg^{-1} fresh weight) <'Jet Set' (7.94 mg.kg^{-1}) fresh weight <'Thelma Sanders' (10.34 mg.kg^{-1} fresh weight) <'Table Gold' (26.74 mg.kg^{-1}). The content of TC in particular squash cultivars was closely depending on the intensity of fruit pulp colour. The expressly highest content of TC was found in the Acorn cultivar 'Table Gold' which fruits was characterized by intensive orange pulp colour.

Regarding to the tested patisson cultivar 'Orfeus', **Kołota and Balbierz (2015)** found relatively comparable carotenoid content which varied from 0.55 mg.kg^{-1} to 1.05 mg.kg^{-1} f. w. in dependence on the patisson cultivar. On the contrary, **Balbierz and Kołota (2017)** found markedly lower content of total carotenoids (0.27 mg.kg^{-1} f. w.) in patisson fruits, compared to the tested cultivar 'Orfeus'.

Similar value of carotenoid content in Acorn squash, compared to our results, was presented in the study of **Wyatt et al. (2016)**, concretely 10,1 mg.kg^{-1} f. w. **Murkovic, Mülleder and Neunteufl (2002)** monitored the carotenoid content (α-carotene, β-carotene, lutein and zeaxanthin) in different squash varieties in experiment realised in Austria. In fruits of Acorn cultivars, the average content of TC was 27.65 mg.kg^{-1} f. w. This value is significantly higer than the average content of TC in our experiment (13.08 mg.kg^{-1} f. w.). The second tested *Cucurbita* species in study of mentioned authors was *Cucurbita moschata* which is known for ther intensive orange colour of fruit pulp. The TC content in the fruits of this species was varied from 41.6 mg.kg^{-1} to 130.4 mg.kg^{-1} f. w. what indicate that *Cucurbita moschata* is richer source of carotenoids than *Cucurbita pepo* (Acorn varieties, patisson, courgette, spaghetti squash etc.). This fact was also presented in the study of **Mendelová et al. (2017)** who found variability of carotenoid content in fruits of *Cucurbita moschata* in the range from 28.2 mg.kg^{-1} to

Table 1 Climate characteristics in experiment area in 2016 (Košice).

Month	Temperature (°C)		Rainfall (mm)	
	2016	Evaluation	2016	Evaluation
May	15.7	normal	75	normal
June	20.7	very hot	58	dry
July	21.2	normal	113	wet
August	19.6	normal	83	normal
September	17.4	hot	26	dry

Note: evaluation of months according to the climatic normal (long-term average 1961 – 2010) for Košice.

Table 2 Agrochemical characteristics of the soil before the experiment establishment in 2016 (Košice).

Humus	pH/KCl	Nutrient content in the soil (mg.kg^{-1})					
(%)		N	P	K	S	Ca	Mg
3.41 G	6,47 SA	18.9 M	85.8 G	152.3 M	28.7 M	605.1 H	632.5 VH

Note: SA – slightly acidic, M – medium content, G – good content, H – high content, VH – very high content.

71.8 mg.kg^{-1} f. w.

Ascorbic acid content (AA)

The ascorbic acid content in fruits of all Acorn squash cultivars was statistically significantly higher compared to the patisson cultivar 'Orfeus'. The statistically significant differences were also found among individual Acorn squash cultivars. The highest AA content was found in cultivar 'Table Gold' (238.79 mg.kg^{-1} f. w.). Difference of AA content between mentioned Acorn cultivar and patisson cultivar 'Orfeus' presented value of 181.49 mg.kg^{-1}. In other Acorn cultivars, differences of AA content, in comparison with patisson cultivar, were ranged from 67.50 mg.kg^{-1} to 136.87 mg.kg^{-1} f. w.

Compared to obtained results, **Kołota and Balbierz (2015)** found markedly higher values of AA content in patisson fruits which varied from 157.90 mg.kg^{-1} to 267.30 mg.kg^{-1} f. w., dependent on the cultivar. Similar value of AA content in patisson (232.50 mg.kg^{-1} f. w.) was also presented in the study of **Balbierz and Kołota (2017)**. According to the **USDA (2017)**, the AA content in Acorn squash is 110 mg. kg^{-1} f. w. All Acorn cultivars, tested in this study, were expressed by higher content of AA in the fruit pulp. After comparison of obtained results with study of **Andrejiová et al. (2016)**, Acorn squash (*Cucurbita pepo*) can be marked as a slightly richer source of AA in comparison with *Cucurbita moschata*, another important squash species. Authors found a variability of AA content in the fruits of *Cucurbita moschata* (six cultivars) in the range from 138.8 mg.kg^{-1} to 186.9 mg.kg^{-1} f. w. Similar value of AA content in *Cucurbita moschata* (139.9 mg. kg^{-1} f. w.) was also presented in the study of **Chua (2007)**.

Antioxidant activity

Antioxidants are wide group of various substances present in vegetables, fruits or other crops. They are usually defined as substances which help to prevent human organism in small amount against negative influence of free radicals, responsible for many serious diseases, e. g. various cancer types, cardiovascular and neurological diseases **(Sindhi et al., 2013)**. In the group of antioxidants, following substances are classified: flavanoids, vitamins C, E or K, carotenoids, phenolic acids, selenium etc. **(Nahak, Suhar, Sahu, 2014)**.

From aspect of antioxidant activity, statistical differences among Acorn cultivars and patisson cultivar were founded. The values of antioxidant acitivity (AOA) were varied from 5.29% (Acorn 'Jet Set') to 10.80% (patisson 'Orfeus'). Obtained results are relatively comparable to the study of **Hamissou et al. (2013)** who found the AOA (DPPH) of *Cucurbita pepo* fruits (zucchini) on the level of 12.19%. **Oleárová et al. (2013)** found the variability of AOA in *Cucurbita pepo* fruits in the range from 2.72% to 6.24%. These values are, in average lower than the AOA of patisson and Acorn cultivars in realised study. The study of **Gajewski et al. (2008)** was focused on the comparison of various Cucurbita species (*Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita maxima*) and quality of their fruits. The AOA of *Cucurbita pepo* fruits (DPPH) was the lowest from all tested species (9.5 – 13.6%); on the other side, values were higher compared to our obtained results. Other tested species, *Cucurbita maxima* and *Cucurbita moschata*, were characterized by markedly higher AOA compared to the *Cucurbita pepo*. The values of AOA in *Cucurbita maxima* were ranged from 56.7% to 73.2%. The AOA in the fruits of *Cucurbita moschata* was on the level of 63.1%. The fact that *Cucurbita moschata* and *Cucurbita maxima* are characterized by expressively higher AOA, compared to the *Cucurbita pepo*, was also presented by **Altemini et al. (2016)**, **Dinu et al. (2016)** or **Zhao et al. (2015)**.

Total soluble solid (TSS)

The content of soluble solids (mainly sugars) in vegetable extracts is oftenly presented and marked as a total soluble (refractometric) solid **(Hegedüsová, Mezeyová, Andrejiová, 2015)**. The statistically

Table 3 Quantitative parameters of patisson and Acorn pumpkin cultivars.

Species/ cultivar	Yield (t.ha^{-1} ±SD)	AW (g ±SD)
Patisson 'Orfeus'	9.5 ±0.12^{c}	780.7 ±37.2^{a}
Acorn 'Thelma Sanders'	11.8 ±0.13^{c}	584.6 ±38.4^{b}
Acorn 'Jet Set'	12.0 ±0.10^{bc}	769.2 ±40.2^{a}
Acorn 'Table Gold'	14.7 ±0.11^{b}	532.4 ±37.0^{b}
Acorn 'Cream of The Crop'	17.8 ±0.13^{a}	708.8 ±33.1^{a}

AW – average fruit weight; SD – standard deviation.
Note: Values with different italics letters are significantly different at p <0.05 by LSD in ANOVA.

Table 4 Qualitative parameters of patisson and Acorn pumpkin cultivars.

Species/cultivar	TC (mg.kg^{-1} ±SD)	AA (mg.kg^{-1} ±SD)	AOA (% ±SD)	TSS (BRIX ±SD)
Patisson 'Orfeus'	1.10 ±0.21^{d}	57.30 ±2.82^{e}	10.80 ±0.21^{a}	0.5 ±0.08^{e}
Acorn 'Thelma Sanders'	10.34 ±0.45^{b}	124.84 ±4.56^{d}	5.57 ±0.20^{d}	3.4 ±0.16^{b}
Acorn 'Jet Set'	7.94 ±0.23^{c}	194.17 ±3.78^{b}	5.29 ±0.12^{d}	3.8 ±0.22^{a}
Acorn 'Table Gold'	26.74 ±0.68^{a}	238.79 ±3.55^{a}	8.57 ±0.43^{c}	2.0 ±0.16^{c}
Acorn 'Cream of The Crop'	7.31 ±0.43^{c}	144.71 ±3.31^{c}	10.23 ±0.33^{b}	1.6 ±0.12^{d}

TC – total carotenoids; ascorbic acid (vitamin C); AOA – antioxidant activity; TSS – total soluble solids; SD – standard deviation.

significant differences of TSS among individual Acorn squash cultivars and patisson cultivar were found. Values of TSS were varied from 0.5 °BRIX (patisson 'Orfeus') to 3.4 °BRIX (Acorn 'Thelma Sanders').

The significantly higher TSS in *Cucurbita pepo* fruits (3.0 – 4.5 °BRIX), compared to this study, were presented by **Gajewski et al. (2008)**. Compared to the tested patisson cultivar 'Orfeus', **Kołota and Balbierz (2015)** found higher values of TSS in the range from 1.57 °BRIX to 1.91 °BRIX, in dependency on the patisson cultivar. **Silva and Bruce (2016)** evaluated the content of TSS in fruits of six Acorn squash cultivars and its values were varied from 8.27 °BRIX to 10.42 °BRIX. **Loy (2006)** found variability of TSS in Acorn squash cultivars in the range from 5.9 °BRIX to 15.0 °BRIX. The significant differences among TSS values were caused by various harvest date and storage period of squash fruits. In all metioned studies, the content of TSS in squash fruits was expressively higher compared to the *Cucurbita pepo* cultivars tested in realised experiment.

Several studies indicate that *Cucurbita pepo* fruits are characterized by lower content of TSS in comparison with some other *Cucurbita* species, e. g. *Cucurbita moschata* or *Cucurbita maxima* **(Gajewski et al., 2008; Iacuzzo, Dalla Costa, 2009; de Carvalho et al., 2015)**.

CONCLUSION

The Acorn squash (*Cucurbita pepo*) is less-known cultivar type in Slovak Republic or Middle European region generally. It is wide-spread and grown on the large areas mainly in USA. The aim of this study was to evaluate the important quantitative (yield per hectare, average fruit weight) and qualitative (total carotenoids, vitamin C, antioxidant activity, total soluble solids) yield parameters of Acorn squash fruits (four cultivars) in comparison with patisson which is typical squash type of *Cucurbita pepo* in Slovak Republic. The average weight of patisson fruits (780.7 g) was higher than its value in Acorn squash cultivars (584.6 – 769.2 g). On the contrary, yield of Acorn squash fruits (11.8 – 17.8 $t.ha^{-1}$) was markedly higher compared to the patisson cultivar (9.5 $t.ha^{-1}$). Acorn squash cultivars were showed the higher total carotenoid content (7.31 – 26.74 $mg.kg^{-1}$ f. w.) in comparison with patisson (1.10 $mg.kg^{-1}$ f. w.). Similarly, the higher content of vitamin C (124.84 – 238.79 $mg.kg^{-1}$ f. w.) and total soluble solids (1.6 – 3.8 °BRIX) in Acorn fruits compared to the tested patisson cultivar (57.30 $mg.kg^{-1}$ f. w.; 0.5°BRIX) was found. On the contrary, the higher antioxidant activity was found in patisson fruits (10.8%) in comparison with Acorn cultivar fruits (5.29 – 10.23%) Obtained results indicate that Acorn cultivars are very interesting squash type with promising yield potential for growing in conditions of Slovak Republic. The significant aspect of Acorn squash cultivar is also quality of fruits, which was expressively higher in several parameters compared to the patisson - typical species of *Cucurbita pepo* in conditions of Slovak Republic, or Middle Europe region generally.

REFERENCES

Altemini, A., Watson, D. G., Choudhary, R., Dasari, M. R., Lightfoot, D. A. 2016. Ultrasound Assisted Extraction of Phenolic Compounds from Peaches and Pumpkins. *Plose One*, vol. 11, no. 2, p. 1-20.

Andrejiová, A., Hegedűsová, A., Šlosár, M., Barátová, S. 2016. Dynamics of selected bioactive substances changes in *Cucurbita moschata* Duch. ex Poir. after storage and different methods of technological processing. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 64, no. 2, p. 387-393. https://doi.org/10.11118/actaun201664020387

Balbierz, A., Kołota, E. 2017. The effects of nitrogen fertilization and stage of fruit maturity at harvest on yield and nutritional value of scallop squash. *Acta Scientiarum Polonorum Hortorum Cultus*, vol. 16, no. 4, p. 33-43. https://doi.org/10.24326/asphc.2017.4.4

Barátová, S., Uher, A., Štefunko, M. 2011. Evaluation of yield and quality characteristics of new selected varieties of *Cucurbita pepo* conver. *patissonina* and *Cucurbita pepo* convar. *giromontiina*. *Acta horticulturae et regiotecturae*, vol. 14, no. 2, p. 29-31.

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. https://doi.org/10.1016/S0023-6438(95)80008-5

Cazzonelli, C. I., Pogson, B. J. 2010. Source to sink: regulation of carotenoid biosynthesis in plants. *Trends in Plant Science*, vol. 15, no. 5, p. 266-274. https://doi.org/10.1016/j.tplants.2010.02.003 PMid:20303820

Chua, J. Y. 2007. *Quantitative analysis of vitamin A, vitamin C, and minerals in pumpkins (Cucurbita moschata)* : dissertation theses. Kota Kinabalu, Sabah, Malaysia : Universiti Malaysia Sabah. 60 p.

de Carvalho, L. M. J., de Carvalho, J. L. V., Faustino, R. M. E. B., Kaser, I. M., Lima, V. G., de Sousa, D. S. F. 2015. Variability of Total Carotenoids in *C. moschata* Genotypes. *Chemical Engineering Transactions*, vol. 44, p. 247-252.

Dinu, M., Soare, R., Hoza, G., Becherescu, A. D. 2016. Biochemical Composition of Some Local Pumpkin Population. *Agriculture and Agricultural Science Procedia*, vol. 10, p. 185-191. https://doi.org/10.1016/j.aaspro.2016.09.051

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

FAOSTAT (Food and Agriculture Organization of the United Nations), 2016. [online] s.a. [cit. 2017-07-10]. Available at: http://faostat3.fao.org/browse/Q/*/E.

Gajewski, M., Radzanowska, J., Danilcenko, H., Jariene, E., Cerniauskiene, J. 2008. Quality of Pumpkin Cultivars in Relation to Sensory Characteristics. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 36, no. 1, p. 73-79.

Gonzalez, M. J., Miranda-Massari, J. R. 2014. *New Insights on Vitamin C and Cancer*. 1st ed. New York, USA : Springer-Verlag. 69 p. ISBN 978-1-4939-1890-4.

Grosso, G., Bei, R., Mistretta, A., Marventano, S., Calabrese, G., Masuelli, L., Giganti, M. G., Modesti, A., Galvano, F., Gazzolo, D. 2013. Effects of Vitamin C on health: a review of evidence. *Frontiers in Bioscience*, vol. 18, no. 3, p. 1017-1029. https://doi.org/10.2741/4160

Hacişevki, A. 2009. An overview of ascorbic acid biochemistry. *Journal of Faculty of Pharmacy of Ankara*,vol. 38, no. 3, p. 233-255.

Hamissou, M., Smith, A. C., Carter, R. E., Triplett, J. K. 2013. Antioxidative properties of bitter gourd (*Momordica charantia*) and zucchini (*Cucurbita pepo*). *Emirates Journal*

of Food and Agriculture, vol. 25, no. 9, p. 641-647. https://doi.org/10.9755/ejfa.v25i9.15978

Hegedűsová, A., Mezeyová, I., Andrejiová, A. 2015. *Determination methods of selected biologically active substances (Metódy stanovenia vybraných biologicky aktívnych látok)*. Nitra : Slovenská poľnohospodárska univerzita. 72 p. ISBN 978-80-552-1420-7.

Iacuzzo, F., Dalla Costa, L. 2009. Yield performance, quality characteristics and fruit storability of winter sqush cultivars in sub-humid areas. *Scientia Horticulturae*, vol. 120, no. 3, p. 330-335. https://doi.org/10.1016/j.scienta.2008.11.026

Kim, M. Y., Kim, E. J., Kim, Y. N., Choi, C., Lee, B. H. 2012. Comparison of the chemical compositions and nutritional values of Various Pumpkin (Cucurbitaceae) species and parts. *Nutrition Research and Practice*, vol. 6, no. 1, p. 21-27. https://doi.org/10.4162/nrp.2012.6.1.21 PMid:22413037

Kołota, E., Balbierz, A. 2015. Yield potential and fruit quality of scallop suash (*Cucurbita pepo* L. var. *patissonina* Greb. f. radiate Nois.) cultivars frown for processing. *Acta Agrobotanica*, vol. 68, no. 3, p. 261-266. https://doi.org/10.5586/aa.2015.030

Loy, J. B. 2006. Interaction of harvest time and storage on the attributes of eating quality in Acorn squash. *HortScience*, vol. 41, no. 3, p. 496.

Maiani, G., Periago Castón, M. J., Catasta, G., Toti, E., Cambrodón, I. G., Bysted, A., Granado-Lorencio, F., Olmedilla-Alonso, B., Knuthsen, P., Valoti, M., Böhm, V., Mayer-Miebach, E., Behsnilian, D., Schlemmer, U. 2009. Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Molecular Nutrition & Food Research*, vol. 53, suppl. no. 2, p. 194-218. https://doi.org/10.1002/mnfr.200800053 PMid:19035552

Mendelová, A., Mendel, Ľ., Fikselová, M., Mareček, J., Vollmanová, A. 2017. Winter squash (*Cucurbita moschata* Duch) fruit as a source of biologically active components after its thermal treatment. *Potravinarstvo Slovak Journal of Food Science*, vol. 11, no. 1, p. 489-495. https://doi.org/10.5219/788

Meravá, E. 2016. *Zelenina : Situačná a výhľadová správa k 31.12.2015* (Vegetables : Situation and perspective report to the 31st December 2015). Bratislava : Výskumný ústav ekonomiky poľnohospodárstva a potravinárstva. 50 p. ISSN 1338-8010.

Murkovic, M., Mülleder, U., Neunteufl, H. 2002. Carotenoid Content in Different Varieties of Pumpkins. *Journal of Food Composition*, vol. 15, no. 6, p. 633-638. https://doi.org/10.1006/jfca.2002.1052

Nahak, G., Suar, M., Sahu, R. K. 2014. Antioxidant Potential and Nutritional Values of Vegetables: A Review. *Research Journal of Medicinal Plants*, vol. 8, no. 2, p. 50-81. https://doi.org/10.3923/rjmp.2014.50.81

Oleárová, H., Chlebo, P., Monka, A., Brindza, J. 2013. Antioxidant activity of juvenile fruit pumpkin (*Cucurbita* spp.) and their use in nutrition (Antioxidačná aktivita juvenilných plodov tekvíc (*Cucurbita* spp.) a ich využitie vo výžive). In *XXX. Zoborský deň a XI. Západoslovenský deň o osteoporóze 2013: zborník z vedeckej konferencie s medzinárodnou účasťou konanej pri príležitosti 65 rokov činnosti Špecializovanej nemocnice sv. Svorada a Zobor, n. o. a v rámci Cyrilo-metodského roku v Nitre pri príležitosti 1150. výročia príchodu sv. Cyrila a Metoda na Veľkú Moravu*. Nitra : Špecializovaná nemocnica sv. Svorada, p. 170-176. ISBN 978-80-552-1011-7.

Pal, J., Sanal, M. G., Gopal, G. J. 2011. Vitamin-C as anti-Helicobacter pylori agent: More prophylactic than curative-Critical review. *Indian Journal of Pharmacology*, vol. 43, no. 6, p. 624-627.

Silva, E., Bruce, D. 2016. *Organic Acorn and Delicata Winter Squash* . Madison, USA : University of Wisconsin. 11 p.

Sindhi, V., Gupta, V., Sharma, K., Bhatnagar, S., Kumari, R., Dhaka, N. 2013. Potential applications of antioxidants - A review. *Journal of Pharmacy Research*, vol. 7, no. 9, p. 828-835. https://doi.org/10.1016/j.jopr.2013.10.001

Šlosár, M., Barátová, S., Hegedűsová, A. 2013. Carotenoids as important component of human nutrition. *Slovak Journal of Health Sciences*, vol. 4, no. 2, p. 13-20.

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

Strang, J., Bale, K., Snyder, J., Smigell, C. 2006. *Winter Squash & Pumpkin Variety Evaluation*. West Lafayette, USA : Purdue Agriculture. 6 p.

Strang, J., Satanek, A., Snyder, J., Slone, D., Lowry, D., Blandford, L., Helsabeck, S., Holden, J. 2001. *Acorn and specialty winter squash variety evaluation : Midwest Vegetable Trial Report for 2001*. West Lafayette, USA : Purdue Agriculture. 6 p.

Tang, G. 2010. Bioconversion of dietary provitamin A carotenoids to vitamin A in humans. *The American Journal of Clinical Nutrition*, vol. 91, no. 5, p. 1468-1473. https://doi.org/10.3945/ajcn.2010.28674G PMid:20200262

USDA, 2016. USDA National Nutrient Database for Standard Reference, release 28, slightly revised May, 2016, United States Department of Agriculture, Agricultural Research Service [online] s.a. [cit.2017-11-16] Available at: http://ndb.nal.usda.gov/ndb/search.

USDA, 2017. USDA National Nutrient Database for Standard Reference, release 28, U.S. Department of Agriculture, Agricultural Research Service [online] s.a. [cit.2017-11-16] Available at: http://ndb.nal.usda.gov/ndb/search.

Wyatt, L. E., Strickler, S. R., Mueller, L. A., Mazourek, M. 2015. An acorn squash (*Cucurbita pepo* ssp. *ovifera*) fruit and seed transcriptome as a resource for the study of fruit traits in *Cucurbita*. *Horticulture Research*, vol. 2, p. 14070. https://doi.org/10.1038/hortres.2014.70 PMid:26504561

Zhao, J., Liu, W., Chen, D., Song, Y., Zhang, Y., Ni, Y., Li, Q. 2015. Physicochemical and Antioxidant Properties of Different Pumpkin Cultivars Grown in China. *Advance Journal of Food Science and Technology*, vol. 9, no. 4, p. 308-316. https://doi.org/10.19026/ajfst.9.2014

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DEVELOPMENT AND CHARACHTERIZATION OF BARELY SUPLLEMENTED FLAVORED CHAPATTIS

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ABSTRACT

Food scientist are actively involved to improve the quality of wheat through composite flour technology by supplementing wheat flour with other grain flours. Barley grains are outstanding source of total dietary fibers (TDF) and offers remarkable quantity of active ingredients for health elevation and disease prevention. Purposely, the current research work was designed to improve the nutritional potential of wheat chapattis by including barley flour 10%, 20%, 30% along with the addition of functional blend (Methi powder and garlic paste) 2%, 4%, 6% levels respectively. Wheat and barley composite flour were analyzed for its chemical, mineral, antioxidant and total dietary composition. The supplementation of barley flour and functional blend into wheat flour enhanced the mineral. Addition of barley flour and functional blend increased total phenolic in composite flour 0.41 (control) to 0.69 mg GAE.100g^{-1} and DPPH from 20.95 – 23.82%. Total dietary fiber in composite flour varied form 3.11% (control) to 7.69% (30% barley flour with 6% functional blend). Total dietary fiber in chapattis ranged from 6.04 (control) to 8.21% (30% barley flour with 6% functional blend). 30% supplementation of barley flour and 4% addition of functional blend presented better sensory response of the prepared chapattis. All the outcomes revealed that nutritionally rich chapattis should be incorporated in daily diet to explore the dietary worth of barley.

Keywords: Composite flour technology; antioxidant; total dietary fiber; chapatti

INTRODUCTION

Cereals are known to have a positive influence on the general state of human body. Healthier diet can be provided by consuming cereal grains containing high fiber that are low in sugar content and high in fiber and fiber foods has been suggested to control over the health issues such as cardiovascular diseases, hypertension, colon cancer and diabetes **(Sudha et al., 2015)** Based on the recent state of the science, there is reasonable indication that risk of obesity can be minimized by taking a diet that is a combination of whole grains and bran or abundant in cereal fiber. The nutritional gains of whole grain foods are mainly credited due to the occurrence of bioactive compounds **(Edge et al., 2005)**.

Predominantly consumption of wheat is for the purpose of production of unleavened flat bread usually known as chapatti in Pakistan and entitled as primary cereal crop in the world **(Gujral and Pathak, 2002)**. Wheat grain is characterized by elevated amount of carbohydrate content (about 70%), comparatively low protein content (9 to 13%), low moisture content, little amounts of lipids, minerals, vitamins and fiber **(Dholakia, 2001)**.

Barley (*Hordeum vulgare* L.) is used as porridge by human beings, forage for cattles, in making fine superiority beers, alcoholic beverages and used in poultry feeds. Due to its various applications, barley has occupied vital position among cereals at global level **(Wahid, 2006)**. Barley provides number of health benefits and contains complex carbohydrate generally starch for the purposes to gain energy, adequate amount of protein that fulfill the requirement of amino acids, vitamins particularly vitamin E, low fat, total fiber, antioxidants mainly polyphenolics and minerals **(Frost et al., 2011)**. Nutritionally important at least fourteen mineral elements have been existed in fluctuating amounts in whole barley flour **(Jilal, 2011)**. Secondary metabolites present in barley grains are known as phenolic compounds. They are antioxidant provide protection against cardiovascular diseases and collectively these properties are called as biological properties **(Han, 2007)**.

The procedure of mingling whole wheat flour with other cereals and legumes flours to attain better nourishment, to impart functional characteristics, to reduce cost of production and to make the usage of locally available raw materials is known as composite flour technology **(Butt et**

al., 2011). **Hussein et al. (2011)** conducted a study as an effort to unravel the scarcity in wheat production by replacing a share of wheat flour (WF) with gelatinized corn flour (GCF), whole meal barley flour (WBF) and both of them in bread. It was found that the incorporation of gelatinized corn flour and whole meal barley into bread improved ash, protein, fiber, fat, β-glucan and minerals (P, K, Fe and Ca). Nutritional worth and protein percentage of wheat flour foodstuffs might be upgraded by using composite flours **(Ajithkumar et al., 2005)**. Fenugreek (Trigonella foenum-graecum) is well recognized for imparting flavor to several traditional foods. Besides, it provides tremendous amount of active ingredients for health promotion and disease prevention. Methi powder is added in chapatti as a taste adjusts for chapatti. Supplementation of fenugreek seed powder in bread serve as functional food accredited to rich nutritional, antioxidant and sensory quality **(Afzal et al., 2016)**. Addition of 5% methi powder enlarged the alimentary worth of flour principally in terms of higher intake of fibers and minerals e.g. iron and calcium **(Dhingra and Jood, 2004)**.

Chapatti prepared from composite flour can be included in the diet for the better management of diabetes and also beneficial to keep away from further secondary complications. To yield suitable chapattis corn, oat, sorghum and barley flour has also been assimilated in wheat flour **(Gujral and Pathak 2002)**. Addition of 15 – 20% barley flour in wheat flour was acceptable for bread preparation. Overall appearance, texture, and flavor was good but poor sensory characteristics like poor brown color, hard crumb texture and reduced loaf volume was observed due to increased level of barley flour **(Dhingra and Jood, 2004)**. **Lagasse et al. (2006)** also reported that better quality bread can be made from 15 – 30% barley flour with minor alteration in texture, shape and color. The color and appearance of chapattis were found to be suitable with the substitution of wheat flour by 30% of barley flour whereas flavor and texture were acceptable even at 40% substitution levels. So, the people requirements of chapattis which is staple food are fulfilled by making composite flours of other cereals and legumes.

Planned actions were required to improve the nutritional profile of people consuming wheat flour chapattis only. Massive population can be easily covered if we assume staple food as a source of supplementation **(Butt et al., 2007)**.

Scientific hypothesis

The recent research was conducted to assess the nutritional properties of composite flour prepared by adding barley flour and to select the best suitable flavored chapattis prepared with barley and functional blend.

MATERIAL AND METHODOLOGY

Procurement of raw material

The study was carried out at National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Commercially available wheat variety named Galaxy 2013 and barley variety named B 9008 was procured from Wheat Research Institute, Ayub Agriculture Research Institute (AARI), Faisalabad. Chemicals were purchased from local market.

Sample Preparation

Wheat and barley grains was thoroughly cleaned to remove dirt, dust, insect, moldy seeds and foreign matter. The raw wheat sample and barely sample were milled to flour sample and stored in airtight container before use. To prepare functional blend fresh leaves of methi were washed and directly dried in the sun for 4 – 5 days. The dried leaves ground by grinder to make powder. For the preparation of garlic paste, garlic was washed and ground to make paste. Methi powder and garlic paste were mixed together in equal ratio to form functional blend.

Analysis of wheat and barley flour samples

The wheat and barley flour samples were analyzed for moisture, ash, crude fat, crude protein, crude fiber and nitrogen free extract according to their respective methods as described in **AACC (2000)**.

Preparation of composite flours

Wheat flour was blended with barley flour and functional blend in different combinations as mentioned in Table 1. Each treatment of composite flour was thoroughly mixed in order to achieve the uniform dispersion of barley flour in wheat flour.

Chemical analysis of composite flour

The wheat and barley composite flour samples were analyzed for moisture, ash, crude fat, crude protein, crude fiber and nitrogen free extract according to the respective methods as described in **AACC (2000)**.

Mineral contents

Sodium and potassium were measured through flame photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK), while calcium, magnesium, zinc, copper, iron and manganese measured by using Atomic Absorption Spectrophotometer (Varian AA 240, Victoria, Australia) by following the procedure of **AOAC (2006)**.

Determination of anti-oxidant profile

To determine the antioxidant profile of composite flour total phenolic content was determined by following methods.

Determination of Total phenolic content (TPC)

The total phenolic compounds in composite flour were estimated by Folin-Ciocalteu method (FCM) described by **Kahkonen et al. (1999)**.

Radical Scavenging Activity by using DPPH Method.

The antioxidant activity of composite flour was determined based on the radical scavenging ability in reacting with a stable DPPH free radical **(Afify et al., 2012)**.

Dietary fiber of composite flour

The flours were analyzed for total dietary fiber content according to method No. 32-05 as described in **(AACC, 2000)** by employing Megazyme Assay Kit. The samples were dispersed in a buffer solution and incubated with heat-stable α-amylase at 95 – 100°C for 35 minutes. After cooling the samples these were incubated at 60°C for 30 minutes by adding 100 µL protease solution. Furthermore, α-amylase and protease treated samples were incubated with amylo glucosidase at 60°C for 30 min. The fiber contents were precipitated by the addition of alcohol in 1 : 4 ratios and filtered. Residue was washed with alcohol and acetone. A blank was run in a similar manner. TDF was determined by applying formula.

Preparation of chapattis

Different blends along with 100% wheat flour control were used to make the chapattis. Dough was made by mixing samples with water for few minutes in a mixer and allowed to rest. The dough was then rolled up manually and turned into chapattis, the dough was be baked on hot plate **(Shahzadi, 2004)**.

Dietary fibers in chapattis

Dietary fiber content of chapattis prepared from the

Table 1 Treatment Plan for wheat-barley composite flours.

Treatments	Wheat flour %	Barley flour %	Functional ingredients blend %
T_0	100	-	-
T_1	88	10	2
T_2	78	20	2
T_3	68	30	2
T_4	86	10	4
T_5	76	20	4
T_6	66	30	4
T_7	84	10	6
T_8	74	20	6
T_9	64	30	6

Table 2 Chemical composition (%) of Wheat and barley flour.

Treatments	Moisture (%)	Ash (%)	Protein (%)	Fiber (%)	NFE
Wheat	9.08 ±0.18	1.38 ±0.04	11.54 ±0.24	1.33 ±0.05	76.26 ±0.37
Barley	7.14 ±0.46	3.05 ±0.24	13.63 ±0.14	3.51 ±0.18	72.14 ±0.10

Table 3 Chemical composition (%) of different supplemented flour.

Treatments	Moisture Content (%)	Ash (%)	Protein (%)	Crude Fat (%)	Fiber (%)	NFE (%)
T_0	9.03 ±0.44^{a}	1.38 ±0.04^{d}	11.54 ±0.24^{e}	1.74 ±0.04^{ab}	1.33 ±0.04^{h}	74.98 ±0.37^{a}
T_1	8.65 ±0.32^{ab}	1.50 ±0.01^{cd}	11.68 ±0.22^{de}	1.87 ±0.05^{ab}	1.51 ±0.07^{gh}	74.91 ±0.29^{ab}
T_2	8.17 ±0.25^{abc}	1.72 ±0.03^{bcd}	12.32 ±0.17^{bcd}	2.17 ±0.01^{ab}	1.85 ±0.02^{def}	74.11 ±0.50^{abcd}
T_3	7.60 ±0.36^{c}	1.98 ±0.01^{ab}	12.84 ±0.08^{ab}	2.26 ±0.07^{ab}	2.0 ±0.03^{abc}	73.70 ±0.45^{bcde}
T_4	8.71 ±0.44^{ab}	1.55 ±0.02^{cd}	11.74 ±0.10^{de}	1.88 ±0.01^{ab}	1.63 ±0.05^{fg}	74.68 ±0.36^{ab}
T_5	8.35 ±0.40^{abc}	1.79 ±0.01^{bc}	12.48 ±0.11^{abc}	2.13 ±0.03^{ab}	1.92 ±0.04^{cde}	73.69 ±0.39^{bcde}
T_6	7.82 ±0.39^{bc}	2.09 ±0.18^{ab}	12.99 ±0.37^{ab}	2.33 ±0.09^{a}	2.23 ±0.06^{ab}	73.10 ±0.61^{de}
T_7	8.83 ±0.33^{ab}	1.60 ±0.03^{cd}	11.79 ±0.33^{cde}	1.94 ±0.03^{ab}	1.75 ±0.07^{efg}	74.37 ±0.15^{abc}
T_8	8.51 ±0.30^{abc}	1.656 ±0.02^{bc}	12.57 ±0.41^{ab}	2.21 ±0.02^{ab}	1.95±0.01^{bcd}	73.31 ±0.60^{cde}
T_9	8.01 ±0.32^{abc}	1.792 ±0.02^{a}	13.1 ±0.084^{a}	2.37 ±0.62^{a}	2.54 ±0.03^{a}	72.57 ±0.25^{e}

Note: Values expressed are means ± standard deviation; T0: whole Wheat Flour (Control); T_1: 88% whole wheat flour +10% barley flour +2% Functional blend; T_2: 78% whole wheat flour +20% barley flour +2% Functional blend, T_3: 68% whole wheat flour +30% barley flour +2% Functional blend, T_4: 86% whole wheat flour +10% barley flour +4% Functional blend, T_5: 76% whole wheat flour +20% barley flour +4% Functional blend, T_6: 66% whole wheat flour +30% barley flour +4% Functional blend, T_7: 84% whole wheat flour +10% barley flour +6% Functional blend, T_8: 74% whole wheat flour +20% barley flour +6% Functional blend, T_9: 64% whole wheat flour +30% barley flour +6% Functional blend.

different treatments of composite flour was determined by following method described **(Prosky et al., 1987)**.

Sensory evaluation of chapattis

Sensory evaluation of chapattis was carried out for various sensory attributes like flavor, texture, color, taste, chewingability and foldingabiity by the panel of 5 trained judges from the National Institute of Food Science and Technology having expertise in Cereal Technology according to the 9-point hedonic scale as described according to the protocol of **Meilgard et al. (2006)**.

Statisic analysis

All analyses were carried out in triplicate and the data was reported as means ±standard deviation computed through Microsoft Excel 2013. Significant difference among treatments was evaluated through analysis of variance (ANOVA) under completely randomized design (CRD).

The results obtained from different parameters of all the treatments were exposed to statistical analysis. Completely Randomized Design (CRD) was used, followed by the Analysis of Variance Technique (ANOVA) and the results were interpreted according to the Least Significant Difference Test (LSD) at 5% level of significance as described by **(Steel et al., 1997)**.

RESULTS AND DISCUSSION

Characterization of wheat and barley flour

The means for proximate composition of both flours given in Table 2. Moisture, crude fat, total ash, crude protein, crude fiber and nitrogen free extract was 9.08, 1.74, 1.33, 11.54%, 76.26% in whole wheat flour and 7.08%, 13.63%, 4.04%, 3.11%, 3.05% and 72.14 in whole barley flour respectively. The whole barley flour possessed minimum moisture content and nitrogen free extract (NFE) as compared to wheat flour. Whole barley flour yielded higher contents of protein, fat, ash and crude fiber as compared to wheat flour sample.

The outcomes of current analysis are in accordance with **Yalmlahi and Ouhuuine (2013)**. whose result supports that moisture content in wheat flour is greater than

Table 4 Sodium and potassium, calcium, magnesium minerals composition of wheat and barley supplemented flours.

Treatments	Na (mg.100g^{-1})	K (mg.100g^{-1})	Ca (mg.100g^{-1})	Mg (mg.100g^{-1})
T_0	2.02 ±0.36^{e}	684.00 ±5.56^{d}	23.09 ±2.38	152.33 ±3.11^{f}
T_1	2.71 ±0.33^{e}	697.00 ±8.54^{cd}	24.68 ±0.94	158.33 ±3.05^{ef}
T_2	3.68 ±0.22^{cd}	712.67 ±5.50^{bc}	25.94 ±1.97	167.63 ±2.07^{bcde}
T_3	4.73 ±0.77^{ab}	728.00 ±3.00^{ab}	27.02 ±2.21	175.78 ±3.86^{ab}
T_4	2.81 ±0.25^{de}	685.80 ±5.30^{d}	24.74 ±1.14	159.77 ±2.92^{def}
T_5	3.70 ±0.14^{cd}	698.53 ±6.30^{cd}	26.04 ±2.74	168.67 ±4.72^{abcd}
T_6	4.77 ±0.17^{a}	729.88 ±2.80^{ab}	27.14 ±3.24	177.13 ±3.72^{ab}
T_7	2.88 ±0.20^{cde}	687.21 ±5.93^{d}	24.83 ±3.37	161.47 ±3.83^{cdef}
T_8	3.78 ±0.22^{bc}	700.07 ±10.8^{cd}	26.19 ±2.79	169.93 ±4.02^{abc}
T_9	4.79 ±0.11^{a}	731.77 ±10.76^{a}	27.21 ±2.9	178.67 ±2.66^{a}

Note: Values expressed are means ± standard deviation; T0: whole Wheat Flour (Control); T_1: 88% whole wheat flour +10% barley flour +2% Functional blend; T_2: 78% whole wheat flour +20% barley flour +2% Functional blend, T_3: 68% whole wheat flour +30% barley flour +2% Functional blend, T_4: 86% whole wheat flour +10% barley flour +4% Functional blend, T_5: 76% whole wheat flour +20% barley flour +4% Functional blend, T_6: 66% whole wheat flour +30% barley flour +4% Functional blend, T_7: 84% whole wheat flour +10% barley flour +6% Functional blend, T_8: 74% whole wheat flour +20% barley flour +6% Functional blend, T_9: 64% whole wheat flour +30% barley flour +6% Functional blend.

Table 5 Manganese, iron, copper and zinc composition of wheat and barley supplemented flours.

Treatments	Mn (mg.100g^{-1})	Fe (mg.100g^{-1})	Cu (mg.100g^{-1})	Zn (mg.100g^{-1})
T_0	3.80 ±0.19^{d}	1.71 ±0.48^{c}	0.31 ±0.01^{f}	2.90 ±0.38^{c}
T_1	3.95 ±0.15^{cd}	2.47 ±0.49^{bc}	0.34 ±0.02^{ef}	3.64 ±0.21^{bc}
T_2	4.37 ±0.28^{bcd}	3.83 ±1.16^{ab}	0.39 ±0.04^{cde}	4.74 ±0.41^{ab}
T_3	4.97 ±0.29^{ab}	4.76 ±0.49^{a}	0.46 ±0.07^{abc}	5.32 ±0.38^{a}
T_4	3.99 ±0.19^{cd}	2.49 ±0.34^{bc}	0.35 ±0.03^{def}	3.67 ±0.08^{bc}
T_5	4.40 ±0.14^{bcd}	3.88 ±0.31^{ab}	0.41 ±0.06^{bcd}	4.76 ±0.05^{ab}
T_6	5.00 ±0.20^{ab}	4.78 ±0.46^{a}	0.47 ±0.1^{ab}	5.36 ±0.28^{a}
T_7	4.24 ±0.24^{cd}	2.54 ±0.31^{bc}	0.36 ±0.08^{def}	3.70 ±0.25^{bc}
T_8	4.46 ±0.19^{bc}	3.90 ±0.26^{ab}	0.43 ±0.05^{abc}	4.81 ±0.43^{ab}
T_9	5.18 ±0.33^{a}	4.83 ±0.42^{a}	0.49 ±0.02^{a}	5.42 ±0.56^{a}

Note: Values expressed are means ± standard deviation; T0: whole Wheat Flour (Control); T1: 88% whole wheat flour +10% barley flour +2% Functional blend; T2: 78% whole wheat flour +20% barley flour +2% Functional blend, T3: 68% whole wheat flour +30% barley flour +2% Functional blend, T4: 86% whole wheat flour +10% barley flour +4% Functional blend, T5: 76% whole wheat flour +20% barley flour +4% Functional blend, T6: 66% whole wheat flour +30% barley flour +4% Functional blend, T7: 84% whole wheat flour +10% barley flour +6% Functional blend, T8: 74% whole wheat flour +20% barley flour +6% Functional blend, T9: 64% whole wheat flour +30% barley flour +6% Functional blend.

Table 6 Total dietary fiber composition of wheat and barley supplemented flour and chapattis.

Treatments	TDF in flour (%)	TDF in chapattis (%)
T_0	3.11 ±0.10^{d}	6.04 ±0.075^{f}
T_1	5.81 ±0.06^{c}	7.34 ±0.046^{e}
T_2	6.52 ±0.28^{b}	7.76 ±0.040^{d}
T_3	7.47 ±0.06^{a}	7.98 ±0.074^{bc}
T_4	5.89 ±0.04^{c}	7.42 ±0.046^{e}
T_5	6.63 ±0.06^{b}	7.81 ±0.050^{cd}
T_6	7.58 ±0.05^{a}	8.08 ±0.09^{ab}
T_7	5.95 ±0.08^{c}	7.49 ±0.06^{e}
T_8	6.71 ±0.16^{b}	7.87 ±0.078^{cd}
T_9	7.69 ±0.05^{a}	8.21 ±0.095^{a}

Note: Values expressed are means ± standard deviation; TDF: Total dietary fiber, T0: whole Wheat Flour (Control); T_1: 88% whole wheat flour +10% barley flour +2% Functional blend; T_2: 78% whole wheat flour +20% barley flour +2% Functional blend, T_3: 68% whole wheat flour +30% barley flour +2% Functional blend, T_4: 86% whole wheat flour +10% barley flour +4% Functional blend, T_5: 76% whole wheat flour +20% barley flour +4% Functional blend, T_6: 66% whole wheat flour +30% barley flour +4% Functional blend, T_7: 84% whole wheat flour +10% barley flour +6% Functional blend, T_8: 74% whole wheat flour +20% barley flour +6% Functional blend, T_9: 64% whole wheat flour +30% barley flour +6% Functional blend.

Table 7 Antioxidants in different supplemented flours**.**

Treatments	TPC (mg GAE.g^{-1})	DPPH (%)
T_0	0.41±0.08^{d}	20.95 ±0.82^{c}
T_1	0.50 ±0.02^{c}	21.85 ±0.77^{bc}
T_2	0.60 ±0.03^{b}	22.79 ±0.04^{ab}
T_3	0.67 ±0.04^{a}	23.74 ±0.05^{a}
T_4	0.52 ±0.05^{c}	21.88 ±0.03^{bc}
T_5	0.61 ±0.07^{b}	22.82 ±0.26^{ab}
T_6	0.68 ±0.02^{a}	23.77 ±0.06^{a}
T_7	0.53 ±0.09^{c}	21.93 ±0.13^{bc}
T_8	0.62 ±0.04^{b}	22.87 ±0.05^{ab}
T_9	0.69 ±0.06^{a}	23.82 ±0.07^{a}

Note: Values expressed are means ± standard deviation; TPC: Total phenolic content, GAE: Gallic acid equivalents (Folin-Ciocalteu method), DPPH: 2,2-diphenyl-1-picrylhydrazyl, T_0: whole Wheat Flour (Control); T_1: 88% whole wheat flour +10% barley flour +2% Functional blend; T_2: 78% whole wheat flour +20% barley flour +2% Functional blend, T_3: 68% whole wheat flour +30% barley flour +2% Functional blend, T_4: 86% whole wheat flour +10% barley flour +4% Functional blend, T_5: 76% whole wheat flour +20% barley flour +4% Functional blend, T_6: 66% whole wheat flour +30% barley flour +4% Functional blend, T_7: 84% whole wheat flour +10% barley flour +6% Functional blend, T_8: 74% whole wheat flour +20% barley flour +6% Functional blend, T_9: 64% whole wheat flour +30% barley flour +6% Functional blend.

moisture content of barley flour. Moisture content was influenced by milling techniques. **Khan (2009)** revealed the same results for fat in whole wheat flour. In another research analysis **Hussein et al. (2013)** observed 4% fat in whole barley flour. As for barley flour, the value obtained is judged too high. This is due to the fact that the separation of germ from bran is not so fine during the barley milling as compared to wheat grain milling. The consequence of existing study are in close agreement with previous research work of **Hussein et al. (2013)**. They found 1.47% ash in wheat flour and 3.08% ash in barley flour. **Ejaz (2014)** noticed less protein content in wheat flour and higher in composite flour. **Ragaee et al. (2006)** made similar observation for protein content present in wheat flour. The results showed the higher percentage of crude fiber in whole barley flour as compare to the whole wheat flour. The results of present study regarding the fiber composition of whole wheat flour and whole barley flour are in close agreement with earlier research work of **Elzamzamy (2014)**. **Hussein et al. (2013)** observed the 1.65% of crude fiber in wheat flour and 3.35% crud fiber in whole barley flour. These results are in close agreement with present research analysis. **Khan (2009)** made similar observation for NFE in whole wheat flour. **Elzamzamy (2014)** made observation that NFE for whole wheat flour was greater than whole barley flour.

Analysis of Composite flour

Chemical composition of composite flours

The mean values regarding proximate composition of varying treatments have been revealed in table 3. The proximate composition of composite varied due to the varying amount of barley flour and functional blend supplemented into the wheat flour.

The highest moisture content (9.03%) was found in T_0 and minimum moisture content (7.6%) was noted in T_3 (68% whole wheat flour +30% barley flour +2% Functional blend). The current conclusions of existing research work are in agreement with **Yalmlahi and Ouhuuine (2013)**. Moisture content reduced by increasing the amount of barley flour moisture and this was attributed

due to a greater water holding capacity of wheat flour than the barley flour.

Minimum ash content was found in control i.e. wheat flour while maximum in treatment T_9. **Shahzadi (2004)** observed the same outcomes in her. It was due to the fact because barley flour usually contains visible specks of bran and subsequently appears darker and is higher in ash content than wheat flour.

The mean value (Table 3) revealed that protein content in composite flour was ranged from 11.54 to 13.1%. Least protein content was observed in wheat flour and maximum in composite flour with 30% barley flour and 6% functional blend. **Beswa (2010)** found similar protein content in wheat-millet composite flour 10, 20 and 30% substitution levels. The present results are close enough to **Ejaz (2014)**. **Ragaee et al. (2006)** found higher protein content in barley and less protein content in hard and soft wheat. They explained the reason of higher protein content in barley. It was due to the reason because high nitrogen fertilization, in most instances, increases storage proteins (that are higher in barley that wheat) and thus total protein of barley.

The fat content varied from 1.74 to 2.37%. The significant increase in the fat content of composite flour with increasing levels of barley flour substitution may be explained by the fact that, the higher content of fat in whole grain product is due to the presence germ in which oil is concentrated and germ portion of barley grain is higher than wheat grain. Fat contents in wheat, sorghum, millet, rye and barley flour are observed by **Ragaee et al. (2006)** whose results are much closer with the discoveries of current outcomes. **Khan (2009)** and **Arab et al. (2010)** revealed same results for fat content in whole wheat flour and composite flours.

The fiber content varied from 2.54% to 1.33%. The significant (p <0.05) increase in the fibre content was due the reason that, wheat flour had lower fibre content values compared to barley flour. Barley contains higher amount of cellulose and lignins and both of these are mainly consisted in crude fiber and fiber portions are mainly

Table 8 Effect of various treatment on color, texture, folding ability and Chew ability of wheat and barley supplemented flavored chapattis.

Treatments	Color	Texture	Folding ability	Chew ability
T_0	7.41 ±0.050^{d}	8.02 ±0.074^{a}	7.91 ±0.04^{d}	8.53 ±0.04^{a}
T_1	6.46 ±0.042^{f}	7.53 ±0.047^{b}	7.27 ±0.08^{f}	5.51 ±0.23^{f}
T_2	8.01 ±0.061^{c}	6.13 ±0.096^{c}	8.32 ±0.05^{c}	6.52 ±0.13^{d}
T_3	8.75 ±0.129^{a}	4.79 ±0.031^{d}	8.68 ±0.07^{ab}	7.59 ±0.040^{b}
T_4	7.03 ±0.036^{e}	7.04 ±0.046^{e}	7.64 ±0.06^{e}	6.05 ±0.08^{e}
T_5	8.51 ±0.064^{b}	5.72 ±0.057^{f}	8.51 ±0.04^{bc}	7.15 ±0.04^{c}
T_6	8.85 ±0.08^{a}	4.54 ±0.04^{g}	8.79 ±0.05^{a}	8.25 ±0.05^{a}
T_7	5.14 ±0.06^{i}	6.52 ±0.05^{h}	6.04 ±0.06^{i}	5.31 ±0.06^{f}
T_8	5.60±0.061 ^{h}	5.31 ±0.050^{i}	6.36 ±0.05^{h}	4.58 ±0.02^{g}
T_9	6.05 ±0.012^{g}	4.147 ±0.06^{j}	6.76 ±0.1^{g}	4.13 ±0.07^{h}

Note: Values expressed are means ± standard deviation; T0: whole Wheat Flour (Control); T_1: 88% whole wheat flour +10% barley flour +2% Functional blend; T_2: 78% whole wheat flour +20% barley flour +2% Functional blend, T_3: 68% whole wheat flour +30% barley flour +2% Functional blend, T_4: 86% whole wheat flour +10% barley flour +4% Functional blend, T_5: 76% whole wheat flour +20% barley flour +4% Functional blend, T_6: 66% whole wheat flour +30% barley flour +4% Functional blend, T_7: 84% whole wheat flour +10% barley flour +6% Functional blend, T_8: 74% whole wheat flour +20% barley flour +6% Functional blend, T_9: 64% whole wheat flour +30% barley flour +6% Functional blend.

Table 9 Mean scores for the effect of various treatment on taste, breakability and overall acceptability of wheat and barley supplemented flavored chapattis.

Treatments	Taste	Breakability	Overall acceptability
T_0	6.98 ±0.06^{d}	5.31 ±0.06^{g}	7.02 ±0.17^{d}
T_1	5.94 ±0.04^{f}	4.75 ±0.1^{h}	6.05 ±0.06^{f}
T_2	7.45 ±0.07^{c}	6.70 ±0.06^{d}	7.56 ±0.08^{c}
T_3	8.47 ±0.03^{a}	7.71 ±0.070^{b}	8.50 ±0.09^{a}
T_4	6.48 ±0.23^{e}	4.43 ±0.08^{i}	6.58 ±0.16^{e}
T_5	7.95 ±0.10^{b}	7.10 ±0.05^{c}	8.01 ±0.20^{b}
T_6	8.7 ±0.22^{a}	8.21 ±0.04^{a}	8.90 ±0.25^{a}
T_7	4.58 ±0.13^{i}	4.03 ±0.08^{j}	4.54 ±0.13^{i}
T_8	5.03 ±0.09^{h}	6.30 ±0.06^{e}	5.09 ±0.10^{h}
T_9	4.57 ±0.03^{g}	5.80 ±0.07^{f}	5.53 ±0.05^{g}

Note: Values expressed are means ± standard deviation; T_0: whole Wheat Flour (Control); T_1: 88% whole wheat flour +10% barley flour +2% Functional blend; T_2: 78% whole wheat flour +20% barley flour +2% Functional blend, T_3: 68% whole wheat flour +30% barley flour +2% Functional blend, T_4: 86% whole wheat flour +10% barley flour +4% Functional blend, T_5: 76% whole wheat flour +20% barley flour +4% Functional blend, T_6: 66% whole wheat flour +30% barley flour +4% Functional blend, T_7: 84% whole wheat flour +10% barley flour +6% Functional blend, T_8: 74% whole wheat flour +20% barley flour +6% Functional blend, T_9: 64% whole wheat flour +30% barley flour 6% Functional blend.

concentrated in bran portion that are higher in barley flour due to poor sepration of bran during milling. Results of **Huma (2004)** are found to be similar to the analysis of current research results. The results are alike to the conclusions of previous researchers **(Butt et al., 2011; Sharma et al., 2011)**. They found greater percentage of crude fiber in composite flour as compared to the crude fiber content in wheat flour. This is due to the higher portion of bran in barley that contain higher content of fiber. Due to this reason crude fiber in barley increased the fiber content of wheat and barley composite flour.

NFE in composite flour was ranged from 72.57 to 74.98. As Nitrogen free extract is generally determined by subtracting sum of moister, protein, fat and fiber from 100. Maximum value was observed in whole wheat flour because it has lower value of protein, fat and fiber content as compared to the other treatments. While maximum value was found in T_3. It is due to the reason of having maximum percentage of barley flour and minimum percentage of functional blend among all treatment. **Khan (2009)** observed 74.64% NFE in whole wheat flour. Similarly, the consequences of existing work are sustained greatly by the judgements by **Ejaz (2014)** who reported decreasing trend for nitrogen free extract with the addition of barley and oatmeal flour.

Mineral composition

The mean value regarding macro and micro nutrients have been expressed in Table 4. The mean values for sodium content was described in table 4. The significant increase in sodium, potassium, magnesium, iron, copper, zinc, managanese content of composite flour with increasing levels of barley flour and functional blend was observed while calcium content did not differ significantly.

The judgements of **Arab et al. (2010)** are related to the consequences of existing research analysis who reported comparable results for sodium content in wheat flour. The potassium content was ranged from 570 mg.100g^{-1} to 976.19 mg.100g^{-1}. According to the recent analysis, potassium content in whole wheat flour are found to be closer enough to research analysis of **Niazi (2015)** and **Ejaz (2014)**. The highest calcium content (27.21 mg.100g^{-1}) was found in T_9 while minimum value (23.09%) was observed in T_0. The effects of existing results of recent research are in accordance with the outcomes of **Hussein et al. (2013)** who reported similar results for calcium content in wheat flour. The results showed that as the supplementation of barley flour and functional blend increased, magnesium content also increased. The results of present study are in accordance with the findings of **Ejaz et al. (2014)** who reported similar magnesium content in whole wheat flour and similar increase in mineral content in composite flour (wheat flour supplemented with oat and barley flour). Highest managanese content (5.18 mg/100g) was found in T_9 while minimum value (3.8 mg.100g^{-1}) was observed in T_0. The findings of **Khan (2009)** are in agreement with the consequences of present research analysis. Khan observed the effect of soy supplementation on manganese content (mg.100g^{-1}) of composite flours. The variation in iron content is evident with an increase in the supplementation rate of barley flour, garlic paste and methi leaves. Highest iron content (4.83 mg.100g^{-1}) was found in T_9 while minimum value (1.71 mg.100g^{-1}) was observed in whole wheat flour. The outcomes of current study are in agreement with the conclusions of **Arab et al. (2010)** who described similar iron content in whole wheat flour. **Hussein et al. (2013)** observed the mineral content of whole barley flour (WBF) and wheat flour (WF) and found closer results. The copper content in composite flour was ranged from 0.31 mg.100g^{-1} to 0.49 mg.100g^{-1}.

Copper content was improved by increasing the supplementation rate of barley flour and functional blend (methi leaves and garlic paste). The analysis of current work have interpreted same results that are strongly supported by work of **Hussein et al. (2013)**. Zinc content in composite flour was ranged from 2.9 mg.100g^{-1} to 5.42 mg.100g^{-1}. The results of present study are in accordance with the findings of **Khan (2009)** who reported that zinc content (mg.100g^{-1}) increased by increasing the supplementation of soy composite flour.

The difference in mineral composition was may be attributed to more mineral content in whole barley flour as compared to the wheat flour in which bran portion in removed more easily during milling and minerals or ash are mainly concentrated in bran portion. While barley kernel are more hard and it is difficult to separate the bran portion.

Dietary fiber composition

Mean values for total dietary fiber of different composite flour and chapattis are presented in table 6. Total dietary fiber content of composite flour was ranged from 3.1% to 7.7% and it was ranged from 6.04% to 8.21% in composite flour chapattis. The result showed that maximum total dietary fiber content was found in treatment which contain highest amount of barley flour (30%) and highest percentage of functional blend (6%) while lowest in wheat flour.

Ragaee et al. (2006) reported the higher composition of total dietary fiber in barley than sorghum, rye and millet as compared to the wheat flour. The results of **Butt et al. (2011)** were closely related to the findings of present study who observed higher percentage of total dietary fiber in composite flour chapattis as compared to control. They observed that chapattis supplemented with 5% chickpea and 1% guar gum (CP5% +GG1%), 3% guar gum (GG 3%) and 2% guar gum (GG 2%) have higher composition of dietary fiber. Results regarding total dietary fiber content in composite flour and chapattis are in line with work of **Ejaz (2014)** who observed the total dietary fiber composition of barley and oatmeal supplemented chapattis.

Dietary fiber are not hydrolyzed in GI track because of absence of particular enzyme but partially hydrolyzed by microflora in the large intestine and produce short chain fatty acids. These short chain fatty acids prevent the cholesterol synthesis so help to reduce heart diseases and this is the main reason of using barley to reduce several heart diseases. The reason that why wheat flour chapattis had relatively low content of total dietary fiber is due to easy removal of bran or the outer kernel layers form wheat grain during milling and dietary fiber are mainly concentrated in bran portion.

Antioxidant analysis

The data related to mean values for total phenolic content and DPPH of composite flour are shown in Table 7. The result showed that higher total phenolic content (0.69 mg GAE.g^{-1}) was found in T_9 while minimum value (0.41 mg GAE.g^{-1}) was observed in T_0. Antioxidant properties of wheat and composite flours were evaluated on the basis of measuring scavenging activity for DPPH radicals. DPPH of composite flour was ranged from 20.95% to 23.82%. The outcomes of current research work are supported by the judgements of **Elzamzamy (2005)**. **Afzal et al. (2016)** designed the research work to elucidate nutritional and antioxidant potential of fenugreek seeds. **Sharma (2012)** reported antioxidant activity (17 – 24%) in barley flour that is higher than wheat flour. It ratifies that addition of barley flour and functional blend in whole wheat flour enhanced the total phenolic content.

The analysis specifies that rich basis of antioxidants are cereals especially barley. Before consumption, cereals are treated with different processing like milling, heat extraction, cooking, parboiling or other technique and most researcher found that processing of barley grains does not remove biologically important compounds and provide protection against free radical that attack on DNA, lipids and protein and thought to be an initiating factor for several chronic diseases **(Slavin et al. 2001)**. **Verardo et al. (2010)** used the barley that help to diminish the oxidation of lipid in bakery foodstuffs. They used barley as a source of phenolic compounds. So, decrease in peroxide value and increase in antioxidant activity is evident with the increase in supplementation rate of barley flour.

The score for acceptability of chapattis of different treatments ranged from 4.54 to 8.9. The highest acceptability (8.9) was found in chapattis prepared from T6 (66% whole wheat flour +30% barley flour +4% Functional blend) due to best color, finest taste, good foldingabiity and breakability, followed by T_3 , T_5, T_2, T_0, T_4, T_1 and lowest score (4.54) was found in chapattis prepared from T_7. T_6 acquired highest score in overall acceptability while nutritionally in all other parameters excerpt sensory T_9 scored best. In the present research, composite flour samples affected the overall acceptability due the variation in sensory attributes of barley flour, methi leaves and garlic paste. The outcomes of recent analysis are compatible with the judgements of **Ejaz (2014)**. **Shahzadi (2004)** also established similar overall acceptability score for wheat-chickpea composite flour.

CONCLUSION

Whole wheat flour supplemented with whole barley flour is a vital source of fibrous food. To improve the nutritional status of many food products, there is a requirement to explore the hidden sources of dietary fiber. In conclusion, barley flour can be a good option to obtain the nutritional significance and health expansions of wheat-based products because scheme that is dependent on diet is an exact approach as it is cost-effective and measureable to escape from health hazards. By incorporating barley flour into popularly consumed wheat-based products such as chapattis it could help consumers to improve their health. As wheat products become healthier by incorporating barley flour, it is expected to see continued and sustainable growth in barley consumption. So it is concluded that for the reason of having high fiber and dietary fiber content, more antioxidants and improved minerals profile as compared to the wheat flour, barley is considered as a desired food ingredient. Thus, intake of chapattis made by selected quantity of composite flours offers an additional health gains that would be helpful for normal humans to avoid diseases.

REFERENCES

AACC, 2000. Approved Methods of American Association of Cereal Chemists. American Association of Cereal Chemists, MN, USA : MN, USA.

Afify, A. M. R., El-Beltagi, H. S., EL-Salam, S. M. A., Omran, A. A. 2012. Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. *Journal of Tropical Biomedicine*, vol. 2, no. 3, p. 203-209. https://doi.org/10.1016/S2221-1691(12)60042-2

Afzal, B., Pasha, I., Zahoor, T., Nawaz, H. 2016. Nutritional potential of fenugreek supplemented bread with special reference to antioxidant profiling. *Pakistan Journal of Agriculture Science*, vol. 53, p. 217-223. https://doi.org/10.21162/PAKJAS/16.4664

Ajithkumar, A., Anderson, R., Aman, P. 2005. Content and molecular behaviour of extractable -glucan in American and Swedish oat samples. *Journal of Agriculture of Food Chemistry*, vol. 53, no. 4, p. 1205-1209. https://doi.org/10.1021/jf040322c PMid:15713042

AOAC. 2006. Official Methods of Analysis of Association of Official Analytical Chemists International. 18th ed. Arlington, VA, USA : AOAC Press.

Arab, E. A. A., Helmy, I. M. F., Bareh, G. F. 2010. Nutritional evaluation and functional properties of chickpea (*Cicer arietinum* L.) flour and the improvement of spaghetti produced from its. *Journal ofAmerican Science*, vol. 6, no. 10, p. 1055-1072.

Beswa, D. 2008. *Assessment of the feasibility of using a wheat-Finger millet composite flour for bread making* : dissertation theses. South Africa : University of Johannesburg.

Butt, M. S., Shahzadi, N., Suleria, H. A. R., Sultan, T., Chohan, M. I. 2011. Effect of dietary fiber in lowering serum glucose and body weight in sprague dawley rats. *Functional Foods in Health Disease*, vol. 8, no. 2011, p. 261-278.

Dhingra, S., Jood, S. 2004. Effect of flour blending on functional, baking and organoleptic characteristics of bread. *International Journal of food Science Technology*, vol. 39, no. 2, p. 213-222. https://doi.org/10.1046/j.0950-5423.2003.00766.x

Dholakia, B. B. 2001. *Molecular approaches to decipher quantitative traits governing grain quality in hweats* : dissertation theses. India : University Pune.

Edge, M., Jones J. M., Marquart. L. 2005. A new life for whole grians. *Journal of the American Diet Association*, vol. 105, no. 12, p. 1856-1860. https://doi.org/10.1016/j.jada.2005.10.022 PMid:16321586

Ejaz, R. 2014. *Preparation of barley and oatmeal supplemented chapaties and its impact on serum biochemical profile in normal individuals* : M.Sc. Thesis. Faisalabad, Pakistan : University of Agriculture.

Elzamzamy, F. M. 2014. Chemical, Sensory and Microbiological Evaluation of Novel two Products Manufactured from Germinated Barley and Wheat Grains.

British Journal of Applied Science and Technology, vol. 4 no. 14, p. 2100-2113. https://doi.org/10.9734/BJAST/2014/4439

Frost, D. J., Adhikari, K., Lewis D. S. 2011. Effect of barley flour on physical and sensory characteristics of chocolate chip cookies. *Journal of food science and Technology*, vol. 48, no. 5, p. 569-576. https://doi.org/10.1007/s13197-010-0179-x PMid:23572789

Gujral, H. S., Pathak, A. 2002. Effect of composite flours and additives on the texture of chapati. *Journal of Food Engineering*, vol. 55, no. 2, p. 173-179. https://doi.org/10.1016/S0260-8774(02)00061-4

Hussein, A. M. S., Abd El-Azeem, A. S., Hegazy, A. M., Afifi, A. A., Gamal, H. R. 2011. Physiochemical, Sensory and Nutritional Properties of corn-fenugreek Flour composite biscuits. *Austutrilian Journal of Basic and Applied Science*, vol. 5, no. 4, p. 84-95.

Hussein, A. M. S., Kamil, M. M., Hegazy, N. A., Abo El-Nor, S. A. H. 2013. Effect of Wheat Flour Supplemented with Barely and/or Corn Flour on Balady Bread Quality. *Polish Journal of Food Nutrition*, vol. 63, no. 1, p. 11-18. https://doi.org/10.2478/v10222-012-0064-6

Jilal, A. 2011. *Assessment of genticaly diverse international barley germplasm for development of food product applications* : dissertation theses. Lismore, NSW : Southern Cross University.

Han, X., Shen, T., Lou, H. 2007. Dietary Polyphenols and Their Biological Significance. *International Journal of Molecule Science*, vol. 8, no. 9, p. 950-988. https://doi.org/10.3390/i8090950

Huma, N. 2004. *Fortification of whole flour with iron flour the production of unleavened flat bread (Chapattis)* : dissertation theses. Faisalabad, Pakistan : National Institute of Food Science and Technology, University of Agriculture.

Kahkonen, M. P., A. I. Hopia, H. J. Vuorela, J. P. Rauha, Khan, M. I. 2009. *Utilization of soy bean as a functional food.* Dissertation theses. Faisalabad, Pakistan : University of Agriculture.

Lagasse, S. L., Hatcher, D. W., Dexter, J. E., Rossnagel, B. G., Izydorczyk, M. S. 2006. Quality characteristics of fres and dried white salted noodles enriched with flours from hull-less barley genotypes of diverse amylose content. *Cereal Chemistry*, vol. 83, no. 2, p. 202-210. https://doi.org/10.1094/CC-83-0202

Meilgard, M. M., Civille, G. V., Carr, T. 2007. *Sensory evaluation techniques*. 4th ed. New York, NY, USA : CRC Press. 600 p. ISBN 9781482216905.

Niazi, S. 2015. M.Sc. Thesis. Faisalabad, Pakistan : University of Agriculture.

Pihlaja, K., Kujala, T. S., Heinonen, M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agriculture and Food Chemistry*, vol. 47, no. 10, p. 3954-3962. https://doi.org/10.1021/jf990146l PMid:10552749

Prosky, L., Asp, N.G., Schweizer, T., Devries, J. Furda, I. 1987. Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. *Journal of Association of Analls Chemistry*, vol. 71, no. 5, p. 1017-1023.

Ragaee, S., Abdel-Aal, E. S. M., Noaman, M. 2006. Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry*, vol. 98, no. 1, p. 32-38. https://doi.org/10.1016/j.foodchem.2005.04.039

Shahzadi, N. 2004. *Use of composite flour for chapatti preparation in relation to serum profile* : dissertation theses. Faisalabad, Pakistan : University of Agriculture.

Shahzadi, N. 2004. *Use of composite flours for chapati preparation in relation to serum profile* : dissertation theses. Faisalabad, Pakistan : University of Agriculture.

Slavin, J. L., Jacobs, D., Marquart, L. 2001. Grain processing and nutrition. *Critical Reviews in Biotechnology*, vol. 21, p. 49-66. https://doi.org/10.1080/20013891081683 PMid:11307845

Sharma, P., Gujral, H. S. 2011. Effect of sand roasting and microwave cooking on antioxidant activity of barley. *Food Research International*, vol. 44, no. 1, p. 235-240. https://doi.org/10.1016/j.foodres.2010.10.030

Sharma, P., Gujral, H. S., Singh, B. 2012. Antioxidant activity of barley as affected by extrusion cooking. *Food Chemistry*, vol. 131, no. 4, p. 1406-1413. https://doi.org/10.1016/j.foodchem.2011.10.009

Sudha, M. L., Eipson, S. W., Hafeeza, K., Naidu, M. M., Rao, G. V. 2015. Effect of normal/dehydrated greens on the rheological, microstructural, nutritional and quality characteristics of paratha-an Indian flat bread. *Journal of Food Science and Technology*, vol. 52, no. 2, p. 1-9. https://doi.org/10.1007/s13197-013-1062-3 PMid:25694692

Verardo V., Ylenia, R., Trivisonno, M. C., Marconi, E., Caboni, M. F. 2010. Effect of the addition of air-classiffed barley flours on the lipid stability of bakery products. *European Food Research Technology*, vol. 231, no. 2, p.309-319. https://doi.org/10.1007/s00217-010-1288-5

Yamlahi, A. E., Ouhssine, M. 2013. Utilization of barley (*Hordeum vulgare* L.) flour with common wheat (*Triticum aestivum* L.) flour in bread-making. *Annual of Biological Research*, vol.4, p. 119-129.

Wahid, A. 2006. Productivity losses in barley attributable to ambient atmospheric pollutants in Pakistan. *Atmosphere Environment*, vol. 40, no. 28, p. 5342-5354. https://doi.org/10.1016/j.atmosenv.2006.04.050

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BACTERIA AND YEASTS ISOLATED FROM DIFFERENT GRAPE VARIETIES

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ABSTRACT

The aim of this study was to isolate and identify bacteria and yeasts in different grape samples. The samples were collected in September 2017. Used 13 grape samples in this study (9 white and 4 red) were from the local Slovak winemakers. Alibernet, Irsai Oliver, Dornfelder, Blue Frankish, Feteasca regala, Green Veltliner, Pálava, Müller Thurgau, Rhinriesling, Cabernet Savignon, Pinot Blanc, Savignon Blanc and Welschriesling. Two cultivation media were used for detection of bacteri and yeasts in grape samples. Malt extract agar base (MEA) and Tryptone Soay agar (TSA) were used for the cultivation of bacteria and 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 identification of bacteria and yeasts. In total, 8 genera of yeasts, 8 genera of Gram-negative bacteria and 10 of Gram-positive bacteria were identified. Together 333 isolates, yeasts, Gram-negative and Gram-positive bacteria were identified.

Keywords: bacteria; yeasts; grape; mass spectrometry

INTRODUCTION

Different physical and chemical parameters of environment determine the growth of plants in geographical region (e.g., temperature, humidity, precipitation, soil nutrient concentrations and solar radiation) **(Droždž et al., 2015)**. These factors also have a significant impact on the biogeography of the bacteria and fungi in the ecosystems. Studies focused on the bacteria associated with grapes by directly sampling during the initial stage of fermentation of the wine must were underatken **(Bokulich et al., 2014; Nedemová et al., 2016)**. Although not previously determined, it stands to reason that the same

The most common bacteria of grapes were *Oenococcus oeni, Leuconostoc mesenteroides, Pediococcus parvulus, P. pentosaceus, P. damnosus,* and different species of *Lactobacillus* (*L. brevis, L. plantarum, L. fermentum, L. buchneri, L. hilgardii, and L. trichodes*) **(Fleet, 2007; Du Toit et al., 2010)**. Malolactic fermentation, in addition to deacidification, contributes the favor characteristics of wine and has a impact on microbial stabilization **(Pozo-Bayon et al., 2005)**.

Previous studies of grapes and grape musts microflora revealed valuable indigenous yeast strains, which could serve as the contributors to the regional character of wines specific to different winemaking regions **(Varela and Borneman, 2016; Raymond Eder et al., 2017)**.

Non-*Saccharomyces* were the predominant yeast species isolated at the early stages of the spontaneous fermentation of *Vitis vinifera* L. grape musts **(Padilla et al., 2016)**. Among these, *Hanseniaspora, Candida, Pichia*, and *Metschnikowia* were the most important genera **(Jolly et al., 2013; Varela and Borneman, 2016).** The population of non-*Saccharomyces* species decreases in fermentation processes and the wine yeast *Saccharomyces cerevisiae* completes the fermentation **(Albergaria and Arneborg, 2016)**. The ability of *S. cerevisiae* to replace non-*Saccharomyces* species is associated with its higher fermentative power, alcohol tolerance and secretion of killer-like compounds **(Albergaria and Arneborg, 2016)**. Previous studies provide an overview on yeast microbiota of *Vitis vinifera* L. grape musts **(Padilla et al., 2016)**, but still is less known about the microorganisms present on grapes from other species of *Vitis*. The potential existence of various grapevine and microbial species communities is an aim of research interest **(Wolfe and Dutton, 2015)**.

A rapid and high-throughput identification method based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been introduced in bacterial taxonomy and in yeast and mold identification **(Pan et al. 2011; Hendrickx et al. 2011)**. MALDI-TOF MS idenfification is bases on measuring complex mixtures of proteins showing a unique fingerprint

for each species. Basically the ribosomal proteins, which are expressed at high level, the phenotypic technique is less influenced by expression variability **(Wieser et al. 2012)**. ‚Nowaday, MALDI-TOF became an important method for microorganism identification.

The aim of our study was to find bacteria and yeasts from the surface of grape berries and identify them by MALDI-TOF Mass Spectrometry.

Scientific hypothesis

The scientic hypothesis of this study was that the surfaces of different grape beries were contaminated with different bacterial and yeasts species, which could be found and identified with mass spectrometry.

MATERIAL AND METHODOLOGY

Grape collection samples

In total, 13 grape samples were used in experiment. Ripe grape bunches were collected into sterile polyethylene bags and transported to laboratory for microbiological analysis. The grape samples were collected from the Lesser Carpathian wine region. The grape samples of following varieties were investigated: Alibernet, Irsai Oliver, Dornfelder, Blue Frankish, Fateasca reagla, Grüner Veltliner, Pálava, Mūller Thurgau, Rheinriesling, Cabernet Savignon, Pinot Blanc, Savignon Blanc and Welschriesling. One sample consisted from one grape.

Microbiological analyses of grape berries samples

Five grams of berries from each grape variety were diluted with 45 ml of sterile physiological saline (0.85%). Berries were stirred on a horizontal shaker for 30 minutes. After that, the dilutions of 10^{-2} and 10^{-3} were prepared for cultivation with spread plate method. A 0.1 ml of each dilution (10^{-2}, 10^{-3}) was placed on the surface of a solid cultivation medium. Bacteria were cultivated on Plate count agar (PCA) (Oxoid, UK), yeasts on Malt extract agar base (MEA) (Oxoid, UK) supplemented with bromocresol green (0.020 $g.L^{-1}$) (Centralchem®, Slovakia). Bacteria were cultivated at 37 °C for 24 – 48 h in aerobic condition, but yeasts at 25 °C for five days in aerobic conditions. Growing colonies with macroscopic morphological differences were recultivated on TSA (Tryptic Soya agar, Oxoid®). Inoculated plates were cultivated at 30 °C for 48 h (TSA). After cultivation, the proteins were extracted from fresh bacterial colonies.

Sample preparation and MALDI-TOF MS measurement

One colony of each bacterial and yeast isolate was transferred into an Eppendorf vial and mixed with 300 µL of sterile water. After addition of ethanol (900 µL), the suspension was mixed and centrifuged (13 000 g, 2 min). After removal of supernatant, the pellets were dried at room temperature at least for 5 min. The bacterial and yeast pellets were resuspended in 20 – 50 µL of formic acid (70 %) and the same amount of acetonitrile. After centrifugation (2 min at 13 000 g), a 1 µL of supernatant was spotted onto a sample position of a polished steel MALDI target plate and dried at room temperature. A 1 µL of MALDI matrix (solution of α-cyano-4-hydroxycinnamic acid (HCCA) in 50 % acetonitrile/2.5 % trifluoro-acetic acid) was added to the spot and dried.

The MALDI target plate was introduced into the MALDI-TOF mass spectrometer for automated measurement and data interpretation. MALDI-TOF profile mass spectra were imported into the MALDI Biotyper 3.0 software and processed automatically after measurement. The logarithm of the score (log[score]) was displayed as the matching result. The MALDI Biotyper output was a log(score) between 0 and 3.0, which was calculated from a comparison of the peak list from an unknown isolate with the reference MSP in the database. A log(score) ≥1.7 indicated identification at the genus level, log(score) ≥2.0 was set as the threshold for a match at the species level. Isolates with ≥2.0 were accepted as a correct identification.

RESULTS AND DISCUSSION

From the surface of grape berries a total of 33 species of 18 bacterial genera (8 Gram negative G^- and 10 Gram positive G^+) and 10 species of yeasts belonging to 8 genera were identified with MALDI-TOF Mass Spectrometry. From a total of 333 isolates, the percentage representation of each microbial group (G^-, G^+ and yeasts) reached the following values: 69 isolates of G- (20.72%), 105 isolates of G^+ (31.53%), and 159 isolates of yeasts (47.74%). Table 1 shows that the most common microorganisms isolated from grapes were yeasts. The highest number of yeast species were identified from grape varieties Irsai Oliver (10.06%), Pálava, Pinot Blanc and Rheinriesling (9.43%). Yeast and bacteria were isolated from each grape variety. Bacterial species were identified in highest counts. The number of species of the three main groups of microorganisms in different grape varieties are given in Table 1.

Yeasts and bacterial genera were identified by MALDI-TOF. Percentages of the number of isolates of each genus are shown in Figure 1 for yeasts, in Figure 2 for G^- and in Figure 3 for G^+. The most abundant G^- bacterium was *Stenotrophomonas maltophilia* and *Ignatzschineria indica.* Within 22 different species of G^+ bacteria, the highest percentage representation (of isolates) was found for *Bacillus endophyticus, Paenibacillus glucanolyticus, Paenibacillus lautus* and *Staphylococcus succinus*. *Rhodotorula mucilaginosa* was the most abundant among of yeasts.

Kántor et al. (2017) found in 19 Slovak grape samples 11 genera of G^-, 11 of G^+ bacteria and nine of yeasts. Among 200 isolates, G^-, G^+ bacteria and yeasts represented 11%, 27% and 62% of the total number of isolates studied. The most common genera of isolated yeasts were *Hanseniaspora* (37%), *Metschnikowia* (31%), and *Rhodotorula* (10%). The most frequently isolated among G^- bacteria were *Acinetobacter* (22%), *Pseudomonas* (22%) and *Sphingomonas* (13%). The most common genera of G^+ bacteria were *Bacillus* (20%), *Lactobacillus* (19%), *Leuconostoc* and *Staphylococcus* (11%), respectively. In our study, from 333 isolates both different and similar species of microorganisms to **Kántor et al. (2017)** results were identified.

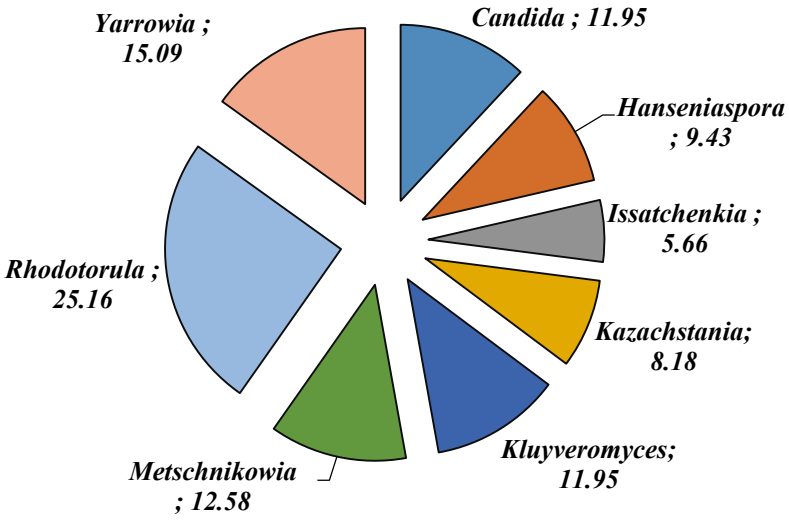

Figure 1 Yeast were isolated from grapes (%).

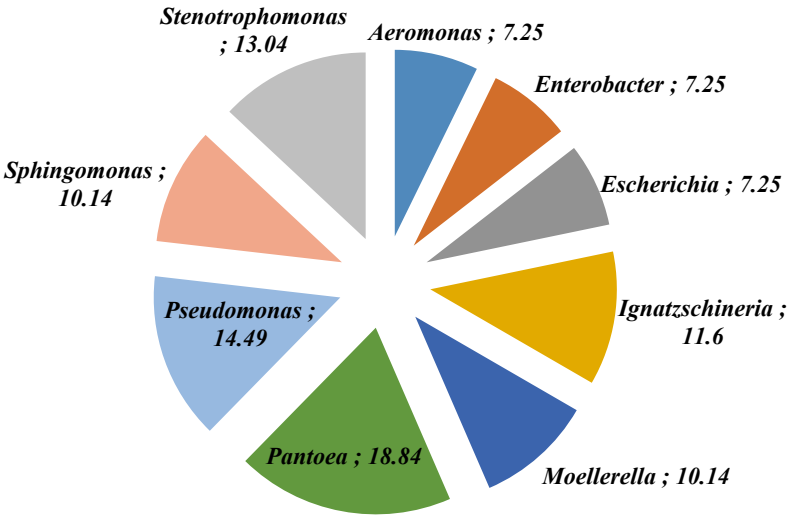

Figure 2 G^- bacteria were isolated from grapes (%).

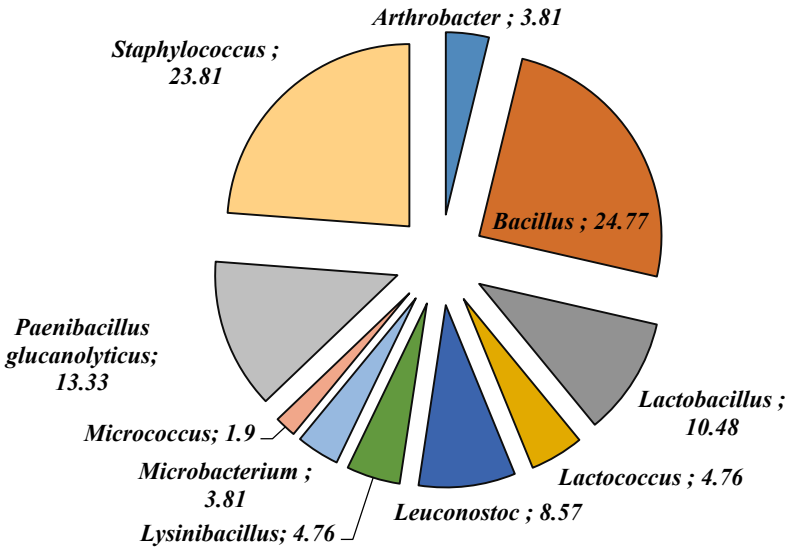

Figure 3 G^+ bacteria were isolated from grapes (%).

Similar results were decribed in research of **Kántor et al. (2016)**, where the microorganisms in similar grape wine varieties were studied. The most dominant species was *Saccharomyces cerevisiae* isolated from all 15 new wine samples, that was a very good wine quality indicator. With mass spectrometry, were identified seven different *S. cerevisiae* strains. The second most common species was *Kloeckera apiculata (Hanseniaspora uvarum)* found in seven new wine samples (2 strains). They also identified other non – *Saccharomyces* yeasts such as *Metschnikowia pulcherrima* (1 strain), *Pichia occidentalis* (1 strain) and *Pichia kluyveri* (1 strain).

Lactic acid bacteria are a minor part of grape microbiota. They are the typical microorganisms of malolactic fermentation and they representatives, including *Oenococcus oeni*, has been seldom isolated from grapes in the vineyard. *Enterobacter* spp., *Enterococcus* spp., *Bacillus* spp., *Burkholderia* spp., *Serratia* spp., *Staphylococcus* spp. are widely distributed in the environment and are among others have been isolated from grapes, while a wine s a not suitable substrat for their growth **(Barata et al., 2012)**.

The community of microorganisms found by **Renouf et al. (2007)** was complex and diverse. It could be divided into 3 groups: 1) species without fermentation ability, e.g. *Auresbasidium* and *Burkholderia*, previously had not been isolated from wine; 2) species with some fermentation ability, e.g. *Lactobacillus, Pichia, Candida, Metschnikowia,* which could act during the first stages of winemaking; and 3) species that are the main fermentation microorganisms - *Saccharomyces cerevisiae* and *Oenococcus oeni*.

Kántor and Kačániová (2015) identified 12 yeasts and 30 species of bacteria species by MALDI TOF MS Biotyper. The dominant genera of microorganisms were *Bacillus*, *Candida, Lactobacillus, Staphylococcus* and *Aureobasidium.* They also identified 4 different strains of *Saccharomyces cerevisiae* (**Kántor and Kačániová, 2015)**.

Table 1 Microorganismus in different grape berries.

Grape variety	Gram positive bacteria	Gram negative bacteria	Yeasts	Total
Alibernet	8	3	5	16
Blue Frankish	12	2	12	26
Cabernet Savignon	3	5	10	18
Dornfelder	5	6	12	23
Feteasca regala	7	5	12	12
Green Veltliner	8	4	12	12
Irsai Oliver	11	8	16	35
Mūller Thurgau	6	5	13	24
Pálava	8	7	15	15
Pinot Blanc	9	9	15	33
Savignon Blanc	12	5	12	29
Rheinriesling	10	7	15	32
Welschriesling	6	3	10	19
Total	105	69	158	333

Table 2 Number of isolates identified with maldi tof ms biotyper in grape.

Microorganisms	White grape	Red grape	Total
Candida magnoliae	5	4	9
Candida parapsilosis	5	5	10
Hanseniaspora uvarum	8	7	15
Issatchenkia orientalis	4	5	9
Kazachstania exigua	7	6	13
Kluyveromyces marxianus	12	7	19
Metschnikowia pulcherrima	15	5	20
Rhodotorula glutinis	10	8	18
Rhodotorula mucilaginosa	15	7	22
Yarrowia lipolytica	18	6	24

Table 2 (continue)

Yeast	**99**	**60**	**159**
Aeromonas hydrophila	3	2	5
Enterobacter cloacae	2	3	5
Escherichia coli	3	2	5
Ignatzschineria indica	4	4	8
Moellerella wisconsensis	5	2	7
Pantoea agglomerans	2	4	6
Pantoea dispersa	2	5	7
Pseudomonas frederiksbergensis	3	2	5
Pseudomonas sp.	2	3	5
Sphingomonas sp.	5	2	7
Stenotrophomonas maltophilia	7	2	9
Gram negative bacteria	**38**	**31**	**69**
Arthrobacter koreensis	2	2	4
Bacillus cereus	3	2	5
Bacillus endophyticus	5	2	7
Bacillus licheniformis	2	0	2
Bacillus safensis	4	2	6
Bacillus simplex	1	2	3
Bacillus thuringiensis	3	0	3
Lactobacillus acidophilus	2	0	2
Lactobacillus fermentum	1	2	3
Lactobacillus paracasei	2	4	6
Lactococcus lactis	4	1	5
Leuconostoc mesenteroides ssp. mesenteroides	7	2	5
Lysinibacillus fusiformis	2	3	5
Microbacterium oxydans	2	2	4
Micrococcus luteus	2	0	2
Paenibacillus glucanolyticus	5	2	7
Paenibacillus lautus	6	1	7
Staphylococcus epidermidis	3	2	5
Staphylococcus hominis	2	4	6
Staphylococcus saprophyticus	2	2	4
Staphylococcus succinus	5	2	7
Staphylococcus warneri	2	1	3
Gram positive bacteria	**67**	**38**	**105**
Total	**204**	**129**	**333**

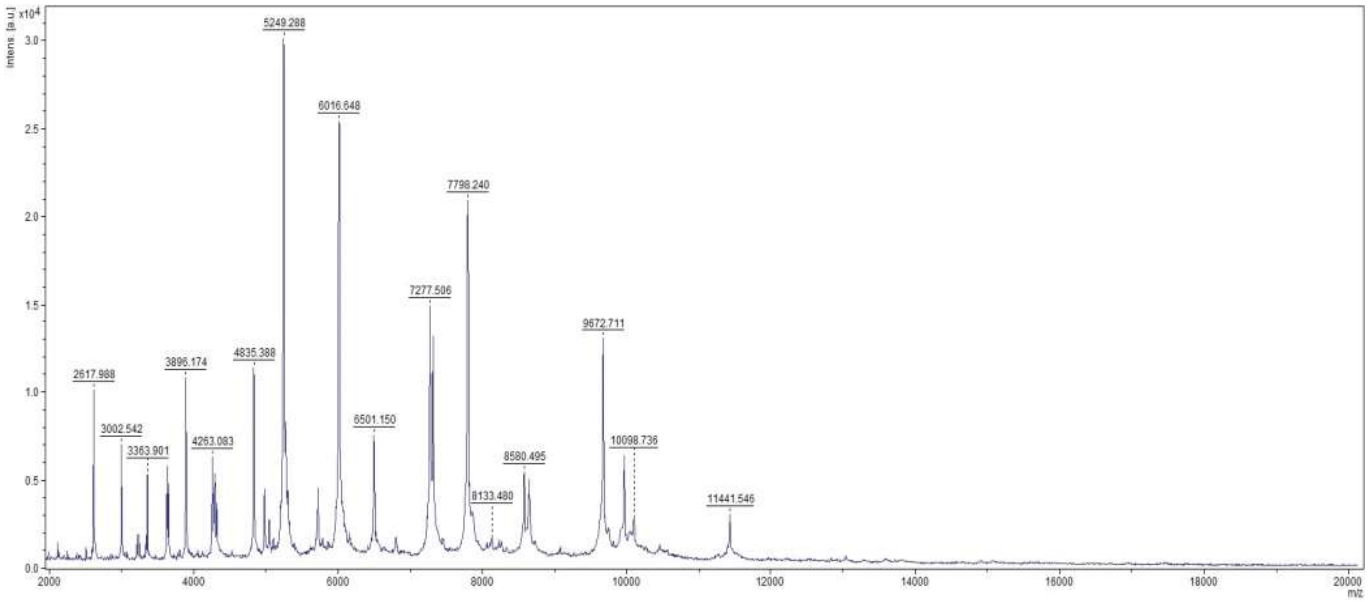

Figure 4 Spectrum of G$^-$ bacteria *Ignatzschineria indica* identified with MALDI TOF mass spectrometry.

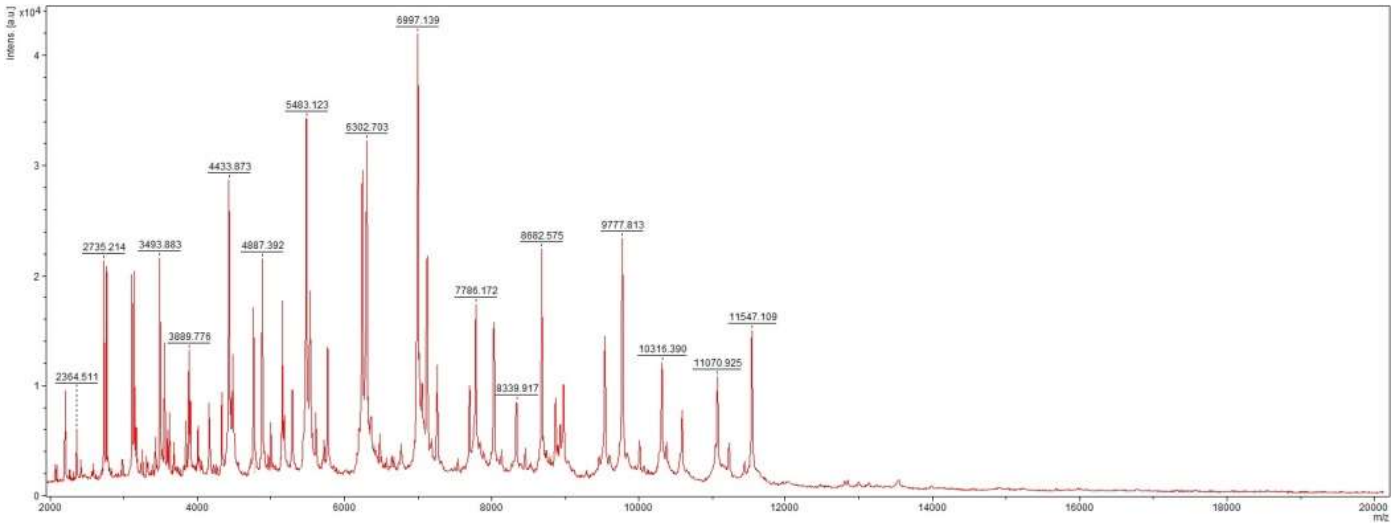

Figure 5 Spectrum of G$^-$ bacteria *Moellerella wisconsensis* identified with MALDI TOF mass spectrometry.

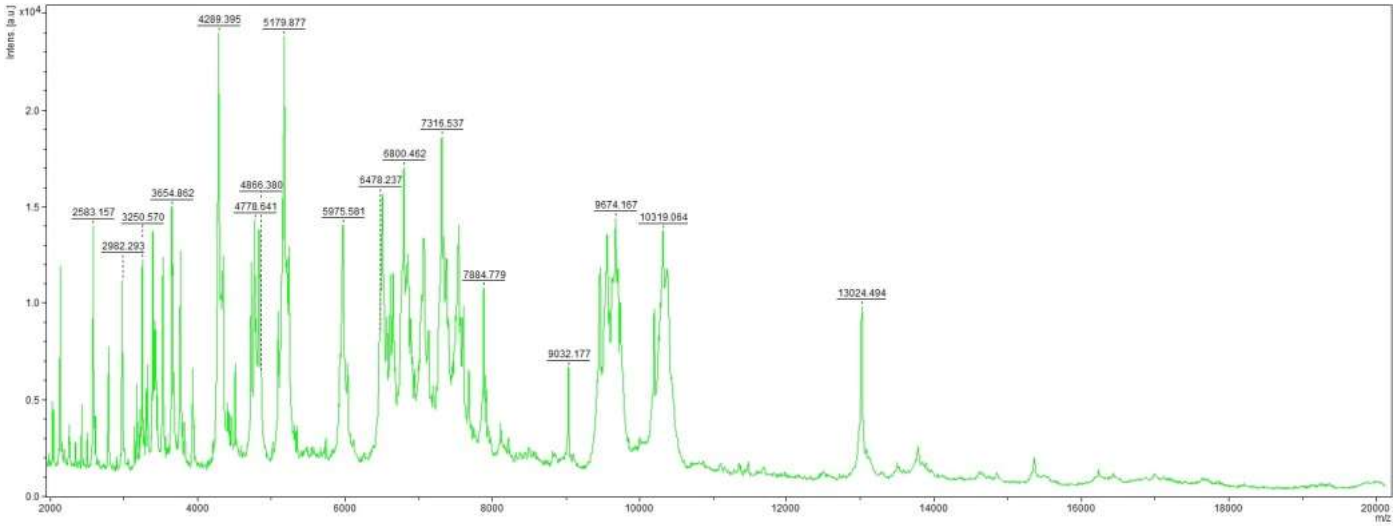

Figure 6 Spectrum of G$^+$ bacteria *Paenibacillus glucanolyticus* identified with MALDI TOF mass spectrometry.

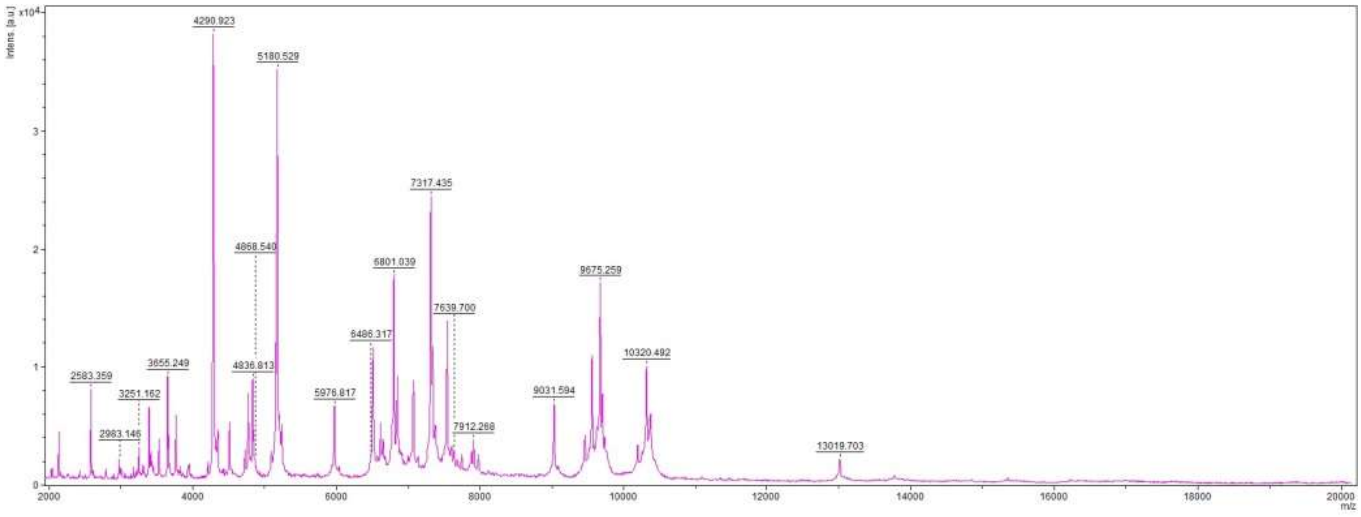

Figure 7 Spectrum of G^+ bacteria *Paenibacillus lautus* identified with MALDI TOF mass spectrometry.

CONCLUSION

Microbiological analysis of 13 grape samples revealed the three main groups of microorganisms:11 species of G^- and 22 species of G^+ bacteria and 10 species of yeasts. In total, 333 isolates were analysed by MALDI-TOF. From white grapes 204 microbial species and 129 from blue grape varieties were isolates, among which the yeasts, representing 47.74% of the all isolates, were the most abundant group.

REFERENCES

Albergaria, H., Arneborg, N. 2016. Dominance of *Saccharomyces cerevisiae* in alcoholic fermentation processes; role of physiological fitness and microbial interactions. *Applied Microbiology and Biotechnology,* vol. 100, no. 5, p. 2035-2046. https://doi.org/10.1007/s00253-015-7255-0 PMid:26728020

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

Bokulich, N. A., Thorngate, J. H., Richardson, P. M., Mills, D. A. 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences*, vol. 111, no. 1, p. E139-E148. https://doi.org/10.1073/pnas.1317377110

Du Toit, M., Engelbrecht, L., Lerm, E., Krieger-Weber, S. 2010. *Lactobacillus*: the next generation of malolactic fermentation starter cultures – an overview. *Food Bioprocess Technology*, vol. 4, no. 6, p. 976-906. https://doi.org/10.1007/s11947-010-0448-8

Drożdż, 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. https://dx.doi.org/10.5219/484.

Fleet G. H. 2007. Wine, p. 863-890. In: Doyle M. P. and L. R. Beuchat (eds). *Food Microbiology Fundamentals and Frontiers*. 3rd ed. Washington, USA : ASM Press. https://doi.org/10.1128/9781555815912.ch41

Hendrickx, M., Goffinet, J-S., Swinne, D., Detandt, M. 2011. Screening of strains of the *Candida parapsilosis* group of the BCCM/IHEM collection by MALDI-TOF MS. *Diagnostic Microbiology and Infectious Disease*, vol. 70, no. 4, p. 544-548. https://doi.org/10.1016/j.diagmicrobio.2011.04.006 PMid:21767712

Jolly, N. P., Varela, C., Pretorius, I. S. 2013. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Research*, vol. 14, no. 2, p. 215-237. https://doi.org/10.1111/1567-1364.12111

Kántor, A., Kačániová M. 2015. Diversity of Bacteria and Yeasts on the Surface of Table Grapes. *Scientific Papers: Animal Science and Biotechnologies*, vol. 48, p. 149-155

Kántor A., Petrová J., Hutková J., Kačániová M. 2016. Yeast diversity in new, still fermenting wine „federweisser". *Potravinarstvo*, vol. 10, no. 1, p. 120-125. https://doi.org/10.5219/547

Kántor, A., Mareček, J., Ivanišová, E., Terentjeva, M., Kačániová, M. 2017. Microorganisms of grape berries. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences,* vol. 71, no. 6, p. 502-508. https://doi.org/10.1515/prolas-2017-0087

Nedomová, Š., Kumbár, V., Pavloušek, P., Pytel, R., Začal, J., Buchar, J. 2016. Influence of harvest day on changes in mechanical prorties of grape of grape. *Potravinarstvo*, vol. 10, no. 1, p. 181-187. https://doi.org/10.5219/600

Padilla, B., García-Fernández, D., González, B., Izidoro, I., Esteve-Zarozo, B., Beltran, G., Mas A. 2016. Yeast biodiversity from DOQ priorat uninoculated fermentations. *Frontiers in Microbiology,* vol. 7, p. 930, https://doi.org/10.3389/fmicb.2016.00930

Pozo-Bayon, M. A., Alegria, E. G., Polo, M. C., Tenori, C., Martin-Alvarez P.J ., Calvo de la Banda, M. T., Ruiz-Larrea, F., Moreno-Arribas, M. V. 2005. Wine volatile and amino acid composition after malolactic fermentation: effect of *Oenococcus oeni* and *Lactobacillus plantarum* starter cultures. *Journal of Agricultural and Food Chemistry*, vol. 53, no. 22, p. 8729-8735. https://doi.org/10.1021/jf050739y

Pan, Y. L., Chow, N. H., Chang, T. C., Chang, H. C. 2011. Identification of lethal *Aspergillus* at early growth stages based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Diagnostic Microbiology and Infectious Disease*, vol. 70, no. 3, p. 344-354. https://doi.org/10.1016/j.diagmicrobio.2011.03.007

Raymond Eder, M. L., Reynoso, C., Lauret, S. C., Rosa, A. L. 2017. Isolation and identification of the indigenous yeast population during spontaneous fermentation of Isabella (*Vitis*

labrusca L.) grape must. *Frontiers in Microbiology*, vol. 8. https://doi.org/10.3389/fmicb.2017.00532

Renouf, V., Strehaiano, P., Lonvaud-Funei, A. 2007. Yeast and bacteria analysis of grape, wine and cellar equipments by PCR–DGGE. *International Journal of Vine and Wine Sciences*, vol. 41, no. 1, p. 51-61. https://doi.org/10.20870/oeno-one.2007.41.1.858

Varela, C., Borneman, A. R. 2016. Yeast found in vineyards and wineries. *Yeast,* vol. 34, no. 3, p. 111-128. https://doi.org/10.1002/yea.3219

Wieser, A., Schneider, L., Jung, J., Schubert, S. 2012. MALDI-TOF MS in microbiological diagnostics identification of microorganisms and beyond (mini review). *Applied Microbiology and Biotechnology*, vol. 93, no. 3, p. 965-974. https://doi.org/10.1007/s00253-011-3783-4

Wolfe, B. E., Dutton, R. J. 2015. Fermented foods as experimentally tractable microbial ecosystems. *Cell*, vol. 161, no. 1, p. 49-55. https://doi.org/10.1016/j.cell.2015.02.034

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Impact of somatic cell count and lameness on the production and composition of ewe's milk

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ABSTRACT

High somatic cell count (SCC) in milk and lameness are two very serious problems on the farms. The aim of the study was to evaluate the impact of lameness, SCC, month and order of entry into the milking parlour on the milk production and its composition. The relationship between lameness and SCC and their impact on the order of entry was also evaluated. The experiment was carried out at the farm, located in northern Slovakia. The farm keeps sheep crossbred of Improved Valachian and Lacaune. Milking was performed two times a day in milking parlor 1x24. Samples of milk were taken once a month by evening milking: May, July. In May, individual milk samples were taken from 214 random sampling ewes with milk yield minimum 300 mL per milking. In July, only from selected ewes in May, the milk samples, milk yield and lameness were recorded. Order of ewes entry into the milking parlour in milking row (one milking row is 24 animals) was recorded in both months. In total 23 milking rows were recorded. Ewes was divided by lameness (non-lame, slightly lame, strong lame), by SCC (A1 = to $2x10^5$ cells, A2 = from $2x10^5$ to $4x10^5$ cells, A3 = from $4x10^5$ to $7x10^5$ cells, A4 = from $7x10^5$ to $10x10^5$ cells, A5 = over $10x10^5$ cells.mL^{-1}) and by the order of entry of ewes into the milking parlour (in first group of ewes were milked in 1-5 rows, second 6-11, third 12-17, fourth 18-23 ones). No effect of lameness was found out on milk yield. Lameness in July affected the order of entry into milking parlour in July as compared with their order of entry recorded in May. The strong lame ewes entered 4.19 ±1.07 milking rows later in July than in May. Only 11.2% and 4.2% of milk samples were found out in a group with SCC >$10x10^5$ cells.mL^{-1} during May and July respectively. In both months, the production of lactose was lower in groups with higher SCC. Ewes entering into the milking parlour earlier had higher SCC as ewes entering into milking parlour later in July but no effect was seen in May. In conclusion the studies under practical conditions deserve continuous research attention to identify risk factors of management affecting lameness and udder health for further improvement of sheep breeding.

Keywords: ewes; lameness; somatic cell count; order of entry

INTRODUCTION

Mastitis is inflammatory disease of mammary gland, which is mainly presented as high somatic cell count in milk (SCC) **(Gonzalo et al., 2002)**. SCC is influenced by various factors such as age, stage of lactation **(Sitkowska, 2008)** and also depends on the infectious factor **(Ariznabarreta et al., 2002)**. Several works have been published which presented a negative correlation between SCC and milk production in ewes **(Arias et al., 2012; Gonzalo et al., 2002)** and cows **(Tančin et al., 2007)**.

Lameness is considered one of the most important health problems in sheep **(Eze, 2002)**. **Gelasakis et al. (2010)** found out the negative impact of hoof disease (the most common cause of lameness) on the milk yield.

The order of entry into the milking parlor is also important factor which could be related to ewe's **(Villagrá et al., 2007; Antonič et al., 2011)**, cow's **(Rathore, 1982; Stefanowska et al., 2000)** and goat's production **(Gorecki and Wojtowski, 2004)**. The order of entry into the milking parlor is influenced by many factors: lactation number **(Antonič et al., 2011)**, dominance and the weight of the animals **(Margetínová et al., 2003)** which also may affect the milk production. Therefore, it appears that the order of entry into the parlor affects the milk yield, milk components **(Margetínová et al., 2001)** and milkability and the quantity of machine stripping, hand stripping and volume of residual milk **(Villagrá et al., 2007)**.

The research studies related to the possible negative effect of high level of SCC and lameness have of great importance by improving of production and animal welfare. The aim of the study was to evaluate the impact of lameness and somatic cell count and order of entry into the milking parlour on the milk production and its

composition. The relationship between lameness and SCC and their impact on the order of entry were also examined.

Scientific hypothesis

Lameness and high SCC in milk reduce milk yield and affect its composition.

MATERIAL AND METHODOLOGY

The experiment was carried at the farm located in northern Slovakia in year 2015. The farm kept sheep crossbred Improved Valachian and Lacaune. 85% of experimental ewes were on their first to fourth lactations. Ewes were housed on deep litter and they grazed on adjacent pastures during the day. The main components of feed were pasture (*ad libitum*) and feed concentrate during milking (200 g/per animal per day). Milking was performed two times a day in milking parlor 1x24. Lambing began in the middle February and lasted until the middle of March.

Samples of milk were taken once a month during evening milking in May, and July (MONTH). In May, individual milk samples were taken from 214 random sampled ewes with milk yield minimum 300 mL per milking. In July, only from selected ewes in May, the milk samples, milk yield and lameness were recorded. Order of ewes entry into the milking parlour as milking row (one milking row is 24 animals) was recorded in both months. In total 23 milking rows were recorded. Analysis of milk samples (428 in total) for somatic cell count and a basic component has been performed in NPPC-Research Institute for Animal Production in Nitra. Basic milk composition was done by MilkoScan FT 120 (FOSS, Denmark) and somatic cell count was determined using a Somacount 150 (Bentley Czech, USA).

Lameness was evaluated according to modified scale according **Kaler et al. (2009)**. All lameness ewes (LAMENESS) were evaluated during entering the milking parlour and standing during milking. The ewes that lamed during the entering into milking parlour and those been lifted (lighten) their limbs during milking were marked as "strong lame" ewes. Ewes that lamed during entering into milking parlour but did not lift their limbs during milking were marked as "slightly lame". Ewes that neither did not lame during the entering into milking parlour nor did not lift their limbs during milking were marked as "non-lame". On the basis of individual SCC in milk, the ewes were divided into five SCC groups (SOMATIC). The first group (A1) represented ewes with SCC below $2x10^5$ cells, the second (A2) from $2x10^5$ to $4x10^5$ cells, the third (A3) from $4x10^5$ to $7x10^5$ cells, fourth (A4) from $7x10^5$ to $10x10^5$ cells and the fifth one (A5) over $10x10^5$ cells per mL of milk. The logarithm of SCC ($\log_{10}$x.mL^{-1}) have been used for the statistical processing.

The value ,,order of entry" into the milking parlor was considered as number of milking row order, in which ewes was milking. Altogether 23 rows were recorded. On the base of number of row, four groups of entries (ORDER) were created: the first five milking rows (1-5) marked as ,,first" entry, milking rows from 6 to 11 ,,second" entry, from 12 to 17 ,,third" entry and from 18 to 23 ,,fourth" entry. The value ,,change of the entry" represent the difference between number of row in May and the number of row in July.

Statisic analysis

The milk yield per milking (mL), fat (%), protein (%), lactose (%), non-fat dry matter - NFDM (%) and total solids (%) and logarithm of somatic cell count ($\log_{10}$ x mL^{-1}) was evaluated. The results were mathematically processed using the Microsoft Excel program and statistically evaluated by SAS/9.4 (2014). It was used paired t-test when comparing differences variables in two groups (the difference in milk yield or milk components in milk between two months). It was used analysis of variance and within Fisher's LSD test, when comparing more than two groups (the difference in milk yield or milk components in groups by lameness, SCC, the order of entry of ewes into the milking parlour). Used statistical model can be written in the following form:

$y_{ijk} = \mu + SOMATIC_i + LAMENESS_j + ORDER_k + u_p + e_{ijk}$

$y_{il} = \mu + SOMATIC_i\ /MONTH_l/ + u_p + e_{il}$

$y_{kl} = \mu + ORDER_k\ /MONTH_l/ + u_p + e_{jl}$

y_{ijk} = the measurements for milk yield and composition

μ = overall mean,

$SOMATIC_i$ = the fixed effects of SCC classes (i = 1 to 5),

$LAMENESS_j$ = the fixed effect of lameness (j = 1 to 3),

$ORDER_k$ = the fixed effect of order of entry (k = 1 to 4),

$MONTH_l$ = the fixed effect of month (l = 1 and 2)

μ_p = random effect of ewes, $u_p \sim N(0, I\ \sigma_c^2)$,

e_{ijk} = random error, assuming $e_{ijkl} \sim N(0, I\ \sigma_e^2)$.

RESULTS AND DISCUSSION

The months of study significantly influenced all measured traits (Table 1). The changes of milk components and milk yield were caused by numbers of lactating days as it is well documented in literature **(Assan, 2015; Komprej et al., 2012; Oravcová et al., 2015; Peralta-Lailson et al., 2005; Tančin et al., 2011; 2013)**.

In our trial the SCC reduced from May to July. Under practical conditions with Tsigai ewes **Vršková et al. (2015)** did not found out significant effect of season on SCC. Reduction of SCC from May to July could be probably explained by reducing milk yield creating thus more effective immune function of mammary gland.

Statistical significance of factors as lameness, SCC and the order of entry into the milking parlour on the evaluated trials are shown in Table 2.

Table 1 The effect of months on observed trials (n = 214).

	May		July	
Trail	**Mean**	**SD**	**Mean**	**SD**
Milk yield (mL)	558^a	173	349^b	143
logSCC (log x.mL^{-1})	5.49^a	0.47	4.81^b	0.63
Fat (%)	5.83^a	0.97	8.88^b	1.02
Protein (%)	5.90^a	0.50	6.47^b	0.52
Lactose (%)	5.17^a	0.20	4.86^b	0.19
NFDM (%)	11.97^a	0.48	12.26^b	0.52
Total solid (%)	17.54^a	1.05	20.87^b	1.52

Note: a,b – means with different letters are significant (p <0.0001), SD – standard deviation.

Table 2 Statistical significance (P value) of lameness, SCC and the order of entry in the milking parlour on the evaluated trials.

	Lameness		SCC		Order of entry	
Trials	**May**	**July**	**May**	**July**	**May**	**July**
Milk yield (mL)	0.7367	0.2881	0.5502	0.0105	0.1477	0.4477
logSCC (log x.mL^{-1})	0.0699	0.6412	x	x	0.0127	<.0001
Fat (%)	0.0575	0.2184	0.3768	0.3874	0.0074	0.1572
Protein (%)	0.6324	0.4459	0.0165	0.0260	0.2026	0.0997
Lactose (%)	0.7415	0.2038	0.0006	<.0001	0.1454	0.1867
NFDM (%)	0.5563	0.4348	0.0029	0.0070	0.6778	0.1673
Total solid (%)	0.1361	0.3672	0.1373	0.2103	0.0126	0.2748
Change of entry (milking rows)	x	0.0345	x	0.7927	x	x

Lameness

In July, 12.15% slightly lame ewes and 8.41% strong lame ewes were recorded. Lower incidence of lameness was found out by **Gelasakis et al. (2015)** on two farms keeping breed Chios (12.4%, respectively. 16.8% - regardless of the degree of lameness), **Gavojdian et al. (2015)** by breed Tsigaia dorper (2.9% respectively. 8%), and in large long-term studies **Winter et al. (2015)** (4.9% to 10.6%).

Milk yield, SCC and milk composition were not affected by lameness of ewes (Table 2) therefore the data are not shown. However, a lower milk yield compared with non-lame ewes in study **Gelasakis et al. (2015)** was found out. There is limited information about relationship between lameness and milk composition in ewes. In dairy cows' positive correlation **(Peeler et al., 1994)**, negative correlation **(Archer et al., 2011)** or as well as in our study with ewes no effect was detected **(Hultgren et al., 2004)** between lameness and SCC.

Lameness significantly reduced the order of entry of ewes into parlour (Table 2). The same ewes when they were lame in July entered into the parlour later in July than in May. Slightly lame ewes in July entered the parlour 2.87 ±1.03 milking rows later and strong lame ewes even 4.19 ±1.07 milking rows later in July than in May. Also non-lame ewes in July entered the parlour later in July than in May but only about 1.14 ±0.42 milking rows. Later entry of lame ewes into the milking parlour was probably due to the unwillingness of lame ewes to walk. Therefore, the high attention of farmers should be given to last entering groups if the one of the reasons could be caused by lameness.

SCC

There was only slow reduction of milk yield with increasing SCC in May. The significant negative effect of SCC on milk production was found out in July only (Table 2), but statistical differences were demonstrated due to low number of animals in the last three groups in July. Similarly, a tendency to lower milk production by a higher SCC was found out in several works **(Antonič et al., 2013; Arias et al., 2012; Gonzalo et al., 2002; Leitner et al., 2008; Margetín et al., 1996; Vršková et al., 2015)**.

Production of lactose was influenced by SCC in the month May and July (Table 2 and Table 3). In both months, the production of lactose was lower in groups with higher SCC. The same significant impact of SCC on lactose was also found out by **De Olives et al. (2013)**, **Caboni et al. (2017)**, **Margetín et al. (2013)** and **Vršková et al. (2015)**. Protein and NFDM has been affected by somatic cell count in May and in July (Table 2) but these significant effects were not clearly related to the SCC groups (Table 3). The fat content and total solid were not affected by SCC (Table 2).

The frequency of distribution of milk samples in different SCC groups are followed: in May 38.3% ewes were included in group A1, 29.4% ewes in group A2, 15.4% ewes in group A3, 5.6% ewes in group A4 and 11.2% ones in group A5. In July, the distribution among the SCC groups was follow: A1 = 80.4%, A2 = 7.5%, A3 = 3.7%, A4 = 4.2%, A5 = 4.2% of ewes. **Berthelot et al. (2006)** reported healthy ewes with SCC below 0.5x10^6 cells and infected udders with SCC higher than 1x10^6 cells.mL^{-1}. **Arias et al. (2012)** have recommended the limit value of 0.3x10^6 cells.mL^{-1} in determining relationship of SCC to milk production. In our results high number of ewes in A1 (below 2x10^5 cells.mL^{-1}) and low number of animals in group A5 (over 10x10^5 cells.mL^{-1}) indicating a good udder health of experimental animals in both months. **Idriss et al. (2015)**, recorded a higher incidence of ewes in a group with SCC >10x10^5 cells.mL^{-1} cellsxmL^{-1} during May (15.83%) compared to July (11.45%) in experimental farm of our Institute. Under Slovak conditions a similar distribution of milk samples differed by SCC within the flock was found out also in other farms **(Baranovič et al., 2016)**. This distribution of samples among SCC groups explains the reduced SCC in July as compared with May (Table 1).

The order of entry of ewes into the milking parlour

The number of observed ewes entering the parlour in different groups is shown in table 4 with possible effect of months of trial.

In May, higher logSCC was in the first two groups (first, second) in compare with third group. In July higher logSCC was in the first two groups (first, second) and lower in the latter two groups (third, fourth) (Table 2 and Table 4).

Table 3 Evaluated trials (milk yield, fat, protein, lactose, NFDM and total solid) in different groups of SCC.

	Month: May – SCC groups									
	A1 (n = 82)		A2 (n = 63)		A3 (n = 33)		A4 (n = 12)		A5 (n = 24)	
Trials	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Milk yield (mL)	583	184	549	169	536	175	548	193	530	132
Fat (%)	5.71	0.929	5.93	0.865	5.72	0.830	6.14	0.833	5.93	1.455
Protein (%)	5.82^{a}	0.446	5.85^{a}	0.554	6.15^{b}	0.475	6.06	0.471	5.93	0.496
Lactose (%)	5.19^{a}	0.181	5.20^{a}	0.166	5.19^{a}	0.165	5.14^{a}	0.197	5.00^{b}	0.322
NFDM (%)	11.88^{a}	0.444	11.98^{a}	0.475	12.23^{b}	0.446	12.10	0.592	11.83^{a}	0.432
Total solid (%)	17.34	1.012	17.64	0.957	17.68	0.911	17.95	0.826	17.52	1.529
	Month: July – SCC groups									
	A1 (n = 172)		**A2 (n = 16)**		**A3 (n = 8)**		**A4 (n = 9)**		**A5 (n = 9)**	
Trials	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Milk yield (mL)	351	143	378	191	270	53	287	93	377	109
Fat (%)	8.91	1.017	8.95	1.043	8.22	0.896	9.07	1.36	8.7	0.803
Protein (%)	6.50^{a}	0.539	6.22^{b}	0.387	6.27	0.229	6.69^{a}	0.623	6.27	0.344
Lactose (%)	4.88^{a}	0.173	4.90^{a}	0.197	4.69^{b}	0.226	4.67^{b}	0.184	4.72^{b}	0.261
NFDM (%)	12.32^{a}	0.52	12.03^{b}	0.41	11.86^{b}	0.373	12.31	0.637	11.91^{b}	0.401
Total solid (%)	20.95^{a}	1.549	20.67	1.265	19.83^{b}	1.108	21.1	1.746	20.33	1.004

Note: a, b – means with different letters are significant (p <0.05); SD – standard deviation.

Table 4 Evaluate trials (milk yield, logSCC, fat, protein, lactose, NFDM and total solid) in different groups by the order of entry of ewes into the milking parlour

	Order of entry into parlour – May							
	first (n = 65)		second (n = 68)		third (n = 71)		fourth (n = 10)	
Trials	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Milk yield (mL)	590	188	561	158	536	178	482	98
logSCC (log x.mL^{-1})	5.52^{a}	0.439	5.59^{a}	0.492	5.34^{b}	0.446	5.53	0.555
Fat (%)	5.57^{a}	0.895	6.12^{b}	1.044	5.83	0.901	5.52	0.825
Protein (%)	5.78^{a}	0.615	5.93	0.39	5.98^{b}	0.495	5.99	0.236
Lactose (%)	5.22^{a}	0.219	5.14^{b}	0.208	5.15	0.189	5.15	0.126
NFDM (%)	11.93	0.547	11.96	0.401	12.01	0.499	12.02	0.187
Total solid (%)	17.23^{a}	1.003	17.81^{b}	1.129	17.59^{b}	0.976	17.29	0.81
	Order of entry into parlour – July							
	first (n = 53)		**second (n = 66)**		**third (n = 51)**		**fourth (n = 44)**	
Trials	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Milk yield (mL)	362	131	341	158	365	133	324	144
logSCC (log x.mL^{-1})	5.11^{a}	0.528	4.91^{a}	0.489	4.60^{b}	0.638	4.56^{b}	0.732
Fat (%)	8.87	0.96	8.93	0.964	8.64^{a}	1.071	9.11^{b}	1.098
Protein (%)	6.32^{a}	0.493	6.54^{b}	0.567	6.47	0.478	6.53^{b}	0.526
Lact. (%)	4.89^{a}	0.206	4.87	0.165	4.85	0.179	4.81^{b}	0.214
NFDM (%)	12.13^{a}	0.555	12.35^{b}	0.558	12.26	0.453	12.29	0.484
Total solid (%)	20.79	2.057	20.97	1.243	20.6	1.294	21.1	1.344

Note: a, b – means with different letters are significant (p <0.05); SD – standard deviation.

Lower SCC in ewes entering into the milking parlour in second half may be due to the lower age of these animals **(Antonič et al., 2011)** because young ewes have lower SCC **(Baranovič et al., 2016)**. **Villagrá et al. (2007)** and **Antonič et al. (2011)** did not record the influence of order of entry into the parlor on SCC. In the study with the dairy cows, animals entering in the milking parlour as the first had lower SCC in compare with last groups (**Rathore, 1982**).

In May, a significant effect the order of entry into the milking parlour on the fat content and total solids was found out (Table 2 and Table 4) but changes were not related to the order of entry. These results are consistent with the **Antonič et al. (2011)**.

The remaining trials were not affected by entry of ewes into the milking parlour (Table 2).

CONCLUSION

The high incidence of lame ewes (over 20%) was found out but no effect on milk yield and its compostion was calculated. Lameness significantly postponed the entry of ewes into parlour (p <0.0345). However, on the SCC basis a relatively good health of udders was found in flock. Only 11.2% and 4.2% of milk samples were found out in a group with SCC >10x10^5 cells.mL^{-1} during May and July respectively. The lactose in milk significantly reduced in milk with high SCC in both months (p = 0.0006 and <0.0001, respectively). The studies under practical conditions deserve research attention to identify risk factors of management affecting lameness and udder health for further improvement of sheep breeding.

REFERENCES

Antonič, J., Jackuliaková, L., Uhrinčať, M., Mačuhová, L., Oravcová, M., Tančin, V. 2013. Changes in milk yield and composition after lamb weaning and start of machine milking in dairy ewes. *Slovak Journal of Animal Science*, vol. 46, no. 3, p. 93-99.

Antonič, J., Tančinová, D., Uhrinčať, M., Tančin, V. 2011. Effect of order of sheep entry into milking parlour on udder health and milk composition. In *Risk factors of food chain: 11th international conference.* Iwonicz, Poland, September 5-6. 2011. Rzeszów: University of Rzeszow.

Archer, S. C., Green, M. J., Madouasse, A., Huxley, J. N. 2011. Association between somatic cell count and serial locomotion score assessments in UK dairy cows. *Journal of Dairy Science.* vol. 94, no. 9, p. 4383-4388. https://doi.org/10.3168/jds.2011-4276 PMid:21854911

Arias, R., Oliete, B., Ramon, M., Arias, C., Gallego, R., Montoro, V., Gonzalo, C., Perez-Guzman, M. D. 2012.Long-term study of environmental effects on test-day somatic cell count and milk yield in Manchega sheep. *Small Ruminant Research*, vol. 106, no. 2-3, p. 92-97. https://doi.org/10.1016/j.smallrumres.2012.03.019

Ariznabarreta, A., Gonzalo, C., San Primitivo, F. 2002. Microbiological quality and somatic cell count of ewe milk with special reference to staphylococci. *Journal of Dairy Science*, vol. 85, no. 6, p. 1370-1375. https://doi.org/10.3168/jds.S0022-0302(02)74203-3

Assan, N. 2015. Consequences of stage of lactation on yield and milk composition in sheep. *Scientific Journal of Pure and Applied Sciences*, vol. 4, no. 1, p. 1-6.

Baranovič, Š., Tančin, V., Mačuhová, L., Uhrinčať, M., Sláma, P. 2016. Somatic cells count in milk of ewes during lactation on selected farms. In: *Safety and quality of milk and plant commodities : 37th international conference*. Štrbské Pleso, Slovakia, May 18-20. 2016, Košice: The University of Veterinary Medicine.

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, 2006, p. 27-31. https://doi.org/10.1016/j.smallrumres.2005.07.047

Caboni, P., Manis, C., Ibba, I., Contu, M., Coroneo, V., Scano, P. 2017. Compositional profile of ovine milk with a high somatic cell count: A metabolomics approach. *International Dairy Journal*, vol. 69, p. 33-39. https://doi.org/10.1016/j.idairyj.2017.02.001

De Olives, A. M., Díaz, J. R., Molina, M. P., Peris, C. 2013. Quantification of milk yield and composition changes as affected by subclinical mastitis during the current lactation in sheep. *Journal of Dairy Science*, vol. 96, no. 12, p. 7698-7708. https://doi.org/10.3168/jds.2013-6998 PMid:24119805

Eze, C. A. 2002. Lameness and reproductive performance in small ruminants in Nsukka Area of the Enugu State, Nigeria. *Small Ruminant Research*, vol. 44, no. 3, p. 263-267. https://doi.org/10.1016/S0921-4488(02)00030-5

Idriss, S. E., Tančin, V., Margetín, M., Tančinová, D., Sláma, P., Havlíček, Z. 2015. The frequency of somatic cell count in dairy ewe's milk. *The Journal of Microbiology, Biotechnology and Food Sciences*, vol. 4, no. 3, p. 148-151. https://doi.org/10.15414/jmbfs.2015.4.special3.148-151

Gavojdian, D., Budai, C., Cziszter, L. T., Csizmar, N., Javor, A., Kusza, S. 2015. Reproduction efficiency and health traits in Dorper, White Dorper, and Tsigai sheep breeds under temperate European conditions. *Asian-Australasian Journal of Animal Sciences*, vol. 28, no. 4, p. 599-603. https://doi.org/10.5713/ajas.14.0659 PMid:25656193

Gelasakis, A. I., Arsenos, G., Valergakis, G. E., Banos, G. 2015.Association of lameness with milk yield and lactation curves in Chios dairy ewes. *The Journal of Dairy Research*, vol. 82, no. 2, p. 193-199. https://doi.org/10.1017/S0022029915000059 PMid:25650132

Gelasakis, A. I., Arsenos, G., Valergakis, G. E., Fortomaris, P., Banos, G. 2010. Effect of lameness on milk production in a flock of dairy sheep. *Veterinary Record*, vol. 167, no. 14, p. 533-534. https://doi.org/10.1136/vr.c4828 PMid:21257401

Gonzalo, C., Ariznabarreta, A., Carriedo, J. A., San Primitivo, F. 2002. Mammary pathogens and their relationship to somatic cell count and milk yield losses in dairy ewes. *Journal of Dairy Science*, vol. 85, no. 6, p. 1460-1467. https://doi.org/10.3168/jds.S0022-0302(02)74214-8

Gorecki, M. T., Wojtowski, J. 2004. Stability of milking order in goat over a long period. *Archiv für tierzucht*, vol. 47, no. 2, p. 203-208.

Hultgren, J., Manske, T., Bergstern, C. 2004. Associations of sole ulcer at claw trimming with reproductive performance, udder health, milk yield, and culling in Swedish dairy cattle. *Preventive Veterinary Medicine*, vol. 62, no. 4, p. 233-251. https://doi.org/10.1016/j.prevetmed.2004.01.002 PMid:15068889

Kaler, J., Wassink, G. J., Green, L. E. 2009. The inter- and intra-observer reliability of a locomotion scoring scale for

sheep. *The Veterinary Journal*, vol. 180, no. 2, p. 189-194. https://doi.org/10.1016/j.tvjl.2007.12.028 PMid:18308594

Komprej, A., Gorjanc, G., Kompan, D., Kovač, M. 2012. Lactation curves for milk yield, fat, and protein content in Slovenian dairy sheep. *Czech Journal of Animal Science*, vol. 57, no. 5, p. 231-239. https://doi.org/10.17221/5921-CJAS

Leitner, G., Silanikove, N., Merin, U. 2008. Estimate of milk and curd yield loss of sheep and goats with intrammamary infection and its relation to somatic cell count. *Small Ruminant Research*, vol.74, no. 1, p. 221-225. https://doi.org/10.1016/j.smallrumres.2007.02.009

Margetín, M., Čapistrak, A., Španik, J., Foltys, V. 1996. Somatic cells in sheep milk relation to milk production and composition during sucking and milking. *Animal Production-UZPI*, vol. 41, no. 12, p. 543-550.

Margetín, M., Milerski, M., Apolen, D., Čapisták, A., Oravcová, Debreceni, O. 2013. Relationships between production, quality of milk and udder health status of ewes during machine milking. Journal of *Central European Agriculture*, vol. 14, no. 1, p. 328-340. https://doi.org/10.5513/JCEA01/14.1.1203

Margetínová, J., Brouček, J., Apolen, D., Mihina, S. 2001. Order of goats during automatic milking. *Slovak Journal of Animal Science*, vol. 34, p. 89-96.

Margetínová, J., Brouček, J., Apolen, D., Mihina, S. 2003. Relationship between age, milk production and order of goats during automatic milking. *Czech Journal of Animal Science*, vol. 48, no. 6, p. 257-263.

Oravcová, M., Margetín, M., Tančin, V. 2015. The effect of stage of lactation on daily milk yield and milk fat and protein content in Tsigai and Improved Valachian ewes. *Mljekarstvo,* vol. 65, no. 1, p. 48-56. https://doi.org/10.15567/mljekarstvo.2015.0107

Peeler, E. J., Otte, M. J., Esslemont, R. J. 1994. Inter-relationships of periparturient diseases in dairy cows. *The Veterinary Record*, vol. 134, no. 6, p. 129-132. https://doi.org/10.1136/vr.134.6.129 PMid:8171781

Peralta-Lailson, M., Trejo-González, A. Á., Pedraza-Villagómez, P., Berruecos-Villalobos, J. M., Vasquez, C. G. 2005. Factors affecting milk yield and lactation curve fitting in the creole sheep of Chiapas-Mexico. *Small Ruminant Research*, vol. 58, no. 3, p. 265-273. https://doi.org/10.1016/j.smallrumres.2004.11.005

Rathore, A. K. 1982. Order of cow entry at milking and its relationships with milk yield and consistency of the order. *Applied Animal Ethology*, vol. 8, no. 1-2, p. 45-52. https://doi.org/10.1016/0304-3762(82)90131-6

Sitkowska, B. 2008. Effect of the cow age group and lactation stage on the count of somatic cells in cow milk. *Journal of Central European Agriculture*, vol. 9, no. 1, p. 57-62.

Stefanowska, J., Plavsic, M., Ipema, A. H., Hendriks, M. M. W. B. 2000. The effect of omitted milking on the behaviour of cows in the context of cluster attachment failure during automatic milking. *Applied Animal Behaviour Science*, vol. 6, no. 4, p. 77-291. https://doi.org/10.1016/S0168-1591(00)00087-3

Tančin, V. 2013. Somatic cell counts in milk of dairy cows under practical conditions. *Slovak Journal of Animal Science*, vol. 46, no. 1, p. 31-34.

Tančin, V., Ipema, A. H., Hogewerf, P. 2007. Interaction of somatic cell count and quarter milk flow patterns. *Journal of Dairy Science*, vol. 90, no. 5, p. 2223-2228. https://doi.org/10.3168/jds.2006-666 PMid:17430921

Tančin, V., Mačuhová, L., Oravcová, M., Uhrinčať, M., Kulinová, K., Roychoudhury, S. H., Marnet, P. G. 2011. Milkability assessment of Tsigai, Improved Valachian, Lacaune and F1Crossbred ewes (Tsigai× Lacaune, Improved Valachian× Lacaune) throughout lactation. *Small Ruminant Research*, vol. 97, no. 1, p. 28-34. https://doi.org/10.1016/j.smallrumres.2011.01.007

Villagrá, A., Balasch, S., Peris, C., Torres, A., Fernández, N. 2007. Order of sheep entry into the milking parlour and its relationship with their milkability. *Applied Animal Behaviour Science,* vol. 108, no. 1-2, p. 58-67. https://doi.org/10.1016/j.applanim.2006.11.016

Vršková, M., Tančin, V., Kirchnerová, K., Sláma, P. 2015. Impact of selected parameters on milk production in Tsigai breed. The Journal of Microbiology, *Biotechnology and Food Sciences*, vol. 4, no. 3, p.185-187. https://doi.org/10.15414/jmbfs.2015.4.special3.185-187

Winter, J. R., Kaler, J., Ferguson, E., KilBride, A. L., Green, L. E. 2015. Changes in prevalence of, and risk factors for, lameness in random samples of English sheep flocks: 2004-2013. *Preventive Veterinary Medicine*, vol. 122, no. 1, p. 121-128. https://doi.org/10.1016/j.prevetmed.2015.09.014 PMid:26435034

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RHEOLOGICAL BEHAVIOUR OF CHOCOLATE AT DIFFERENT TEMPERATURES

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ABSTRACT

The rheological behaviour of the chocolate at different temperatures was studied using a concentric cylinder viscometer with precision small samples adapter, temperature sensor and standard spindle. BIO chocolate (100% organic cocoa) has been used for the whole types of experiments. At the first, the range of temperature has been chosen 36 °C, 38 °C, 40 °C, 42 °C, and 44 °C. The shear deformation rate was established from the 0.1 s^{-1} up to 68 s^{-1}. Rheological behaviour was non–Newtonian (plastic) with inconsiderable yield stress in all temperatures. The chocolate unambiguously demonstrated plastic behaviour and flow curves were fitted by the power law model (Herschel–Bulkley model), Bingham model, and Casson model with taking into account the coefficient of determination R^2. The obtained results of rheological behaviour of chocolate can be best described as Casson fluid. Exactly coefficients of models can be used for modelling of flow velocity, volume flow, friction factor, Reynolds number, two dimensional and three dimensional velocity profiles and much more for flow in the real technical elements e.g. pipes, trough, tubes. Finally, temperature dependence of apparent viscosity of chocolate was also continuously measured in the range from 35 °C up to 62 °C. The apparent viscosity decreased in the temperature range. This decrease was fitted using power law equation. The knowledge of the plastic flow behaviour of chocolate is very important, because it is not quite common flow behaviour of foodstuffs.

Keywords: plastic fluid; yield stress; flow model; chocolate; temperature

INTRODUCTION

In the paper **(Lapčík et al., 2017)** states that chocolate is unique as a food in that fact which is solid at normal room temperatures however it melts easily in the mouth. Since the properties of the main fat component, cocoa butter, is essentially solid at temperatures below 25 °C when it holds all the solid sugar and cocoa particles together. However, this fat is almost entirely liquid at body temperature, enabling the particles to flow past one another, thus the chocolate becomes a smooth liquid by heating in the mouth.

There are many methods for testing properties of chocolate e.g. colour and hardness measuring **(Machálková et al., 2015)**, in this paper we are focused on rheological measuring.

Precision knowledge of the rheological properties of foodstuffs is essential for the product development, sensory evaluation and design, quality control, and evaluation of the process equipment. The flow behaviour of a fluid and semisolid food can be varied from Newtonian to time dependent non-Newtonian depending on its origin, composition and structure behaviour **(Trávníček et al., 2016; Hlaváč et al., 2016; Kumbár et al., 2017)**. This behaviour is necessary to model. The rigorous knowledge of rheological behaviour is also very important for chocolate **(Bozkurt and Icier, 2009; Gonçalves and da Silva Lannes, 2010)**. Especially, the temperature dependence of flow properties is very important for processing liquid chocolate as a topping or filling **(Quiñones-Muñoz et al., 2011; Božiková and Hlaváč, 2013; Glicerina et al., 2013)**. Most of the researchers studied the rheological characteristics of chocolate reported as non-Newtonian plastic fluid with inconsiderable yield stress **(Ačkar et al., 2015; Cikrikci et al., 2017)**. Many papers deals with rheological behaviour blends of cocoa and supplements (hydrocolloids, milk, butter or other fat) and used many mathematical models e.g. Casson model, Windhab model, Carreau model, and Power Law model **(Fernandes et al., 2013; Barbosa et al., 2016; Glicerina et al., 2016)**.

Protective effect of cocoa flavonoids on the heart and blood vessels is declared for longer time, and is associated with their ability to change the course of many pathological processes at the development of cardiovascular diseases **(Adriefdjohan et al., 2005; Ding et al., 2006)**.

There is strong evidence that high cocoa intake lowers blood pressure, improves vascular endothelial function, and potentially increases insulin sensitivity **(Kozelová et al., 2014)**. With increased calories in chocolate consumption,

further careful risk-benefit analysis is needed to assess whether consuming cocoa in the form of energy-dense chocolate products may yield a net benefit on cardiovascular risks **(Bauer et al., 2011).**

Scientific hypothesis

The main hypothesis of this work is to determine rheological behaviour of the pure chocolate for five different temperatures. The flow curves will be modeled using several mathematical models. Herschel-Bulkley model, Bingham model, and Casson model will be used for description of chocolate flow behaviour. With these mathematical models is possible to get value of yield stress.

MATERIAL AND METHODOLOGY

The research was focused on evaluating rheological behaviour of pure BIO chocolate (100% cocoa) without lecithin and without traces of milk (Puratos Belcolade, Belgium). The original chocolate sample was in the form of chocolate chips. Samples of chocolate were slowly heating to required temperatures using water bath with a digital controllable thermostat TC-650 (Brookfield, USA).

Rheological measurements were carried out using the DV-3P rotary viscometer (Anton Paar, Austria) equipped with a coaxial cylinder sensor system with precision small samples adapter, temperature sensor pt100, and standard spindle TR9 according to Anton Paar (number 27 according to Brookfield). The geometry of the measuring device it can be seen in **Kumbár and Dostál (2014)**.

In the first step flow curves (shear strain rate versus shear stress) of chocolate were measured in shear strain rate between 0.1 s^{-1} and 68 s^{-1} for five different temperatures 36 °C, 38 °C, 40 °C, 42 °C, and 44 °C, see Table 1.

In the second step temperature dependence of apparent viscosity of chocolate was measured in the range from 35 °C up to 62 °C.

Statisic analysis

Statistical analysis were carried out using MATLAB® R2012a with Statistics toolbox (MathWorks, USA) - analysis of variance (ANOVA) with interaction, testing on the significance level of $p = 0.05$.

RESULTS AND DISCUSSION

In this section, it is provided a careful analysis of the rheological behaviour of pure chocolate in the different temperatures. Very important is selecting of a suitable mathematical flow model. As **Rao (2014)** written, flow model may be considered to be a mathematical equation that can describe rheological data, such as shear strain rate versus shear stress, in a basic shear diagram, and that provides a convenient and concise manner of describing the data.

Occasionally, such as for the (apparent) viscosity versus temperature data, more than one equation may be necessary to describe the rheological data. In addition to mathematical convenience, it is important to quantify how magnitudes of model parameters are affected by state variables, such as temperature, and the effect of structure or composition of foods and establish widely applicable relationships that may be called functional models **(Rao, 2014)**.

Obtained flow curves were modelled using three mathematical flow models which can possible to get value of yield stress, see Figure 1.

The first flow model was Herschel-Bulkley model **(Konar et al., 2015)**:

$$\tau = \tau_0 + K\dot{\gamma}^n, \quad (1)$$

Where τ is shear stress, τ_0 is yield stress, K is consistency index, $\dot{\gamma}$ is shear strain rate, n is flow index. It is noted here that the concept of yield stress has been challenged **(Barnes and Walters, 1989)** because a fluid may deform minutely at stress values lower than the yield stress. Nevertheless, yield stress may be considered to be an engineering reality and plays an important role in many food products **(Rao, 2014)**. If $\tau < \tau_0$ the Herschel-Bulkley fluid behaves as a solid, otherwise it behaves as a fluid. For $n < 1$ the fluid is shear-thinning, whereas for $n > 1$ the fluid is shear-thickening. If $n = 1$ and $\tau_0 = 0$, this model reduces to the Newtonian fluid **(Kumbár et al., 2015)**.

The second flow model was Bingham plastic model **(Zzaman et al., 2014)**:

$$\tau = \tau_0 + \eta_B\dot{\gamma}, \quad (2)$$

Where τ is shear stress, τ_0 is yield stress, η_B is the Bingham plastic viscosity, and $\dot{\gamma}$ is shear strain rate. Bingham plastic model can be described by straight lines in terms of shear rate and shear stress, and the former can be described by two parameters η_B and τ_0. However, the shear rate–shear stress data of shear-thinning and shear-thickening fluids are curves that require more than one parameter to describe their data. Given that the equation of a straight line is simple, it is easy to understand attempts to transform shear rate–shear stress data in to such lines (Rao, 2014).

The third flow model was Casson model (De Graef et al., 2011):

$$\sqrt{\tau} = \sqrt{\tau_0} + \sqrt{\eta_c\dot{\gamma}}, \quad (3)$$

Where τ is shear stress, τ_0 is yield stress, η_c is the Casson viscosity, and $\dot{\gamma}$ is shear strain rate. The Casson model is a structure-based model that, although was developed for characterizing printing inks originally, has been used to characterize a number of food dispersions **(Rao, 2014)**.

The International Office of Cocoa and Chocolate has adopted the Casson model as the official method for interpretation of flow data on chocolates. However, it was suggested that the vane yield stress would be a more reliable measure of the yield stress of chocolate and cocoa products **(Servais et al., 2004)**.

The Figure 2 shows flow curves created by Casson model in five different temperatures of samples. In the Table 2 there are all regression coefficients of three used flow curve models – Herschel-Bulkley, Bingham, and Casson model.

The temperature dependence of apparent viscosity of pure chocolate was measured at constant shear strain rate 50 s^{-1}. This dependence was fitted using power law model (Figure 3) according to **Alvarez et al. (2006)** and **Kumbár and Nedomová (2015)**:

Table 1 Values of shear stress of pure chocolate.

Shear strain rate, s^{-1}	Shear stress, Pa				
	36 °C	38 °C	40 °C	42 °C	44 °C
0.10	9.13	9.08	8.74	8.40	8.05
0.17	10.08	9.83	9.49	9.08	8.86
0.20	10.45	10.00	9.55	9.26	9.04
0.34	11.61	11.05	10.71	10.30	10.08
0.51	12.78	12.10	11.70	11.22	11.09
0.68	13.80	13.03	12.55	12.17	11.92
0.85	14.72	13.78	13.29	12.79	12.54
1.02	15.44	14.42	13.88	13.39	13.09
1.36	16.86	15.63	15.04	14.49	14.14
1.70	18.06	16.62	15.99	15.32	14.92
2.04	19.10	17.62	16.94	16.25	15.80
3.40	23.13	21.04	20.10	19.19	18.58
4.08	24.71	22.46	21.44	20.49	19.77
6.80	31.24	28.40	27.00	25.66	24.70
10.2	37.66	34.11	32.27	30.53	29.30
17.0	49.96	45.15	42.47	40.09	38.13
20.4	55.22	49.98	46.84	44.10	42.00
34.0	76.87	69.12	64.84	60.93	57.63
51.0	98.89	92.06	86.09	80.68	76.04
68.0	***	***	106.08	99.14	93.16

Note: *** denotes out of range.

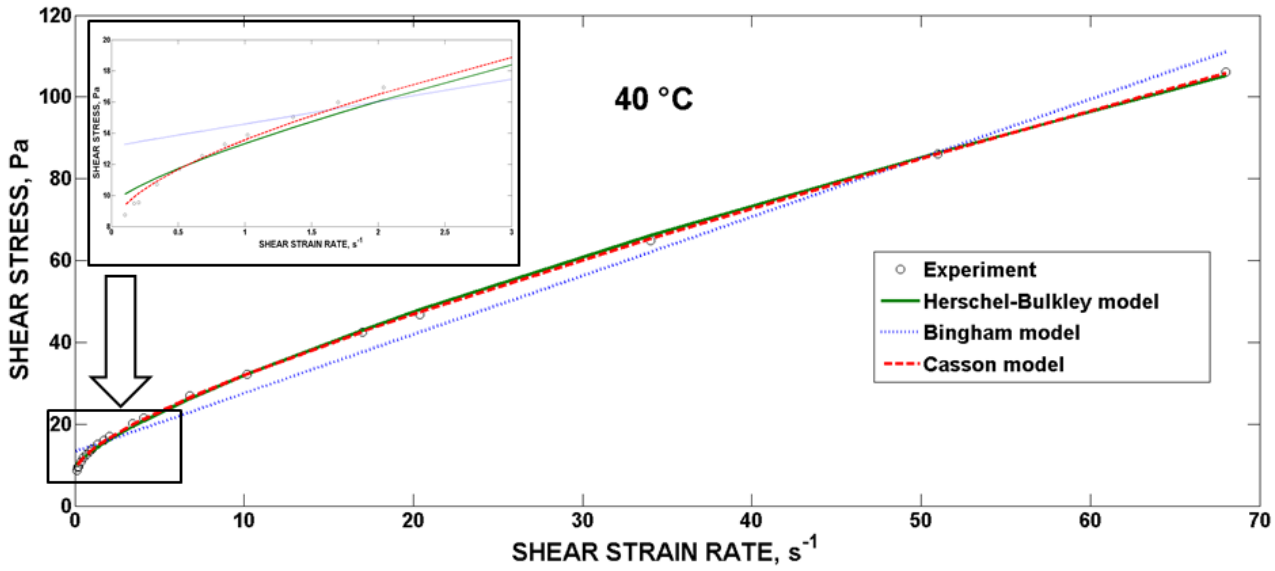

Figure 1 Comparison of the flow curve models of pure chocolate at the temperature 40 °C.

Table 2 Regresion coefficients of the flow models.

Temperature	Herschel-Bulkley model				Bingham model			Casson model		
	τ_0	K	n	R^2	τ_0	η_B	R^2	τ_0	η_c	R^2
°C	Pa	Pa·s^n	–	–	Pa	Pa·s	–	Pa	Pa·s	–
36	9.096	5.764	0.6973	0.9994	14.23	1.792	0.9774	8.549	0.987	0.9991
38	9.339	4.525	0.7359	0.9991	13.17	1.638	0.9831	7.906	0.899	0.9998
40	9.386	3.939	0.7560	0.9992	13.15	1.438	0.9866	7.679	0.830	0.9998
42	9.053	3.755	0.7506	0.9991	12.67	1.340	0.9851	7.489	0.764	0.9998
44	8.838	3.651	0.7414	0.9990	12.41	1.254	0.9847	7.450	0.703	0.9997

Note: R^2 denotes coefficients of determination.

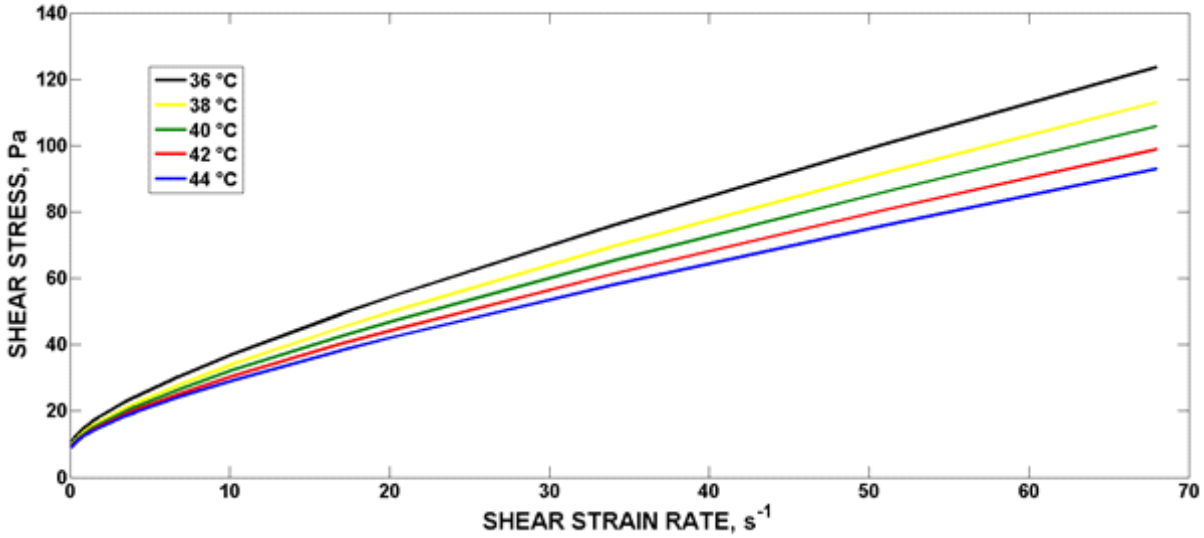

Figure 2 Flow curves (using Casson model) of pure chocolate at five different temperatures.

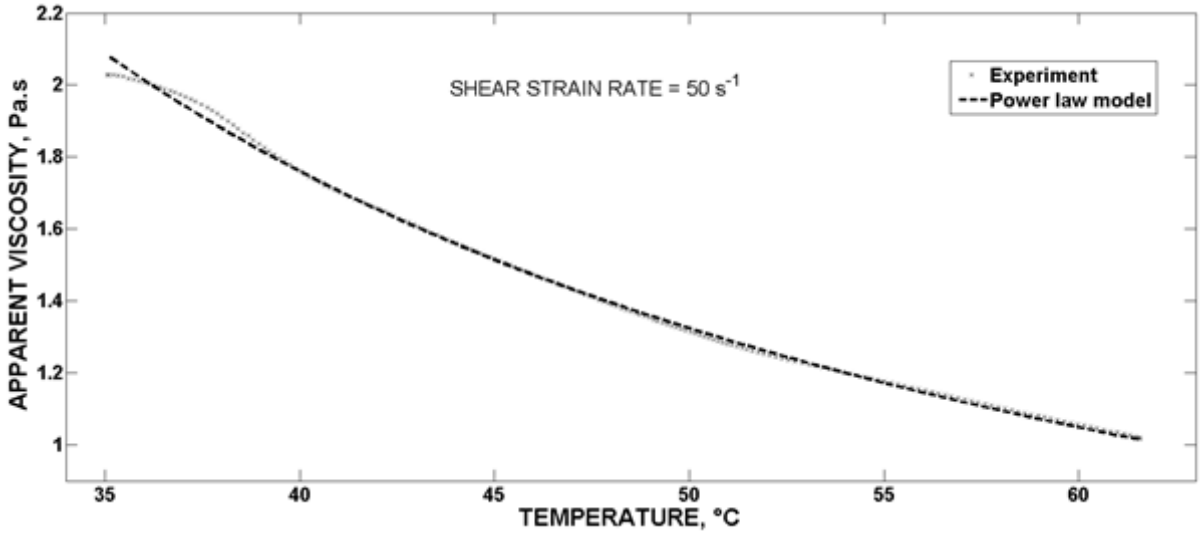

Figure 3 Temperature dependence of apparent viscosity of pure chocolate.

$$\eta_{app} = at^b, \quad (4)$$

Where η_{app} is apparent viscosity, t is temperature, a and b are coefficients. The values of coefficients for Eq. (4) are a = 195.6 Pa·s·°C^{-b}, b = -1.277, and R^2 = 0.9983.

It was found out that all of the calculated regression coefficients of all used models are statisticaly significantly different (p <0.05).

It is evident that apparent viscosity of pure chocolate decreased with increasing temperature (p <0.05). This decrease **Aguilera et al. (2004)** described that chocolate microstructurally regarded as a particulate medium formed by an assembly of fat-coated particles. Within this matrix the liquid fraction of cocoa fat (which increases with temperature) is likely to move under capillary forces through interparticle passages and connected pores.

CONCLUSION

Obtained results demonstrated effect of temperature on the rheological behaviour of pure chocolate. The significant yield stress τ_0 (p <0.05) and plastic behaviour of pure chocolate were observed and described in the five different temperatures between 36 °C and 44 °C.

Experimental data was successfully fitted using Herschel-Bulkley model (R^2 ranged from 0.9990 up to 0.9994), Bingham model (R^2 ranged from 0.9774 up to 0.9866), and Casson model (R^2 ranged from 0.9991 up to 0.9998). The best model for fitted flow curves of chocolate was chosen Casson model, which also had the best values of coefficient of determination R^2 (average R^2 of five models is 0.99964). At the other hand, Herschel-Bulkley model gives also very accurate results of pure chocolate flow curve modelling (average R^2 of five models is 0.99916).

Finally, the liquid pure chocolate shows behaviour of Casson fluid. We can also conclude that apparent viscosity of pure chocolate decreased with increasing temperature (p <0.05).

REFERENCES

Ačkar, D., Škrabal, S., Šubarić, D., Babić, J., Miličević, B., Jozinović, A. 2015. Rheological properties of milk chocolates as influenced by milk powder type, emulsifier, and cocoa butter equivalent additions. *International Journal of Food Properties*, vol. 18, no. 7, p. 1568-1574.

Adriefdjohan, M. W., Savaiano, D. A. 2005. Chocolate and cardiovascular health: is it too good to be true? *Nutrition Reviews*. vol. 63, no. 12, p. 427-430. https://doi.org/10.1111/j.1753-4887.2005.tb00118.x

Aguilera, J. M., Michel, M., Mayor, G. 2004. Fat migration in chocolate: Diffusion or capillary flow in a particulate solid? - A hypothesis paper. *Journal of Food Science*, vol. 69, no. 7, p. 167-174. https://doi.org/10.1111/j.1365-2621.2004.tb13615.x

Alvarez, E., Cancela, M. A., MacEiras, R. 2006. Comparison of rheological behaviour of salad sauces. *International Journal of Food Properties*, vol. 9, no. 4, 907-915. https://doi.org/10.1080/10942910600840870

Barbosa, C., Diogo, F., Alves, M. R. 2016. Fitting mathematical models to describe the rheological behaviour of chocolate pastes. *AIP Conference Proceedings 1738*. vol. 370016. https://doi.org/10.1063/1.4952161

Barnes, H. A., Walters, K. 1989. The yield stress myth? *Rheologica Acta*, vol. 24, p. 323-326. https://doi.org/10.1007/BF01333960

Bauer, S. R., Ding, E. L, Smit, L. A. 2011. Cocoa Consumption, Cocoa Flavonoids, and Effects on Cardiovascular Risk Factors: An Evidence-Based Review. *Current Cardiovascular Risk Reports*, vol. 5, no. 2, p 120-127. https://doi.org/10.1007/s12170-011-0157-5

Božiková, M., Hlaváč, P. 2013. Temperature and storing time influence on selected physical properties of milk and acidophilus milk. *Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis*, vol. 61, no. 6, p. 1589-1595. https://doi.org/10.11118/actaun201361061589

Bozkurt, H., Icier, F. 2009. Rheological characteristics of quince nectar during ohmic heating. *International Journal of Food Properties*, vol. 12, no. 4, p. 844-859. https://doi.org/10.1080/10942910802102962

Cikrikci, S., Yucekutlu, M., Mert, B., Oztop, M. H. 2017. Physical characterization of low-calorie chocolate formulations. *Journal of Food Measurement and Characterization*, vol. 11, no. 1, 41-49. https://doi.org/10.1007/s11694-016-9369-1

De Graef, V., Depypere, F., Minnaert, M., Dewettinck, K. 2011. Chocolate yield stress as measured by oscillatory rheology. *Food Research International*, vol. 44, no. 9, p. 2660-2665. https://doi.org/10.1016/j.foodres.2011.05.009

Ding, E. L., Hutfless, S. M., Ding, X., Girotra, S. 2006. Chocolate and Prevention of Cardiovascular Disease: A Systematic Review. *Nutrion Metabolism (Lond)*. vol. 3, no. 2-3.

Fernandes, V. A., Müller, A. J., Sandoval, A. J. 2013. Thermal, structural and rheological characteristics of dark chocolate with different compositions. *Journal of Food Engineering*, vol. 116, p. 97-108. https://doi.org/10.1016/j.jfoodeng.2012.12.002

Glicerina, V., Balestra, F., Rosa, M. D., Romani, S. 2016. Microstructural and rheological characteristics of dark, milk and white chocolate: A comparative study. *Journal of Food Engineering*, vol. 169, p. 165-171. https://doi.org/10.1016/j.jfoodeng.2015.08.011

Glicerina, V., Balestra, F., Rosa, M. D., Romani, S. 2013. Rheological, textural and calorimetric modifications of dark chocolate during process. *Journal of Food Engineering*, vol. 119, p. 173-179. https://doi.org/10.1016/j.jfoodeng.2013.05.012

Gonçalves, E. V., da Silva Lannes, S. C. 2010. Chocolate rheology. *Food Science and Technology*, vol. 30, no. 4, p. 845-851. https://doi.org/10.1590/S0101-20612010000400002

Hlavač, P., Božikova, M., Cviklovič, V. 2016. Dynamic viscosity and activation energy of wort during fermentation and storing. *Acta Technologica Agriculturae*, vol. 19, no. 1, p. 6-9. https://doi.org/10.1515/ata-2016-0002

Konar, N., Poyrazoglu, E. S., Artik, N. 2015. Influence of calcium fortification on physical and rheological properties of sucrose-free prebiotic milk chocolates containing inulin and maltitol. *Journal of Food Science and Technology*, vol. 52, no. 4, p. 2033-2042. https://doi.org/10.1007/s13197-013-1229-y

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

Kumbár, V., Dostál, P. 2014. Temperature dependence density and kinematic viscosity of petrol, bioethanol and their blends. *Pakistan Journal of Agricultural Sciences*, vol. 51, no. 1, p. 175-179.

Kumbár, V., Nedomová, Š. 2015. Viscosity and analytical differences between raw milk and UHT milk of Czech cows. *Scientia Agriculturae Bohemica*, vol. 46, no. 2, p. 78-83. https://doi.org/10.1515/sab-2015-0020

Kumbár, V., Nedomová, Š., Pytel, R., Kilián, L., Buchar, J. 2017. Study of rheology and friction factor of natural food hydrocolloid gels. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 203-209. https://doi.org/10.5219/735

Kumbár, V., Trnka, J., Nedomová, Š., Buchar, J. 2015. On the influence of storage duration on rheological properties of liquid egg products and response of eggs to impact loading - Japanese quail eggs. *Journal of Food Engineering*, vol. 166, p. 86-94. https://doi.org/10.1016/j.jfoodeng.2015.05.030

Lapčík, L., Lapčíková, B., Žižková, H., Peng, L., Vojteková, V. 2017. Effect of cocoa fat content on wetting and surface energy of chocolate. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 410-416. https://doi.org/10.5219/732

Machálková, L., Hřivna, L., Nedomová, Š., Jůzl, M., The effect of storage temperature on the quality and formation of

blooming defects in chocolate confectionery. *Potravinarstvo*, vol. 9, no. 1, p. 39-47. https://doi.org/10.5219/425

Quiñones-Muñoz, T., Gallegos-Infante, J. A., Rocha-Guzmán, N. E., Ochoa-Martinez, L. A., Morales-Castro, J., González-Laredo, R. F., Medina-Torres, L. 2011. Mixing and tempering effect on the rheological and particle size properties of dark chocolate coatings. *CYTA - Journal of Food*, vol. 9, no. 2, p. 109-113. https://doi.org/10.1080/19476337.2010.482748

Rao M. A. 2014. *Rheology of Fluid, Semisolid, and Solid Foods. Food Engineering Series*. 3rd ed. BOSTON, USA: Springer Science+Business Media. 461 p. ISBN 978-1-4614-9229-0.

Servais, C. Ranc, H., Roberts, I. D. 2004. Determination of chocolate viscosity. *Journal of Texture Studies*, vol. 34, no. 5–6, p. 467-498. https://doi.org/10.1111/j.1745-4603.2003.tb01077.x

Trávníček, P., Burg, P., Krakowiak-Bal, A., Junga, P., Vítěz, T., Ziemiańczyk, U. 2016. Study of rheological behaviour of wines. *International Agrophysics*, vol. 30, no. 4, p. 509-518. https://doi.org/10.1515/intag-2016-0018

Zzaman, W., Issara, U., Febrianto, N. F., Yang, T. A. 2014. Fatty acid composition, rheological properties and crystal formation of rambutan fat and cocoa butter. *International Food Research Journal*, vol. 21, no. 3, p. 983-987.

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ADDITION OF RUTIN/QUERCETIN MIXTURE TO SPREADABLE PROCESSED CHEESE: ANTIOXIDANT AND TEXTURAL CHARACTERISTICS

Libor Červenka, Tomáš Hájek, Richardos Nikolaos Salek, Michaela Černíková, Helena Velichová, František Buňka

ABSTRACT

Spreadable processed cheese is a traditional product made from a mixture of cheese, fat, water and emulsifying salts.The aim of this research was prepared spreadable processed cheese with new functional properties. Spreadable processed cheese enriched with the mixture (1:1) of rutin and quercetin (1.0 g.100g^{-1}) was prepared at two melting temperature (80°C and 90°C) for three holding times (1, 5 and 10 min). The effect of melting temperature and holding time on the quercetin and rutin content was assessed using liquid chromatography with UV detection after ultrasonic-assissted extraction to methanol. The corresponding antioxidant characteristics were determined using spectrophotometric assays for total phenolics (TPC) and radical scavenging activities DPPH and ABTS. The extraction yield for quercetin varied from 45.8 to 66.4% and from 12.8 to 40.8% for rutin. The level of quercetin significantly descrased with the increase of holding time, while rutin content has increased with the increase of melting temperature. TPC values ranged from 10.8. to 14.8 mg GAE·g^{-1} in SPC sample enriched with rutin/quercetin mixture, and the increase of melting temperature resulted in the decrease of TPC values. DPPH and ABTS assays did not reveal any statistically significant pattern using Kruskal-Wallis ANOVA. The addition of the mixture of flavonoids into the processed cheese significantly reduced the complex modulus in comparison with the control sample (without flavonoids). This indicate that the structure of enriched SPC sample was more flexible than those in control processed cheese samples. Both melting temperature and holding time increased the complex modulus. Spreadable processed cheese are scarcely used as a carrier of flavonoids in scientific researches probably due to very complex matrices. Our research proved that spreadable processed cheese containing rutin/quercetin mixture can be used as a functional food.

Keywords: processed cheese; flavonoid; antioxidant; technology; rheometry

INTRODUCTION

Quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-chromen-4-on) and rutin (quercetin-3-rutinoside) are major dietary flavonoids examined in functional food. Buckwheat, as a natural source of those substances, is usually used in preparation of various bakery **(Krejzová et al., 2017; Lin and Zhou, 2018; Wang et al., 2017)** and pasta products **(Cho and Lee, 2015; Jambrec et al., 2015)**. Both flavonoids has a potential to be beneficial to human health. Quercetin and its derivates may act as antioxidant and anti-inflammatory agents **(Lesjak et al., 2018)** when consume in quercetin-rich diet. Pharmacological potential of rutin including vasoprotective, antidiabetic or neuroprotective effects has been extensively studied **(Rauf et al., 2017)**. Enrichment of food with plant extracts or pure chemical substances was investigated in order to develop new functional food products. Cheese is one of the promising carrier of health-related compounds. Cheese prepared using various lipophilic compounds such as retinyl palmitate, α-tocopherol, CoQ_{10} **(Stratulat et al., 2017)** and soy phytosterols **(Giri et al., 2014)** were examined. Phenolics such as catechin and epigallocatechin were frequently used as functional ingredients for manufacturing cheese with enhanced antioxidant properties **(Rashidinejad et al., 2016; Lamothe et. al, 2016; Han et al., 2011)**. Quercetin and its inclusion compounds with cyclodextrins were added to fresh cheese enhancing its nutraceutical properties **(Pereira et al., 2017)**. However, low-fat cheese or ripened cheese were assessed in above cited research papers. Processed cheese spread is scarcely used in experiments, probably due to the high content of fat and high temperature of processing. Spreadable processed cheese are multicomponent mixture comprising of cheese, fat, water and emulsifying salts. This mixture is stirred and melted at temperatures ranged from 85°C to 110°C for

holding times from 1 to 10 minutes. In our recent work, the content of quercetin in spreadable processed cheese (SPC) decreased with the increase of holding time but stable at different melting temperature. On the other hand, the level of rutin incorporated into the SPC showed decreasing trend with the increase of melting temperature **(Přikryl et al., 2018)**. In this research, SPC samples with mixture of rutin/quercetin was prepared and the effect of processing condition on the recovery of quercetin and rutin, antioxidant properties and textural characteristics was determined.

Scientific hypothesis

Both melting temperature and holding time significantly affect the level of rutin and quercetin as well as antioxidant and textural properties of enriched spreadable processed cheese.

MATERIAL AND METHODOLOGY

All solvent and chemicals were purchased from Sigma-Aldrich (Prague, Czech Republic).

Processed cheese manufacturing

Processed cheese was manufactured from the following ingredients per 100g of final product: Eidam cheese (Kromilk PLC, Kroměříž, Czech Republic, 8-week maturity, 50 g), butter (Madeta PLC, České Budějovice, Czech Republic, 84 g), water, emulsifying salts (Fosfa PLC, Břeclav, Poštorná, Czech Republic, 2.3 g) and the mixture of quercetin hydrate/rutin hydrate (1.0 g, 1:1, w/w). The amount of butter and water was adjusted in order to obtain product with 37 g.100g^{-1} dry matter content and 50 g.100g^{-1} of fat in dry matter content. Control sample (without quercetin/rutin mixture) was also prepared.

The SPC samples were prepared according to **Přikryl et al. (2018)** at two melting temperatures (80°C and 90°C) and three holding times (1, 5 and 10 min).

The preparation of extracts

SPC samples (1.0 g) was extracted by 10.0 mL of methanol in ultrasonic bath Sonorex TK52 (Bandelin Electronic, Berlin, Germany) for 30 min. After centrifugation at 1400 × g for 10 min (Vintrum NF400, Nűve, Ankara, Turkey), a clear supernatant was filtered using 0.45 µm syringe polytetrafluoroethylene membrane filter (Labicom, Olomouc, Czech Republic).

Determination of antioxidant characteristics

Antioxidant assays

The total phenolic assay (TPC) using Folin-Ciocalteau reagent was applied according to Santos et al. (2012). The results were expressed as amount of gallic acid per gram of SPC sample measured at 765 nm (DU 530, Beckman Coulter Inc., Brea, CA, USA). The extraction solvent used instead of SPC sample extract served as a blank.

The DPPH (2,2-diphenyl-1-picrylhydrazil) and ABTS (2,2´-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging assays were performed as described by Mišan et al. (2011). The increase of absorbance was measured at 515 and 734 nm for DPPH and ABTS radical scavenging assay, respectively. The scavenging activity was plotted against various concentration of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and expressed as Trolox antioxidant capacity ($TEAC_{DPPH}$ and $TEAC_{ABTS}$) in amount of Trolox per g of SPC sample.

HPLC analysis of quercetin and rutin

Agilent 1100 Series (Agilent Technologies, Santa Clara, CA, USA) equipped with a quarternary pump, a degasser, an autosampler, a termostetted column compartment, and UV-MS detector Agilent 1100 Series LC/MSD Trap SL. A Gemini 5 µm column (150 × 3.0 mm) was used (Phenomenex®, Torrance, CA, USA). The gradient flow rate 0.7 mL/min (formic acid: acetonitrile from 900 mL:100 mL to 500 mL: 500 mL for 0 – 15 min) was applied. Formic acid (0.21% v/v) was acidifed to pH 3.05.

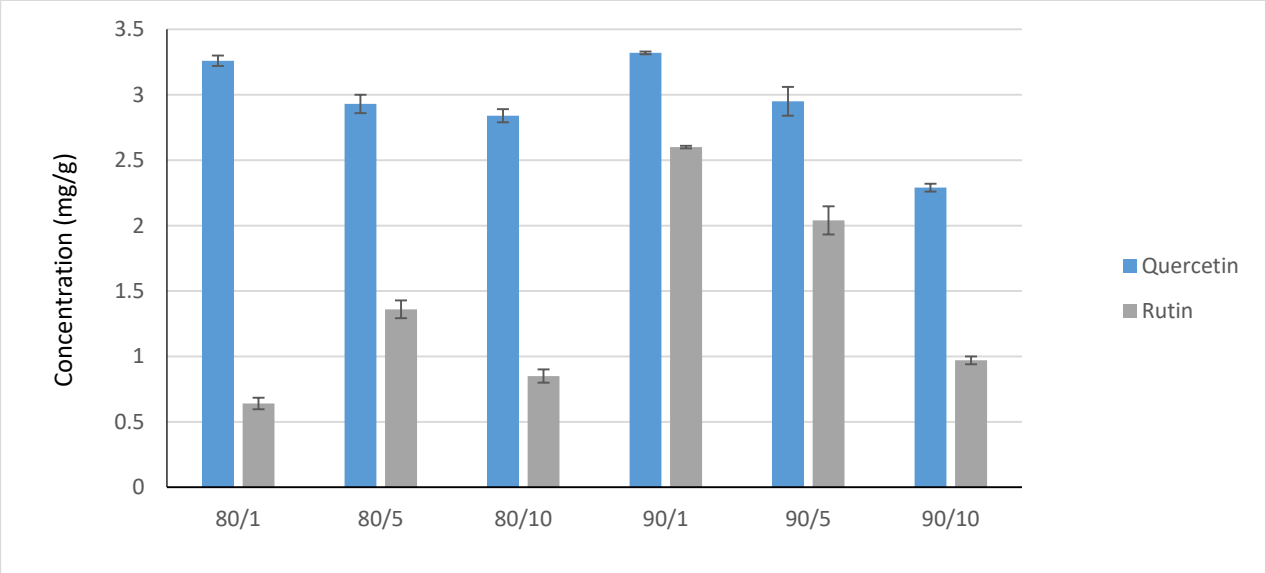

Figure 1 The effect of melting temperature and holding time (80/1 means 80°C for 1 min, 80/5 means 80°C at 5 min, etc.) on the quercetin and rutin levels extracted from spreadable processed cheese. Average mean ± standard deviation (n = 4).

The analysis was performed at 40°C and both peaks of quercetin a rutin were detected at 360 nm. Quantification was based upon the separation of standard solution of flavonoids from 1 to 100 μg.mL^{-1} and plotting the peak area against concentration.

Determination of rheologial properties

A dynamic oscillatory shear rheometer (RheoStress 1, Haake, Bremen, Germany) with a plate-plate geometry (diameter 35 mm, gap 1 mm) were used. The complex modulus (G^*) at reference frequency 1 Hz was calculated based on the values uf storage (G´) and loss (G´´) moduli:

$$G^* = \sqrt{(G')^2 + (G'')^2} \quad (1)$$

Increasing values of the complex moduli (G*) indicated rigid consistency of processed cheese and rising gel strength **(Černíková et al., 2017).** All rheological measurements were done at 20.0°C.

Statisic analysis

All the measurements were performed in four repetitions (n = 4) and the average mean with standard deviation (SD) was calculated. Non-parametric Kruskall-Wallis ANOVA was used for determination of the effect of melting temperature and holding time on the content of quercetin/rutin and antioxidant properties. Spearman correlation coefficients (r) were calculated in order to find mutual associations between variables. All the statistical procedures were done at the probability level of p = 0.05 (Statistica 12, StatSoft CR s.r.o., Prague, Czech Republic).

RESULTS AND DISCUSSION

Rutin and quercetin content in SPC samples

Extraction of flavonoids from solids into alcohols and aqueous-alcoholic solutions belongs to the frequently used methods in plant-based material prior to their determination. As can be see from **Figure 1**, higher concentrations (p <0.001) of quercetin were determined in SPC samples extracted to 100% methanol in comparison with rutin levels. The extraction yield for quercetin ranged from 45.8 to 66.4% and from 12.8 to 40.8% for rutin. As was expected, higher levels of quercetin than rutin was recovered from SPC samples extracted to methanol. This is probably due to the higher hydrophobic properties of quercetin. It should be noted that cheese is a very complex matrix and various biochemical reaction may occur between phenolics and cheese proteins as was suggested in a study of **Rashidinejad et al. (2016)**. The recovery of green tea catechins from full-fat cheese varied from 3.2 to 29.4% in their experiments. In our previous work **(Přikryl et al., 2018)**, we prove that quercetin and rutin added separately to the formulation of SPC samples, may undergo thermal degradation or chemical reaction with the constituents of cheese during melting. Quercetin was more sensitive to holding time whereas rutin level decreased with the increase of melting temperature. In this research, the level of quercetin significantly descreased with the increase of holding time (p = 0.013). Kruskal-Wallis ANOVA procedure revealed that rutin concentration was influenced by the melting temperature but in the opposite manner in comparison with our previous research **(Přikryl et al., 2018)**; i.e. rutin level was higher in SPC samples processed at 90°C than 80°C (p = 0.025). Rutin level usually decreased with the increase of processing temperature. For instance, **Chaaban et al. (2016)** determined the degradation kinetics of rutin in solution at various temperatures and found that half life time for rutin has decreased from 19.25 to 1.99 h at 70°C and 90°C, respectively. **Krejzová et al. (2017)** found that the amount of rutin in buckwheat flour and seeds substantially decreased when thermall treatment exceeded 150°C. On the other hand, rutin content significantly increased with the elevated temeparture during baking of Tartary buckwheat enriched bread **(Wang et al., 2017)**. However, rutin was released from the cell wall of buckwheat during baking procedure in this research, which should not be expected in SPC samples enriched with rutin/queretin. We

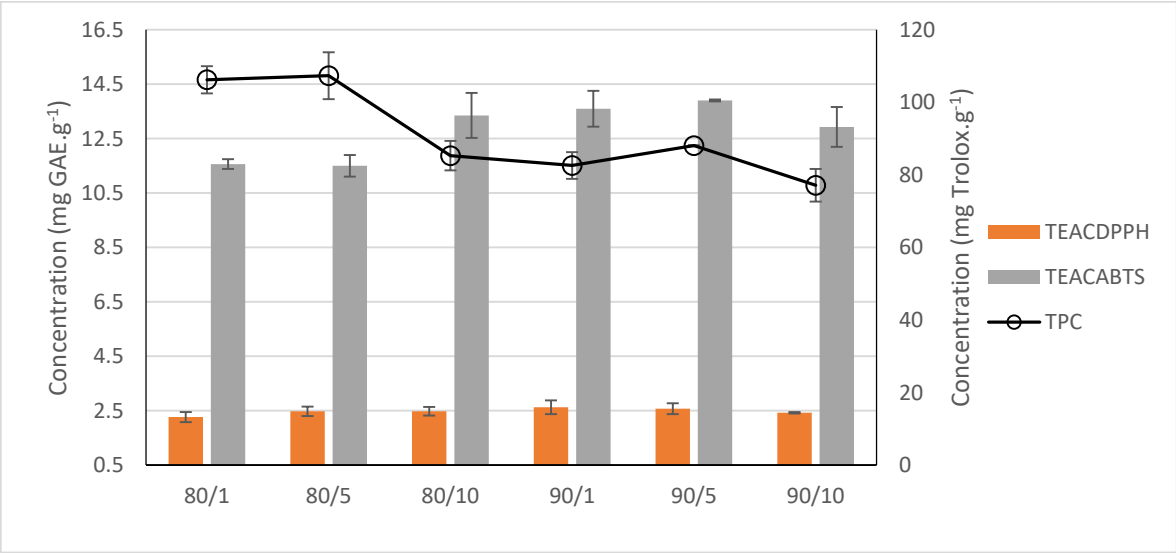

Figure 2 The effect of melting temperature and holding time (80/1 means 80°C for 1 min, 80/5 means 80°C at 5 min, etc.) on antioxidant properties of spreadable processed cheese enriched with quercetin and rutin. Average mean ± standard deviation (n = 4).

may hypothesise that different rheological properties of processed cheese prepared at 90°C could enhanced the extraction yield of rutin.

Antioxidant properties of SPC samples

SPC samples enriched with the mixture (1:1) of rutin and quercetin at 1.0 g/kg exhibited total phenolics content in the range from 11.9 to 14.8 mg GAE·g^{-1} when melted at 80°C, and from 10.8 to 12.2 mg GAE·g^{-1} when melted at 90°C **(Figure 2)**. According to Kruskal-Wallis ANOVA procedure, increasing melting temperature resulted in significant decrease of TPC values (p = 0.020). Regarding the holding time for each temperature treatment, lower TPC value (11.9 ±0.5 mg GAE·g^{-1}) was obtained after melting SPC sample at 80°C for 10 min whereas constant TPC values were determined at 90°C. As can be seen from **Figure 2**, antioxidant properties of SPC samples measured in terms of ABTS assay showed remarkable higher values (82.5 – 100.5 mg Trolox·g^{-1}) than those obtained using DPPH assay (13.2 – 15.5 mg Trolox·g^{-1}). This discrepanse was maily attributed to the steric hindrance and due to the different mechanism of radical scavenging **(Schaich et al., 2015)**. The overall effect of both melting temperature and holding time on $TEAC_{DPPH}$ and $TEAC_{ABTS}$ values was negligible (p >0.05) but significantly higher $TEAC_{ABTS}$ value (96.4 ±6.2 mg Trolox·g^{-1}) was observed for SPC samples melted at 80°C for 10 min than for 1 and 5 min at the same melting temperature (p <0.01). Correlation analysis revealed both positive and negative weak but non-significant associations between variables. The highest one was for TPC/$TEAC_{ABTS}$ (r = -0.410, p = 0.186). Nevertheless, it is interesting that low TPC values was associated with the high $TEAC_{ABTS}$ value at 80°C and 10 min **(Figure 2)**. The same pattern was determined in tomatoe boiled in water-oil mixture in comparsion with raw counterparts **(Ramírez-Anaya et al., 2015)**. They stated that some hydrosoluble comounds without true antioxidant properties may contribute to the opposite effect. In addition, irreversible higher oxidation products of ABTS and milk protein (particularly with thiol groups) may cause the additional increase of $TEAC_{ABTS}$ **(Çekiç et al., 2015)**.

Rheological properties of SPC samples

The results of the complex modulus G" of processed cheese with quercetin/rutin (1:1) content at different melting temperatures and holding times are presented in Table 2. With the increase of both processing parameters, G" values increased indicating the formation of denser net structure (p <0.05). As can be seen from the differences between control and enriched SPC samples, the addition of quercetin/rutin mixture significantly decreased G" parameters under all the processing conditions (showing negative values). As the complex modulus G" is related to the strenght of the intermolecular interaction among proteins in cheese, our findings confirmed that addition of quercetin/rutin mixture reduced the protein network. This is in agreement with the study of **He et al. (2018)** who found that the addition of quercetin and rutin decreased the cohesivness of Tartary buckwheats starch gel. Different rheological properties of SPC samples manufactured under different conditions may also influence the extractibility of both flavonols. For instance, the Young´s modulus of elasticity (textural characteristic of seed) showed negative correlation with the extraction yield for catechin, epicatechin and procyanidin B_1, but exhibited positive correlation with epicatechin gallate **(Segade et al., 2016)**.

CONCLUSION

The addition of the mixture of rutin/quercetin to the processed cheese spread resulted in its enhanced antioxidant capacity, as was expected. We used rutin and quercetin in combination as these flavonoids ale likely to be occure in plant material together. It is evident from the results that the mixture of rutin and quercetin significantly decreased viscoelastic properties, i.e. enriched processed cheese were more flexible than those in control samples. Increasing melting temperature or holding time increased the complex modulus thus SPC samples became more rigid in texture. The effect of processing parameters on the content of rutin/quercetin and corresponding antioxidant properties was not so unambiguous. While quercetin level decreased with the increase of holding time, rutin levels were affected by melting temperature. However, higher rutin levels were observed in SPC samples prepared at higher temperature. This may be attributed to the changes of consistency and better extractability of rutin, although no correlation was found between rutin content and complex modulus G". Based on the results, we may conclud that scientific hypothesis was not confirmed regarding the impact of processing technology on the antioxidant characteristic but is valid for rheologial properties. Further research is needed to elucidate the effect of rheologial properties on the extractability of phenolic substances from processed cheese matrix.

Table 2 The effect of melting temperature and holding time on the complex modulus at the reference frequency 1 Hz ($G^{"}$, kPa).

	Melting temperature (°C)	Holding time (min)		
		1	5	10
SPC with rutin/quercetin mixture (1:1)	80	2533 ±123^{a} A	6028 ±341^{b} A	7465 ±375^{c} A
	90	3706 ±145^{a} B	7585 ±424^{b} B	11019 ±514^{c} B
Difference from control SPC sample	80	-910***	-720*	-1703**
	90	-695**	-914*	-1019*

Note: Means ±standard deviation (n = 4); significant differences between means for melting temperatures are indicated by capital letters; the means within a row followed by different superscript letters (p <0.05); the differences from control SPC samples are statistically significant at * <0.05, ** <0.01 and *** <0.001.

REFERENCES

Çekiç, S. D., Demir, A., Başkan, A. S., Tütem, E., Apak, R. 2015. Determination of total antioxidant capacity of milk by CUPRAC and ABTS method with separate characterisation of milk protein fraction. *Journal of Dairy Research*, vol. 82, p. 177-184. https://doi.org/10.1017/S0022029915000114

Černíková M., Salek, R. N., Kozáčková, D., Běhalová, H., Luňáková, L., Buňka, F. 2017. The effect of selected processing parameters on viscoelastic properties of model processed cheese spreads. *International Dairy Journal*, vol. 66, p. 84-90. https://doi.org/10.1016/j.idairyj.2016.11.007

Chaaban, H., Ioannou, I., Chebil, L., Slimane, M., Gérardin, C., Paris, C., Charbonnel, C., Chekir, L., Ghoul, M. 2016. Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. *Journal of Food Processing and Preservation*, vol. 41, e13203. https://doi.org/10.1111/jfpp.13203

Cho, I. J., Lee, S. 2015. Extraction of rutin from Tartary buckwheat milling fractions and evaluation of its thermal stability in an instant fried noodle system. *Food Chemistry*, vol. 176, p. 40-44. https://doi.org/10.1016/j.foodchem.2014.12.020

Giri, A., Kanawjia, S. K., Rajoria, A. 2014. Effect of phytosterols on textural and melting characteristics of cheese spread. *Food Chemistry*, vol. 157, p. 240-245. https://doi.org/10.1016/j.foodchem.2014.01.127

Han, J., Britten, M., St-Gelais, D., Champagne, C. P., Fustier, P., Salmieri, S., Lacroix, M. 2011. Polyphenolic compounds as functional ingredients in cheese. *Food Chemistry*, vol. 124, p. 1589-1594. https://doi.org/10.1016/j.foodchem.2010.08.021

He, C., Zhang, Z., Liu, H., Gao, J., Li, Y., Wang, M. 2018. Effect of rutin and quercetin on the physicochemical properties of Tartary buckwheat starch. *Starch/Stärke* vol. 70, 1700038. https://doi.org/10.1002/star.201700038

Jambrec, D., Sakač, M., Mišan, A., Mandić, A., Pestorić, M. 2015. Effect of autoclaving and cooking on phenolic compounds in buckwheat-enriched whole wheat tagliatelle. *Journal of Cereal Science*, vol. 66, p. 1-9. https://doi.org/10.1016/j.jcs.2015.09.004

Krejzová, E., Bittová, M., Kráčmar, S., Vojtíšková, P., Kubáň, V, Golian, J. 2017. Effect of thermal treatment on rutin content in selected buckwheat products using calcium as an internal tracer. *Potravinarstvo Slovak Journal of Food Science*, vol. 11, no. 1, p. 679-684. https://doi.org/10.5219/853

Lamothe, S., Langlois, A., Bazinet, L., Couillard, C., Britten, M. 2016. Antioxidant activity and nutrient release from polyphenol-enriched cheese in a simulated gastrointestinal environment. *Food and Functions*, vol. 7, p. 1634-1644. https://doi.org/10.1039/c5fo01287b

Lesjak, M., Beara, I., Simin, N., Pintać, D., Majkić, T., Bekvalac, K., Orčić, D., Mimica-Dukić, N. 2018. Antioxidant and anti-inflammatory activities of quercetin and its derivates. *Journal of Functional Foods*, vol. 40, p. 68-75. https://doi.org/10.1016/j.jff.2017.10.047

Lin, J., Zhou, W. 2018. Role of quercetin in the physicochemical properties, antioxidant and antiglycation activities in bread. *Journal of Functional Food*, vol. 40, p. 299-306. https://doi.org/10.1016/j.jff.2017.11.018

Pereira, A. B., da Silva, A. M., Barroca, M. J., Marques, M. P. M., Braga, S. S. 2017. Physicochemical properties, antioxidant action and practical application in fresh cheese of the solid inclusion compound γ-cyclodextrin·quercetin, in comparison with β-cyclodextrin·quercetin. *Arabian Journal of Chemistry*, in press. https://doi.org/10.1016/j.arabjc.2017.04.001

Přikryl, J., Hájek, T., Švecová, B., Salek, R. N., Černíková, M., Červenka, L., Buňka, F. 2018. Antioxidant properties and textural characteristics of processed cheese spreads enriched with rutin and quercetin: The effect of processing conditions. *LWT-Food Science and Technology*, vol. 87, p. 266-271. https://doi.org/10.1016/j.lwt.2017.08.093

Ramírez-Anaya, J. P., Samaniego-Sánchez, C., Castaneda-Saucedo, M. C., Villalón-Mir, M., Serrana, H. L.-G. 2015. Phenols and the antioxidant capacity of Mediterranean vegetables prepared with extra virgin oil using different domestic cooking techniques. *Food Chemistry*, vol. 188, p. 430-438. https://doi.org/10.1016/j.foodchem.2015.04.124

Rashidinejad, A., Birch, E. J., Everett, D. W. 2016. Antioxidant activity and recovery of green teen catechins in full-fat cheese following gastrointestinal simulated digestion. *Journal of Food Composition and Analysis*, vol. 48, p. 13-24. https://doi.org/10.1016/j.jfca.2016.02.004

Rauf, A., Imran, M., Patel, S., Muzaffar, R., Bawazeer, S. S. 2017. Rutin: Exploitation of the flavonols for health and homeostasis. *Biomedicine and Pharmacotherapy*, vol. 96, p. 1559-1561. https://doi.org/10.1016/j.biopha.2017.08.136

Segade, S. R., Torchio, F., Gerbi, V., Quijada-Morín, N., García-Estévez, I., Giacosa, S., Escribano-Bailón, M. T., Rolle, L. 2016. Impact of postharvest dehydration process of winegrapes on mechanical and acoustic properties of the seeds and their relationship with flavanol extraction during simulated maceration. *Food Chemistry*, vol. 199, p. 893-901. https://doi.org/10.1016/j.foodchem/2015.12.072

Schaich, K. M., Tian, X., Xie, J. 2015. Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assay. *Journal of Functional Foods*, vol. 14, p. 111-125. https://doi.org/10.1016/j.jff.2015.01.043

Stratulat, I., Britten, M., Salmieri, S., Fustier, P., St-Gelais, D., Champagne, C. P. & Lacroix, M. 2014. Enrichment of cheese with bioactive lipophilic compounds. *Journal of Functional Foods*, vol. 6, 48-59. https://doi.org/10.1016/j.jff.2013.11.023

Wang, X., Fan. D., Zhang, T. 2017. Effects of hydrothermal processing on rutin retention and physicochemical properties of Tartary buckwheat enriched dough and Chinese steamed bread. *International Journal of Food Science and Technology*, vol. 52, p. 2180-2190. https://doi.org/10.1111/ijfs.13497

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SQUARE WAVE VOLTAMMETRY AT CARBON PASTE ELECTRODE MODIFIED WITH SURFACTANT FOR ALPHA TOCOPHERYL ACETATE DETERMINATION IN COSMETICS

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ABSTRACT

The aim of this study was describe electrochemical properties of a carbon paste electrode (CPE) bulk modified with 30% (w/w) surfactant sodium dodecyl sulphate (CPE/SDS) and demonstrates its application in the determination of α-tocopheryl acetate (α-TAc), known as vitamin E acetate, in selected cosmetic products, especially body creams. In addition to anionic SDS, cationic hexadecylpyridinium chloride monohydrate (CPC) was also tested as possible modifier. It was found that selection of surfactant type and its content significantly affect an electrical conductivity and mechanical stability of these heterogeneous electroanalytical sensors in pure organic solvents. Under this study, it was found that CPC is a totally inappropriate mediator due to very high backgroundcurrent. Together with other lipophilic vitamins characterized by antioxidant activity (dominantly retionoids), this completely synthetic substance is widely used as significant cosmetic additive due its preservative properties. Monitoring of its content in cosmetic products is usually performed by high-performance liquid chromatography (HPLC) with UV detection. This standard analytical protocol is always burdened with the complex and time-consuming preparation of the sample before analysis. For that reason, robust and simple electroanalytical method based on anodic oxidation of the α-TAc at CPE/SDS by square wave voltammetry (SWV) performed in pure organic electrolyte (99.8% acetonitrile containing 0.1 mol·L^{-1} $LiClO_4$) was developed. Moreover, simple dissolution of sample in supporting electrolyte using ultrasonic bath and subsequent filtering through a stacked filter included all the necessary procedures for sample preparation. The linear range from 0.1 to 1.2 mmol·L^{-1}and limit of detection 37 μmol·L^{-1} were found at pulse amplitude 10 mV and frequency 10 Hz as optimum. In analysis of selected cosmetics, the developed electroanalytical method was not validated using comparison with standard HPLC. At least, the recovery was verified by analysis of model sample and value 95.8% was calculated.

Keywords: carbon paste electrode; surfactant; tocopheryl acetate; square wave voltammetry; cosmetics

INTRODUCTION

The first description of the carbon paste electrode (CPE) was recorded in short communicaton **(Adams, 1958)**. At first, it was thought as a voltammetric alternative to, in those times a very popular, dropping mercury electrode (DME) used in polarography. The attempt to construct a dropping electrode which can be polarized in an area of positive potentials has not been successful. However, the CPE has found a wider application in electroanalysis together with the progression of modern voltammetric techniques, especially with the development of stripping analysis **(Švancara et al., 2012)**. From a physical point of view, CPE is a dispersion of carbon particles (spectrographic carbon, glassy carbon, pyrolytic carbon, etc.) in a viscous lipophilic binder (paraffin or silicone oil, Vaseline, paraffin wax, etc.). After the precise homogenization, the resulting dispersion is pressed into Teflon cavity of electrode holder. A simple modification of the carbon paste (by mixing another components, e.g. mediators in the form of platinum and metal oxide powders **(Labuda et al., 1998)**, enzymes **(Gorton, 1995)**, carbon nanoparticles **(Punbusayakul, 2012)**, surfactants **(Digua et al., 1994)**, etc., can achieve different desired properties. Carbon paste electrodes modified with the anionic surfactant sodium dodecyl sulphate (CPE/SDS) or the cationic surfactant hexadecylpyridinium chloride monohydrate (CPE/CPC) represent specific sensors which can be used for voltammetric measurements in pure organic solvents.

These electrodes can be utilized for sensitive determination of lipophilic vitamins with antioxidant

properties and their analogues (most often esters of acetic, propionic or palmitic acid) in many cosmetic products **(Thiele and Hsieh, 2005; Ramos-e-Silva et al., 2001)**. These substances are able to prevent the rancidity. Thus they function as preservatives and prolong the expiration date **(Sýs et al., 2017)**.

Retinol acetate (vitamin A) and α-tocopherol (vitamin E) have been already electrochemically studied and several voltammetric methods focusing on their voltammetric determination were described **(Atuma et al., 1974; Wilson et al., 2006)**. Generally, both of these substances have strong antioxidant properties. In previous studies, it was found that α-tocopherol provides only one oxidation peak in a non-aqueous solution **(Sýs et al., 2016)**. On the other hand, retinol acetate provides three oxidation steps under the same conditions **(da Silva et al., 2015)**. Fortunately, potential values of these three oxidation peaks of the retinol acetate are sufficiently distant from the potential value of α-tocopherol peak. For that reason, the peaks of retinol acetate and α-tocopherol can be distinguished well. Nevertheless, it is necessary to mention that synthetic α-tocopheryl acetate (α-TAc) is more common additive in the cosmetic products than pure α-tocopherol due to its higher chemical stability.

This substance is usually determined by standard high-performance liquid chromatography (HPLC) with UV or electrochemical detection **(Wang and Wang, 2001; Almeida et al., 2009; Nada et al., 2010)**. The HPLC with fluorescence detection has been used for determination of tocopherol acetate in nutritional supplements **(Iwase, 2000)**, vegitable oils **(Yang et al., 2018)**, dairy products **(Sunarič et al, 2017)** infant formulas, breakfast cereals, multivitamin juices, and isotonic beverages **(Balz et al., 1993)**. The α-TAc is also used as an internal standard in liquid chromatography for the determination of its analogues **(Hewavitharana et al., 2004)**. **Rodas Mendoza et al. (2003)** applied HPLC on reverse phases to determinate of retinyl acetate and tocoferyl acetate in infant formulas.

The aim of this work is presented simple electrochemical method based on anodic oxidation of α-TAc utilizing the square wave voltammetry at CPE modified by ionic surfactant sodium dodecyl sulfate (SDS) performed in a non-aqueous medium. Except for individual optimization steps, an electrochemical behaviour of the α-TAc is also included.

Scientific hypothesis

Sodium dodecyl sulphate represents common surfactant which is widely used as CPE modifier. Whithin sciethific hypothesis, it was necessary to verify the fact that anionic surfactants are better choice than cationic surfactants. In this case, CPC was used as suitable example. Generally, organic compounds carry a certain charge depending on the pH of the medium used. It is known that value of acetonitrile (ACN) pH decreases with higher content of water **(Barbosa and Sanz-Nebot, 1992)**. Therefore, it can be assumed that 99.8% ACN represents weak acidic medium.

A determination of the electrical charge of α-TAc (ester) in such a medium can be very difficult because it does not contain any well dissociating functional groups in its molecular structure. It should be evident that only one of this surfactant can be usefull due to electrostatic interactions.

MATERIAL AND METHODOLOGY

Chemicals and reagents

All the reagents such as α-tocopheryl acetate, acetonitrile (ACN) suitable for HPLC of purity 99.8 %, and anhydrous $LiClO_4$ were purchased from Sigma-Aldrich, spol.sr.o. (Prague, Czech Republic). Spectrographic carbon powder with a particle size >5 µm from Graphite Týn, s. r. o. (Týnec nad Vltavou, Czech Republic), paraffin oil (PO) from Merck (Darmstadt, Germany), and surfactants sodium dodecyl sulphate (SDS) with cetylpyridinium chloride (CPC) from mentioned Sigma-Aldrich were used for preparation appropriate modified CPEs. Other chemicals were of the required analytical purity.

Electrochemical setup

A three electrode system consisting of modified CPE (working), Ag/AgCl/3.0 mol·L^{-1} KCl (reference) from Methrom, Prague, Czech Republic, and platinum wire (counter electrode) connected to Autolab PGSTAT 101 compatible with software Nova 1.11 from the above company was used for all electrochemical measurements.

Preparation of modified carbon paste electrode

To prevent a demage of the electrode material by presence of an organic solvent, the CPE has to contain a sufficient quantity of surfactant **(Digua et al., 1994)**. In our case, 0.5 g of the carbon powder, 0.2 g of the paraffin oil and 0.3 g of the SDS were used for the preparation of working electrode (CPE/SDS). All the components were homogenized in a ceramic mortar for 20 min. The resulting homogeneous paste was packed into the Teflon piston holder (2 mm inner diameter). Generally, fresh electrodes, especially modified ones, should not be used for any measurement due to their unstable characteristics. This negative phenomenon is attributed to the incomplete homogenization of all components. Therefore, it is recommended to allow the CPE to rest at laboratory conditions for one day. After this time, the auto-homogenization process is completed and the electrode can be used for analysis. It is known that surface of CPE can be regenerated by renewing and polishing using wet filter paper before each measurement. Surprisingly, it was found that this procedure is not necessary in our case.

Procedure

All experiments were carried out in 0.1 mol·L^{-1} $LiClO_4$ in 99.8% ACN as supporting electrolyte. The cyclic voltammetry (CV) was used for study of α-TAc electrochemical behaviour at CPE/SDS. Conditions of CV were as follows: potential range from -0.4 to +1.6 V, scan rate 50 mV·s^{-1}, minimaly 5 repetative cycles. Analysis of cosmetic products was done by square wave voltammetry (SWV) with potential window from 0 to +1.6 V, at potential step 5 mV, potential amplitude 10 mV and frequency 10 Hz. A volume of 10 mL 0.01 mol·L^{-1} α-TAc in pure ACN without content of salt was prepared as stock solution. Measurement of calibration curve was done by addition of appropriate volume of this stock solution into 10 mL of supporting electrolyte. All changes in parameters

of CV or SWV are mentioned in legends under corresponding figures.

Analysis of cosmetics

Severeal cosmetic products, especially body creams (hand cream Diamonds and Pearls from Oriflame; Sweden, refreshing cleansing milk from Nivea; Germany, and suntan cream with protective factor UVA + UVB 30 from Astrid Cosmetics; Czech Republic), commonly available in the Czech stores were selected for analysis. Usually, 5 g of sample were dissolved in 50 mL volumetric flask using supporting electrolyte and ultrasonicated for 30 min at 25°C. The resulting sample solution had to be filtered through a stacked filter. After that 1 mL of resulting filtrate was added into 9 mL of supporting electrolyte and analysed by standard addition method (at least three additions of 200 µL α-TAc stock solution in pure ACN only). Each sample analysis was minimally three times repeated ($n = 3$). To verify the accuracy of the results obtained, the RSD values were compared with calculated value of significance level $\alpha = 0.05$.

Generally, body/hand creams can be classified as complex mixture developed by producers. In a narrower sense, they represent emulsions of water in several oils which are completely soluble in used electrolyte. For that reason, all complicated steps needed in the HPLC as standard reference method are not necessary.

For the desired rheological properties, they usually contain many accompanying substances such as glycerin, caprylic/capric triglyceride, cetyl alcohol, dimethicone, stearyl alcohol, glyceryl stearate, several emulsifiers etc. Most of these substances are not electroactive so they cannot interfere the determination.

Statistic analysis

Aritmetic mean ($\bar{x}$) and standard deviation (σ) of minimally five repetitives ($N = 5$), and slop of linear calibration curve (k) represent data which are necessary for the calculation of important parameters (limit of detection; LOD, limit of quantification; LOQ, accuracy, and correctness) of each developed analytical method. Values of LOQ and LOD were calculated according to follow equations $LOQ = 10\sigma/k$ and $LOD = 3\sigma/k$, respectively, where σ is the standard deviation of five repetitive measurements of the concentration for lowest concetration of calibration curve **(MacDougall and Crummett, 1980)**.

There is necessary to mentioned that only three examples of body/hand cream were analysed. Due to the low number of samples (N), standard reference method was not applied. For that reason, any statistical methods for comparison of obtained results such as ANOVA or t-test were not needed. A precision of developed voltammetric method was validated using recovery (%) of model sample.

RESULTS AND DISCUSSION

Characterisation of surfactant modified electrodes

Surfactants as SDS and CPC contain a long non-branched aliphatic chains with 12 or 16 carbons in their structure, respectively. Therefore, they are well soluble in lipophilic pasting liquid like PO which behaves like electric insulators. It was experimentally confirmed that an increasing content of surfactant caused significant reduction of carbon paste viscosity (η). Evidently, distance between each carbon particle increased due to increasing of pasting liquid volume. The dependency of ohmic resistance (R) on the content of surfactant in CPE is very similar to curves contributed to R on the volume of pasting liquid **(Mikysek et al., 2009)**. Generally, increasing content of any electric isolant in each electrode material usually worse electrochemical properties, especially capacitance current current which is needed to charging of working electrode.

However, it was found that presence of organic solvents has much more influence because carbon particles are more exposed to ACN with decrasing content of surfactant. It can be stated that modified CPEs with content of surfactant low than 30 % provided satisfactory electrochemical properties.

Under repetitive CV of pure 0.1 $mol \cdot L^{-1}$ $LiClO_4$ in 99.8% ACN, it was observed that an increasing content of SDS significantly decreases the current response of base line. Peak current (I_p) of clearly visible reduction peak at voltage (E_p) +1.1 V obtained at CPE with 20% SDS (w/w) increased with each repetition (not shown). This parasitic peak is probably attributed to the presence of water (2%; v/v) in the basic electrolyte. Lower contents of water did not have any significant effect on shapes of voltammograms.

Selection of a suitable surfactant

Two different kinds of surfactants were tested, namely anionic SDS and cationic CPC. Although CPEs modified by monohydrate of CPC provided better electrical conductivity (G), extremely high background current response has been observed at SWV (not shown). An explanation can be the isolation of the electrode surface that is essentially represents a capacitor. For that reason, the CPC cannot be used as suitable modifier of CPE for the determination of lipophilic compounds in cosmetic products. Unlike this, satisfactory base line current responses (>0.5 µA) at CPEs with 30 and 40% content of SDS (R from 90 to 100 Ω) were observed.

In addition, evidently better plotting of peak shape (more narrow) was obtained at CPE with 40% SDS than those with a lower content. Nevertheless, higher content than 30% SDS caused dramatically decreasing of peak current (sensitivity). For that reason value 30% SDS was chosen as optimum.

The scientific hypothesis in the previously mentioned paragraph was confirmed. A surface of CPE/SDS is evidently negatively charged due to presence of HSO_3 functional group in the chosen weak acidic medium (99.8% ACN). Unlike this, the α-TAc is a neutral form of vitamin E (pH 6-8) which has the role of base in the acidic media and its repulsion from the electrode surface can not become like in the case of CPE/CPC.

Electrochemical behaviour of α-tocopheryl acetate

Electrochemical behaviour of α-TAc in an organic medium at CPE/SDS was studied by cyclic voltametry with potential window from -0.4 to +1.6 V. Only one oxidation peak at +1.35 V (none in reverse scan) was observed (see Figure 1). Herein, it is necessary to state that this voltage is only approximate value because exact determination is quite difficult in the base line escape.

From chemical point of view, the α-TAc is a synthetic analogue of α-tocopherol (α-TOH) which is known as the most biologically active form vitamin E (**Sýs et al., 2017**). As ester of acetic acid and α-TOH, it lacks a free hydroxy group in its structure which can be involved in an electrochemical reaction **(Sýs et al., 2016)**. Moreover, the ester functional group is not electron-rich for anodic oxidation as the delocalized electron system of benzene ring. It should be remembered that all tocopherols are oxidized by radical mechanisms in non-aqueous media **(Wilson et al., 2006)**. The oxidation of the α-TAc at CPE/SDS occurs in one electron step and is probably irreversible. **Mikheeva and Anisimova (2007)** supposed that possible formation of the intermediate and its subsequent chemical conversion to the final products is proceeded.

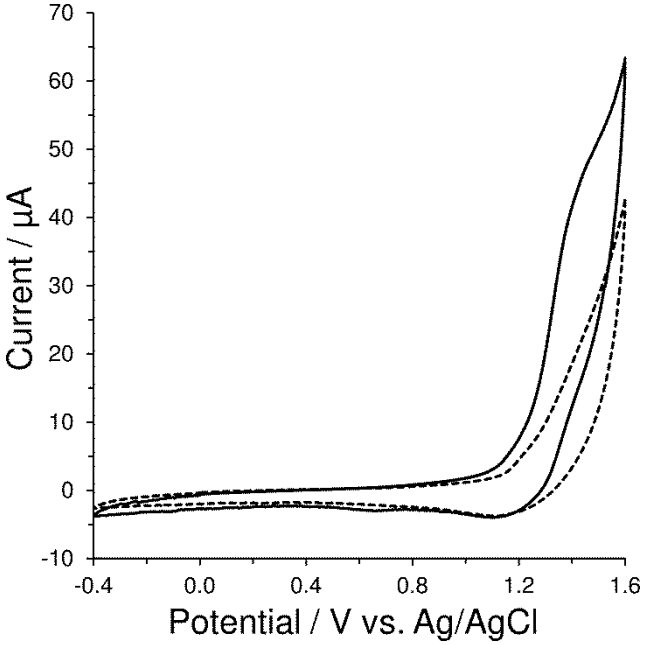

Figure 1 CV of 0 (dashed line) and 0.1 mmol·L^{-1} α-tocopheryl acetate at CPE/SDS (solid line) performed in 99.8% ACN containing 0.1 mol·L^{-1} $LiClO_4$ at 50 mV·s^{-1}.

Optimization of square wave voltammetry

At the beginning, it was necessary to find optimum working conditions of used pulse voltammetric technique. In the case of the SWV, the sensitivity is affected by two main parameters, namely potential amplitude (E_{ampl}) and frequency (f). It is generally known that height of peak current usually increases with higher values of these parameters (see Figures 2 and 3). Setting of amplitude higher than 10 mV and frequency 10 Hz significantly worsened the shape of corresponding voltammograms and thus determination their peak heights. For that reason, these values were chosen as optimum. For demonstration, typical voltammetric record with corresponding calibration curve is shown in Figure 4. Thanks to the extremely high base line current from +1.15 V, it was not possible to achieve lower value of detection limit than 17 mg·L^{-1}. All other important analytical parameters together with comparison with already described electroanalytical methods are included in the Table 1. It appears that the developed voltammetric method does not provide such sensitivity as those based on utilization of glassy carbon electrode (GCE). From scientific point of view, this paper represents the first more detailed work where particular type of CPE was used in the monitoring of α-TAc.

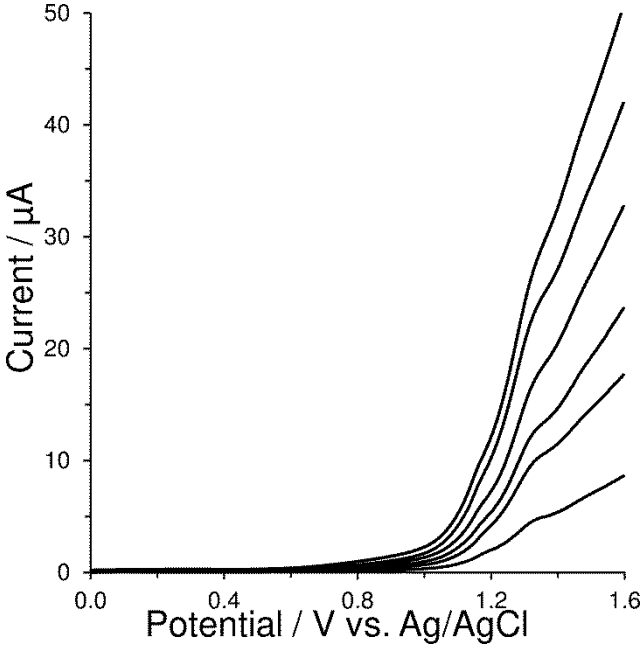

Figure 2 SWV of 0.2 mmol·L^{-1} α-tocopheryl acetate at CPE/SDS (solid line) performed in 99.8% ACN containing 0.1 mol·L^{-1} $LiClO_4$ at potential amplitudes 5, 10, 15, 20, 25, 30 mV, and frequency 10 Hz.

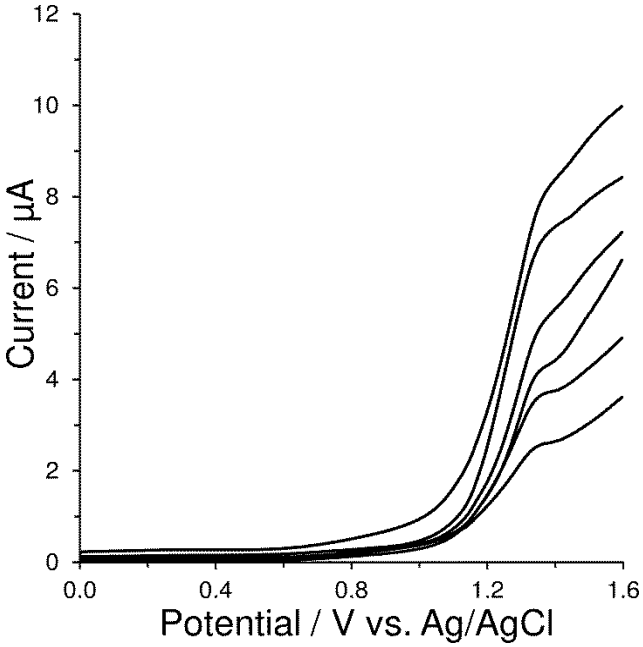

Figure 3 SWV of 0.2 mmol·L^{-1} α-tocopheryl acetate at CPE/SDS (solid line) performed in 99.8% ACN containing 0.1 mol·L^{-1} $LiClO_4$ at potential amplitude 25 mV, frequencies 5, 10, 20, 30, 40, and 50 Hz.

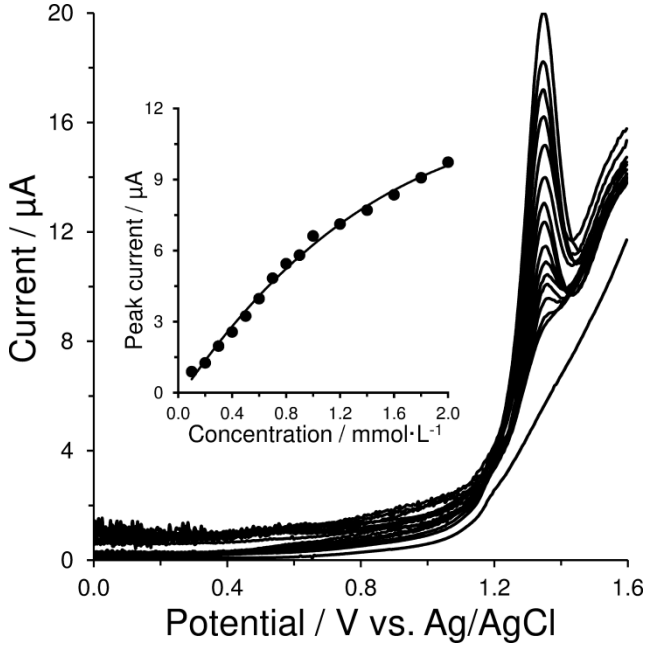

Figure 4 Voltammograms for 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 mmol·L^{-1} α-tocopheryl acetate with corresponding calibration curve (inserted one) obtained at CPE/SDS. Measured by SWV at E_{step} = 5 mV, E_{ampl} = 10 mV, and f = 10 Hz.

Analysis of cosmetics products

From chemical point of view, all body/hand creams are always claasified as complex emulsion which can be defined as a mixture of oily and watery liquids (the main componets). Emulsifiers, fragrances, preservatives can be considered as secondary components **(Moravkova and Filip, 2013)**. The preparation of samples for electroanalysis depends on their solubility in organic solvents.

Within this work, three typical examples of cosmetic products were selected for analysis by developed voltammetric method. Herein, it is necessary to mentioned that presence of the α-TAc in all selected samples is declared by producers. Unfortunately, its content is not listed on the products label. It is no wonder that this information is not known because the exact composition of body/hand creams is often subject to the production secret.

Generally, content of α-tocopheryl acetate in cosmetics products usually occurs up to four concentration ranges. The lowest amount from 0.0001 to 25% (w/w) can be found in bath products and bath products like shampoos. Values lower than 0.3% are typical for deodorats, hair products, and after-shave lotion. In the body/hand creams from 0.001 to 25%, suntan gels and creams from 0.05 to 1%, makeup liquids, eye shadows, lipticks, and face powders from 0.02 to 0.8% reperesent common values **(Thiele and Hsieh, 2005)**.

Common body/hand creams were well soluble in used supporting electrolyte except of suntan cream, probably due to high content of nonpolar components. Therefore, it has to be counted with a certain error of determination.

With respect to analytical method selectivity, it should be stated that other accompanying substances, especially esters of all-*trans* retinol **(Tan et al., 2014)** may not be oxidized together with the α-Tac at 1.32 V because oxidation of retinyl acetate and retinyl palmitate occur at voltages 0.85 and 1.05 V, respectively. Moreover, their presence was not declared by the manufacturers.

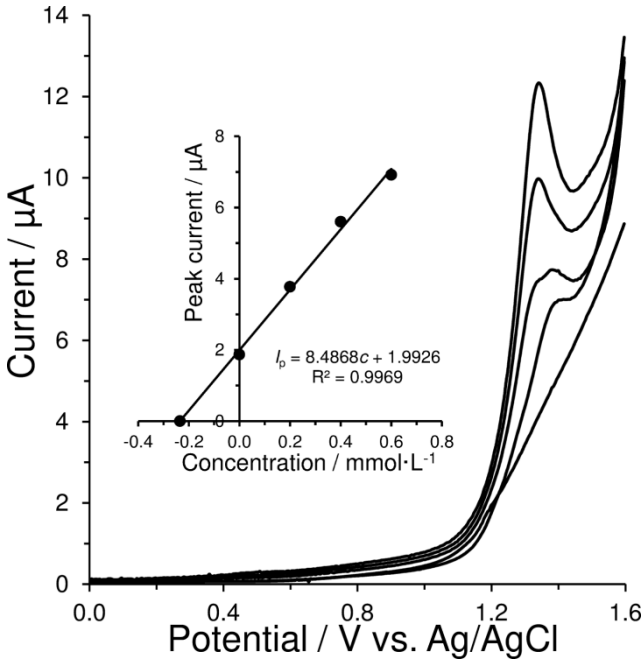

Figure 5 Analysis of refreshing cleansing milk from company Nivea (Germany) by standard addition method.

Figure 5 and Figure 6 show typical voltammograms obtained during analysis of refreshing cleansing milk using standard addition method. Three additions 200 µL of 0.01 mol·L^{-1} α-TAc into 10 mL of usually ten times diluted sample solution were performed. Calculated contents of α-TAc in creams are shown in Table 2. The content ranged from 0.5 to 2% for these samples which is in line with commonly found quantities. Nevertheless, it is necessary to mention that verification of developed method was done only by calculation of the recovery 95.8% using analysis of model sample.

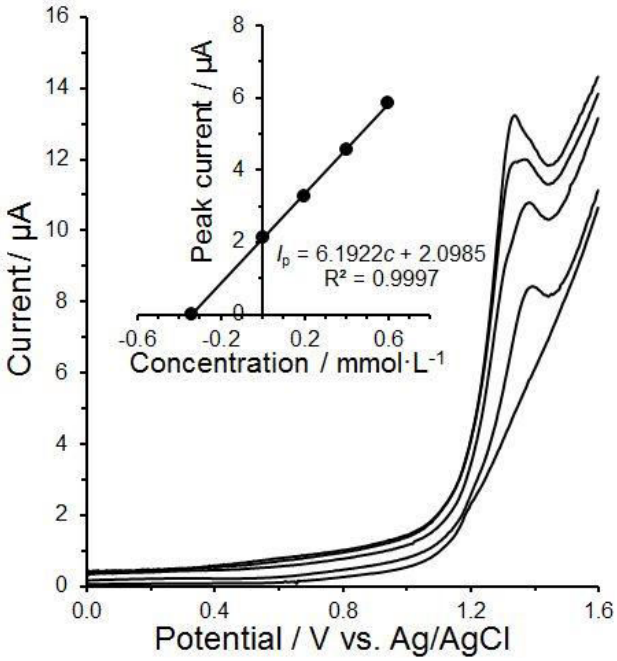

Figure 6 Analysis of hand cream company Oriflame (Sweden) by standard addition method.

Table 1 Comparison of conventional voltammetric methods developed for determination of α-tocopheryl acetate.

Sensor	Technique	Supporting electrolyte	Linear range ($\mu mol \cdot L^{-1}$)	LOD ($\mu mol \cdot L^{-1}$)	References
mPtE	LSV	0.1 $mol \cdot L^{-1}$ $NaClO_4$	90 to 2300	27	**(Michalkiewicz et al., 2004)**
mPtE	DPV	0.1 $mol \cdot L^{-1}$ $NaClO_4$	70 to 2200	21	**(Michalkiewicz et al., 2004)**
mPtE	SWV	0.1 $mol \cdot L^{-1}$ $NaClO_4$	60 to 2100	18	**(Michalkiewicz et al., 2004)**
GCE	DPV	0.1 $mol \cdot L^{-1}$ $NaClO_4$ in ACN	—	0.74	**(Mikheeva and Anisimova, 2007)**
GCE	DPV	0.1 $mol \cdot L^{-1}$ BRB (pH 2.8)	0.11 to 8.46	0.03	**(Hassan et al., 2008)**
CPE/SDS	SWV	0.1 $mol \cdot L^{-1}$ $LiClO_4$ in ACN	100 to 1200	37	**(Present work)**

ACN; acetonitrile; BRB; Britton-Robinson buffer, CPE/SDS; carbon paste electrode modified by sodium dodecyl sulfate, DPV; differential pulse voltammetry, GCE; glassy carbon electrode, LSV; linear sweep voltammetry, LOD; limit of detection, mPtE; microdisc platinum electrode, SWV; square wave voltammetry.

Table 2 Analysis of selected cosmetic products.

Sample	Distributor	SWV (g per 100 g)	Declared amount (g per 100 g)	Recovery (%)
Model	—	0.23 ±0.02	0.24	95.8
Refreshing cleansing milk	Nivea, Germany	0.56 ±0.04	—	—
Hand cream	Oriflame, Sweden	1.60 ±0.08	—	—
Suntan cream	Astrid, Czech Republic	2.22 ±0.14	—	—

Note: Values given as arithmetic means with corresponding standard deviations for three analyses. SWV; square wave voltammetry.

CONCLUSION

Herein, it can be concluded that CPE modified by 30% (w/w) ionic surfactant SDS represents a sophisticated voltammetric sensor suitable for simple and rapid determination of vitamin E acetate in cosmetic products. Unlike very complicated sample preparation in the HPLC as standard reference method usually consisting many consecutive steps (saponification, extraction into organic solvent, and itself separation), only dissolution in the supporting electrolyte and subsequent filtration represented the all steps necessary in the electroanalysis. The developed method provides sufficient sensitivity (LOD = 17 $mg \cdot L^{-1}$) for routine analysis of cosmetics because lipophilic vitamins are present in quantity of units to tens of milligrams per 100 g of sample.

REFERENCES

Adams, R. N. 1958. Carbon paste electrodes. *Analytical Chemistry*, vol. 30, no. 9, p. 1576-1576. https://doi.org/10.1021/ac60141a600

Almeida, M. M., Alves, J. M, Patto, D. C. S., Lima, C. R. R. C., Quenca-Guillen, J. S., Santoro, M. I. R. M., Kedor.Hackmann, E. R. M. 2009. Determination of tocopheryl acetate and ascorbyl tetraisopalmitate in cosmetic formulations by HPLC. *Journal of Cosmetic Science*, vol. 31, no. 6, p. 445-450. https://doi.org/10.1111/j.1468-2494.2009.00514.x

Atuma, S. S., Lindquist, J., Lundström, K. 1974. The electrochemical determination of vitamin. A part I. Voltammetric determination of vitamin A in pharmaceutical preparations. *Analyst,* vol. 99, no. 1183, p. 683-689. https://doi.org/10.1039/an9749900683

Balz, M. K., Schulte, E., Their, H. P. 1993. Simultaneous determination of α-tocopheryl acetate, tocopherols and tocotrienols by HPLC with fluorescence detection in foods. *European Journal of Lipid Science and Technology*, vol. 95, no. 6, p. 215-220. https://doi.org/10.1002/lipi.19930950603

Barbosa, J., Sanz-Nebot, V. 1992. pH measurements in acetonitrile-water mixtures by use of a glass electrode. *Journal of Pharmaceutical & Biomedical Analysis*, vol. 10, no. 10-12, p. 1047-1051.https://doi.org/10.1016/0731-7085(91)80117-R

Da Silva, F. L. O., Da Silva Tinoco, L. M., Ferreira, L. A. M., Malagutti, A. R., Carneiro, G. 2015. Determination of all-trans retinoic acid loaded in solid liquid nanoparticles by differential pulse voltammetry at glassy carbon electrode. *Electrochimica Acta*, vol. 182, p. 929-934. https://doi.org/10.1016/j.electacta.2015.10.018

Digua, K., Kauffmann, J. M., Delplancke, J. L. 1994. Surfactant modified carbon paste electrode: Part 1: Electrochemical and microscopic characterization. *Electroanalysis*, vol. 6, no. 5-6, p. 451-458. https://doi.org/10.1002/elan.1140060515

Digua, K., Kauffmann, J. M., Khodari, M. 1994. Surfactant modified carbon paste electrode: Part 2: Analytical performances. *Electroanalysis*, vol. 6, no. 5-6, p. 459-462. https://doi.org/10.1002/elan.1140060516

Gorton, L. 1995. Carbon paste electrodes modified with enzymes, tissues, and cells. *Electroanalysis*, vol. 7, no. 1, p. 23-45. https://doi.org/10.1002/elan.1140070104

Hassan, E. M., Khamis, E. F., El-Kimary, E. I.,Barary, M. A. 2008. Development of a differential pulse voltammetric method for the determinativ of Silymarin/Vitamin E, p. acetate mixture in pharmaceuticals. *Talanta*, vol. 74, p. 773-778. https://doi.org/10.1016/j.talanta.2007.07.006

Hewavitharana, A. K., Lanari, M. C., Becu, C. 2004. Simultaneous determination of vitamin E homologs in chicken meat by liquid chromatography with fluorescence detection. *Journal of Chromatography A*, vol. 1025, no. 2, p. 313-317. https://doi.org/10.1016/j.chroma.2003.10.052

Iwase, H. 2000. Determination of tocopherol acetate in emulsified nutritional supplements by solid-phase extraction and high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography A*, vol. 881, no. 1-2, p. 243-249. https://doi.org/10.1016/S0021-9673(00)00058-3

Labuda, J., Meister, A., Gläser, P., Werner, G. 1998. Metal oxide-modified carbon paste electrodes and microelectrodes for the detection of amino acids and their application to capillary electrophoresis. *Fresenius' Journal of Analytical Chemistry,* vol. 360, no. 6, p. 654–658. https://doi.org/10.1007/s002160050775

MacDougall, D., Crummett, W. B. 1980. Guidelines for data acquisition and data quality evaluation in environmental chemistry. *Analytical Chemistry*, vol. 52, no. 14, p. 2242-2249. https://doi.org/10.1021/ac50064a004

Michalkiewicz, S., Pryciak, M., Malyszko, J., Oszczudlowski, J. 2004. Voltammetric determination of α-tocopheryl acetate in pharmaceutical dosage form. *Electroanalysis*, vol. 16, no. 11, p. 961-965. https://doi.org/10.1002/elan.200302893

Mikheeva, E. V., Anisimova, L. S. 2007. Voltammetric determination of vitamin E (α-tocopherol acetate) in multicomponent vitaminized mixtures. *Journal of Analytical Chemistry,* vol. 62, no. 4, p. 373-376. https://doi.org/10.1134/S1061934807040144

Mikysek, T., Švancara, I., Kalcher, K., Bartoš, M., Vytřas, K., Ludvík, J. 2009. New approaches to the characterization of carbon paste electrodes using the ohmic resistance effect and qualitative carbon paste indexes. *Analytical Chemistry*, vol. 81, no. 15, p. 6327-6333. https://doi.org/10.1021/ac9004937

Moravkova, T., Filip, P. 2013. The influence of emulsifier on rheological and sensory properties of cosmetic lotions. *Advances in Materials Science and Engineering*, vol. 2013, no. 1, p. 1-7. https://doi.org/10.1155/2013/168503

Nada, A., Krishnaiah, Y. S., Zaghloul, A. A., Khattab, I. 2010. Analysis of vitamin E in commercial cosmetic preparations by HPLC. *Journal of Cosmetic Science*, vol. 61, no. 5, p. 353-365. PMid:20939975

Punbusayakul, N. 2012. Carbon nanotubes architectures in electroanalysis. *Procedia Engineering*, vol. 32, no. 1, p. 683-689. https://doi.org/10.1016/j.proeng.2012.01.1327

Ramos- E Silva, M., Hexsel, D. M., Rutowitsch, M. S., Zechmeister, M. 2001. Hydroxy acids and retionids in cosmetics. *Clinics in Dermatology*, vol. 19, no. 4, p. 460-466. https://doi.org/10.1016/S0738-081X(01)00189-4

Rodas Mendoza, B., Morera Pons, S., Castellote Bargalló, A. I., López-Sabater, M. C. 2003. Rapid determination by reverse-phase high-performance liquid chromatography of vitamins A and E in infant formulas. *Journal of Chromatography A*, vol. 1018, no. 2, p. 197-202. https://doi.org/10.1016/j.chroma.2003.08.018,

Sunarič, S., Lalič, J., Spasič, A. 2017. Simultaneous determination of alpha-tocopherol and alpha-tocopheryl acetate in dairy pdoructs, plant milks and health supplements by using SPE and HPLC method. *Food Analytical Methods*, vol. 10, no. 12, p. 3880-3901. https://doi.org/10.1007/s12161-017-0943-x

Sýs, M., Metelka, R., Stočes, M., Vytřas, K. 2016. Electrochemical properties of α-tocopherol in aqueous electrolytes after its previous extraction into the glassy carbon paste from aqueous-acetonic mixture. *Monatschefte für Chemie*, vol. 147, no. 1, p. 31-38. https://doi.org/10.1007/s00706-015-1620-7

Sýs, M., Švecová, B., Švancara, I., Metelka, R. 2017. Determination of vitamin E in margarines and edible oils using square wave anodic stripping voltammetry with glassy carbon paste electrode. *Food Chemistry*, vol. 229, p. 621-627. https://doi.org/10.1016/j.foodchem.2017.02.068

Sýs, M., Žabčíková, S., Červenka, L., Vytřas, K. 2016. Adsorptive stripping voltammetry in lipophilic vitamins determination. *Potravinarstvo*, vol. 10, no. 1, p. 260-264. https://doi.org/10.5219/587

Tan, Y. S., Urbančok, D., Webster, R. D. 2014. Contrasting voltammetric behaviour of different forms of vitamin A in aprotic organic solvents. *The Journal of Physical Chemistry B*, vol. 118, no. 29, p. 8591-8600. https://doi.org/10.1021/jp505456q

Thiele J. J., Hsieh, S. N., Ekanayake-Mudiyanselage, S. 2005. Vitamin E: critical review of its current use in cosmetic and clinical dermatology. *Dermatologic Surgery*, vol 31, no. 1, p. 805-813. https://doi.org/10.1111/j.1524-4725.2005.31724

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. https://doi.org/10.1016/S0731-7085(01)00381-8

Wilson, G. J., Lin, Ch. Y., Webster, R. D. 2006. Significant differences in the lectrochemical behavior of the α-, β-, γ-, and δ-tocopherols (vitamin E). *Journal of Physical Chemistry B*, vol. 110, no. 23, p. 11540-11548. https://doi.org/10.1021/jp0604802

Yang, Y., Lu, D., Yin, S., Yang, D., Chen, Y., Li, Y., Sun, Ch. 2018. A convenient ultrasound-assisted saponification for the simultaneous determination of vitamin E isomers in vegetable oil by HPLC with fluorescence detection. *Journal of Separation Science*, (in editing) http://doi.org/10.1002/jssc.201701393

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GENETIC DIVERSITY AND POPULATION STRUCTURE IN TUNISIAN CASTOR GENOTYPES (*RICINUS COMMUNIS* L.) DETECTED USING SCOT MARKERS

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ABSTRACT

Due to the chemical and physical properties of castor oil (*Ricinus communis* L.) that make it a valuable raw material for numerous industrial applications, including the production of biofuel, interest to develop more and better varieties has been increased. In the present study, the representatives of the genus castor collected from 12 different parts of Tunisia were differentiated by the DNA fingerprinting patterns using 37 SCoT primers. PCR amplification of DNA using 37 primers for SCoT analysis produced 268 DNA fragments that could be scored in all 56 genotypes of Tunisian castor. The number of amplified fragments varied from 4 (SCoT 45, SCoT 31 and ScoT 17) to 10 (SCoT 3, SCoT 11, SCoT 14, SCoT 18 and SCoT 12). Of the 268 amplified bands 230 were polymorphic, with an average of 6.22 polymorphic bands per primer. To determine the level of polymorphism in the analysed group of Tunisian castor genotypes polymorphic information content (PIC) was calculated. The lowest values of polymorphic information content were recorded for SCoT 17 (0.411) and the the highest PIC values were detected for SCoT 14 (0.868) with an average of 0.751. A dendrogram was constructed from a genetic distance matrix based on profiles of the 37 SCoT primers using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 56 Tunisian castor genotypes were clustered into two main clusters (1 and 2). Of the 56 genotypes of Tunisian castor, 2 unique genotypes were separated (BA-5 and K-4). Genetically the closest were two genotypes from Tunisian region Souassi (S-2 and S-5) in subclaster 2bc. Results showed the utility of SCoT markers for estimation of genetic diversity of castor genotypes leading to genotype identification.

Keywords: castor; DNA; PCR; dendrogram; SCoT marker

INTRODUCTION

The castor-oil plant (*Ricinus communis* L.), a member of the spurge family (*Euphorbiaceae*), is a versatile industrial oil crop that is cultivated in many tropical and subtropical regions of the world **(Anjani, 2012)**. Due to the distinctive characteristics of castor oil, such as its high percentage of ricinoleic acid, unusual chemical structure and low freezing point, castor oil is widely utilised in paints, nylon, aviation oil, lubricants, soaps, inks, dyes, cosmetics, adhesives, biodiesel, and other novel castor-bean-derived studied

using molecular techniques, including random amplified polymorphism DNA (RAPD) **(Vivodík et al., 2015)**, amplified fragment length polymorphism (AFLP) **(Allan et al. 2008)**, simple sequence repeat (SSR) **(Gálová et al., 2015)**, inter-simple sequence repeat (ISSR) **(Wang et al., 2013)**, single nucleotide polymorphism (SNP) markers products **(Scholz and Silva, 2008)**. Castor is cultivated on commercial scale in an area of 1,525,000 ha in 30 countries with 1,581,000 MT seed production. India, China, Brazil, USSR, Thailand, Ethiopia and Philippines are the major castor growing countries in the world **(Damodaram and Hegde, 2010)**.

Knowledge of genetic variability is important for breeding programs to provide the basis for developing desirable genotypes. Genetic variability in castor bean has been

(Foster et al. 2010), start codon targeted polymorphism (SCoT) **(Kallamadi et al., 2015),** target region amplification polymorphism (TRAP) **(Simões et al., 2017)** and using protein markers **(Cheema et al., 2010; Malook et al., 2016)**. The polymerase chain reaction (PCR) has been used by many authors, such as **Žiarovská et. al.,**

(2015); **Kanti et. al., (2015)**; **Vyhnánek et. al., (2015)**; **Bošeľová and Žiarovská (2016)**; **Ražná et. al., (2016)**; **Žiarovská et. al., (2017)**; **Simões et. al., (2017)**.

With initiating a trend away from random DNA markers towards gene-targeted markers, a novel marker system called SCoT **(Collard and Mackill, 2009)** was developed based on the short conserved region flanking the ATG start codon in plant genes. SCoT markers are generally reproducible, and it is suggested that primer length and annealing temperature are not the sole factors determining reproducibility. They are dominant markers like RAPDs and could be used for genetic analysis, quantitative trait loci (QTL) mapping and bulk segregation analysis. In principle, SCoT is similar to RAPD and ISSR because the same single primer is used as the forward and reverse primer **(Collard and Mackill, 2009)**. Suitability of SCoT markers system has been successfully employed in genetic diversity analysis and fingerprinting of a number of agricultural and horticultural crop species, such as oat **(Balážová et al., 2017)**, rye **(Petrovičová et al., 2017)**, maize **(Vivodík et al., 2016)**, date palm **(Al-Qurainy et al., 2015)**, orchardgrass **(Jiang et al., 2014)**, pepper **(Tsaballa et al., 2015)**, ramie **(Satya et al., 2015)**, castor **(Kallamadi et al., 2015)**, sugarcane **(Que et al., 2014)** and mango (**Gajera et al., 2014)**.

Scientific hypothesis

The present study is focused on estimation of genetic distance between 56 Tunisian castor genotypes, based on 37 SCoT markers. Although the information gathered here would be helpful in future for genomic mapping studies leading to development of castor cultivars with broader genetic background to obtain improved crop productivity.

MATERIAL AND METHODOLOGY

Fifty-six castor (*Ricinus communis* L.) genotypes were used in the present study. Seeds of castor were obtained from the University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGREF), Regional Station of Gabès, Tunisia. The ricin genotypes were obtained from 12 regions of Tunisia: S- Souassi (5 genotypes), BT- Bouthay (4 genotypes), GH- Ghomrassen (5 genotypes), BA- Sidi bou ali (5 genotypes), MT- Matmata (4 genotypes), AG- Mateur (5 genotypes), N- Nefza (4 genotypes), MD- Mednine (5 genotypes), M- Mornag (5 genotypes), G- Gabes (4 genotypes), K- Kebili (5 genotypes), KJ- Ksar jedid (5 genotypes). Genomic DNA of castor cultivars was extracted from leaves of 14-day old plantlets with GeneJET Plant Genomic DNA Purification Mini Kit according to the manufacturer's instructions. DNA concentrations were estimated by UV-Vis spectrophotometer Q5000, Quawell.

SCoT amplification: A total of 37 SCoT primers developed by **Collard and Mackill (2009)** were selected for the present study (Table 1). 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.

Statisic analysis

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 the present study, the representatives of the genus *Ricinus communis* collected from 12 different parts of Tunisia were differentiated by the DNA fingerprinting patterns using 37 SCoT primers. The efficacy of the SCoT technique in this study is further supported by the obtained PIC values of the primers used in the analysis. The PIC value of the SCoT marker system was found to be 0.751 which are at par with the optimal PIC. PCR amplification of DNA using 37 primers (Table 1) for SCoT analysis produced 268 DNA fragments that could be scored in all 56 genotypes of Tunisian castor (Figure 1). The number of amplified fragments varied from 4 (SCoT 45, SCoT 31 and ScoT 17) to 10 (SCoT 3, SCoT 11, SCoT 14, SCoT 18 and SCoT 12), and the amplicon size ranged from 200 to 2500 bp. Of the 268 amplified bands 230 were polymorphic, with an average of 6.22 polymorphic bands per primer. Results indicated the presence of wide genetic variability among different genotypes of Tunisian castor. From these 37 primers, primers SCoT 3, SCoT 14 and SCoT 15 were the most polymorphic, where 9 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (3) were detected by primers SCoT 2, SCoT 17, SCoT 31 and SCoT 45. The percentage of polymorphism ranged from 50.00% (SCoT 2) to 100% (SCoT 13, SCoT 15, SCoT 20, SCoT 30, SCoT 44, SCoT 65 and SCoT 66). To determine the level of polymorphism in the analysed group of Tunisian castor genotypes polymorphic information content (PIC) was calculated. The lowest values of polymorphic information content were recorded for SCoT 17 (0.411) and the the highest PIC values were detected for SCoT 14 (0.868) with an average of 0.751.

A dendrogram was constructed from a genetic distance matrix based on profiles of the 37 SCoT primers using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 56 Tunisian castor genotypes were clustered into two main clusters (1 and 2) (Figure 2). Of the 56 genotypes of Tunisian castor, 2 unique genotypes were separated: BA-5 – genotypes from Tunisian region Sidi bou ali and K-4 – genotypes from Tunisian region Kebili. Cluster 1 was divided into subclasters 1a and 1b. Subclaster 1a contained 5 genotypes of castor from different regions of Tunisia and subclaster 1b contained 6 genotypes of castor from different regions of Tunisia. Cluster 2 was divided into subclasters 2a and 2b. Subclaster 2a contained 6 genotypes of Tunisian castor, all 5 genotypes from the region Ksar jedid (KJ-1, KJ-2, KJ-3, KJ-4, KJ-5) and one genotype

from Tunisian region Kebili (K-5). Subclaster 2b was divided into 3 subclasters (2ba, 2bb and 2bc). Subclaster 2 ba contained 2 genotypes from Tunisian region Ghomrassen (GH-2 and GH-5). Subclaster 2bb contained 6 genotypes from different regions of Tunisia and subclaster 2 bc contained 29 gentypes of castor from different regions of Tunisia. Genetically the closest were two genotypes from Tunisian region Souassi (S-2 and S-5) in subclaster 2bc (Figure 2).

Lower average polymorphism (21%) obtained by SCoT technique was detected by **Kallamadi et al. (2015)** who analysed molecular diversity of castor (*Ricinus communis* L.). Out of a total of 108 bands, 23 (21%) were polymorphic with an average of 2.1 polymorphic bands per primer. The total number of bands per primer varied from 5 and 20 in the molecular size range of 100 – 3000 bp. The PIC/DI varied from 0.06 for SCoT28 to 0.45 for SCoT12 with an average of 0.24. On the other side, higher polymorphism with SCoT primers has been reported in crops like peanut **(Xiong et al., 2011)**, cicer **(Amirmoradi et al., 2012)**, mango **(Luo et al., 2010)**, ramie **(Satya et al., 2015)**, sugarcane **(Que et al., 2014)**, Chinese bayberry (**Fang-Yong and Ji-Hong, 2014)**, pepper **(Tsaballa et al., 2015)**, castor **(Kallamadi et al., 2015)**, maize **(Vivodík et al., 2016)**, durum wheat **(Etminan et al., 2016)**, oat **(Balážová et al., 2017)**, rye **(Petrovičová et al., 2017)**, vetch **(Chai et al., 2017)**.

Chai et al. (2017) investigate the optimal number of individuals that may represent the genetic diversity of a single population, using Start Codon Targeted (SCoT) markers. Two cultivated varieties and two wild accessions were evaluated using five SCoT primers, also testing different sampling sizes: 1, 2, 3, 5, 8, 10, 20, 30, 40, 50, and 60 individuals. Cluster analysis by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and structure placed the 240 individuals into four distinct clusters. **Balážová et al. (2017)** used eighteen primers to study polymorfism of 8 oat genotypes.

Altogether 153 different fragments were amplified of which 67 were polymorphic with an average number of 3.72 polymorphic fragments per genotype. The number of polymorphic fragments ranged from one (SCoT9, SCoT62) to nine (SCoT40). The polymorphic information content ranged from 0 (SCoT9, SCoT62) to 0.876 (SCoT40) with an average of 0.524. **Petrovičová et al. (2017)** study genetic variability among the set of 45 rye genotypes using 8 SCoT markers. Amplification of genomic DNA of 45 genotypes, using SCoT analysis, yielded 114 fragments, with an average of 14.25 polymorphic fragments per primer. The hierarchical cluster analysis showed that the rye genotypes were divided into 2 main clusters. In the present study, **Etminan et al. (2016)** analyzed genetic variation in a mini-core collection of durum wheat germplasm, including 25 breeding lines and 18 landraces, using six start codon targeted (SCoT) markers. High levels of polymorphism were observed; 98.70% (ISSR) and 100% (SCoT), which indicated that these markers are useful tools for detection of genetic variation in the collection. In the present investigation, **Vivodík et al. (2016)** analyzed 40 genotypes of maize from Czechoslovakia, Hungary, Poland, Union of Soviet Socialist Republics, Slovakia and Yugoslavia 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.

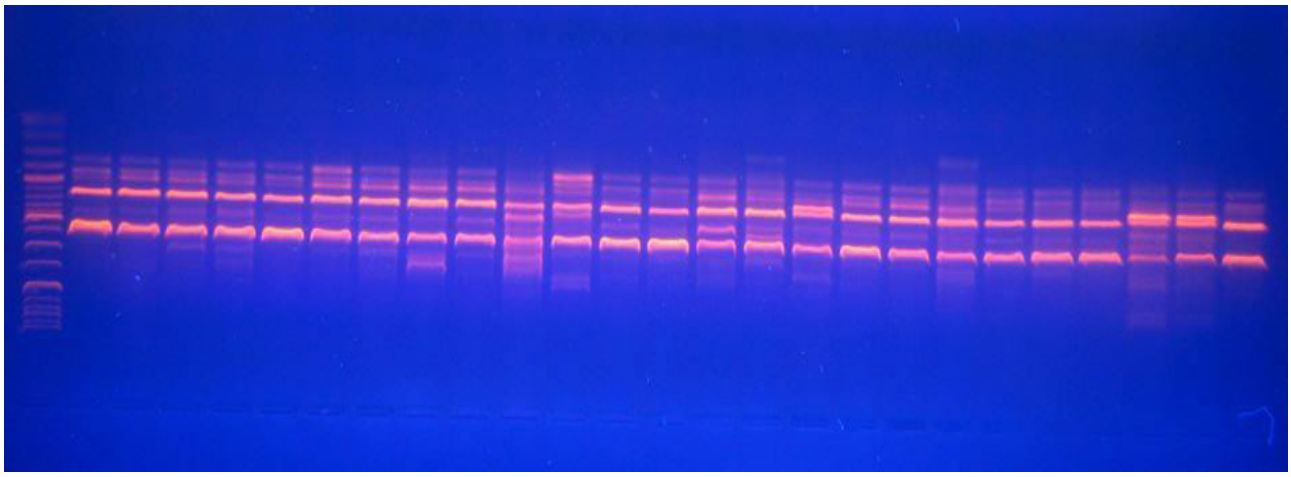

Figure 1 PCR amplification products of 25 genotypes of Tunisian castor produced with primer SCoT-12. Lane M is Quick-Load® 100 bp DNA ladder and lanes 1-25 are Tunisian castor genotypes.

Table 1 Statistical characteristics of the SCoT markers used in Tunisian castor genotypes

SCoT Primers	Primer sequence (5´-3´)	TNoB	NoPB	PoPB	PIC
ScoT 2	CAACAATGGCTACCACCC	6	3	50.00	0.605
ScoT 3	CAACAATGGCTACCACCG	10	9	90.00	0.850
SCoT 6	CAACAATGGCTACCACGC	8	6	75.00	0.758
SCoT 8	CAACAATGGCTACCACGT	8	7	87.50	0.815
SCoT 9	CAACAATGGCTACCAGCA	8	6	75.00	0.772
SCoT 11	AAGCAATGGCTACCACCA	10	8	80.00	0.839
SCoT 13	ACGACATGGCGACCATCG	7	7	100.00	0.793
SCoT 14	ACGACATGGCGACCACGC	10	9	90.00	0.868
SCoT 15	ACGACATGGCGACCGCGA	9	9	100.00	0.843
SCoT 16	ACCATGGCTACCACCGAC	8	7	87.50	0.822
SCoT 17	ACCATGGCTACCACCGAG	4	3	75.00	0.411
SCoT 18	ACCATGGCTACCACCGCC	10	8	80.00	0.852
SCoT 19	ACCATGGCTACCACCGGC	9	8	88.89	0.854
SCoT 20	ACCATGGCTACCACCGCG	8	8	100.00	0.841
SCoT 21	ACGACATGGCGACCCACA	8	7	87.50	0.772
SCoT 22	AACCATGGCTACCACCAC	6	4	66.67	0.717
SCoT 12	ACGACATGGCGACCAACG	10	8	80.00	0.811
SCoT 23	CACCATGGCTACCACCAG	7	6	85.71	0.822
SCoT 26	ACCATGGCTACCACCGTC	7	6	85.71	0.731
SCoT 28	CCATGGCTACCACCGCCA	6	5	83.33	0.731
SCoT 29	CCATGGCTACCACCGGCC	7	6	85.71	0.816
SCoT 30	CCATGGCTACCACCGGCG	8	8	100.00	0.851
SCoT 31	CCATGGCTACCACCGCCT	4	3	75.00	0.438
SCoT 33	CCATGGCTACCACCGCAG	8	7	87.50	0.828
SCoT 34	ACCATGGCTACCACCGCA	6	5	83.33	0.706
SCoT 36	GCAACAATGGCTACCACC	6	5	83.33	0.742
SCoT 40	CAATGGCTACCACTACAG	7	6	85.71	0.726
SCoT 44	CAATGGCTACCATTAGCC	5	5	100.00	0.765
SCoT 45	ACAATGGCTACCACTGAC	4	3	75.00	0.477
SCoT 54	ACAATGGCTACCACCAGC	8	7	87.50	0.830
SCoT 59	ACAATGGCTACCACCATC	6	5	83.33	0.705
SCoT 60	ACAATGGCTACCACCACA	7	6	85.71	0.726
SCoT 61	CAACAATGGCTACCACCG	9	8	88.89	0.815
SCoT 62	ACCATGGCTACCACGGAG	6	5	83.33	0.742
SCoT 63	ACCATGGCTACCACGGGC	5	4	80.00	0.533
SCoT 65	ACCATGGCTACCACGGCA	8	8	100.00	0.834
SCoT 66	ACCATGGCTACCAGCGAG	5	5	100.00	0.739
Average		**7.24**	**6.22**	**85.20**	**0.751**
Total		**268**	**230**	**-**	**-**

Note: TNoB-Total number of bands, NoPB- Number of polymorphic bands, PoPB- Percentage of polymorphic bands (%), PIC- Polymorphic information content.

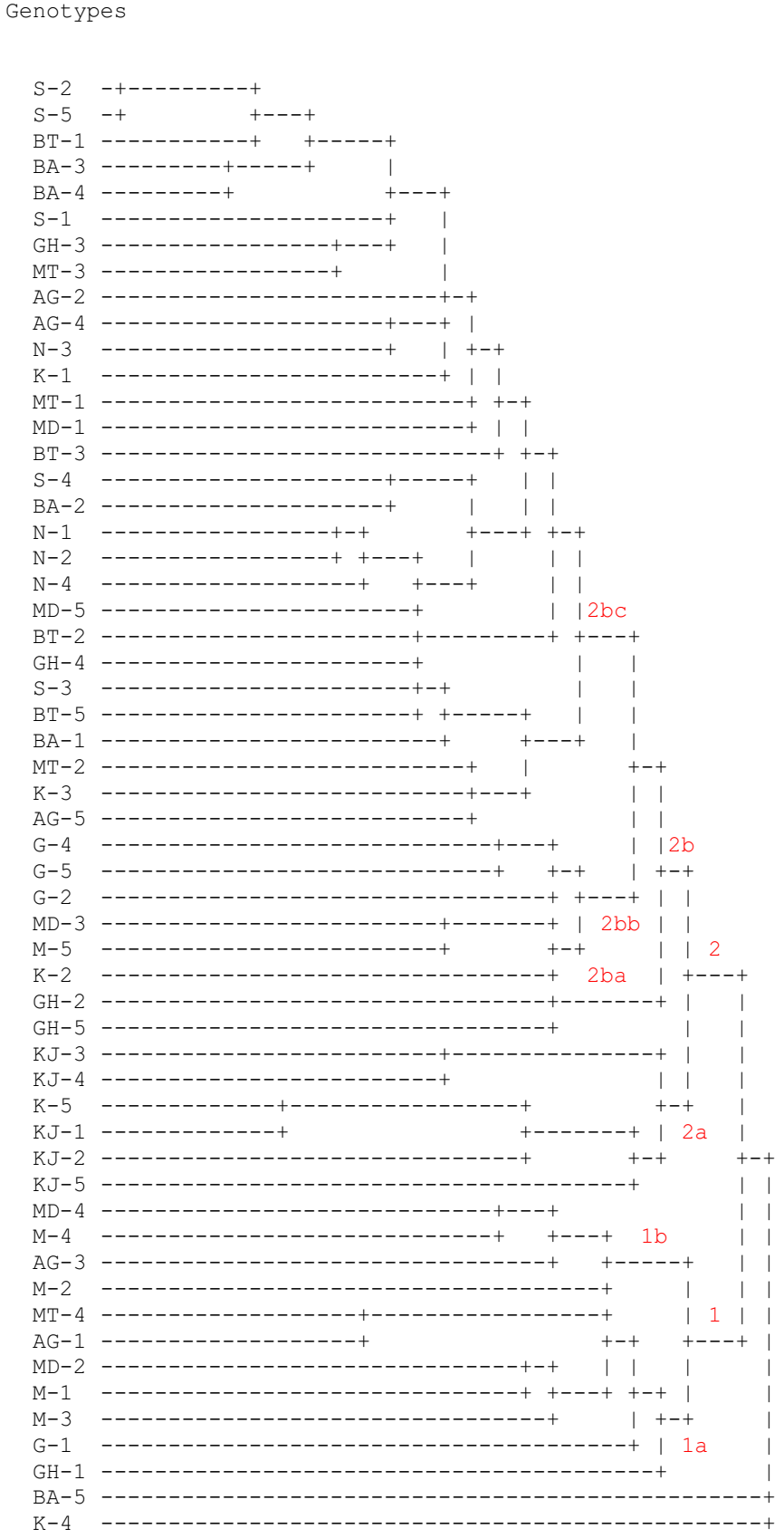

Figure 2 Dendrogram of 56 Tunisian castor genotypes prepared based on 37 SCoT markers. S- Souassi (5 genotypes), BT- Bouthay (4 genotypes), GH- Ghomrassen (5 genotypes), BA- Sidi bou ali (5 genotypes), MT- Matmata (4 genotypes), AG- Mateur (5 genotypes), N- Nefza (4 genotypes), MD- Mednine (5 genotypes), M- Mornag (5 genotypes), G- Gabes (4 genotypes), K- Kebili (5 genotypes), KJ- Ksar jedid (5 genotypes).

CONCLUSION

The present work reported utilization of SCoT markers for the detection of genetic variability of castor genotypes. In summary, SCoT marker analysis was successfully developed to evaluate the genetic relationships among the genus of castor accessions originated from various regions of Tunisia. The hierarchical cluster analysis divided castor genotypes into 2 main clusters. SCoT markers are generated from the functional region of the genome; the genetic analyses using these markers would be more useful for crop improvement programs. Polymorphism revealed by SCoT technique was abundant and could be used for molecular genetics study of the castor accessions, providing high-valued information for the management of germplasm, improvement of the current breeding strategies, construction of linkage maps, conservation of the genetic resources of oat species and QTL mapping.

REFERENCES

Al-Qurainy, F., Khan, S., Nadeem, M., 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. 637-640.

Allan, G., Williams, A., Rabinowicz, P. D., Chan, A. .P., Ravel, J., Keim P. 2008. Worldwide genotyping of castor bean germplasm (*Ricinus communis* L.) using AFLPs and SSRs. *Genet. Resour. Crop Evol.,* vol. 55, p. 365-378. https://doi.org/10.1007/s10722-007-9244-3

Amirmoradi, B., Talebi, R., Karami E. 2012. Comparison of genetic variation anddifferentiation among annual Cicer species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. *Plant Systematics and Evolution*, vol. 298, no. 9, p. 1679-1688. https://doi.org/10.1007/s00606-012-0669-6

Anjani, K. 2012. Castor genetic resources: a primary gene pool for exploitation. *Ind Crops Prod*., vol. 35, p. 1-14. https://doi.org/10.1016/j.indcrop.2011.06.011

Balážová, Ž., Gálová, Z., Vivodík, M., Petrovičová, L., Hornyák Gregáňová, R. 2017. Molecular variability of oat based on gene specific markers. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 332-337. https://dx.doi.org/10.5219/774

Bošeľová, D. and Žiarovská, J. 2016. Direct PCR as the platform of *Hedera helix*, L. genotypying without the extraction of DNA. *Journal of Central European Agriculture*, vol. 17, no. 4, p. 941-949. https://doi.org/10.5513/jcea01/17.4.1795

Collard, B. C. Y., 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 Report,* vol. 27, p. 86-93. https://doi.org/10.1007/s11105-008-0060-5

Damodaram, T., Hegde, D. M. 2010. Oilseeds Situation: A Statistical Compendium. *Directorate of Oilseeds Research*, Hyderabad.

Etminan, A., Pour-Aboughadareh, A., Mohammadi, R., Ahmadi-Rad, A., Noori, A., Mahdavian Z. and Moradi Z. 2016. Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes. *Biotechnology & Biotechnological Equipment*, vol. 30, no. 6, p. 1075-1081, https://doi.org/10.1080/13102818.2016.1228478

Fang-Yong, Ch., 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. https://doi.org/10.1016/j.scienta.2014.03.010

Foster J. T., Allan, G. J., Chan, A. P., Rabinowicz, P. D., Ravel, J., Jackson, P. J., Keim, P. 2010. Single nucleotide polymorphisms for assessing genetic diversity in castor bean (*Ricinus communis*). *BMC Plant Biol.,* vol. 10, p. 13-18. https://doi.org/10.1186/1471-2229-10-13 PMid:20082707

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, p. 1011-1020. https://doi.org/10.1007/s00606-013-0939-y

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. https://doi.org/10.5219/516

Chai, X., Dong, R., Liu, W., Wang, Y. and Liu, Z. 2017. Optimizing sample size to assess the genetic diversity in common Vetch (*Vicia sativa* L.) populations using Start Codon Targeted (SCoT) markers. *Molecules*, vol. 22, p. 567-577. https://doi.org/10.3390/molecules22040567

Cheema, N. M., Malik, M. A., Qadir, G. and Rabbani, M. A. 2010. Characterization of castor bean genotypes under various environments using SDS-PAGE of total seed storage proteins. *Pak. J. Bot*., vol. 42, no. 3, p. 1797-1805.

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. *Genet. Mol. Res.,*vol. 13, no. 2, p. 4406-4418. http://dx.doi.org/10.4238/2014.June.11.4

Kallamadia, P. R., Ganga Rao Nadigatlab, V. P. R., Mulpuriba, 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

Kanti, M., Anjani, K., Usha Kiran, B. and Vivekananda, K. 2015. Agro-morphological and molecular diversity in Castor (*Ricinus communis* L.) germplasm collected from Andaman and Nicobar Islands, India. *Czech J. Genet. Plant Breed*., vol. 51, no.3, p. 96–109. https://doi.org/10.17221/205/2014-cjgpb

Luo, C., He, X. H., Chen, H., Ou, S. J., Gao, M. P. 2010. Analysis of diversity and relationships among mango cultivars using start codon targeted (SCoT) markers. *Biochemical Systematics and Ecology*, vol. 38, no. 6, p. 1176-1184. https://doi.org/10.1016/j.bse.2010.11.004

Malook, I., Jamil, M., Fayyaz, M., Rehman, H., Irshad, M., Bashar, K. U., Khan, S., Akhter, N., Aslam M. M. and Rha, E. S. 2016. Biochemical and molecular characterization of Castor bean (*Ricinus Communis* L.) collected from different climatic zones of Pakistan. *Journal of Bio-Molecular Sciences*, vol. 4, no. 1, p. 16-23.

Petrovičová, L., Balážová, Ž., Vivodík, M., Gálová, Z. 2017. Detection genetic variability of *Secale cereale* L. by SCoT markers. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 197-202. https://dx.doi.org/10.5219/726

Ražná, K., Bežo, M., Hlavačková, L., Žiarovská, J., Miko, M., Gažo, J., Habán, M. 2016. MicroRNA (miRNA) in food resources and medicinal plant. *Potravinarstvo*, vol. 10, no. 1, p. 188-194. https://doi.org/10.5219/583

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. https://doi.org/10.1016/j.mgene.2015.01.003 PMid:2575086

Simões, K. S., Silva, S. A., Machado, E. L. and Silva, M. S. 2017. Genetic divergence in elite castor bean lineages based on TRAP markers. *Genetics and Molecular Research*, vol. 16, no. 3, p. 100-112. http://dx.doi.org/10.4238/gmr16039776

Simões, K. S., Silva, S. A., Machado, E. L. and Brasileiro, H. S. 2017. Development of TRAP primers for *Ricinus communis* L. *Genetics and Molecular Research,* vol. 16, no. 2, p. 100-113. http://dx.doi.org/10.4238/gmr16029647

Scholz, V., Silva, J. N. 2008. Prospects and risks of the use of castor oil as a fuel. *Biomass Bioenerg*, vol. 32, p. 95-100. https://doi.org/10.1016/j.biombioe.2007.08.004

Tsaballa, A., Ganopoulos, I., Timplalexi, A., Aliki, X., Bosmali, I., Irini, N., Athanasios, T., Madesis, P. 2015. Molecular characterization of Greek pepper (*Capsicum annuum* L.) landraces with neutral (ISSR) and gene-based (SCoT and EST-SSR) molecular markers. *Biochemical Systematics and Ecology*, vol. 59, p. 256-263. http://dx.doi.org/10.1016/j.bse.2015.02.005

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. 150-160. http://dx.doi.org/10.1155/2014/468375

Vivodík, M., Balážová, Ž., Gálová, Z. and Kuťka Hlozáková, T. 2015. Differentiation of ricin using RAPD markers. *Pak. J. Bot.*, vol. 47, no. 4, p. 1341-1345.

Vivodík, M., Gálová, Z., Balážová, Ž., Petrovičová, L. 2016. Start codon targeted (SCoT) polymorphism reveals genetic diversity in European old maize (*Zea mays* L.) genotypes. *Potravinarstvo*, vol. 10, no. 1, p. 563-569. https://doi.org/10.5219/660

Vyhnánek,T., Trojan, V., Štiasna, K., Presinszká, M., Hřivna, L., Mrkvicová, E., Havel, L. 2015. Testing of DNA isolation for the identification of Hemp. *Potravinarstvo*, vol. 9, no. 1, p. 393-397. https://doi.org/10.5219/509

Wang, Ch., Li, G., Zhang, Z., Peng, M., Shang, Y., Luo, R., Chen, Y. 2013. Genetic diversity of castor bean (*Ricinus communis* L.) in Northeast China revealed by ISSR markers. *Biochemical Systematics and Ecology*, vol. 51, p. 301-307. https://doi.org/10.1016/j.bse.2013.09.017

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

Xiong, F., Zhong, R., Han, Z., Jiang, J., He, L., Zhuang, W., Tang, R. 2011. Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) genotypes. *Molecular Biology Reports*, vol. 38, no. 5, p. 3487-3494. https://doi.org/10.1007/s11033-010-0459-6 PMid:21104441

Žiarovská, J., Grygorieva, O., Zeleňáková, L., Bežo, M., Brindza, J. 2015. Identification of sweet chesnut pollen in bee pollen pellet using molecular analysis. *Potravinarstvo*, vol. 9, no. 1, p. 352-358. https://doi.org/10.5219/497

Žiarovská, J., Kyseľ, M., Cimermanová, R., Knoteková, L. 2017. Effect of DNA extraction method in the *Rosa Canina* L. identification under different processing temperature. *Potravinarstvo Slovak Journal of Food Sciences,* vol. 11, no. 1, p. 190-196. https://dx.doi.org/10.5219/717

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COMPARISON OF THE CONTENT OF SELECTED MINERAL SUBSTANCES IN CZECH LITURGICAL AND COMMON WINES

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ABSTRACT
The article deals with the content of selected mineral substances in Czech liturgical wines and compares them with common wines. Sulphur, phosphorus, boron, potassium, calcium, magnesium, iron, cadmium and lead were selected as evaluated minerals, and they were all found in all the analyzed varieties – Pinot Noir, Red Traminer and Chardonnay. Mineral substances were determined using a quadrupole mass spectrometer in the inductively coupled plasma variant of the Thermo Scientific ICAP Q ICP-MS. Generally, measured results did not show significant differences between the mineral content in liturgical and common wines. Therefore the influence of specific production technologies of liturgical wines on the mineral contents was not proved either. One of the highest mineral concentration was in communion Red Traminer, which, besides beneficial minerals, also contained a high amount of toxic elements. On the contrary, common Red Traminer had one of the lowest concentrations of toxic compounds. The content of permitted lead levels was exceeded in a single case, namely in the Chardonnay kosher sample. For cadmium, no sample exceeded the allowed limit.

Keywords: communion wine; kosher wine; mineral substances; mass spectrometry; cadmium

INTRODUCTION
Plutarch (c. 46 – c. 127), Greek writer, historian and philosopher said that, the wine is "the most useful of all beverages, the tastiest of all medications, and the most pleasant of all food". Wine has been used since time immemorial in various religious ceremonies for worshipping a deity in the ritual of a given church. For the Christian religion in the **Bible** the wine symbolizes the blood of Jesus Christ. According to the Torah, wine, along with bread, "gladdens the living" and "brings joy to the God and man" **(Torah, Psalm 104:15; Torah, Koheles 10:19; Torah, Pesachim 109a; Divecký, 2005; Bondyová and Sliva, 2008)**.

Liturgical wine, used for religious purposes, must meet the conditions of a particular religion and must be approved by a given ecclesiastical authority. The rules for the production of communion wine used in the Eucharist as the "blood of the Lord" for the Czech Republic are set up by the Czech Bishops' Conference **(Koudelka, 2010)**. The Congregation for Worship and the Sacrament states that, the communion wine must be only of natural origin, made from grapevine, unaltered, unmixed with other ingredients, chemically untreated, and according to the Czech Bishops' Conference, the grapes have to come from Bohemia or Moravia. Production is similar to organic farming. Other conditions include the prohibition of the use of additives such as dyes or flavorings, and at least 20 degrees of the sugar content of the grapes used. The content of alcohol in wine is not limited, so it may vary **(Železný, 2010)**.

In the Jewish religion, wine is used much more than in the Christian religion. For example, during Tu BiShvat, the feast of trees, it is obligatory to drink gradually white, pink, light red and a bold red wine **(Tvarůžek, 1948)**. On Purim, celebrating the rescue of the Jews in Persian exile by Queen Ester, the Talmud commands the orthodox Jew to get drunk so much to "not being able to distinguish Mordecai from Haman" (two adversaries). Wine is divided into several groups - cooked or pasteurized ("yayin mewushal"), uncooked or unpasteurised ("yayin lo mewushal"), wine for Pesach, which must not come in contact at all with grain, bread or dough, and wine "mehadrin" for ultra-orthodox Jews **(Nádeníčková, 2014)**. In order to preserve kosher quality, the wine must not be touched or opened by a non-Jew, except for the boiled wine. Strict rules also apply to the cultivation and processing of such wine, e.g. the vineyard is left to rest every seventh year (sabbatical year) as commanded in the Third Book of Moses.

Wine is an interesting source of biologically active substances, such as polyphenols, antioxidants and minerals **(Bajčan et al., 2016; Škrovánková et al., 2017)**. The soil

and its geological origin, the fertilization, the variety, the weather in the given year and the processing technology all have a great influence on the content of minerals in grapes and wine. The influence of nutrition in the conditions of a particular vineyard is also significant. The roots of the vine receive minerals with water from the soil. The mineral content of the must is reduced by their crystallization, precipitation and utilization by yeasts **(Jedlička, Novotná, et Valšíková, 2014)**. The total amount of minerals determined as the ash content in the wine is 1500 – 4000 mg.L^{-1} **(Steidl, 2010)**. Sulphur, phosphorus, boron, potassium, calcium and magnesium are the basic minerals evaluated in this article. Harmful for the aroma, color and taste of the wine are mainly iron, copper, nickel, tin, aluminium, zinc and toxic metals. This work focused on iron and toxic metals - arsenic, chromium, lead and cadmium. Mass spectrometry was used to determine them. Mass spectrometry methods used for analyzing elements in the wine can detect not only the content of individual elements, but also determine the authenticity of the wine or the dilution of the wine with water **(Čížková et al., 2012)**.

Scientific hypothesis

Scientific hypothesis is: The content of the selected minerals evaluated by the mass spectrometry is different in Czech liturgical and common wines.

The aim of the work was to compare the content of selected minerals in Czech liturgical and common wines. The samples were chosen with regard to the comparability of vintage, sub-area and special attributes.

MATERIAL AND METHODOLOGY

Wine samples

To analyze the selected minerals in the communion wines, Pinot Noir and Red Traminer was chosen. Chardonnay was used to compare the minerals in kosher wine. For each variety, 2 samples of common wine and 2 samples of liturgical wine were tested. Samples were chosen to ensure the highest comparability possible (vintage, sub-area and attribute), but different producers.

Due to the difficulty of acquiring the comparable samples, their gathering took 15 months. Samples were bought gradually in the common market, specialized wine shops and directly from the producers. Two bottles of each wine were bought and analyzed. The samples in **Table 1** were tested.

Chemicals

There were used:

- HNO_3 p. a., Mr. 63.01, Penta, Praha, CZ,
- Deionized water, 18.2 MOhm.cm, Millipore.

All chemicals were of analytical reagent grade or equivalent analytical purity.

Evaluation of the mineral content

The quadrupole mass spectrometer in an inductively coupled plasma variant of Thermo Scientific iCAP Q ICP-MS (Thermo Scientific, MA, USA) was used to determine the mineral elements. The device is computer controlled by Thermo Scientific ™ Qtegra ™ Intelligent Scientific Data Solution ™ (ISDS) platform software (Thermo Scientific, MA, USA).

1 mL of sample with 1 mL of nitric acid was put into a 100 mL volumetric flask and refilled up to the mark with pure deionized water. For each sample the measurement was repeated 3 times. The content of individual elements is expressed in mg.L^{-1}.

Statistical analysis

The data were analyzed using Excel 2013 (Microsoft Corporation, USA) and STATISTICA Cz version 12 (StatSoft, Inc., USA). Results were expressed by average ± standard deviation. Comparison of the results was

Table 1 Wine origin and category.

Sample	Category	Vintage	Sub-area, village, track	Quality
Pinot Noir				
PN1	M	2012	Znojemská, Stošíkovice na louce, U tří dubů	VB
PN2	M	2012	Velkopavlovická, Havraníky, Staré vinice	VH
PN3	B	2012	Znojemská, Miroslavské Knínice, Stará hora	VH
PN4	B	2012	Velkopavlovická, Velké Bílovice	VH
Red Traminer				
TR1	M	2013	Velkopavlovická	VH
TR2	M	2013	Znojemská, Stošíkovice na louce, U tří dubů	VH
TR3	B	2013	Znojemská, Bzenec,	PS
TR4	B	2013	Znojemská, Sedlec, Nad Nesytem	PS
Chardonnay				
CH1	K	2010	Izrael, Samson	Q
CH2	K	2011	Slovácká, Hýsly / Moštěnsko	PS
CH3	B	2010	Mikulovská, Perná, Purmice	PS
CH4	B	2011	Znojemská, Bzenec,	VH

Note: K – kosher wine, M – communion wine, B – common wine, VB – special selection of berries, VH – special selection of grapes, PS – Late harvest, Q - quality.

performed using a Kruskal-Walllis test (α = 0.05). The samples of individual varieties were compared to each other. Furthermore, all samples of individual varieties of liturgical wines were compared against common wines.

RESULTS AND DISCUSSION

Sulphur

The sulphur content in the wine may range from 400 to 1000 mg.L^{-1}. The greatest amount of sulphur comes into the wine in the form of sulphur dioxide during the sulfation of the must or wine. The first references regarding the use of antimicrobial effects of sulphur are found not only in the Bible, but also in Greek and Roman literature **(Amâncio et al., 2009)**. Furthermore, sulphur can be found in the wine as a residue of nitrogenous or magnesium fertilizers in the form of sulphates.

In Pinot Noir the sulphur content varied between 60.30 ±1.62 mg.L^{-1} and 62.69 ±3.26 mg.L^{-1}. Red Traminer contained 66.05 ±1.79 mg.L^{-1} to 69.14 ±3.63 mg.L^{-1}. In Chardonnay the values ranged from 66.97 ±0.51 mg.L^{-1} to 71.24 ±5.83 mg.L^{-1} of sulphur. Other results are shown in **Table 2**. There was no statistically significant difference (p >0.05) in sulphur content between liturgical and common wines. No statistically significant difference was found either between individual samples of individual varieties (p >0.05).

According to **Annex IB of the Commission Regulation (EC) No 606/2009** of 10 July 2009, laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions, the maximum content of SO_2 in red wine is 150 mg.L^{-1} and 200 mg.L^{-1} in white wine. For selected Czech wines with special attributes, there is an exception for up to 400 mg.L^{-1} (special election of berries, special selection of botrysided berries, ice wine or straw wine).

In this research, the content of sulphur varied from 60.30 mg.L^{-1} to 71.24 mg.L^{-1}. **Sandler et al. (2003)** states, that to make the sulfation effective, the dose must be high enough to ensure the content of SO_2 in the final product at least 30 mg.L^{-1}. Doses higher than 70 – 80 mg.L^{-1} are not recommended because of influencing the taste properties of wine. This was the case of the sample CH2, which may be considered slightly too much sulfated, which can influence its taste properties. There is no other significant difference between liturgical or common wines.

Phosphorus

Phosphorus is one of the key elements for cultivating grapevine. It gets into the soil as a part of phosphate fertilizers. However, it is also included in the soil as a part of the parent rocks. This mineral is found in the wine both in inorganic and organic forms, such as glycerofosphates, phosphorus esters or pectin compounds. Another important role of phosphorus is during the fermentation, when it is utilized by yeast. There are soils rich in phosphorus in the Czech Republic. The content of phosphorus in wine is from 60 mg.L^{-1} to 1000 mg.L^{-1} **(Fic, 2015)**.

In Pinot Noir the content of phosphorus varied from 982.39 ±19.57 mg.L^{-1} to 1389.32 ±5.27 mg.L^{-1}. Red Traminer had the value of 974.89 ±5.69 mg.L^{-1} to 1460.46 ±27.07 mg.L^{-1}. For Chardonnay the content of phosphorus was from 862.20 ±20.46 mg.L^{-1} to 1493.15 ±25.51 mg.L^{-1}. Unlike with sulphur, a statistically significant difference (p <0.05) in the content of phosphorus was found between samples of liturgical and common wines for all the varieties analyzed. Comparison of the individual samples of the different varieties revealed a statistically significant difference (p <0.05) for the Pinot Noir variety samples PN2 and PN4, for the Red Traminer variety between TR1 and TR4 samples and for the

Table 2 The content of selected mineral substances (boron, magnesium, phosphorus, sulphur, potassium) in czech liturgical and common wines (Pinot Noir, Red Traminer, Chardonnay) in mg.L^{-1}.

Sample	Boron		Magnesium		Phosphorus		Sulphur		Potassium	
	M	SD	M	SD	M	SD	M	SD	M	SD
Pinot Noir										
PN1	84.86	2.24	2335.60	17.10	1342.86	23.97	61.47	2.51	20494.84	440.7
PN2	105.46	1.20	2428.03	38.92	1389.32	5.27	60.49	1.35	12052.24	251.4
PN3	83.23	1.15	2462.59	0.85	1329.18	10.92	62.69	3.26	15373.05	144.3
PN4	99.63	1.40	2569.93	67.84	982.39	19.57	60.30	1.62	18051.27	523.3
Red Traminer										
TR1	107.87	0.37	3064.03	31.32	1460.46	27.07	66.05	1.79	17927.10	183.0
TR2	79.98	1.30	2147.20	23.20	1417.65	9.52	68.75	2.32	7081.77	69.4
TR3	71.26	0.93	2122.85	17.22	1251.15	7.22	69.14	3.63	9561.64	53.7
TR4	76.96	1.25	2338.48	30.10	974.89	5.69	67.48	2.44	6658.59	149.3
Chardonnay										
CH1	66.49	0.83	2463.27	11.56	1493.15	25.51	69.51	1.54	4148.21	67.0
CH2	83.42	1.08	1949.67	15.77	1087.55	22.60	71.24	5.83	10367.63	135.2
CH3	77.98	1.49	2147.99	14.12	1075.18	5.63	70.04	1.80	9439.80	57.1
CH4	151.24	0.34	2055.87	47.88	862.20	20.46	66.97	0.51	9115.07	238.1

Chardonnay variety between CH1 and CH4 samples. No statistically significant difference was found between the other samples ($p > 0.05$).

Table 2 shows different content of phosphorus in wine samples, ranging from 862.20 mg.L^{-1} to 1493.15 mg.L^{-1}. In liturgical wine the content is higher than in the common wine. The reason may be the using of phosphate fertilizers in some vineyards, or the high concentration in Czech soils. However, the results are higher than the range stated by **Fic (2015)**.

Boron

Boron is a very important microelement in grapevine nutrition. It participates in pollination, fertilization of inflorescences, photosynthesis and transport of glycides. Its content is lower in toxic or calcareous soils. It is most commonly found in the form of boric acid, which content in wine can be 10 – 120 mg.L^{-1} **(Fic, 2015)**.

In Pinot Noir the content of boron was between 83.23 ±1.15 mg.L^{-1} and 105.46 ±1.20 mg.L^{-1}. Red Traminer contained from 71.26 ±0.93 mg.L^{-1} to 107.87 ±0.37 mg.L^{-1}. In Chardonnay the content of boron varied from 66.49 ±0.83 mg.L^{-1} to 151.24 ±3.44 mg.L^{-1}. A statistically significant difference ($p < 0.05$) in the content of boron was found between samples of liturgical and common wines in the Red Traminer variety. Comparison of the individual samples of the different varieties revealed a statistically significant difference ($p < 0.05$) between PN2 and PN3 samples of the Pinot Noir, between TR1 and TR3 samples of Red Traminer and between CH1 and CH4 samples of Chardonnay. No statistically significant difference was found between the other samples ($p > 0.05$).

The content of boron in wines was 66.49 – 151.24 mg.L^{-1}, as can be seen in the **Table 2**. **Fic (2015)** states the content of boron is influenced by calcareous and toxic soils. It was supposed, that due to the contamination of soil with toxic elements and the high content of calcium in the soil within the observed area, the content of boron would be lower. Despite this fact, the content of boron is approaching rather the upper limits of the range 10 – 120 mg.L^{-1}, or even exceeds it (in the last sample).

Potassium

Potassium plays an important role in the exchange of K^+ ionts for H_3O^+ oxonium ionts and it is quite mobile in the soil. The grape vine is most absorbing it during the lush growth. It occurs in must mostly as potassium hydrogen tartrate or potassium sulphate. Higher quantities occur in red wines and varieties of dry white wines such as Chardonnay or Pinot Blanc. During ripening, its concentration in grapes increases in relation to the accumulation of sugars. Potassium also affects the acid content and the pH of must and wine. This mineral element softens the taste of the wine, and its higher quantity can also indicate the age of the wine. Low-potassium wines taste sour and bitter. By precipitating "wine stone" (potassium hydrogen tartrate) through fermentation the potassium content can be reduced by up to 1000 mg.L^{-1}. Wines contain potassium in the range of 160 – 2500 mg.L^{-1} **(Fic, 2015)**.

In Pinot Noir the potassium content ranged within 1205.22 ±25.14 mg.L^{-1} and 2049.48 ±44.07 mg.L^{-1}. Red Traminer contained from 665.86 ±14.93 mg.L^{-1} to 1792.71 ±18.30 mg.L^{-1} of potassium. In Chardonnay the level of potassium was between 414.82 ±6.70 mg.L^{-1} and 1036.76 ±13.52 mg.L^{-1}. For potassium, no statistically significant difference ($p > 0.05$) was found between the liturgical and common wines. Comparison of the individual samples of different varieties revealed a statistically significant difference ($p < 0.05$) between the PN1 and PN2 samples of Pinot Noir, between the TR1 and

Table 3 The content of selected mineral substances (calcium, iron, cadmium, lead) in czech liturgical and common wines (Pinot Noir, Red Traminer, Chardonnay) in mg.L^{-1}.

Sample	Calcium		Iron		Cadmium		Lead	
	M	SD	M	SD	M	SD	M	SD
Pinot Noir								
PN1	771.24	10.95	3.54	0.11	0.0036	0.0002	0.0289	0.0004
PN2	1177.55	7.81	9.33	1.65	0.0047	0.0001	0.0965	0.0019
PN3	1194.80	10.51	13.35	1.11	0.0034	0.0006	0.0369	0.0010
PN4	1069.77	23.87	13.06	0.17	0.0023	0.0001	0.0259	0.0005
Red Traminer								
TR1	1872.26	20.01	18.32	0.52	0.0056	0.0001	0.1387	0.0009
TR2	1152.93	12.81	9.84	0.09	0.0064	0.0003	0.1115	0.0021
TR3	1282.98	17.50	9.81	0.32	0.0051	0.0004	0.0815	0.0010
TR4	691.03	8.70	12.39	0.43	0.0059	0.0005	0.1025	0.0012
Chardonnay								
CH1	1048.66	14.01	10.05	0.21	0.0101	0.0011	0.0371	0.0011
CH2	1091.78	4.82	9.43	0.17	0.0023	0.0001	0.0557	0.0009
CH3	833.32	2.25	9.50	0.21	0.0033	0.0004	0.0713	0.0009
CH4	1063.36	12.85	6.80	0.03	0.0076	0.0008	0.1054	0.0023

TR4 samples of Red Traminer and between the CH1 and CH2 samples of the Chardonnay variety. No statistically significant difference was found between the other samples (p >0.05).

Table 2 shows the content of potassium in samples, which was between 414.82 mg.L^{-1} and 2049.48 mg.L^{-1}. The concentration of potassium in wine reflects its content in the final stages of berry ripening, which may explain the big difference between the values. High potassium levels affect the stability of the wine with regard to its precipitation in the form of potassium hydrogen tartrate. Higher levels of potassium, especially in the formation of these crystals, may indicate the age of the wine. This aspect was relatively well observable in red wines, which were 4 years old at the time of measurement. As stated by **Stafilov and Karadjova (2009)**, red wines usually have higher content of potassium than white wines. This is suggested also in this research (**Table 2**). The results are within the range declared by **Fic (2015)**.

Calcium

Calcareous soils are very abundant in the Czech Republic‘s wine-growing sub regions, thus the Czech wines contain enough calcium. However, excess calcium in the soil may result in chlorosis of the vine. Calcium has a positive influence on the taste and aroma of the wine. The amount of calcium increases when deacidated. It must also be expected that it will "fall out" in the form of calcium hydrogen tartrate. The wine may be in the range of 100 – 220 mg.L^{-1} **(Fic, 2015)**.

In Pinot Noir the calcium content was between 771.24 ±10.95 mg.L^{-1} and 1194.80 ±10.51 mg.L^{-1}. Red Traminer contained from 691.03 ±8.70 mg.L^{-1} to 1872.26 ±20.01 mg.L^{-1} of calcium. In Chardonnay the values ranged between 833.32 ±2.25 mg.L^{-1} and 1091.78 ±4.82 mg.L^{-1} of calcium. There was no statistically significant difference (p >0.05) in the content of calcium between the liturgical and common wine. Comparison of the individual samples of each of the varieties revealed a statistically significant difference (p <0.05) between the PN1 and PN3 samples of Pinot Noir, between the TR1 and TR4 samples of the Red Traminer, and between the CH2 and CH3 samples of Chardonnay. No statistically significant difference was found between the other samples (p >0.05).

Content of calcium in wine was between 691.03 mg.L^{-1} and 1872.26 mg.L^{-1}, as shown in **Table 3**. Higher content of calcium in TR1 sample can be caused by long term storage in concrete tanks, where the calcium could permeate from the walls into the wine. This was mentioned by **Jackson (2008)**. High level of calcium can cause crystal formation in the wine. The results are highly above the values declared by **Fic (2015)**. This may be due to the difference between values declared by different authors. E.g. **Rupasinghe and Clegg (2007)** declared the content of calcium 620 mg.L^{-1}.

Magnesium

Magnesium’s most important role is as the part of chlorophyll. This mineral may be deficient in calcareous and sandy soils. In high concentration, magnesium may cause bitter taste of the wine. Wine can content 50 – 2000 mg.L^{-1} of magnesium **(Fic, 2015)**.

In Pinot Noir the content of magnesium varied from 2335.60 ±17.10 mg.L^{-1} to 2569.93 ±67.84 mg.L^{-1}. Red Traminer contained between 2122.85 ±17.22 mg.L^{-1} and 3064.03 ±31.32 mg.L^{-1}. Chardonnay contained from 1949.67 ±15.77 mg.L^{-1} to 2463.67 ±11.56 mg.L^{-1} of magnesium. For magnesium, a statistically significant difference (p <0.05) was found between samples of liturgical and common wines only for the Pinot Noir variety. Comparison of the individual samples of the different varieties revealed a statistically significant difference (p <0.05) between the samples PN2 and PN4 of Pinot Noir, between the TR1 and TR4 samples of Red Traminer and between the CH1 and CH4 samples of the Chardonnay variety. No statistically significant difference was found between the other samples (p >0.05).

Table 2 shows different magnesium content in wine samples, which varied from 1949.67 mg.L^{-1} to 3064.03 mg.L^{-1}. As declared by **Avram et al. (2014)** the level of magnesium depends on the similar conditions as potassium, and it is possible to claim that, the content of magnesium could correlate with content of potassium. This can be supported by comparison of the **Table 2**. Higher level of magnesium can also indicate the use of the different fertilizers and production processes. Furthermore, the samples come from areas known to be rich in magnesium. All of the above may be the reason why the results are not within the range stated by **Fic (2015)**.

Iron

This mineral causes turbidity, thus the higher concentration of iron in wine is undesirable. It can be removed by clarifying (fining), but the content can significantly increase due to contact with iron tools during processing or storage. In Czech Republic the iron occurs especially in soils in Znojmo and Brno Districts. The content is around 0.3 – 10 mg.L^{-1} **(Kraus, 1999)**.

In Pinot Noir the iron content ranged from 3.54 ±0.11 mg.L^{-1} to 13.35 ±1.11 mg.L^{-1}. Red Traminer contained between 9.81 ± 0.32 mg.L^{-1} and 18.32 ±0.52 mg.L^{-1} of iron. Content of iron in Chardonnay varied from 6.80 ±0.03 mg.L^{-1} to 10.05 ±0.21 mg.L^{-1}. A statistically significant difference (p <0.05) in the content of iron was found between samples of liturgical and common wines only for the Pinot Noir variety. Comparison of the individual samples of all the varieties revealed a statistically significant difference (p <0.05) for the Pinot Noir variety between the samples PN1 and PN4 and for the Chardonnay variety between samples CH1 and CH4. No statistically significant difference was found between the other samples (p >0.05).

Iron level in samples was 3.54 – 18.32 mg.L^{-1}, as shown in **Table 3**. The significantly higher content of iron in the sample of white communion wine could be the result of wine contact with a corroded winery device. Besides causing turbidity, the higher iron content can catalyze oxidative reactions, e.g. conversion of ascorbic acid to dehydroascorbic acid, or cause wine browning. Higher iron content may also promote polymerization of phenolic compounds with acetaldehyde, as reported by **Jackson (2008)**. Analyzed samples of the common red wines, first white communion wine and the second white communion

wine can be considered problematic for long-term storage regarding the higher content of iron. The results are within the range declared by **Kraus (1999)**.

Cadmium

Cadmium occurs in nature as a part of the minerals and organic compounds of the soil solution. In Czech soil the common content is 0.2 – 1.5 mg.kg^{-1} soil. During the last 150 years its content has increased by 55 %. The main limiting factor for cadmium content in the soil is the chemical composition of the mother rock. High doses of cadmium can damage soil microflora **(Fic, 2015)**.

The content of cadmium in Pinot Noir was between 0.0023 ±0.0001 mg.L^{-1} and 0.0047 ±0.0001 mg.L^{-1}. Red Traminer contained from 0.0051 ±0.0004 mg.L^{-1} to 0.0064 ±0.0003 mg.L^{-1} of cadmium. The content of cadmium in Chardonnay varied from 0.0023 ±0.0001 mg.L^{-1} to 0.0101 ±0.0011 mg.L^{-1}. A statistically significant difference ($p < 0.05$) in the content of cadmium was found between samples of liturgical and common wines only for the Pinot Noir variety. Comparison of the individual samples of the different varieties revealed a statistically significant difference ($p < 0.05$) for the Pinot Noir variety between the samples PN2 and PN4, for the Red Traminer variety between the TR2 and TR3 samples and for the Chardonnay variety between the CH1 and CH2 samples. No statistically significant difference was found between the other samples ($p > 0.05$).

Table 3 shows the content of cadmium in wine samples, which was between 0.023 mg.L^{-1} and 0.0101 mg.L^{-1}. In the first kosher wine and the second common wine (Chardonnay) the content of cadmium was higher than in the rest of the samples. This may be due to the air pollution, postfermental contamination or the contact of wine with stainless steel during the process, as declared by **Dehelean and Voica (2012)** and **Stafilov and Karadjova (2009)**. OIV (International Organization of Vine and Wine) set the limit of cadmium in wine 0.01 mg.L^{-1}. All analyzed samples fulfilled this requirement.

Lead

The natural lead content in the soil is 2 – 300 mg per kilogram of soil in the form of Pb_2^+ in acidic igneous rocks. As a result of anthropogenic activity, the amount of lead in the soil increases above the limit value set by the Ministry of Agriculture **(Fic, 2015)**.

In Pinot Noir the content of lead ranged from 0.0259 ±0.0005 mg.L^{-1} to 0.0965 ±0.0019 mg.L^{-1}. Red Traminer contained between 0.0815 ±0.0010 mg.L^{-1} and 0.1387 ±0.0009 mg.L^{-1} of lead. In Chardonnay the content of lead varied among 0.0371 ±0.0011 mg.L^{-1} and 0.1054 ±0.0023 mg.L^{-1}. For lead, a statistically significant difference ($p < 0.05$) was found between samples of liturgical and common wines only for the Red Traminer and Chardonnay. Comparison of the individual samples in the different varieties revealed a statistically significant difference ($p < 0.05$) for the Pinot Noir variety between the samples PN2 and PN4, for the Red Traminer variety between the TR1 and TR3 samples, and for the Chardonnay variety between the CH1 and CH4 samples. No statistically significant difference was found between the other samples ($p > 0.05$).

The range of lead content in the samples was 0.0259 – 0.1387 mg.L^{-1}, as shown in **Table 3**. Lead is a contaminant that could get into the wine from the soil. The most frequent reasons are emissions, agricultural chemicals and industrial pollution. In the more traditional manufacturers the contamination may be caused by brass fittings and faucets that wine comes into contact with during the secondary fermentation. In rare cases, due to long-term storage in crystal containers that release lead into the wine. This is also declared by **Jackson (2008)**. Although lead is a toxic metal, it precipitates together with other metals and is excluded during fermentation with turbidities. The OIV set the lead content in wine to 0.15 mg.L^{-1} **(OIV, 2016)**. All analyzed sample meet this criteria.

CONCLUSION

This study dealt with content of mineral substances in communion and kosher wines, compared to content in common wines. Generally, mass spectrometry analysis did not prove that the liturgical wines have better composition regarding the mineral content than the common wines. A statistically significant difference between samples of liturgical and common wines was found in only one third of the analyzed samples. A statistically significant difference between the samples of individual varieties was proved in only one sample of the whole set for the individual variety. There was no statistically significant difference between the other samples. Therefore, this study demonstrates that no significant influence of the specific technology of the production of liturgical wines has been proved for the selected samples. Communion wine Red Traminer (sample TR1), had one of the highest content of beneficial minerals, but also contained high amounts of toxic elements, which however, did not exceed any legally set limit. On the contrary, the limit for lead content was exceeded in a single case, by Chardonnay kosher (sample CH1). None of the samples exceeded the limit for cadmium. It would be suitable to conduct further research on this issue, as the up to date sources or analyses, which would cover this issue with complexity, is scarce.

REFERENCES

Amâncio, S., Tavares, S., Fernandes, J., Sousa, C. 2009. Grapevine & Sulfur: Old Partners, New Achievements. In Roubelakis-Angelakis, K.A. *Grapevine Molecular Physiology & Biotechnology*. Dordrecht, Germany : Springer Science+Business Media B.V., p. 31-52. ISBN-13: 978-90-481-2304-9.

Avram, V., Voica, C., Hosu, A., Cimpoiub, C., Măruțoiuc, C. 2014. ICP-MS Characterization of some Roumanian White Wines by Their Mineral Content. *Revue Roumaine de Chimie*, vol. 59, no. 11-12, p. 1009-1019.

Bajčan, D., Vollmannová, A., Šimanský, V., Bystrická, J., Trebichalský, P., Árvay, J., Czako, P. 2016. Antioxidant activity, phenolic content and colour of the Slovak cabernet sauvignon wines. *Potravinarstvo,* vol. 10, no. 1, p. 89-94. http://dx.doi.org/10.5219/534

Bible. 1991. *Bible. Písmo svaté Starého a Nového zákona*. (*Bible. Holy Scriptures of the Old and New Testaments*). 2nd ed. Prague Czech Repulic : Zvon. ISBN-10: 8071130095. (In Czech)

Bondyová, R., Sliva, J. 2008. *Boží Hody: Jak jedli Židé v Čechách a na Moravě*. (*God's Feast: How the Jews ate in Bohemia and Moravia*). 1st ed. Prague, Czech Republic : Franz Kafka. ISBN: 978-80-86911-16-8. (In Czech)

Čížková, H., Ševčík, R., Rejchl, A., Pivoňka, J., Voldřich, M. 2012. Trendy v autenticitě potravin a v přístupech k detekci falšování. (Trends in Food Authenticity and Detection of Food Adulteration). *Chemické Listy*, vol. 106, no. 10, p. 903-910. (In Czech)

Dehelean, A., Voica, C. 2012. Determination of Lead and Stronicum Isotope Ratios in Wines by Inductively Coupled Plasma Mass Spectrometry. *Romanian Journal of Physics*, vol. 57, no. 7-8, p. 1194-1203.

Divecký, J. 2005. *Židovské svátky: [kalendář od Pesachu do Purimu]*. (*Jewish holidays: [calendar from Pesach to Puri]*). 1st ed. Prague, Czech Republic: P3K, ISBN: 80-903584-3-8. (In Czech)

FIC, V.2015. *Víno: analýza, technologie, gastronomie.* (*Wine: analysis, technology, gastronomy*). 1st ed. Český Těšín: 2 THETA. ISBN: 978-80-86380-77-3. (In Czech)

Jackson, R. S. 2008. *Wine science: principles and applications*. 3rd ed. Amsterdam, Nethrerland : Elsevier/Academic Press. ISBN-13: 978-0123736468.

Jedlička, J., Novotná, B., Valšíková, M. 2014. Evaluation of influence of the locality, the vintage year, wine variety and fermentation process on volume of cooper and lead in wine. *Potravinarstvo,* vol. 8, no. 1, p. 290-295. http://dx.doi.org/10.5219/403

Koudelka, Z. 2010. Košer a mešní víno. (Kosher and Masswine). *Vinařský obzor*, vol. 103, no. 5, p. 236-239. (In Czech)

Kraus, V. 1999. *Réva a víno v Čechách a na Moravě*. (*Grapevine and wine in Bohemia and Moravia*). 1st ed. Praha: Radix. 280 p. ISBN: 80-86031-23-3. (In Czech)

Nádeníčková, B. 2014. *Technologie speciálních vín*. (*The technology of special wines*). Brno, Czech Republic: Mendel University in Brno, 87 p. ISBN: 978-80-7509-019-5. (In Czech)

Commission Regulation (EC) No 606/2009 of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions. OJ L 193, 24.7.2009, p. 1–59.

Rupasinghe, H. P. V., Clegg, S. 2007. Total antioxidant capacity, total phenolic content, mineral elements, and histamine concentrations in wines of different fruit sources. *Journal of Food Composition and Analysis*, vol. 20, no. 2, p. 133-137. http://dx.doi.org/10.1016/j.jfca.2006.06.008

Sandler, M., Pinder, R. 2003. *Wine: a scientific exploration.* New York, USA : Taylor a Francis. ISBN-10: 0415247349. https://doi.org/10.4324/9780203361382

Stafilov, T., Karadjova, I. 2009. Atomic Absorption Spectrometry in Wine Analysis. *Macedonian Journal of Chemistry and Chemical Engineering,* vol. 28, no. 1, p. 17-31. http://dx.doi.org/10.20450/mjcce.2009.218

Steidl, R. 2010. *Sklepní hospodářství*. (*Cellar Management*). 2nd ed. Valtice: Národní vinařské centrum. 309 p. ISBN: 978-80-903201-9-2. (In Czech)

Škrovánková, S., Mlček, J., Orsavová, J., Juríková, T., Dřímalová, P. 2017. Polyphenols content and antioxidant activity of paprika and pepper spices. *Potravianarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 52-57. http://dx.doi.org/10.5219/695

Torah. 2012. *Chamiša chumšej Tora: im ha-haftarot*. Praha: Sefer. ISBN-13: 978-80-85924-67-1. (In Czech)

Tvarůžek, R. 1948. *Réva a víno v Bibli*. (*Grapevine and wine in the Bible*). Brno: Vinařské družstvo v Bzenci, 225 p. (In Czech)

Železný, V. 2010. *Dobré rady milovníka vína*. (*Good advice of a wine lover*). 1st ed. Prague, Czech Republic : Mladá fronta, 243 p. ISBN: 978-80-204-2217-0. (In Czech)

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STUDY OF POLYMORPHISM OF MAIZE USING DNA AND PROTEIN MARKERS

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ABSTRACT

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, 10 simple sequence repeat (SSR) markers, 13 random amplified polymorphic (RAPD) markers and using SDS-PAGE markers. Twenty SCoT primers produced 114 DNA fragments with an average of 5.7 bands per primer. Out of the total of 114 amplified fragments, 86 (76.43 %) were polymorphic, with an average of 4.30 polymorphic bands per primer. Ten SSR primers revealed a total of 65 alleles ranging from 4 (UMC1060) to 8 (UMC2002 and UMC1155) alleles per locus with a mean value of 6.50 alleles per locus. 20 SCoT 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 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 were screened. 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. The dendrogram of 40 old maize genotypes based on SSR, SCoT, RAPD and SDS-PAGE markers using UGMA algorithm was constructed.

Keywords: RAPD; SSR; SDS-PAGE; SCoT; old maize; dendrogram

INTRODUCTION

With the advent of the first maize hybrids, in 1933 in the US and around 1950 in Europe, maize cultivation has undergone a complete change **(Gay, 1984; Dubreuil and Charcosset, 1998)**. Since 1990, random amplified polymorphic DNA (RAPD) markers have been successfully applied for identification of DNA polymorphism in various plant species **(Williams et al., 1990).** RAPD technique requires only small amounts of DNA sample without involving radioactive labels and are simpler as well as faster **(Masojc´ et al., 2001).** Molecular markers based on polymerase chain reaction (PCR) methods, such as simple sequence repeats (SSRs) or microsatellites, have become important genetic markers in a wide range of crop species, including maize **(Elçi et al., 2015)**. SSRs markers have many advantages over other types of molecular markers, such as co-dominance, abundant in genomes, highly polymorphisms, locus specificity, good reproducibility and random distribution throughout the genome **(Sun et al., 2011)**. These features, coupled with their ease of detection, make them ideal for identifying and distinguishing between accessions that are genetically very similar **(Saker et al., 2005)**. 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 **(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. 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)**. 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 et al., 2006)**. Zein is major seed storage protein of maize and consists of one major and three minor classes and these four classes constitute approximately 50 – 70%

of maize endosperm **(Freitas et al., 2005; Vasal, 1999)**. The non-zein protein consists of globulins (3%), glutelins (34%) and albumins (3%). Zein is specific to maize endosperm 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 **(Prasanna et al., 2001; Hamoud et al., 2005)**. 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; 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 **(Ranjan et al., 2013)**. 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)**. For the analysis of genetic diversity of maize genotypes were used several dominant molecular markers: amplified fragment length polymorphism (AFLP) **(Roy and Kim, 2016)**, random amplified polymorphic DNA (RAPD) **(Balážová et al., 2016)**, start codon targeted (SCoT) **(Vivodík et al., 2016)**, inter-simple sequence repeat (ISSR) **(Idris et al., 2012; Žiarovská et al., 2013)** and sequence-related amplified polymorphism (SRAP) **(Abd El-Azeem et al., 2015)**. And codominant molecular markers were also used for the analysis of maize genotypes: simple sequence repeat (SSR) **(Shiri et al., 2014)**, expressed sequence tag (EST)-SSR **(Galvão et al., 2015)**, single nucleotide polymorphism (SNP) **(Sa et al., 2012)** and using protein markers (SDS-PAGE) **(Vivodík et al., 2016)**. The polymerase chain reaction (PCR) has been used by many authors, such as **Žiarovská et. al., (2015)**; **Kanti et. al., (2015)**; **Vyhnánek et. al., (2015)**; **Bošeľová and Žiarovská (2016)**; **Ražná et. al., (2016)**; **Žiarovská et. al., (2017)**; **Simões et. al., (2017)**.

Scientific hypothesis

The present study aimed to examine the genetic variability within and among old 40 maize genotypes cultivated in the Europe, using SSR, SCoT, RAPD and SDS-PAGE markers. The data collected will contribute to identification, rational exploitation and conservation of germplasms of maize genotypes.

MATERIAL AND METHODOLOGY

Maize genotypes (40) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the Gene Bank in Piesťany, the Slovak Republic. DNA of 40 genotypes of maize was extracted from leaves of 10 day old seedlings using the Gene JET Plant Genomic DNA Purification Mini Kit.

SSR analysis: Amplification of SSR fragments was performed according to **Elçi et al. (2015)** (Table 1). Polymerase chain reaction (PCR) were performed in 20 µl of a mixture containing 7.5 µl H_2O, 10.0 µl Master Mix (Genei, Bangalore, India), 0.75 µl of each primer (10 pmol) and 1 µl DNA (100 ng). Amplification was performed in a programmed thermocycler (Biometra, Germany) and amplification program consisted of an initial denaturing step at 94 °C for 2 min, followed by 35 cycles of amplification [95 °C (30 s), 1 min at the 55 °C, 72 °C (30 s)] and a final elongation step at 72 °C for 10 min. Amplification products were confirmed by electrophoresis in 7% denaturing polyacrylamide gels and silver stained and documented using gel documentation system Grab-It 1D for Windows. Data analysis: For the assessment of the polymorphism between maize genotypes and usability of SSR markers in their differentiation diversity index (DI) **(Weir, 1990)**, the probability of identity (PI) **(Paetkau et al., 1995)** and polymorphic information content (PIC) **(Weber, 1990)** were used.

SCoT analysis: 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. For the assessment of the polymorphism between genotypes maize and usability SCoT markers in their differentiation we used polymorphic information content (PIC) **(Weber, 1990)**.

RAPD analysis: Amplification of RAPD fragments was performed according to **Gajeraa et al. (2010)** (Table 3) using decamer arbitrary primers (Operon technologies Inc, USA; SIGMA-D, USA). Polymerase chain reactions (PCR) were carried out in 25 µl of following mixture: 10.25 µl deionized water, 12.5 µl Master Mix (Promega, USA), 1.25 µl of genomic DNA, 1 µl of 10 pmol of primer. Amplification was performed in a thermocycler (Biometra, Germany) with initial denaturation at 94 °C for 5 min, 42 cycles of denaturation at 94 °C for 1 min, primer annealing at 38 °C for 1 min, extension at 72 °C for 1 min, and 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 Grab-It 1D pre Windows. For the assessment of the polymorphism between genotypes maize and usability RAPD markers in their differentiation we used diversity index (DI) **(Weir, 1990)**, the probability of identity (PI) **(Paetkau et al., 1995)** and polymorphic information content (PIC) **(Weber, 1990)**.

SDS-PAGE analysis: SDS-PAGE was carried out according to the standard reference ISTA method **(Wrigley, 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

Table 1 List of SSR primers of maize.

SSR markers	F primer	R primer
UMC1363	AAAGGCATTATGCTCACGTTGATT	TCTCCCTCCCCTGTACATGAATTA
UMC1004	CTGGGCATACAAAGCTCACA	TGCATAAACCGTTTCCACAA
UMC2002	TGACCTCAACTCAGAATGCTGTTG	CACAAAATCCTCGAGTTCTTGATTG
UMC1117	AATTCTAGTCCTGGGTCGGAACTC	CGTGGCCGTGGAGTCTACTACT
UMC1587	ATGCGTCTTTCACAAAGCATTACA	AGGTGCAGTTCATAGACTTCCTGG
UMC1060	ACAGGATTTGAGCTTCTGGACATT	GGCCTCTCCTTCATCCTATTCAA
UMC1155	TCTTTTATTGTGCCCGTTGAGATT	CCTGAGGGTGATTTGTCTGTCTCT
UMC1072	GAGGAGACCGCCTCTGGTTC	CTTCGGGTTCCTGGACCTTCT
UMC1133	ATTCGATCTAGGGTTTGGGTTCAG	GATGCAGTAGCATGCTGGATGTAG
UMC1413	CATACACCAAGAGTGCAGCAAGAG	GGAGGTCTGGAATTCTCCTCTGTT

Table 2 SCoT markers used in maize**.**

SCoT Primers	Primer sequence (5´-3´)
SCoT 6	CAACAATGGCTACCACGC
SCoT 8	CAACAATGGCTACCACGT
SCoT 9	CAACAATGGCTACCAGCA
SCoT 12	ACGACATGGCGACCAACG
SCoT 23	CACCATGGCTACCACCAG
SCoT 26	ACCATGGCTACCACCGTC
SCoT 28	CCATGGCTACCACCGCCA
SCoT 29	CCATGGCTACCACCGGCC
SCoT 30	CCATGGCTACCACCGGCG
SCoT 36	GCAACAATGGCTACCACC
SCoT 40	CAATGGCTACCACTACAG
SCoT 44	CAATGGCTACCATTAGCC
SCoT 45	ACAATGGCTACCACTGAC
SCoT 54	ACAATGGCTACCACCAGC
SCoT 59	ACAATGGCTACCACCATC
SCoT 60	ACAATGGCTACCACCACA
SCoT 61	CAACAATGGCTACCACCG
SCoT 62	ACCATGGCTACCACGGAG
SCoT 63	ACCATGGCTACCACGGGC
SCoT 65	ACCATGGCTACCACGGCA

Table 3 List of RAPD primers and their sequences.

Primers	Primer sequence (5´-3´)
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-13	CAGCACCCAC
OPB-08	GTCCACACGG
OPD-02	GGACCCAACC
OPD-07	TTGGCACGGG
OPD-08	GTGTGCCCCA
OPD-13	GGGGTGACGA
OPE-07	AGATGCAGCC
OPF-14	TGCTGCAGGT
SIGMA-D-01	AAACGCCGCC
SIGMA-D-14	TCTCGCTCCA
SIGMA-D-P	TGGACCGGTG

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.

Statisic analysis

The SSR, SCoT, RAPD and SDS-PAGE bands were scored as present (1) or absent (0), each of which was treated as an independent character regardless of its intensity. A dendrogram based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) with the SPSS professional statistics version 17 software package was constructed.

RESULTS AND DISCUSSION

SSR analysis: Ten maize SSR primers were used for identification and estimation of the genetic relations among 40 old European maize genotypes. All 10 SSR primers generated clear banding patterns with high polymorphism. Ten SSR primers revealed a total of 65 alleles ranging from 4 (UMC1060) to 8 (UMC2002 and UMC1155) alleles per locus with a mean value of 6.50 alleles per locus (Table 4). Variations in DNA sequences lead to polymorphism. Greater polymorphism is indicative of greater genetic diversity. The PIC values ranged from 0.713 (UMC1060) to 0.842 (UMC2002) with an average value of 0.810 and the DI value ranged from 0.734 (UMC1060) to 0.848 (UMC2002) with an average value of 0.819 (Table 4). 100% of used SSR markers had PIC and DI values higher than 0.7 that means high polymorphism of chosen markers used for analysis.

Similar results were detected by other authors (**Kanagarasu et al., 2013; Molin et al., 2013; Al-Badeiry et al., 2014; Shiri et al., 2014; Efendi et al., 2015; Ignjatovic-Micic et al., 2015; Salami et al., 2016)** and these results presented a high level of polymorphism of old maize genotypes detected by SSR markers. **Kanagarasu et al. (2013)** used 10 SSR molecular markers to analysis of 27 maize inbred lines. Ten SSR markers produced 23 polymorphic alleles with an average of 2.3 alleles per locus and mean polymorphic information content (PIC) of 0.45. The aim of **Molin et al. (2013)** was study the genetic diversity across 48 varieties of maize landraces cultivated at different locations in the States of Rio Grande do Sul and Paraná by 47 simple sequence repeat (SSR) markers. SSR analysis resulted in amplification of 105 polymorphic fragments and a polymorphic index of 78.3%. **Al-Badeiry et al. (2014)** detected 41 alleles among the tested maize varieties using 10 Simple Sequence Repeat (SSR).The molecular size of bands obtained from amplification of SSR products ranged from 91 to 288 bp. Alleles ranged from one in umc1653 to ten in bnlg1189 loci. **Shiri et al. (2014)** study genetic diversity of 38 maize

hybrids using 12 SSR primers. The total number of PCR-amplified products was 40 bands, all of them polymorphic. Primer Phi031 generated the highest number of bands (6). Among the studied primers, UMC2359, PHI031 and UMC1862 showed the maximum polymorphism information content (PIC) and the greatest diversity. The aim of **Efendi et al. (2015)** was to select homozygosity and analyze genetic diversity of 51 maize inbreds using 36 SSRs markers. The research was aimed to select among 51 maize inbreds with high homozygosity and to investigate the genetic diversity using 36 SSRs markers. The result shows that there were 30 inbreds indicating homozygosity level of more than 80%. **Ignjatovic-Micic et al. (2015)** analyzed nine flint and nine dent accessions from six agro-ecological groups, chosen on the basis of diverse pedigrees. Ten SSR primers revealed a total of 63 alleles. High average PIC value (0.822) also supports informativeness and utility of the markers used in this study. The aim of study **Salami et al. (2016)** was to evaluate the genetic diversity of Benin's maize accessions by SSR marker. Thus, 187 maize accessions from three areas were analyzed using three SSR markers. A total of 227 polymorphic bands were produced and showed high genetic diversity. The polymorphic information content (PIC) values for the SSR loci ranged from 0.58 to 0.81, with an average of 0.71.

Table 4 List of SSR primers, total number of bands and the statistical characteristics of the SSR markers used in maize.

Marker name	Number of alleles	DI	PIC	PI
UMC1363	7	0.808	0.799	0.011
UMC1004	6	0.830	0.823	0.005
UMC2002	8	0.848	0.842	0.005
UMC1117	5	0.794	0.780	0.010
UMC1587	7	0.835	0.827	0.006
UMC1060	4	0.734	0.713	0.022
UMC1155	8	0.835	0.830	0.007
UMC1072	7	0.845	0.839	0.004
UMC1133	6	0.818	0.808	0.007
UMC1413	7	0.846	0.841	0.005
Average	**6.50**	**0.819**	**0. 810**	**0.008**

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

SCoT analysis

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 with an average of 5.7 bands per primer (Table 5). 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 5). The polymorphic information content (PIC) value 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 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. **Fang-Yonga 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.

Table 5 Statistical characteristics of the SCoT markers used in maize.

SCoT Primers	TNoB	NoPB	PoPB	PIC
SCoT 6	5	4	80.00	0.729
SCoT 8	4	4	100.00	0.652
SCoT 9	6	4	66.66	0.780
SCoT 12	7	5	71.43	0.715
SCoT 23	7	5	71.43	0.816
SCoT 26	5	4	80.00	0.714
SCoT 28	8	5	62.50	0.846
SCoT 29	6	4	66.66	0.810
SCoT 30	7	6	85.71	0.825
SCoT 36	7	7	100.00	0.812
SCoT 40	6	5	83.33	0.731
SCoT 44	4	2	50.00	0.710
SCoT 45	2	2	100.00	0.374
SCoT 54	5	3	60.00	0.717
SCoT 59	6	3	50.00	0.794
SCoT 60	6	3	50.00	0.790
SCoT 61	6	5	83.33	0.808
SCoT 62	4	4	100.00	0.618
SCoT 63	8	7	87.50	0.832
SCoT 65	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.

RAPD analysis

Our study dealt with detection of genetic polymorphism in maize cultivars using RAPD markers. For the differentiation of forty maize genotypes thirteen RAPD markers (Table 6) were chosen according to **Gajeraa et al. (2010)**.

Table 6 Statistical characteristics of the RAPD markers.

Primers	Number of alleles	DI	PIC	PI
OPA-02	5	0.768	0.755	0.041
OPA-03	7	0.826	0.820	0.007
OPA-13	10	0.874	0.872	0.006
OPB-08	5	0.718	0.709	0.032
OPD-02	6	0.765	0.751	0.049
OPD-07	5	0.725	0.723	0.026
OPD-08	8	0.834	0.829	0.006
OPD-13	9	0.856	0.849	0.005
OPE-07	7	0.835	0.829	0.006
OPF-14	8	0.865	0.862	0.003
SIGMA-D-P	7	0.839	0.833	0.005
SIGMA-D-01	8	0.854	0.849	0.004
SIGMA-D-14	7	0.741	0.728	0.023
Average	**7.08**	**0.808**	**0.801**	**0.016**

Note: SDS-PAGE analysis.

Chosen primers amplified DNA fragments across 40 maize genotypes studied, with the number of amplified fragments ranged from 5 (OPA-02, OPB-08, OPD-07) to 10 (OPA-13). The polymorphic information content (PIC) values varied from 0.709 (OPB-08) to 0.872 (OPA-13), with an average of 0.801 and index diversity (DI) value varied from 0.718 (OPB-08) to 0.874 (OPA-13) with an average of 0.808 (Table 6). Similar values of DI and the PIC were detected by other authors and these values presented a high level of polymorphism of maize genotypes detected by RAPD markers. **Osipova et al. (2003)** used RAPD markers to analyse the genetic divergence between the regenerated plants derived from callus cultures and the original maize line A188. Specific polymorphism revealed with random primers was completely confirmed using five SCAR markers. **De Vasconcelos et al. (2008)** used the RAPD technique to evaluate somaclonal variation in maize plants derived from tissue culture from the maize inbred line L48. Forty seven different decamer oligonucleotide primers generated 221 amplification products, 130 of them being polymorphic. **Mukharib et al. (2010)** used RAPD markers to fingerprint 20 varieties of maize. The primers of the most interest of this purpose were those that produced more variety specific DNA profiles, such as OPD-03, OPE-18, OPF-05, OPL-11 and OPX-04. Much higher number of alleles (7) compared to **Al-Badeiry et al. (2013)**, who detected only one allele, can be caused by diverse set of maize varieties used for analysis. **Mrutu et al. (2014)** assessed the genetic diversity of maize hybrids grown in Southern highlands of Tanzania by using RAPD markers. Twelve maize samples were collected and used in this study. The aim of **Molin et al. (2013)** was to estimate the genetic diversity across 48 varieties of maize landraces cultivated at different locations in the States of Rio Grande do Sul and Paraná by means of different marker system including random amplified polymorphic DNA. Maize landrace accessions were genotyped using the 30 RAPD primers. Similar level of polymorphism (84.44%) obtained also **Bruel et al. (2007)**.

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 were screened. 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. 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. 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.

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 (**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. 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).

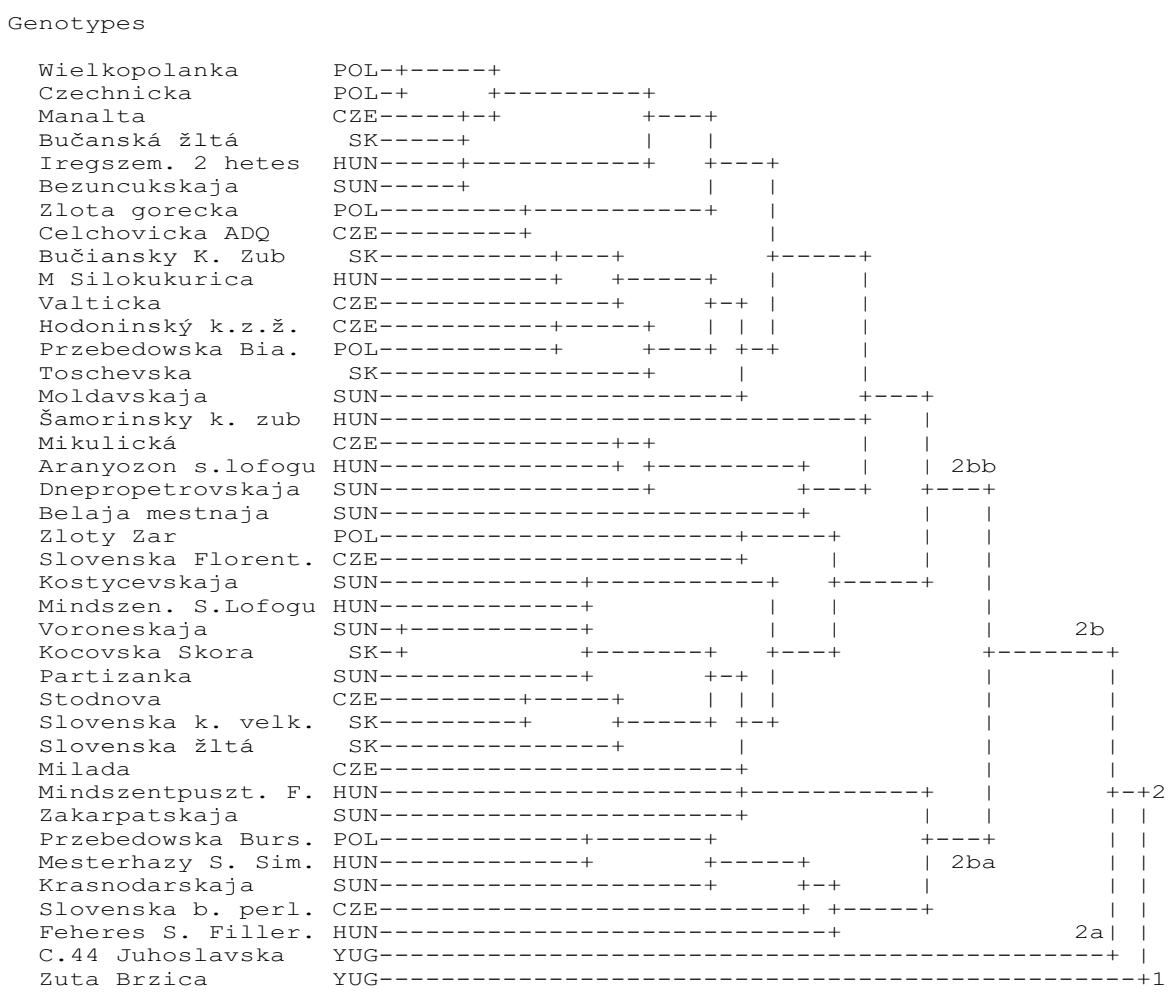

Figure 1 Dendrogram of 40 maize genotypes prepared based on SSR, SCoT, RAPD and SDS-PAGE markers. CZE – Czechoslovakia, HUN – Hungary, POL – Poland, SUN – Union of Soviet Socialist Republics, SK – Slovakia, YUG – Yugoslavia.

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%.

The dendrogram of 40 maize genotypes based on SSR, SCoT, RAPD and SDS-PAGE markers using UGMA algorithm was constructed (Figure 1). 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.

CONCLUSION

The present study indicates the validity of PCR technique for estimating genetic diversity among old maize genotypes. The current data will enhance the breeding efficiency and will add the strength of Marker Assisted Selection (MAS). In the light of information about the genetic diversity in 40 European maize genotypes, it is suggested that the breeding programs with the help of DNA fingerprinting technology will be helpful to utilize the genotypes to produce cultivars/varieties by crossing them with different elite. Genetic diversity plays a key role in crop improvement. In conclusion, a high level of genetic diversity exists among the old maize accessions analyzed. A SSR, SCoT, RAPD and SDS-PAGE markers system are a rapid and reliable method for cultivar identification that might also be used in quality control in certified seed production programs, to identify sources of seed contamination, and to maintain pure germplasm collections. The present study shows effectiveness of employing SCoT, RAPD, SSR and SDS-PAGE markers in analysis of maize, and would be useful for further studies in population genetics, conservation genetics and genotypes improvement.

REFERENCES

Abd El-Azeem, R. M., Hashem, M. H. and Abd-El-Haleem, S. H. M. 2015. Detection of genetic variability in *Zea mays* inbred lines using SSRs and SRAP markers. *Egypt. J. Genet. Cytol.*, vol. 44, p. 291-307.

Al-Badeiry, N. A. H., Merza, T. K. and Al-Saadi, A. H. 2013. Assessment of genetic diversity and relationships among maize *(Zea mays* L.) varieties in Iraq using random amplified polymorphic DNA (RAPD) markers. *J. Life Sci.*, vol. 7, no. 12, p. 1260-1271.

Al-Badeiry, N. A. H., Al-Saadi, A. H., Merza, T. K. 2014. Analysis of Genetic Diversity in Maize (*Zea mays* L.) Varieties Using Simple Sequence Repeat (SSR) Markers. *Journal of Babylon University*, vol. 22, no. 6, p. 1768-1774.

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. https://doi.org/10.1155/2015/874906

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.

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. https://doi.org/10.9755/ejfa.2015-05-204

Bošeľová, D. and Žiarovská, J. 2016. Direct PCR as the platform of *Hedera helix*, L. genotypying without the extraction of DNA. *Journal of Central European Agriculture*, vol. 17, no. 4, p. 941-949. https://doi.org/10.5513/jcea01/17.4.1795

Bruel, D. C., Carpentieri-Pípolo, V., Ruas, C. F., Gerage, A. C. and de Souza, S. G. H. 2007. Assessment of genetic diversity in maize inbred lines using RAPD markers. *Crop Breed. Appl. Biotechnol.*, vol. 7, p. 173-178. https://doi.org/10.12702/1984-7033.v07n02a09

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. https://doi.org/10.1007/s11105-008-0060-5

De Vasconcelos, M. J. V., Antunes, M. S., Barbosa, S. M. and De Carvalho, C. H. S. 2008. RAPD analysis of callus regenerated and seed grown plants of maize (*Zea mays* L.). *Rev. Bras. Milho e Sorgo*, vol. 7, no. 2, p. 93-104. https://doi.org/10.18512/1980-6477/rbms.v7n2p93-104

Dubreuil, P. and Charcosset, A. 1998. Genetic diversity within and among maize populations: a comparison between isozyme and nuclear RFLP loci. *Theor Appl Genet.*, vol. 96, p. 577-587. https://doi.org/10.1007/s001220050776

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. https://doi.org/10.1016/j.lwt.2011.12.019

Efendi, R., Sunarti, S., Musa, Y., Farid, B. M., Danial Rahim, M. and Azrai, M. 2015. Selection of Homozygosity and Genetic Diversity of Maize Inbred using Simple Sequence Repeats (SSRs) Marker. *Int. J. Curr. Res. Biosci. Plant Biol.*, vol. 2, no. 3, p. 19-28.

Elçi, E. and Hançer, T. 2015. Genetic analysis of Maize (*Zea mays* L.) hybrids using microsatellite markers. *Tarim Bilimleri Dergisi – Journal of Agricultural Sciences,* vol. 21, p. 192-198.

Fang-Yong, 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. https://doi.org/10.1016/j.scienta.2014.03.010

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.

Gajeraa, B. B., Kumara, N., Singha, A. S., Punvara, B. S., Ravikirana, R., Subhasha, N. and Jadejab, G. C. 2010. Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. *Ind. Crops Prod.*, vol. 32, p. 491-498. https://doi.org/10.1016/j.indcrop.2010.06.021

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. https://doi.org/10.1007/s00606-013-0939-y

Galvão, K. S., Ramos, H. C., Santos, P. H., Entringer, G. C., Vettorazzi, J. C., Pereira, M. G. 2015. Functional molecular markers (EST-SSR) in the full-sib reciprocal recurrent selection program of maize (*Zea mays* L.). *Genet Mol Res.,* vol. 14, no. 3, p. 7344-7355. https://doi.org/10.4238/2015.july.3.10

Gay, J. P. 1984. Fabuleux mais—Histoire et avenir d'une plante. AGPM, Pau; 295 p.

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.

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. https://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.

Ignjatovic-Micic, D., Ristic, D., Babic, V., Andjelkovic, V. and Vancetovic, J. 2015. A simple SSR analysis for genetic diversity estimation of maize landraces. *Genetika*, vol. 47, no. 1, p. 53-62. https://doi.org/10.2298/GENSR1501053I

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.

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. https://doi.org/10.4238/2014.June.11.4

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. https://doi.org/10.1016/j.indcrop.2014.12.061

Kanagarasu, S., Nallathambi, G., Ganesan, K. N., Kannan, S., Shobhana, V. G. and Senthi, N. 2013. Determination of genetic polymorphism among indigenous and exotic maize inbreds using microsatellite markers. *African Journal of Biotechnology*, vol. 12, no. 39, p. 5723-5728.

Kanti, M., Anjani, K., Usha Kiran, B. and Vivekananda, K. 2015. Agro-morphological and molecular diversity in Castor (*Ricinus communis* L.) germplasm collected from Andaman and Nicobar Islands, India. *Czech J. Genet. Plant Breed*., vol. 51, no. 3, p. 96-109. https://doi.org/10.17221/205/2014-CJGPB

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.

Lawton, J. W. 2006. Isolation of zein using 100% ethanol. *Cereal Chemistry*, vol. 83, no. 5, p. 565-568. https://doi.org/10.1094/CC-83-0565

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. https://doi.org/10.1016/j.bse.2015.07.017

Masojc´, P., Mysków, B. and Milczarski, P. 2001. Extending a RFLP-based genetic map of rye using random amplified polymorphic DNA (RAPD) and isozyme markers. *Theor. Appl. Genet.*, vol. 102, no. 8, p. 1273-1279. https://doi.org/10.1007/s001220000512

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. https://doi.org/10.4238/2013.January.22.8

Mrutu, B. A., Feyissa, T. and Ndunguru, J. 2014. Assessment of genetic diversity of maize inbred lines and hybrids in Southern Highlands of Tanzania by using Random amplified Polymorphic DNA (RAPD) markers. *Am. J. Res. Commun.*, vol. 2, no. 4, p. 84-99.

Mukharib, D. S., Patil, V, C., Biradar, D. P., Salimath, P. M. and Chimmad, V. P. 2010. Assessment of molecular diversity in selected maize inbreds. *Karnataka J. Agric. Sci.*, vol. 23, no. 3, p. 409-412.

Osipova, E. S., Koveza, O. V., Troitskij, A. V., Dolgikh, Y. I., Shamina, Z. B. and Gostimskij, S. A. 2003. Analysis of specific RAPD and ISSR fragments in maize (*Zea mays* L.) somaclones and development of SCAR markers on their basis. *Russ. J. Genet.*, vol. 39, no. 12, p. 1412-1419. https://doi.org/10.1023/B:RUGE.0000009156.74246.bc

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. https://doi.org/10.5897/ajb12.2644

Paetkau, D., Calvert, W., Stirling, I., Strobeck, C. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.*, vol. 4, p. 347-354. https://doi.org/10.1111/j.1365-294X.1995.tb00227.x PMid:7663752

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.

Ražná, K., Bežo, M., Hlavačková, L., Žiarovská, J., Miko, M., Gažo, J., Habán, M. 2016. MicroRNA (miRNA) in food resources and medicinal plant. *Potravinarstvo*, vol. 10, no. 1, p. 188-194. https://doi.org/10.5219/583

Roy, N. S. and Kim, N. S. 2016. Genetic diversity analysis of maize lines using AFLP and TE-based molecular marker systems. *Genes Genom*., vol. 38, p. 1005-1012. https://doi.org/10.1007/s13258-016-0461-z

Sa, K. J., Park, J. Y., Park, K. C. et al., 2012. Analysis of genetic mapping in a waxy/dent maize RIL population using SSR and SNP markers. *Genes Genom*., vol. 34, p. 157-164. https://doi.org/10.1007/s13258-011-0208-9

Saker, M., Nagchtigall, M., Kuehne, T. A. 2005. Comparative assessment of DNA fingerprinting by RAPD, SSR and AFLP in genetic analysis of some barley genotypes. *Egypt J Genet Cytol.*, vol. 34, p. 81-97.

Salami, H. A., Sika, K. C., Padonou, W., Aly, D., Yallou, C., Adjanohoun, A., Kotchoni, S. and Baba-Moussa, L. 2016.Genetic Diversity of Maize Accessions (*Zea mays* L.) Cultivated from Benin Using Microsatellites Markers. *American Journal of Molecular Biology*, vol. 6, p. 12-24. https://doi.org/10.4236/ajmb.2016.61002

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. https://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. https://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. https://doi.org/10.12692/ijb/5.2.14-22

Shiri, M. R., Choukan, R. and Aliyev, R. T. 2014. Study of genetic diversity among maize hybrids using SSR markers and morphological traits under two different irrigation conditions. *Crop Breeding Journal*, vol. 4, no. 1, p. 65-72.

Simões, K. S., Silva, S. A., Machado, E. L. and Brasileiro, H. S. 2017. Development of TRAP primers for *Ricinus communis* L. *Genetics and Molecular Research,* vol. 16, no. 2, p. 100-113. http://dx.doi.org/10.4238/gmr16029647

Sun, D. F., Ren, W. B., Sun, Gl., Peng, J. H. 2011. Molecular diversity and association mapping of quantitative traits in Tibetan wild and worldwide originated barley. *Euphytica*, vol. 178, p. 31-43. https://doi.org/10.1007/s10681-010-0260-6

Vasal, S. K. 1999. Quality protein maize story. Proceedings of workshop on improving human nutrition through agriculture: the role of international agricultural research. IRRI, Los Banos, Philippines, p. 1-16.

Vivodík, M., Gálová, Z., Balážová, Ž., Petrovičová, L. 2016. Start codon targeted (SCoT) polymorphism reveals genetic diversity in European old maize (*Zea mays* L.)

genotypes. *Potravinarstvo*, vol. 10, no. 1, p. 563-569. https://doi.org/10.5219/660

Vivodík, M., Gálová, Z., Balážová, Ž., Petrovičová, L., Kuťka Hlozáková, T. 2016. Genetic variation and relationships of old maize genotypes (*Zea mays* L.) detected using SDS-PAGE. *Potravinarstvo*, vol. 10, no. 1, p. 532-536. https://doi.org/10.5219/661

Vyhnánek,T., Trojan, V., Štiasna, K., Presinszká, M., Hřivna, L., Mrkvicová, E., Havel, L. 2015. Testing of DNA isolation for the identification of Hemp. *Potravinarstvo*, vol. 9, no. 1, p. 393-397. https://doi.org/10.5219/509

Weber, J. L. 1990. Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics*, vol. 7, p. 524-530. https://doi.org/10.1016/0888-7543(90)90195-Z

Weir, B. S. 1990. Genetic data analysis. Methods for discrete population genetic data, 1990 p. ISBN 0-87893-872-9

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, vol. 18, p. 6531-6535. https://doi.org/10.1093/nar/18.22.6531 PMid:1979162

Wrigley, C. W. 1992. Identification of cereal varieties by gel electrophoresis of the grain proteins. Heidelberg: Springer – Verlag, p. 17-41. https://doi.org/10.1007/978-3-662-01639-8_2

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. https://doi.org/10.3390/molecules20045987

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. https://doi.org/10.5513/JCEA01/14.2.1227

Žiarovská, J., Grygorieva, O., Zeleňáková, L., Bežo, M., Brindza, J. 2015. Identification of sweet chesnut pollen in bee pollen pellet using molecular analysis. *Potravinarstvo*, vol. 9, no. 1, p. 352-358. https://doi.org/10.5219/497

Žiarovská, J., Kyseľ, M., Cimermanová, R., Knoteková, L. 2017. Effect of DNA extraction method in the *Rosa Canina* L. identification under different processing temperature. *Potravinarstvo Slovak Journal of Food Sciences,* vol. 11, no. 1, p. 190-196. https://dx.doi.org/10.5219/717

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Voltammetric determination of cholecalciferol at glassy carbon electrode performed in water-ethanol mixture

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ABSTRACT

To confirm or disprove previous hypotheses, cyclic voltammetry of 0.5 mM cholecalciferol (vitamin D_3) at glassy carbon electrode (GCE) and platinum disk electrode (PtE) in pure acetonitrile and water-ethanol mixture at 50 $mV \cdot s^{-1}$ has been used to investigate the oxidation mechanism. The oxidation occurs in two one-electrone steps. According to calculation of the highest electron density in cholecalciferol molecule which is evidently delocalized over carbon atoms of the three conjugated double bonds (C19, C10, C5-C8) points to part of the molecule involved in oxidation processes. An oxidation peak (at +0.925 V vs. Ag/AgCl) was used to develop direct voltammetric method based on differential pulse voltammetry for the vitamin D_3 determination at GCE performed in 40% ethanol containing 0.1 M $LiClO_4$. Under optimization of analytical procedure, it was found that a composition of the supporting electrolyte used significantly affects a current response of oxidation peak obtained. Satisfactory sensitivity was achieved in the 1:1 water-ethanol mixture containing 0.05 M lithium perchlorate as as supporting electrolyte. The linear range for vitamin D_3 determination was $2.4 \times 10^{-6} - 3.5 \times 10^{-4}$ M with the detection limit of 8.0×10^{-7} M. This work demonstrates a fact that the GCE is suitable electroanalytical device for analysis of various food supplements and medicaments.

Keywords: cholecalciferol; anodic differential pulse voltammetry; glassy carbon electrode; vitamin food supplements

INTRODUCTION

As known, vitamin D belongs to the group of lipophilic vitamins **(Webster, 2012)**. In a narrower sense, it is included into the group of fat-soluble steroid substances liable for increasing of intestinal absorption of calcium and other minerals **(Wang, 2013)**. Vitamin D occurrs in two different forms **(Thacher et al., 2009)** such as vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Generally, cholecalciferol is naturally generated in human body during exposure of skin to UV radiation **(Wacker and Holick, 2013)**. In contrast, the ergocalciferol present in muschrooms is produced by activation of ergosterol at the same radiation **(Bikle, 2014)**.

In case of avitaminosis, sufficient intake of vitamin D-rich food is necessary. Due to low content of vitamin D in foodstuffs usually µg per 100 g **(Sabolová et al., 2016)**, it can be ingested from commercial supplements which were one of the targets of this work. Already mentioned avitaminosis has a direct influence on the absorption of calcium and phosphorus in the body, especially in the proximal section of the small intestine **(Christakos et al., 2014)**. It is related with symptoms of several diseases such as bone thinning and rhinitis in children **(Kutluğ et al., 2017)**. From the medical environmental point of view, several reports appeared in scientific papers refering the fact that deficiency of vitamin D may also cause cardiovascular disease, immunological abnormalities, and cancer **(Zittermann et al., 2012; Lappe et al., 2007)**. From previous paragraphs, it is evident that development of sensitive analytical methods for determination of cholecalciferol is important in several branges such as food quality control, medicine, and pharmacy.

Nowadays, standard analytical method for determination of ergocalciferol and cholecalciferol in foodstuffs ČSN EN 12821 (560047) is used. After saponification from selected foodstuff, vitamins are extracted with a suitable solvent. Determination of these vitamins in suitable sample extracts is usually performed by normal phase semipreparative high-performance liquid chromatography (HPLC) followed by reverse phase analytical HPLC. Subsequent detection is spectrometric in the UV-area. Nevertheless, it is necessary to state that the existing standard method is characterized by some disadvantages such as time analysis including complex sample preparation, high consumption of organic solvents, and high acquisition costs.

Especially, the last mentioned disadvantage represents the main problem for small routine laboratories. For that

reason, the aim of this work was to develop a simple electroanalytical procedure to determine cholecalciferol based on direct anodic oxidation at glassy carbon electrode (GCE) performed in water-ethanol mixture using pulse voltammetric technique, namely different pulse voltammetry (DPV). The main objective of this work was to verify whether the direct voltammetric method, if applied, can achieve the necessary sensitivity required for quantitative analysis of food supplements and become therefore an alternative way.

Scientific hypothesis

Two different reaction mechanisms of anodic oxidation of cholecalciferol were proposed in the literature. Probably just one of these two versions is true. Older hypothesis argues that cholecalciferol is anodically hydroxylated on C8 and C7 which are part of a conjugated system of three double bonds **(Webster, 2012; Canevari et al., 2014)**. In contrast, a completely different reaction mechanism is proposed in a recent study **(Filik and Avan, 2017)**. Authors claim that hydroxylation of C25 located in the aliphatic happens. One of the aims of this work was to select one of these claims as more credible.

MATERIAL AND METHODOLOGY

Chemicals and reagents

Analytical standard of crystalline cholecalciferol was purchased from Merck (Darmstadt, Germany). Anhydrous lithium perchlorate with 99.9% acetonitrile (ACN) from Sigma Aldrich (Prague, Czech Republic), 96% ethanol from Lach-Ner, s.r.o. (Neratovice, Czech Republic), and demineralized water (ρ = 18.3 MΩ·cm; Milli-Q system, Millipore) and were used for preparation of supporting electrolytes (50% ethanol containing 0.05 M $LiClO_4$ and 0.1 M $LiClO_4$ in pure ACN). Due to relatively high chemical stability, only the 0.01 M cholecalciferol stock solution in pure ethanol was stored in a refrigerator at 5 C.

Instrumentation

All electrochemical experiments were carried out in the 20 mL supporting electrolyte at 25 °C. Typical three-electrode system consisting of solid GCE (or platinum disk electrode; PtE), Ag/AgCl/3.0 M KCl (reference) and platinum wire (auxiliary) electrodes was connected to potentiostat Autolab PGSTAT101 from Metrohm (Prague, Czech Republic) operating with software Nova version 1.11.

Pretreatment of glassy carbon electrode

Due to the fact that products of cholecalciferol electrochemical oxidation remain adsorbed on the surface of the GCE, it was necessary to renovate the electrode surface by rinsing with pure hexane and subsequently by polishing on a furry pad with presence of wet alumina powder (particle size 1.0 μm) for 10 s. This time duration is also recommended by the accompanying manual (Metrohm). After subsequent rinsing of the surface by distilled water, the GCE was ready for further electrochemical experiments.

Sampling

Two different food supplements Optisana (calcium + vitamin D) available in Tesco Stores Czech Republic ČR a. s. and PRO formula (calcium, D_3 & K) from Lidl Czech Republic v.o.s. were analysed. Usually eight tablets were dissolved in the supporting electrolyte in a 50 mL volumetric flask and subsequently sonicated for 30 minutes at laboratory conditions. The resultant dispersion was filtered using stacked filter paper. After that, a volume of 10 mL resulting filtrate was inserted into voltammetric cell and analysed by standard addition methods where three 50 μL aliquots of 0.001 M cholecalciferol were used. Analyses of each sample were repeated at least three times.

Procedure

Cyclic voltammetry (CV) of 500 μM cholecalciferol at GCE and PtE in 0.1 M $LiClO_4$ in pure ACN as supporting electrolyte was performed for study of its electrochemical behaviour. Potential window from -0.2 to +1.6 V (or +0.2 to -0.6 V), step potential (E_{step}) 5 mV, scan rate (v) 50 mV·s^{-1}, and five repetitive cycles were chosen as working conditions. Differential pulse voltammetry (DPV) of cholecalciferol at GCE was used under following conditions: the applied potential from 0 to +1.2 V, E_{step} = 5 mV, potential of amplitude (E_{ampl}) 140 mV, interval time (t_{int}) 0.5 s, and v = 50 mV·s^{-1}. All voltammetric measurements were repeated at least 5 times. If not stated, otherwise all changes in the experimental conditions are shown in the legends below the corresponding figures.

Validation of differential pulse voltammetry

Each analytical method is defined by three main parameters, namaly sensitivity, accuracy, and precission. The sensitivty is defined as the lowest detectable concentration of an analyte (limit of detection; LOD) and is given by a slope of corresponding calibration curve (k). From a practical point of view, limit of quantification (LOQ) is a more important parameter than the theoretical value of LOD because it usually represent the lowest value of the calibration curve. Values of LOQ and LOD were calculated according to the following equations LOQ = 10σ/k and LOD = 3σ/k, respectively, where σ is the standard deviation of measurement (n = 5) of the concentration for lowest concetration of the calibration curve.

The accuracy is usually defined as a consistency of repeated measurements under the same conditions and it can be calculed as RSD = σ/$\bar{x}$*100, where $\bar{x}$ is the arithmetic mean of minimally five repetitions.

The precission represents an accordance between the real concentration of analyte and that found by an analytical method used. This analytical parameter is often verified using a model sample (recovery), declared amount, or by comparison with a reference method based on another physicochemical principle.

Statisical analysis

Within the optimization, calculation of p-value (X) to determine statistical significance of evidence was used. The p-value is widely used in statistical hypothesis H testing, specifically in null hypothesis H_0 significance testing. Before performing an experiment, the null hypothesis is chosen for threshold value which is called the significance level of the test, traditionally 5% and

denoted as α. The smaller the p-value, the higher the significance because it tells the investigator that the *H* under consideration may not adequately explains the observation. The H_0 is rejected if any of these probabilities is less than or equal to a small, fixed but arbitrarily pre-defined threshold value α which is referred to as the level of significance.

Within analysis of vitamin supplements, a standard reference analytical method ČSN EN 12821 (560047) did not used for comparison. Due to analysis of only two samples (n = 2), normality test, ANOVA or t-test were not required to apply. In this case, calculation of the recovery (%) for model sample was found to be sufficient.

RESULTS AND DISCUSSION

Electrochemical behaviour of cholecalciferol

One of the main objectives of this study was to confirm or refute the previous allegations regarding to cholecalciferol oxidation mechanism due to the major inconsistencies previously published. Two different electrode reaction mechanisms are listed in the literature, namely hydroxylation of C25 located in the aliphatic chain **(Filik and Avan, 2017)** or hydroxylation of C8 and C7 located in a conjugated system of double bonds **(Canevari et al., 2014; Webster, 2012)**.

Theoretically, the mechanism of the electrode reaction can be predicted according to quantum chemical calculations because every redox process is generally associated with an electron exchange. Knowledges of the distribution of the electron charges in certain molecule can help to determine individual reaction centres. It means that molecular orbitals with an excess of electrons are more easily oxidized than those with a deficiency. Thus, the prediction of cholecalciferol electrochemical behaviour can be facilitated by theoretical calculation of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO). However, steric hindrances, especially accessibility of the reaction center to the surface, should be taken into account. Calculated orbitals for cholecalciferol are shown in Figure 1.

Program Gaussian 09 with density functional method B3LYP was used for calculation. Based on the results obtained, it can be predicted that an electrochemical oxidation is delocalized over carbon atoms of the three conjugated double bonds (C8-C19), where the highest current density has been found. Due to this delocalized electron system, it is not possible to decide with certainty which carbon of the conjugate system is oxidized primarily. It is also evident that carbon atoms of the side aliphatic chain (C20-C27) are not energy efficient for electrochemical oxidation.

Cyclic voltammetry as a frequently used analytical technique for study of electrochemical processes has been preferred. Cholecalciferol provided two oxidation peaks at +1.05 and +1.15 V and two reduction peaks at +0.13 and -0.02 V in the back-cathodic scan at PtE in non-aqueous supporting electrolyte (pure ACN). It was confirmed that theese two cathodic signals corresponded to the electrochemical reduction of the resulting oxidation products because no reduction peak was observed for cathodic scan form +0.2 to -0.6 V (see Figure 2). Very similar electrochemical behaviour of cholecalciferol was obtained at GCE at the same conditions. Thanks to the worst charge transfer, only one oxidation (at +1.18 V) and one reduction peaks (at -0.14 V) were observed (not shown). Moreover, it is very important to mention that no reduction peak was obtained in water-organic mixures.

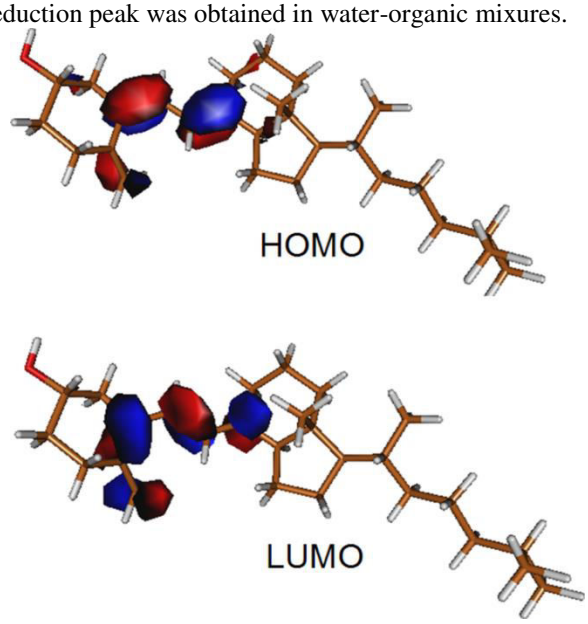

Figure 1 Calculated orbitals for cholecalciferol.

Within scan rate study (not shown), it was found that oxidation peak current linearly ($R^2 = 0.9996$) increased with the square root of the scan (*v*). Moreover, slope (*k*) value 0.419 for linear dependency of the peak current logarithm on log *v* was obtained. For this finding, diffusion-controlled electrochemical oxidation reaction can be accepted.

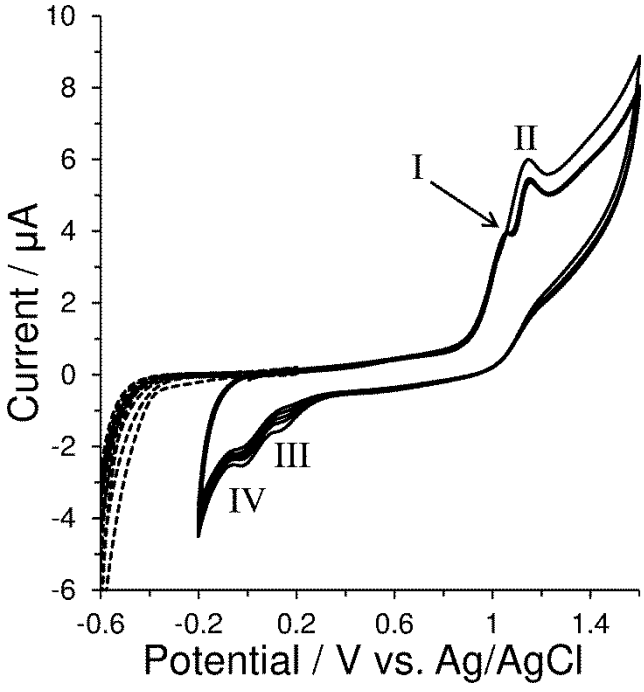

Figure 2 Cyclic voltammetry of 0.5 mM cholecalciferol at PtE performed in 98% ACN containg 0.1 M $LiClO_4$ at 50 $mV \cdot s^{-1}$.

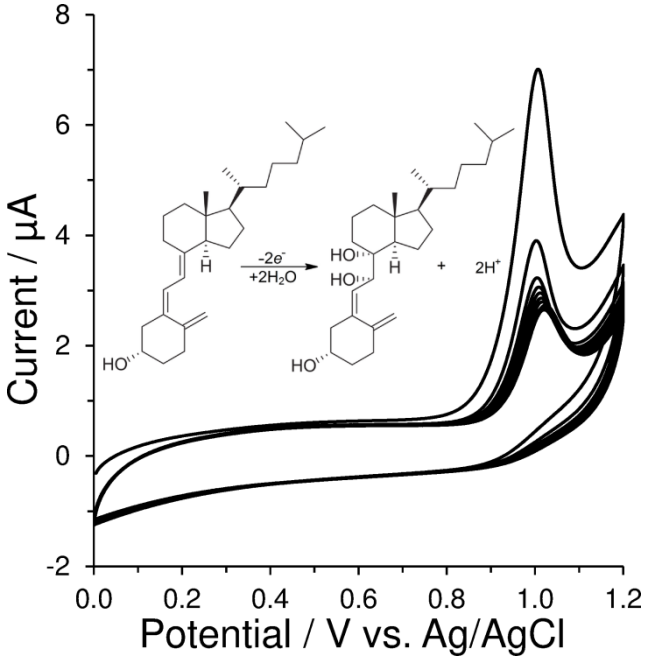

Figure 3 Repettive cyclic voltammetry (10 cycles) of 0.2 mM cholecalciferol at GCE performed in 50% ethanol containg 0.1 M $LiClO_4$ at 50 $mV \cdot s^{-1}$.

Thus, it can be concluded that the cholecalciferol undergoes chemically irreversible anodic oxidation processes at GCE in used water-ethanol mixture (see Figure 3), which corresponds to previously published results **(Chan et al., 2014; Sýs et al., 2016)**.

Thus, it can be concluded that a reaction mechanism (see Figure 3 insert) proposed by **Webster (2012)** is more probable than that from **Filik and Avan (2017)**. However, it seems that electrochemical techniques do not represent sufficient tools to determine the cholecalciferol oxidation mechanism. This should be considered as the identification of the voltammetric oxidation product is a necessary way to design a mechanism with certainty.

Optimization of supporting electrolyte composition

Selection of the working electrode, supporting electrolyte, and setting of the electrochemical teqnique represent the main factors influencing the final analytical parameters of the direct voltammetric method. Due to insolubility of cholecalciferol in strictly aqueous solutions, it was necessary to find optimum content of ethanol and $LiClO_4$. The highest value of anodic peak current (I_p) was obtained when 50% content of ethanol was used. For the higher content of ethanol, evidently lower constant peak current responses were observed (see Figure 4). For that reason, 50% content of ethanol was chosen as the optimum.

Generally, solubility of the lipophilic substance in the water-organic mixture decreases with a higher salt content due to the increasing of ionic strength (I) defined as the sum of all electrically charged particles (positive and negative ions) present in the solution. Nevertheless, it is important to mention that the conductivity (G) of the supporting electrolyte increases with higher salt content. Concentrations higher than 50 mM did not cause peak heights to increase. The above salt content was therefore used as the optimum (see Figure 5).

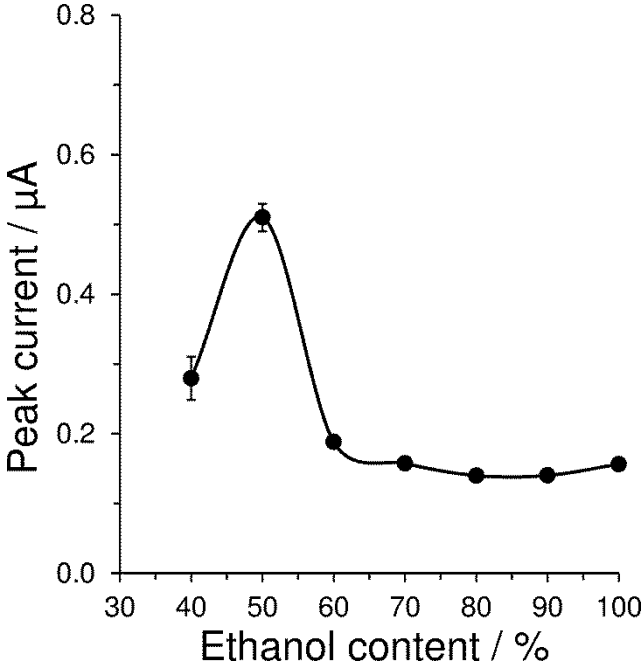

Figure 4 Dependency of peak current on amount of ethanol in supporting electrolyte containing always 0.1 M $LiClO_4$. For concetration of 100 µM cholecalciferol measured at GCE by DPV at E_{step} = 5 mV, E_{ampl} = 25 mV, t_{int} = 0.5 s, and v = 50 $mV \cdot s^{-1}$.

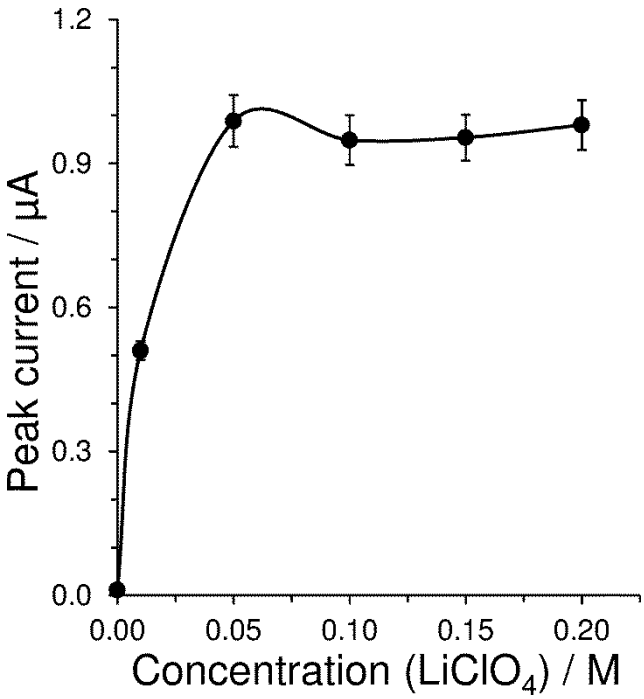

Figure 5 Dependency of peak current on amount of $LiClO_4$ in supporting electrolyte containing always 50% ethanol. For concetration of 100 µM cholecalciferol measured at GCE by DPV at E_{step} = 5 mV, E_{ampl} = 25 mV, t_{int} = 0.5 s, and v = 50 $mV \cdot s^{-1}$.

Electrochemical detection of cholecalciferol using DPV

The optimization of differential pulse voltammetric detection focused on finding the proper pulse amplitude and scan rate, which influence the peak current the most importantly. It was found that setting pulse amplitude higher than 140 mV did not significantly increase the peak current response, because it was calculated that values of corresponding peak current belonged to the interval for *p*-value 0.05.

Analogous behaviour was found for the effect of the scan rate higher than 50 mV·s^{-1}. Typical records of voltammograms together with the corresponding calibration curve are shown in Figure 6.

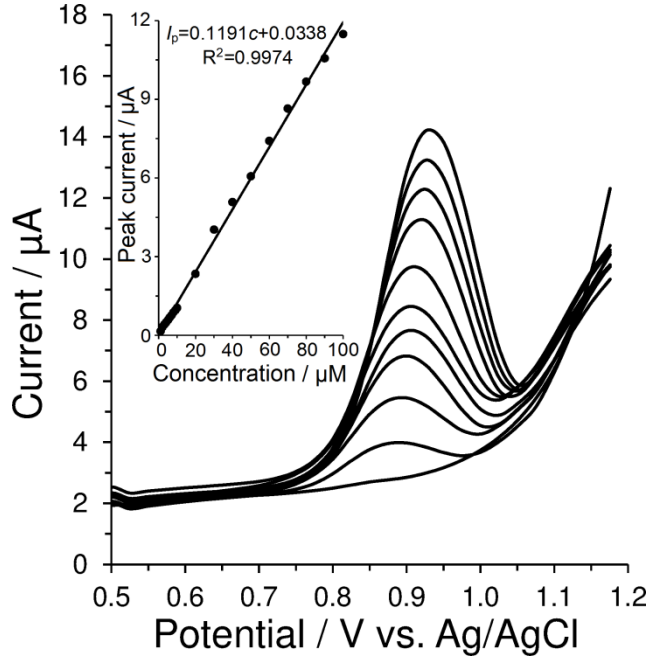

Figure 6 Voltammograms for 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µM cholecalciferol with corresponding calibration curve obtained at GCE. Measured by DPV at E_{step} = 5 mV, E_{ampl} = 140 mV, t_{int} = 0.5 s, and v = 50 mV·s^{-1}.

All analytical parameters are demonstrated together with those of previously published direct voltammetric method (see Table 1). Evidently, this voltammetric method based on direct anodic oxidation in water-ethanol mixture is sensitive for analysis of samples (fish oils, food supplements, pharmaceutical products like Vigantol etc.) with relatively high content of vitamin D_3. Unfortunately, it cannot be applied in the analysis of milk and products derived from it, clinical samples, etc.

Herein, it can be concluded that voltammetric method developed by **Cincoto et al. (2014)** based on anodic oxidation at GCE in water-ethanol mixtures was thoroughly verified. Fortunately, significant progress in linear range of calibration curve was achieved after setting completely new optimum working conditions. Therefore, it seems that some improvement has been achieved in the already published method (**Cincoto et al., 2014**).

Using repeated voltammetric measurements of 10 µM cholecalciferol, value of RSD was calculated. Values 3.2% from peak height and 3.9% from peak area were found. Both these values indicate a satisfactory repeatibility of voltammetric measurements because they are lower than 5%, and are therefore considered optimal at a significance level $\alpha = 0.05$.

Voltammetric determination of cholecalciferol in vitamin food supplements

First, the developed voltammetric method was validated by analysis of 10 mL model 5.0 µM cholecalciferol sample. It was analyzed by the standard addition method (three 10 µL consecutive additions of 0.01 M cholecalciferol stock solution in 98% ethanol). A recovery of 94.9 % was calculated from three repettions.

Two different types of food supplements such as Optisana (calcium + vitamin D) available in Tesco Stores Czech Republic ČR a. s. and PRO formula (calcium, D_3 & K) from Lidl Czech Republic v.o.s. were analyzed by this developed voltammetric standard addition method

Corresponding analyses of food supplements were repeated minimally five times, the evaluated results showed correlation coefficients (R^2) of minimally 0.9992 (not shown). The final results from DPV measurements of these food supplements on the content of vitamin D were compared with declared amounts listed by distributors (see Table 2). With pure consciency, it can be stated that the developed voltammetric method provides satisfactory results, and could be used for routine food analysis in more simply equipped laboratories.

Table 1 Comparison of conventional voltammetric methods developed for determination of cholecalciferol.

Sensor	Techniq ue	Supporting electrolyte	Linearity (µM)	LOD (µM)	References
rotating GCE	DPV	75 mM $LiClO_4$ in Me-OH	2.0 to 200	2.0	**(Hernández Mendez et al., 1988)**
GCE/SiO_2/GO/$Ni(OH)_2$	DPV	0.1 M NaOH	5.0 to 50	0.003	**(Canavari et al., 2014)**
GCE	DPV	0.1 M $LiClO_4$ in 40% Et-OH	0.3 to 4.3	0.12	**(Cincoto et al., 2014)**
GCE/poly(ARS)MWCNTs	SWV	0.05% SDS in 1.5 M AAS	8.0 to 160	5.0	**(Filik et al., 2017)**
GCE	DPV	50 mM $LiClO_4$ in 50% Et-OH	2.4 to 350	0.8	**(Present work)**

Note: AAS; ammonium acetate solution, DPV; differential pulse voltammetry, Et-OH; ethanol, GCE/poly(ARS)MWCNTs; poly (Alizarin red S)/multi-walled carbon nanotubes modified glassy carbon electrode, GCE/SiO_2/GO/$Ni(OH)_2$; amorphous nickel (II) hydroxide particles onto a hybrid material composed of silica and graphene oxide modified glassy carbon electrode, LOD; limit of detection, Me-OH; methanol, SDS; sodium dodecyl sulfate, SWV; square wave voltammetry.

Table 2 Analysis of selected food supplements.

Food supplements	Distributor	DPV (µg per tablet)	Declared amount (µg per tablet)	Recovery (%)
Optisana (calcium + vitamin D)	Tesco Stores Czech Republic ČR a. s.	5.8 ±0.3	5.0	116
PRO formula (calcium, D_3 & K)	Lidl Czech Republic v.o.s.	5.3 ±0.4	5.0	106

Note: Values given as arithmetic means with appropriate standard deviations for five analyses.

CONCLUSION

In this contribution, the cyclic voltammetry of cholecalciferol using GCE as well as disk PtE in water-ethanol mixture and in pure ACN was performed and evaluated to clarify its electrochemical reaction mechanism. Under this study, it was found that cholecalciferol is electrochemically oxidized in which probably correspond to oxidation in delocalized electron system of conjugated double bonds. This is confirmed by the results obtained with quantum chemical calculations.

This paper also represents a secondary study on already developed electroanalytical method to determine cholecalciferol, commonly known as vitamin D_3. In this case, it can be concluded that that certain improvements in analytical parameters were achieved, especially evident extension of the linear range. On the other hand, an analysis of more complex samples can not be done without a time-consuming sample preparation consisting a hydrolysis and subsequent extraction into a suitable organic solvent as it is as is the case with standard analytical method ČSN EN 12821 (560047) based on HPLC.

REFERENCES

Bikle, D. D. 2014. Vitamin D metabolism, mechanism of action, and clinical applications. *Chemical Biology*, vol. 21, no. 3, p. 319-329. https://doi.org/10.1016/j.chembiol.2013.12.016

Canevari, T. C., Cincotto F. H., Landers, R., Machado, S. A. S. 2014. Synthesis and characterization of a-nickel (II) hydroxide particles on organic-inorganic matrix and its application in a sensitive electrochemical sensor for vitamin D determination. *Electrochimica Acta*, vol. 147, no. 1, p. 688-695. https://doi.org/10.1016/j.electacta.2014.10.012

Chan, Y. Y., Yue, Y., Webster, R. D. 2014. Voltammetric studies on vitamins D_2 and D_3 in organic solvents. *Electrochimica Acta*, vol. 138, no. 20, p. 400-409. https://doi.org/10.1016/j.electacta.2014.06.133

Christakos, S., Lieben, L., Masuyama, R., Carmeliet, G. 2014. Vitamin D endocrine system and the intestine. *Bonekey Reports*, vol. 3, no. 1, p. 496-453. https://doi:10.1038/bonekey.2013.230

Cincotto, F. H., Canevari, T. C., Machado, S. A. S. 2014. Highly sensitive electrochemical sensor for determination of vitamin D in mixtures of water-ethanol. *Electroanalysis*, vol. 26, no. 12, p. 2783-2788. http://dx.doi.org/doi:10.1002/elan.201400451

Filik, H., Avan, A. A. 2017. Simultaneous electrochemical determination of vitamin K_1 and vitamin D_3 by using poly (Alizarin red S)/multi-walled carbon nanotubes modified glassy electrode. *Current Analytical Chemistry*, vol. 13, no. 5, p. 350-360. http://dx.doi.org/doi:10.2174/1573411013666170105143113

Hernández-Méndez, J., Sánchez-Pérez, A., Delgado-Zamarreño, M., Hernández-Garcia, M. L. 1988. Voltammetric determination of vitamin D_3 with a rotating glassy carbon electrode. *Journal of Pharmaceutical and Biomedical Analysis*, vol. 6, no. 6-8, p. 737-741. https://doi.org/10.1016/0731-7085(88)80085-2

Kutluğ, S., Kılıç, M., Bilgici, B., Paksu, Ş., Yıldıran, A., Sancak, R. 2017. An evaluation of vitamin D levels in children with seasonal allergic rhinitis during pollen season. *Pediatric Allergy and Immunology,* vol. 28, no. 5, p. 446-451. https://doi.org/10.1111/pai.12728

Lappe, J. M., Travers-Gustafson, D., Davies, K. M., Recker, R. R., Heaney, R. P. 2007. Vitamin D and calcium supplementation reduces cancer risk: Results of a randomized trial. *The American Journal of Clinical Nutrition,* vol. 85, no. 6, p. 1586-1591. PMid:17556697

Sabolová, M., Adámková, A., Kouřimská, L., Chrpová, D., Pánek, J. 2016. Minor lipophilic compounds in edible insects. *Potravinarstvo*, vol. 10, no. 1, p. 400-406. https://doi.org/10.5219/605

Sýs, M., Žabčíková, S., Červenka, L., Vytřas, K. 2016. Adsorptive stripping voltammetry in lipophilic vitamins determination. *Potravinarstvo,* vol. 10, no. 1, p. 260-264. https://doi:10.5219/587

Thacher, T. D., Obadofin, M. O., O'Brien, K. O., Abrams, S. A. 2009. The effect of vitamin D_2 and vitamin D_3 on intestinal calcium absorption in nigerian children with rickets. *The Journal of Clinical Endocrinology & Metabolism,* vol. 94, no. 9, p. 3314-3321. https://doi.org/10.1210/jc.2009-0018

Wacker, M., Holick, M. F. 2013. Sunlight and Vitamin D a global perspective for health. *Dermato-Endocrinology*, vol. 5, no. 1, p. 51-108. https://doi.org/10.4161/derm.24494

Wang, Ch. 2013. Role of vitamin D in cardiometabolic diseases. *Journal of Diabetes Research*, vol. 2013, no. 1, p. 1-10. https://doi.org/10.1155/2013/243934

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. https://doi.org/10.1002/tcr.201100005

Zittermann,A., Borgermann, J., Gummert, J. F., Pilz, S. 2012. Future directions in vitamin D and cardiovascular research. *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 22, no. 7, p. 541-546. https://10.1016/j.numecd.2012.02.004

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Conflict of interest:

All authors declare no conflict of interest.

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EVALUATION OF QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF SELECTED CELERY (*APIUM GRAVEOLENS* VAR. *DULCE*) VARIETIES IN THE CONTEXT OF JUICES PRODUCTION

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ABSTRACT

Celery, *Apium graveolens* var. *Dulce* (Mill.) is a biennial plant, lesser used in Slovakia in comparison with celeriac, but with increasing popularity because of its very health beneficial properties. The aim of the study was to evaluate quantitative (yields) and qualitative (chlorophyll *a* and *b*, soluble solids) parameters in selected patioles of celery in the context of raw juice production in a small-plot field experiment. Six varieties of celery (var. *Dulce*) were planted - ´Helios´, ´Red Soup´, ´Malachit´, ´Verde Pascal´, ´Golden Self- Blanching´ and ´Celebrity´. The harvest was carried out twice a season in terms of August and September. The chlorophyll *a* and chlorophyll *b* were determined spectrophotometrically, Total soluble solids were estimated by the help of refractometer. The lowest values in yields reached ´Malachit´variety, the highest ´Red Soup´ variety. In the evaluation of both harvests the ´Celebrity´ and ´Golden S.´ varieties showed the lowest chlorophyll content. The total soluble solid content reached values ranging from 5.00 to 8.77 °BRIX, in the following order: ´Celebrity´ <´Helios´ <´Golden S.´ < ´Red Soup´ <´Verde Pascal´. The term of harvest had significant influence on chlorophyll *a* and *b*. The effect wasn´t confirmed on yields and soluble solids according to used statistical analyse. There were mainly other kind of characteristics followed in previous scientific studies about celery (var. *Dulce*), such a flavonoids, vitamins, minerals, fibre, the essential oils and phenolic acids, etc. Processing to juices or smoothies allow consuming of other antioxidant – chlorophyll.

Keywords: celery; yields; chlorophyll; total soluble solids

INTRODUCTION

Celery, originating from the Mediterranean area of southern Europe and from the swamps of Egypt and Sweden **Helaly et al. (2013)** is comprised of three cultivated forms: a) celeriac, *Apium graveolens* var. *rapaceum* (Mill.) Gaud. Beaup., which is widespread mainly in Central Europe; b) celery, *Apium graveolens* var. *Dulce* (Mill.) DC., grown primarily in Western Europe and the USA; and the least known c) leaf celery, *Apium graveolens* L. var. *secalinum* Alef., which is mainly used as a spice plant **Rožek (2013)**. *Apium graveolens* L. is particularly popular in Western Europe, Japan and in the USA, where no celeriac is ever grown. It is a biennial plant and it belongs to lesser known vegetables in Slovakia. In the first year, a rosette of upright leaves and long, sharpened stems is created, coloured to white, yellow or green **Uher et al. (2009)**. These long, three to seven centimetres wide, strong flashy and juicy leaf stems (petioles) are utility part of the plant **Pekárková (2004)**. Celery petioles are an excellent part of healthy nutrition. Celery is rich in a variety of nutrients like vitamins, minerals and proteins and it has many pharmacological efficacies, which make it an increasing popular vegetable to consumers **Fu et al. (2013)**. Compared to other types of vegetable, it has low calorie content. It contains substances that act anti-inflammatory. It is suitable for rheumatism and arthritis **Mandžuková (2014)**. Herb contains aromatic essential oil, which together with asparagine activates diuretic effects **Vániková (2006)**. Celery contains vitamins, minerals and aromatics and is considered to be healthy and energy-rich vegetables. 100 g of celery provides about 20 kcal, but 32 mg of vitamin C and 0.2 mg of vitamin A (**Rizzo and Muratore, 2009**). It contains many aromatic compounds, and some flavone glycosides and volatile oils that provide a typical flavour of celery. In the celery there are strong antioxidants, vitamins A, C and E, in high representation also flavones **Hostetler et al. (2012)**. The content of flavonoids and other secondary metabolites is high. Studies have shown that celery has a high content of flavonols (glycosides), especially apigenin-7-O-apiosylglucoside (apiin) **Lin et al. (2007)** and malonylapiin, which can be transformed into apiine **Hostelter et al. (2012)**. **Mandžuková (2014)** states that celery is a good source of calcium, it contains vitamin C, certain vitamins from B group, beta-carotene (provitamin A), calcium, iron, magnesium, phosphorus. According to

Vaughan et al. (2009) celery contains 95 percent of water, therefore there are few proteins, fat and sugar, but many of minerals, some carotenes, vitamin E, and vitamin B complex, the vitamin C content is low 8 mg.100 g^{-1}. Celery is considered to be beneficial for the digestive tract and the cardiovascular system. It has the ability to reduce the level of bad LDL cholesterol in the body due to substances called phthalides. Phthalides trigger the production of bile acids that lower the level of LDL cholesterol in the blood **Rizzo and Muratore (2009)**. In addition, flavonoid luteolin prevents the growth of tumours due to its anti-inflammatory and diuretic effects **Hostelter et al. (2012)**. Freshly pressed fruit and vegetable juices are an excellent source of minerals and vitamins that catalyse chemical reactions that occur in the body. These enzymes also produce energy for the digestion, absorption and conversion of food into the body tissues. An increased intake of fruit and vegetable juices ensures that the human body will absorb more minerals and vitamins. Another useful benefit of fruit and vegetable juices is their ability to promote detoxification of the human body **(Gbasouzor and Okonkwo 2014)**. Juices supplies antimicrobials, antioxidants, diuretics, chlorophyll. Celery juice is strongly alkaline and helps to prevent acidosis, high blood pressure, headaches, heartburn, constipation and flatulency; it has also strong anti-inflammatory properties **(Hostetler, 2012)**. It has also been reported celery juices are reducing systolic and diastolic blood pressure **(Tabassum, 2011, Liu, 2016)**. The aim of the study was to evaluate quantitative (yields) and qualitative (chlorophyll *a* and *b*, soluble solids) parameters in selected patioles of celery in the context of raw juice production.

MATERIAL AND METHODOLOGY

Location and description of the experiment

Small plot field experiment with celery was conducted in areal of the Department of Vegetable production, SUA Nitra, in 2016. The total area of the field trials was 32.5 m^2. Sowing and growing of seedlings were carried out in greenhouses of Botanical Garden of Slovak University of Agriculture, in Nitra.

Climate conditions

The city of Nitra belongs to mild climate area with varying weather characteristics. The meteorological data from the site of trial were provided by the Department of Biometeorology and Hydrology, HLEF, SUA, in Nitra are presented in Table 1.

Characteristics of selected celery varieties

Helios - It is a medium-sized variety of celery (*Apium graveolens* var. *Dulce*). It produces strong, long, meat stems. It is suitable for salads, soups, sauces and meat.

Red Soup - It is an attractive red type of celery (*Apium graveolens* var. *Dulce*) that, as the name suggests, adds a delicious flavor. It is also excellent with shoots or with leaf roots.

Malachit – It is a semi-high non-hybrid variety with dark green leaves. The stems are strong, meaty, long and upright, weak to medium green without anthocyanins. Vegetation time is 80 to 90 days from planting. The recommended growing spacing is 40 x 35 cm or 40 x 40 cm.

´Verde Pascal´ - It is a world-wide grown variety. It produces coarse, long, medium-green coloured petioles with dark leaves. It grows to a height of 60 cm. It requires sandy – aluminous to aluminous soils, lighter to moderate with high humus content and a good supply of calcium. Vegetation time is 80 – 85 days.

´Golden Self- Blanching´ - This is a dwarf variety with tasty, golden-yellow heads and one requiring no earthing-up.

´Celebrity´ - A self-blanching type with short stalks. Quick to mature for the earliest outdoor crops, it has consistently high yields of superb quality stalks with a lovely flavour. Has very little stringiness.

Planting

Healthy, well developed celery seedlings were planted on pre-prepared soil on 16th of May, 2016 into a cultivation spacing 0.50 x 0.60 m. Six rows were planted (one row of each variety), with 12 plants planted in one row.

Treatment of the crop

During vegetation a manual trapping was carried out against weeds and for removal of the soil crust. There was regular irrigation in the morning. During the vegetation, no herbicide was applied; on 24th of July, 2016 fungicide (Kuprikol 40g / 5 l water) against septoriosis was done.

Table 1 Monthly assessments based on climatic normal temperatures and long-term precipitation averages (1961-1990), Nitra, 2016.

Month	t [ºC]	characteristic	Z [mm]	characteristic
V.	15.0	normal	91	very wet
VI.	20.3	very hot	14	extra wet
VII.	21.4	hot	135	extra wet
VIII.	19.5	normal	35	dry
IX.	17.5	hot	37	normal

Table 2 Agrochemical characteristics of the soil before the experiment establishment in mg.kg^{-1}, Nitra, trial place, 2016.

pH	Nan	Nutrient content in mg.kg^{-1} (Mehl.III)				%
	mg.kg^{-1}	P	K	Ca	Mg	of humus
7.17 N	13.0 S	142.5 V	565 VV	14750 VV	740.9 VV	4.14 V
7.14 N	13.0 S	198.8 VV	487.5 VV	14900 VV	767.5 VV	4.17 V

Note: pH: N – neutral, nutrients: VN – very low content, N – low content, S – medium content, D – good content, V – high content, VV – very high content.

Fertilization of the crop

On the basis of agrochemical analysis of the soil the nitrogen was added (Table 2). It was applied in the form of a fertilizer DASA 26/13 in dosage 1.4 kg/ 32.5m² before the planting, after the planting and after the first harvest, it was applied in the form of ammonium (LAD 27) in dosages 0.45 kg/32.5m².

Harvesting and post-harvest treatment

The harvest of the celery was carried out twice a season (en block). The first harvest took place on 4th of August, 2016 and the second one on 6th of September, 2016. The harvest was carried out mechanically (by knife); the whole plants were cut just above the surface of the soil. After harvest each variety was prepared for analysis according to the chosen methodology **Hegedűsová et al. (2015)**.

Determination of quantitative and qualitative parameters

Determination of yields

After harvest the petioles were weighed in the handling room of the Department of Vegetable Production. The weighed samples were recalculated to the yields in $t.ha^{-1}$.

Estimation of chlorophyll a and chlorophyll b content

The chlorophyll *a* and chlorophyll *b* were determined spectrophotometrically (Spektralquant PHARO 200) laterally in the acetone extract on the wavelengths 649 nm and 665 nm in homogenisated fresh plant (150 – 200 g) **Hegedűsová et al. (2015)**. Number of analysed samples for average content of chlorophyll *a* and *b* was 10 in case of each variety.

Total soluble solids estimation

The juice from the homogenized sample was squeezed on the dry block of the digital hand-held refractometer (Kern ORD 45BM, Balingen, Germany). The value of soluble solids was directly read. Measurement was performed at room temperature according to **Hegedűsová et al. (2015)**. Ten samples were analysed for average content soluble solids in case of each variety.

Statistical analysis

A statistical analysis was performed by using of the Statgraphic Centurion XVII (StatPoint Inc. USA). Obtained results were evaluated by analysis of variance (ANOVA) and average values were tested by LSD test performed at the significance level of 95%.

RESULTS

Values of the celery yields ranged from 27.99 $t.ha^{-1}$ (´Malachit´) to 47.33 $t.ha^{-1}$ (´Golden S.´) in first harvest and from 21.01 $t.ha^{-1}$ (´Malachit´) to 51.03 $t.ha^{-1}$ (´Red Soup´) in second harvest as it is figured in Table 3. When comparing data from both harvests in average there was noticed statistically significant difference (p <0.05) between tested varieties. The lowest values in yields reached ´Malachit´ variety (24.50 $t.ha^{-1}$), the highest ´Red Soup´ variety (47.01 $t.ha^{-1}$). Based on the crop yields there was found that for the earlier term of harvest (in August) were more suitable varieties ´Malachit´ and ´Golden Self-Blanching´, as they had a higher yields - ´Malachit´ about 24.93% and the ´Golden S.´ variety about 11.22% compared to the their second harvest. In later harvest term (in September) ´Helios´, ´Red Soup´, ´Verde Pascal´ and ´Celebrity´ reached higher yields than from the first harvest about 6.91%, 18.70%, 5.20% band 9.17% in the following order. By the statistical analysis of all the data, differences between the first and the second harvest weren´t evaluated as significant (Table 3).

The chlorophyll content ranged from 7.21 to 76.55 $mg.kg^{-1}$ (Table 4) in the first harvest, where the lowest content reached the ´Celebrity´ variety and the highest ´Helios´ variety. In the second harvest, the lowest values of chlorophyll *a* were again in the case of the ´Celebrity´ variety (9.52 $mg.100g^{-1}$). The changes in the chlorophyll content were in case of Malachite variety, where it reached the highest value 97.70 $mg.100g^{-1}$. In the evaluation of both harvests, the ´Celebrity´ and ´Golden S.´ varieties showed the lowest chlorophyll values. The differences in the chorophyll content of these varieties were significantly lower compared to the other estimated varieties according to the statistical analysis. In terms of chlorophyll *a* values, there were significant differences between the observed two harvests. For all tested varieties (except of the ´Helios´) the chlorophyll *a* content was increased in the second harvest, the most visibly in case of Malachit variety (increase about 152.13% in the second harvest).

According to Table 4, the content of chlorophyll *b* in observed varieties of celery ranged from 3.46 $mg.100\ g^{-1}$ (´Celebrity´) to 50.06 $mg.100\ g^{-1}$ (´Helios´) in the first harvest and from 3.38 mg.100 $mg.100\ g^{-1}$ (´Celebrity´) to 51.04 $mg.100\ g^{-1}$ (´Malachite´) in the second harvest. The white petioles of the celery are usually grown to obtain the smallest amount of chlorophyll because of delicious taste. From this point of view, based on our results, the ´Celebrity´ and ´Golden S.´ varieties were the most suitable in case of both harvests, as well as in case of both observed pigments (chlo *a* and chlo *b*). Due to the different purpose of the study - aimed to increasing of the chlorophyll content as an antioxidant with using in processing to juices or smoothies, the ´Helios´ variety should be harvested in August, because there was significant reduction (p <0.05) of chlorophyll *b* (about 42.47%) in the second harvest (in September), as well as in case of chlorophyll *a* (decrease about 41.39 %). On the contrary, ´Red Soup´, ´Malachite´ and ´Verde Pascal´ varieties are more interesting during the second harvest, because there was a significantly increase of chlorophyll *b* content during the second harvest about 19.17%, 82.35% and 38.74% respectively at tested p value <0.05. The similar increase was found also in case of chlorophyll *a*.

The total soluble solid content according the Table 5 reached values ranging from 5.00 to 8.77 °BRIX, the average values counted from both harvests moved in the following order: ´Celebrity´ (5.30 °BRIX) <´Helios´ (6.83 °BRIX) <´Golden S.´ (7.18 °BRIX) <´Malachite´ (7.58 °BRIX) <´Red Soup´ (7.82 °BRIX) <´Verde Pascal´ (8.47 °BRIX). The harvest term did not affect the soluble solids content since the differences between the first and the second harvest were not statistically significant (p <0.05) according to the chosen methodology.

Table 3 Influence of variety and term of harvest on the the crop yields of tested celery varieties (t.ha^{-1}).

Variety	1th harvest^{A}	2nd harvest ^{A}	Average
´Helios´	35.00 ±2.59^{b}	37.42 ±1.41^{bc}	36.21 ±2.62^{bc}
´Red Soup´	42.99 ±2.66^{c}	51.03 ±0.93^{d}	47.01 ±1.72^{d}
´Malachit´	27.99 ±0.88^{a}	21.01 ±2.68^{a}	24.50 ±1.42^{a}
´Verde Pascal´	32.33 ±1.42^{ab}	34.01 ±3.10^{b}	33.17 ±0.83^{b}
´ Golden S.´	47.33 ±4.23^{c}	42.02 ±1.08^{c}	44.68 ±3.43^{d}
´Celebrity´	36.66 ±1.50^{b}	40.02 ±2.22^{c}	38.34 ±1.73^{c}

Note: a, b, A, B – Different letters in the upper index represent a statistically proven difference (p <0.05, LSD test, ANOVA), Statgraphic XVII.

Table 4 Average content of chlorophyll a (mg.kg^{-1}) in selected varieties of tested celery varieties.

Variant	1th harvest ^{A}	2nd harvest ^{B}	Average
´Helios´	76.55 ±3.73^{d}	54.14 ±3.57^{b}	67.59 ±12.68^{c}
´Red Soup´	39.39 ±3.66^{c}	67.25 ±6.64^{b}	50.54 ±15.83^{bc}
´Malachit´	38.75 ±3.62^{c}	97.70 ±16.08^{c}	62.33 ±33.37^{bc}
´Verde Pascal´	35.92 ±0.75^{c}	59.00 ±0.65^{b}	45.15 ±12.66^{b}
´ Golden S.´	13.50 ±1.90^{b}	16.01 ±1.47^{a}	14.50 ±2.06^{a}
´Celebrity´	7.21 ±0.99^{a}	9.52 ±0.73^{a}	8.14 ±1.49^{a}

Note: a, b, A, B – Different letters in the upper index represent a statistically proven difference (p <0.05, LSD test, ANOVA), Statgraphic XVII
n = average content = 10 measurements.

Table 5 Average content of chlorophyll b (mg.kg^{-1}) in selected varieties of tested celery varieties.

Variant	1th harvest ^{A}	2nd harvest ^{B}	Average
´Helios´	50.06 ±2.20^{b}	28.80 ±3.05^{b}	41.56 ±11.84^{d}
´Red Soup´	27.23 ±2.23^{b}	32.45 ±3.10^{b}	29.32 ±3.61b^{c}
´Malachit´	27.99 ±2.43^{c}	51.04 ±7.32^{c}	37.21 ±13.26^{cd}
´Verde Pascal´	22.43 ±0.27^{b}	31.12 ±0.63^{b}	25.90 ±4.78^{b}
´ Golden S.´	7.80 ±0.62^{a}	9.06 ±0.69^{a}	8.31 ±0.89^{a}
´Celebrity´	3.46 ±0.58^{a}	3.38 ±0.34^{a}	3.43 ±0.45^{a}

Note: a, b, A, B – Different letters in the upper index represent a statistically proven difference (p <0.05, LSD test, ANOVA), Statgraphic XVII
n = average content = 10 measurements.

Table 6 Average content of total soluble solids (°BRIX) in selected varieties of tested celery varieties.

Variant	1th harvest ^{A}	2nd harvest ^{A}	Average
´Helios´	6.47 ±0.74^{b}	7.20 ±0.10^{b}	6.83 ±0.62^{b}
´Red Soup´	7.97 ±0.31^{c}	7.67 ±0.06^{bc}	7.82 ±0.26^{d}
´Malachit´	7.07 ±0.25^{b}	8.10 ±0.30^{c}	7.58 ±0.62^{cd}
´Verde Pascal´	8.17 ±0.15^{c}	8.77 ±0.57^{d}	8.47 ±0.50^{e}
´ Golden S.´	7.07 ±0.60^{b}	7.30 ±0.20^{b}	7.18 ±0.42^{bc}
´Celebrity´	5.00 ±0.10^{a}	5.60 ±0.17^{a}	5.30 ±0.35^{a}

Note: a, b, A, B – Different letters in the upper index represent a statistically proven difference (p <0.05, LSD test, ANOVA), Statgraphic XVII
n = average content = 10 measurements.

DISCUSSION

Celery (*Apium graveolens* var. *Dulce*) is harvested as a whole plant followed by the root removing. The petioles are used for consumption. According to **Petříková (2012)** the weight of one plant is 500 - 800 g, yield from 1 ha is 35 – 50 t.ha^{-1}, which corresponds to our results. Differences in yields in the two harvests were not significant (p <0.05), which is in accordance to the research of quantitative characteristics in case of **Guerra et al. (2010)**, where no differences in the morphological characteristics measured (total weight, total length, total leaf number and petiole length) were found between the two maturity stages (HD1 – 93 days after transplantation and HD2 – 124 days after transplantation). Varietal differences in crop yields have been confirmed, it is necessary to monitor also the qualitative characteristics of celery in terms of using for juices and smoothies. There were mainly other kind of characteristics followed in

previous scientific studies about celery (var. *Dulce*), such flavonoids **(Li et al. 2014)**, vitamins **(Valšíková et al. 2016, Mezeyová et al. 2017)**, minerals (**Sheng et al. 2009, Moghadam et al., 2016**), fibre **Abd El-Mageed (2011)**, essential oils and phenolic acids **Helaly et al. (2013)**, etc.

Processing to juices or smoothies allow consuming of other antioxidant – chlorophyll. That is the reason of five lesser known varieties testing. Self-blanching petioles of

Figure 1 Plants in the stage of transplantation, Botanical Garden, Nitra, 2016, photo: Gubovičová.

Figure 2 The arrangement of vegetation, Nitra, 2016, photo: Gubovičová.

celery were not in the attention of scientists in the frame of chlorophyll monitoring, the data are not available. The content of chlorophyll was on the other hand tested in leafy celery (var. *Secalinum*). In research of **Helaly et al. (2015)** they tested 3 leaf celeries. Their values moved from 1.20 to 2.15 $mg.g^{-1}$ FW in the first harvest year and from 1.24 to 2.20 $mg.g^{-1}$ FW in the second harvest year. In comparison with our results their values are higher, but in juices the petioles are occurred in higher amount than in case of the herb using for cooking. Variability influence plays the role in increasing of the celery (var. *Dulce*) quality, but importatnt is also using of suitable processing ways (storage, cutting, squeezing conditions, etc.) to conserve the chlorophyll as well as other antioxidants and nutritionally valuable compounds. Such as according to **Manzocco et al. (2009)** by the help of light treatment, which has been reported as a novel preservative approach that is cheap, nontoxic, free of residuals and environment-friendly in comparison with traditional methods. In study of **Zhan et al. (2013)** exposing fresh-cut celery to light preserved 47% and 48% more chlorophyll *a* and chlorophyll *b* content than in darkness during storage, respectively, light exposure significantly maintained sugar content of fresh-cut celery during storage. Exposing petioles to light resulted in 17%, 25% and 67% more sucrose, reducing sugar and glucose contents than in darkness at the end of storage. Our values of total soluble solids content were compared with the values of celeriac, as the studies of celery are not very extensive. According to **Kreck et al. (2006)** celery juices were made of different *Apium graveolens* L. cultivars cultivated at different irrigation levels. The soluble solid was highly dependent on the cultivar. In genuine juices from cultivar "Monarch" a lower concentration of soluble solids (7.83 °BRIX) was detectable, whereas in juices from "Bergers weiße Kugel" higher Brix values (10.25 °BRIX) were obtained. This is reflected by the sugar content, i.e. glucose; fructose and saccharose concentrations were dependent on the variety. Variation in soluble sugars (sucrose, glucose, and fructose) was observed in the celery accessions according to **Helaly et al. (2015).** Fructose was the most abundant sugar detected in the accessions during the first harvest and glucose was the most abundant in the second year. In study of **Nadwodnik et al. (2008) c**elery and common plantain were selected because much of what is known about the transport of mannitol and sorbitol has come from studies of these plant species, and more recently, the relevant transporters for sucrose as well as for sugar alcohols were cloned from these species.

CONCLUSION

Celery (*Apium graveolens* var. *Dulce*) is used in salads, smoothies or in juices because of its high content of health positive compounds such vitamins (A, D, E, K, C, group of B vitamins), minerals, mainly calcium, iron, potassium, sodium or phosphorus as well as carbohydrates in small amount. In common the consumer is looking for white kind of celery, because of its mild and pleasant taste, but with more intensive popularity of juices and smoothies, varieties with higher content of chlorophyll started to be also interesting. Varieties ´Helios´, ´Red Soup´, ´Malachit´ and ´Verde Pascal´ can be consumed in combination with other ingredients of fruity – vegetable juices with benefit of higher antioxidant impact of chlorophyll *a* and *b*. The stalks of ´Golden S.´ and ´Celebrity´ varieties with lower chlorophyll content are more suitable for classical raw or lightly cook using.

REFERENCES

Abd El-Mageed, N. M. 2011. Hepatoprotective effect of feeding celery leaves mixed with chicory leaves and barley grains to hypercholesterolemic rats., *Pharmacognosy Magazine*, vol. 7, no. 26, p. 151-156. http://doi.org/10.4103/0973-1296.80675

Fu, N., Wang, Q., Shen, H. L. 2013. De Novo Assembly, Gene Annotation and Marker Development Using Illumina Paired-End Transcriptome Sequences in Celery (*Apium graveolens* L.), *PLoS ONE*, vol. 8, no. 2., p. 1-12. http://doi.org/10.1371/journal.pone.0057686

Gbasouzor, A. I., Okonkwo, C. A. 2015. Design and Characterization of a Model Fruit Juice Extracting Machine for Healthy and Vibrant Life in Today's Modern. In: Kim H., Amouzegar M., Ao S. (eds) *Transactions on Engineering Technologies.* Dordrecht, Germany : Springer, p. 445-469. https://doi.org/10.1007/978-94-017-7236-5_32

Guerra, N., Carrozzi, L., Goni, M. G., Roura, S., Yommi, A. 2010. Quality Characterization Of Celery (*Apium Graveolens* L.) By Plant Zones And Two Harvest Dates, *Journal Of Food Science*, vol. 75, no. 6, p. 1-6. https://doi.org/10.1111/j.1750-3841.2010.01701.x

Hegedűsová, A., Mezeyová, I., Andrejiová, A. 2015. *Metódy stanovenia vybraných biologicky aktívnych látok (Determination methods of selected biologically active substances)*. Nitra, Slovakia : Slovak University of Agriculture in Nitra, 72 p. ISBN 978-80-552-1420-7

Helaly, A. A., Baek, J. P. Mady, E., Eldekashy, M. H. Z., Craker, L. 2015. Phytochemical Analysis of Some Celery *Accessions. Journal of Medicinally Active Plants*, vol. 4, no. 1, p. 1-7. https://doi.org/10.7275/R5542KJF

Helaly, A., El-Refy, A., Mady, E., Mosa, K. A., Craker, L. 2013. Morphological and molecular analysis of three celery accessions, *Journal of medicinally active plants*, vol. 2, no. 3, p. 27-32.

Hostetler, G. L., Riedl, K. M., Schwartz, S. J. 2012. Endogenous Enzymes, Heat, and pH Affect Flavone Profiles in Parsley (*Petroselinum crispum var. neapolitanum*) and Celery (*Apium graveolens*) during Juice Processing, *Journal of Agricultural and Food Chemistry*, vol. 60, no. 1, p. 202-208. https://doi.org/10.1021/jf2033736

Kreck, M., H., Dietrich, C. D. Patz, M. Ludwig, P. J., Paschold, S., Both, A., Baumgart, H. J., Schmitz, D., Schrenk. 2006. Characterization of celery juices made of different celery varieties at different irrigation levels. *Deutsche Lebensmittel Rundschau*, vol. 102, no. 1, p. 17-24.

Li, P., Jia, J., Zhang, D., Xie, J., Xu, X., Wei, D. 2014. In vitro and in vivo antioxidant activities of a flavonoid isolated from celery (*Apium graveolens* L. *Var. Dulce*). *Food Funct.*, vol. 5, no. 1, p. 50-56. https://doi.org/10.1039/c3fo60273g

Lin, L. Z., Lu, S., Harnly, J. M. 2007. Detection and Quantification of Glycosylated Flavonoid Malonates in Celery, Chinese Celery, and Celery Seed by LC-DAD-ESI/MS. *Journal of Agricultural and Food Chemistry*, vol. 55, no. 4, p. 1321-1326. http://doi.org/10.1021/jf0624796

Liu, C., Huang, Y. 2016. Chinese Herbal Medicine on Cardiovascular Diseases and the Mechanisms of Action. *Frontiers in Pharmacology*, vol. 7, 469 p. http://doi.org/10.3389/fphar.2016.00469

Mandžuková, J. 2014. Miraculous nature drugs (Natural magic medicine), Slovakia : Príroda, 200 p., ISBN 978800702304

Manzocco, L., Quarta, B., Dri, A. 2009. Polyphenoloxidase inactivation by light exposure in model systems and apple derivatives. *Innovative Food Science & Emerging Technologies*, vol.10, no. 4, p. 506-511. https://doi.org/10.1016/j.ifset.2009.02.004

Mezeyová, I., Hegedűsová, A., Gubovičová, S., Mezey, J., Farkaš, J. 2017. Influence of varietal variability and harvest time on the content of vitamin C in the stem and stalk of celery (*Apium graveolens* L. var. *dulce*), *Horticulture*, p. 174-182. ISBN 978-80-552-1725-3

Moghadam, M. H., Imenshahidi, M., Mohajeri, S. A. 2013. Antihypertensive Effect of Celery Seed on Rat Blood Pressure in Chronic Administration. J*ournal of Medicinal Food*, vol. 16, no. 6, p. 558-563. http://doi.org/10.1089/jmf.2012.2664

Nadwodnik, J., Lohaus, G. 2008. Subcellular concentrations of sugar alcohols and sugars in relation to phloem translocation in *Plantago major, Plantago maritima, Prunus persica, and Apium graveolens*., *Planta,* vol. 227, no. 5, p. 1079-1089. https://doi.org/10.1007/s00425-007-0682-0

Pekárková, E. 2004. *We grow carrots, radishes, celery and other root vegetables*, Prague, Czech Republic : Grada Publishing,a.s, 100 p. ISBN 80-247-0744-6

Petříková, K. 2006. *Vegetables - Cultivation, economics, sales*. Prague, Czech Republic : Profi Press, s.r.o., 240 p. ISBN 80-86726-20-7

Rizzo, V., Muratore, G. 2009. Effects of packaging on shelf life of fresh celery. *Journal of Food Engineering*, vol. 90, no. 1, p. 124-128. https://doi.org/10.1016/j.jfoodeng.2008.06.011

Rożek, E. 2013. Yielding of leaf celery Apium graveolens L. var. secalinum Alef. depending on the number of harvests and irrigation, *Modern Phytomorphology*, vol. 3, p. 83-86. https://doi.org/10.5281/zenodo.166571

Sheng, J. P., Liu, C., Shen, L. 2018. Comparative study of minerals and some nutrients in organic celery and traditional celery, *Spectroscopy and Spectral Analysis* vol. 29, p. 247-249.

Tabassum, N., Ahmad, F. 2011. Role of natural herbs in the treatment of hypertension, *Pharmacogn Rev*. vol. 5, no. 9, p. 30-40. https://doi.org/10.4103/0973-7847.79097

Uher, A., Kóňa, J., Valšíková, M., Andrejiová, A. 2009. *Vegetables - Field Cultivation, University Textbook*, Slovak University of Agriculture in Nitra, 212 p., ISBN 978-80-552-0199-3. (In Slovak)

Valšíková, M., Mezeyová, I., Rehuš, M., Šlosár, M. 2016. Changes of vitamin C content in celery and parsley herb after processing, *Potravinarstvo*, vol. 10, no. 1, p. 637-642. https://doi.org/10.5219/687

Vániková, A. 2006. Celery and leaf celery, Research institute of vegetables, [cit. 2018-01-15]. Available at: http://old.agroporadenstvo.sk/rv/zelenina/zeler_stopkovy.htm

Vaughan, J., Geissler, J., 2009. *The New Oxford Book of Food Plants*, 2nd Edition, Oxford, UK : OUP Oxford, 288 p., ISBN-13: 978-0199549467

Zhan, L., Hu, J., Pang, L., Li, Y. and Shao, J. 2014. Effects of light exposure on chlorophyll, sugars and vitamin C content of fresh-cut celery (*Apium graveolens var. Dulce*) petioles. *Int. J. Food Sci. Technol*., vol. 49, p. 347-353. https://doi.org/10.1111/ijfs.12292

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INTERACTION OF POLYPHENOLS AND WINE ANTIOXIDANTS WITH ITS SULFUR DIOXIDE PRESERVATIVE

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ABSTRACT

Wine is considered to be a significant alcoholic beverage, which is the result of fermentation of grape must or mash. Wine is a must when the substances contained in it play a major role, which are essential inhibiting water, carbohydrates, acids, minerals, nitrates, polyphenols and aromatics. These biochemical components are an important tracking element in wine evaluation in terms of chemical analyzes. An important parameter of monitoring is polyphenolic substances. Polyphenol substances are identified in plant materials as several thousand pieces with a very diverse structure. However, they have a common feature up to one or more aromatic rings substituted with hydroxyl groups. These substances may be present in plant material in a small or large amount. The total daily intake of polyphenols is estimated at 1 g. This is a higher intake than antioxidant vitamin intakes and it is confirmed that their antioxidant activity is higher than that of antioxidant vitamins. When monitoring the content of all polyphenols (TPC) in selected samples using a spectrophotometric method, a higher TPC content of red wines against white white wines can be observed. Total antioxidant activity is introduced to compare antioxidant effects of different mixtures and is based on the ability to eliminate radicals. Antioxidant activity and effects of polyphenols can be inhibited by the addition of preservatives to wine. The preservative is sulfur dioxide (SO_2), which has antimicrobial and antioxidant effects. This compound is not harmless because it is a strong allergen, blocks bacteria in the digestive tract and prevents the conversion of sugars and alcohol derivatives in the liver by blocking vitamin B. In the normal life, SO_2 is consumed under the E 220 mark. The aim of this work is to monitor the change in the total polyphenols content related to free and bound sulfur dioxide (SO_2) content using accredited OIV-MA-AS323-O4B: R, 2009 samples in wine samples. Comparison of organic wines and wines produced by classical, it was found that organic wine have a higher content of biologically active substances and have a strong correlation factor TAA - total SO_2 (r = 0.77 to 0.91), depending on the wine variety.

Keywords: total polyphenol, antioxidant aktivity, organic, red wine, white wine

INTRODUCTION

Grape vines and, above all, their product of production - wine is considered to be a significant alcoholic beverage resulting from the fermentation of grape musts or grapes, which has retained its popularity for many millennia **(McIntyre et al. 2015)**. Production is divided into several technological steps, including grape harvesting, cropping and crushing, pressing, fermentation, apple fermentation, training and bottling. Wines are characterized by the high content and essential role of inhibiting water, carbohydrates, acids, minerals, nitrogenous substances, polyphenols and aromatics. For example, aromatic substances can be classified into aromatic substances from grapes resulting from fermentation and occurring during wine maturation. They play a significant and notable part in the choice of wine by the consumer, as this is the first impression the wine customer will acquire. Important biologically active substances of the wine are polyphenols **(Del Pino-García et al. 2016)**. These are mostly contained in red wines, to a lesser extent in white wines **(Bajčan et al. 2016)**. For example, the content of significant polyphenol resveratrol is reported in white wines ranging from 0.2 – 0.8 $mg.L^{-1}$, on the other hand in red wines 2 – 6 $mg.L^{-1}$ **(Kyseláková et al. 2003)**. Red wine contains many bioactive polyphenols such as resveratrol, anthocyanins, catechins, and tannins that do not originate in grapes, but in oak barrels, where red wines often mature **(Panchal et al., 2013)**. Polyphenolic substances, especially reseratrol, in cooperation with other components of wine (alcohol, etc.) are attributed to a positive effect on human

health. Some studies show positive effects on cardiovascular system, oxidative stress, cholesterol, and others **(Gea et al., 2014; Karadeniz et al. 2014)**. This research is carried out using spectrophotometric techniques using for example the DPPH method, the reaction of the test substance with a stable free radical of 1,1-diphenyl-2-picrylhydrazyl, for antioxidant capacity **(Bajčan et al. 2017)**. An important component of the wine is the preservative and inhibiting agent sulfur dioxide (SO_2) or E220. In wine we can find it both in the form of endogenous, which is created during fermentation, and above all as exogenous. Exogenous, non-bound SO_2 is added in various technological operations. Bonded sulfur dioxide is formed by the enzymatic transformation of sulfur compounds (sulfur amino acids - cysteine, cystine, methionine, glutathione, free elemental sulfur, etc.) by the action of Saccharomyces, in addition to a number of other metabolites, in the unsaturated grape juice itself, but mainly during the alcoholic fermentation cerevisiae. SO_2 has both strong antimicrobial effects but also primarily reductive (antioxidant) effects. Most antimicrobial and antioxidant effects are usually attributed to free SO_2. Sulfur dioxide is mainly used in the form of gas, but also an aqueous solution of sulfuric acid or hydrogen sulphide or a powder. Due to its properties it is very well soluble in water. At 20 °C, 39 liters of SO_2 are dissolved in 1 liter **(Valášek et al. 2014)**. The formation and development of bound sulfur dioxide depends on a number of factors (formation during fermentation of wine) **(Romano et al., 1993)** and may range from several mg. L^{-1} to 30 mg. L^{-1} in extreme conditions, bound sulfur dioxide may occur at concentrations up to 100 mg. L^{-1} **(Rankine et al., 1969; Eschenbruch 1974; Dott et al., 1976; Suzzi et al., 1985)**. Concentration of bound SO_2 along with free SO_2 produced microorganisms during alcoholic fermentation is often critical to the course of malolactic fermentation **(Henick-Kling et al. 1994)**. Endogenous sulfur dioxide is present mainly in the form of bound but in small amounts also free sulfur dioxide **(Wells et al., 2011)**. The presence of both forms should be taken into account when exogenous sulfur dioxide is dosed. By classical iodometric titration using accredited methods OIV-MA-AS323-O4B: R, 2009 **(OIV, 1990)** the content of free and bound sulfur dioxide in wine was monitored. At the same time corrections were made for the presence of reducing agents (reducers).

Scientific hypothesis

Wine is a very popular alcoholic beverage spread throughout the world. The aim of this research was to present the results of analyzes in monitoring the interaction of sulfur dioxide and biologically active substances contained in wine. In the experiment were used three varieties of white wine and red wine standard or made oraganic form in the wine region of Moravia, Czech Republic.

MATERIAL AND METHODOLOGY

Samples of wine

Samples of used white and red wines come from different wineries from the wine region of the Moravian, Mikulov, Slovácko and Velkopavlovice subregions, which includes more than 13 000 hectares of vineyards. There are approximately 18,000 small, recreational or professional growers here. The average annual temperature is 9.42 °C, the annual precipitation diameter is 510 mm and the average annual sunshine is 2244 hours according to the 78-year average found at the Winery Brewery in Velké Pavlovice. The climate is transient with an incline towards the inland, with occasional invasions of humid Atlantic air or even ice from the inland. The growing season is a bit shorter than in Western Europe. White wines produced according to classic methods of Riesling, Pinot Blanc and Veltliner. Red wines of Pinot Noir, André and Frankovka. In addition, organic wines were used, namely white wines of the Veltliner, Pinot Blanc and Riesling wines, red wines of the Pinot Noir, André and Frankovka varieties. All of these wines were produced and are produced in the year 2016. Five samples of an identical batch of wine were collected and analyzed from each wine variety. In total, sixty samples of classical and organic wines were analyzed.

Chemicals and laboratory equipments

Standardization of iodine solution

Sulfuric acid (H_2SO_4), starch (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic), Potassium iodide (KI), Potassium dichromate ($K_2Cr_2O_7$), and Sodium thiosulfate ($Na_2S_2O_3$) (Ing. Petr Lukeš, Uherský Brod, Czech Republic)

Determination of SO_2 by OIV-MA-AS323-04B : R 2009

Sulfuric acid (H_2SO_4), starch (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic), Sodium hydroxide (NaOH), EDTA 3, Acetaldehyde, Iodine (I_2) (Ing. Petr Lukeš, Uherský Brod, Czech Republic), ordinary laboratory glassware and equipment, stopwatch, 25 mL burette digital, lamp.

Determination of total polyphenol compounds (TPC)

Distilled water, Folin–Ciocalteu reagent (FCR) (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic), Sodium carbonate (Na_2CO_3) (Ing. Petr Lukeš, Uherský Brod, Czech Republic).

Determination of total antioxidant ativity by DPPH metod

Methanol, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) (Penta s.r.o. Ing. Petr Švec, Prague, Czech Republic)

Spekctrophotometric methods

Spectrophotometric measurements were performed on a Lambda 25 UV-VIS spectrophotometer (PerkinElmer, USA) in 10 mm optical quartz cuvettes.

Methods

Determination of free SO_2

50 mL of wine sample is pipetted Into a 500 mL volumetric flask, we add 3 mL of 16% H_2SO_4, 1 mL EDTA 3 solution having a concentration of 1%, 5 mL of starch solution is titrated against a white background I_2 solution having a concentration of 0.02 mol.L^{-1} to blue color. The obtained power consumption is used in the final calculation (V1).

Determination of total SO_2

After titration of free SO_2 we add to a sample 8 mL NaOH solution at a concentration of 4 mol.L^{-1}, after 5 minutes we add 10 mL of 16% H_2SO_4 solution titrated with iodine. We use final consumption to calculate (V2). Then we add 20 mL of NaOH, and 200 mL of distilled water after 5 minutes, 30 mL of 16% H_2SO_4 solution and titrate with iodine to a blue color. We get V3 consumption.

Correction for reductones

We measure out 50 mL wine sample, 1 mL of 1% formaldehyde, and after 30 minutes add 3 mL of 16% H_2SO_4, 1 mL EDTA 3 solution having a concentration of 1%, 5 mL of starch solution and titrate against a white background I_2 solution having a concentration of 0.02 mol.L^{-1} to blue color. With this step, we get V4 consumption.

Calculation concentration SO_2 (mg.L^{-1})
Concentration of free SO_2 c = (V1-V2).f.12,8
Concentration of total SO_2 c = (V1+V2+V3-V2).f.12,8
12,8 – coefficient for conversion to SO_2 when used 0,025 M I_2

Determination of total polyphenol content (TPC)

To determine the total content of phenolic compounds (TPC), a spectrophotometric method using Folin-Ciocalteau reagent based on to reduce the phosphomolybdate-tungsten complex by phenolic substances in an alkaline medium. The modified method of **Singleton and Rossi (1965)** according to **Sumczynski et al. (2015)** was used. Determination was performed at a wavelength of 765 nm after a 30 min incubation. The total content of phenolic substances was expressed as gallon (GAE) in mg.L^{-1}. The repeatability of the assay was verified on 10 parallel determinations for cm = 0.5 g.L^{-1} of tannin. The calibration dependence A = f (cm) was constructed using six calibration solutions. For the preparation of calibration solutions, we dispense approximately 20 mL of distilled water into four 50 mL volumetric flasks and pipette 0.4; 0.6; 0.8; 1.0 mL of standard solution, add 1 mL of Folin-Ciocalteau and mix. After 3 minutes add 5 mL of 20% Na_2CO_3 solution, make up to the mark with distilled water and mix. After 60 minutes, we measure the intensity of the staining in a 10 mm cuvette at 765 nm against the blank spectrophotometrically. In the same way, determine the absorbance of the samples. According to the regression curve equation we calculate the polyphenol content expressed as mg gallic acid (GAE).L^{-1}.

Determination of total antioxidant ativity by DPPH metod

Total antioxidant activity was assessed by modification method of **Rop et al. (2010)**. First, a stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of metanol. A working solution is then prepared from the prepared stock solution by mixing 10 mL of the stock solution and 45 mL of methanol. Subsequently, the working solution thus prepared is spectrophotometrically measured at a wavelength of 515 nm against methanol as blank. A sample of 450 μL of wine was pipetted into a test tube and then 8.55 mL DPPH working solution was added. After 60 minutes of incubation in the dark, the sample was measured spectrophotometrically at said wavelength. Absorption loss was recalculated using the linear regression equation to equivalent Trolox (TE).L^{-1}.

Statisic analysis

Results are reported as mean values with standard deviation (SD). Differences between observed results were detected by t-test (Statistica, 2018, StatSoft, Inc., USA). A p <0.05 (*) and p <0.01 (**) was considered statistically significant.

RESULTS AND DISCUSSION

Free SO_2

Free sulfur dioxide (Table 1) in test samples of standard white wines ranges from 5.37 to 11.14 mg.L^{-1}. The smallest content was found in the white wine of the Riesling variety, on the contrary, the white wine of the Pinot Blanc variety. The content of free SO_2 in white organic wines ranged from 0.41 to 0.69 mg.L^{-1}. The least free SO_2 was determined in the white organic wine of the Veltliner variety, most notably Pinot Blanc. There is already a distinction between standard and organic wine with a different free SO_2 content of up to 10.73 mg.L^{-1}. The most significant difference can be seen (Table 1; Table 3) for the Pinot Blanc and organic Pinot Blanc varieties (p <0.01), with a difference of 10.45 mg.L^{-1} free SO_2. If we compare the achieved values (Table 1) with a study of sulfur dioxide **(Valášek et al., 2014)**, which gives the value of free SO_2 in Riesling 23 mg.L^{-1}, Veltliner 33 mg.L^{-1} and Pinot Blanc 28 mg.L^{-1}, our analyzed samples achieves significantly lower free SO_2 values in the same wine samples from the same wine region and sub-region. For samples of red wines (Table 2) we can observe free SO_2 content in the range of 0.83 - 32.19 mg.L^{-1}. Both of these values are recorded for red wine of the Pinot Noir variety, the lower of which was determined for organic wines. **Ivanova et al. (2015)** provides a comparison of free SO_2 in Pinot Noir, the conclusion of their study suggests a free SO_2 content of 11.52 mg.L^{-1}.

Total SO_2

Total SO_2 was determined after deduction of reductons. In standard wines (Table 1) the content ranges from 13.00 – 53.40 mg.L^{-1} total SO_2. The lowest content was recorded for organic Pinot Blanc wine (13.00 mg.L^{-1}), on the other hand, most of the Riesling organic wine (25.10 mg.L^{-1}). For standard production wines, the lowest value is found for Riesling wine (29.78 mg.L^{-1}), most notably for Veltliner (53.40 mg.L^{-1}). In red vines, large differences in total SO_2 content can be seen. **Valášek et al. (2014)** shows the values of Pinot Blanc 148 mg.L^{-1}, Riesling 119 mg.L^{-1} and Veltliner 236 mg.L^{-1} as compared to the established values (Table 1). The difference between the smallest and the highest content is about 110 mg.L^{-1} total SO_2. At the same time, the highest content was determined at Frankovka (135.95 mg.L^{-1}), at least at Frankovka organic (24.40 mg.L^{-1}). The maximum permitted amount of total SO_2 as laid down in Commission Regulation (EC) No. 606/2009, which sets the maximum SO_2 content in silent white wines at 200 mg.L^{-1}, in red wines at 150 mg.L^{-1}. For wines with residual sugar greater than 5 g.L^{-1}, the maximum value for white wine is

250 mg.L^{-1} and in red for 200 mg.L^{-1}. Therefore, if we compare all the aspects with the wine samples mentioned above, it is necessary to state that all the set values are inferior and above all organic wines, which show up to 10 times lower SO_2 than the allowed limit.

Total polyphenol

A spectrophotometric method using the Folin-Ciocalteau reagent was used to determine the total polyphenols in wine and organic wine samples, using gallic acid as the standard. For the wines tested, the higher value of the total polyphenols was predominantly for organic wines, for all their samples, both white and red. For whites of white wines, Veltliner organic predominates with 678.78 mg GAE.L^{-1}, at least Riesling 203.06 mg GAE.L^{-1}. As a result, the difference with wine with the highest total polyphenols, Veltliner and Veltliner organic, is more than 200 mg GAE.L^{-1}. Red wines have up to 2 to 3 times the white content of total polyphenols against white wines, this being determined by the production process and, above all, the biological properties of the grape vine itself. As can be seen (Table 2), the highest value was determined for the André organic sample (1349.12 mg GAE.L^{-1}), more than 400 mg GAE.L^{-1} was the lowest value of the total polyphenols determined for the Frankovka wine produced by the standard procedure. **Špakovska et al. (2012)** indicates the value of 256 mg of GAE.L^{-1} as the average content of total polyphenols in selected white wine samples. Pinot Blanc in the research reached an average value, according to the results obtained (Table 1) it is possible to observe a higher content of polyphenols in grape varieties mentioned by about 100 mg GAE.L^{-1}, Pinot Blanc organic records almost double the content. On the contrary, **Lapčíková et al. (2017)** presents the content of total polyphenols in samples Riesling (1085 mg GAE.L^{-1}) and Veltliner (732 mg GAE.L^{-1}). These very high values of total polyphenols in our research do not reach even the favored organic wines. The reason for this can be laid to the south wine region, different soil composition and meteorological conditions.

Antioxidant activity

To determine the antioxidant activity of wine samples, a DPPH method was used which is based on the reaction of the test substance with the stable 1,1-difenyl-2-picrylhydrazyl radical and the trolox standard. Results from Table 1 and Table 2 confirm the statement in the previous chapter that higher antioxidant activity is noted in

Table 1 Comparison of free, fixed and total sulfur dioxide, total polyphenols and antioxidant activity in samples of standard and organic white wines (n = 5).

White wine	Free SO_2 (mg.L^{-1})±SD	Fixed SO_2 (mg.L^{-1}) ±SD	Total SO_2 (mg.L^{-1}) ±SD	TPC (mg GAE.L^{-1}) ±SD	TAA (mg TE.L^{-1}) ±SD
Pinot Blanc	11.14 ±1.88	50.59 ±2.21	42.13 ±2.24	317.76 ±14.26	559.85 ±65.05
Pinot Blanc organic	0.69 ±0.20	18.85 ±0.38	13.00 ±0.18	405.12 ±6.07	674.55 ±6.61
Riesling	5.37 ±0.32	34.80 ±1.10	29.78 ±1.08	203.06 ±7.79	445.75 ±1.60
Riesling organic	0.63 ±0.07	28.51 ±0.62	25.10 ±1.28	319.72 ±6.62	508.50 ±3.62
Veltliner	9.33 ±3.96	58.40 ±2.58	53.40 ±2.32	445.45 ±6.53	685.13 ±15.81
Veltliner organic	0.41 ±0.02	21.19 ±0.66	15.16 ±0.66	678.78 ±18.65	806.28 ±11.65

Table 2 Comparison of free, fixed and total sulfur dioxide, total polyphenols and antioxidant activity in samples of standard and organic red wines (n = 5).

Red wine	Free SO_2 (mg.L^{-1})±SD	Fixed SO_2 (mg.L^{-1}) ±SD	Total SO_2 (mg.L^{-1}) ±SD	TPC (mg GAE.L^{-1}) ±SD	TAA (mg TE.L^{-1}) ±SD
Frankovka	28.52 ±4.98	154.73 ±3.83	135.95 ±4.90	905.21 ±3.85	2123.31 ±24.91
Frankovka organic	0.87 ±0.07	30.00 ±0.82	24.40 ±0.56	1020.52 ±14.75	2570.92 ±78.41
André	26.88 ±1.56	125.97 ±4.52	111.11 ±4.30	1130.96 ±35.37	2312.99 ± 18.17
André organic	1.04 ±0.06	35.11 ±1.28	30.01 ±1.23	1349.12 ±28.01	2529.25 ± 33.73
Pinot Noir	32.19 ±0.80	134.52 ±3.06	103.54 ±4.03	1046.30 ±57.81	1862.01 ±47.89
Pinot Noir organic	0.83 ±0.10	37.5 ±0.82	32.17 ±0.80	1300.04 ±12.89	2039.22 ±49.29

Note: Table 1 and Table 2: SO_2 – sulfur dioxide; Total SO_2 after deduction of reductons; TPC – total polyphenol content; TAA - total antioxidant ativity using DPPH - radical scavenging activity; TE – trolox equivalent; GAE - gallic acid equivalent; ±standard deviation.

Table 3 Statistically significant differences between classic and organic wines

	Free SO_2	Fixed SO_2	Total SO_2	TPC	TAA
Pinot Blanc classic / organic	**	**	**	**	*
Riesling classic / organic	**	**	**	**	**
Veltliner classic / organic	**	**	**	**	**
Frankovka classic / organic	**	**	**	**	**
André classic / organic	**	**	**	**	**
Pinot Noir classic / organic	**	**	**	**	**

Note Table 3: *p <0.05; **p <0.01.

organic wines. Here the highest antioxidant activity was determined for white wine Veltliner organic with a value of 806.28 mg TE.L^{-1}, for red wine Frankovka organic 2570.92 mg TE.L^{-1}. The lowest antioxidant activity was recorded in Riesling white wine of 445.75 mg TE.L^{-1}, the highest grade of antioxidant activity in red wine André 2312.99 mg TE.L^{-1}, while the lowest in Pinot Noir 1862.01 mg TE.L^{-1}. From the above results it is evident that the antioxidant activity in red wines is generally higher up to 5 times compared to white wines. This is confirmed by the assertion used for the determination of total polyphenols. **Stratil et al. (2008)** evaluate the antioxidant activity of different wines Czech wine regions. Veltliner antioxidant activity (614 mg TE.L^{-1}) achieves the same results as our sample (Table 1). **Lachman et al. (2009)** gives the result of Frankovka of 1230 mg TE.L^{-1} as compared to Table 2, we find more than double the antioxidant activity values.

Correlation of sulfur dioxide content of biologically active substances

These results show a strong correlation of total sulfur dioxide antioxidant activity with Pinot Blanc organic, where the correlation coefficient was r = 0.91, Veltliner r = 0.81 and Veltliner organic r = 0.77. For André organic, this correlation coefficient was the strongest of all red wines r = 0.91. A slight correlation in relation to antioxidant activity and total SO_2 achieved results for Pinot Blanc bovine varieties (r = 0.51) and Riesling (r = 0.22). A slight correlation also results in red wines and Frankovka where the correlation coefficient r = 0.36 was here. With the strong correlations we can conclude that the total SO_2 influences the antioxidant activity of the red wines. Here, however, there is a strong correlation with white wines before red wine.

CONCLUSION

The study, which examined the effect of sulfur dioxide on total polyphenols and antioxidant activity in samples of white and red grape wines produced by the strandart route and organic wines. A strong relationship between SO_2 content and antioxidant activity was observed, especially in organic wines. It is possible to see the significant difference in total amount of sulfur dioxide in white wine Pinot Noir organic and classic samples (p <0,01) and red wine Frankovka (p <0,01). The lowest amount total SO_2 was recorded in organic Pinot Blanc (13,00 mg.L^{-1}) and organic Frankovka (24,4 mg.L^{-1}). The highest total amount of SO_2 was determined by Veltliner (53,40 mg.L^{-1}) and Frankovka (135,95 mg.L^{-1}). Organic André achieved the highest content of TPC (1349.12 mg.GAE.L^{-1}) and Frankovka organic highest content of TAA (2570.92 mg.TE.L^{-1}). The fact, however, is that SO_2 in wine serves as an antioxidant and protects wine from oxidation and acts as an antimicrobial agent. Therefore, we can state, according to the results, that organic wine provides higher biologically active values and contains less allergen. However, their sensory properties may differ from the standard as well as wines made shorter shelf life and quality may decrease during storage.

REFERENCES

Bajčan, D., Vollmannová, A., Šimanský, V., Bystrická, J., Trebichalský, P., Árvay, J., Czako, P. 2016. Antioxidant activity, phenolic content and colour of the Slovak cabernet sauvignon wines. *Potravinarstvo*, vol. 10, no. 1, p. 89-94. https://doi.org/10.5219/534

Bajčan, D., Árvay, J., Vollmannová, A., Bystrická, J., Trebichalský, P., Harangozo, L., Šimanský, V. 2017. Antioxidant properties, total phenolic and total flavonoid content of the Slovak white wines – Welschriesling and Chardonnay. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 266-271. https://doi.org/10.5219/730

Del Pino-García, R., González-SanJosé, M. L., Rivero-Pérez, M. D., García-Lomillo, J., and Muñiz, P. 2017. The effects of heat treatment on the phenolic composition and antioxidant capacity of red wine pomace seasonings. *Food chemistry*, vol. 221, p. 1723-1732. https://doi.org/10.1016/j.foodchem.2016.10.113

Dott, W., & Trüper, H. G. 1976. Sulfite formation by wine yeasts. *Archives of Microbiology*, vol. 108, no. 1, p. 99-104. https://doi.org/10.1007/BF00425098

Eschenbruch, R. 1974. Sulfite and sulfide formation during winemaking--a review. *American journal of Enology and Viticulture*, vol. 25, no. 3, p. 157-161.

Gea, A., Bes-Rastrollo, M., Toledo, E., Garcia-Lopez, M., Beunza, J. J., Estruch, R., & Martinez-Gonzalez, M. A. 2014. Mediterranean alcohol-drinking pattern and mortality in the SUN (Seguimiento Universidad de Navarra) Project: a prospective cohort study. *British Journal of Nutrition*, vol. 111, no. 10, p. 1871-1880. https://doi.org/10.1017/S0007114513004376 PMid:24480368

Henick-Kling, T., and Park, Y. H. 1994. Considerations for the use of yeast and bacterial starter cultures: SO_2 and timing of inoculation. *American Journal of Enology and Viticulture*, vol. 45, no. 4, p. 464-469.

Ivanova, V., Petruseva, D., Mitrev, S. 2015. Methods for determination of SO_2 and reducing sugars in wines and alcoholic beverages. *Yearbook of Faculty of Agriculture*, vol. 13, no. 1, p. 119-127.

Karadeniz, M., Akçay, Y. D., Yıldırım, H. K., Yılmaz, C., and Sözmen, E. Y. 2014. Effect of red wine consumption on serum oxidation and adiponectin levels in overweight and healthy individuals. *Polish Journal of Food and Nutrition Sciences*, vol. 64, no. 3, p. 201-207. https://doi.org/10.2478/pjfns-2013-0016

Kyseláková, M., Balík, J., Veverka, J., Tříska, J., Vrchotová, N., Totušek, J., and Lefnerová, D. 2003. Resveratrol v červených vínech. *Vinařský obzor*, vol. 7, no. 8, p. 357-358.

Lachman, J., Šulc, M., Faitová, K., and Pivec, V. 2009. Major factors influencing antioxidant contents and antioxidant activity in grapes and wines. *International Journal of Wine Research*, vol. 1, no. 1, p. 101-121. https://doi.org/10.2147/IJWR.S4600

Lapčíková, B., Lapčík, L., and Hupková, J. 2017. Physico-chemical characterisation of Slovak wines. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 216-222. https://doi.org/10.5219/727

McIntyre, J. 2015. Inventing wine: a new history of one of the world's most ancient pleasures. *Journal of Wine Research*, vol. 26, no. 3, p. 243-245. https://doi.org/10.1080/09571264.2015.1042965

OIV. 2O15. International Organisation of Vine and Wine, Compendium of international Methods of wine and

must analysis, vol. 2 [online] s.a. [cit. 2018 -02-09] Available at: http://www.oiv.int/en/technical-standards-and-documents/methods-of-analysis/compendium-of-international-methods-of-analysis-of-wines-and-musts-2-vol

Panchal, S. K., and Brown, L. 2013. Cardioprotective and hepatoprotective effects of ellagitannins from European oak bark (*Quercus petraea* L.) extract in rats. *European journal of nutrition*, vol. 52, no. 1, p. 397-408. https://doi.org/10.1007/s00394-011-0277-1

Rankine, B. C., and Pocock, K. F. 1969. Influence of yeast strain on binding of sulphur dioxide in wines, and on its formation during fermentation. *Journal of the Science of Food and Agriculture*, vol. 20, no. 2, p. 104-109. https://doi.org/10.1002/jsfa.2740200210

Romano P., Suzzi G. 1993. Sulfur dioxide and wine microorganisms. *Fleet GH (Ed) Wine Microbiology and Biotechnology, Harwood Academic Publishers*, p. 373-393.

Rop, O., Mlček, J., Juríková, T., Valšíková, M., Sochor, J., Řezníček, V., and Kramářová, D. 2010. Phenolic content, antioxidant capacity, radical oxygen species scavenging and lipid peroxidation inhibiting activities of extracts of five black chokeberry (*Aronia melanocarpa* (Michx.) Elliot) cultivars. *Journals of Medicinal Plants Research*, vol. 4, no. 22, p. 2431-2437. https://doi.org/10.5897/JMPR10.576

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.

Stratil, P., Kuban, V., and 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. https://doi.org/10.17221/1119-CJFS

Sumczynski, D., Bubelova, Z., Sneyd, J., Erb-Weber, S., and Mlcek, J. 2015. Total phenolics, flavonoids, antioxidant activity, crude fibre and digestibility in non-traditional wheat flakes and muesli. *Food chemistry*, vol. 174, p. 319-325. https://doi.org/10.1016/j.foodchem.2014.11.065

Suzzi, G., Romano, P., and Zambonelli, C. 1985. Saccharomyces strain selection in minimizing SO_2 requirement during vinification. *American journal of enology and viticulture*, vol. 36, no. 3, p. 199-202.

Špakovská, E., Marcinčák, S., Bača, M., and Turek, P. 2012. Polyphenolic content and antioxidative activity of wines from the sobrance wine region. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 6, no. 3, p. 32-35. https://doi.org/10.5219/204

Valášek, P., Mlček, J., Fišera, M., Fišerová, L., Sochor, J., Baroň, M., and Juríková, T. 2014. Effect of various sulphur dioxide additions on amount of dissolved oxygen, total antioxidant capacity and sensory properties of white wines. *Mitteilungen Klosterneuburg, Rebe und Wein, Obstbau und Früchteverwertung*, vol. 64, no. 4, p. 193-200.

Wells, A., and Osborne, J. P. 2011. Production of SO_2 binding compounds and SO_2 by Saccharomyces during alcoholic fermentation and the impact on malolactic fermentation. *South African Journal of Enology and Viticulture*, vol. 32, no. 2, p. 267-279. http://doi.org/10.21548/32-2-1387

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EFFECT OF ADDITIVES TO MICROBIOLOGICAL QUALITY OF YOGURTS

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ABSTRACT

The objective of this work was to study the effect of addition chia flour, quinoa flour, nopal powder, apple fibre and bamboo fibre BAF 40 in yogurt to microbiological quality. Yogurts were made with 1, 3 and 5% of addition of these additives. The milk used for manufacturing was heated up to 85 °C for 5 min and flour, powder and fiber were irradiated for 20 min in three replicates. It was monitored: Lactic acid bacteria (LAB) – 72 hours at 30 °C (ISO 13721:1998) and yeasts and moulds – 5 days at 25 °C (ISO 21527-1:2009). During storage, the number of LAB was increased to match the initial concentration of yogurt with addition chia flour (concentration 1, 3, 5%) and quinoa flour (1%). The addition of nopal powder, apple or bamboo fiber to yogurt showed a tendency to decrease the number of LAB compared with its initial concentration. All samples were compared with the control yogurt without addition whatever flour, powder or fiber. The amount of yeasts and moulds was increased with the increasing addition of fiber in yogurts. The lowest amount of yeasts and moulds was in yogurt with the bamboo fiber. On the other hand the highest amount was in yogurt with chia flour.

Keywords: chia; quinoa; nopal; fiber; dairy product

INTRODUCTION

The term yogurt encompasses a wide range of products. Yogurt is a fermented dairy product, which is generally manufactured from pasteurized milk. High-temperature pasteurization of the yogurt mix is employed to obtain a smooth and firm body. Non-fat dry milk or stabilizers may also be added to increase the water-holding capacity and therefore improve its body **(Marth and Steele, 2001)**. Yogurt is manufactured from the milk and common commercial cultures composite from *Streptococcus thermophilus* and *Lactobacillus delbruckii* spp. *bulgaricus.* The microorganisms used in the production of yogurt accomplish briefly two tasks: production of lactic acid and flavour components **(Yildiz, 2010)**. Regarding the chemical composition of milk and yogurt, there is no significant difference between gross composition of milk and fermented milk. However, the fermentation process causes a beneficial effect on yogurt **(Walstra et al., 2006).** Fermentation is carried out by bacteria, moulds and yeasts which produced the enzymes. These enzymes caused that organic substances are broken down to smaller compounds. As a result, these processes cause that milk is more digestible, stable and flavoured **(Yousef and Carlstrom, 2003).** The addition of oat fiber did not significantly influence fermentation time, pH evolution, or orotic acid consumption by the starter bacteria during fermentation **(Fernández-García et al., 1998)**. Orange fibers presence in fermented camel milks also enhanced bacterial growth and survival of probiotic bacteria **(Ibrahimand and Khalifa, 2015)**. Food fortification is one of the processes which have influence to increase food quality and quantity. Fortification of yogurt is very effective because consumption rate of dairy products such as yogurt is very high **(Hashemi Gahruie et al., 2015)**. More authors add the fiber to yogurts but most of these works follow up rheological properties and sensory profiles as **Staffolo et al. (2004)**. They studied the effect of apple, wheat, bamboo fibers, or inulin on sensory and rheological properties of yogurt. The chia seed is good source of valuable protein fraction and antioxidant compounds **(Kačmárová et al., 2016)**. Chia seed is the best known plant source to maintain a balanced serum lipid profile **(Nitrayová et al., 2014)**. According **Remeňová et al. (2017)** yogurt with addition of pressed flax seed and honey can have beneficial effects on human body, but addition of pressed flax had no effect on sensory properties of yogurt. **Hashim et al. (2009)** shown, that fortifying yogurt with 3% of date fiber produced an acceptable product with potential beneficial health effects.

The set yogurts are more safe from the microbial aspect than stirred yogurt. The set yogurt fermented at higher temperature and shorter time, producing more lactic acid and thus prohibited the growth of contaminating

microbiota eg. coliforms. The stirred yogurt fermented at lower temperature and longer time, the fermentation is slower and thus allowed a growth of mesophilic and coliforms bacteria **(Görner and Valík, 2004)**. According to **Decree no. 397/2016 Coll.** yogurt must contain a minimum 7 log CFU.g^{-1} and must be made from protosymbiotic mixture of *Steptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Moulds contamination is almost exclusively caused by Mucoraceae family which had very strong proteolytic and lipolytic activity which leads to intensive odour. The spores of Mucoreceae are spreading very rapidly **(Holec et al., 1989)**. *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* are one of the most common yeasts which changed the taste of yogurt. The typical yogurt which is contaminant by yeasts is yeast taste and bubble in the coagulum. These changes are most often seen in flavoured yogurt **(Görner and Valík, 2004)**. If the yogurt is by the "good manufacturing practice", it should contain no greater than 1 yeast cell.g^{-1}. When products are correctly stored in refrigerator (5 °C), the shelf life of this yogurt is 3 or 4 weeks **(Suriyarachchi and Fleet, 1981)**. Presence of yeasts or moulds in yogurt also indicates poor sanitary practices in manufacturing or packaging. Yogurts with added sugar or fruits especially are susceptible to yeast growth **(Arnott et al., 1974)** but the consummation of flavoured cream yogurts is increases **(Habánová et al., 2010)**.

Scientific hypothesis

There was monitored the effect of addition chia flour, quinoa flour, nopal powder, apple fibre and bamboo fibre BAF 40 in yogurt to Lactic acid bacteria (LAB) and yeasts and moulds during the storage.

MATERIAL AND METHODOLOGY

This research was carried out at the Department of Food Technology at Mendel University in Biotechnology Pavilion M, financed by the OP VaVpI CZ.1.05/4.1.00/04.0135 project.

The yogurt for the research was prepared from milk of Holstein dairy cows from South Moravia region. The bovine milk content: 3.50% of fat by Gerber´s acidobutyrometric method **(ISO 2446:2008)**, 3.42% of protein by Kjeldahl´s method **(EN ISO 8968-1:2002)**, 4.50% of lactose and titratable acidity 6.7 SH according **Czech state standart no. 57 0530 (1974)**. The milk was heated up to 85 °C for 5 min and then cooled down to 36 °C. Then cooling was added into pasteurized milk of starter for making original Bulgarian yogurt (bulgaricus.cz, GENESIS LABORATORIES, Bulgary). It was used a lyophilized started for preparing original Bulgarian yogurt. Before used was prepared the starter (1 g of this starter was inoculated in 1 L of milk), after fermentation was added 2% of this prepared starter. The addition of starter to the milk was such that the resulting concentration in the yogurt was 8 log CFU.g^{-1}. This mixture was fermented at 36 °C for 18 hours. After fermentation, the coagulum was stirred for 5 min and divided into 16 groups. In these groups were added chia flour, quinoa flour, nopal powder, apple fiber and bamboo fiber. Each addition was made from three concentrations: 1, 3 and 5% of addition of these fibers. Before the addition, fibers in yogurt were irradiated for 20 min in three replicates. One group of yogurt was made as natural (control). The samples of final yogurt were stored at the 4 °C for 28 days.

The microbiological analysis was carried out in microbiological lab at the Department of Food Technology at Mendel University. Samples were taken from three different crucibles.

For all samples were determined: Lactic acid bacteria (LAB) – 72 hours at 30 °C **(ISO 15214:1998)** and yeasts and moulds – 5 days at 25 °C **(ISO 21527-1:2009)**.

The 1st analysis was carried out 24 hours after yogurt manufacturing, 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage).

Statisic analysis

The results were statistically processed by program MS EXCEL version 2010 (Microsoft) and STATISTICA CZ version 12 (StatSoft, Czech Republic). It was used: the calculation of basic statistical parameters, the simple sorting method of analysis of variance (ANOVA, Duncan's test) and regression analysis.

RESULTS AND DISCUSSION

Chia flour addition

The control yogurt had 8.6 log CFU.g^{-1} at the start and, during the storage, the amount of LAB has fallen to 7.9 log CFU.g^{-1}. The amount of lactic acid bacteria was higher than is given by legislation (more than 7 log CFU.g^{-1} in **Decree no. 397/2016 Coll)**. According the **Lengyelová et al., 2010** the amount of LAB in tested yogurt was higher than Slovak Food codex limits. The addition 1, 3 or 5% of chia flour had influence to lower amount of LAB than in the control yogurt at the start sampling, one week later the amount of LAB was higher than amount of LAB in control yogurt. The third week sampling the amount of LAB was reduced, the tendency for bacterial growth therefore decreased as well as in the control yogurt (Figure 1). There was no statistically significant difference ($p > 0.05$) between addition of 1, 3 or 5% of chia flour to amount of LAB into yogurt.

The total of yeasts and moulds in control yogurt during three weeks storage were undetectable. The amount the yeasts and moulds was higher than 2 log CFU.g^{-1} in 1% addition in yogurt. The addition of 3% of chia flour in the yogurt had influence on total amount of yeasts and moulds in yogurt. After three weeks of storage, the amount of yeasts and moulds was higher than 3.2 log CFU.g^{-1}. This amount is comparable with 3.5 log CFU.g^{-1} which was detectable in the yogurt with 5% addition of chia flour after three weeks of storage (Figure 2). The shelf life of yogurt with chia flour was affected with high contamination of chia flour by the yeasts and moulds. The shelf life of these yogurts was 2 weeks. This storage time is shorter than presented **Suriyarachchi and Fleet, 1981**. The total amount of yeasts and moulds were increased during all time of storage. These changes were statistically significant ($p < 0.05$).

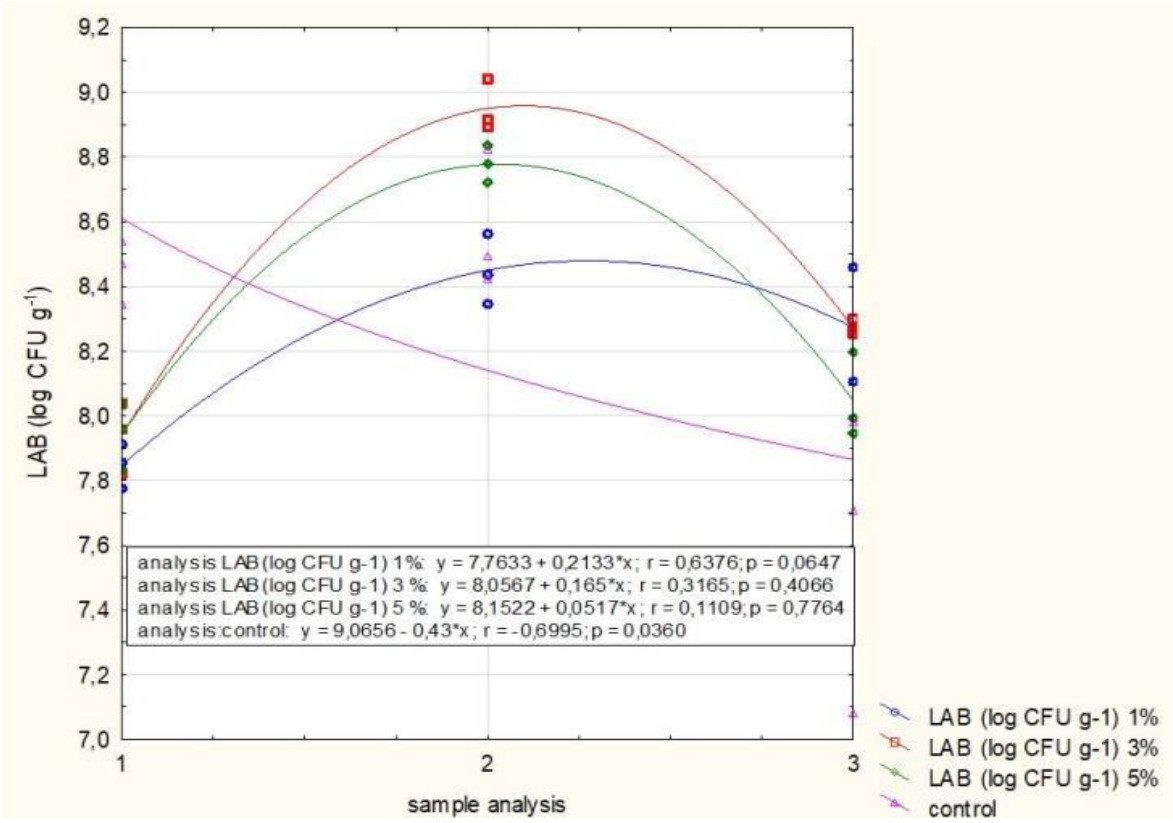

Figure 1 Changes in the amount LAB (log CFU.g-1) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage) in yogurt with chia flour.

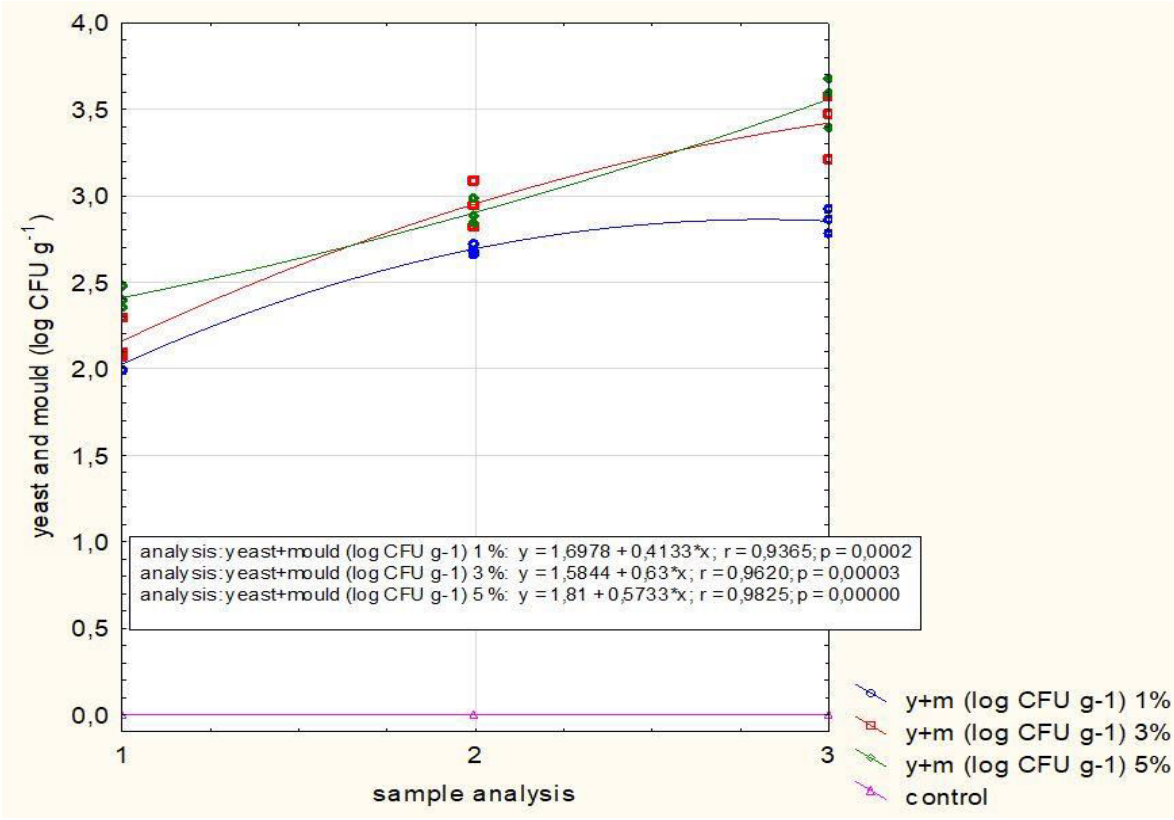

Figure 2 Changes in the amount yeasts and moulds (log CFU.g^{-1}) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage) in yogurt with chia flour.

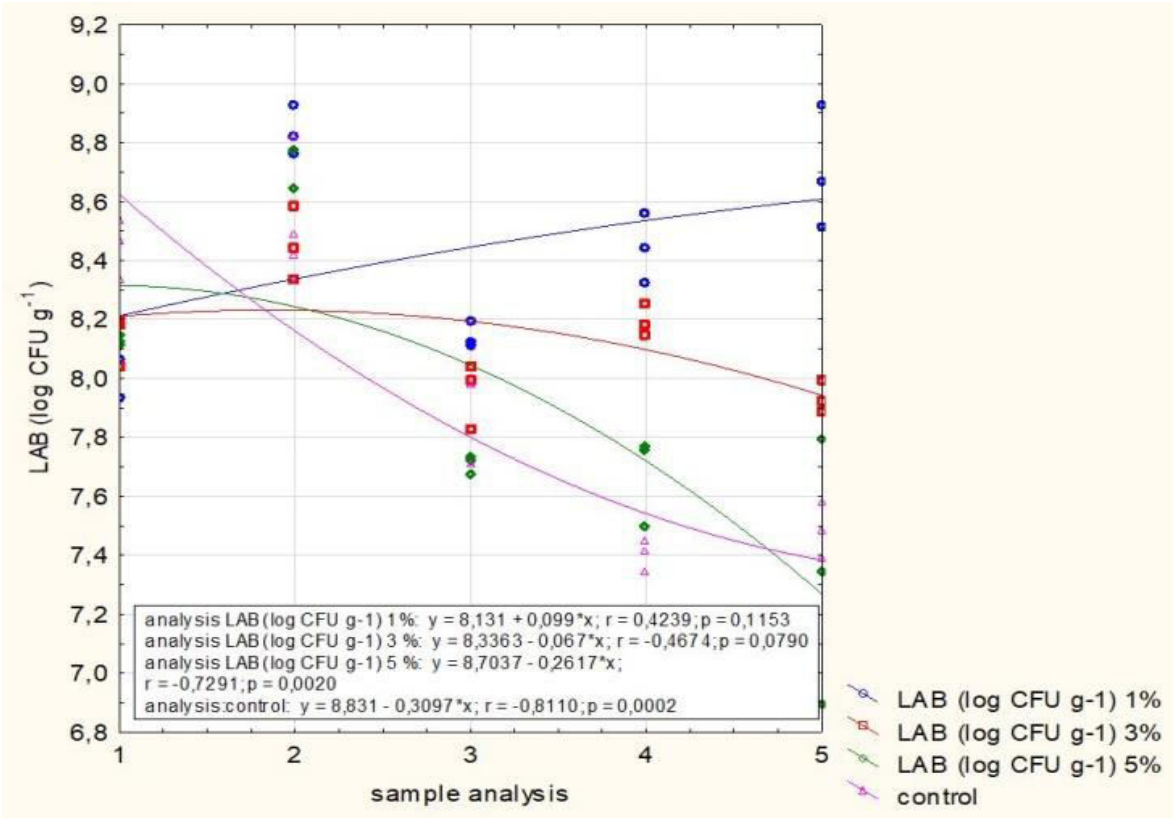

Figure 3 Changes in the amount LAB (log CFU.g^{-1}) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with quinoa flour.

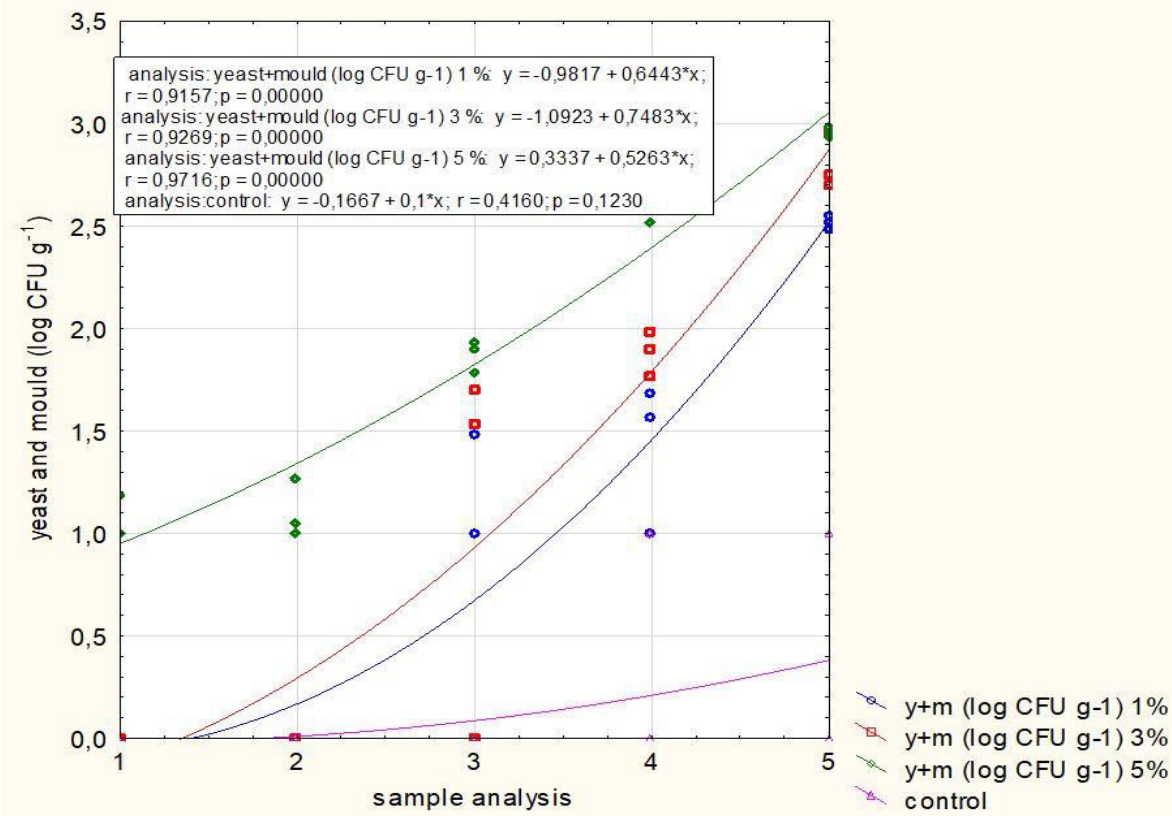

Figure 4 Changes in the amount yeasts and moulds (log CFU.g^{-1}) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with quinoa flour.

Quinoa flour addition

The addition 1% of quinoa flour in yogurt caused that during the storage, the amount of LAB has increased (Figure 3). In the beginning, the amount of LAB was 8.2 log CFU.g^{-1} and at the end of shelf life, the amount of LAB was 8.6 log CFU.g^{-1}. Addition of 3 or 5% caused that the amount of LAB has fallen during storage. With addition of 3% quinoa flour, the amount of LAB decreased from 8.2 log CFU.g^{-1} to 7.9 log CFU.g^{-1}. When there was added more quinoa flour (5%), that caused bigger decrease from the 8.3 log CFU.g^{-1} to 7.3 log CFU.g^{-1}. Saponins are bitter compounds that are naturally present in quinoa located in the outer layers of quinoa seeds. The content saponin in quinoa is in range 0.1 to 5.0% **(Valencia-Chamorro, 2003)**. This bigger decrease of LAB in yogurt can be caused by higher content of saponins. This decrease was statistically significant (p <0.05).

There was no statistically significant difference between the amount of LAB in control and 5% addition quinoa flour in yogurt (p >0.05). But the statistically significant difference (p <0.05) was between 1 and 3% of quinoa flour added in yogurt.

The total amount of yeasts and moulds in control yogurt during the whole storage time was very low (less than 0.4 log CFU.g^{-1} after five weeks of storage). This change was statistically insignificant (p >0.05).

The amount the yeasts and moulds at the beginning was not detected. The amount of yeasts and moulds was increased to 2.5 log CFU.g^{-1} in yogurt with 1% of quinoa flour addition, respectively 2.9 log CFU.g^{-1} in yogurt with 3% of quinoa flour addition during the storage. The most yeasts and moulds were in yogurt with 5% addition of quinoa flour. At the beginning was 1 log CFU.g^{-1} yeasts and moulds in yogurt and, after five weeks of storage, the amount of yeasts and moulds increased to 3.1 log CFU.g^{-1}. There was statistically significant growth trend (p <0.05) of yeasts and moulds in yoghurt with 1, 3 or 5% quinoa flour addition (Figure 4). The least yeasts and moulds were in the control yogurt, while the most yeasts and moulds were in yogurt with 5% quinoa flour added regardless of the other observed factors (storage time).

Nopal powder

The control yogurt had 8.6 log CFU.g^{-1} at the start and, during the storage the amount of LAB has fallen to 7.4 log CFU.g^{-1}. The amount of lactic acid bacteria was higher after five weeks of storage than is given by legislation – more than 7 log CFU.g^{-1} **(Decree no. 397/2016 Coll.)**. At the beginning was amount of LAB lower in yogurt with 1, 3 or 5% nopal powder addition, but after 1 week of storage was the amount of LAB higher than the amount of LAB in control yogurt. After one week of storage the amount of LAB decreased also in the control yogurt. The tendency for bacteria growth decreased. For yogurt with 5% nopal powder addition, there was a typical, almost constant decline of the amount of LAB during storage (Figure 5). There was no statistically significant difference (p >0.05) between the control yogurt and yogurt with 1, 3 or 5% nopal powder addition to amount of LAB in these yogurts regardless of the other observed factors (storage time).

The change of the amount of yeasts and moulds in control yogurt was not statistically significant (p >0.05). The amount of yeasts and moulds at the beginning was not detected regardless of the nopal powder addition. The amount of yeasts and moulds was increased to 2.5 log CFU.g^{-1} in yogurt with 1% nopal powder addition, respectively 2.3 log CFU.g^{-1} in yogurt with 3% nopal powder addition during the storage. The most yeasts and moulds were in yogurt with 5% nopal powder addition. At the beginning were not detected yeasts and moulds in yogurt and after five weeks of storage was amount of yeasts and moulds increased to 3.5 log CFU.g^{-1}. There was statistically significant growth trend (p <0.05) yeasts and moulds in yogurt with 1, 3 and 5% nopal powder addition (Figure 6). The least yeasts and moulds were in the control yogurt, while the most yeasts and moulds were in yogurt with 5% nopal powder addition, regardless of the other observed factors (storage time).

Apple fiber

The control yogurt and yogurts with apple fiber addition had at the beginning more than 8 log CFU.g^{-1} of LAB. Yogurt with 1% (8.5 log CFU.g^{-1}) and 5% (8.3 log CFU.g^{-1}) apple fiber addition showed that amount of LAB in these yogurts was decreased until the third sampling when the amount of LAB was increased. At the end of sampling was in yogurt with 1% apple fiber addition 8 log CFU.g^{-1} and yogurt with 5% apple fiber addition 7.8 log CFU.g^{-1}. On the other hand, the yogurt with 3% apple fiber addition showed the opposite trend. The amount of LAB was increased until the third sampling and, after the third sampling, the amount of LAB has decreased (Figure 7). **Staffolo et al. (2004)** there were found the highest differences between control and yogurt with apple fiber in rheological and sensory characteristics.

There was no significant difference (p <0.05) between control yogurt and yogurt with 1, 3 or 5% apple fiber addition to amount of LAB in these yogurts regardless of the other observed factors (storage time).

The amount of yeasts and moulds at the beginning was not detected regardless of the apple fiber addition. The amount of yeasts and moulds increased to 1.9 log CFU.g^{-1} in yogurt with 1% apple fiber addition, 2.6 log CFU.g^{-1} in yogurt with 3% apple fiber addition and 3.9 log CFU.g^{-1} yogurt with 5% apple fiber addition after five weeks of storage. With increasing the addition of apple fiber has increased the amount yeasts and moulds in yogurts. There was statistically significant growth trend (p <0.05) of yeasts and moulds in yoghurt with 1, 3 and 5% apple fiber addition (Figure 8). The least amount of yeasts and moulds was in the control yogurt, while the most yeasts and moulds were in yogurt with 5% apple fiber added regardless of the other observed factors – storage time (p <0.05).

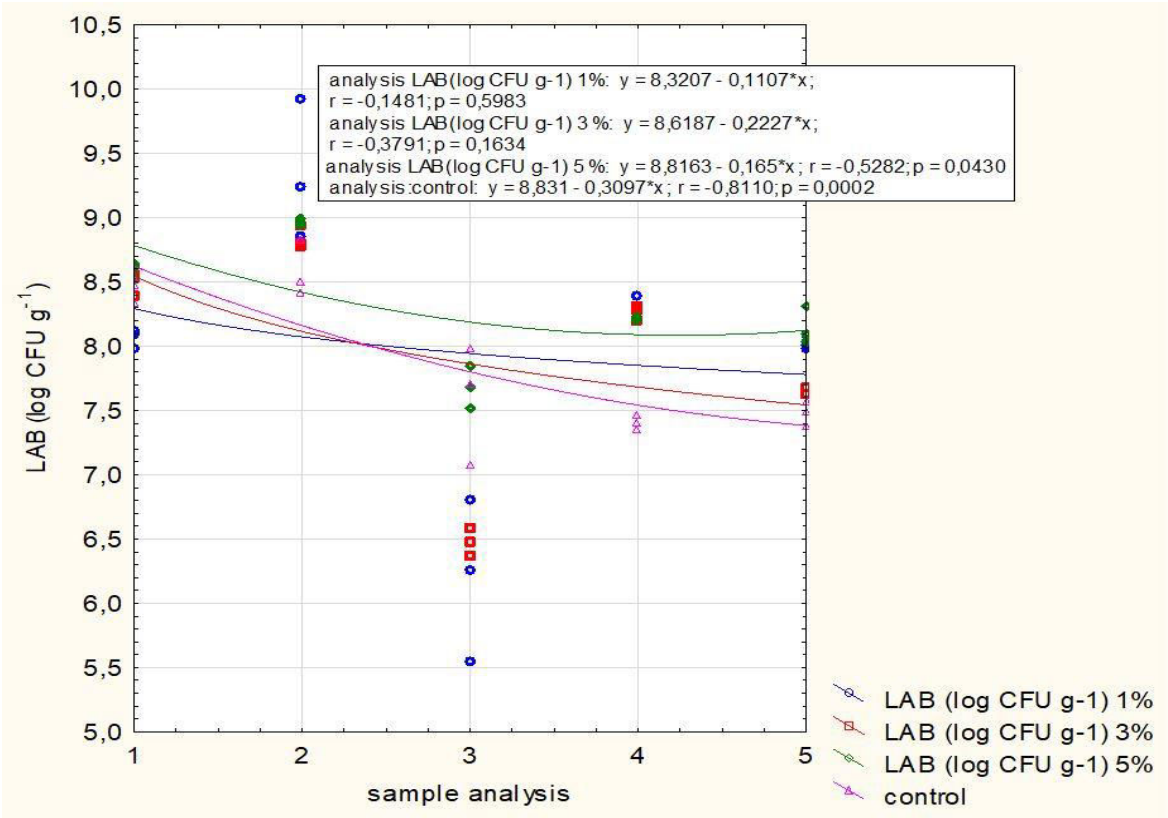

Figure 5 Changes in the amount LAB (log CFU.g^{-1}) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with nopal powder.

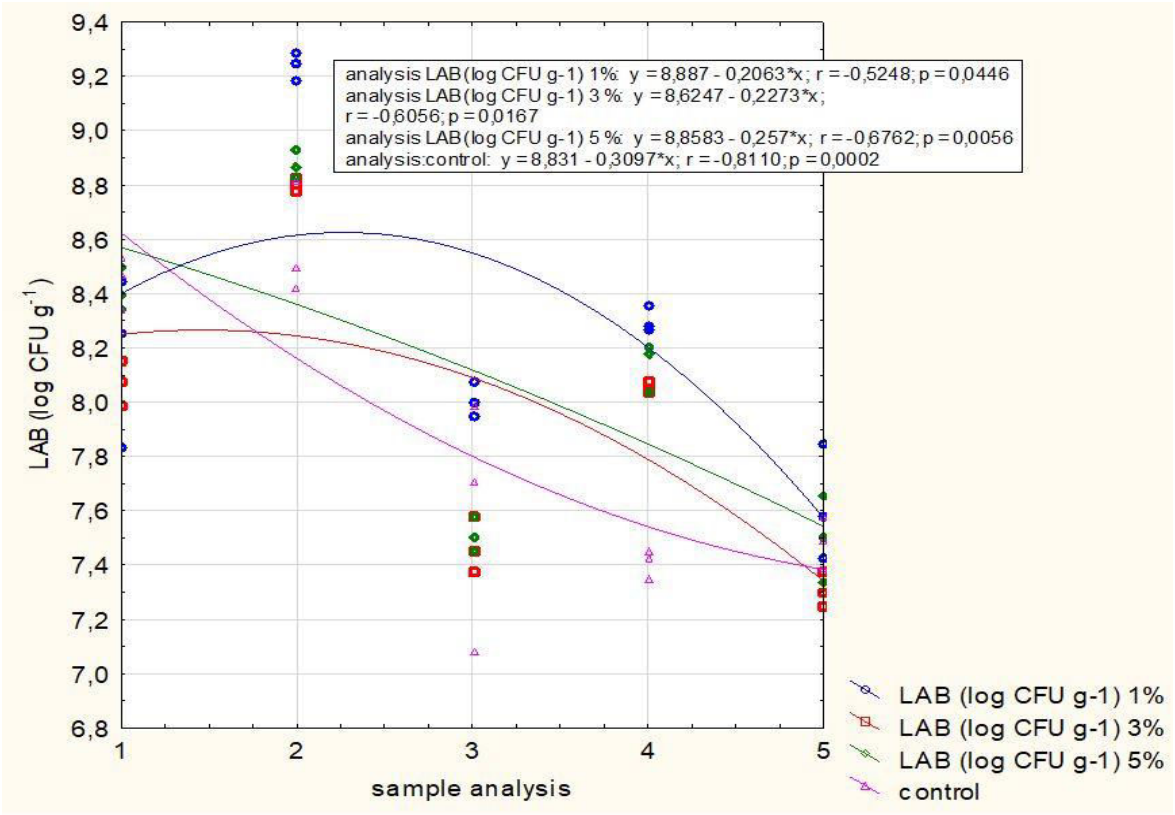

Figure 6 Changes in the amount yeasts and moulds (log CFU.g^{-1}) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with nopal powder.

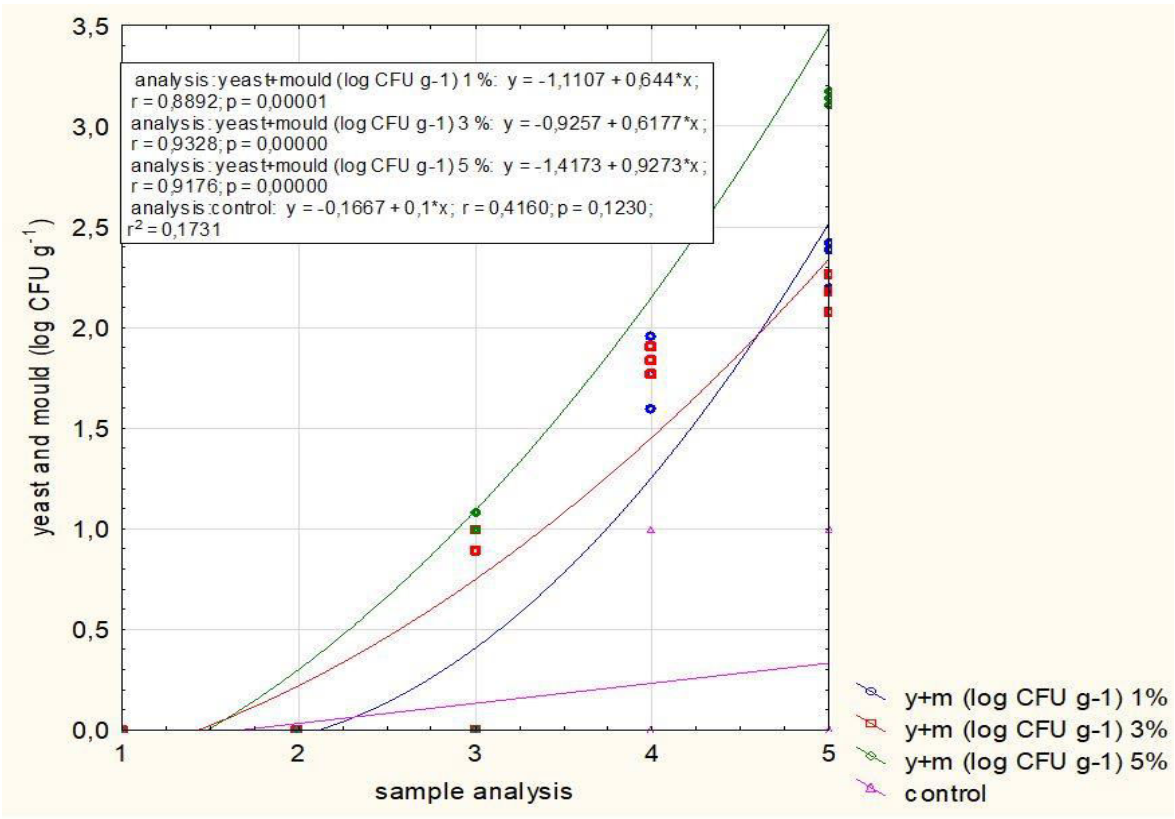

Figure 7 Changes in the amount LAB (log CFU.g^{-1}) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with apple fiber.

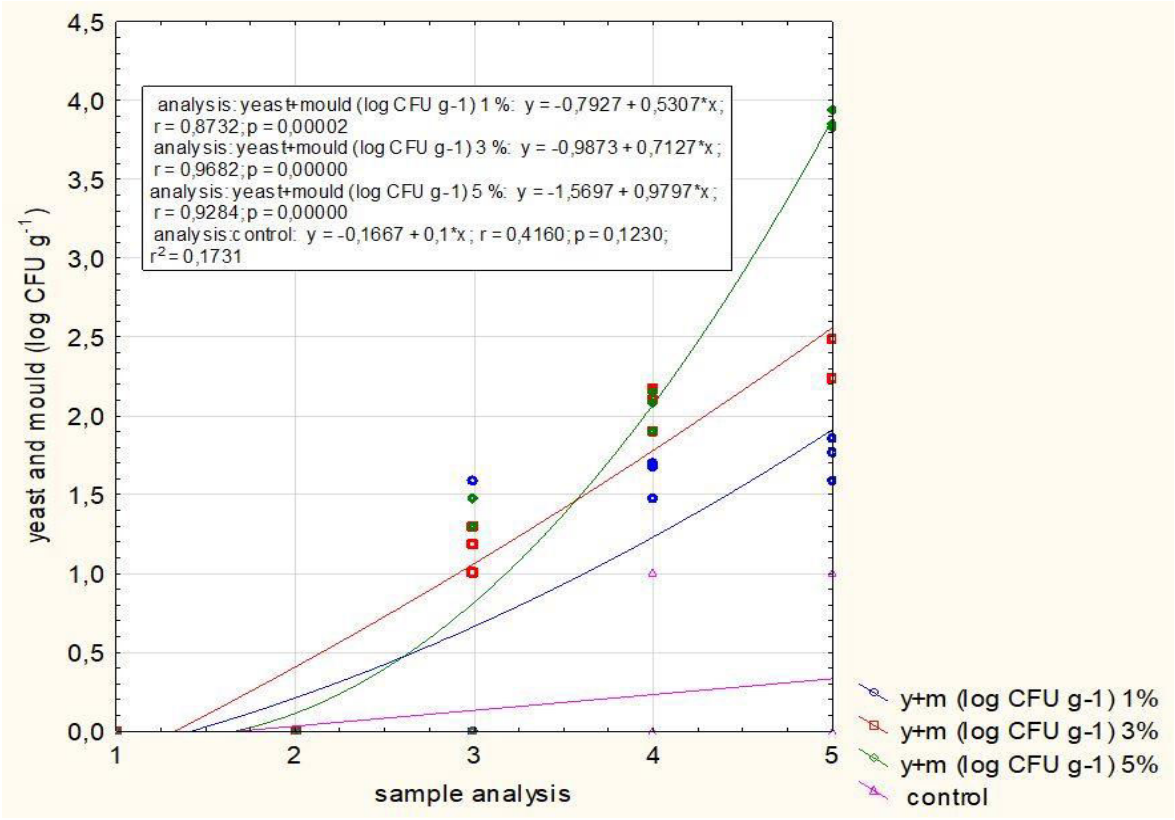

Figure 8 Changes in the amount yeasts and moulds (log CFU.g^{-1}) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with apple fiber.

Bamboo fiber

The addition of 1% of bamboo fiber had an influence on lower amount of LAB than in the control yogurt at the start of sampling. This situation changed after three weeks of storage when the amount of LAB increased in yogurt with 1% bamboo fiber addition. The amount of LAB in yogurt with 3% bamboo fiber addition was changed during storage time. At the beginning and at the end had this yogurt more LAB than control yogurt. These changes in yogurt with 1 and 3% addition were not statistically significant ($p > 0.05$). But yogurt with 5% bamboo fiber addition had, for the whole storage time, more LABs in yogurt than the control yogurt. Addition of 5% bamboo fiber in yogurt made a good condition for growth LAB (Figure 9). There was no statistically significant difference ($p > 0.05$) between addition of 1, 3 or 5% of bamboo fiber to amount of LAB into yogurt.

The amount of yeasts and moulds at the beginning was not detected regardless of the bamboo fiber added. This amount of yeasts and moulds was same for the whole storage time. There was no statistically significant difference ($p > 0.05$) between the control and 1, 3 or 5% bamboo fiber addition samples, regardless of the other observed factors (storage time). Just as **Ibrahimand and Khalifa (2015)** in their work, they did not detect any yeasts and moulds in yogurt with date and orange fiber during storage.

CONCLUSION

There was monitored the effect of addition of chia flour, quinoa flour, nopal powder, apple fibre and bamboo fibre BAF 40 to Lactic acid bacteria (LAB) and the amount of yeasts and moulds in these yogurts.

The addition of chia flour, apple fiber or bamboo fiber in yogurts caused that the amount of Lactic acid bacteria was higher than the amount of LAB in control samples of yogurt. The addition of 1 or 3% of quinoa flour in yogurt showed higher amount LAB in yogurt too. These changes were not statistically significant ($p > 0.05$). But addition of 5% of quinoa flour caused a decrease of LAB in yogurt due to a possible higher presence of saponins that may affect the condition of growth for LAB (statistically significant difference $p < 0.05$). Yogurt with the nopal powder had the same or higher amount of LAB during the storage. The statistically significant difference ($p < 0.05$) was for yogurt with 3 and 5% nopal powder addition.

The amount of LAB was not decreased below the limit 7 log CFU.g^{-1} during the storage. All monitored yogurts fulfilled the requirements of the Decree no. 397/2016 Coll.

The amount of yeasts and moulds in the control yogurt was very low during the whole storage time. It confirmed a "good manufacturing practice" because the total amount of yeasts and mould was not greater than 1 yeast cell.g^{-1}. However, the problem was with the added flour, powder and fiber. The amount of yeasts and moulds increased with the higher addition of fiber in yogurts. The lowest amount of yeasts and moulds was in the yogurt with bamboo fiber. On the other hand, the highest amount was in the yogurt with chia flour.

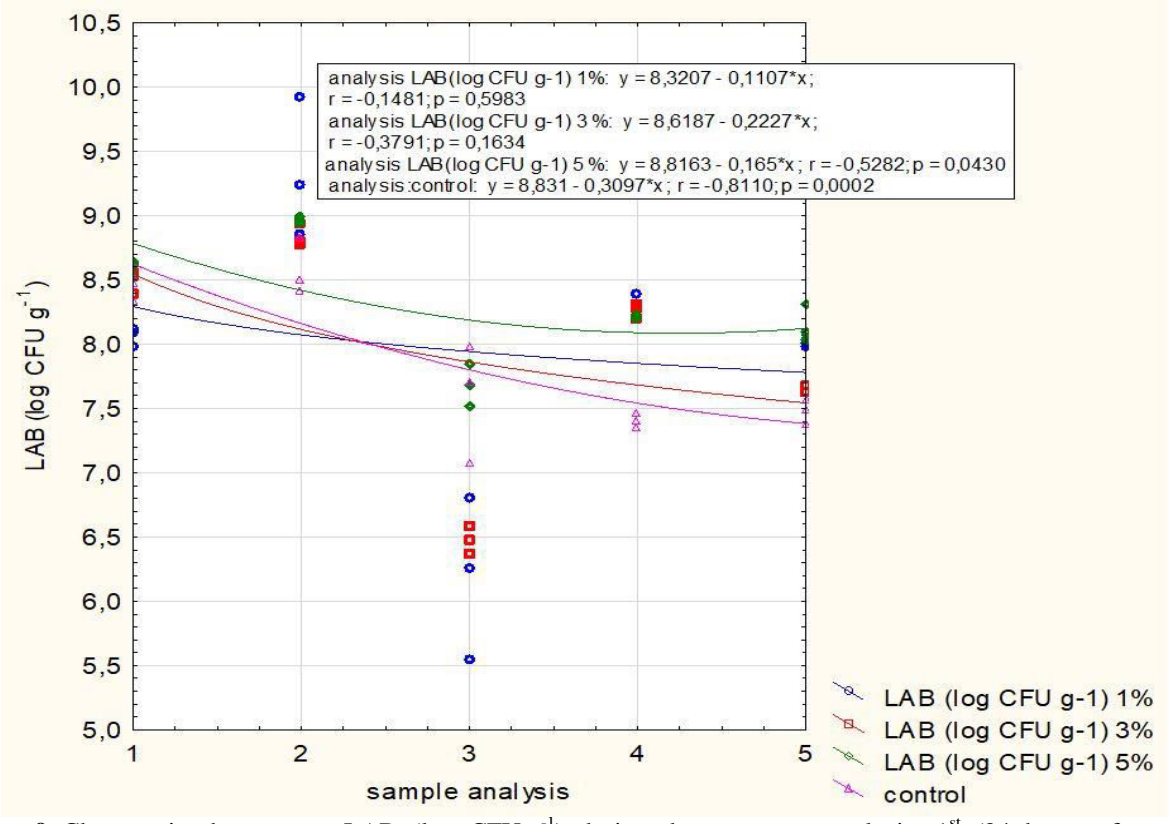

Figure 9 Changes in the amount LAB (log CFU.g^{-1}) during the storage – analysis: 1st (24 hours after yogurt manufacturing), 2nd (7 days after storage), 3rd (14 days after storage), 4th (21 days after storage), 5th (28 days after storage) in yogurt with bamboo fiber.

REFERENCES

Arnott, D. R., Duttschaever, C. L., Bullock, D. H. 1974. Microbiological evaluation of yogurt produced commercially in Ontario. *Journal of Milk and Food Technology,* vol. 37, no. 1. p. 11-13. https://doi.org/10.4315/0022-2747-37.1.11

Czech state standart no. 57 0530:1974. Methods for testing of milk and milk products (Metody zkoušení mléka a tekutých mléčných výrobků.

Decree no. 397/2016 Coll., Ministry of Agriculture of Czech Republic on requirements for milk and dairy products, frozen creams and edible fats and oils. Collection of Laws, no.162, 12.12.2016 (Vyhláška Ministerstva zemědělství o požadavcích na mléko a mléčné výrobky, mražené krémy a jedlé tuky a oleje. Sbírka zákonů ČR, částka 162/2016, 12.12.2016). Decree of the Ministry of Agriculture of the Czech Republic on requirements for milk and dairy products, milled creams and edible fats and oils. Collection of Laws of the Czech Republic, amount 162/2016, 12.12.2016

EN ISO 8968-1:2002. Milk and milk products – Determination of nitrogen content. Part 1, Kjeldahl method and crude protein calculation.

Fernández-García, E., McGregor, J. U., Traylor, S. 1998. The addition of oat fiber and natural alternative sweeteners in the manufacture of plain yogurt. *Journal of Dairy Science,* vol. 81, no. 3. p. 655-663. https://doi.org/10.3168/jds.S0022-0302(98)75620-6

Görner, F., Valík, L. 2004. *Applied food microbiology. (Aplikovaná mikrobiológia poživatín)*. Bratislava, Slovakia : Malé Centrum, PPA. 528 p. ISBN 80-967064-9-7. (In Slovak)

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. 4, no. 3, p. 19-23. http://dx.doi.org/10.5219/26

Hashemi Gahruie, H., Hadi Eskandari, M., Mesbahi, G., Amin Hanifpour, M. 2015. Scientific and technical aspects of yogurt fortification: A review. *Food Science and Human Wellness,* vol. 4, p. 1-8. http://dx.doi.org/10.1016/j.fshw.2015.03.002

Hashim, I. B., Khalil A. H, Afifi, H. S. 2009. Quality characteristics and consumer acceptance of yogurt fortified with date fiber. *Journal of Dairy Science,* vol. 92, no. 11, p. 5403-5407. DOI: https://doi.org/10.3168/jds.2009-2234

Holec, J. 1989. *Milk and dairy products hygiene and technology (Hygiena a technologie mléka a mléčných výrobků)*. 2nd ed. Brno: Státní pedagogické nakladatelství Praha. 362 p. ISBN 80-85114-60-7. (In Czech)

Ibrahimand, A. H., Khalifa, S. A. 2015. Improve Sensory Quality and Textural Properties of Fermented Camel's Milk By Fortified With Dietary Fiber. *Journal of American Science,* vol. 11, no. 3, p. 42-54.

ISO 15214:1998 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 degrees C.

ISO 21527-1:2009. Microbiology of food and animalfeeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0.95.

ISO 2446:2008. Milk – Determination of fat content.

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

Lengyelova, L., Kozelová, D., Trstenovičová, L., Pinterová, S. 2010. Comparison of occurence lactic acid bacteria in chosen yogurts. *Potravinarstvo*, vol. 4, no. 4, p. 38-43. http://dx.doi.org/10.5219/31

Marth, E. H., Steele J. L. 2001. *Applied dairy microbiology*. 2nd ed. NEW YORK: M. Dekker, Food science and technology (Marcel Dekker, Inc,). 110 p. ISBN: 0-8247-0536-x.

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

Remeňová, Z., Čanigová, M., Kročko, M., Ducková., V., Šimková, A. 2017. Effects of floral honey and pressed flax seeds on intensity of yogurt aroma, sweetness and sour taste of yogurts during storage. *Potravinarstvo*, vol. 11, no. 1, p. 436-440. https://dx.doi.org/10.5219/760

Staffolo, M. D., Bertolaa, N., Martinoa, M., Bevilacquaa, A. 2004. Influence of dietary fiber addition on sensory and rheological properties of yogurt. *International Dairy Journal*, vol. 14, p. 263-268. DOI: https://dx.doi.org/10.1016/j.idairyj.2003.08.004

Suriyarachchi, V. R., Fleet, G. H. 1981. Occurence and Growth of Yeasts in Yogurts. *Applied and Environmental Microbiology*, vol. 42, no. 3, p. 574-579.

Valencia-Chamorro, S. A. 2003. Quinoa. In Encyclopedia of Food Sciences and Nutrition. 2nd ed.: Academic Press, p. 4895-4902. ISBN: 978-0-12-227055-0.

Walstra, P., Wouters, J. T. M., Geurts, T. J. 2006. *Dairy science and technology*. 2nd ed. New York : CRC/Taylor & Francis. 763 p. ISBN: 0-8247-2763-0.

Yildiz, F. 2010. Development and manufacture of yogurt and other functional dairy products. BOCA RATON, FL : CRC Press/Taylor & Francis. 451 p. ISBN: 978-1-4200-8207-4.

Yousef, A. E., Carlstrom, C. 2003. *Food Microbiology*: *A Laboratory Manual.* John Wiley and Sons. 277 p. ISBN: 0471391050.

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UTILIZATION OF PUMPKIN POWDER IN BAKED ROLLS

Lucia Minarovičová, Michaela Lauková, Jolana Karovičová, Zlatica Kohajdová

ABSTRACT

Bakery products are good vehicles for dietary fiber incorporation. Traditionally, the source of dietary fiber has been mainly from cereals. However, vegetable can be used as potential source of dietary fiber. Pumpkin powder was characterized with good hydration properties (water holding and water retention capacity and swelling capacity), but fat absorption capacity was very low. Wheat flour was substituted with pumpkin powder at different level (2.5; 5; 7.5; and 10%). Addition of pumpkin powder modified the farinographic properties in various ways (water absorption and dough development time increased, while dough stability and mixing tolerance index decreased). Also it was concluded, that addition of pumpkin powder affected the qualitative properties of baked rolls. Enriched baked rolls had lower volume and specific volume. Moreover, the cambering values were significantly decreased as contain of pumpkin powder increased. The effect of incorporation of pumpkin powder in baked rolls on firmness values was also evaluated. It was also observed that enriched rolls were firmer during storage compared to control sample. Baked rolls containing pumpkin powder at higher addition levels had vegetable taste and flavour. From the results also concluded that the overall acceptance of baked rolls with 2.5 and 5% addition levels of pumpkin powder were comparable with control sample (without pumpkin powder).

Keywords: pumpkin powder; hydration property; Farinograph; bakery product; texture

INTRODUCTION

Bakery products are widely consumed as staple food all over the world. In last few decades, bakery products have been explored extensively for development of functional foods via fortification of active ingredients such as dietary fiber, bioactive peptides, minerals, vitamins etc. to increase its therapeutic values **(Mudgil et al., 2016)**. Bakery products are varied by addition of value added ingredients. Among the added ingredients, dietary fibre has gained tremendous attention **(Noor Aziah and Komathi, 2008)**.

Dietary fibre is naturally present in cereals, vegetables, fruits and nuts. The amount and composition of fibres differ from food to food. A fibre-rich diet is lower in energy density, often has a lower fat content, is larger in volume and is richer in micronutrients. This larger mass of food takes longer to eat and its presence in the stomach may bring a feeling of satiety sooner, although this feeling of fullness is short term. It is suggested that healthy adults should eat between 20 and 35 g of dietary fibre each day **(Dhingra et al., 2012)**. The use of specific fibres in food products is largely determined by their functionality, which depends on physicochemical properties, and by food processing conditions. Additional factors that must be considered in many applications include fibre colour and flavour because of their impact on sensory characteristics **(Tosh and Yada, 2010)**.

Fibre can even be produced from sources that might otherwise be considered waste products. For example, wheat straw, soy hulls, oat hulls, peanut and almond skins, corn stalks and cobs, spent brewer's grain and waste portions of fruits and vegetables processed in large quantities can be converted into fibre ingredients, which may be highly functional in certain food applications. Dietary fibre holds all the characteristics required to be considered as an important ingredient in the formulation of functional foods, due to its beneficial health effects **(Dhingra et al., 2012)**.

Pumpkin is consumed in a variety of ways such as fresh or cooked as well as being stored, frozen or canned. Pumpkin is a good source of β-carotene, fibre, pectin, mineral salts, vitamins and other substances that are beneficial to health. These facts lead to the processing of pumpkin into various food products **(Kuchtová et al., 2016)**. Pumpkin can be processed into flour which has a longer shelf-life. Pumpkin flour is used because of its highly-desirable flavour, sweetness and deep yellow-orange colour. It has been reported to be used to supplement cereal flours in bakery products like cakes, cookies, bread, for soups, sauces, instant noodle and spice

as well as a natural colouring agent in pasta and flour mixes **(Minarovičová et al., 2017)**.

Scientific hypothesis

The purpose of this study was to evaluate the effect of pumpkin powder addition on rheological properties (water absorption, dough development time, dough stability and mixing tolerance index) of wheat dough and qualitative parameters of baked rolls.

MATERIAL AND METHODOLOGY

Fine wheat flour (wet gluten 24.16%, dry gluten 8.75% and moisture 11.32%) and other ingredients (vegetable oil, salt, sugar and yeast) were obtained in local market. Pumpkin powder (PP) was prepared according **Minarovičová et al. (2017)**.

Functional properties

Hydration properties of PP were determined according to methods described by **Sowbhagya et al. (2007)** with slight modifications. Water holding capacity (WHC) was determined by accurately weighing dry sample (1 g) into a graduated test tube, and adding around 30 mL of water, and it was allowed to hydrate for 18 h at ambient temperature. The supernatant was removed, the hydrated residue weight was recorded and it was dried at 105 °C for 2 h to obtain the residue dry weight. WRC was expressed as grams of retained water per gram of sample.

Water retention capacity (WRC) was determined by accurately weighing dry sample (1 g) into a graduated centrifuge tube, adding 30 mL of water and it was hydrated for 18 h, centrifuged (3000 g, 20 min)and the supernatant solution was removed by passing through a sintered glass crucible (G4) under applied vacuum. The hydrated residue weight was recorded and then sample was dried at 105 °C for 2 h to obtain its dry weight. WHC was expressed as grams of retained water per gram of sample **(Sowbhagya et al., 2007)**.

Swelling capacity (SC) is defined as the ratio of the volume occupied when the sample is immersed in excess of water after equilibration to the actual weight. Accurately weighed dry sample (0.2 g) was placed in a graduated test tube, around 10 mL of water was added and it was hydrated for 18 h, and the final volume attained by the sample was measured. SC was expressed as mL of swollen sample per gram of sample **(Sowbhagya et al., 2007)**.

Fat absorption capacity (FAC) was measured according to method used by **Adeleke and Odedeji (2010)** with slight modifications. The 10 mL of refined corn oil was added to 1 g of the flour in a weighed 25 or 80 mL centrifuge tube. The tube was agitated on a vortex mixer for 2 min and kept at room temperature for 1 h. It was centrifuged at 2500 rpm for 20 min. The volume of free oil was recorded and decanted. FAC is expressed as mil of oil bound by 1 g dried sample.

Dough rheology

Farinographic parameters (water absorption - WA, dough stability - DS, dough development time - DDT and mixing tolerance index - MTI) of wheat dough with addition of pumpkin powder (2.5, 5, 7.5, and 10%) was determined using Farinograph Brabender (Duisburg, Germany) according to method **ISO 5530-1:2013**.

Rolls preparation

Wheat rolls were prepared using a recipe described by **Kohajdová and Karovičová (2007)**. The control recipe included: wheat flour 300 g, vegetable oil 7.5 g, yeast 12.06 g, salt 5.63 g, sugar 3.22 g and water to farinographic consistency 400 Brabender units (BU). The dough was prepared and mixed during 6 min in farinographic mixing bowl. After first fermentation (20 min), the dough was divided into 100 g loaves and formed on dough former (Extensograph Brabender, Duisburg, Germany). After second fermentation (45 min), the loaves were baked during 13 min at 230 °C. The rolls were cooled and after 2 h were packed in plastic bags. Baked rolls are presented in Figure 1.

Qualitative parameters of baked rolls

Qualitative parameters of baked rolls were evaluated 2 h after baking. The volume of baked rolls was measured by rapeseeds displacement method **(AACC Method 10-05.01)**. Specific volume (cm^3 per 100 g of loaf) was calculated by dividing the values of volume by weight. Cambering of baked rolls was calculated as a ratio of loaf height and width **(Lauková et al., 2016a)**. Baking loss (%) is characterized as the bakery product weigh reduction after baking. It is determined according to dough weight before baking and product weight, which was detected one hour after baking **(Korczyk-Szabó and Lacko-Bartošová, 2012)**.

Textural analysis

Baked rolls firmness was determined according to method described by **Al-Saleh and Brennan (2012)** using a texture analyzer (TA-XT Plus, Stable Micro Systems, Godalming, Surrey UK). The results were calculated using Exponent Texture Analysis software 2011. Firmness (the maximum force obtained during compression) was recalculated using macro and measurement was repeated 8

Figure 1 Baked rolls enriched with PP. Note: PP – pumpkin powder.

Table 1 Functional properties of PP.

	WHC (g.g^{-1})	WRC (g.g^{-1})	SC (mL.g^{-1})	FAC (g.g^{-1})
WF	1.78 ±0.08	0.65 ±0.03	2.47 ±0.10	0.94 ±0.04
PP	9.29 ±0.34	3.62 ±0.01	8.49 ±0.31	1.01 ±0.05

Note: FAC – fat absorption capacity, PP – pumpkin powder, SC – swelling capacity, WF – wheat flour, WHC – water holding capacity, WRC – water retention capacity.

times. Baked rolls were sliced mechanically into 12.5 mm slice thickness. Two slices were stacked together for each test, discarding two end slices of the rolls. Rolls firmness was measured with a probe P/36 R (setting: pre-test speed 1.0 mm.s^{-1}, test speed 1.7 mm.s^{-1}, post-test speed 10.0 mm.s^{-1}) at 40% compression on three successive days. Baked rolls were stored in plastic bags at 25 °C during 72 h.

Sensory evaluation

The sensory evaluation of final products was evaluated by 11 trained judges using 9-point hedonic scale. The scale of values ranged from a high score of 9, "like extremely", to a low score of 1, "dislike extremely" **(Gómez et al., 2011)**. The attributes evaluated were: shape of product, crust and crumb colour, flavour and taste of baked rolls, adhesiveness to palate on longer chewing. Overall acceptability of baked rolls was assessed using 100 mm graphic non-structured line segment with the description of extremes (100% - maximal intensity and 0% - minimal intensity) **(Kuchtová et al., 2016)**.

Statistic analysis

All analyses were carried out in triplicate unless otherwise stated and the average values were calculated. The results were expressed as mean value ± standard deviation. Significant differences between mean values at significance level $p = 0.05$ were establish using the One way analysis of variance and Student`s test. Microsoft Excel version 2010 was used as the statistical analysis software.

RESULTS AND DISCUSSION

The chemical composition of pumpkin powder and fine was presented in previous study by **Minarovičová et al. (2017)**.

Plant fibres show some functional properties, such as WHC and SC which have been more useful for understanding the physiological effect of dietary fibre, than the chemical composition alone **(Sharoba et al., 2013)**. These properties are also important for stating the usefulness of fiber fractions obtained as bulking, swelling and/or thickening agents in formulations or foods of relatively high water activity **(de Escalada Pla et al., 2007)**. The maximum amount of water that the fiber may hold is dependent on the fiber source, method of measurement and preparation as well as its physico-chemical and structural characteristics. The hydration properties of dietary fiber determine their optimal usage levels in food because a desirable texture must be retained **(Raghavendra et al., 2006)**. Hydration properties of PP are summarized in Table 1.

WHC is defined by the quantity of water that is bound to the fiber without the application of any external force (except for gravity and atmospheric pressure) **(Sowbhagya et al., 2007)**. The PP was characterized by high value of WHC (9.29 g.g^{-1}) which was higher than those described for spinach (6.40 g.g^{-1}) and apple pomace powder (8.54 g.g^{-1}) observed by **Saraç and Dogan (2016)** and **O´Shea et al. (2015)**. WRC is defined as the quantity of water that remains bound to the hydrated fiber following the application of an external force (pressure of centrifugation) **(Sowbhagya et al., 2007)**. It was observed that WRC value of PP (3.62 g.g^{-1}), which was higher than those published by **Kuchtová et al. (2016)** for PP. SC indicates how much the fiber matrix swells as water is absorbed **(de Escalada Pla et al., 2007)**. SC value of PP (8.49 mL.g^{-1}) was lower than was described for potato (12.0 mL.g^{-1}) and cabbage powder (16.37-21.41 mL.g^{-1}) by **Kaack et al. (2006)** and **Jongaroontaprangsee et al. (2007)**.

FAC is an important property as it improves the mouth feel and retains the flavor **(Kaur et al., 2015)**. The PP was characterized by low FAC value (1.01 g.g^{-1}) (Table 1).

Dough rheological characterization is imperative for both the milling and baking industry. It predicts flour dough characteristics during processing and the quality of end products. Rheological studies are extensively utilized and acknowledged by cereal technologists as useful techniques in evaluation of flour quality **(Iqbal et al., 2015)**. The influence of fibres on dough consistency and elasticity could be due to their effect on the internal structure of doughs **(Martínez et al., 2014)**. The rheological parameters of dough with addition of PP are presented in Table 2.

Table 2 Farinographic parameters of dough.

	Water absorption (%)	Dough development time (min)	Dough stability (min)	Mixing tolerance index (BU)
WF	54.48 ±0.00	3.00 ±0.00	16.11 ±0.08	38.00 ±0.00
PP 2.5%	54.42 ±0.05	4.05 ±0.13*	13.68 ±0.16 *	30.10 ±0.50*
PP 5%	57.45 ±0.14 *	3.77 ±0.03 *	10.67 ±0.14 *	36.97 ±0.55*
PP 7.5%	57.55 ±0.05 *	4.60 ±0.10 *	12.55 ±0.05 *	31.17 ±1.04*
PP 10%	57.61 ±0.11 *	4.48 ±0.08 *	10.55 ±0.05 *	20.13 ±0.23*

Note: BU – brabender units, PP – pumpkin powder, WF – wheat flour, * denotes statistically significant difference at $p < 0.05$ level.

Table 3 Qualitative parameters of baked rolls.

	Volume (cm^3)	Specific volume ($cm^3.100g^{-1}$)	Cambering	Baking loss (%)
control	294.25 ±7.37	327.20 ±6.93	0.66 ±0.02	10.12 ±0.50
PP 2.5%	195.25 ±2.22*	214.38 ±2.39*	0.58 ±0.02*	9.97 ±0.12
PP 5%	155.50 ±4.73*	169.65 ±5.33*	0.42 ±0.02*	8.37 ±0.20*
PP 7.5%	131.00 ±1.15*	144.13 ±1.05*	0.42 ±0.01*	9.13 ±0.45*
PP 10%	129.25 ±4.99*	141.52 ±5.61*	0.43 ±0.00*	8.73 ±0.12*

Note: PP – pumpkin powder, * denotes statistically significant difference at $p < 0.05$ level.

WA is the amount of water required to make dough of proper consistency for bread baking when mixed to optimum conditions based on the feel and appearance of the dough. Water absorption is an important quality factor to the baker as it is related to the amount of bread that can be produced from a given weight of flour **(Bojňanská and Mocko, 2014)**. From the results concluded that addition of PP significantly increased the WA. The highest WA value (57.61%) was recorded at addition level 10%. The increase in WA could be explained by the important number of hydroxyl groups existing in the fiber structure, which allow more water interactions through hydrogen bonding **(Lauková et al., 2016b)**. High WA is important from the economical point of view and avoiding staling **(Mosharraf et al., 2009)**. These results were in agreement with those described by **Turksoy and Özkaya (2011)** after addition pumpkin and carrot powder to wheat dough.

DS is related to the quality of the protein matrix, which is easily damaged by the incorporation of other ingredients, due to gluten dilution **(Minarovičová et al., 2017)**. It is an indicator of the strength, which higher values suggesting stronger dough **(Kohajdová et al., 2011)**. It was observed that addition of PP significantly decreased the DS from 16.11 (control) to 10.55 min (10% PP). The decrease in DS value could be due to dilution of gluten **(Rawat and Darappa, 2015)**.

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 **(Lauková et al., 2016b)**. The DDT values of dough with addition of PP ranged from 4.05 min (2.5% PP) to 4.48 min (10%). This change could have been due to differences in physiochemical properties between the constituents of pumpkin powder and wheat flour **(Kundu et al., 2012)**.

MTI value is the difference in BU from the top of the curve at the peak to the top of curve measured at 5 min. after the peak is reached **(Kundu et al., 2012)**. From the results concluded that addition of PP significantly decreased MTI from 38.00 BU (control) to 20.13 BU (10% PP). MTI is inversely proportional to the strength of the dough, higher values indicate lower strength or tolerance to mixing **(Rawat and Drappa, 2015)**. Similar decrease in MTI was also described when PP was added in semi coarse wheat flour **(Minarovičová et al., 2017; Kuchtová et al., 2016)**.

Qualitative parameters of baked rolls are summarized in Table 3. Loaf volume is used as a criterion to measure the quality of fresh bread in the industrial quality control, and by consumers. Specific volume of loaves of bread provides a uniform basis for comparing results of various studies. It is an indication of the gluten content of the bread but other constituents such as starch and fibre also contribute to the specific volume of bread **(Lauková et al., 2016a)**. It was observed that with increasing addition level significantly decreased the volume and specific volume of baked rolls from 294.25 cm^3 (control) to 129.25 cm^3 (10% PP) and from 327.20 $cm^3.100\ g^{-1}$ (control) to 141.52 $cm^3.100\ g^{-1}$ (10% PP), respectively. Similar effect on loaf volume was described by **Gül and Şen (2017)** when rosehip seed powder was used as flour substituent. In general, breads obtained from dough with a lower consistency achieved higher specific volumes, whereas more consistent dough produced breads with lower specific volumes **(Martínez et al., 2014)**. This phenomenon was possibly a result of the fiber weakening or crippling dough structure and reducing CO_2 gas retention. Moreover, appreciable amounts of water could have strongly bound to the added fibers during bread-making, so less water was available for the development of the starch-gluten network, causing an underdeveloped gluten network and reduced loaf volume **(Sivam et al., 2010)**.

Cambering (loaf height/width ratio) shows the loaf height to width ratio of loaves. Its higher value is desirable and predicts the product with better shape. Ideally the loaf should have an arched shape, rounded at transition from the bottom to the upper crust, and have a high ratio of width to height **(Bojňanská and Mocko, 2014)**. It is known that cambering values between 0.60 and 0.70 are deemed to be good and cambering values below 0.50 are considered insufficient **(Kohajdová et al., 2013)**. From the results concluded that increasing of proportion level of PP

Table 4 Sensory parameters of baked rolls with addition of PP.

	Appearance	Crumb colour	Crust colour	Flavour	Taste	Adhesiveness	Overall acceptance
control	8.40 ±0.39	8.35 ±0.41	8.16 ±0.34	8.12 ±0.27	8.21 ±0.22	8.05 ±0.37	87.50 ±4.25
PP 2.5%	8.20 ±0.13	8.32 ±0.15	8.21 ±0.07	7.52 ±0.12*	8.11 ±0.10	7.31 ±0.17*	88.60 ±3.13
PP 5%	8.39 ±0.22	8.78 ±0.16	8.49 ±0.06	7.43 ±0.08*	8.14 ±0.10*	7.54 ±0.07*	87.20 ±1.75
PP 7.5%	8.07 ±0.09*	8.31 ±0.13	8.20 ±0.13	7.30 ±0.18*	7.40 ±0.15*	7.05 ±0.11*	82.40 ±2.95*
PP 10%	7.70 ±0.08*	8.20 ±0.13*	8.03 ±0.08*	7.40 ±0.15*	7.52 ±0.12*	6.76 ±0.19*	82.00 ±2.05*

Note: PP – pumpkin powder, * denotes statistically significant difference at $p < 0.05$ level.

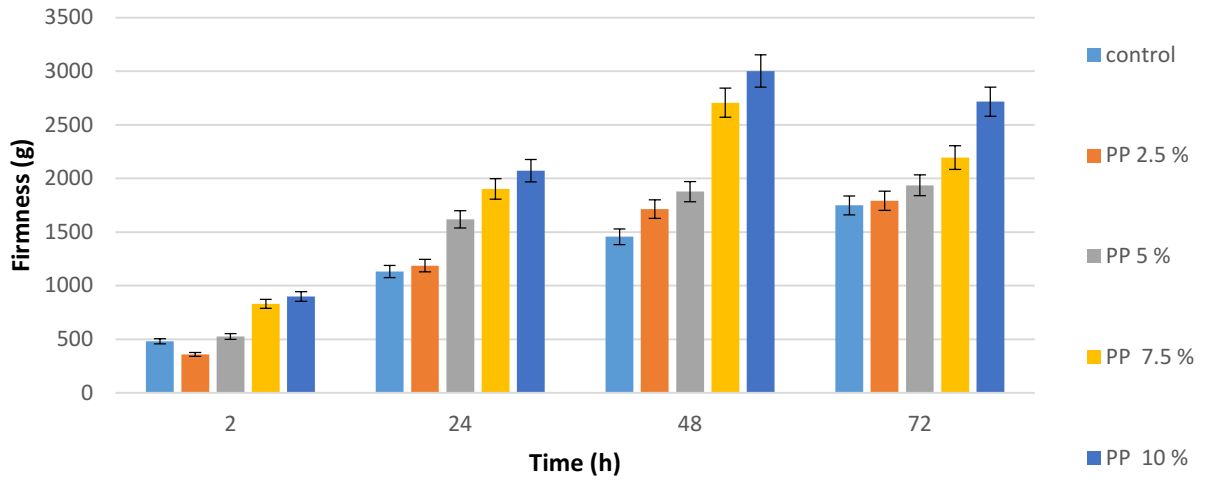

Figure 2 Firmness of PP enriched rolls during storage. Note: PP – pumpkin powder.

in caused lower values of cambering. Similar findings were described by **Kohajdová et al. (2012)** for carrot powder enriched bakery products.

Determining the actual baking losses is very important as the finished product after baking must have a defined weight. The loss by baking is influenced mainly by the weight of the product, by shape and moisture content **(Bojňanská and Mocko, 2014)**. It can be noticed that baking loss decreased with addition of PP.

Crumb texture is an important attribute of bread quality, and the protein fraction plays a key role in the formation of the structure, gas retention, and volume of breads **(Conte et al., 2016)**. Firming of bread crumb during storage is a common phenomenon and leads to a crumbly texture, and lower consumer acceptance. This parameter is the preferred parameter used to evaluate staling development **(Kohajdová and Karovičová, 2007)**. The textural properties of baked rolls are presented in Figure 2. From the results concluded that incorporation of PP increased the rolls firming 2h after baking. Moreover, firmness of rolls increased during storage. The hardening effect observed after addition of DF results from the dilution of gluten content and also due to the thickening of the walls surrounding the air bubbles in the crumb **(Kohajdová et al., 2012)**.

The effects of PP incorporation on sensory properties of baked rolls are showed in Table 4. It was observed that addition of PP in baked rolls had no significant effect on sensory attributes. The rolls enriched by PP had acceptable shape and crust colour. The highest score of crust colour was observed at 5% addition level. It was also concluded that PP enriched rolls had pleasant taste and flavour. On the other hand, incorporation of PP caused higher values of adhesiveness. The overall acceptances of enriched rolls were comparable with control sample. Furthermore, products with addition of 2.5% PP were the most acceptable for assessors.

CONCLUSION

This study showed that pumpkin powder had good hydration properties (high water holding and swelling capacity). Incorporation of pumpkin powder caused higher water absorption and longer dough development time. Addition of pumpkin powder significantly (p <0.05) affected the qualitative parameters of baked rolls (volume, cambering and baking loss decreased). Firmness of baked rolls increased with increasing level of pumpkin powder. Moreover, firmness values increased during 72 h. Sensory evaluation showed that most acceptable baked rolls were obtained by addition of 2.5% pumpkin powder. Enriched rolls were characterized with pumpkin flavour and after taste.

REFERENCES

AACC (2000). Method 10-05.01 Guidelines for measurement of volume by rapeseed displacement.

Adeleke, R. O., Odedeji, J. O. 2010. Functional properties of wheat and sweet potato flour blends. *Pakistan Journal of Nutrition*, vol. 9, no. 6, p. 535-538. http://dx.doi.org/10.3923/pjn.2010.535.538

Al-Saleh, A., Brennan, C. S. 2012. Bread wheat quality: some physical, chemical and rheological characteristics of Syrian and English bread wheat samples. *Foods*, vol. 1, no. 1, p. 3-17. http://dx.doi.org/10.3390/foods1010003

Aziah, N. A. A., Komathi, C. A. 2009. Acceptability attributes of crackers made from different types of composite flour. *International Food Research Journal*, vol. 16, p. 479-482.

Bojnanská, T., Mocko, K. 2014. Bread-making quality of Slovak and Serbian wheat varieties. *The Journal of Microbiology, Biotechnology and Food Sciences*, vol. 3, no. 3, p. 190-194. Available at: http://www.jmbfs.org/wp-content/uploads/2014/01/54_jmbfs_bojnanska_2014_fs.pdf

Conte, P., Fadda, C., Piga, A., Collar, C. 2016. Techno-functional and nutritional performance of commercial breads available in Europe. *Food Science and Technology International*, vol. 22, no. 7, p. 621-633. http://dx.doi.org/10.1177/1082013216637724

de Escalada Pla, M. F., Ponce, N. M., Stortz, C. A., Gerschenson, L. N., Rojas, A. M. 2007. Composition and functional properties of enriched fiber products obtained from pumpkin (*Cucurbita moschata Duchesne ex Poiret*). *LWT-Food Science and Technology*, vol. 40, no. 7, p. 1176-1185. http://dx.doi.org/10.1016/j.lwt.2006.08.006

Dhingra, D., Michael, M., Rajput, H., Patil, R. T. 2012. Dietary fibre in foods: a review. *Journal of food science and technology*, vol. 49, no. 3, p. 255-266. http://dx.doi.org/10.1007/s13197-011-0365-5

Gómez, M., Jiménez, S., Ruiz, E., Oliete, B. 2011. Effect of extruded wheat bran on dough rheology and bread quality. *LWT-Food Science and Technology*, vol. 44, no. 10, p. 2231-2237. http://dx.doi.org/10.1016/j.lwt.2011.06.006

Gül, H., Şen, H. 2017. The influence of rosehip seed flour on bread quality. *Scientific Bulletin. Series F. Biotechnologies*, vol. 21, p. 336-342. Available at: http://biotechnologyjournal.usamv.ro/pdf/2017/Art54.pdf

Iqbal, Z., Pasha, I., Abrar, M., Masih, S., Hanif, M. S. 2015. Physico-chemical, functional and rheological properties of wheat varieties. *Journal of Agricultural Research*, vol. 53, no. 2, p. 253-267.

ISO 5530-1:2013. Wheat flour – Physical characteristic of doughs – Part 1: Determination of water absorption and rheological properties using a farinograph.

Jongaroontaprangsee, S., Tritrong, W., Chokanaporn, W., Methacanon, P., Devahastin, S., Chiewchan, N. 2007. Effects of drying temperature and particle size on hydration properties of dietary fiber powder from lime and cabbage by-products. *International Journal of Food Properties*, vol. 10, no. 4, p. 887-897. http://dx.doi.org/10.1080/10942910601183619

Kaack, K., Pedersen, L., Laerke, H. N., Meyer, A. 2006. New potato fibre for improvement of texture and colour of wheat bread. *European Food Research and Technology*, vol. 224, no. 2, p. 199-207. http://dx.doi.org/10.1007/s00217-006-0301-5

Kaur, M., Sandhu, K. S., Arora, A., Sharma, A. 2015. Gluten free biscuits prepared from buckwheat flour by incorporation of various gums: physicochemical and sensory properties. *LWT-Food Science and Technology*, vol. 62, no. 1, p. 628-632. http://dx.doi.org/10.1016/j.lwt.2014.02.039

Kohajdova, Z., Karovicova, J. 2007. Effect of incorporation of spelt flour on the dough properties and wheat bread quality. *Żywność Nauka Technologia Jakość*, vol. 53, no. 4, p. 36-45. Available at: http://www.pttz.org/zyw/wyd/czas/2007,%204(53)/03_Kohajdova.pdf

Kohajdová, Z., Karovičová, J., Magala, M. 2013. Effect of lentil and bean flours on rheological and baking properties of wheat dough. *Chemical Papers*, vol. 67, no. 4, p. 398-407. http://dx.doi.org/10.2478/s11696-012-0295-3

Korczyk-Szabó, J., Lacko-Bartošová, M. 2012. Direct baking quality of spelt wheat (*Triticum spelta* L.). *Research Journal of Agricultural Science*, vol. 44, no. 1, p. 86-89.

Kuchtová, V., Karovičová, J., Kohajdová, Z., Minarovičová, L. 2016. Chemical composition and functional properties of pumpkin pomace-incorporated crackers. *Acta Chimica Slovaca*, vol. 9, no. 1, p. 54-57. http://dx.doi.org/10.1515/acs-2016-0009

Lauková, M., Kohajdová, Z., Karovičová, J. 2016a. Effect of incorporating potato dietary fibre to wheat dough on the quality of baked rolls. *Acta Chimica Slovaca*, vol. 9, no. 1, p. 14-18. http://dx.doi.org/10.1515/acs-2016-0003

Lauková, M., Kohajdová, Z., Karovičová, J. 2016b. Effect of hydrated apple powder on dough rheology and cookies quality. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 10, no. 1, p. 506-511. http://dx.doi.org/10.5219/597

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, no. 12, p. 49-56. http://dx.doi.org/10.1016/j.jfoodeng.2014.06.020

Minarovičová, L., Lauková, M., Kohajdová, Z., Karovičová, J., Kuchtová, V. 2017. Effect of pumpkin powder incorporation on cooking and sensory parameters of pasta. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 373-379. http://dx.doi.org/10.5219/743

Mosharraf, L., Kadivar, M., Shahedi, M. 2009. Effect of hydrothermaled bran on physicochemical, rheological and microstructural characteristics of Sangak bread. *Journal of cereal science*, vol. 49, no. 3, p. 398-404. http://dx.doi.org/10.1016/j.jcs.2009.01.006

Mudgil, D., Barak, S., Khatkar, B. S. 2016. Optimization of bread firmness, specific loaf volume and sensory acceptability of bread with soluble fiber and different water levels. *Journal of Cereal Science*, vol. 70, no. 7 p. 186-191. http://dx.doi.org/10.1016/j.jcs.2016.06.009

O'Shea, N., Ktenioudaki, A., Smyth, T. P., McLoughlin, P., Doran, L., Auty, M. A. E., Arendt, E., Gallagher, E. 2015. Physicochemical assessment of two fruit by-products as functional ingredients: Apple and orange pomace. *Journal of food engineering*, vol. 153, no. 5, p. 89-95. http://dx.doi.org/10.1016/j.jfoodeng.2014.12.014

Raghavendra, S. N., Swamy, S. R., Rastogi, N. K., Raghavarao, K. S. M. S., Kumar, S., Tharanathan, R. N. 2006. Grinding characteristics and hydration properties of coconut residue: a source of dietary fiber. *Journal of Food Engineering*, vol. 72, no. 3, p. 281-286. http://dx.doi.org/10.1016/j.jfoodeng.2004.12.008

Rawat, N., Darappa, I. 2015. Effect of ingredients on rheological, nutritional and quality characteristics of fibre and protein enriched baked energy bars. *Journal of Food Science and Technology*, vol. 52, no. 5, p. 3006-3013. http://dx.doi.org/10.1007/s13197-014-1367-x

Saraç, M. G., Dogan, M. 2016. Incorporation of dietary fiber concentrates from fruit and vegetable wastes in butter: effects on physicochemical, textural, and sensory properties. *European Food Research and Technology*, vol. 242, no. 8, p. 1331-1342. https://dx.doi.org/10.1007/s00217-016-2637-9

Sharoba, A. M., Farrag, M. A., Abd El-Salam, A. M. 2013. Utilization of some fruits and vegetables waste as a source of dietary fiber and its effect on the cake making and its quality attributes. *Journal of Agroalimentary Processes and Technologies*, vol. 19, no. 4, p. 429-444.

Sowbhagya, H. B., Suma, P. F., Mahadevamma, S., Tharanathan, R. N. 2007. Spent residue from cumin–a potential source of dietary fiber. *Food chemistry*, vol. 104, no. 3, p. 1220-1225. http://dx.doi.org/10.1016/j.foodchem.2007.01.066

Tosh, S. M., Yada, S. 2010. Dietary fibres in pulse seeds and fractions: Characterization, functional attributes, and applications. *Food Research International*, vol. 43, no. 2, p. 450-460. http://dx.doi.org/10.1016/j.foodres.2009.09.005

Turksoy, S., Özkaya, B. 2011. Pumpkin and carrot pomace powders as a source of dietary fiber and their effects on the mixing properties of wheat flour dough and cookie quality. *Food Science and Technology Research*, vol. 17, no. 6, p. 545-553. http://dx.doi.org/10.3136/fstr.17.545

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THERMO-MECHANICAL PROPERTIES OF DOUGH ENRICHED WITH WHEAT BRAN FROM DIFFERENT WHEAT VARIETY

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ABSTRACT

Wheat bran is the by-product derived from the wheat milling and represents a good source of dietary fiber. Consumption of wheat bran is associated with many health benefits. The hydration properties (water holding, water retention and swelling capacity) and oil binding capacity of bran from various wheat variety were investigated. It was showed that the water holding capacity of bran ranged from 2.27 to 2.98 $g.g^{-1}$, which were approximately four times higher compared to wheat flour. Also, it was observed that commercial wheat bran was characterised with the highest swelling capacity (5.21 $mL.g^{-1}$) and the lowest water retention and oil binding capacities (1.38 and 1.35 $g.g^{-1}$, respectively). Mixing and pasting properties of wheat dough with addition of bran at different level (5, 10 and 15%) were studied using Mixolab. From the results it was concluded that water absorption and dough development time increased with addition of different bran, while dough stability decreased. Moreover, with increasing addition level of different bran significantly affected the thermo-mechanical properties of wheat dough. The lowest effect on protein weakening was found after addition of spelt bran. The higher starch pasting ability of enriched dough was recorded after incorporation of bran from crossbreed Lubica. Furthermore, it was found that dough enriched with the commercial wheat bran was characterized by the lowest values of C3 (lower starch pasting ability), C4 (lower stability of hot formed gel) and C5 (lower starch retrogradation) parameters.

Keywords: wheat bran; spelt bran; functional properties; dough rheology; Mixolab

INTRODUCTION

Triticum aestivum L. is one of the major crop worldwide and mainly in the Mediterranean-type temperate zones, it is used for production of staple food **(Gotti et al., 2017)**. The quality of wheat is genetically conditioned and influenced by soil conditions, climate, technology, diseases and pest attack. Therefore, in order to have the chance to harvest quality wheat it is absolutely necessary to cultivate a variety that has the potential to develop that quality and for people to provide conditions for the wheat to achieve its potential **(Constantinescu et al., 2011)**.

Interest in the development of dietary fiber enriched foods has grown significantly as a result of increasing health awareness among consumers and food industry. As a complement of wheat flour in milling, one of the most obvious sources of dietary fiber in the baking industry is wheat bran **(Jacobs et al., 2015)**. Wheat bran is mainly composed of epidermis, peel, seed, nucellus layer, and aleurone layer **(Yan et al., 2015)**. Regarding the different types of bran fraction, there are quantitative and qualitative differences among the different cereal grains. Different types of bran have a different chemical composition; it depends on grain genetics, the agricultural background, and the milling process **(Jefremova et al., 2015)**. The wheat bran is rich in total dietary fiber (36.5 – 52.4%, w/w), and >90% of that is water-insoluble, thus the wheat bran might have significant prebiotic and antioxidant activities in lowing the risk of cardiovascular diseases, and provide the best protections against tumor, cancer and neurodegenerative diseases mainly because of the phenol compounds **(Jefremova et al., 2015)**. However, addition of bran has negative consequences on bread volume and organoleptic properties, depending on wheat cultivar, particle size, and the other treatment applied to bran **(Gómez et al., 2011)**.

Rheological tests on dough can predict their behaviour in a bakery, although only if the applied stress and the extent of the deformation are in the same range as those encountered during dough processing **(Rodriguez-Sandoval et al., 2012)**. The traditional instruments, which provide practical information for the cereal industry, measure the power input during dough development caused by a mixing action (farinograph, mixograph) and determine the extensional deformation of a prepared dough (extensigraph, alveograph) **(Minarovičová et al., 2017)**. Mixing and pasting properties of wheat flour dough can be

studied by Mixolab, which is a new tool capable of giving empirical rheological measurements of flour quality. The instrument allows analysing the quality of the protein network and the starch behaviour during heating and cooling **(Jia et al., 2011)**. The incorporation of wheat bran into wheat dough greatly interferes with protein association and its further aggregation during heating. Presumably, fibers occupy the space of the proteins in the gluten network. In addition, a fiber also affect pasting characteristics of starch such as peak viscosity, breakdown and final viscosity **(Xhabiri et al., 2016)**.

Scientific hypothesis

The objective of this study was to determine the effect of substituting wheat flour with different bran on pasting and mixing properties of dough. The functional properties of different bran were also evaluated.

MATERIAL AND METHODOLOGY

The bran from wheat Lubica (LB) (crossbreed *Triticum aestivum* x *Triticum spelta*) and different wheat cultivars B1 and B2 (wheat variety with purple colour of grain) were obtained from Research and Breeding Station, Vígľaš Pstruša, Slovakia and Research Institute of Plant Production Piešťany, Slovakia. Commercial wheat bran (B3) (PRO-BIO, s.r.o., Staré mesto, Czech Republic), spelt bran (SB) (PRO-BIO, s.r.o., Staré mesto, Czech Republic) and wheat flour (WF) (Penam Slovakia, a. s., Nitra, Slovak Republic) (moisture 10.53%, wet gluten 31.24% and dry gluten 10.17%) were purchased in local market.

Functional properties

The Hydration properties (water holding capacity, water retention capacity and swelling capacity) of bran were determined according to the method described by **de Escalada Pla et al. (2010)** with slight modifications.

Water-holding capacity (WHC). An accurately weighed dry sample (1.000 g) was hydrated in a graduated conical tube with 20.0 mL of water for 18 h at room temperature. The supernatant was decanted and the weight of the hydrated residue was recorded. After drying at 105°C to constant weight, the residual dry weight was obtained.

WHC ($g.g^{-1}$) = (Hydrated residue weight - dry residue weight)/dry residue weight

Water retention capacity (WRC). An accurately weighed dry sample (1.000 g) was hydrated in a graduated centrifuge tube with 20.0 mL of water for 18 h at room temperature. Centrifugation for 20 min at 2500 xg was then performed into the same tube. The supernatant was separated and the weight of the hydrated residue after centrifugation was recorded. After drying at 105°C to constant weight, the residual dry weight was obtained.

WRC ($g.g^{-1}$) = (hydrated residue weight after centrifugation - dry residue weight)/dry residue weight

Swelling capacity (SC). An accurately weighed dry sample (1.000 g) was placed in a graduated conical tube and 10.0 mL of water was added. It was hydrated for 18 h and after this time, the final volume attained by the fiber product was measured. Swelling capacity was calculated using:

SC ($mL.g^{-1}$) = volume occupied by the sample / original sample weight

Oil binding capacity (OBC) of bran were determined using method reported by **Sangnark et al. (2004)** with slight modification. A dried sample (1.000 g) was mixed with vegetable oil in a centrifugal tube and left for 1 h at room temperature. The mixture was then centrifuged at 2500xg for 15 min. The supernatant was separated and the weight of the residue was recorded. OBC was expressed as follows:

OBC ($g.g^{-1}$) = (residue weight-dry weight) / dry weight

Rheological properties

Wheat flour was replaced with various brans at different levels (5, 10 and 15 %). Rheological properties of wheat

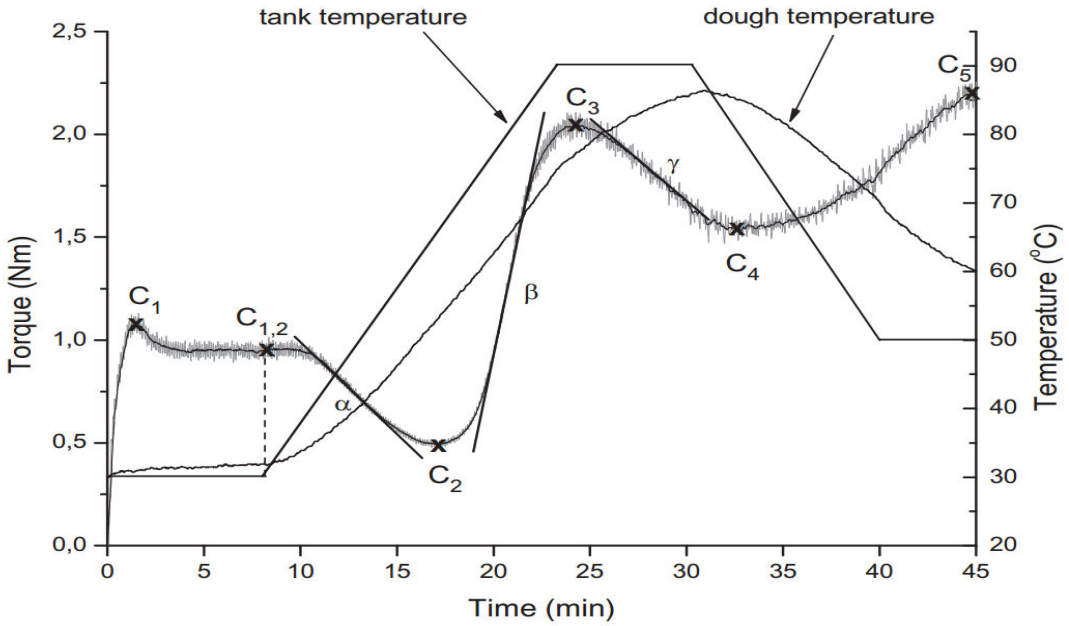

Figure 1 Typical Mixolab Curve **(Dapčević Hadnađev et al., 2011)**.

dough enriched with bran were determined using Mixolab (Chopin Villeneuve-la-Garenne, France) according to **Jia et al. (2011)**. The settings used in the test were 8 min at 30°C with a temperature increase of 4°C/min until the mixture reached 90°C. There was a 7-min holding period at 90°C, followed by a temperature decrease of 4°C/min until the mixture reached 55°C, and then 6 min of holding at 55°C. The mixing speed during the entire assay was 80 rpm.

The typical Mixolab curve (Figure 1), showing the following parameters: water absorption (%) – WA or the percentage of water required for the dough to produce a torque of 1.1; dough development time (min) – DDT or the time to reach the maximum torque at 30 °C; stability (min) S or time until the loss of consistency is lower than 11% of the maximum consistency reached during the mixing; initial maximum consistency (Nm) – C1, used to determine the water absorption; torque at the end of the holding time at 30 °C (Nm) – C1.2; mechanical weakening (Nm) – the torque difference between C1 and C1.2; minimum consistency (Nm) – C2, the minimum value of torque produced by dough passage while being subjected to mechanical and thermal constraints; thermal weakening (Nm) – the difference between the C1.2 and C2 torques; pasting temperature (°C) – the temperature at the onset of this rise in viscosity; peak torque (Nm) – C3, the maximum torque produced during the heating stage; peak temperature (°C) – the temperature at the peak viscosity; minimum torque (Nm) – C4, minimum torque reached during cooling to 50°C; breakdown torque (Nm) – calculated as the difference between C3 and C4; final torque(Nm) – C5, the torque after cooling at 50°C; setback torque (Nm) – the difference between C5 and C4 torque **(Dapčević Hadnađev et al., 2011)**.

Statisic analysis

All measurement were carried out in triplicate and the results were expressed as mean standard deviation. Significant differences between mean values at significance level p <0.05 were compared using Student`s test using MS Excel version 2010.

RESULTS AND DISCUSSION

The hydration properties of dietary fiber determine their optimal usage levels in food because a desirable texture must be retained. Usually, the hydration properties are described by three different measurable parameters such as water holding, water retention and swelling capacity **(Raghavendra et al., 2006)**. The hydration properties and oil absorption capacity of bran from different wheat variety are presented in Table 1.

WHC is defined by the quantity of water that is bound to the fibers without the application of any external force, except for gravity and atmospheric pressure **(Raghavendra et al., 2006)**. Generally, it was observed that the WHC values for bran were higher than WHC for wheat flour. The polysaccharide constituents of dietary fibers are strongly hydrophilic. Water is held on the hydrophilic sites of the fiber itself or within void spaces in the molecular structure **(Mudgil and Barac, 2013)**. The highest WHC value (2.98 $g.g^{-1}$) was determined for SB, while the lowest WHC value was for B2 (2.27 $g.g^{-1}$). Higher WHC values were reported by **Lebesi and Tzia (2012)** for oat and rice bran (3.13 – 4.53 $g.g^{-1}$). The high WHC values could indicate a lightness improve of food products where they were added **(Sharoba et al., 2003)**.

The WRC is the quantity of water that remains bound to the hydrated fiber following the application of an external force (pressure or centrifugation) **(Chantaro et al., 2008)**. It was concluded that WRC ranged from 1.38 $g.g^{-1}$ (B3) to 1.92 $g.g^{-1}$ (B1), which were similar to data presented by **Esposito et al. (2005)** for wheat bran (1.5 – 2.1 $g.g^{-1}$). Dietary fibres with high WRC can be used as functional ingredients which can modify the viscosity and texture of some formulated foods **(Nandi and Ghosh, 2015)**.

SC indicates how much the fiber matrix swells as water is absorbed, including loosely associated water. It is a consequence of the macromolecule relaxation during hydration, which leads to an increment in the occupied volume by the fiber product. The greater capacity to swell is the most desirable parameter for the physiological functionality of DF **(Lebesi and Tzia, 2012)**. The SC values of bran determined in this study (3.97 – 5.21 $mL.g^{-1}$) were higher than those presented by **He et al. (2018)** for hard white winter wheat bran (1.6 – 2.8 $mL.g^{-1}$).

OBC is another important property for the ingredients used in the formulation and stabilisation of food with high percentage of fat and emulsion. In fact, insoluble fibres can retain up to five times of their mass in oil. It can be beneficial for flavour retention and yield improvement technology **(Yaich et al., 2015)**. Furthermore, the OBC of fiber sources samples are also related to the particle size, overall charge density and hydrophilic nature of the individual particles **(Sharoba et al., 2003)**. From the results concluded that OBC values of different bran (1.35 – 1.79 $g.g^{-1}$) were higher than were reported for crude and extruded wheat bran (1 – 1.25 $g.g^{-1}$) **(Yan et al., 2015)**.

Table 1 Functional properties of different wheat bran.

	WHC ($g.g^{-1}$)	WRC ($g.g^{-1}$)	SC ($mL.g^{-1}$)	OBC ($g.g^{-1}$)
WF	0.65 ±0.04	0.52 ±0.02	2.21 ±0.01	0.28 ±0.02
SB	2.98 ±0.07	1.68 ±0.02	5.19 ±0.01	1.73 ±0.01
LB	2.68 ±0.02	1.71 ±0.09	4.58 ±0.02	1.79 ±0.03
B1	2.59 ±0.09	1.92 ±0.04	3.97 ±0.04	1.41 ±0.01
B2	2.27 ±0.01	1.86 ±0.02	4.38 ±0.08	1.52 ±0.02
B3	2.44 ±0.08	1.38 ± 0.01	5.21 ±0.02	1.35 ±0.01

Note: B1 – bran from new variety of wheat, B2 – bran from variety with purple colour of grain, B3 – commercial wheat bran, LB – bran from crossbreed Lubica, OBC – oil binding capacity, SB – spelt bran, SC – swelling capacity, WF – wheat flour, WHC – water holding capacity, WRC – water retention capacity.

Rheological properties of dough are very important indices for product development in terms of product quality and process efficiency **(Minarovičová et al., 2017)**. Incorporation of fiber into wheat flour interacts directly with structural elements of the three dimensional gluten network and disrupts the starch-gluten matrix, affecting the rheological behaviour of blended dough during mixing, sometime causing negative effect on the finished bread quality **(Mironeasa and Codină, 2013)**. The mixing properties of wheat dough with addition of different bran are summarized in Figure 2.

WA, which is expressed as the quantity of water needed to reach the defined dough consistency, is mainly reflected the contents of gluten protein in wheat flour **(Teng et al., 2015)**. It was observed that WA increased with addition of bran at all addition levels compared to wheat flour (55.9%). High WA is important from the economical point of view and avoiding staling **(Mosharraf et al., 2009)**. From the results also concluded that WA significantly increased with addition of SB, LB, B1 and B2 at addition level 10 and 15%, while incorporation of B3 resulted in significantly higher WA at all addition levels. This effect can be explained by the high fibre content of bran, specifically of pentosans, which have a high water-absorption capacity, as the addition of fibre had a similar effect on water-absorption. The explanation of this phenomenon is based partly on the fact that the fibre structure contains a large number of hydroxyl groups, which interact with the hydrogen bonds of water **(Gómez et al., 2011)**. Similar observations were found in case of addition of wheat bran by other autors **(Aravind et al., 2011, Xiong et al. 2017)**.

DS is related to the quality of the protein matrix, which is easily damaged by the incorporation of other ingredients, due to gluten dilution **(Minarovičová et al., 2017)**. This study also showed that DS decreased with addition of different bran. Furthemore, the highest DS values were observed after addition of SB (10.40, 10.35 and 10.18 min). Similar effect was also previously observed by **Boita et al. (2016)** and **El-Sharnouby et al. (2012)** after addition of wheat bran and mixture of wheat bran and date powder to wheat dough.

DDT is an important factor because it reflects the time between the first addition of water and the time when the dough seems to have optimum elastic and viscous properties for the retention of gas. DDT depends on the water absorption speed of flour constituents to form a smooth and homogenous appearance **(Vizittiu et al., 2011)**. It was observed that DDT values significantly prolonged with increasing addition level of different bran except of addition B1 at level 5%. This effect could be attributed to a fiber-gluten interaction, which prevents protein hydration **(Messia et al., 2016)**. These results were in agreement with those obtained by **Gómez et al. (2011)** and **Xiong et al. (2017)** after incorporation of wheat bran to wheat dough.

The thermo-mechanical properties of composed dough were presented in Table 2. Parameter C2 shows that with increase in temperature and mixing stress, the dough strength decreases as a result of protein weakening **(Sharma et al., 2017)**. From the results it was stated, that with increasing addition level of different bran parameter C2 significantly decreased except of incorporation of SB at level 5 and 10%. The lower C2 torque demonstrating greater weakening in proteins **(Gulia and Khatkar, 2014)**. The high decrease in parameter C2 may be attributed to the gluten dilution, thus losing some of its elasticity and becoming more extensible and less resistant **(Mironeasa et al., 2016)**.

After C2 the torque starts to increase and this is the stage

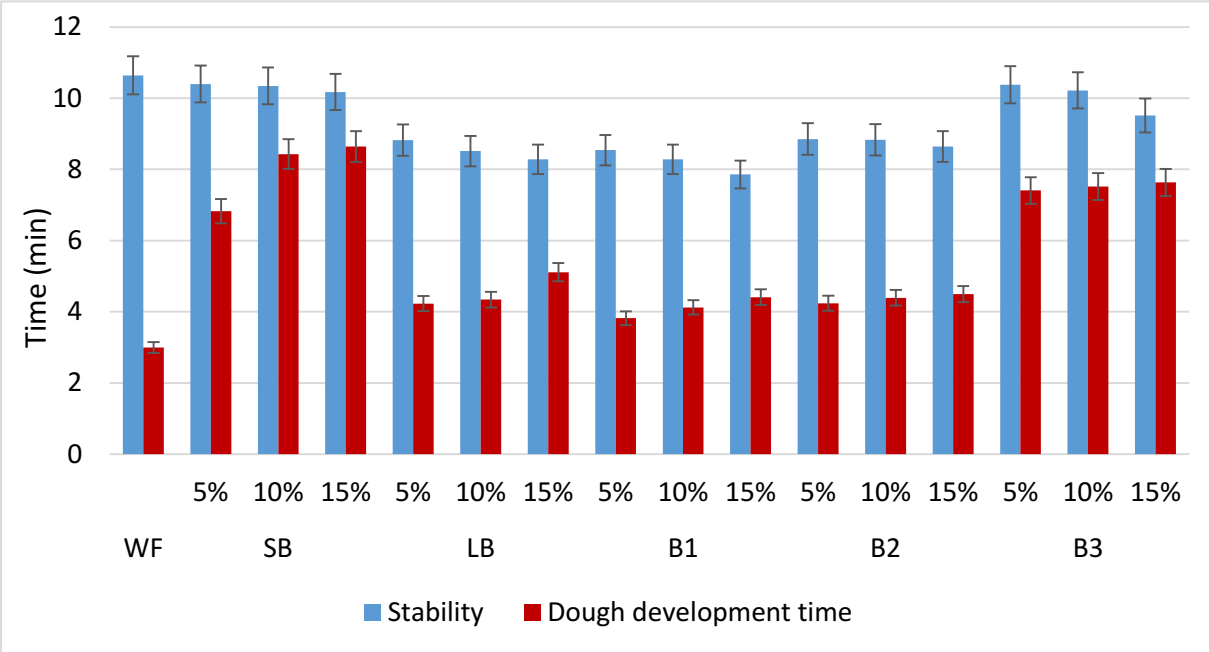

Figure 2 Dough development time and dough stability.
Note: B1 – bran from new variety of wheat, B2 – bran from variety with purple colour of grain, B3 – commercial wheat bran, LB – bran from crossbreed Lubica, SB – spelt bran, WF – wheat flour.

Table 2 Thermo-mechanical properties of dough with addition od different wheat bran.

		WA (%)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
WF	0%	55.9 ±0.00	0.575 ±0.030	2.109 ±0.022	1.895 ±0.039	2.956 ±0.091
SB	5%	59.50 ±0.03	0.572 ±0.004	1.867 ±0.009*	1.599 ±0.021*	2.701 ±0.135*
	10%	61.50 ±0.00*	0.570 ±0.005*	1.841 ±0.013*	1.553 ±0.021*	2.618 ±0.045*
	15%	62.78 ±0.04*	0.569 ±0.009*	1.814 ±0.009*	1.496 ±0.022*	2.576 ±0.068*
LB	5%	56.60 ±0.00	0.458 ±0.011*	2.061 ±0.013*	1.808 ±0.065*	2.940 ±0.065
	10%	57.20 ±0.01*	0.451 ±0.016*	2.023 ±0.022*	1.753 ±0.035*	2.877 ±0.035*
	15%	57.48 ±0.05*	0.448 ±0.012*	1.992 ±0.024*	1.712 ±0.021*	2.744 ±0.021*
B1	5%	56.20 ±0.02	0.446 ±0.010*	2.010 ±0.018*	1.715 ±0.036*	2.806 ±0.089*
	10%	57.35 ±0.02*	0.430 ±0.017*	1.930 ±0.024*	1.594 ±0.025*	2.563 ±0.055*
	15%	57.75 ±0.03*	0.428 ±0.018*	1.891 ±0.028	1.507 ±0.047*	2.489 ±0.117*
B2	5%	56.33 ±0.08	0.462 ±0.013*	2.055 ±0.019*	1.833 ±0.081*	2.973 ±0.190
	10%	57.00 ±0.00*	0.455 ±0.008*	2.028 ±0.019*	1.766 ±0.020*	2.810 ±0.050*
	15%	58.00 ±0.00*	0.458 ±0.012*	1.984 ±0.013*	1.673 ±0.018*	2.742 ±0.017*
B3	5%	59.60 ±0.00*	0.563 ±0.011*	1.839 ±0.027*	1.537 ±0.039*	2.452 ±0.070*
	10%	61.40 ±0.01*	0.546 ±0.006*	1.801 ±0.003 *	1.460 ±0.026*	2.347 ±0.062*
	15%	61.87 ±0.00*	0.531 ±0.023*	1.751 ±0.045*	1.373 ±0.018*	2.300 ±0.043*

Note: B1 – bran from new variety of wheat, B2 – bran from variety with purple colour of grain, B3 – commercial wheat bran, LB – bran from crossbreed Lubica, SB – spelt bran, WA – water absorption, WF – wheat flour, * denotes statistically significant difference at p <0.05 level.

when gelatinization process starts and it keeps on increasing till C3 is reached. The C3 is the maximum torque obtained during the heating stage resulting in increase in consistency due to bursting of the starch granules **(Sharma et al., 2017)**. WF contributed to a better starch performance of the samples (higher starch gelatinization, C3) than composite flour with bran. This could be ascribed to the competence for water established between the starch and the bran **(Hadnadev et al., 2011)**. The C3 value for WF was 2.11 Nm. As can be seen from the Table 2, values C3 decreased with increasing addition level of bran. The lowest values were observed for dough with addition of B3.

The further viscosity reduction in C4 is the result of the physical breakdown of the granules due to the mechanical shear stress and the temperature constraint **(Hadnadev et al., 2011)**. It was observed that addition of different bran significantly decreased C4 values at all addition levels. A lower torque indicates lower stability of hot-formed gel **(Gulia and Khatkar, 2014)**. Bran is mainly from the grain mantle that contains also large quantity of α-amylases the cause of the reduction of the above mentioned values **(Xhabiri et al., 2013)**.

The C5 is the maximum torque of the cooling stage, which reflects starch retrogradation **(Wang et al., 2017)**. This study also showed, that addition of SB and B3 significantly decreased the C5 values at all addition levels. Furthermore, it was found that the lowest values were recorded after incorporation of bran B3. Lowering in starch recrystallization rate could progressively prolong a shelf-life of end product **(Švec and Hrušková, 2015)**.

CONCLUSION

In this study the functional properties of different wheat bran (spelt bran, wheat bran from new wheat variety and bran from crossbreed of *Triticum aestivum* x *Triticum spelta*) were evaluated. Rheological properties of dough with addition of bran were also examined. In general, bran from different wheat variety were characterised with good hydration properties. Spelt bran was characterized with the highest water absorption value. From the results also concluded, that addition of different bran had significant (p <0.05) effect on mixing and pasting properties of wheat dough. The water absorption increased and dough development time prolonged with addition of bran, while dough stability decreased. The highest value of water absorption was observed after incorporation of spelt bran. This study also showed that increasing of addition level of different bran significantly (p <0.05) affected the weakening of protein structure (C2), stability of hot-formed gel (C4) and retrogradation of starch (C5).

REFERENCES

Aravind, N., Sissons, M., Egan, N., Felows, Ch. 2012. Effect of insoluble dietary fibre addition on technological, sensory, and structural properties of durum wheat spaghetti. *Food Chemistry*, vol. 130, no. 2, p. 299-309. https://dx.doi.org/10.1016/j.foodchem.2011.07.042

Boita, E. R. F., Oro, T., Bressiani, J., Santetti, G. S., Bertolin, T. E., Gutkoski, L. C. 2016. Rheological properties of wheat flour dough and pan bread with wheat bran. *Journal of Cereal Science*, vol. 71, p. 177-182. https://dx.doi.org/10.1016/j.jcs.2016.08.015

Chantaro, P., Devahastin, S., Chiewchan, N. 2008. Production of antioxidant high dietary fiber powder from carrot peels. *LWT - Food Science and Technology*, vol. 41, no. 10, p. 1987-1994. https://dx.doi.org/10.1016/j.lwt.2007.11.013

Constantinescu, G., Dabija, A., Buculei, A., Rebenciuc, I. 2011. Evaluation of cereal cultivar impact on bread quality. *Journal of Agroalimentary Processes and Technologies*, vol. 17, no. 4, p. 473-476.

Dapčević Hadnađev, M., Torbica, A., Hadnađev, M. 2011. Rheological properties of wheat flour substitutes/alternative

crops assessed by Mixolab. *Procedia Food Science*, vol. 1, p. 328-334. https://dx.doi.org/10.1016/j.profoo.2011.09.051

de Escalada Pla, M. F., Uribe, M., Fissore, E. N., Gerschenson, L. N., Rojas, A. M. 2010. Influence of the isolation procedure on the characteristics of fiber-rich products obtained from quince wastes. *Journal of Food Engineering*, vol. 96, no. 2, p. 239-248. https://dx.doi.org/10.1016/j.jfoodeng.2009.07.018

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

Esposito, F., Arlotti, G., Bonifati, A. M., Napolitano, A., Vitale, D., Fogliano, V. 2005. Antioxidant activity and dietary fibre in durum wheat bran by-products. *Food Research International*, vol. 38, no. 10, p. 1167-1173. https://dx.doi.org/10.1016/j.foodres.2005.05.002

Gómez, M., Jiménez, S., Ruiz, E., Oliete, B. 2011. Effect of extruded wheat bran on dough rheology and bread quality. *LWT – Food Science and Technology*, vol. 44, no. 10, p. 2231-2237. https://dx.doi.org/10.1016/j.lwt.2011.06.006

Gotti, R., Amadesi, E., Fiori, J., Bosi, S., Bregola, V., Marotti, I., Dinelli, G. 2018. Differentiation of modern and ancient varieties of common wheat byquantitative capillary electrophoretic profile of phenolic acids. *Journal of Chromatography A*, vol. 1532, p. 208-215. https://dx.doi.org/10.1016/j.chroma.2017.11.058

Gulia, N., Khatkar, B. S. 2014. Relationship of dough thermomechanical properties with oil uptake, cooking and textural properties of instant fried noodles. *Food Science and Technology International*, vol. 20, no. 3, p. 171-182. https://dx.doi.org/10.1177/1082013213476076

He, S., Li, J., He, Q., Jian, H., Zhang, Y., Wang, J., Sun, H. 2018. Physicochemical and antioxidant properties of hard white winter wheat (*Triticum aestivm* L.) bran superfine powder produced by eccentric vibratory milling. Powder Technology, vol. 325, p. 126-133. https://dx.doi.org/10.1016/j.powtec.2017.10.054

Jacobs, P. J., Hemdane, S., Dornez, E., Delcour, J. A., Courtin, Ch. M. 2015. Study of hydration properties of wheat bran as a function of particle size. *Food Chemistry*, vol. 179, p. 296-304. https://dx.doi.org/10.1016/j.foodchem.2015.01.117

Jefremova, O., Radenkovs, V., Kunkulberga, D., Klava, D. 2015. Technological properties of dough from wheat flour and fermented bran. *Chemical Technology*, vol. 66, no. 1, p. 13-18.

Jia, Ch., Huang, W., Abdel-Samie, M. A.-S., Huang, G., Huang, G. Dough rheological, Mixolab mixing, and nutritional characteristics of almond cookies with and without xylanase. *Journal of Food Engineering*, vol. 105, no. 2, p. 227-232. https://dx.doi.org/10.1016/j.jfoodeng.2011.02.023

Lebesi, D. M., Tzia, C. 2012. Use of endoxylanase treated cereal brans for development of dietary fiber enriched cakes. *Innovative Food Science & Emerging Technologies*, vol. 13, p. 207-214. https://dx.doi.org/10.1016/j.ifset.2011.08.001

Messia, M. C., Reale, A., Maiuro, L., Candigliota, T., Sorrentino, E., Marconi, E. 2016. Effects of pre-fermented wheat bran on dough and bread characteristics. *Journal of Cereal Sience*, vol. 69, p. 138-144. https://dx.doi.org/10.1016/j.jcs.2016.03.004

Minarovičová, L., Lauková. M., Kohajdová, Z., Karovičová, J., Kuchtová, V. Effect of pumpkin powder incorporation on cooking and sensory parameters of pasta. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 373-379. https://dx.doi.org/10.5219/743

Mironeasa, S., Codină, G. G. 2013. Effect of citrus fibers addition on wheat flour dough rheological properties. *Food and Environment Safety*, vol. 12, no. 4, p. 322-327.

Mironeasa, S., Codina, G. G. 2016. The mixolab rheological properties and dough microstructure of defatted mustard seed–wheat composite flours. *Journal of Food Processing and Preservation*, vol. 41, no. 5, e13130. https://dx.doi.org/10.1111/jfpp.13130

Mosharraf, L., Kadivar, M., Shahedi, M. 2009. Effect of hydrothermaled bran on physicochemical, rheological and microstructural characteristics of Sangak bread. *Journal of Cereal Science*, vol. 49, no. 3, p. 398-404. https://dx.doi.org/10.1016/j.jcs.2009.01.006

Mudgil, D., Barak, S. 2013. Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: A review. *International Journal of Biological Macromolecules*, vol. 61, p. 1-6. https://dx.doi.org/10.1016/j.ijbiomac.2013.06.044

Nandi, I., Gosh, M. 2015. Studies on functional and antioxidant property of dietary fibre extracted from defatted sesame husk, rice bran and flaxseed. *Bioactive Carbohydrates and Dietary Fibre*, vol. 5, no. 2, p. 129-13. https://dx.doi.org/10.1016/j.bcdf.2015.03.001

Raghavendra, S. N., Ramachandra Swamy, S. R., Rastogi, N. K., Raghavarao, K. S. M. S., Kumar, S., Tharanathan, R. N. 2006. Grinding characteristics and hydration properties of coconut residue: A source of dietary fiber. *Journal of Food Engineering*, vol. 72, no. 3, p. 281-286. https://dx.doi.org/10.1016/j.jfoodeng.2004.12.008

Rodriguez-Sandoval, E., Sandoval, G., Cortes-Rodríguez, M. 2012. Effect of Quinoa and Potato Flours on the Thermomechanical and Breadmaking Properties of Wheat Flour. *Brazilian Journal of Chemical Engineering*, vol. 29, no. 3, p. 503-510. https://dx.doi.org/10.1590/S0104-66322012000300007

Sangnark, A., Noomhorm, A. 2004. Chemical, physical and baking properties of dietary fiber prepared from rice straw. *Food Research International*, vol. 37, no. 1, p. 66-74. https://dx.doi.org/10.1016/j.foodres.2003.09.007

Sharoba, A. M., Farrag, M. A., Abd El-Salam, A. M. 2013. Utilization of some fruits and vegetables waste as a source of dietary fiber and its effect on the cake making and its quality attributes. *Journal of Agroalimentary Processes and Technologies*, vol. 19, no. 4, p. 429-444.

Švec, I., Hrušková, M. 2015. The Mixolab parameters of composite wheat/hemp flour and their relation to quality features. *LWT – Food Science and Technology*, vol. 60, no. 1, p. 623-629. https://dx.doi.org/10.1016/j.lwt.2014.07.034

Teng, Y., Liu, C., Bai, J., Liang, J. 2015. Mixing, tensile and pasting properties of wheat flour mixed with raw and enzyme treated rice bran. *Journal of Food Science and Technology*, vol. 52, no. 5, p. 3014-3021. https://dx.doi.org/10.1007/s13197-014-1366-y

Vizitiu, D., Danciu, I. 2011. Evaluation of farinograph and mixolab for prediction of mixing properties of industrial wheat flour. *Acta Universitatis Cibiniensis Series E: Food Technology*, vol. 15, no. 2, p. 31-38.

Xhabiri, G. Q., Durmishi, N., Idrizi, X., Ferati, I., Hoxha, I. 2016. Rheological Qualities of Dough from Mixture of Flour and Wheat Bran and Possible Correlation between Bra Bender and Mixolab Chopin Equipments. *MOJ Food Processing & Technology*, vol. 2, no. 4, p. 1-7. https://dx.doi.org/10.15406/mojfpt.2016.02.00042

Xiong, L., Zhang, B., Niu, M., Zhao, S. 2017. Protein polymerization and water mobility in whole-wheat dough influenced by bran particle size distribution. *LWT - Food Science and Technology*, vol. 82, p. 396-403. https://dx.doi.org/10.1016/j.lwt.2017.04.064

Yaich, H., Garna, H., Bchir, B., Besbes, S., Paquot, M., Richel, A., Blecker, Ch., Attia, H. 2015. Chemical composition and functional properties of dietary fibre extracted by Englyst and Prosky methods from the alga Ulva lactuca collected in Tunisia. *Algal Research*, vol. 9, p. 65-73. https://dx.doi.org/10.1016/j.algal.2015.02.017

Yan, X., Ye, R., Chen, Y. 2015. Blasting extrusion processing: The increase of soluble dietary fiber content and extraction of soluble-fiber polysaccharides from wheat bran. *Food Chemistry*, vol. 180, p. 106-115. https://dx.doi.org/10.1016/j.foodchem.2015.01.127

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EFFECT OF DIFFERENT REVERSE TRANSCRIPTION APROACHES IN Pru p 3 TRANSCRIPTS SEMIQUANTITATIVE AMPLIFICATION

Jana Žiarovská, Matúš Kyseľ, Lucia Zeleňáková, Eloy Fernández Cusimamani

ABSTRACT

Reverse transcriptase transcribes the cDNA based on its previous extraction and standardization. Reverse transcription step is considered to be critical in the workflow of quantification of transcribed genes. The aim of the study was to extract total RNA by different methods and to analyse the results of the subsequent reverse transcription reaction when different commercial RT kits were used to process RNA extracted from pulp of matured peach fruit. Mature peach pulp was used in the study. The fruit of variety Vistarich was collected in summer 2017 in the orchard of Dvory nad Žitavou. Two RNA extraction methods, TRIzol® Reagent and GeneJET Plant RNA Purification Kit, were tested in to determine the suitable method for peach fruit RNA extraction. Three different cDNA reagent sets were used to transcribe 115 ng/500 ng total RNA or 11 ng/115 ng, respectively. Both variants of the primers, random hexamers as well as oligo (dT) 18, were used to anneal the target mRNA of Pru p 3 allergen following the manufacturer instructions. No specific effect was obtained in the case of peach fruit when using ethanol-extracted tissue treatment and the effect of the used extraction method was more significant. The A260/230 ratios were similar for three from four tested methods. In the case of these three methods, the A260/A230 ratios for all the extracted samples were higher than 1.9 which indicates high purity without contamination by polyphenols and polysaccharides. The specificity of obtained amplicons was proved by restriction cleavage using Tse I restriction endonuclease. This provided the cleavage of the 179 bp long product in all amplicons. Working with mature fruit meet a specific situation in the field of RNA extraction and subsequently all the downstream applications. That is, why choosing the most fitting methods and kits is a crucial step. Here, the method for the semi-quantitative analysis of the Pru p 3 allergen expressions was set up in the way that will be directly applicable for Pru p 3 expression analyses.

Keywords: reverse transcription; peach; RNA extraction; Pru p 3; semiquantitative amplification

INTRODUCTION

The variable types of specific analytical procedures are used to describe plant genome variability and plant transcriptomic characteristics actually. Different DNA markers are used for the purpose of the genome mapping and revealing their natural variability **(Vivodík et al., 2015; Ražná et al., 2016)**. Quantifying of gene expression is one of the well establishing methods that are a part of a research in many different area of interest **(Kačániová et al., 2012; Žiarovská et al., 2013)**. RT-PCR (reverse transcriptase polymerase chain reaction) transcribes the cDNA based on its previous extraction and standardization. Reverse transcription step is considered to be critical in the workflow of quantification especially for the low copy transcribed genes **(Sanders et al., 2014)**. The process of reverse transcription optimizing comprises from a several steps (Figure 1) that conditioned the final efficiency of the analysis.

The research strategy based on the RT method is a very reproducible one, gives a very high precision and allows amplification of different types of mRNA **(Nicot et al., 2005)**.

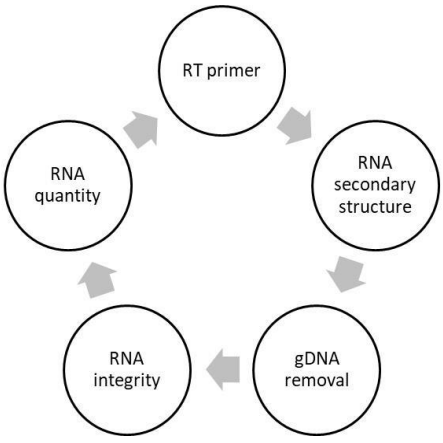

Figure 1 Components of reverse transcription process optimization.

The aim of the study was to extract total RNA by different methods and to analyse the results of the subsequent reverse transcription reaction when different commercial RT kits were used to process RNA extracted from pulp of matured peach fruit.

Scientific hypothesis

Here, two premises were set up for the experiments.
1) The secondary metabolites content in the peach fruit is well-drained by water content that allow use the standard extraction method, even those of commercially available.
2) Effectivity of reverse transcription will be different for the same peach extracted RNA for different cDNA synthesis kits used to process it.

Statistical analysis

The primary testing for both of the hypothesis data was based on the qualitative analysis by resolution through an agarose gel. Statistical evaluation of the results was used for data obtained for RNA extraction method and for results of reverse transcription. It was realized by ezANOVA software for Windows (http://www.cabiatl.com/mricro/ezanova/) Measurements of repeating of samples were expressed as means ± standard deviation. The data were subjected to the one factorial ANOVA pairwise comparisons with Tukey HSD with the level of significance associated to the statistical test 0.01. The null hypothesis was tested that a difference exists among the amounts of extracted RNA depending on the extraction method used as well as in effectivity of reverse transcription.

MATERIAL AND METHODOLOGY

Biological material

Mature peach pulp was used in the study. The fruit of variety Vistarich was collected in summer 2017 in the orchard of Dvory nad Ţitavou. Collected fruit were stored in -20°C until the processing.

RNA extraction method and quality/quantity checking

Two RNA extraction methods, TRIzol® Reagent (Invitrogen) and GeneJET Plant RNA Purification Kit – (Thermo Fisher Scientific), were tested in to determine the suitable method for peach fruit RNA extraction. Both of the methods were tested in two ways – without any change of the manufacturer´s instruction and with the initial step of ethanol-extracted method of the peach tissue preparation following the protocol according the **Asif et al. (2006)**. The samples were signed as determined in the table 1. Extracted RNA quantity was analysed by Nanodrop spectrophotometer (Thermo Scientific) with absorbances at 230 nm, 260 nm and 280 nm. Contamination level of the extracted RNA by protein and polysaccharides and phenolic compounds was determined as the ratio of the A260/A280 and A260/A230 absorbances. Integrity of the extracted RNA was analysed in 1% agarose gel stained with GelRed™ (Biotium).

Reverse transcription

Three different cDNA reagent sets were used to transcribe 115 vs. 500 ng of total RNA or 11 vs. 115 ng, respectively as follows: Tetro cDNA Synthesis Kit (Bioline), Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase (Thermo Scientific) and AccuScript High Fidelity 1st Strand cDNA Synthesis Kit (Agilent Technologies). Both variants of the primers, random hexamers as well as oligo (dT) 18, were used to anneal the target mRNA of Pru p 3 allergen following the manufacturer instructions. The reverse transcription reactions were performed at the time and temperature settings recommended by the supliers, too. A half of the obtained transcription product was cleaned by AgencourtAMPure XP purification system (Beckman Coulter) following the manufacturer´s instructions, dissolved in water subsequently and measured for the quantity and quality by NanodropNanophotometer™. The second half of the transcription product was subjected to semi-quantitative amplification.

Table 1 Codes of samples used in the RNA extraction method testing.

RNA extraction method	Codes of 10 samples extracted in total
GeneJET Plant RNA Purification Kit without change	A1 – A10
GeneJET Plant RNA Purification Kit with ethanol-extracted step	B1 – B10
TRIzol® Reagent method without change	C1 – C10
TRIzol® Reagent with ethanol-extracted step	D1 – D10

Semi-quantitative amplification and product specificity checking

Amplification of Pru p 3 allergen transcripts were performed by Combi PPP Master Mix (Top-Bio) using the 300 nmoL × dm^{-1} of the specific primers and 100 ng of transcribed cDNA. Primers for the amplification of Pru p 3 allergen were designed by Primer3web version 4.0.0 (http://primer3.ut.ee/) on the base of sequence from NCBI under the accession AY620230.1. Thermal profile of PCR reactions was as follows: 94 °C, 1 minute, 35 x (94 °C for 20 seconds; 60 °C for 20 seconds; 72 °C for 30 seconds) and final 72 °C 7 minutes. PCR products specificity was checked using the 2% AGE and confirmed subsequently by Tse I (NEB Enzymes) restriction cleavage.

RESULTS AND DISCUSSION

RNA isolation is often the most serious difficulty to solve in the workflow of gene expression analysis during fruit development and ripening. This obstacle is caused by the biochemical nature of secondary metabolite concentrations in fruit and its changes that occur during the process of ripening. That is, what affect both the quantity and quality of isolated RNA **Gudenschwager et al. (2012)**.

Here, four protocols were used to extract total RNA from the pulp of peach that is known to contain high levels of polysaccharides and polyphenolic compounds **(Gil et al., 2002; Hu et al., 2002)**.

The A260/230 ratios were similar for three from four tested methods (Table 2).

Table 2 Purity and yield analysis of total extracted RNA from peach pulp using different methods.

Method	Quantity and quality parameters			Number of samples
	A260/A208 ±SD	A260/A230 ±SD	RNA yield ng.µL^{-1}±SD	
A	2.00 ±0.22	2.12 ±0.10	340 ±71	10
B	1.98 ±0.13	1.88 ±0.37	400 ±20	10
C	1.84 ±0.25	1.86 ±0.16	35 ±28	10
D	1.95 ±0.18	1.98 ±0.03	18 ±7	10

Note: A – GeneJET Plant RNA Purification Kit without change; B – GeneJET Plant RNA Purification Kit with ethanol-extracted step; C – TRIzol® Reagent method without change; D – TRIzol® Reagent with ethanol-extracted step

Table 3 ANOVA analysis of the yield of RNA extraction methods used.

Descriptive details	Extraction method			
	A	B	C	D
Mean	340	400	35	18
StDev	71	20	28	7
SE	40.99	11.55	16.17	4.04
Var	5041	400	784	49
CI95%	52.73	52.73	52.73	52.73
N	10	10	10	10
Skew	0	0	0	0
zSkew	0	0	0	0

PAIRWISE COMPARISONS [Q=TukeyHSD: **=p <0.01]
[A] vs [B] t(4) = 1.41 p <0.2317 Q = 2.6240
[A] vs [C] t(4) = 6.92 p <0.0023 Q = 13.3388**
[A] vs [D] t(4) = 7.82 p <0.0014 Q = 14.0823**
[B] vs [C] t(4) = 18.37 p <0.0001 Q = 15.9629**
[B] vs [D] t(4) = 31.22 p <0.0001 Q = 16.7064**
[C] vs [D] t(4) = 1.02 p <0.3653 Q = 0.7435

Note: A – GeneJET Plant RNA Purification Kit without change; B – GeneJET Plant RNA Purification Kit with ethanol-extracted step; C – TRIzol® Reagent method without change; D – TRIzol® Reagent with ethanol-extracted step.

Table 4 Influence of the priming method on the cDNA yield using different kits.

Variant of the reverse transcription	Amount of transcribed product in 1 µL
Tetro cDNA Synthesis Kit/ oligo dT(18) primers/ 115 ng RNA in reverse transcription	311
Tetro cDNA Synthesis Kit/ random primers/ 115 ng RNA in reverse transcription	353
Tetro cDNA Synthesis Kit/ oligo dT(18) primers/ 500 ng RNA in reverse transcription	1589
Tetro cDNA Synthesis Kit/ random primers/ 500 ng RNA in reverse transcription	1568
Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase/ primer mix/ 115 ng RNA in reverse transcription	1587
Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase/ primer mix/ 500 ng RNA in reverse transcription	5847
AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / oligo dT(18) primers/ 11 ng RNA in reverse transcription	1571
AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / random primers/ 11 ng RNA in reverse transcription	1469
AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / oligo dT(18) primers/ 115 ng RNA in reverse transcription	14870

Table 5 ANOVA analysis of the yield obtained by different transcriptions.

Descriptive details	cDNA synthesis kit		
	Tetro	**Maxima**	**Accu**
Mean	322	1539.75	14603.75
StDev	22.88	50.7	432.85
SE	11.44	25.35	216.42
Var	523.33	2570.92	18358.92
CI95%	284.97	284.97	284.97
N	4	4	4
Skew	0.992	-0.005	-1.687
zSkew	0.81	-0.005	-1.378

PAIRWISE COMPARISONS [Q = TukeyHSD: ** = p <0.01]
[Tetro] vs [Maxima] t(6) = 43.78 p <0.0001 Q = 9.6662**
[Tetro] vs [Accu] t(6) = 65.90 p <0.0001 Q = 113.3647**
[Maxima] vs [Accu] t(6) = 59.95 p <0.0001 Q = 103.6986**

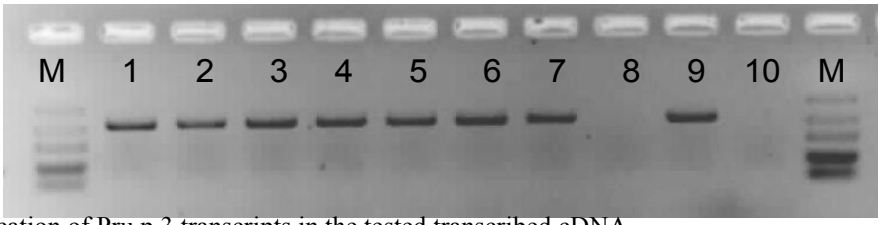

Figure 2 Amplification of Pru p 3 transcripts in the tested transcribed cDNA.

Code in the electrophoreogram	Variant of the reverse transcription
1	Tetro cDNA Synthesis Kit/ oligo dT(18) primers/ 115 ng RNA in reverse transcription
2	Tetro cDNA Synthesis Kit/ random primers/ 115 ng RNA in reverse transcription
3	Tetro cDNA Synthesis Kit/ oligo dT(18) primers/ 500 ng RNA in reverse transcription
4	Tetro cDNA Synthesis Kit/ random primers/ 500 ng RNA in reverse transcription
5	Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase/ primer mix/ 115 ng RNA in reverse transcription
6	Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase/ primer mix/ 500 ng RNA in reverse transcription
7	AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / oligo dT(18) primers/ 11 ng RNA in reverse transcription
8	AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / random primers / 11 ng RNA in reverse transcription
9	AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / oligo dT(18) primers/ 115 ng RNA in reverse transcription
10	AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / random primers / 115ng RNA in reverse transcription

Note:
Code in the electrophoreogram — Variant of the reverse transcription
1 — Tetro cDNA Synthesis Kit/ oligo dT(18) primers/ 115 ng RNA in reverse transcription
2 — Tetro cDNA Synthesis Kit/ random primers/ 115 ng RNA in reverse transcription
3 — Tetro cDNA Synthesis Kit/ oligo dT(18) primers/ 500 ng RNA in reverse transcription
4 — Tetro cDNA Synthesis Kit/ random primers/ 500 ng RNA in reverse transcription
5 — Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase/ primer mix/ 115 ng RNA in reverse transcription
6 — Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase/ primer mix/ 500 ng RNA in reverse transcription
7 — AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / oligo dT(18) primers/ 11 ng RNA in reverse transcription
8 — AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / random primers / 11 ng RNA in reverse transcription
9 — AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / oligo dT(18) primers/ 115 ng RNA in reverse transcription
10 — AccuScript High Fidelity 1st Strand cDNA Synthesis Kit / random primers / 115ng RNA in reverse transcription

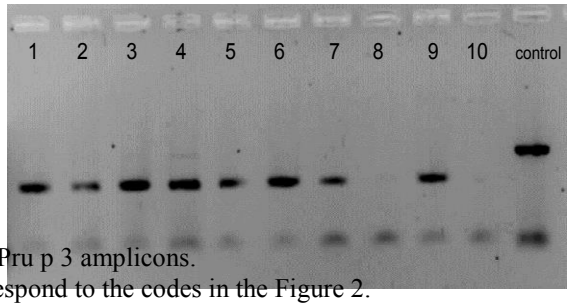

Figure 3 Restriction analysis of Pru p 3 amplicons.
Note: Codes of the samples correspond to the codes in the Figure 2.

In the case of these three methods, the A260/A230 ratios for all the extracted samples were higher than 1.9 which indicates high purity without contamination by polyphenols and polysaccharides. Here, the A260/A280 ratios varied between 1.88 and 2.12 for the extracted samples with the lack of contamination by proteins. In contrast, the samples extracted by TRIzol® Reagent method without change showed protein contamination indicated by the lower A260/280 ratios. Extraction protocols tested in the study resulted in much higher RNAyield in the case of GeneJET Plant RNA Purification Kit with/without change in the manufacturer´s workflow when compared to the TRIzol® Reagent method.

Ethanol-extracted step was added to the protocol because of the removal of water and carbohydrates from fruit were critical for obtaining high-quality and sufficient quantities of RNA **(Davis et al., 2006)**. Here, no specific effect was obtained in the case of peach fruit when using ethanol-extracted tissue treatment and the effect of the used extraction method was more significant (Table 3). Setting of the RNA extraction protocol efficiency differ highly for the individual plant species, because **Da Luz et al. (2016)** reported, that TIzol® Reagent/ice protocol is preferred for extracting of *P. edulis* RNA. This method eliminates polyphenols very effectively and a high amount of extracted RNA was obtained for the reported species.

Extracted RNA with the best parameters of quality and quantity was processed by different reverse transcription strategies further. All the transcriptomic reactions actually used are very dependent on the reliability of the reverse transcription and the accuracy of this steps both, in the experiments as well as in the diagnostics **(Mannonen et al., 2011; Huggett and Bustin, 2011)**. The reverse transcription is still not completely understood **(Ståhlberg et al., 2004)** and in spite of its importance, it is considered as an uncertain step of the transcriptomic analysis. Reverse transcriptases possess a much higher error rates when comparing them to other DNA polymerases **(Roberts et al., 1988)**. The sucsess here a mix of the effect of secondary and tertiary structure of mRNA, priming variability and effectivity, and finally the characteristics of reverse transcriptase that is used. All this is strongly affected by inhibitors that can persist in minor after RNA extraction, especially in plant biological material **(Lekanne et al., 2002; Polumuri et al., 2002)**. Actually, no unified method exists for plant species.

Three different cDNA synthesis kits were used to transcribe 500 ng, 115 ng or 11 ng of extracted RNA respectively. All of them are suitable for the RNA extracted from plants and possess a certain range of the starting amount of RNA. Tetro cDNA Synthesis Kit and Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase were used in the 500 ng and 115 ng of RNA and AccuScript High Fidelity 1st Strand cDNA Synthesis Kit was used with the 115 ng and 11 ng of RNA, because the manufacturer declares a lower amount of RNA that is needed for the reverse transcription. Tetro cDNA Synthesis Kit and AccuScript High Fidelity 1st Strand cDNA Synthesis Kit was tested in the both, random hexamers as well as oligo (dT) 18 primers. Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase provides a primer mix that is prepared and mixed by the supplier.

First, the sensitivity of the reverse transcription kits was analysed. When comparing all three cDNA synthesis kit, the starting amount specific differences were obtained dependent in the amount of transcribed product among variants of different amount of RNA used for the transcription (Table 4). Further, the differences among the individual kits were obtained. The lowest amount of transcribed product measured in the case of Tetro cDNA Synthesis Kit / 115 ng of RNA and the highest for the Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase / 500 ng. All the three cDNA synthesis kits are suitable for further processing of the transcribed product with gene-specific primers. Tetro cDNA Synthesis Kit contains MMLV reverse transcriptase and is designed to be used with the range of RNA from 100 pg up to the 2 µg. Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase contains *in vitro* derivate of MMLV reverse transcriptase and is designed to be used with the range of DNA from 5 ng up to the 0.5 fg. AccuScript High Fidelity 1st Strand cDNA Synthesis Kit contains a derivate of MMLV reverse transcriptase, too and is designed to be used with a range of 10 ng up to the 5 µg. When comparing data from the reverse transcription from 115 ng that was realized by all the three tested cDNA kits, statistical differences exist in the obtained amounts of transcribed product (Table 5).

Second, the applicability and the incorporation of the cDNA protocol to the workflow of Pru p 3 semiquantitative PCR were analysed. Individual transcribed products were diluted and unified to the 100 $ng.\mu L^{-1}$ and the semiquantitative reactions were performed. The PCR resulted in the negative amplification only in the case of using the random primers for both of the tested different starting amount of RNA with AccuScript High Fidelity 1st Strand cDNA Synthesis Kit (Figure 2).

The specifity of obtained amplicons was proved by restriction cleavage using Tse I restriction endonuclease. This provided the cleavage of the 179 bp long product in all amplicons (Figure 3).

Reverse transcription PCR is accepted as a very sensitive and specific approach that is used widely for the transcripts detection and their subsequently quantification. Despite the accuracy of absolute or quantitative techniques, semi-quantitative methods are still widely used and appropriate for many purposes **(Marone et al., 2001)**, when the specific transcripts quantification and detection of any variation in their expression levels under different experimental conditions is needed. Semi quantitative approach was applied previously successful in the expression analysis of the genes in different plant species **(Hirose and Terao, 2004; Zou et al., 2008)**. The expression patterns of different starch synthase genes **(Hirose and Terao, 2004)** and nine heat shocks protein genes **(Zou et al., 2008)** were obtained by semi quantitative RT–PCR analysis in *Oryza sativa*, L.

Quantifying of plant allergen expression is still limited mainly to its analysis of the presence/absence in the food matrix and only a few studies exist where the methods for RNA extraction or RT-PCR can be found **(Žiarovská and Zeleňáková, 2016)**. **Knoteková and Žiarovská (2017)** used the semi quantitative approach to analyse the Mal d 1.03 allergen in the varieties Golden and Spartan during

the ripening. This technique was proved to be sensitive and effective in all of these studies.

CONCLUSION

Working with mature fruit meet a specific situation in the field of RNA extraction and subsequently all the downstream applications. That is, why choosing the most fitting methods and kits is a crucial step. Here, the initial step of ethanol-extracted method of the peach tissue preparation was not proved as a statistical significant in the workflow with the p values $p < 0.0023$ and $p < 0.3653$. Subsequently, the method for the semi-quantitative analysis of the Pru p 3 allergen expression was set up in the way that will be directly applicable for Pru p 3 expression analysis with the amplicon specificity analysis with Tse I restriction endonuclease.

REFERENCES

Asif, M., Trivedi P., Solomos, T., Tucker, M. 2006. Isolation of High-Quality RNA from Apple (*Malus domestica*) Fruit. *Journal of Agricultural and Food Chemistry*, vol. 54, no. 15, p. 5227-5529.

Davis, A. R., Levi, A., Kim, S., King, S. R., Hernandez, A. 2006. RNA Extraction Method from Fruit Tissue High in Water and Sugar. *Horticulture Science*, vol. 41, no. 5, p. 1292-1294.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Kader, A. A. 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 17, p. 4976-4982. https://doi.org/10.1021/jf020136b
PMid:12166993

Gudenschwager, O., González-Agüero, M., De Filippi, B.G. 2012. A general method for high-quality RNA isolation from metabolite-rich fruits. *South African Journal of Botany*, vol. 83, p. 186-192. https://doi.org/10.1016/j.sajb.2012.08.004

Hu, C. G., Honda, C., Kita, M., Zhang, Z., Tsuda, T., Moriguchi, T. 2002. A simple protocol for RNA isolation from fruit trees containing high levels of polysaccharides and polyphenol compounds. *Plant Molecular Biology Reporter*, vol. 20, no. 1, p. 69a-69g. https://doi.org/10.1007/BF02801935

Hirose, T., Terao, T. 2004. A comprehensive expression analysis of the starch synthase gene family in rice (*Oryza sativa* L.). *Planta*, vol. 220, no. 1, p. 9-16. https://doi.org/10.1007/s00425-004-1314-6

Huggett, J. B., Bustin, S. A. 2011. Standardisation and reporting for nucleic acid quantification. *Accreditation and Quality Assurance*, vol. 16, no. 8, p. 399-405. https://doi.org/10.1007/s00769-011-0769-y

Kačániová, M., Hleba, L., Pochop, J., Kádasi-Horáková, M., Fikselová, M., Rovná, K. 2012. Determination of wine microbiota using classical method, polymerase chain method and Step One Real-Time PCR during fermentation process. *Journal of Environmental Science and Health*, vol. 47, no. 6, p. 571-578. https://doi.org/10.1080/03601234.2012.665750

Knoteková, Ľ., Ţiarovská, J. 2017. Comparative analysis of Mal d 1.03A expressed transcripts in pulp of apple cultivars Golden and Spartan. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 7, no. 3, p. 221-225. https://doi.org/10.15414/jmbfs.2017/18.7.3.221-225

Lekanne Deprez, R. H., Fijnvandraat, A. C., Ruijter, J. M., Moorman, A. F. M. Sensitivity and accuracy of quantitative real-time polymerase chain reaction using SYBR green I depends on cDNA synthesis conditions. *Analytical Chemistry*, vol. 307, no. 1, p. 63-69.

Mannonen, L., Markkula, E., Puolakkainen, M. 2011. Analysis of *Chlamydia pneumoniae* infection in mononuclear cells by reverse transcription-PCR targeted to chlamydial gene transcripts. *Medical Microbiology and Immunology*, vol. 200, no. 3, p. 143-154. https://doi.org/10.1007/s00430-011-0184-3

Marone, M., Mozzetti, S., De Ritis, D., Pierelli, L., Scambia, G. 2001. Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts fromthe same sample. *Biological Procedures Online*, vol. 3, p. 19-25. https://doi.org/10.1251/bpo20
PMid:12734582

Nicot, N., Hausman, J. F., Hoffmann, L., Evers, D. 2005. Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. *Journal of Experimental Botany*, vol. 56, no. 421, p. 2907-2914. https://doi.org/10.1093/jxb/eri285

Polumuri, S. K., Ruknudin, A., Schulze, D. H. 2002. RNase H and its effects on PCR. *Biotechniques*; vol. 32, no. 6, p. 1224-1225.
PMid:12074147

Raţná, K., Beţo, M., Hlavačková, L., Ţiarovská, J., Miko, M., Gaţo, J., Habán, M. 2016. MicroRNA (miRNA) in food resources and medicinal plant. *Potravinarstvo*, vol. 10, no. 1, p. 188-194. https://doi.org/10.5219/583

Roberts, J. D., Bebenek, K., Kunkel, T. A. 1988. The accuracy of reverse transcriptase from HIV-1. *Science*, vol. 242, no. 4882, p. 1171-1173. https://doi.org/10.1126/science.2460925
PMid:2460925

Sanders, R., Mason, D. J., Foy, C. A., Huggett, J. F. 2014. Considerations for accurate gene expression measurement by reverse transcription quantitative PCR when analyzing clinical samples. *Analytical Bioanalytical Chemistry*, vol. 406, no. 26, p. 6471-6483. https://doi.org/10.1007/s00216-014-7857-x
PMid:24858468

Ståhlberg, A., Håkansson, J., Xian, X., Semb, H., Kubista, M. 2004. Properties of the Reverse Transcription Reaction in mRNA Quantification. *Clinical Chemistry*, vol. 50, no. 3, p. 509-515.https://doi.org/10.1373/clinchem.2003.026161
PMid:14726469

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

Zou, J., Liu, A., Chen, X., Zhou, X., Gao, G., Wang, W., Zhang, Z. 2009. Expression analysis of nine rice heat shock protein genes under abiotic stresses and ABA treatment. *Journal of Plant Physiology*, vol. 166, no. 8, p. 851-861. https://doi.org/10.1016/j.jplph.2008.11.007
PMid:19135278

Ţiarovská, J., Labajová, M., Raţná, K., Beţo, M., Štefúnová, V., Shevtsova, T., Garkava, K., Brindza, J. 2013. Changes in expression of BetV1 allergen of silver birch pollen in urbanized area of Ukraine. *Journal of environmental science and health*, vol. 48, no. 12, p. 1479-1484. https://doi.org/10.1080/10934529.2013.796788
PMid:23802156

Ţiarovská, J., Zeleňáková, L. 2016. Central and Eastern European spring pollen allergens and their expression analysis-state of the art. *Diversity*, vol. 8, no. 4, p. 1-11. https://doi.org/10.3390/d8040019

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INFLUENCE OF COMPOSITION OF FEED AND LACTATION PERIOD ON MINERAL COMPOSITION OF MARE'S

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ABSTRACT

Effects of lactation period and feed on essential minerals composition of mare's milk were studied. Average Ca, P, Na and Mg concentrations in feed DM were 0.66, 4.30, 0.13 and 2.21 $g.kg^{-1}$ of DM (dry matter), respectively. In regard to milk all elements concentrations were not similar to each other due to the changes of the lactation day differences. Average Ca, P, Na and Mg concentrations (in DM) caused by breed differences and lactation days were 1.95, 1.08, 0.53 and 0.22 $g.kg^{-1}$, respectively. During the milk period, a high-quality feed were maintaining the major mineral composition of mare's milk in 1.5 – 2 times higher amount than milk of mare fed with pasture and a low-quality feed. The colostrum stage of mare was much shorter than other animals, the mare's milk on the 2nd day of lactation showed properties of initial milk in which its composition, particularly in minerals, were two times higher when compare to that on milk periods that was started from 5th day after parturition. It seemed that some factors, except well physiological conditions, such as mare's age, number of foaling, sex of foal etc. did not affect milk nutrient composition.

Keywords: Mare's milk; nutrients; minerals; lactation periods; feed; lactating mare

INTRODUCTION

Milk is a fluid secreted by the female of all species of mammals or opaque white liquid produced by the mammary glands of mammals. Milk provides the complete nutritional requirements of the neonate of the species, as well as some immunological protection and other physiological requirements. The milk samples of all species are basically similar but there are very significant species-specific differences. Interspecies differences in the quantitative composition of milk probably reflect differences in the metabolic processes of the lactating mothers and in the nutritive requirements (amino acids, minerals, fatty acid, vitamins etc.) of the suckling. In regard with horse, now there are approximately 260 of different breeds of *Equus ferus caballus* all over world. The mare's milk is one of the basic foodstuffs for human populations in Kazakhstan, Kirghizia, Tadzhikistan, and Uzbekistan and mainly in Mongolia. Milk-alcoholic beverages, called Koumiss, Airag and Kumis, are traditionally produced through fermentation **(Montanari et al., 1996, Malacarne et al., 2002, Raffaela et al., 2004, Pikul and Wojtowski, 2008)**. To the lesser extent, horses have been used as dairy herds **(Doreau et al., 2007)** in Eastern Europe (Belarus, Ukraine and Bulgaria) and Central Europe (especially Hungary).

The composition of mare's milk fat, in addition to the properties of its protein fractions and amino acid composition, suggests that this milk is more similar to human milk than cow's milk. For this reason, and because of the low cross-reactivity between cow's and mare's milk proteins, a clinical study has suggested that mare's milk could be used as a valid replacement for cow's milk in children with severe IgE-mediated cow's milk protein allergy **(Barello et al., 2008)**. More recently, the consumption of mare's milk and other dairy foods, by virtue of their mineral, bioactive lipids, and protein components, have been shown to help reduce the risk of chronic disease disorders including osteoporosis, hypertension, excess body weight and body fat, dental caries, and some cancers **(Businco et al., 2000, Guradi et al., 2001, Official Methods, 1990)**. Important nutrients are secreted by the mare to supply her foal with energy, protein, fat, carbohydrates, vitamins and minerals for optimal development and growth. To correct these nutrient losses and at the same time support maintenance requirements, lactating mares must consume adequate amounts of quality feeds. It is known that intake of minerals and amino acids are particularly important for the growth foals.

In the last decade, many studies have been carried out on equine milk and colostrums composition and, especially on

proteins, lipids and minerals for above-mentioned reasons. Essential nutrients, such as minerals and amino acid composition, and vitamins of mare's milk have been reported, however, there is no complete study regarding mare's milk properties. Moreover, there are a few studies of relationship between changes of mare's milk main components and affecting factors such as lactation stage and other factors components.

Scientific hypothesis

This paper is focused on changes of contents of essential nutrients of mare's milk depending on lactation stages, feeding system and some breeds. Aims were to analyze the content of significant nutrient elements in mare's milk and feed for the lactating mare, as well as to characterize effects of several factors that affect milk properties such as lactation periods, breed and mare's diet. More specifically, the aims were i) to analyze changes in major minerals composition of mare's milk caused by differences of broodmares and periods of the first month after parturition, ii) to analyze the major mineral and dry matter of the feed used to feed the lactating broodmares on the lactation days after parturition, and iii) to study the relations between mineral composition of milk and feed. The final goal was to improve on knowledge of the relationship between minerals in mare's milk and their feed, and, if possible, to expand the findings on equine dairy science.

MATERIAL AND METHODOLOGY

Reagents and solutions:

Nitric acid (≥ 65%) and water (both Trace Select Ultra, Ultra Trace ACS, for trace analysis, Fluka, Buchs, Switzerland) were used. Standards of elements Ca, Na, Mg and P (Astasol-Ca, Astasol-Na, Astasol-Mg and Astasol-P were purchased from Analytika (Prague, Czech Republic). The working standard solutions were prepared by diluting the Ca, Na, Mg stock solutions 1.000 ±0.002 $g.L^{-1}$ in 2% nitric acid and P stock solution in 0.005% sulphuric acid. All working standard solutions were stored in polypropylene bottles. Deionised water was used for the preparation of all solutions.

All glassware was initially washed with detergent and water, and then the glassware was rinsed several times with deionised water and dried. Argon (purity 4.8) and nitrogen (purity 4.6) was from Messer (Prague, Czech Republic). All other reagents and solutions were of analytical grade purity (Sigma-Aldrich, St. Louis, MO, USA).

Equipments:

Microwave oven MarsXpress (CEM Corporation, Matthews, NC, USA) equipped with 20 mL PTFE high pressure vessels was used for microwave digestion (power 800 W). A Prodigy ICP-OES (Teledyne Leeman Labs, Hudson, NH, USA) was used for determination of calcium, phosphorus, sodium and magnesium contents in milk and feed. A vacuum lyophiliser Christ Alpha 1–4 (Martin Christ GmbH, Osterode am Harz, Germany) was used for sample vacuum lyophilisation.

Selection of broodmares and broodmare's milk samples

In order to collect mare's milk and colostrum samples, eight different broodmares were selected. All the mares of age from seven to sixteen years (10 ±3 parities) were mature and well-developed live weight between 500 and 600 kg. They were kept indoor and outdoor individually, and fed with 1.3 kg wheat bran and 2.1 kg homily, 5.0 kg oats in every day. Water was available months.

The milk samples (1P – 8P, approximately 40 – 50 mL each) were taken in plastic containers at days 2, 5, 10, and 28 days postpartum (Table 1, and in some cases also at 56th day for 1F and 2P). Before milking, foals were separated from their mothers for approximately 2 hours to prevent from suckling. First and second milking of mares was undertaken with hand milking as deep as possible. Some mares had not previously been subjected to any milking procedures. Collected milk samples were taken directly to the laboratory and frozen at -80 °C for 4 hours, and then lyophilised at 12 Pa and -40 °C using vacuum lyophilisation. All lyophilised samples were stored in fridge at -34°C until use for analysis.

The feed samples (in amount of approximately 500 g) were collected in polyethylene bags from feed mixture for the lactating mares at same days as milk sample collecting (Table 1). Then all samples were taken to the laboratory and stored at room temperature. All feed samples were homogenized and grounded. The feed samples were put into plastic containers and stored at room temperature until use for analysis.

Procedures

Analysis of dry matter

Dry matter (DM) was determined according to the standard procedure of AOAC. Grounded feed samples were dried in oven at 105 ±1 °C to constant weight for 5 h **(Official Methods, 1990, Nat. Res. Council, 1989).**

Element analysis

The minerals contents of all milk and feed samples were determined by inductively coupled argon plasma optical emission spectrometry (ICP-OES). The lyophilized milk (20 mg) and feed samples (20 mg) were accurately weighted and put into a 20 mL high pressure PTFE containers and 4 mL digestion mixture (2 mL deionised water and 2 mL 65% nitric acid) were added and placed in the microwave oven for mineralization. Program of decomposition consisted of three steps i) temperature increase to180 °C in 25 minutes, ii) maintaining the temperature at 180 °C for 10 minutes and iii) cooling at room temperature. Afterwards, mineralized samples were put into 10 mL volumetric flasks and diluted with deionised water up to 10 mL for mineral analysis.

Calcium, phosphorus, sodium and magnesium contents (on DM basis) of milk and feed were determined by using ICP-OES. After scanning a blank, a standard solution and a sample solution in the programmed wavelength range, the background correction wavelengths were selected manually at appropriate background positions for each analyte signal. The detection limits of the method at the pre-selected wavelengths (Ca – 317.933 nm, P – 214.914 nm, Na – 588.995 nm and Mg – 280.271 nm) were high enough and permitted the determination. For each sample three determinations were performed. Instrument configuration and general experimental conditions are summarized in Table 2.

Statisic analysis

All data on the content of four minerals in DM of all samples are expressed as arithmetic mean accompanied by a standard deviation (mean ± SD), with outlying results excluded by performing a Dean-Dixon test (Q-test). To calculate arithmetic average and standard deviation was used by Office Excel® Microsoft. Data were evaluated by producing summary statistics and analyzing the variance using an ANOVA. Single Factor Scattering and Scale Comparisonthe Scheffé's method was implemented using the statistical program QC Expert 3.3 (Trilobite Statistical Software, Pardubice, CZ). Level of significance single factor analysis of scattering and pair comparison by Scheffé's method was set to 5% (p <0.05). In regard to lyophilized milk samples, all data revealed by ICP-OES were converted to the mean on fresh milk.

RESULTS AND DISCUSSION

Dry matter of the feed

The dry matter (DM) content of all feed samples for the lactating mares is showed in Table 3. The percentage of DM content of every feed sample was a little different from each other due to the preparation conditions of feed mixture and the ratio of forages in feed mixture. The average DM content of the feed was 89.2 ±0.7 % w/w (p <0.05) or in the range from 88.6 to 90.6%.

According to some authors **(Berg, 2009)** the DM content of some high quality feed types for the lactating mare such as grains, hay and chaff ranged from 87 to 91% (w/w). These results of DM content of feed samples also were in agreement with National Research Council (NRC) values **(Nat. Res. Council, 1989)**. DM content of all feeds, therefore, had the standard level of DM of feed and forage for the lactating mare.

Mineral composition of feed

The concentration ranges of major four elements (Ca, P, Na and Mg) determined in the 29 feed samples for lactating broodmares on the 2nd, 5th, 10th, 28th and 56th days after parturition are summarized in Tables 4 – 7. These data were expressed as mean ± SD (p <0.05). There were significant differences among the feeds with regard to four elements content. For all feed samples, significant changes of contents of minerals were observed causing by days after parturition. Ca, P, Na and Mg concentrations (on a DM basis) were respectively 0.66 – 0.78, 4.95 – 5.05, 0.13 – 0.15 and 2.59 – 2.61 g.kg^{-1} in feed. Also these tables show the average content of these four elements in feed samples related to same lactation days.

As shown in Table 4, generally, the average concentrations of Ca in feeds for the lactating mares on 5th day after parturition was higher 0.72 ±0.18 g.kg^{-1} than other days, and on 28th, was the lowest 0.57 ±0.05 g.kg^{-1}. Calcium average concentration of feeds caused by kinds of forage and feed for horse ranges from 0.4 to 17.1 g.kg^{-1} and the feed type of grains such as oats and wheat, in, particular, ranges from 0.5 to 0.9 g.kg^{-1} **(Berg, 2009)**. Ca concentrations of 1F and 3F on 5th and, the on 10th days after parturition, were increased gradually and then decreased. In regard to 2F, 5F, 7F and 8F, slight decrease, from the 2nd to 28th, was observed. Concentrations of 4F and 6F minerals were increased on the 5th day, but afterwards, decreased dramatically. The average concentration of Ca in the feed ranged from 0.70 ±0.14 to 0.57 ±0.05 g.kg^{-1}. Generally, Ca concentration increased gradually at interval between the 2nd and 5th days 0.70 ±0.14 and 0.72 ±0.18 g.kg^{-1}, and on 10th and 56th days, then decreased slightly 0.61 ±0.23 and 0.57 ±0.05 g.kg^{-1}. The average Ca concentration of all feed samples depending on days was 0.66 ±0.09 g.kg^{-1} and these all results of Ca concentration in feed samples were in agreement with NRC values to feed the lactating mare **(Berg, 2009, Saastamoinen and Koskinen, 1993)**.

Table 5 presents P concentrations of feeds. As it results from the table, feeds for mares on the 2nd day were characterized by the highest P concentrations (4.54 ±0.69) g.kg^{-1} when compared with the 5th, 10th, 28th and 56th days, 4.03 ±0.32, 4.05 ±0.51, 4.41 ±0.52 and 4.44 ±0.01 g.kg^{-1}, respectively. According to NRC **(Nat. Res. Council, 1989)**, daily P requirement of the lactating mare is 31 – 51 g.kg^{-1} and average P concentration of feeds caused by kinds of forage and feed for horse ranges from 1.0 to 12.7 g.kg^{-1} and the feed type of grains such as oats and wheat, pasture and hay/chaff, particularly, ranges from 2.0 to 4.1 g.kg^{-1}. P concentrations of 1F, 2F, 4F and 6F on the 5th day decreased, although on the 10th day, increased slightly again. In regard to 3F, 5F, 7F and 8F, P concentrations decreased continuously and slightly. The average P concentration of all feed samples depending on experiment days was 4.30 ±0.26 g.kg^{-1}, and these all results of P concentration in feed samples were in agreement with NRC to feed the lactating mare **(Berg, 2009, Saastamoinen and Koskinen, 1993)**.

Na concentrations of feeds are shown in Table 6. It was observed that the highest Na concentration was in 8F on 10th day as 0.21 ±0.01 g.kg^{-1}, and it was 0.12 ±0.01 g.kg^{-1}, on the 2nd day. The Na concentration of all feed samples, except 5F, 6F and 7F, increased gradually during lactation days. The average Na concentrations of all feeds on lactation days were 0.12 ±0.01, 0.13 ±0.02, 0.13 ±0.03 and 0.14 ±0.02 g.kg^{-1}, respectively. On the other hand, Na content rose slightly till 56th day and approximately constant on the 5th and 10th days. The average Na content was 0.13 ±0.01 g.kg^{-1}. Mg concentrations of feeds were not similar to each others, which are shown in Table 7. On the other hand, an increase and decrease of Mg content of all feeds were observed in different ranges caused by lactating days, and the average Mg concentration was 2.21 ±0.12 g.kg^{-1}.

According to NRC **(Nat. Res. Council, 1989)**, daily Mg requirement of the lactating mare is 12.0 – 15.2 g.kg^{-1}. Mg contents of 1F and 6F on the 10th day, 2F, 3F, 4F, 7F an 8F on the 2nd day and 5F on the 5th day were the highest compare to that on other days, 2.59 ±0.01, 2.59 ±0.01, 2.44 ±0.01, 2.78 ±0.01, 2.45 ±0.03, 2.16 ±0.03 and 2.26 ±0.01 g.kg^{-1}, respectively. The average Mg concentrations of feeds for lactating mares demonstrated that Mg contents on the 5th an 10th days were constant and, further on the 28th, increased gradually again. Overall, the feed mineral composition is influenced by many factors such as storage condition, contamination, and ratio of feed mixtures **(Csapó-Kiss et al., 1995)**.

Table 1 Milk and feed samples characteristics.

Milk	Days postpartum				Feed	Days postpartum			
	2	5	10	28		2	5	10	28
1P[a]	+	+	+	+	1F	+	+	+	+
2P[b]	+	+	+	+	2F	+	+	+	+
3P	+	+	+	+	3F	+	+	+	+
4P	+	+	+	+	4F	+	+	+	+
5P	+	+	+	+	5F	+	+	+	-
6P	+	+	+	-	6F	+	+	+	-
7P	+	+	+	-	7F	+	+	+	-
8P	+	+	+	+	8F	+	+	+	-

Note: +, sample was taken; -, sample was not taken, a a sample on 56th day was also collected for 1P, b a sample on 56th day was also collected for 2P.

Table 2 ICP-OES operating conditions.

Operating conditions		Operating conditions	
Radio frequency	27.12 Hz	Outer gas flow rate	Ar 17 L/min
Radio frequency power	2.5kW	Intermediate gas flow rate	Ar 1 L/min
Plasma's temperature	8000 – 9000 K	Carrier gas flow rate	Ar 1 L/min

Table 3 The dry matter content of feed (%, w/w).

Feed	Dry matter (mean value ±SD)			
	2nd	5th	10th	28th
1F[a]	89.38 ±0.19	89.32 ±0.15	89.04 ±0.51	88.96 ±0.66
2F	90.56 ±0.67	88.64 ±0.34	89.54 ±0.37	89.36 ±0.55
3F	90.32 ±0.54	89.55 ±0.86	88.69 ±0.98	89.04 ±0.22
4F	88.89 ±0.42	89.14 ±0.51	89.06 ±0.20	88.90 ±0.08
5F	89.64 ±0.40	88.99 ±0.70	88.65 ±0.68	n.a.
6F	90.12 ±0.39	88.80 ±0.57	88.81 ±0.58	n.a.
7F	90.47 ±0.31	89.37 ±0.37	88.81 ±0.53	n.a.
8F	90.00 ±0.63	88.80 ±0.39	88.7 ±0.51	n.a.

Note: F – feed samples; SD – standard deviation; n.a. – not analyzed, a DM = (89.49 ±0.51) on 56th day.

Mineral composition of milk

The nutritive minerals found in mare's milk as mainly ionized form are essential for nutritional and metabolic functions of newborn neonate. The data on major essential mineral composition of milk from different eight broodmares fed with supplement feed mixture are given in Tables 3 – 7. All minerals concentrations were described as mean ±SD ($g.kg^{-1}$). The results of mineral concentration of milk samples demonstrated that Ca, P, Na and Mg contents of broodmares' milk were not similar to each other due to breed differences and lactation days. As shown in Table 4, Ca concentrations of 5P 2.82 ±0.01 $g.kg^{-1}$ and 8P 2.72 ±0.01 $g.kg^{-1}$ on the 2nd lactation day were the highest in that of others, which were two times higher than that reported by Csapo-Kiss **(Csapó-Kiss et al., 1995)**, while the lowest Ca concentration 0.66 ±0.01 $g.kg^{-1}$ in 1P on the 56th day which was comparable to the mean of literature values. As reported in the literature, the mineral level of mare's milk caused by lactation stage differs greatly, which ranges 0.8 – 1.3 $g.kg^{-1}$ on 4 – 180 lactation days. In many papers, Ca level is higher in colostral stage, in which milk is rich in proteins, than milk stage because of Ca ions play an important role in the structure and stability of casein micelles **(Csapó et al., 2009)**. From dependence on lactation days, Ca concentrations of 1P and 5P decreased gradually from the 2nd to 5th and 10th days and then increased on the 28th day. In regard to others, a decrease on the 5th and 28th days and an increase on the 10th day were observed. The observations were in agreement with major papers **(Csapó-Kiss et al., 1995, Schryver et al., 1986, Summer et al., 2004, Cisla et al., 2009, Nascimento et al., 2010)**.

With regard to phosphorus concentration of studied broodmares milk, the highest amounts were observed in all milk on the 2nd day during the lactation periods, which ranged from 1.93 $g.kg^{-1}$ to 0.94 $g.kg^{-1}$ caused by breed of broodmares. P concentration of 5P was the highest on the 2nd day while the lowest in 1P, but it was declined continuously on other days. Some authors reported that P content ranged from 0.7 to 0.9 $g.kg^{-1}$ on 0 – 45 days of the lactation **(Schryver et al., 1986)**. Although, mineral content is caused significantly by type of breed and feed which are the most powerful affecting factors on it **(Summer et al., 2004)**.

Table 4 Ca content in feed and milk ($g.kg^{-1}$).

Feed	ADM (%)	Ca content (mean value ±SD)				AC[2]	Milk	Ca content (mean value ±SD)				AC[2]
		2nd	5th	10th	28th	2-56th days		2nd	5th	10th	28th	2-56th days
1F[3]	89.24 ±0.23	0.83 ±0.02	1.03 ±0.01	1.13 ±0.00	0.64 ±0.00	0.86 ±0.22	1P	1.43 ±0.03	0.77 ±0.01	0.66 ±0.00	0.97 ±0.01	0.99 ±0.31
2F	89.53 ±0.79	0.95 ±0.00	0.80 ±0.00	0.55 ±0.01	0.59 ±0.00	0.72 ±0.19	2P	1.81 ±0.01	2.06 ±0.00	2.75 ±0.02	1.26 ±0.01	1.97 ±0.62
3F	89.40 ±0.71	0.68 ±0.00	0.69 ±0.00	0.73 ±0.00	0.52 ±0.00	0.65 ±0.09	3P	2.38 ±0.00	2.35 ±0.00	2.67 ±0.00	n.a.	2.47 ±0.18
4F	88.99 ±0.12	0.62 ±0.01	0.89 ±0.00	0.61 ±0.00	0.55 ±0.01	0.67 ±0.15	4P	2.44 ±0.01	2.06 ±0.01	2.15 ±0.02	1.97 ±0.01	2.16 ±0.20
5F	89.09 ±0.50	0.68 ±0.01	0.62 ±0.00	0.45 ±0.00	n.a.	0.58 ±0.12	5P	2.82 ±0.01	2.35 ±0.02	1.43 ±0.01	2.57 ±0.02	2.29 ±0.60
6F	89.24 ±0.76	0.56 ±0.00	0.75 ±0.00	0.47 ±0.00	n.a.	0.59 ±0.14	6P	n.a	1.91 ±0.01	n.a.	n.a.	-
7F	89.55 ±0.84	0.53 ±0.01	0.51 ±0.00	0.49 ±0.00	n.a.	0.50 ±0.03	7P	2.35 ±0.02	n.a.	1.82 ±0.03	n.a.	2.09 ±0.37
8F	89.17 ±0.72	0.76 ±0.01	0.51 ±0.00	0.48 ±0.00	n.a.	0.58 ±0.15	8P	2.72 ±0.00	n.a.	n.a.	n.a.	-
AC[1]	**89.28 ±0.20**	**0.70 ±0.14**	**0.72 ±0.18**	**0.61 ±0.23**	**0.57 ±0.05**	**0.66 ±0.10[a]**	**AC[1]**	**2.28 ±0.50**	**1.92 ±0.59**	**1.91 ± 0.79**	**1.70 ±0.72**	**1.95 ±0.24[a]**

Note: ADM – average dry matter of feed; [3] (0.66 ±.01) and (0.661 ± 0.002) for 1F and 2P on 56th day; AC[1] – average content in feeds at same lactation days; AC[2] – average content in feeds at different lactation days; [a] – average content of AC[1]; n.a. – not analyzed.

Table 5 P content in feed and milk ($g.kg^{-1}$).

Feed	ADM (%)	P content (mean value ±SD)				AC[2]	Milk	P content (mean value ±SD)				AC[2]
		2nd	5th	10th	28th	2-56th days		2nd	5th	10th	28th	2-56th days
1F[3]	89.24 ±0.23	4.39 ±0.01	4.29 ±0.01	4.93 ±0.03	4.52 ±0.01	4.51 ±0.25	1P	0.94 ±0.01	0.39 ±0.00	0.30 ±0.00	0.39 ±0.00	0.50 ±0.26
2F	89.53 ±0.79	5.12 ±0.02	3.99 ±0.01	4.30 ±0.02	3.76 ±0.00	4.29 ±0.59	2P	1.10 ±0.01	1.00 ±0.00	1.30 ±0.02	0.52 ±0.00	0.98 ±0.33
3F	89.40 ±0.71	4.59 ±0.01	3.40 ±0.01	3.83 ±0.02	4.35 ±0.01	4.19 ±0.34	3P	1.41 ±0.00	0.96 ±0.01	1.46 ±0.01	n.a.	1.28 ±0.28
4F	88.99 ±0.12	5.61 ±0.04	3.68 ±0.01	4.10 ± .00	5.02 ±0.02	4.60 ±0.88	4P	1.85 ±0.01	1.14 ±0.01	1.00 ±0.00	0.83 ±0.00	1.20 ±0.45
5F	89.09 ±0.50	3.27 ±0.01	4.46 ±0.01	3.43 ±0.01	n.a.	3.72 ±0.65	5P	1.93 ±0.00	1.31 ±0.00	0.69 ±0.00	1.70 ±0.00	1.41 ±0.54
6F	89.24 ±0.76	4.30 ±0.01	4.20 ±0.00	4.42 ±0.03	n.a.	4.31 ±0.11	6P	n.a	1.66 ±0.00	n.a.	n.a.	-
7F	89.55 ±0.84	4.84 ±0.03	4.13 ±0.01	3.98 ±0.03	n.a.	4.32 ±0.46	7P	1.56 ±0.00	n.a.	0.63 ±0.01	n.a.	1.09 ±0.54
8F	89.17 ±0.72	4.22 ±0.03	3.49 ±0.03	3.44 ±0.02	n.a.	3.72 ±0.44	8P	1.69 ±0.00	n.a.	n.a.	n.a.	-
AC[1]	**89.28 ±0.20**	**4.54 ±0.69**	**4.03 ±0.32**	**4.05 ±0.51**	**4.41 ±0.52**	**4.30 ±0.26**	**AC[1]**	**1.50 ±0.37**	**1.08 ±0.42**	**0.90 ±0.44**	**0.86 ±0.59**	**1.08 ±0.29[a]**

Note: ADM – average dry matter of feed; [3] (4.44 ±0.01) and (0.481 ±0.001) for 1F and 1P on 56th day; AC[1] – average content in feeds at same lactation days; AC[2] – average content in feeds at different lactation days; [a] – average content of AC[1]; n.a. – not analyzed.

P content of 1P was lowest on all days when compared to others which ranged from 0.39 to 0.94 $g.kg^{-1}$, but it was in agreement with data on previous studies **(Summer et al., 2004, Solaroli et al., 1993)**.

Generally, it is reported that P content is declined after colostral period, and in the case of present study, P concentration of all milk, except 3P and 5P, decreased gradually after the 2nd day which is shown in Table 5.

The concentration of Na in milk exists in lower amount than that of Ca and P, because Ca and P are associated in form of the colloid calcium phosphate, which are a large class of milk proteins **(Solaroli et al., 1993)**.

Table 6 Na content in feed and milk ($g.kg^{-1}$).

Feed	ADM (%)	Na content (mean value ±SD)				AC[2]	Milk	Na content (mean value ±SD)				AC[2]
		2nd	5th	10th	28th	2-56th days		2nd	5th	10th	28th	2nd	2-56th days
1F[3]	89.24 ±0.23	0.12 ±0.00	0.13 ±0.00	0.13 ±0.00	0.13 ±0.00	0.13 ±0.01	1P	0.60 ±0.01	0.18 ±0.00	0.15 ±0.00	0.32 0.00	0.31 ±0.18	0.60 ±0.01
2F	89.53 ±0.79	0.13 ±0.00	0.14 ±0.00	0.12 ±0.00	0.17 ±0.00	0.14 0.02	2P	0.68 ±0.01	0.34 ±0.00	0.36 ±0.01	0.21 ±0.00	0.40 ±0.20	0.68 ±0.01
3F	89.40 ±0.71	0.13 ±0.00	0.12 ±0.00	0.14 ±0.00	0.14 ±0.00	0.13 ±0.01	3P	0.81 ±0.00	0.64 ±0.01	0.82 ±0.00	n.a.	0.76 ±0.11	0.81 ±0.00
4F	88.99 ±0.12	0.13 ±0.00	0.11 ±0.00	0.11 ±0.00	0.12 ±0.00	0.12 0.01	4P	0.97 ±0.00	0.49 ±0.01	0.33 ±0.01	0.55 ±0.00	0.58 ±0.28	0.97 ±0.00
5F	89.09 ±0.50	0.11 ±0.00	0.14 ±0.00	0.11 ±0.00	n.a.	0.12 ±0.02	5P	0.87 0.00	0.41 ±0.00	0.33 ±0.00	0.73 ±0.01	0.59 ±0.26	0.87 ±0.00
6F	89.24 ±0.76	0.13 ±0.00	0.15 ±0.00	0.11 ±0.00	n.a.	0.13 ±0.02	6P	n.a.	0.88 ±0.01	n.a.	n.a.	-	n.a.
7F	89.55 ±0.84	0.11 ±0.00	0.14 ±0.00	0.11 ±0.00	n.a.	0.12 ±0.02	7P	0.79 ±0.01	n.a.	0.34 ±0.00	n.a.	0.57 ±0.32	0.79 ±0.01
8F	89.17 ±0.72	0.12 ±0.00	0.10 ±0.00	0.21 ±0.00	n.a.	0.14 ±0.06	8P	0.87 ±0.01	n.a.	n.a.	n.a.	-	0.87 ±0.01
AC[1]	**89.28 ±0.20**	**0.12 ± .01**	**0.129 ±0.02**	**0.13 ±0.03**	**0.14 ±0.02**	**0.13 ± .01[a]**	**AC[1]**	**0.80 ±0.13**	**0.49 ±0.24**	**0.39 ±0.23**	**0.45 ±0.23**	**0.53 ±0.18[a]**	**0.80 ±0.13**

Note: ADM – average dry matter of feed; [3] (0.13 ±0.01) and (0.28 ±0.00) for 1F and 2P on 56th day; AC[1] – average content in feeds at same lactation days; AC[2] – average content in feeds at different lactation days; [a] – average content of AC[1]; n.a. – not analyzed.

Table 7 Mg contents in feed ($g.kg^{-1}$).

Feed	ADM (%)	Mg contents (mean value ±SD)				AC[2]	Milk	Mg contents (mean value ±SD)				AC[2]
		2nd	5th	10th	28th	2-56th days		2nd	5th	10th	28th	2-56th days
1F[3]	89.24 ±0.23	2.36 ±0.04	2.26 ±0.02	2.59 ±0.01	2.46 ±0.00	2.39 ±0.14	1P	0.16 ±0.00	0.08 ±0.00	0.05 ±0.00	0.08 ±0.00	0.09 ±0.04
2F	89.53 ±0.79	2.59 ±0.01	2.06 ±0.00	2.16 ±0.02	1.95 ±0.01	2.19 ±0.28	2P	0.24 ±0.00	0.22 ±0.00	0.28 ±0.00	0.11 ±0.00	0.21 ±0.07
3F	89.40 ±0.71	2.44 ±0.01	2.04 ±0.01	1.89 ±0.01	2.31 0.01	2.19 ±0.25	3P	0.27 ±0.00	0.22 ±0.00	0.34 ±0.00	n.a.	0.28 ±0.06
4F	88.99 ±0.12	2.78 ±0.01	2.00 ±0.01	2.03 ±0.01	2.50 ±0.01	2.33 ±0.38	4P	0.33 ±0.00	0.23 ±0.00	0.19 ±0.00	0.179 ±0.00	0.23 ±0.07
5F	89.09 ±0.50	1.73 ±0.01	2.26 ±0.01	1.76 ±0.01	n.a.	1.91 ±0.30	5P	0.33 ±0.00	0.26 ±0.00	0.14 ±0.00	0.30 ±0.00	0.26 ±0.08
6F	89.24 ±0.76	2.26 ±0.00	2.18 ±0.00	2.26 ±0.02	n.a.	2.23 ±0.04	6P	n.a.	0.35 ±0.00	n.a.	n.a.	-
7F	89.55 ±0.84	2.45 ±0.03	2.10 ±0.00	1.96 ±0.01	n.a.	2.17 ±0.26	7P	0.31 ±0.00	n.a.	0.17 ±0.00	n.a.	0.24 ±0.10
8F	89.17 ±0.72	2.16 ±0.03	1.76 ±0.00	1.81 ±0.01	n.a.	1.91 ±0.22	8P	0.33 ±0.01	n.a.	n.a.	n.a.	-
AC[1]	**89.28 ±0.20**	**2.34 ±0.31**	**2.08 ±0.16**	**2.06 ±0.28**	**2.30 ±0.25**	**2.210 ±0.122[a]**	**AC[1]**	**0.28 ±0.06**	**0.22 ±0.09**	**0.20 ±0.10**	**0.16 ±0.10**	**0.22 ±0.05[a]**

Note: ADM - average dry matter of feed; [3] (2.26 ±0.01) and (0.09 ±0.00) for 1F and 2P on 56th day; AC[1] – average content in feeds at same lactation days; AC[2] – average content in feeds at different lactation days; [a] – average content of AC[1]; n.a. – not analyzed.

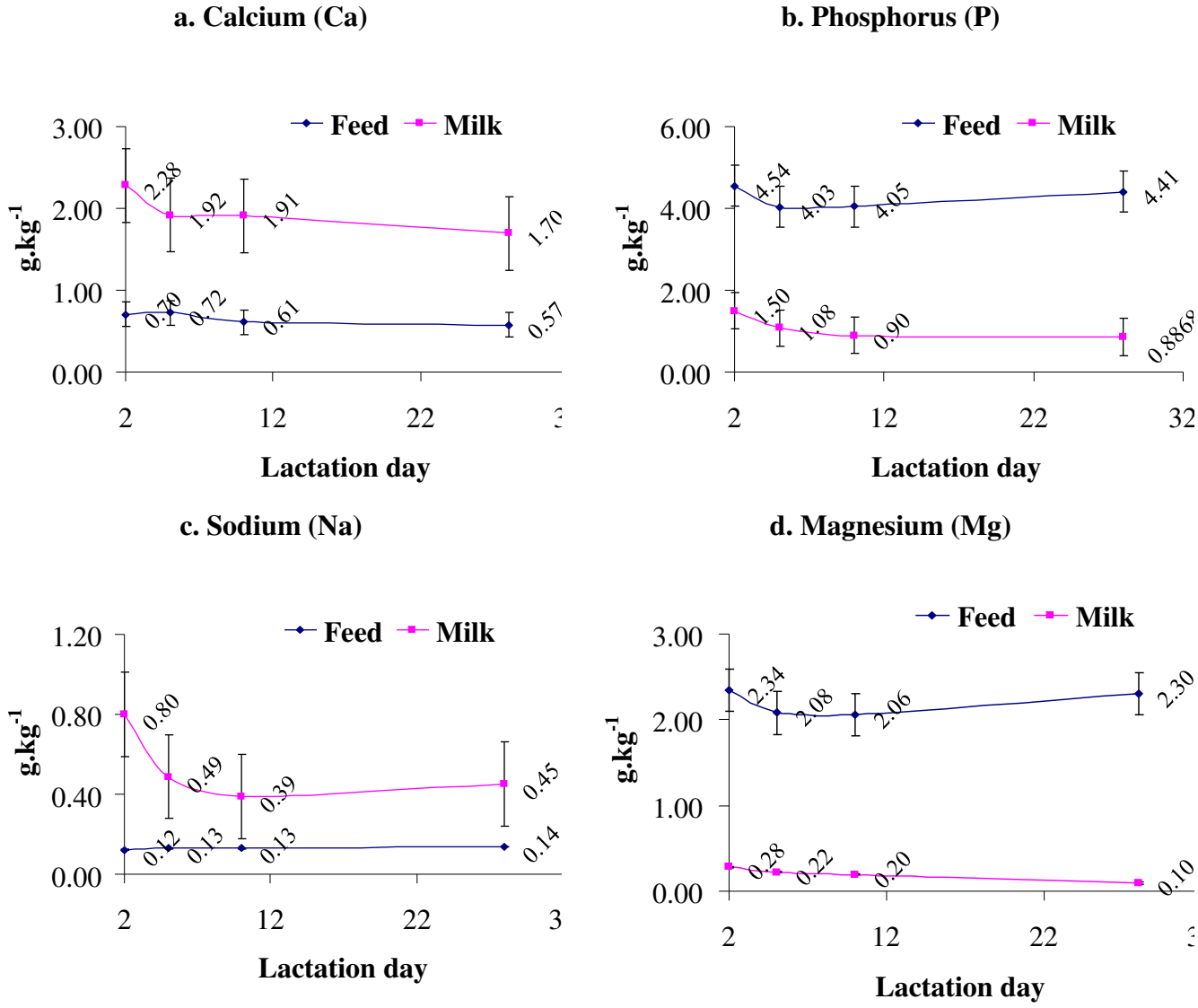

Figure 1a-d. Relation between average Ca, P, Na, Mg contents in milk and feed.

Na content of mare's milk is lower than other farm animals **(Sarwar et al., 1998)**. According to the data shown in Table 6, significantly differences among Na contents of milk during lactation days were observed. Na concentration of all milk on the 2nd day was the highest and the lowest on the10th day. The highest Na amount 0.97 g.kg^{-1} was in 4P which was three times higher than the value reported in literature and the lowest 0.21 g.kg^{-1} in 2P, which was in agreement with some authors **(Sarwar et al., 1998)**. However, many papers have shown different values of Na content of equine milk caused by breed and feed **(Schryver et al., 1986, Gálik et al., 2012)**. Na concentration of 1P per each day of lactation was lower than others and of 2P, 4P and 5P was almost similar to each other during lactation days. In regard to 3P, the highest concentration 0.82 g.kg^{-1} was on the 10th day. According to some reports in the literature that Na concentration is high at the beginning of colostrum and then declines gradually, 1P, 2P and 7P were comparable to it, but of 3P, 4P and 5P decreased till the 10th and then increased on the 28th day of lactation.

Table 7 shows Mg concentration of all mares' milk during lactation periods. The only Mg concentration of 1P on all
days, which ranged from 0.16 g.kg^{-1} to 0.05 g.kg^{-1}, was in agreement with earlier studies 50 – 180 mg.L^{-1} of fresh milk **(Summer et al., 2004, Nascimento et al., 2010)**, but also Mg concentrations of 2P and 3P on the 2nd day were less higher, 0.24 g.kg^{-1} and 0.27 g.kg^{-1} respectively. It was two times higher than the mean of the literature values in the case of others on the 2nd, 5th and 28th days. The highest Mg concentrations were observed in all milk on the 2nd day of the lactation, after that it was decreased dramatically.

The average mineral concentrations depended on breed during the lactation periods is also shown in Tables 4 – 7. Average Ca concentration in all broodmare's milk caused by breed difference was much higher (ranged from 1.70 to 2.28 g.kg^{-1}) than other three major elements P, Na and Mg in milk and ratios of Ca to the others were 1.8:1, 3.7:1 and 9:1, respectively which was comparable to the some literature values **(Schryver et al., 1986, Summer et al., 2004)**.

Figure 2 Mare with foal (source: https://zlinsky.denik.cz/galerie/).

P was the second major element in mare's milk in regard to its amount and the average P concentration ranged from 0.86 to 1.50 $g.kg^{-1}$ during the 1st month during lactation. With regard to Na, the highest amount was observed on 2nd day as 0.80 $g.kg^{-1}$ and then two times dropped on the 5th day until 0.49 $g.kg^{-1}$. The average Mg decreased gradually after the 2nd day of lactation until 0.16 $g.kg^{-1}$ on the 28th day and it was 1.5 times lower than the 2nd day of lactation.

Overall, Ca, P, Na and Mg concentrations in mare's milk were high in the beginning of the lactation periods and then declined as approximately 1.5 times on the 5th day of lactation and continuously till the 28th day. These results on four minerals were in agreement with and comparable to results of previous researches **(Csapó-Kiss et al., 1995, Schryver et al., 1986, Summer et al., 2004, Nascimento et al., 2010, Solaroli et al., 1993, Sarwar et al., 1998, Gálik et al., 2012, Martin et al., 1992)**.

Relation between the mineral composition of feed and milk

The changes in average content of major four elements in different eight broodmares' milk and feed for them depended on the 2nd, 5th, 10th and 28th days of the lactation and the relations between them are given in Figure 1. All data were expressed as mean ± SD, which indicated significant differences between changes in mineral content of milk and feed. Generally, lactation period is the most important factor influencing on mare's milk composition. The results conducted by changes in mineral content of milk showed that except Na, other three mineral Ca, P and Mg contents decreased gradually from 2.28, 4.54 and 0.28 to 1.92, 4.03 and 2.08 $g.kg^{-1}$ during lactation periods, in particular on the 5th day of lactation, all minerals contents were declined significantly and further, decreased slightly. Some author reported that the ash content did not vary or decreased only slightly and the effect of the lactation period on mare's milk composition was similar for different breed **(Martin and Doreau, 2006)**. However, the results of present study on milk mineral were not in agreement with above report. Moreover, it was in agreement with many results demonstrated in the literature that with regard to ash composition of mare's milk, the highest ash content is observed during the 1st week of lactation period. Afterward, total ash content regularly decreased, due to a decline of all minerals, the ash content in the later stages of lactation wais about 39% lower than that of the earlier stages **(Summer et al., 2004, Martuzzi et al., 1997)**. In regard to Na concentration of milk decreased till the 10th day of lactation and then, on 28th day, a slight increase was observed. Also it was similar to results reported by some authors **(Summer et al., 2004, Martuzzi et al., 1997)**. The changes in mineral concentration of feeds for the lactating broodmares during lactation were not similar to each other. Ca and Mg concentration decreased during lactation period while P and Na increased gradually, except a slight decrease on the 5th day of lactation. In fact, the mineral composition of these feeds depended greatly on preparation of feed mixture.

As shown in Figure 1a-d, there was no significant relation between changes in average mineral content of milk and feed. Although, P and Mg concentrations in milk and feed on the 5th day of lactation decreased dramatically, perhaps it was due to, in the case of milk, ending of colostral period. In contrast, Ca and Na concentration of milk decreased considerably while Ca and Na in feed increased. Further, Ca, P, Na and Mg concentration in milk on the 2nd, 5th, 10th and 28th days after parturition to feed ratios were found in amounts of Ca - 3.2:1, 2.6:1, 3:1 and 3:1, P - 1:3, 1:3.7, 1:4.2 and 1:5.1, Na - 6.6:1, 3.8:1, 3:1 and 3.2:1, Mg - 1:8.4, 9.5:1, 10.3:1 and 23:1, respectively. Overall, changes in milk mineral content caused by lactation periods were not correlated with that of feed, it was in agreement with some authors **(Martuzzi et al., 1997, Hidiroglou and Proulx, 1982)**, perhaps, due to milk production metabolic functions of the lactating mare and mineral transfer on it **(Kavazik et al., 2002)**.

CONCLUSION

All results revealed indicated significant relations and changes in major minerals composition of different lactating mares' milk depending on the lactation periods and feed used to feed the mares. The average DM content of the feed was 89.2 ±0.72 % w/w (p <0.05). The highest Ca, P, Na and Mg concentration of feed were 1.13 $g.kg^{-1}$ in 1F on the 10th day, 5.02 $g.kg^{-1}$ in 4F on the 28th, 0.21 $g.kg^{-1}$ in 8F 10th and 2.59 $g.kg^{-1}$ in 1F 10th, respectively while the lowest concentration were 0.47 $g.kg^{-1}$ in 5F on 10th, 3.27 $g.kg^{-1}$ in 5F on 2nd, 0.11 $g.kg^{-1}$ in 5F on 10th and 1.73 $g.kg^{-1}$ in 5F on 2nd day, respectively. Average Ca, P, Na and Mg concentrations of all feeds caused by all days were 0.66, 4.30, 0.13 and 2.21 $g.kg^{-1}$, respectively. In regard to milk minerals, the highest Ca, P, Na and Mg concentration were observed in amounts of 2.82 $g.kg^{-1}$ in 5P on 2nd, 1.93 $g.kg^{-1}$ in 5P on 2nd, 0.97 $g.kg^{-1}$ in 4P on 2nd and 0.32 $g.kg^{-1}$ in 5P and 8P on 2nd day, respectively, when compared to the lowest were 0.66 $g.kg^{-1}$, 0.30 $g.kg^{-1}$, 0.05 $g.kg^{-1}$ and 0.15 $g.kg^{-1}$ in 1P on 10th day, respectively. All minerals concentrations were not similar to each other due to changes of the lactation days and breed differences. Average Ca, P, Na and Mg concentrations caused by breed differences and lactation days ranged from 1.95, 1.08, 0.53 to 0.22 $g.kg^{-1}$, respectively. Generally Ca and Na concentrations of feed were much lower and P and Mg concentration were higher than that of milk. The ratios of the average Ca, P, Na and Mg concentrations of milk caused by lactation days and breed difference to those of all feeds were 3:1, 1:4, 4:1 and 1:10, respectively.

These ratios, except Mg, were kept during all experienced lactation days, without exception of the 2nd day.

REFERENCES

Barello, C., Garoffo, L. P., Montorfano, G., Zava, S., Berra, B., Conti, A., Giuffrida, M. G. 2008. Analysis of major proteins and fat fractions associated with mare's milk fat globules. *Mol. Nutr. Food Res.,* vol. 52, no. 12, p. 1448-1456. https://doi.org/10.1002/mnfr.200700311 PMid:18727006

Berg van den, B.M. 2009. Feeding the lactating mare. *Horses People Magaz.*, p. 12-14.

Businco, L., Giampietro, P. G., Lucenti, P., Lucaroni, F., Pini, C., Di Felice, G., Iacovacci, P., Guradi, M. C., Orlandi, M. J. 2000, Allergenicity of mare's milk in children with cow's milk allergy. *Allergy Clin. Immunol.,* vol. 105, no. 5, p. 1031-1034. https://doi.org/10.1067/mai.2000.106377

Caroprese, M., Albenzlo, M., Marino, R., Musclo, A., Zezza, T., Sevi, A. 2007. Behavior, milk yield, and milk composition of machine- and hand-milked. Murgese mares. *J. Dairy Sci.,* vol. 90, no. 6, p. 2773-2777. https://doi.org/10.3168/jds.2006-603 PMid:17517717

Cisla, A., Palacz, R., Janiszewska, J., Skorka, D. 2009. Total protein, selected protein fractions and chemical elements in the colostrum and milk of mares. Archiv Tierzucht, 1, 1-6. ISSN: 000-9438.

Csapó, J., Salamon, S., Loki, K., Csapó-Kiss, Z. 2009. Composition of mare's milk colostrum and milk II. Protein content, amino acid composition and contents of macro- and micro-elements. *Acta Univ. Sapientlie, Alim.,* vol. 2, no. 1, 133-148.

Csapó-Kiss, Z., Stefler, J., Martin, T. G., Makray, S., Csapó, J. 1995.Composition of mare's colostrum and milk. Fat content, fatty acid composition and vitamin content. *Inter. Dairy J.,* vol. 5, no. 4, p. 403-415. https://doi.org/10.1016/0958-6946(94)00014-G

Doreau, M., Martin-Rosset, W. 2002. Horse. In *Encyclopedia of Dairy Sci*., New York, USA : Academic Press, p. 630-637. ISBN: 978-0-12-374402-9 https://doi.org/10.1016/B0-12-227235-8/00115-2

Guradi, M. C., Giampietro, P. G., Lucenti, P., Orlandi, M. 2001. Use of mare milk in pediatric allergology. *Proc. Assoc. Sci. Prod. Anim.. XIV Cong.,* Firenze, June 12-15, vol. 14, p. 647-649.

Gálik, B., Šimko, M., Juráček, M., Bíro, D., Buňka, F., Misař, D., Kráčmar, S. 2012. Content changes of selected mineral nutrients in mare´s colostrums in the first 72 hours after foaling. *Acta univ. agric. et silvic. Mendel. Brun.,* vol. 60, no. 1, p. 45-48. https://doi.org/10.11118/actaun201260010045

Hidiroglou, M., Proulx, J. G. 1982. Factors affecting the calcium, magnesium and phosphorus content of beef cow milk. *Canad. J. Comparat. Med.,* vol. 46, no. 2, p. 212-214. PMid:7093815

Kavazik, A. N., Kivipelto, J., Ott, E. A. 2002. Factors affecting the calcium, magnesium and phosphorus content of beef cow milk. *J. Equine Vet. Sci.,* vol. 22, p. 460-464.

Malacarne, M., Martuzzi, F., Summer, A., Mariani, P. 2002. Protein and fat composition of mare's milk: some nutritional remarks with reference to human and cow's milk. *Inter. Dairy J.,* vol. 12, no. 11, p. 869-877. https://doi.org/10.1016/S0958-6946(02)00120-6

Martin, R. G., Mcmeniman, N. P., Dowsett, K. F. 1992. Milk and water intakes of foals sucking grazing mares. *Equine Vet. J.,* vol. 24, no. 4, p. 295-299. https://doi.org/10.1111/j.2042-3306.1992.tb02839.x PMid:1499538

Martuzzi, F., Catalano, A. L., Summer, A., Mariani, P. 1997. Calcium, phosphorus and magnesium in the milk of nursing mares from Italian Saddle Horse breed and their variations during lactation. *Ann. Fac. Med. Vet., Univ. Parma,* vol. 27, p. 205-212.

Martuzzi, F., Doreau, M. 2006. Mare milk composition: Recent findings about protein fraction and mineral content. In *Nutr. Feed. Broodmare* (eds. N. Miraglia and W. Martin-Rosset EAAP Publication No. 120, Wageningen, The Netherlands : Wageningen Academic Publishers, p. 65-76.

Montanari, G., Zambonelli, C., Grazia, L., Kamesheva, G. K., Shigaeva, M. K. 1996. *Saccharomyces unisporus* as the principal alcoholic fermentation microorganism of traditional koumiss. *J. Dairy Res.,* vol. 63, no. 2, p. 327-331. https://doi.org/10.1017/S0022029900031836

Nascimento, I. R., Jesus, R. M., Santos, W. N. L., Souza, W. D. F., Reis, P.S. 2010. Determination of the minerals composition of fresh bovine milk from milk-producing areas located in the state of Sergipe in Brazil and evaluation employing exploratory analysis. *Microchem. J.,* vol. 96, no. 1, p. 37-41. https://doi.org/10.1016/j.microc.2010.01.010

Nat. Res. Council. 1989. Nutrient Requirements of Horses, 5th Ed. 294-304. National Academy Press, Washington DC.

Official Methods of Analysis AOAC. 1990. 15th Ed,, Washington DC.

Pikul J., Wojtowski J. 2008. Fat and cholesterol content and fatty acid composition of mares' colostrums and milk during five lactation months. *Livestock Sci.,* vol. 113, no. 2-3, p. 128-290. https://doi.org/10.1016/j.livsci.2007.06.005

Raffaela, D. C., Antonia, T. G. G., Cosimo, L. M. D. A., Michele, F., Paolo, A., Marco, G. 2004. Uses of mares' milk in manufacture of fermented milks. Inter. *Dairy J.,* vol. 14, no. 9, p.767-775. https://doi.org/10.1016/j.idairyj.2004.02.005

Saastamoinen, M. T., Koskinen, E. 1993. Influence of quality of dietary protein supplement and anabolic steroids on muscular and skeletal growth of foals. *Anim. Prod.,* vol. 56, no. 1, p. 135-144. https://doi.org/10.1017/S0003356100006255

Sarwar, G., Botting, H. C., Davis, T. A., Darling, P., Pencharz, P. B. 1998. Free amino acids in milks of human objects, other primates and non-primates. *Brit. J. Nutr.,* vol. 79, no. 2, p. 129-131. https://doi.org/10.1079/BJN19980023 PMid:9536856

Schryver, H. F., Oftedal, O. T., Williams, J., Soderholm, L. V., Hintz, H. F. 1986. Lactation in the horse: the mineral composition of mare milk. *J. Nutr.,* vol. 116, no. 11, p. 2142-2147. https://doi.org/10.1093/jn/116.11.2142 PMid:3794822

Solaroli, G., Pagliarini, E., Peri, C. 1993. Composition and nutritional quality of mare's milk. *Ital. J. Food Sci.,* vol. 5, no. 1, p. 3-10.

Summer, A., Sabbioni, A., Formaggioni, P., Mariani, P. 2004. Trend in ash and mineral element content of milk from Haflinger nursing mares throughout six lactation months. Livestock *Prod, Sci.,* vol. 88, no. 1-2, p. 55-62. https://doi.org/10.1016/j.livprodsci.2003.11.002

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A THAUMATIN-LIKE GENOMIC SEQUENCE IDENTIFICATION IN *Vitis vinifera* L., STORMY WINES AND MUSTS BASED ON DIRECT PCR

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ABSTRACT

Direct polymerase chain reaction method was use to amplify a thaumatin-like sequence of *Vitis vinifera* L. in grapes as well as in stormy wines and musts. Thaumatin-like proteins (TLPs) of *Vitis vinifera* possess beside its function in abiotic and biotic stress response another one – they are able to cause protein haze in wine unless removed prior to bottling. Direct PCR is an approach where omission of DNA extraction is typical prior the amplification of the target site of plant genome. Crude extract or small pieces of plant tissues are used in the analysis directly without steps of extraction and purification of gDNA. The biological material that was used in analysis was collected during August – October 2017 in local stores and winery Sabo and comprises from cultivars Iršai, Muškát, Savignon Blanc, Svätovavrinecké, Dornfelder and Pálava. Direct PCR was performed by a cutted piece of grape tissue and a dilution buffer was use in 1:2 for stormy wine or must, respectively. Direct amplification of thaumatin-like protein sequence of *Vitis vinifera* was performed along with the control reactions with the primers for conserved region of plant chloroplast. Possitive amplification of thaumatin-like allergen sequence resulted in 570 bp amplicon. The most abundant amplicons were amplified in stormy wines, followed by musts and the amplicons from grapes were weaker when comparing them to others. The amplicon specificity checking of obtained PCR product of thaumatin-like allergen was performed by restriction cleavage by Psi I and resulted in restriction amplicons of the 80 bp, 81 bp, 94 bp and 315 bp in length. Confirmation of the amplicon specificity by restriction cleavage support the potential of direct PCR to become a reproducible method that will be fully applicable in routine analysis of not only plant genomes in the future, but it was demonstrated, that it works in liquids, too.

Keywords: direct PCR; *Vitis vinifera* L.; thaumatin-like sequence; stormy wine; must

INTRODUCTION

Thaumatin protein was firstly described in fruits of *Thaumatococcus danielii* Benth. **(Van der Wel and Loeve, 1972)**. Thaumatin comprises from a typical osmotin-like protein domain and thaumatin-like protein family **(Wang et al., 2011)**.

Thaumatin-like proteins are reported as a very diversified in their function **(Liu et al., 2010)** from the stress related responses to drought, cold or salt **(D´Angeli and Altamura, 2007; Husaini and Abdin, 2008; Parkhi et al., 2009)** or the resistance to different pathogens **(Garcia-Casado et al., 2000; Chu and Ng, 2003; Ho et al., 2007)**. Some authors have demonstated the engagement of thaumatin-like proteins of *Vitis vinifera* L. in the pathogen resistance response to *E. ampelina* and *E. necator* **(Jayasankar et al., 2000; Yan et al. 2017)**.

Thaumatin-like proteins (TLPs) of *Vitis vinifera* L. possess beside its function in plant-pathogen interactions another one – they are able to cause protein haze in wine unless removed prior to bottling **(Marangon et al., 2014)**. This is the most important instability that is not caused by microorganisms especially for white wine production, as the pathogen-related proteins has the potential to aggregate to form a visible haze **(Ferreira et al., 2001; Waters et al., 2005)**.

Here, a direct polymerase chain reaction method was use to amplify a thaumatin-like sequence of *Vitis vinifera* L. in grapes as well as in different liquid products. Direct PCR is an approach where omitting of DNA extraction is typical prior the amplification of the target site of plant genome. Crude extract or small pieces of plant tissues are used in the analysis directly without steps of extraction and purification of gDNA **(Chum et al., 2012)**. If none DNA extraction step is used in the PCR workflow, the benefits of utilization of samples without loss, saving time and reducing the cost of analysis are typical.

Direct PCR can facilitate routine genotyping widely in the future, but to date, its application is not typically used in plant genomes analysis. This is caused by a specific composition of plant cells where much more PCR contaminats exist **(Bošeľová et al., 2016; Bošeľová, Žiarovská, 2016; Žiarovská et al., 2016, 2017).** Direct

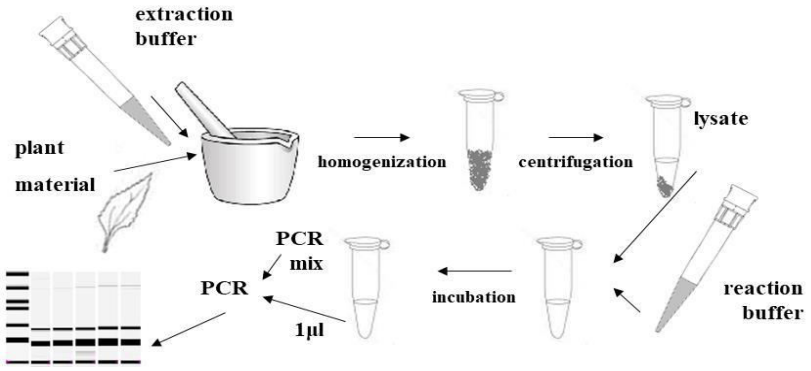

Figure 1 Workflow of direct PCR **(Žiarovská et al., 2016).**

PCR was used in plants firstly to amplify the *Nit1* gene of *Arabidopsis thaliana*, L. **(Young et al., 2007)**. The combination of commercial extraction buffer and in laboratory prepared specific PCR buffer was used to amplify the target (Figure 1).

The aim of the study was to analyse the possibility of amplification of thaumatin-like sequence of *Vitis vinifera* L. in grape and liquid products directly without DNA extraction and to optimize this for routine analysis.

Scientific hypothesis

The concentration of PCR inhibitors in stormy wines and musts can be overcome by direct PCR and a routine amplification protocol can be set-up for the purpose of thaumatin-like allergen DNA screening.

Statisic analysis

Testing of the hypothese based on the reported chemical content **(Aubert and Chalot, 2018)** of *Vitis vinifera* L. is primary the qualitative analysis of obtained (or not) amplicons by resolution through an agarose gel. Statistical evaluation of the results was applied for data of obtained amount of amplicons where the ezANOVA software for Windows (http://www.cabiatl.com/mricro/ezanova/) was used. The analyses of the amount of amplified PCR products were performed by the interpolation of density of pixels to the marker of knowing amounts using the software SynGeneGeneTools 4.01.04. Measurements of triplicate samples were subjected to the multifactorial analysis of variance and pairwise comparisons with Tukey HSD with the level of significance associated to the statistical test 0.05. The null hypothesis was tested that the difference exists among the amounts of obtained PCR amplicons depending on the type of used biological material.

MATERIAL AND METHODOLOGY

Biological material

The biological material that was used in analysis was collected during August – October 2017 in local stores and winery Sabo (Vrbové, Slovak Republic) (Table 1).

Primer design

BLAST alignment **(Zhang et al., 2000)** of the thaumatin-like protein, (NCBI accession code AF227324.1) was done by BLASTtn against *Vitis vinifera* (taxid: 29760) nucleotide sequences in the NCBI database to check the specifity or existing nucleotide differences. Primer design was performed in Primer-BLAST **(Ye et al., 2012)**.

Table 1 Codes of samples used in the study.

Code of sample	*Vitis vinifera* variety	Type of sample
I	Iršai	grape
M	Muškát	grape
SB	Savignon Blanc	stormy wine
SV	Svätovavrinecké	stormy wine
D	Dornfelder	must
P	Pálava	must

Direct PCR

Direct PCR was performed by Phire® Plant Direct PCR Kit (Thermo Scientific). A 0.35 mm cutter was used to obtain a piece of grape tissue and a dillution buffer was use in 1:2 with stormy wine or must, respectively. All the cutted pieces as well as dillutions were prepared in technical triplicates.

The following thermal and time profile was used: 98 °C for 5 min; 39 cycles of : 98 °C for 10 sec; 55 °C for 10 sec; 72 °C for 30 sec with final 72 °C for 5 minutes. All the amplification reactions were performed in C1000 thermocycler (BioRad).

Product specificity verification

Nucleotide sequence of *Vitis vinifera* thaumatin-like protein was uploaded into the NEBcutter v 2.0 **(Vincze et al., 2003)** and the specific restriction enzyme was selected to verify the PCR amplicons. The amplified direct PCR product was inspected for the specificity using the Psi I restriction endonuclease that cleaves in total four restriction sites.

RESULTS AND DISCUSSION

Thaumatin-like allergen of *Vitis vinifera* L. is defined well on both, protein as well as genomic sequence level. To date, a total of twenty-eight sequence accessions are available in the NCBI nucleotide database, from which ten are predicted (Table 2).

Table 2 Names and accessions of known thaumatin-likesequences.

Thaumatin-likesequences in NCBI
Vitis vinifera mRNA for putative thaumatin-likeprotein, partial 612 bp linear mRNA; Accession code: AJ237998.1
Vitis vinifera mRNA for thaumatin-like protein 918 bp linear mRNA; Accession code: AJ237999.1
Vitis vinifera thaumatin-like protein (TL3), mRNA 678 bp linear mRNA; Accession code: NM_001281202.1
Vitis vinifera thaumatin-like protein VVTL1 mRNA, complete cds 954 bplinear mRNA; Accession code: AF003007.1
Vitis vinifera cultivar Regent thaumatin-like protein mRNA, complete cds 678 bplinear mRNA; Accession code: DQ406688.1
Vitis vinifera thaumatin-like protein (Tl3) mRNA, complete cds 915 bp linear mRNA; Accession code: AF532965.1
Vitis vinifera cultivar Riesling thaumatin-like protein mRNA, complete cds 678 bp linear mRNA; Accession code: DQ406687.1
Vitis vinifera thaumatin-like protein gene, complete cds 2,777 bp linear DNA; Accession code: AF227324.1
Predicted Thaumatin-like sequences in NCBI
Vitis vinifera thaumatin-like protein 1 (LOC100265945), mRNA 862 bplinear mRNA; Accession code: XM_002265816.3
Vitis vinifera thaumatin-like protein (LOC100261232), mRNA 981 bp linear mRNA; Accession code: XM_003634158.3
Vitis vinifera thaumatin-likeprotein 1 (LOC100246972), mRNA 1,939 bp linear mRNA; Accession code: XM_010664644.2
Vitis vinifera thaumatin-like protein (LOC100242321), mRNA 2,096 bp linear mRNA; Accession code: XM_010662681.2
Vitis vinifera thaumatin-like protein (LOC100253199), mRNA 1,534 bp linear mRNA; Accessioncode: XM_002273235.4
Vitis vinifera thaumatin-like protein (LOC100265026), mRNA 945 bplinear mRNA; Accession code: XM_002282957.3
Vitis vinifera thaumatin-like protein (LOC100259841), mRNA 903 bp linear mRNA; Accession code: XM_002282994.3
Vitis vinifera thaumatin-like protein (LOC100254732), mRNA 772 bp linear mRNA; Accessioncode: XM_002283006.4
Vitis vinifera thaumatin-like protein (LOC100242737), mRNA 987 bp linear mRNA; Accession code: XM_002282952.4
Vitis vinifera thaumatin-like protein (LOC100244461), mRNA 905 bp linear mRNA; Accession code: XM_010662912.2
Vitis vinifera thaumatin-like protein 1b (LOC100248638), mRNA 890 bp linear mRNA; Accession code: XM_002265565.3
Vitis vinifera thaumatin-like protein (LOC100854994), mRNA 1,176 bp linear mRNA; Accessioncode: XM_003632623.3
Vitis vinifera thaumatin-like protein 1b (LOC100247111), mRNA 1,207 bp linear mRNA; Accessioncode: XM_002265769.3
Vitis vinifera thaumatin-like protein 1b (LOC100264253), mRNA 4,490 bp linear mRNA; Accession code: XM_002265889.4
Vitis vinifera thaumatin-like protein 1b (LOC100265907), mRNA 1,840 bp linear mRNA; Accession code: XM_002277512.4
Vitis vinifera thaumatin-like protein 1b (LOC100232855), mRNA 1,317 bp linear mRNA; Accession code: XM_002277426.3
Vitis vinifera thaumatin-like protein 1b (LOC100257373), mRNA 1,677 bp linear mRNA; Accession code: XM_002274101.4
Vitis vinifera thaumatin-like protein 1b (LOC100242003), mRNA 1,537 bp linear mRNA; Accession code: XM_010666529.2
Vitis vinifera thaumatin-like protein (LOC109124045), mRNA 351 bp linear mRNA; Accession code: XM_019225556.1
Vitis vinifera thaumatin-like protein (LOC100247919), mRNA 1,143 bp linear mRNA; Accession code: XM_002278043.3

Note: Thaumatin-like DNA sequence is written in bold in the table.

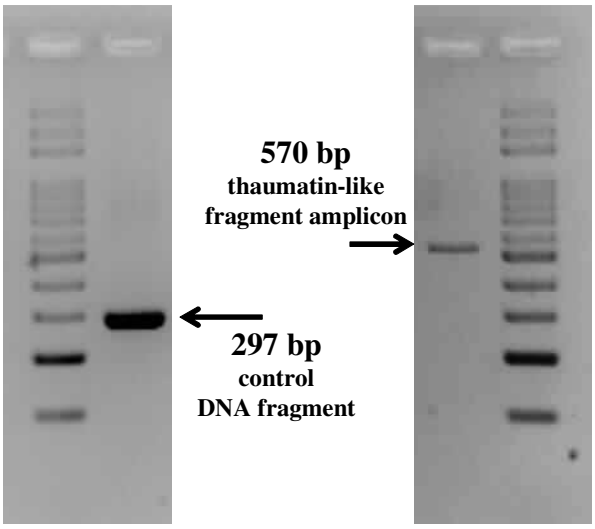

Figure 2 Control and amplified thaumatin-like amplicons lenght checking in agarose gel electrophoresis.

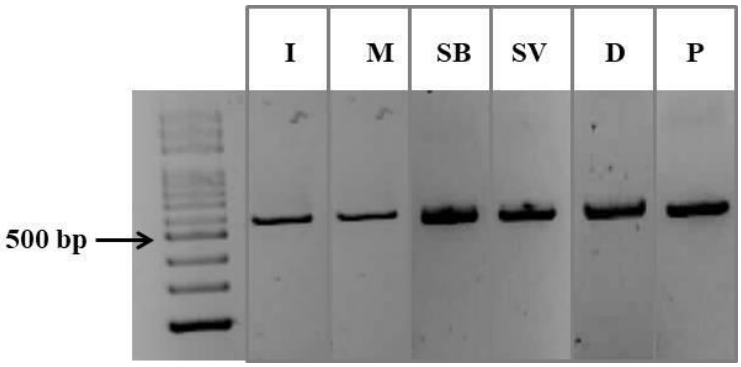

Figure 3 Amplicons of thaumatin-like allergen sequences resulted in direct PCR.

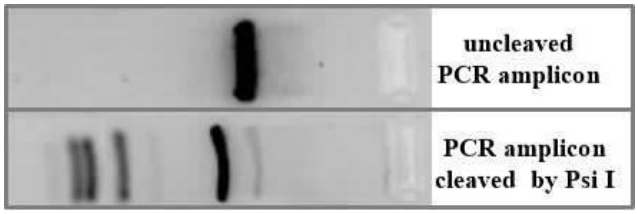

Figure 4 Restriction cleavage of amplified sequence of thaumatin-like allergen of *Vitis vinifera*, L.

Direct amplification of thaumatin-like protein sequence of *Vitis vinifera* was performed along with the control reactions with the primers for conserved region of plant chloroplast. In all the samples, control reaction was positive with the amplicon of 297 bp (Figure 2). This shows the suitability of Phire® Plant Direct PCR Kit for working with liquid products such as stromy wines and musts. Positive amplification of thaumatin-like allergen sequence resulted in amplicon of the length of 570bp (Figure 2).

Subsequent direct PCR was performed when using all the three types of samples – grapes, stormy wines and musts. In all the cases, an amplicon of appropriate length was obtained (Figure 3). The most abundant amplicons were amplified in stormy wines, followed by musts and the amplicons from grapes were the weaker when comparing them to others. The amplicon abundancy was statistical relevant among the types of the biological material, not between different varieties for individual types of biological material except of the must samples (Table 2).

Actually, amplifications of DNA regions of different, not only plant species, is well established method. Molecular and DNA analysis became a standard part of the research in many areas and these analysis are performed in a wide range of different approaches ranged from DNA markers based analysis up to the specific analysis of plant allergens detection or their expression (**Medo et al., 2015; Žiarovská et al., 2015; Oslovičová et al., 2014; Revák et al., 2014; Trebichalský et al., 2013; Židek et al., 2012; Milella et al., 2011**).

Table 2 ANOVA analysis of theyield of ampliconsobtained by direct PCR.

Descriptive details/	Types of samples and varieties					
	grape Iršai	grape Muškát	stormy wine Savignon Blanc	stormy wine Svätovavrinecké	must Dornfelder	must Pálava
Mean	101.75	90.57	179.82	151.26	190.34	191.55
StDev	1.59	1.9	3.73	1.48	1.86	1.71
SE	0.92	1.1	2.16	0.85	1.07	0.99
Var	2.53	3.62	13.95	2.18	3.45	2.92
CI 95%	2.75	2.75	2.75	2.75	2.75	2.75
N	3	3	3	3	3	3
Skew	1.393	-0.314	-1.466	-0.443	0.266	1.708
zSkew	0.985	-0.222	-1.037	-0.313	0.188	1.208

PAIRWISE COMPARISONS

[grape_Iršai]vs[grape_2] t(4) = 7.83 p <0.0014
[grape_1] vs [stormy wine_1] t(4) = 33.30 p <0.0001
[grape_1] vs [stormy wine_2] t(4) =39.49 p <0.0001
[grape_1] vs [must_1] t(4) = 62.71 p <0.0001
[grape_1] vs [must_2] t(4) = 66.58 p <0.0001
[grape_2] vs [stormy wine_1] t(4) = 36.88 p <0.0001
[grape_2] vs [stormy wine_2] t(4) = 43.65 p <0.0001
[grape_2] vs [must_1] t(4) = 64.98 p <0.0001
[grape_2] vs [must_2] t(4) = 68.38 p <0.0001
[stormy wine_1] vs [stormy wine_2] t(4) = 12.32 p <0.0002
[stormy wine_1] vs [must_1] t(4) = 4.37 p <0.0120
[stormy wine_1] vs [must_2] t(4) = 4.95 p <0.0078
[stormy wine_2] vs [must_1] t(4) = 28.53 p <0.0001
[stormy wine_2] vs [must_2] t(4) = 30.91 p <0.0001
[must_1] vs [must_2] t(4) = 0.83 p <0.4530

Note: Codes of *Vitisvinifera* L. varieties in pairwisecoparison are as follows: grape 1 – Iršai, grape 2 – Muškát, stromy wine 1 – Savignon Blanc, stromy wine 2 – Svätovavrinecké, must 1 – Dornfelder, must 2 – Pálava.

Direct PCR method was applied here to amplify a thaumatin-like allergen sequence of *Vitis vinifera* L. This method was succesfully used by **Belstedt et al. (2010)** previously to amplify fragments of plant genomes of thirty-two different plant families. The authors have used the extraction and PCR buffer to one amplicon analysis to be fully function in the species, that possess a wide range of PCR contaminants – *Coffea arabica* L.; *Thymus vulgaris* L. *Olea europea*, L. or *Lauru snobilis* L. PCR amplification in their study was carried out using standard universal primers of the chloroplast-encoded trn L-F locus with a positive amplification in all of the tested plant species except of two – *Cyatheade albata* (G. Forst.) Swartz and *Carpobrotus* sp. **Bošeľová and Žiarovská (2016)** reported direct PCR approach applicable in the marker based analysis of plants, too. They have used the direct PCR protocol to analyse the PBA polymorphism of *Hedera helix*, L.

The amplicon specificity checking of obtained PCR product of thaumatin-like allergen was performed by restriction cleavage by Psi I (Figure 4). In total, four restriction sites are predicted in the amplicon by NebCutterv 2.0 **(Vincze et al., 2003)** that resulted in restriction amplicons of the 80 bp, 81 bp, 94 bp and 315 bp in length.

The wine production is one of the most profitable agricultural activities and a wide diversity of *Vitis vinifera* L. cultivars are involved in the production of wine **(Briciu et al., 2010)**. This need to verify different DNA based techniques to characterize and authentify grapevine genepool. DNA of *Vitis vinifera* was reported previously as to be extracted from different parts of the plant and many studies were aimed to the methods of extracting DNA from grapevine products such as grape juice, musts or wines of different stages of the processing **(Faria et al. 2000; Siret et al. 2002; Baleiras-Couto and Eiras-Dias 2006; Faria et al. 2008; Drábek et al. 2008; Işçi et al., 2014)**, but in this studies it is concluded, that an efficient DNA extraction and subsequent PCR analysis from must and wines is not fully reproducible an still difficult. **Garcia-Beneytez et al. (2002)** suppose these difficulties because of specific processes of wine production. These together resulted completely removing of grapevine DNA. On the other hand, extraction of DNA from must is difficult thank to the presence of high levels of polyphenols and polysaccharides **(Briciu et al., 2010)**.

Confirmation of the amplicon specificity by restriction cleavage support the potential of direct PCR to become a reproducible method that will be fully applicable in routine analysis of not only plant genomes in the future, but it was demonstrated, that it works in liquids, too. The development a proving the efficient and reliable methods for tracing *Vitis vinifera* cultivars and its products is an

important aim as demand grows for origin product and sources of knowledge.

CONCLUSION

Direct PCR can be used well in *Vitis vinifera* L. genomic sequences amplification. Direct PCR was proved here to work on all the three types of tested samples – grapes, stormy wines and musts. In all the cases of qualitative analyse of the results an amplicon of thaumatin-like genomic sequence was obtained with the appropriate length of 570 bp. The most abundant amplicons were amplified in stormy wines, followed by musts and the amplicons from grapes were the weaker when comparing them to others. When regarding the quantitative analyse of results, grape and stormy wine amplicons abundance obtained by direct PCR is statistical relevant for different varieties with the p <0.05 and for must the p value was 0.453. Direct PCR provides a reliable method for rapid screening of allergen genomic sequences and can be utilized in liquid products, too.

REFERENCES

Aubert, Ch., Chalot, G. 2018. Chemical composition, bioactive compounds, and volatiles of six table grape varieties (*Vitis vinifera* L.). *Food Chemistry*, vol. 240, p. 524-533. https://doi.org/10.1016/j.foodchem.2017.07.152 PMid:28946307

Baleiras-Couto, M. M., Eiras-Dias, J. E. 2006. Detection and identification of grape varieties in must and wine using nuclear and chloroplast microsatellite markers. *Analytica Chimica Acta*, vol. 563, no. 1-2, p. 283-291. https://doi.org/10.1016/j.aca.2005.09.076

Bellstedt, D. U., Pirie, M. D., Visser, J. Ch., de Villiers, M. J., Gehrke, B. 2010. A Rapid and Inexpensive Method for the Direct PCR Amplification of DNA from Plants. *American Journal of Botany*, vol. 97, no. 7, p. 65-68. https://doi.org/10.3732/ajb.1000181

Bošeľová, D., Žiarovská, J. 2016. Direct PCR as the platform of *Hedera helix*, L. genotypying without the extraction of DNA. *Journal of Central European Agriculture*. vol. 17, no. 4, p. 941-949. https://jcea.agr.hr/en/issues/article/1795

Bošeľová, D., Žiarovská, J., Hlavačková, L., Ražná, K., Bežo, M. 2016. Comparative analysis of different methods of *Hedera helix* DNA extraction and molecular evidence of the functionality in PCR. *Acta fytotechnica et zootechnica*, vol. 19, no. 4, p. 144-149.

Briciu, D., Pamfil, D., Briciu, A., Curticiu, D., Balazs, E., Taoutaou, A., Pop, I., and Coia, L. 2010. Development of methods for DNA extraction from leaves and must grapes. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies.* vol. 67, no. 1-2, p. 1843-1849.

Drábek, J., Stávek, J., Jalůvková, M., Jurček, T., Frébort, I. 2008. Quantification of DNA during winemaking by fluorimetry and *Vitis vinifera* L. specific quantitative PCR. *European Food Research and Technology*, vol. 226, no. 3, p. 491-497. https://doi.org/10.1007/s00217-007-0561-8

D'Angeli, S., Altamura, M. M. 2007. Osmotin induces cold protection in olive trees by affecting programmed cell death and cytoskeleton organization. *Planta*, vol. 225, no. 5, p. 1147-1163. https://doi.org/10.1007/s00425-006-0426-6 PMid:17086398

Faria, M. A., Magalhães, R., Ferreira, M. A., Meredith, C. P., Monteiro, F. F. 2000. *Vitis vinifera* must varietal authentication using microsatellite DNA analysis (SSR). *Journal Agricultural and Food Chemistry*, vol. 48, no. 4, p. 1096-1100. https://doi.org/10.1021/jf990837h PMid:10775355

Faria, M. A., Nunes, E., Oliveira, M. 2008. Relative quantification of *Vitis vinifera* L. varieties in musts by microsatellite DNA analysis. *European Food Research and Technology* , vol. 227, p. 845-850. https://doi.org/10.1007/s00217-007-0795-5

Ferreira, R. B., Piçarra-Pereira, M. A., Monteiro, S., Loureiro, V. B., Teixeira, A. R. 2001. The wine proteins. *Trends in Food Science and Technology*, vol. 12, no. 7, p. 230-239. https://doi.org/10.1016/S0924-2244(01)00080-2

Garcia-Beneytez, E., Moreno-Arribas, M. V., Borrego, J., Polo, M. C., Ibanez, J. 2002. Application of a DNA analysis method for the cultivar identification of grape musts and experimental and commercial wines of *Vitis vinifera* L. using microsatellite markers. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 21, p. 6090-6096. https://doi.org/10.1021/jf0202077 PMid:12358485

Garcia-Casado, G., Collada, C., Allona, I., Soto, A., Casado, R., Rodriguez-Cerezo, E., Gomez, L., Aragoncillo, C. 2000. Characterization of an apoplastic basic thaumatin-like protein from recalcitrant chestnut seeds. *Physiologia Plantarum*, vol. 110, no. 2, p. 172-180. https://doi.org/10.1034/j.1399-3054.2000.110205.x

Ho., V. S., Wong, J. H., Ng, T. B. 2007. A thaumatin-like antifungal protein from the emperor banana. *Peptides*, vol. 28, no. 4, p. 760-766. https://doi.org/10.1016/j.peptides.2007.01.005

Husaini, A. M., Abdin, M. Z. 2008. Development of transgenic strawberry (*Fragaria* x *ananassa* Duch.) plants tolerant to salt stress. *Plant Science*, vol. 174, no. 4, p. 446-455. https://doi.org/10.1016/j.plantsci.2008.01.007

Chu, K. T., Ng, T. B. 2003. Isolation of a large thaumatin-like antifungal protein from seeds of the Kweilin chestnut *Castanopsis chinensis*. *Biochemical and Biophysical. Research Communication*, vol. 301, no. 2, p. 364-370. https://doi.org/10.1016/S0006-291X(02)02998-4

Chum, P. Y., Haimes, J. D., André, Ch. P., Kuusisto P. K., Kelley, M. L. 2012. Genotyping of plant and animal samples without prior DNA purification. *Journal of Visualized Experiments*, vol. 67, p. e3844. https://doi.org/10.3791/3844

Işçi, B., Yildirim, H. K., Altindisli, A. 2014. Evaluation of methods for DNA extraction from must and wine. *Journal of Institute Brewing*, vol. 120, no. 3, p. 238-243. https://doi.org/10.1002/jib.129

Jayasankar, S., Li, Z., Gray, D. J. 2000. *In-vitro* selection of *Vitis vinifera* `Chardonnay' with *Elsinoe ampelina* culture filtrate is accompaniedby fungal resistance and enhanced secretion of chitinase. *Planta*, vol. 211, no. 2, p. 200-208. https://doi.org/10.1007/s004250000285 PMid:10945214

Liu, J., Sturrock, R., Ekramoddoullah, A. K. 2010. The superfamily of thaumatin-like proteins: its origin, evolution, and expression towards biological function. *Plant Cell Reporter*, vol. 29, no. 5, p. 419-436. https://doi.org/10.1007/s00299-010-0826-8

Marangon, M., Van Sluyter, S. C., Waters, E. J., Menz, R. I. 2014. Structure of Haze Forming Proteins in White Wines: *Vitis vinifera* thaumatin-Like Proteins. *PLOS ONE*, vol. 9, no. 12, e113757 https://doi.org/10.1371/journal.pone.0113757 PMid:25463627

Medo, J., Maková, J., Kovácsová, S., Majerčíková, K., Javoreková, S. 2015. Effect of Dursban 480 EC (chlorpyrifos)

and Talstar 10 EC (bifenthrin) on the physiological and genetic diversity of microorganisms in soil. *Journal of environmental science and health,* vol. 50, no. 12, p. 871-883. https://doi.org/10.1080/03601234.2015.1062659

Milella, L., Martelli, G., Salava, J., Fernández, C. E., Ovesná, J., Greco, I. 2011. Total phenolic content, RAPDs, AFLPs and morphological traits for the analysis of variability in *Smallanthus sonchifolius*. *Genetic Resources and Crop Evolution*, vol. 58, no. 4, p. 545-551. https://doi.org/10.1007/s10722-010-9597-x

Oslovičová, V., Simmonds, J. R., Snape, J. W., Gálová, Z., Balážová, Ž., Matušíková, I. 2014. Molecular marker-based characterization of a set of wheat genotypes adapted to Central Europe. *Cereal research communications,* vol. 42, no. 2, p. 189-198. https://doi.org/10.1556/CRC.42.2014.2.2

Parkhi, V., Kumar, V., Sunilkumar, G., Campbell, L. M., Singh, N. K., Rathore, K. S. 2009. Expression of apoplastically secreted tobacco *osmotin*in cotton confers drought tolerance. *Molecular Breeding*, vol. 23, no. 4, p. 625-639. https://doi.org/10.1007/s11032-009-9261-3

Revák, O., Golian, J., Židek, R., Čapla, J., Zajác, P. 2014. Detection of lupine (*Lupinus* spp. L.) as a food allergen using three methods: end-point PCR, real-time PCR and ELISA. *Potravinarstvo,* vol. 8, no. 1, p. 207-215. https://doi.org/10.5219/384

Siret, R., Gigaud, O., Rosec, J. P., This, P. 2002. Analysis of grape *Vitisvinifera* L. DNA in must mixtures and experimental mixed wines using microsatellite markers. *Journal Agricultural and Food Chemistry*, vol. 50, p. 3822-3827. https://doi.org/10.1021/jf011462e

Trebichalský, A., Kalendar, R., Schulman, A., Stratula, O., Gálová, Z., Balážová, Ž., Chňapek, M. 2013. Detection of genetic relationships among spring and winter triticale (× *Triticosecale* Witt.) and rye cultivars (*Secale cereale* L.) by using retrotransposon-based markers. *Czech journal of genetics and plant breeding*, vol. 49, no. 4, p. 171-174. https://doi.org/10.17221/56/2013-CJGPB

Vincze, T., Posfai, J., Roberts, R. J. 2003. NEBcutter: a program to cleave DNA with restriction enzymes. *Nucleic Acids Research*, vol. 31, no. 13, p. 3688-3691. https://doi.org/10.1093/nar/gkg526 PMid:12824395

Wang, Q., Li, F., Zhang, X., Zhang, Y., Hou, Y., Zhang, S., Wu, Z. 2011. Purification and characterization of a *CkTLP*protein from *Cynanchum komarovii* seeds that confers antifungal activity. *PloS one.* vol. 6, no. 2, e16930. https://doi.org/10.1371/journal.pone.0016930

Waters, E. J., Alexander, G., Muhlack, R., Pocock, K. F., Colby, C., et al. 2005. Preventing protein haze inbottled white wine. *Austrian Journal Grape Wine Research*, vol. 11, no. 2, p. 215-225. https://doi.org/10.1111/j.1755-0238.2005.tb00289.x

Van der Wel, H., Loeve, K. 1972. Isolation and characterization of thaumatin I and II, the sweet-tasting proteins from *Thaumatococcus daniellii* Benth. *European Journal of Biochemistry*, vol. 31, no. 2, p. 221-225. https://doi.org/10.1111/j.1432-1033.1972.tb02522.x PMid:4647176

Yan, X., Qiao, H., Zhang, X., Guo, Ch., Wang, M., Wang, Y., Wang, X. 2017. Analysis of the grape (*Vitis vinifera* L.) thaumatin-like protein (TLP) gene family and demonstration that *TLP29* contributes to disease resistance. *Scientific Reports*, vol. 7, no. 1, p. 4269. https://doi.org/10.1038/s41598-017-04105-w

Yang, G. Y., Kim, J. Y., Moon-Soo, S., Doo-Sik, K. 2007. A Simple and Rapid Gene Amplification from Arabidopsis Leaves Using AnyDirect System. *Journal of Biochemistry and Molecular Biology*, vol. 40, no. 3, p. 444-447. https://doi.org/10.5483/BMBRep.2007.40.3.444

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., Madden, T. L. 2012. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics,* vol. 13, p.134. https://doi.org/10.1186/1471-2105-13-134 PMid:22708584

Zhang, Z., Schwartz, S., Wagner, L. and Miller, W. A. 2000. A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology*, vol. 7, no. 1-2, p. 203-214. https://doi.org/10.1089/10665270050081478 PMid:10890397

Žiarovská, J., Hricová, A., Gálová, Z., Záhorský, M., Bošeľová, D. 2016. Utilization of DNA without isolation approaches in plant genome analyzes (Využitie DNA bez-izolačných prístupov v analýzach genómov rastlín). *Chemické listy*, vol. 110, p. 931-934. (In Slovak)

Žiarovská, J., Grygorieva, O., Zeleňáková, L., Bežo, M., Brindza, J. 2015. Identification of sweet chesnut pollen in bee pollen pellet using molecular analysis. *Potravinarstvo*. vol. 9, no. 1, p. 352-358. https://doi.org/10.5219/497

Žiarovská, J., Bošeľová, D., Zeleňáková, L., Bežo, M. 2016a. Utilization of different markers for *Hedera helix*, L. germplasm evaluation. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 5, no. 1, p. 23-26. https://doi.org/10.15414/jmbfs.2016.5.special1.23-26

Žiarovská, J., Kyseľ, M., Cimermanová, R., Knoteková, Ľ. 2017. Effect of DNA extraction method in the *Rosa canina* L. identification under different processing temperature. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, p. 190-196. https://dx.doi.org/10.5219/695

Židek, R., Bajzík, P., Maršálková, L, Golian, J. 2012. Detection of the beef meat adulteration with Real-Time PCR. *Maso*, vol. 23, no. 1, p. 15-17.

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THE QUALITY OF KETCHUPS FROM THE CZECH REPUBLIC'S MARKET IN TERMS OF THEIR PHYSICO-CHEMICAL PROPERTIES

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ABSTRACT

Ketchup is a tomato-based condiment with a tang contributed by vinegar, sugar, salt and spices. Physical and chemical quality requirements for ketchup are regulated in the Czech Republic by Decree No. 157/2003 as amended. The main monitored parameters determining the quality of ketchups are total tomato content, total soluble solids, total organic acids and total salt content. In this work the following parameters were monitored in a total of eight ketchups from the commercial markets in the Czech Republic: pH, total solids, total soluble solids, citric acid content, acetic acid content, lycopene content, fructose, glucose and sucrose content and content of Ca, K, Mg and Na. In addition to chemical analyses, rheological measurements were performed and dynamic viscosity and yield stress were determined. The results obtained were statistically processed and the hypothesis i) whether the sales price of ketchups is related to the quality of ketchups expressed in chemical composition and ii) whether the chemical composition affects the rheological properties of ketchups has been verified. The Pearson correlation matrix showed very good correlation between the total solids and tomato content in the ketchup (R = 0.8464) as well as between the total soluble solids and tomato content in the ketchup (R = 0.8583). Another significant correlation was found between total soluble solids and total saccharides content in ketchup (R = 0.7309) as well as between potassium content and and tomato content in the ketchup (R = 0.8864). The chemical composition of ketchups did not significantly affect the dynamic viscosity of ketchups, however strong correlation between tomato content in ketchup and between yield stresses was found (R = 0.8436). No correlation was found between the ketchup price and chemical composition of ketchup, however cheaper ketchups contained more salt.

Keywords: tomatoes; ketchup; PCA; chemical analysis; rheology

INTRODUCTION

Vegetables are an essential part of rational human nutrition. The world's most cultivated vegetables include tomatoes that are consumed mainly fresh, but they are also used for production of tomato juice or puree, which is the main raw material for the production of ketchup **(Burton-Freeman and Reimers, 2011)**. Ketchup is one of the most common flavouring agents. In addition to essential nutrients, saccharides and fibre it contains significant amounts of vitamin C, lycopene and other nutritionally important substances **(Canene-Adams et al., 2005)**.

Ketchup means roughly two to four times thickened tomato puree. The taste of ketchup is adjusted with salt, vinegar, sweetener and spice extracts. The stabilization of the resulting product requires the stabilizers (most often modified starches in an amount of about 2 - 5%) to prevent the distribution of the solid and liquid content and simultaneously to modify the consistency of the ketchup, which is to be smooth and glossy **(Hayes et al., 1998)**. Physical and chemical quality requirements for ketchup are regulated in the Czech Republic by Decree No. 157/2003 as amended. This decree states that in ketchups containing at least 12% total soluble solids, determined by refractometry, the refractometric total solid content of tomato raw material must be at least 7%. For ketchups marked as Prima, Extra or Special with refractometric total solid content at least 30%, shall be at least 10% of refractometric solids introduced with tomato raw material. Other ketchup parameters to be followed are the maximum amount of salt (up to 3%) and maximum amount of total acid (2.2% expressed as acetic acid). Rheological properties of ketchups are not regulated by decree or law, however food rheology is important in quality control during food manufacture and processing. Rheological properties of ketchups helps producers to determine ingredient functionality in product development, to predict product performance and product acceptance by consumers or to test the shelf life of product (**Norton et al., 2011**).

Scientific hypothesis

Two hypotheses were tested in this study. First, whether higher product price means higher quality for consumers in connection with the composition of the product and the other, whether the chemical composition of ketchups affects their rheological behavior. In addition, it was verified that all analyzed ketchups complied with the applicable legislation in terms of physical and chemical quality requirements.

MATERIAL AND METHODOLOGY

Sample preparation

For determination of organic acids 2 g of sample was extracted with 20 mL of ultrapure water (Elga pure lab classic, Veolia water systems Ltd., UK), in a 50 mL centrifugation tube placed on vertical skake table (GFL, Germany). After 1h of extraction, samples were centrifuged at 6000 rpm in centrifuge (EBA 21, Hettich, Germany), supernatant was filtered using filter with 0.45 μm pore size (Labicom, Czech Republic) and filled up to 50 mL in a volumetric flask with an ultrapure water.

Sample for analysis of saccharides was prepared by the same way as described in the case of organic acid, with the difference that ethanol (VWR, Germany) and ultrapure water in 4:1 volume ratio was used for the extraction.

Sample for elemental analysis was prepared using wet ashing method in a microwave oven (Milestone 1200, Milestone, Italy). For decomposition of sample matrix a mixture of nitric acid (6 mL, Analytika, Czech Republic) and hydrochloric acid (2 mL, Analytika) was used. After the decomposition sample was filtered using filter with 0.45μm pore size and filled up to 25 mL in a volumetric flask with an ultrapure water.

For a lycopene determination 0.1 g of sample was weighed to a 20 mL centrifugation tube and 8 mL of mixture of hexane, ethanol and acetone (VWR) in 2:1:1 volume ratio was added to the sample. The sample was well mixed on a vortex and left to stay for 10 min in a dark place. After 10 min 1mL of ultrapure water was added to the sample and the sample was well mixed and stored for 10 min in dark place again. The upper layer of the sample was then collected for the analysis.

Chemical analysis

Total solids were determined according to EN method **(CSN EN 12145, 1997)**. The pH value was measured using pH meter with combinated electrodes (WTW, Germany). Total soluble solids were determined by table refractometer (Kruss AR4, Germany). Organic acids were determined using ion chromatography (Metroohm 850 professional IC, Metroohm, Switzerland) with conductivity detector. A Metrosep organic acids column (250/7.8 mm) was used as stationary phase and 15% acetone (VWR) in 0.5 mmol.l^{-1} sulphuric acid (Analytika) was used as a mobile phase. An Agilent Infinity 1260 liquid chromatograph (Agilent Technologies, USA) equipped with ELSD detector was used for determination of saccharides. A Prevail Carbohydrates ES column (250/4.6 mm) was used as a stationary phase and acetonitrile (VWR) mixed with water in 75:25 volume ratio was used as mobile phase. Lycopen content was determined according to the method described by **Suwanaruang (2016)** using Helios gamma spectrophotometer (Spectronic Unicam, Great Britain). An elemental analysis was performed using ICP-OES (Ultima 2, Horiba Scientific, France) according to procedure described by **Diviš et al. (2015)**.

Rheological analysis

The flow properties were determined on a rheometer Discovery HR-2 (TA Instruments) using 25 mm diameter plate-plate steel geometry. The measuring temperature was 25°C, conditioning step was 2 min, measuring slit was 28 μm, shear rate range was 0.1 – 1000 s^{-1}, the number of points per decade in logarithmic mode was 6, measurement time of one point was 10s and the number of measurements per point was 3. After the sample was subjected to the basic flow test, the same sample was again subjected to a flow test with the same rheometer parameters after an 8 minute conditioning step. By this way sample relaxation was detected. From the shear stress to shear rate dependence yield stress and flow index were calculated using the Herschel-Bulkley equation in the form: $\tau = \tau_0 + K \cdot \gamma^n$, where τ is the shear stress, τ_0 is the yield stress, K is the consistency coefficient and n is the flow index.

Statisic analysis

All samples were prepared in duplicates and each sample analysis was performed three times. Before the main data analysis, results were tested for outliners and data distribution. Grubbs test for outliners did not revealed any outlined values within all analyzed parameters and data showed a normal gaussian distribution. All parameters were analyzed by Pearson correlation matrix and independent variables were further classified using principal component analysis (XL Stat, version 2015.5, Addinsoft, France). Tukey's comparative test using the 0.05 significance level has been performed to find means that are significantly different from each other.

RESULTS AND DISCUSSION

Total solids and total soluble solids are an important quality factors in the tomato processing industry **(Thakur et al. 1996)**. According to valid legislation in the Czech Republic, ketchups must contain at least 12% total solids, determined by refractometry. In the case of ketchups marked as "Prima", "Extra" and "Special" the total solids determined by refractometry must be at least 30%. Total solids content of the analyzed samples ranged from 23.6 to 31.4% **(Table 1)**. The results are in agreement with those obtained by **Lehkoživová et al. (2009)**, or **Sharoba et al. (2005)**. All ketchup samples, including samples K2 and K6 marked as "Extra", were in compliance with the requirements of the applicable legislation. There was good correlation (R = 0.8583) between the total solids content and the tomato content in the ketchup as well as between the total soluble solids and the tomato content in the ketchup (R = 0.8464) (**Table 5**). However, ketchups with a smaller amount of tomatoes, which have a relatively high total solids content, in particular samples K3 and K5, do not suit the model of correlation. For these ketchups, it is to be assumed that other vegetables (such as carrots, onions) have been used to produce them, in addition to

Table 1 Basic physicochemical parameters of ketchup samples K1-K8.

Sample	pH (1±SD)	Total solids (mg.kg^{-1}±SD)	TSS* (%±SD)	Tomato content (g.100g^{-1})**
K1	3.98 ±0.05^{a}	25.0 ±0.3^{f}	27.71 ±0.05^{g}	140
K2	3.94 ±0.05^{ab}	31.4 ±0.2^{a}	35.44 ±0.05^{a}	240
K3	3.72 ±0.05^{e}	26.5 ±0.2^{e}	30.50 ±0.05^{f}	148
K4	3.85 ±0.05^{cd}	26.4 ±0.2^{e}	31.55 ±0.05^{c}	170
K5	3.74 ±0.05^{de}	27.8 ±0.2^{c}	31.33 ±0.05^{d}	151
K6	3.98 ±0.05^{a}	28.2 ±0.3^{b}	31.06 ±0.05^{e}	200
K7	3.74 ±0.05^{de}	27.4 ±0.1^{d}	32.17 ±0.05^{b}	210
K8	3.82 ±0.05^{bc}	23.6 ±0.1^{g}	27.12 ±0.05^{h}	140

Note: Values in the same column with different letters are significantly different at p <0.05. * TSS = total soluble solids, ** Declared content on packaging.

Table 2 Saccharide content in ketchup samples K1-K8.

Sample	Saccharides				
	fructose (mg.g^{-1}±SD)	glucose (mg.g^{-1}±SD)	sucrose (mg.g^{-1}±SD)	Σ Saccharides (mg.g^{-1}±SD)	Σ Saccharides* (mg.g^{-1})
K1	47.0 ±0.3^{c}	66.4 ±0.8^{a}	n.d.	113.4 ±1.1^{e}	240
K2	40.9 ±0.5^{e}	57.6 ±0.3^{c}	82.7 ±0.5^{e}	181.2 ±1.3^{c}	344
K3	43.7 ±0.4^{d}	43.2 ±0.4^{f}	115.2 ±0.7^{c}	205.7 ±1.5^{a}	232
K4	53.9 ±0.3^{a}	49.3 ±0.3^{d}	82.6 ±0.6^{e}	185.8 ±1.2^{b}	260
K5	24.4 ±0.2^{g}	24.5 ±0.2^{h}	117.0 ±0.8^{b}	165.9 ±1.2^{d}	250
K6	34.8 ±0.3^{f}	28.9 ±0.2^{g}	121.0 ±0.6^{a}	184.7 ±1.1^{b}	240
K7	48.1 ±0.6^{b}	45.7 ±0.4^{e}	89.3 ±0.4^{d}	183.1 ±1.4^{bc}	240
K8	46.0 ±0.3^{c}	62.8 ±0.5^{b}	n.d.	108.8 ±0.8^{f}	276

Note: The chemical composition is on a wet weight basis. Values in the same column with different letters are significantly different at p <0.05. *Declared content on packaging.

tomatoes, which increased the total solids content. Only ketchup sample K5, however, declares on the package the use of dried vegetables (onion, garlic).

Total soluble solids are generally closely related to saccharide content. In this work a good correlation was also found between the total soluble solids and total saccharides (R = 0.7280) **(Table 5)**. The main saccharides in ketchup samples were glucose and fructose. In most samples sucrose was also determined. The amount of carbohydrates is related to the tomato variety and tomato ripening used to produce ketchup. Other saccharides can be added to the ketchups during their sweetening. The concentration of fructose in ketchup samples varied from 24.4 mg.g^{-1} to 53.9 mg.g^{-1}, while concentration of glucose varied between 24.5 mg.g^{-1} and 66.4 mg.g^{-1} and sucrose between non-detectable quantities and 121 mg.g^{-1} **(Table 2)**. Similar values were measured by **Sharoba et al. (2005).** The total amount of saccharides obtained by the sum of glucose, fructose and sucrose concentration does not agree with the data on the packaging, indicating higher sacharide content. This difference can be explained by the addition of starch or xanthan to ketchup, which affect its texture. These added polysaccharides may make up the difference between the total saccharide content and the declared total saccharide content.

The quantity of tomatoes used for ketchup production correlates fairly well with the lycopene content in the ketchups (R = 0.6704) **(Table 5)**. The amount of lycopene in ketchups ranged between 0.056 and 0.266 mg.g^{-1} **(Table 3)**. **Wawrzyniak et al. 2005** mentioned the amount of lycopene in ketchup in the range of 0.07 – 0.140 mg.g^{-1}. The content of lycopene in ketchup is dependent on lycopene content in tomatoes used for tomato puree production and also on ketchup production process in which tomatoes undergo multistep mechanical and heat-related processing operations that could potentially reduce lycopene content in final product **(Mendelova et al., 2013)**.

The pH value is one of the most important factors affecting the growth and biochemical activity of microorganisms in food. In the case of chemically preserved vegetables, a pH of 4.1 is required under the legislation. The pH of ketchups is affected by the natural organic acid content in tomatoes (e.g. malic or ascorbic acid) and by added preservatives. The pH of the individual samples ranged between 3.72 and 3.98 **(Table 1)**. Similar values were measured by **Sharoba et al. (2005)** while **Lehkoživová et al. (2009)** measured generally higher pH, between 4.1 and 4.3. The most important acids in ketchup are acetic and citric acid. The acetic acid content is related to the technological process of ketchup production, when vinegar is added to the ketchup as a flavoring agent. Citric acid is the most commonly used pH regulator in food. The acetic acid content in analyzed samples varied from 16.8 to

Table 3 Organic acids content and lycopene content in ketchup samples K1-K8.

Sample	Citric acid ($mg.g^{-1}$±SD)	Acetic acid ($mg.g^{-1}$±SD)	Lycopene ($mg.g^{-1}$±SD)
K1	3.7 ±0.2^{d}	16.8 ±0.3^{f}	0.108 ±0.003^{d}
K2	7.7 ±0.3^{a}	31.6 ±0.3^{a}	0.241 ±0.017^{b}
K3	2.9 ±0.1^{e}	26.4 ±0.4^{c}	0.056 ±0.005^{e}
K4	5.4 ±0.3^{c}	29.9 ±0.5^{b}	0.132 ±0.007^{c}
K5	4.3 ±0.2^{d}	22.1 ±0.2^{d}	0.135 ±0.003^{c}
K6	5.9 ±0.4^{c}	22.1 ±0.3^{d}	0.266 ±0.006^{a}
K7	6.9 ±0.3^{b}	29.6 ±0.6^{b}	0.103 ±0.007^{d}
K8	3.9 ±0.2^{d}	19.1 ±0.5^{e}	0.123 ±0.004^{cd}

Note: The chemical composition is on a wet weight basis. Values in the same column with different letters are significantly different at p <0.05.

Table 4 Mineral composition of ketchup samples K1-K8.

Sample	Elemental compositon				
	Ca ($mg.g^{-1}$±SD)	K ($mg.g^{-1}$±SD)	Mg ($mg.g^{-1}$±SD)	Na ($mg.g^{-1}$±SD)	NaCl ($mg.g^{-1}$±SD)
K1	0.308 ±0.009^{e}	2.483 ±0.084^{e}	0.136 ±0.003^{e}	6.383 ±0.138^{c}	16.22 ±0.14^{c}
K2	0.461 ±0.016^{b}	4.979 ±0.033^{a}	0.367 ±0.006^{a}	5.736 ±0.089^{d}	14.61 ±0.09^{d}
K3	0.416 ±0.012^{c}	1.759 ±0.040^{g}	0.137 ±0.003^{e}	4.974 ±0.026^{e}	12.62 ±0.03^{e}
K4	0.417 ±0.004^{c}	3.771 ±0.126^{c}	0.198 ±0.005^{c}	7.816 ±0.161^{b}	19.95 ±0.16^{b}
K5	0.217 ±0.005^{f}	2.103 ±0.083^{f}	0.104 ±0.003^{f}	4.606 ±0.036^{f}	11.71 ±0.04^{g}
K6	0.386 ±0.007^{d}	2.899 ±0.070^{d}	0.175 ±0.004^{d}	3.570 ±0.044^{g}	9.13 ±0.04^{h}
K7	0.910 ±0.013^{a}	4.394±0.074^{b}	0.293 ±0.004^{b}	8.865 ±0.189^{a}	22.52 ±0.19^{a}
K8	0.331 ±0.005^{e}	2.001 ±0.032^{f}	0.111 ±0.003^{f}	4.708 ±0.066^{ef}	12.06±0.07^{f}

Note: The chemical composition is on a wet weight basis. Values in the same column with different letters are significantly different at p <0.05.

Table 5 Pearson correlation matrix.

variables	variables									
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
A	1	0.8583	0.8464	0.6704	0.4997	0.8436	0.0687	0.8864	0.2268	0.1852
B	0.8583	1	0.9476	0.4831	0.7309	0.5870	0.3457	0.7689	0.2012	0.4769
C	0.8464	0.9476	1	0.6409	0.6262	0.6830	0.4164	0.6671	-0.0371	0.3214
D	0.6704	0.4831	0.6409	1	0.1382	0.7735	0.0631	0.4488	-0.3848	-0.1086
E	0.4997	0.7309	0.6262	0.1382	1	0.4286	0.2798	0.3349	0.0941	0.9019
F	0.8436	0.5870	0.6830	0.7735	0.4286	1	-0.0124	0.5673	-0.1030	0.1473
G	0.0687	0.3457	0.4164	0.0631	0.2798	-0.0124	1	-0.2001	-0.5419	0.3437
H	0.8864	0.7689	0.6671	0.4488	0.3349	0.5673	-0.2001	1	0.5920	0.0422
I	0.2268	0.2012	-0.0371	-0.3848	0.0941	-0.1030	-0.5419	0.5920	1	0.0333
J	0.1852	0.4769	0.3214	-0.1086	0.9019	0.1473	0.3437	0.0422	0.0333	1

Note: A = tomato content, B = total soluble solids, C = total solids, D = lycopene, E = Σsaccharides, F = yield stress, G = unit price, H = potassium content, I = salt, J = dynamic viscosity.

31.6 $mg.g^{-1}$ and citric acid content ranged from 2.9 to 7.7 $mg.g^{-1}$ (**Table 3**). The acetic acid content was in agreement with results obtained by **Lehkoživová et al. (2009)** and by **Porretta (1991)**, however **Porreta (1991)** measured higher concentrations of citric acid in ketchup (average value = 16.6 $mg.g^{-1}$). For sample K7 where the second highest citric acid content was measured, the manufacturer declares on the package the use of lemon concentrate for the production of ketchup.

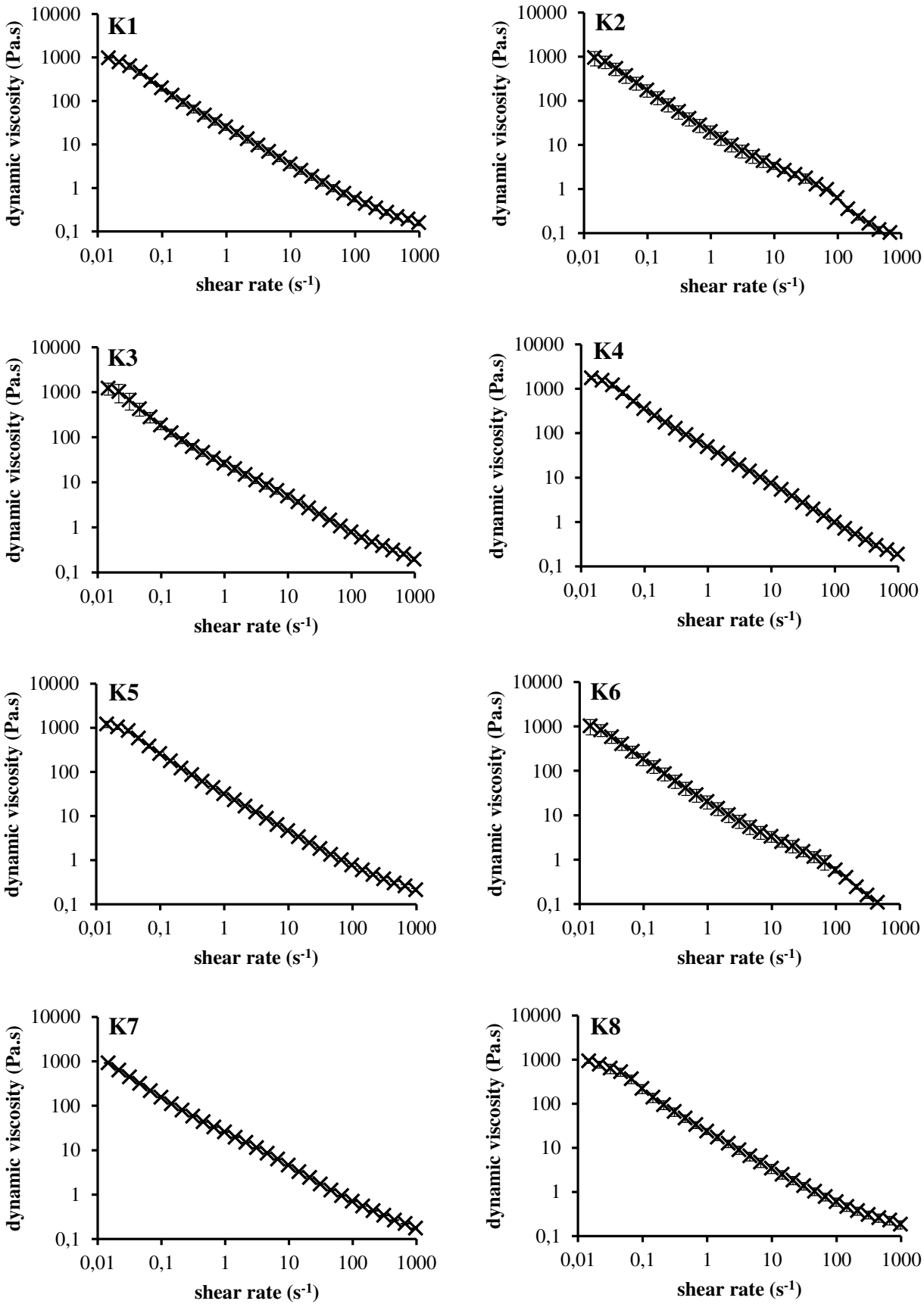

Figure 1 Flow curves of ketchup samples K1-K8.

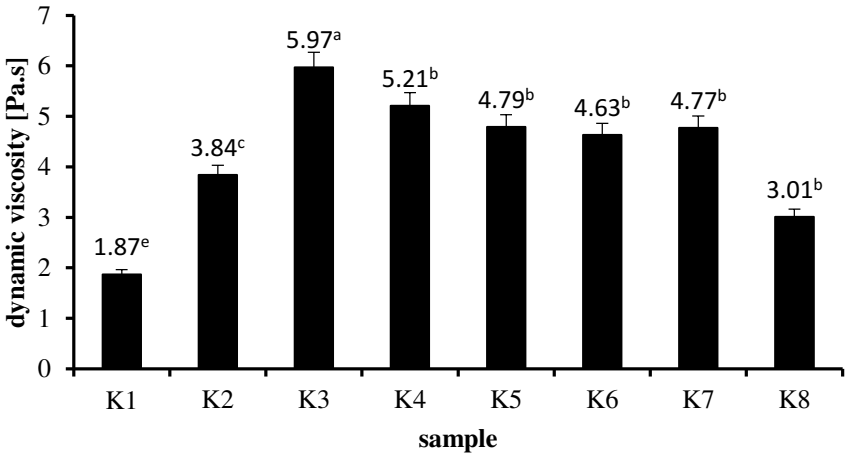

Figure 2 Dynamic viscosity of ketchup samples K1-K8. Values with different letters are significantly different at p <0.05.

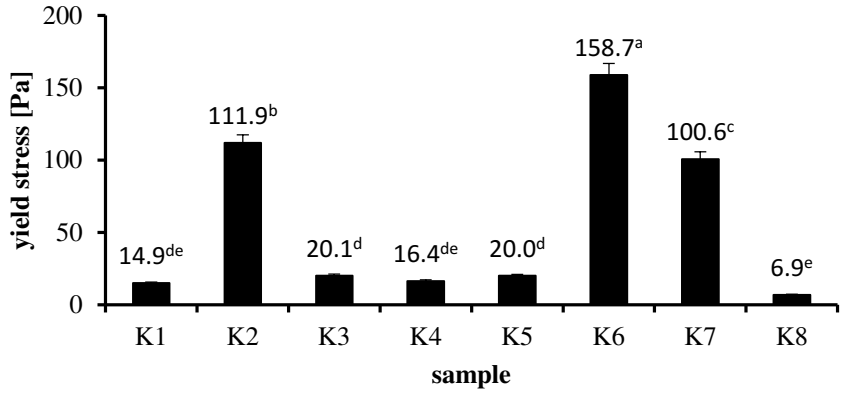

Figure 3 Yield stress of ketchup samples K1-K8. Values with different letters are significantly different at p <0.05.

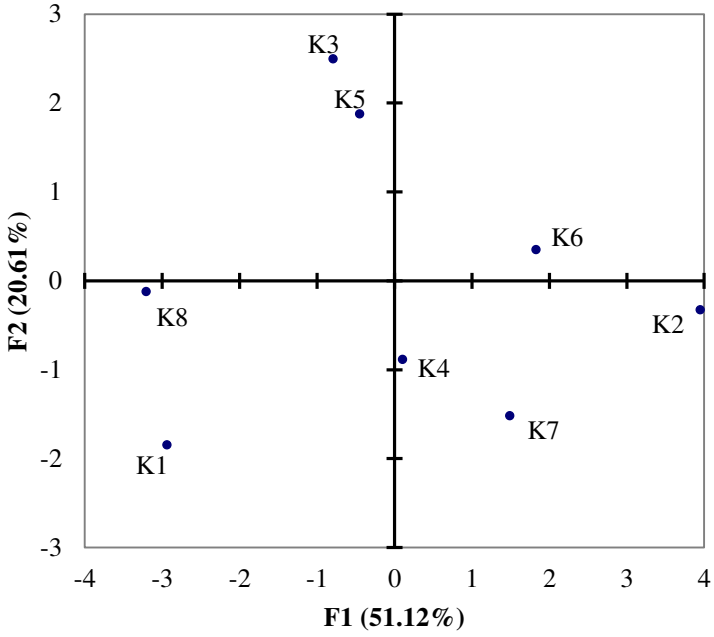

Figure 4 Plots of the first two principal components from Pearson principal component analysis of results from analysis of ketchup samples.

Tomato products are after milk, potatoes, beef, coffe, poultry, and orange juice the most important source of potassium in human nutrition **(Burton-Freeman and Reimers, 2011)**. Tomatoes into ketchup also brings a number of other nutritionally important elements which can also serve as authentic markers. Concentration of potassium in ketchup samples was within the range 1.759 – 4.979 $mg.g^{-1}$ **(Table 4)** and correlated well with tomato content in ketchup (R = 0.8864) **(Table 5)**. Concentration of other mineral elements (Ca, Mg, Na) was as follows: Ca from 0.217 to 0.910 $mg.g^{-1}$, Mg from 0.104 to 0.367 $mg.g^{-1}$ and Na from 3.570 to 8.865 $mg.g^{-1}$ **(Table 4)**. Because tomatoes naturally contain sodium in relatively low concentration **(USDA, 2015)** it can be assumed that the main source of sodium in ketchup is the salt used to flavor ketchup. Salt amount calculated from sodium content in ketchup varied from 9.1 to 22.5 $mg.g^{-1}$ which is in line with valid legislation that sets the maximum salt content in ketchup to be 30 $mg.g^{-1}$.

Viscosity is a principal parameter when any flow measurements of fluids, such as liquids or semi-solids, are made. Viscosity measurements are made in conjunction with product quality and efficiency. All samples exhibited a similar type of behavior: non-Newtonian fluid exhibiting pseudoplasticity **(Figure 1)**. Pseudoplasticity of ketchups lies in the orientation of the solid particles in the direction of flow due to the shear rate exhibited. When comparing the absolute values, there are no significant differences in the individual samples. The only difference can be seen in samples K2 and K6, which show a slight increase in viscosity between 10 – 100 s^{-1}, and there is a sign of Newtonian plateau, which can be caused by structural changes in samples K2 and K8 when subjected to deformation (shear rate). These samples also exhibit the lowest viscosity at the highest shear rates (1000 s^{-1}). Absolute dynamic viscosity values were compared at a shear rate of 10 s^{-1} **(Figure 2)**, which should correspond to typical shear rates when extruding material from the tube (1 – 100 s^{-1}). The rheological behavior of the examined ketchup samples corresponds to the results published by **Sharoba et al. 2005** or **Bayod et al. 2008**.

Ketchup is a fluid with a yield stress, it is necessary to impose some external stress at which the liquid begins to flow. High yield stress of ketchup is not so desirable, leading to dosing problems in gravity-feed systems or an excess of residue on the sides of inverted bottles. Many products are modified to keep them flowing at very low shear stress. Samples K1, K3, K4, K5 and K8 had a low yield stress, while samples K2, K6 and K7 had significantly higher yield stress (F_{crit} = 5.99, F = 65.23, p = 0.0002). For most ketchups, the determined yield stress is consistent with the results published by **Torbica et al., 2016**. Yield stress may be influenced by the amount of tomatoes used in ketchup production and by the amount of added thickeners. A significant correlation was found between tomato content in ketchup and between the yield stress value (R = 0.8436, **Table 5**).

All measured data was processed using Pearson principal component analysis. The result of this analysis is graphically shown in the **Table 5** and in the **Figure 4**. Ketchup samples were divided into four quadrants according to their similarity. The smallest distance was recorded for samples K3 and K5 located in first quadrant and for K1 and K8 samples located in third quadrant. Ketchup samples K2, K4 and K7 were all located into the fourth quadrant, but their distance was larger here. Samples K1 and K8 are ketchups with lowest tomato content (140 $g.100g^{-1}$), samples K3 and K5 are ketchups with a moderately high proportion of tomatoes (150 $g.100g^{-1}$) and samples K2, K7 and K4 are ketchupes with high proportion of tomatoes (>170 $g.100g^{-1}$). From the Pearson correlation matrix **(Table 5)** it can be seen that in addition to the already discussed correlations of some ketchup parameters, the market price of ketchups is not significantly related to their chemical composition. Weak correlation was found only between the ketchup price and the salt content in ketchup. Cheaper ketchups contained more salt.

CONCLUSION

First hypothesis that higher ketchup price means higher quality for consumers in connection with the chemical composition of the ketchup has not been confirmed as no correlation was found between the ketchup price and chemical composition of ketchup. The second hypothesis that chemical composition of ketchup affects the rheological properties of ketchups was confirmed. Ketchups with a higher content of tomatoes had significantly higher yield stress (p = 0.0002). All ketchup samples investigated in this study suit the current legislation in terms of their quality parameters. This comprehensive study, among others, showed that chemical markers as potassium or lycopene are suitable for evaluation of tomato ketchup authenticity.

REFERENCES

Bannwart, G. C. M., Bolini, H. M. A., Toledo, M. C., Kohn, A. P. C., Cantanhede, G. C. 2008. Evaluation of Brazilian light ketchups II: quantitative descriptive and physicochemical analysis. *Cienc. Tecnol. Aliment.*, vol. 28, no. 1, p. 107-115. https://doi.org/10.1590/S0101-20612008000100016

Bayod, E., Willers Pilman, E., Tornberg, E. 2008. Rheological and structural characterization of tomato paste and its influence on the quality of ketchup. *LWT*, vol. 41, no. 7, p. 1289-1300. https://dx.doi.org/10.1016/j.lwt.2007.08.011

Burton-Freeman, B., Reimers, K. 2011. Tomato consumption and health: Emerging benefits. *American Journal of Lifestyle Medicine*, vol. 5, p. 182-191. https://doi.org/10.1177/1559827610387488

Canene-Adams, K., Campbell, J. K., Zaripheh, S., Jeffery, E. H., Erdman, J. W. 2005. Tomato as a functional food. *Journal of Nutrition*, vol. 135, no. 5, p. 1226-1230. https://doi.org/10.1093/jn/135.5.1226
PMid:15867308

CSN EN 12145. 1997. Fruit and vegetable juices - Determination of total dry matter - Gravimetric method with weight loss on drying.

Divis, P., Porizka, J., Vespalcova, M., Matejicek, A., Kaplan, J. 2015. Elemental composition of fruits from different black elder (*Sambucus nigra* L.) cultivars grown in the Czech Republic. *J. Elementol.*, vol. 20, no. 3, p. 549-557. https://doi.org/10.5601/jelem.2015.20.1.758

Hayes, W. A., Smith, F. G., Morris, A. E. J., 1998. The production and quality of tomato concentrates. *Crit. Rev. Food Sci. Nutr.* vol. 38, no. 7, p. 537-564. https://doi.org/10.1080/10408699891274309

Lehkoživová, J., Karovičová, J., Kohajdová, Z. 2009. The quality and authenticity markers of tomato ketchup. *Acta Chimica Slovaca*, vol. 2, p. 88-96.

Mendelova, A., Mendel, L., Fikselova, 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

Norton, I. T., Spyropoulos, F., Cox, P. 2011. *Practical Food Rheology: An Interpretive Approach.* Wiley-Blackwell, 280 p., ISBN-13: 978-1405199780. https://doi.org/10.1002/9781444391060

Porretta, S. 1991. Analytical profiling of ketchup. *J. Sci. Food Agric.*, vol. 57, p. 293-301. https://doi.org/10.1002/jsfa.2740570213

Sharoba, A. M., Senge, B., El-Mansy, H. A., Bahlol, H., Blochwitz, R. 2005. Chemical, sensory and rheological properties of some commercial German and Egyptian tomato ketchups. *Eur. Food Res. Technol.*, vol. 220, no. 2, p. 142-151. https://doi.org/10.1007/s00217-004-0981-7

Suwanaruang, T. 2016. Analyzing lycopene content in fruit. *Agriculture and Agricultural Science Procedia*, vol. 11, p. 46-48. https://doi.org/10.1016/j.aaspro.2016.12.008

Torbica, A., Belovic, M., Mastilovic, J., Kevresan, Z., Pestoric, M., Skrobot, D., Dapcevic Hadnadev, T. 2016. Nutritional, rheological, and sensory evaluation of tomato ketchup woth increased content of natural fibres made from fresh tomato pomace. *Food and Bioproducts Processing*, vol. 98, p. 299-309. https://doi.org/10.1016/j.fbp.2016.02.007

Thakur, B. R., Singh, R. K., Nelson, P. E. 1996. Quality attributes of processed tomato products: A review. *Food Rev. Int.*, vol. 12, no. 3, p. 375-401. https://doi.org/10.1080/87559129609541085

USDA, 2015. United States Department of Agriculture database. Available at: https://ndb.nal.usda.gov/

Wawrzyniak, A., Marciniak, A., Rajewska, J. 2005. Lycopene content of selected foods available on the polish market and estimation of its intake. *Pol. J. Food Nutr. Sci.*, vol. 14/55, p. 195-200.

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CONTENT OF ENDOGENOUS SULFUR DIOXIDE IN WINES

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ABSTRACT

Content of free and total endogenous sulfur dioxide were evaluated by classical iodometric titration in must, during winemaking processes and in bio-wine. No exogenous sulfur dioxide was added in any technological operations to simplify the evaluations. In addition, the results were corrected on the content of reductons (total content of reducing substances). The results confirmed formation of endogenous sulfur dioxide from sulfur containing substances (sulfur containing amino acids etc.) in both experiments. Microbial sulfur dioxide is preferably bound to carbonyl substances. Only minor part is present in the free (active) form of the sulfur dioxide. In addition, total content of polyphenols (TPC) and total antioxidant capacity (TAC) were determined by spectrophotometry at the same time. A procedure OIV-MA-AS323-O4B: R 2009 was used. Contents of "free" and "total" sulfur dioxide (with/without correction on contents of reductons) and total content of reductones were determined after complexing the sulfur dioxide with formaldehyde. A standard spectrophotometric method using Folin-Ciocalteu reagent was applied for determination of total content of polyphenolics (TPC) at 765 nm after 60 min incubation. The results were expressed as tannin equivalents (in $mg.L^{-1)}$. A standard DPPH (2,2´-difenyl-1-picrylhydrazyl dissolved in methanol) spectrophotometric method was applied for determination of total antioxidant capacity (TAC) at 515 nm. Depletion of the color intensity was measured after 60 min incubation against blank (methanol) and absorbance decrease $\Delta(A) = (A_0 - A_1)/A_0$ was calculated and used for construction of calibration curve. The TAC values were expressed as ascorbic acid concentrations (in $mg.L^{-1}$).

Keywords: Endogenous sulfur dioxide; wine; reductons; iodemetric titration; spectrophotometry

INTRODUCTION

Bio-wine is a wine that strictly produced by fermentation of wine musts obtained by pressing of wine grapes grown according the rules of ecological agriculture. In Europe, most of the bio-wines are produced in small wineries. Plantation and subsequent complete technological process, from collection of grapes up to bottling of final wine, forms a fully closed cycle with respect to the abovementioned rules and in accordance with the environmental protection. The special attention must be focused on elimination or substantial reductons of losses of content of biologically active substances and thus to prevent reductons of total antioxidant activity/capacity (TAA/TAC). The appropriate high levels of TAA/TAC thus allow reductons of usage of synthetic additives. Typical and individual natural aroma characteristics of bio-wine could be preserved.

Sulfur dioxide can be present as endogenous (formed during fermentation processes) and especially also as exogenous (added in individual technological operations) in wines, and generally in all fermented drinks (e.g. beers, ciders etc.). Endogenous sulfur dioxide is produced (in addition to the other metabolites) in sulfite non-supplied grape must, but mostly during fermentation by enzymatic transformation of sulfur containing substances (e.g. thioamino acids, i.e. cysteine, cystine, methionine, glutathione, thio-compounds, sulfates, elemental sulfur etc.) by *Saccharomyces cerevisiae* action. Production of endogenous sulfur dioxide depends on many factors (type of microorganism, matrix composition **(Rankine, 1968**; **Eschenbruch, 1974; Romano and Suzzi, 1993; Špakovská et al., 2012; Bajčan et al., 2016)**. Contents of endogenous sulfur dioxide can reach from several $mg.L^{-1}$ up to 30 $mg.L^{-1}$ and its concentrations can be as high as 100 $mg.L^{-1}$ in some extreme conditions (**Rankine and Pocock 1969; Eschenbruch, 1974; Dott et al., 1976; Suzzi et al., 1985)**. Higher concentrations of exogenous SO_2 together with endogenous SO_2 could be critical for malolactic fermentation **(Henick-Kling and Park, 1994)**. Endogenous sulfur dioxide is mostly present in bound forms, but minor concentrations of free (active) form could be also present **(Wells and Osborne, 2011)**. Presence of both forms of the endogenous SO_2 must be known before

addition of the exogenous (technological) sulfur dioxide in any step of technological processes.

Evaluation of the role of reducing substances (reductones) on content of "total" and "free" sulfur dioxide in technological processes of winemaking of bio-wine (from the treatment of wine grape musts up to bio-wine bottling) was the main aim of the study. No exogenous sulfur dioxide was added during the technological process in any form.

Scientific hypothesis

Microorganisms (*Saccharomyces cerevisiae*) produce so called endogenous sulfur dioxide during alcoholic fermentation in the range measurable concentrations (from several $mg.L^{-1}$ up to 50 $mg.L^{-1}$ and under extreme conditions up to 100 $mg.L^{-1}$) directly in the sulfite-supported grape musts. The values presented in literature are controversial, since they probably represent not only the sulfur dioxide, but also so called reductones.

MATERIAL AND METHODOLOGY

A pH meter Level 1 equipped with a combined pH electrode (WTW GmbH, Weilheim, Germany) that was regularly calibrated with a set of buffers of pH 4.01, 7.00 and 9.23. All spectrophotometric measurements were performed using UV-VIS spectrophotometer Helios Delta (Spectronic Unicam, Cambridge, UK) in fused silica rectangular cuvettes with 10 mm optical lengths.

NaOH (c_m = 0.1, 1.0 and 2.0 $mol.L^{-1}$), oxalic acid dihydrate, buffer solution (20 ml acetic acid conc. and 80 ml 27% sodium acetate filled up to 250 ml with distilled water), reagent solution (5 g NH_4VO_3 dissolved in 75 ml of 1 $mol.L^{-1}$ NaOH and after addition of 100 ml 270 $g.L^{-1}$ sodium acetate solution filled up to 250 ml with distilled water), tartaric acid (c_m = 10 $g.L^{-1}$), charcoal, I_2 solution with $c(I_2)$ = 0.01 $mol.L^{-1}$, 16% (v/v) H_2SO_4, 1% (v/v) formaldehyde (CH_2O), EDTA (c_m = 1 $g.L^{-1}$), starch solution, arsonium oxide (all from Pliva-Lachema, Brno, Czech Republic) were used.

Wine grape must and intermediate products in individual steps of the technological processes of winemaking of white bio-wines Veltlinské zelené (VZ) and Ryzlink rýnský (RR) and red bio-wine Rulandské modré (RM) production of moderate climatic region from Marcinčák Winery - Bio-wine Ltd., (Mikulov, Czech Republic) winery sub-region Moravia were analyzed. Contents of sulfur dioxide ("free" and "total") and content of reductones (sum of all substances oxidized by iodine in strongly acidic condition of sulfuric acid, except of sulfur dioxide), in addition to total contents of polyphenols (TPC) and total antioxidant capacity (TAC) were determined in all wines. Samples of the individual wines were collected from storage balloons in repetitive two-week intervals and immediately analyzed.

Determination of SO_2 in wine

A procedure OIV-MA-AS323-O4B: R 2009 that is used for determination of free and total content of sulfur dioxide in grape wines, fruit wines, malt and Tokay wines was used. Contents of "free" and "total" sulfur dioxide (with/without correction on contents of reductons) and total content of reductones (organic substances with hydroxyl groups on double bonds, i.e. 2,3-dihydroxypropenal, L-ascorbic acid, gallic acid, etc. and some inorganic substances having strong reductions properties) were determined.

Determination of the "free SO_2"

A wine sample (50 mL) was pipetted with a pipette touching the bottom into an Erlenmeyer bottle (500 mL) and 16% H_2SO_4 (10 mL), EDTA solution (1 mL, c = 10 $g.L^{-1}$), starch solution (5 mL) were immediately added and mixed. The mixture was titrated quickly with standard I_2 solution ($c(I_2)$ = 0.02 $mol.L^{-1}$) until blue color persisting 30 s was observed against the white background. Based on the standard I_2 solution (V_1) consumption, the concentration (in $mg.L^{-1}$) of "free sulfur dioxide" in wine was calculated. The sum of the "free sulfur dioxide" and total content of reductones was detected.

Determination of the "total SO_2"

A wine sample (50 mL) was pipetted with a pipette touching the bottom into an Erlenmeyer bottle (500 mL) containing NaOH solution (8 mL, c = 4 $mol.L^{-1}$). Erlenmeyer bottle was sealed, solutions were mixed and allowed to stand in dark place. After 5 min, 16% H_2SO_4 (15 mL), EDTA solution (1 mL, c = 10 $g.L^{-1}$), starch solution (5 mL) were added and immediately mixed. The mixture was titrated quickly with standard I_2 solution ($c(I_2)$ = 0.02 $mol.L^{-1}$) until blue color persisting 30 s was observed against the white background. Based on the standard I_2 solution (V_2) consumption, the concentration (in $mg.L^{-1}$) of "total sulfur dioxide" in wine was calculated. The sum of the "total sulfur dioxide" and total content of reductones was detected.

Correction on reductones

A wine sample (50 mL) was pipetted with a pipette touching the bottom into an Erlenmeyer bottle (500 mL) containing formaldehyde solution (1 mL, c = 10 $g.L^{-1}$). Erlenmeyer bottle was sealed, solutions were mixed and allowed to stand in dark place. After 30 min, 16% H_2SO_4 (10 mL), EDTA solution (1 mL, c 10 $g.L^{-1}$) and starch solution (5 mL) were added and immediately mixed. The mixture was titrated quickly with standard I_2 solution ($c(I_2)$ = 0.02 $mol.L^{-1}$) until blue color persisting 30 s was observed against the white background. Based on the standard I_2 solution (V_4) consumption, the concentration (in $mg.L^{-1}$) of "total reductones" in wine was calculated and "total reductones" concentration was subtracted from the "free sulfur dioxide" or "total sulfur dioxide" concentrations in wines. Content of reductones was expressed ascorbic acid and/or sulfur dioxide concentration (in $mg.L^{-1}$).

Determination of the total polyphenols content (TPC)

A standard spectrophotometric method using Folin-Ciocalteu reagent was applied for determination of total content of polyphenolics (TPC) at 765 nm after 60 min incubation. The results (TPC) were expressed as tannin equivalents (in $mg.L^{-1}$). Repeatability was verified by 10 repetitive determination of gallic acid (c_m = 0.4 $mg.L^{-1}$) and tannin (0.5 $mg.L^{-1}$). A six points calibration curve A =

$f(c_m)$ was constructed using standard solutions. Samples were collected regularly in two-week intervals **(Figures. 1 – 5)** and immediately analyzed.

Determination of the total antioxidant capacity/activity (TAC/TAA)

A standard DPPH spectrophotometric method was applied for determination of total antioxidant capacity (TAC) at 515 nm. A precisely weighed sample of 2,2´-difenyl^{-1}-picrylhydrazyl (DPPH, 24 mg) was dissolved in methanol (100 mL) and the standard solution was stored for max. 2 months in refrigerator. Working solution was prepared by dilution of the DPPH standard solution (10 mL) with methanol (45 mL). Absorbance (A_0) of the working solution has to be in the range A_0 = 0.8 – 1.0, at wavelength 515 nm. A six points calibration curve $A = f(c_m)$ was constructed using standard solutions of ascorbic acid (40, 60, 80 a 100 mg.L^{-1}). Samples were collected regularly in two-week intervals **(Figures. 1 – 5)** and immediately analyzed.

The DPPH working solution (8.55 mL) was added to 450 µl of the calibration solution or sample solution and mixture was mixed. The mixture was allowed to stand in cold and dark place. Depletion of the color intensity was measured after 60 min incubation against blank (methanol) and absorbance decrease $\Delta(A) = (A_0 - A_1)/A_0$ was calculated and used for construction of calibration curve. The TAC values were expressed as ascorbic acid concentrations (in mg.L^{-1}).

Statistical methods

The obtained data were subjected to analysis of variance using the Minitab 17 statistical software program (Minitab, Coventry, United Kingdom). Where statistical differences were noted, differences among data were determined, using the Tukey's test. Significance was defined at p <0.05.)

RESULTS AND DISCUSSION

Samples for the determination of acidity (pH), the total antioxidant capacity (TAC) and the total content of polyphenols (TPC) and the "total" and "free" sulfur dioxide were collected in regular two weeks intervals during grapes ripening, production of the grape musts by pressing, in principal steps of the winemaking up to bottling of the final bio-wine and during storage of the bio-wine in bottles. Collection of the samples for the total content of reductones was done immediately after pressing and in accordance with the collections for the other analyses.

pH values **(Figure 1)** of the samples of ripped grapes varied in the interval 2.5 – 2.7 and then pH values rapidly increased up to pH values close to 3. The changes of pH values could be explained by some biochemical a physico-chemical processes (i.e. sugar destruction, production of ethanol by yeast, biotransformation of acids, production of CO_2, temperature changes of fermentation media etc.) taking part in alcoholic fermentation. Acidity of fermentation medium was in the pH intervals 3.0 – 3.4, 3.0 - 3.4 and 3.0 – 3.4 for VZ, RR and RM in the beginning of fermentation. Small decrease of the pH values in individual wines was observed in must samples before its first decantation. More evident decrease of the pH values was observed in samples of RM (from pH 3.41 to 3.23). Increase of the pH was observed in samples of VZ probably due to the more intense contacts with atmospheric oxygen and subsequent oxidation of some wine components. Very small decrease of pH values was observed in samples of RR during the technological process. Less evident changes of pH values were observed during winemaking processes of all wine samples. Thus the processes were „standardized".

As can be seen from **Figsure 2** and **Figure 3**, the values of the total antioxidant capacity (TAC) and the total contents of polyphenols (TCP) were relatively high and their values oscillated around 800 – 1000 gallic acid equivalent (GAE). The values of both parameters close to pressing (ripped grapes) rapidly decreased and oscillated around 500 GAE a 50 ascorbic acid equivalents (AAE) for TPC and for TAC. Both values insignificantly increased after pressing the grape must and were practically constant (in the range of experimental errors) during the whole vinification processes. It means that both values were stabilized.

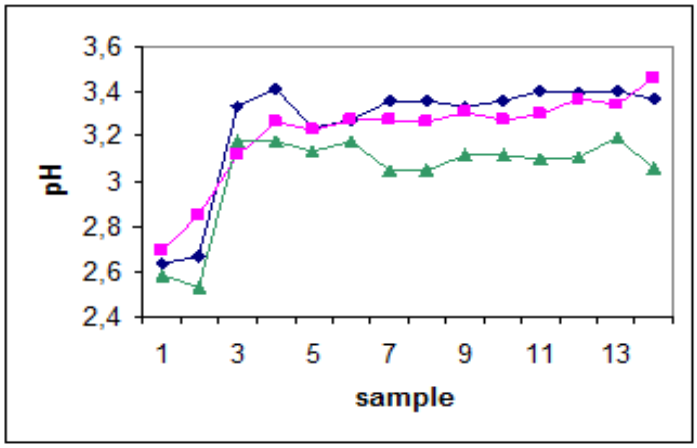

Figure 1 Changes of pH values during technological processes of bio-wine production.
Note: Rulandské modré ♦, Veltlínské zelené ■, Ryzlink rýnský ▲, sample: 1 - must after pressing, 2 - must after first decantation, 3 - 14 samples during fermentation (two-week intervals).

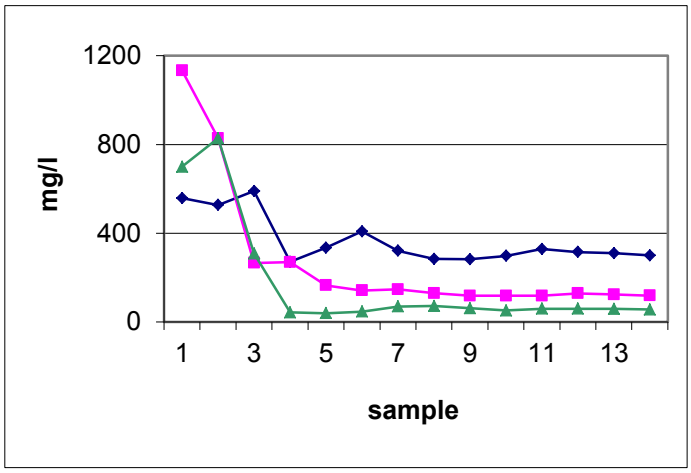

Figure 2 Changes of the total antioxidant capacity TAC in mg.L^{-1} ascorbic acid. Note: Rulandské modré ♦, Veltlínské zelené ■, Ryzlink rýnský ▲, sample: 1 - hard grapes, 2 - soft grapes, 3 – ripped gapes, 4 – must after pressing, 5 - 14 samples during fermentation (two-week intervals).

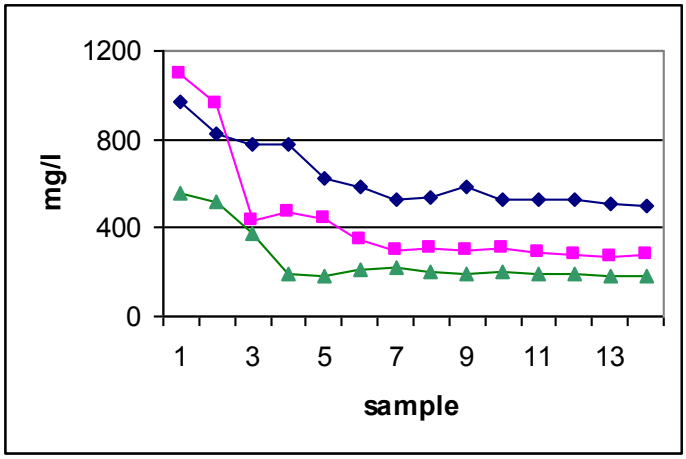

Figure 3 Changes of the total contents of polyphenols during technological processes of bio-wine production. Note: Rulandské modré ♦, Veltlínské zelené ■, Ryzlink rýnský ▲, samples: see **Figure 2.**

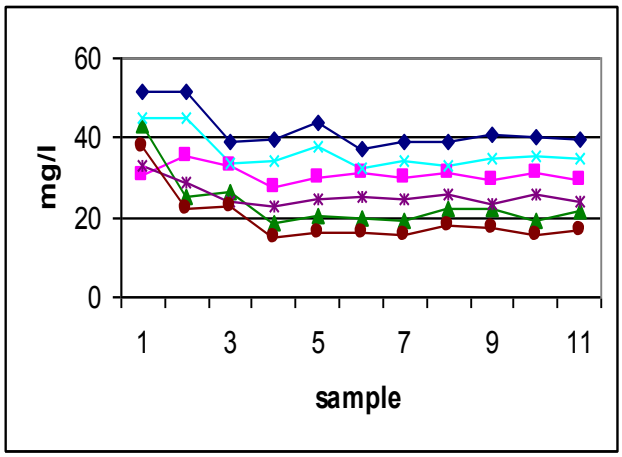

Figure 4 Changes of the contents of endogenous total SO_2 during technological processes of bio-wine without and with the corrections on reductones contents. Note: Rulandské modré ♦, Veltlínské zelené ■, Ryzlink rýnský ▲, samples: see Figure 2.

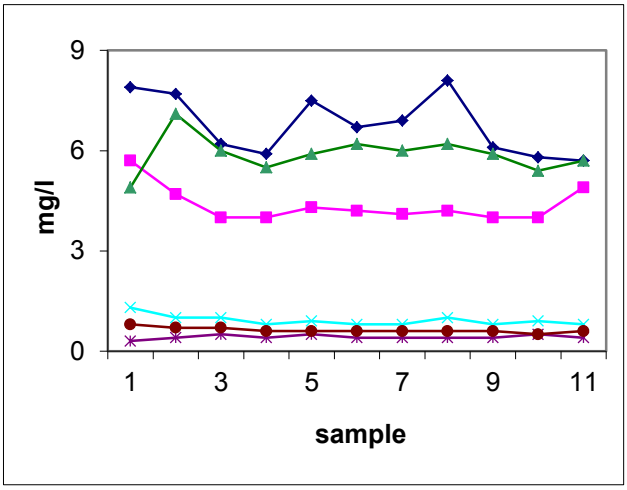

Figure 5 Changes of the contents of endogenous free SO_2 during technological processes of bio-wine without and with the corrections on reductones contents. Note: Rulandské modré ♦, Veltlínské zelené ■, Ryzlink rýnský ▲, samples: see **Figure 2**.

Contents of the „total sulfur dioxide“ and the „free sulfur dioxide“ were controlled **(Figure 4 and Figure 5)** from moment of pressing of ripped grapes. Contents of the „total sulfur dioxide“oscillated around 50 $mg.L^{-1}$ for RM and around 45 – 48 $mg.L^{-1}$ for RR and VZ. Contents of the „free sulfur dioxide“ were 4 – 7 $mg.L^{-1}$ much lower. The slightly higher values were found for RM while lower values were found for RR and VZ. All values decreased approximately of 10% during the following period and with the slight increase of the contents during the all time of the experiments to the levels between 33 – 41, 13 – 22 and 20 – 29 $mg.L^{-1}$ for RM, VZ and RR. Very similar trends were observed for the contents of „free sulfur dioxide“ in the intervals 5 – 7, 2.3 - 5.0 and 5 – 6 $mg.L^{-1}$ for RM, VZ and RR.

The obtained concentrations of the „total sulfur dioxide“ and „free sulfur dioxide“ included also total concentrations of reduced substances (reductones) with red-ox potentials lower than red-ox potential of the reaction of $I_2/2\ I^-$ and thus all substance easily oxidized by iodine under given experimental conditions (strongly acidic medium of H_2SO_4). Organic compounds with hydroxy-groups on the double bounds, having strong reductions properties (i.e. 2,3-dihydroxypropenal, L-ascorbic acid etc.) belong to the typical reductones. The substances interfere in the iodometric determinations of the „total”, “bound“ and „free” sulfur dioxide and they are source of false and seriously over-estimated results. After the corrections on the contents of reductones, the obtained contents of the „total sulfur dioxide“ (5 – 7, 3 – 4 and 4 – 6 $mg.L^{-1}$) and also the „free sulfur dioxide“ (0.1 – 1.3, 0.2 – 0.5 and 0.2 – 0.7 $mg.L^{-1}$) were one order of magnitude lower.

The results confirmed that microorganisms (*Saccharomyces cerevisiae*) produced so called endogenous sulfur dioxide during the alcohololic fermentation in the range measurable concentrations directly in the sulfite non-supplied grape musts **(Rankine, 1968; Eschenbruch, 1974; Romano and Suzzi, 1993; Bajčan et al., 2016)**. Concentrations of the endogenous sulfur dioxide can be from several $mg.L^{-1}$ up to 50 $mg.L^{-1}$ and under some extreme conditions up to 100 $mg.L^{-1}$ **(Rankine and Pocock, 1969; Eschenbruch, 1974; Dott et al., 1976; Suzzi et al., 1985)**. These values presented in the literature are controversial, since they probably represent not only the sulfur dioxide, but also reductones.

The very similar trend was observed in sulfite supplied grape must and wines **(Jančářová et al., 2011)**. In that case, concentrations of „free sulfur dioxide“ were determined for four varieties of white wines (Müller-Thurgau, Rulandské bílé, Sauvignon, Muškát Ottonel) and two varieties of red wines (Dornfelder and Frankovka). Concentrations of reductones were as high as tens of percents of the concentrations of „sulfur dioxide“. In addition to the endogenous sulfur dioxide, microorganisms produce **(Wells and Osborn, 2011)** also a lot of other substances (acetaldehyde, acetoine, carbonyl compounds etc.) that together with the other substances present in wine matrix (sugars, aldehydes, colors etc.) rapidly bound produced endogenous sulfur dioxide.

From the above mentioned facts it can be concluded that classical iodometric titrations produce false and seriously overestimated (in units up to tens percents) results due to the interferences of the reductones with the red-ox potential lower than red-ox potential of the reaction between iodide and iodine ($I_2/2\ I^-$) and thus easily oxidized under strongly acidic conditions (H_2SO_4). Standard iodometric methods for the determination of “free”, “bound” and “total” SO_2 needs revision and it will be necessary to develop a more specific method for determination of different forms of SO_2, free of interference of reductones. The separation techniques (ion chromatography or capillary electrophoresis), gas-diffusion flow injection analysis **(Kubáň et al., 1989)** and spectroscopic methods **(Čmelík et al., 2005)** can be given as an examples.

The very similar problem can be probably expected in the methods for determination of total antioxidant capacities and total contents of polyphenols. These methods produce probably also false and overestimated results due to interferences of presented exogenous and endogenous sulfur dioxide. Both forms are strong antioxidants. Some interactions of reagents for determination of polyphenolics (Folin-Ciocalteu) and antioxidants (FRAP, DPPH, ABTS etc.) with SO_2 and their interferences in results were

reported by earlier authors and also present applicants of the methods (**Abramovic et al., 2015**).

CONCLUSIONS

The values of the total antioxidant capacity (TAC) and the total contents of polyphenols (TCP) were relatively high and their values oscillated around 800 – 1000 gallic acid equivalent (GAE). The values of both parameters close to pressing (ripped grapes) rapidly decreased and oscillated around 500 GAE a 50 ascorbic acid equivalents (AAE) for TPC and for TAC. Both values insignificantly increased after pressing the grape must and were practically constant.

It can be concluded that classical iodometric titrations produce false and seriously overestimated (in units up to tens percents) results due to the interferences of the reductones with the red-ox potential lower than red-ox potential of the reaction between iodide and iodine (I2/2 I-) and thus easily oxidized under strongly acidic conditions (H2SO4). Standard iodometric methods for the determination of "free", "bound" and "total" SO2 needs revision and it will be necessary to develop a more specific method for determination of different forms of SO2, free of interference of reductones. The separation techniques (ion chromatography or capillary electrophoresis), gas-diffusion flow injection analysis and spectroscopic methods can be given as an examples.

The very similar problem can be probably expected in the methods for determination of total antioxidant capacities and total contents of polyphenols. These methods produce probably also false and overestimated results due to interferences of presented exogenous and endogenous sulfur dioxide.

REFERENCES

Abramovic, H., Kosmerl, T., Ulrih, N. P., Cigic B. 2015. Contribution of SO_2 to antioxidant potential of white wine. *Food Chemistry*, vol. 174, no. 1, p. 147-153. http://dx.doi.org/10.1016/j.foodchem.2014.11.030

Alexandre, H., Costello, P. J., Remize, F., Guzzo, J., Guilloux-Benatier, M. 2004. *Saccharomyces cerevisiae* - *Oenococcus oeni* interactions in wine: Current knowledge and perspectives. *International Journal of Food Microbiology*, vol. 93, no. 1, p. 141-154. http://dx.doi.org/10.1016/j.ijfoodmicro.2003.10.103

Bajčan, D., Vollmannová, A., Šimanský, V., Bystrická, J., Trebichalský, P., Árvay, J., Czako, P. 2016. Antioxidant activity, phenolic content and colour of the Slovak cabernet sauvignon wines. *Potravinarstvo*, vol. 10, no. 1, p. 89-94. http://dx.doi.org/10.5219/534

Carrete, R., Vidal, M. T., Bordons, A., Constanti, M. 2002. Inhibitory effect of sulfur dioxide and other stress compounds in wine on the ATPase activity of *Oenococcus oeni*. FEMS Microbiological Letters, vol. 211, no. 1, p. 155-159. http://dx.doi.org/10.1111/j.1574-6968.2002.tb11218.x

Čmelík, J., Machát, J., Niedobová, E., Otruba, V., Kanický, V. 2005, Determination of free and total sulfur dioxide in wine samples by vapour-generation inductively coupled plasma–optical-emission spectrometry. *Analytical and Bioanalytical Chemistry*, vol. 383, no. 3, p. 483-488. http://dx.doi.org/10.1007/s00216-005-3382-2

Eschenbruch, R. 1974. Sulfite and sulfide formation during winemaking – review. *American Journal of Enology and Viticulture*, vol. 25, no. 3, p. 157-161.

Dott, W., Truper, H. G. 1976. Sulfite formation by wine yeasts. 3. Properties of sulfite reductase. *Archivum Microbiology*, vol. 108, no. 1, p. 99-104.

Henick-Kling, T., Park, Y. H. 1994. Considerations fort he use of yeast and bacterial starter cultures – SO_2 and timing of inoculation. *American Journal of Enology and Viticulture*, vol. 45, no. 3, p. 464-469.

Jančářová, I., Jančář, L., Náplavová, A., Kubáň, V. 2014. A role of reductones in monitoring of the content of sulfur dioxide in wines during their maturation and storage. *Czech Journal of Food Science*, vol. 32, no. 3, p. 232-240.

Kubáň, P., Jánoš, P., Kubáň, V. 1998. Gas diffusion-flow injection determination of free and total sulfur dioxide in wines by conductometry. *Collection of Czech Chemical Communication*, vol. 63, no. 1, p. 770-782.

Rankine, B. C., Pocock, K. F. 1969. Influence of yeast strain on binding of sulphur dioxides in wines and on its formation during fermentation. *Journal of the Science Food Agriculture*, vol. 20, no. 2, p. 104.

Rankine, B. C. 1968. The influence of yeasts in determining the composition and quality of wines. *Vitis*, vol. 7, no. 1, p. 22-49. PMid:5777007

Romano, P., Suzzi, G. 1993. Sulfur dioxide and wine microorganism. In: Fleet G. H. (Ed.) *Wine Microbiology and Biotechnology*. Harwood Academic Publishers, Chur, Switzerland. pp. 373-393.

Suzzi, G., Romano, P., Zambonelli, C. 1985. *Saccharomyces* strain selection in minimizing SO_2 requirement during vinification. *American Journal of Enology and Viticulture*, vol. 36, no. 1, p. 199-202.

Špakovská, E., Marcinčák, S., Bača, M., Turek, P. 2012. Polyphenolic content and antioxidative activiny of wines from the Sobrance wine region. *Potravinarstvo*, vol. 6, no. 3, p. 35-35. http://dx.doi.org/10.5219/204

Wells, A., Osborne, J. P. 2011. Production of SO_2 binding compounds and SO_2 by *Saccharomyces* during alcoholic fermentation and the impact on malolactic fermentation. S*outh African Journal of Enology and Viticulture*, vol. 32, no. 2, p. 267-278.

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EFFECT OF DIFFERENT STORAGE CONDITIONS ON THE MICROBIOLOGICAL CHARACTERISTICS OF INSECT

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ABSTRACT
When introducing a novelty food, its safety needs to be monitored. One of the safety aspects of human health is microbial contamination. In this work, microbiological parameters of long-term stored edible insect material – mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), field cricket (*Gryllus assimilis*), and migratory locust (*Locusta migratoria*) were evaluated. The monitored indicators (colony forming units, enterobacteria, lactic acid bacteria, yeasts and moulds) were evaluated using common microbiological methods. All samples of stored insect were determined as safe for human consumption, except for the lesser mealworm sample from 2016, in which case the limit was exceeded. Sample of adult field cricket seems to be suitable for long-term storage, as it contained the lowest amount of microorganism. Sample of dried *Gryllus assimilis* from 2014 had the lowest microbial contamination. Further results suggest that, for long-term storage, the most suitable way of preparation is killing with boiling water, drying at 103 °C for 12 hours and subsequent hermetic packaging.

Keywords: edible insect; microbiota; microbiological safety; colony forming units; enterobacteria

INTRODUCTION
Microorganisms are part of every being, including edible insect. They are on the insect exoskeleton and inside the insect body. This microbiota can be dangerous for human health, thus it is necessary to pay attention to this problem when preparing human foodstuffs using edible insect, which belongs among novelty food **(van Huis et al., 2013; EFSA, 2015)**. Microorganisms produce enzymes with lipolytic and proteolytic abilities. They cause decomposition of fats and proteins in commodities, thus change the nutritional value of food **(Adams et Moss, 2002)**. According to **the Commission Regulation (EC) no. 2073/2005** the main source of human foodborne diseases is microbial danger. According to this regulation „Foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that pose an unacceptable risk to human health."

Microbiota in the insect digestive system is vital for its metabolism. It reflects the lifestyle of insect in the wild or its breeding conditions. In the culinary treatment, edible insect is often processed with intestinal contents, and it is therefore necessary to let the insects starve before further processing. Besides the microbiota found in the intestine, another microbiota is found on the insect's exoskeleton. Even this can be potentially dangerous for humans. This is the major safety risk in eating edible insects **(EFSA, 2015)**. However, microbiological limits have not yet been established for edible insects. Insects have the same allergen (chitin) as crustaceans, and from the culinary perspective, they are often compared with them. Because of this it could be possible to apply microbiological limits according to the food safety criteria defined in the **Commission Regulation (EC) no. 2073/2005** for the production of cooked crustaceans and molluscan shellfish. The limits in this category are set for microorganisms, their toxins and metabolites of *Salmonella* (absence in 25 g). It is also possible to use microbiological limits specified by **ČSN 56 9609**, which sets the M limit for total microorganism count 10^5 CFU.g^{-1}, 5.10^5 CFU.g^{-1} for *Escherichia coli* and absence of *Salmonella* spp. in 25 g.

There are very few specific scientific studies regarding the microbiological safety of edible insect bred in a controlled environment. Available literature declares a high number of bacteria $10^5 – 10^7$ CFU.g^{-1} **(van Huis et al., 2013; Grabowski et al., 2008)**. The most common bacteria in edible insect are: *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Proteus* spp., *Pseudomonas* spp., *Escherichia* spp., *Micrococcus* spp., *Lactobacillus* spp. and *Acetobacter* spp. **(EFSA, 2015)**.

Causal agent of alimentary diseases related to edible insect consumption can also be microscopic fungi (yeasts and moulds). Reason is the production of secondary

metabolites, which may be toxic to both animals and humans. Breeding environment has a very high impact on the concentration of the microscopic fibrous fungi, which are carried by insects. Another factor influencing the concentration of the micromycetes in processed material is further manipulation, processing and storage.

Scientific hypothesis

Scientific hypothesis is: Microbial load of long-term stored materials from selected edible insect species is safe for human consumption with a focus on the treatment. Drying and freezing and long-term storage of edible insect materials will ensure the microbial safety of this commodity for human consumption.

The aim of this work was to detect and compare the microbiological characteristic of long-term stored material produced using selected edible insect species, and based on the results choose the most suitable species for long-term storage.

MATERIAL AND METHODOLOGY

Material

Insect samples for the determination of microbiological parameters were obtained in cooperation with the Mendel University in Brno. The following species were analysed:

1. mealworm (*Tenebrio molitor* – TM) – larva, year of breeding 2012, 2015 and 2016
2. lesser mealworm (*Alphitobius diaperinus* - AD) – larva, year of breeding 2016
3. field cricket (*Gryllus assimilis* – GA) - nymph, year of breeding 2012 and 2016
4. field cricket (*Gryllus assimilis* – GA) – adult, year of breeding 2014
5. migratory locust (*Locusta migratoria* – LM) – adult, year of breeding 2012, 2015 and 2016

Live insect was put into sterile bag that was placed in a freezer box. Here the insect was killed and the microbiological characteristics were determined for the freshly killed samples (year of breeding 2016). This procedure was also used for 2012 samples, which were left in the freezer box for a long time. The rest of the samples were killed with boiling water, dried at 103°C for 12 hours, homogenized and subsequently stored at room temperature until analysis (January 2017).

Methods

Growth media and preparation of the dilution solution

To evaluate microbiological parameters, different types of growth media were used. To evaluate total count of mesophilic microorganism PCA (Plate Count Agar) produced by Hi Media Laboratories Pvt. Ltd., India was used. Dehydrated medium (20.5 g) was dissolved in 1000 mL of distilled water, stirred and sterilized by the procedure below.

Enterobacteria were evaluated using the VRBA (Violet Red Bile Agar) growth medium produced by Hi Media Laboratories Pvt. Ltd., India. Dehydrated medium (38.5 g) was dissolved in 1000 mL of distilled water stirred and sterilized by the procedure below.

Lactic bacteria (especially *Lactobacillus* spp.) were determined using MRS Agar (De Man Rogosa Sharpe Agar) from Oxoid Ltd., Great Britain. According to the instructions, 55.15 g of dehydrated soil and 15 g of agar were dissolved in 1000 mL of distilled water. Prepared growth medium was then stirred and sterilized by the procedure below.

Growth medium CHYGA (Chloramphenicol Yeast Glucose Agar) produced by Oxoid Ltd., Great Britain, was used to determine yeasts and moulds. Dehydrated medium (40 g) was dissolved in 1000 mL of distilled water. Prepared growth medium was then stirred and sterilized by the procedure below.

Sterilization of all growth media was done in autoclave at 121 °C for 20 minutes. After cooling, sterile growth media were put into sterile Petri dishes and after solidifying stored in the fridge upside down.

To prepare 1000 mL of dilution solution PPS (Physiological Peptone Solution) 1 g of peptone (Hi Media Laboratories Pvt. Ltd., India) and 8.5 g NaCl (PENTA, Ing. Petr Švarc, Czech Republic) were used. The weighed components were dissolved in 1000 mL of distilled water and the solution was sterilized in an autoclave at 121 °C for 20 minutes.

Sample processing

Samples were processed in January 2017. Weighing was different because of the different size and weight do specific insect. During subsequent homogenization, the insect was put into the homogenization bag with 50 mL of sterile PPS solution. The homogenization itself took 2 minutes using Stomacher homogenizer (Seward, Great Britain) and decimal dilution with PPS solution were then prepared. Homogenized sample represented dilution 10^0, and the dilution was done until 10^{-5}. Streak-plate inoculation was done out of each dilution, using 0.1 mL of inoculum.

Microbiological analysis

The following tests on microbiological analysis were performed:

1) the total number of aerobic mesophilic microorganisms (TNM) on Plate count agar (HiMedia, Bombai, India) at 30 °C for 48 h **(ISO Standard No. 4833-2; 2013)**;

2) the number of enterobacteria on violet red bile glucose agar (HiMedia) at 37 °C for 24 h **(ISO Standard No. 4832; 2006)**;

3) mesophilic lactic acid bacteria (LAB) on MRS agar (Oxoid, Basingstoke, UK) adjusted to pH 5.7 at 30 °C for 72 h **(ISO Standard No. 15214; 1998)**;

4) the number of yeasts and moulds on Chloramphenicol Yeast Glucose Agar (Oxoid, Basingstoke, UK) at 25 °C for 5 days **(ISO Standard No. 21527-1; 2008)**.

Results expression

Colony forming units, which grew in the Petri dishes on the growth media, were counted after the cultivation period. The following formulas (1, 2) were used **(Suchánková, 2016)**:

$$N = \frac{\frac{\sum c}{n} \times V_2}{V_1 \times d \times m} \quad (1)$$

$$N = \frac{\frac{\sum c}{n} \times V_2}{V_1 \times d \times Ks} \quad (2)$$

where:

N - total microorganisms [CFU.g^{-1}; CFU.mL^{-1}],
∑c - colony forming units (of each group of microorganisms) in all dishes used for counting,
d - diluent factor for each dilution,
V_1 - inoculum volume (pipetted sample or (suspension)) for each plate [mL],
V_2 - PPS volume used for sample homogenization [mL],
n - amount of dishes used for calculation,
m - sample weighing [g],
Ks - number of insect species used for microbiological analysis.

For the lowest dilution the formulas (3) and (4) were used:

$$N < \frac{1 \times V_2}{V_1 \times d_n \times m} \quad (3)$$

$$N < \frac{1 \times V_2}{V_1 \times d_n \times ks} \quad (4)$$

where:

d_n - diluent factor for the lowest dilution used for calculation.

Statisic analysis

The data were analysed using Excel 2013 (Microsoft Corporation, USA) and STATISTICA Cz version 12 (StatSoft, Inc., USA). Results were expressed by average and standard deviation. Each measurement was performed 3 times. Comparison of the results was performed using a Kruskal-Walllis test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Results of microbiological analysis for evaluated insects are shown in **T**Chyba! Nenašiel sa žiaden zdroj odkazov.. CFU did not exceed the limit for microbiological safety, except for the lesser mealworm (*Alphitobius diaperinus*), in whose case the limit was exceeded about 5x (2.3×10^6 CFU.g^{-1}). Selection of suitable thermal processing (in this case drying at 103 °C for 12 hours) leads to a reduction of the water activity **(Adams et Moss, 2002)**, thus reducing the amount of micro-organisms to a safe level. In the case of the CFU, a statistically significant difference (p <0.05) was found for mealworm (*Tenebrio molitor*), between both ways of heat treatment. Drying seems to be more effective as there was 10^5 × reduction (from 10^8 to 10^3), than freezing (10 × reduction). *Gryllus assimilis* and *Locusta migratoria* samples against freshly killed insects. Again, a statistically significant difference (p <0.05) was found between samples of freshly killed and dried insect. For these samples, there was at least 10^2 × reduction of the total number of microorganisms.

Recent studies **(Klunder et al., 2012; Vandeweyer et al., 2015; Stoops et al., 2016; Ssepuuya, 2017)** show microbiological characteristics for insect freshly killed with boiling water. Using this way of killing, our samples exceeded the limits for microbiological safety. Evaluation of the microbiological safety of samples, that were heat-treated and subsequently stored in boxes to prevent contamination from the environment, was done by **Grabowski (2017)**. This study suggests that in case of superworm (*Zophobas atratus*), boiling for 10 minutes and drying for 24 hours at 80 °C lowered CPM below the harmful level for 5 days of storage. When the temperature was 60 °C, CPM did not drop below the limit and the foodstuff remained inappropriate for human consumption. The last heat-treatment **Grabowski (2017)** tested was cooking for 30 minutes with subsequent drying at 80 °C for 12 hours and then drying at 100 °C for another 12 hours. It is interesting, that after this treatment, the CPM dropped below the limit only on the first day of storage, and for the rest of days it exceeded the limit. *Staphylococcus* spp. was not detected in these samples, on the contrary to other samples. In two-spotted cricket *(Gryllus bimaculatus)* **Grabowski (2017)** detected CPM above the limit in all samples.

When evaluating the microbial quality of 55 insect products in the Netherlands, 59 % of them were found to exceed the sanitary limits for aerobic bacteria

Table 1 Microbiological characteristic of material from selected insect species - Total microorganism count (CFU.g^{-1}), Enterobacteria (CFU.g^{-1}).

Species	Breeding year	Live cycle stage	Total microorganism count (CFU.g^{-1} ±SD)	Enterobacteria (CFU.g^{-1} ±SD)
Freshly killed				
Tenebrio molitor	2016	Larvae	$2.2 \times 10^8 \pm 2.4 \times 10^7$	$1.9 \times 10^8 \pm 7.1 \times 10^6$
Gryllus assimilis	2016	Nymphs	$3.3 \times 10^6 \pm 3.1 \times 10^5$	$3.5 \times 10^4 \pm 1.2 \times 10^3$
Locusta migratoria	2016	Nymphs	$2.8 \times 10^5 \pm 1.2 \times 10^4$	$1.5 \times 10^5 \pm 1.2 \times 10^4$
Frozen				
Tenebrio molitor	2012	Larvae	$3.4 \times 10^7 \pm 3.7 \times 10^6$	$4.2 \times 10^6 \pm 1.9 \times 10^5$
Gryllus assimilis	2012	Nymphs	$4.7 \times 10^6 \pm 3.9 \times 10^5$	$2.6 \times 10^5 \pm 1.2 \times 10^4$
Locusta migratoria	2012	Nymphs	$1.9 \times 10^6 \pm 1.4 \times 10^5$	$6.0 \times 10^4 \pm 2.5 \times 10^3$
Dried				
Tenebrio molitor	2015	Larvae	$6.6 \times 10^3 \pm 4.4 \times 10^2$	<10
Tenebrio molitor	2016	Larvae	$5.4 \times 10^3 \pm 1.9 \times 10^2$	<10
Alphitobius diaperinus	2016	Larvae	$2.3 \times 10^6 \pm 1.9 \times 10^5$	$1.6 \times 10^6 \pm 1.2 \times 10^5$
Gryllus assimilis	2014	Adults	$<10^2$	<10
Gryllus assimilis	2016	Nymphs	$7.1 \times 10^3 \pm 3.7 \times 10^2$	<10
Locusta migratoria	2015	Adults	$7.3 \times 10^3 \pm 3.9 \times 10^2$	<10

Table 2 Microbiological characteristic of material from selected insect species - Lactic acid bacteria [CFU.g^{-1}], Yeasts and moulds [CFU.g^{-1}].

Species	Breeding year	Live cycle stage	Lactic acid bacteria	Yeasts and moulds
Freshly killed				
Tenebrio molitor	2016	Larvae	$7.2 \times 10^7 \pm 3.1 \times 10^6$	$8.9 \times 10^3 \pm 3.7 \times 10^2$
Gryllus assimilis	2016	Nymphs	$5.8 \times 10^6 \pm 2.4 \times 10^5$	$4.4 \times 10^5 \pm 2.5 \times 10^4$
Locusta migratoria	2016	Nymphs	$1.5 \times 10^4 \pm 1.2 \times 10^3$	$< 2.2 \times 10^2$
Frozen				
Tenebrio molitor	2012	Larvae	$2.4 \times 10^5 \pm 1.2 \times 10^4$	$3.3 \times 10^4 \pm 2.4 \times 10^3$
Gryllus assimilis	2012	Nymphs	$5.0 \times 10^5 \pm 3.1 \times 10^4$	$5.1 \times 10^5 \pm 3.9 \times 10^4$
Locusta migratoria	2012	Nymphs	$1.5 \times 10^4 \pm 1.2 \times 10^3$	$1.5 \times 10^4 \pm 1.2 \times 10^3$
Dried				
Tenebrio molitor	2015	Larvae	$3.8 \times 10^3 \pm 2.1 \times 10^2$	$1.7 \times 10^4 \pm 1.9 \times 10^3$
Tenebrio molitor	2016	Larvae	$2.6 \times 10^3 \pm 1.9 \times 10^2$	$1.5 \times 10^3 \pm 1.2 \times 10^2$
Alphitobius diaperinus	2016	Larvae	$2.8 \times 10^6 \pm 2.5 \times 10^5$	$2.6 \times 10^6 \pm 1.9 \times 10^5$
Gryllus assimilis	2014	Adults	$<10^2$	$7.0 \times 10^2 \pm 5.5 \times 10^1$
Gryllus assimilis	2016	Nymphs	$2.2 \times 10^3 \pm 1.2 \times 10^2$	$6.0 \times 10^3 \pm 3.2 \times 10^2$
Locusta migratoria	2015	Adults	$1.6 \times 10^4 \pm 1.4 \times 10^3$	$2.6 \times 10^3 \pm 2.1 \times 10^2$

(10^6 CFU.g^{-1}). Analysed samples were freeze-dried with no further culinary treatment or processing. This study also evaluated the content of *Bacillus cereus* spores and the presence of bacteria *Clostridium perfringens*, *Salmonella* and *Vibrio*. *Clostridium perfringens*, *Salmonella* spp. and *Vibrio* spp. were not detected in samples and *Bacillus cereus* spores content was lower than 10^2 CFU.g^{-1} **(EFSA, 2015)** in 93 % of samples.

Klunder et al. (2012) did a microbiological evaluation of mealworm larvae (*Tenebrio molitor*), after he put them under the boiling temperature for several minutes. He found out, that *Enterobacteriaceae* were killed, but the spores survived the process and can be active again under optimal growth condition. The requested elimination of spores was done only by subsequent roasting. The case was similar for the house cricket (*Acheta domesticus*), where double culinary treatment (cooking and subsequent roasting) deactivated both the spore-forming bacteria and the *Enterobacteriaceae* bacteria. Dangerous limit ($> 10^5$ CFU.g^{-1}) was not exceeded in the samples, that were only boiled or only fried (10^3 CFU.g^{-1}.), but bacteria can multiply due to inappropriate storage, and the foodstuff may become harmful for consumers **(Hanboonsog et Durst, 2014)**. Belgian study, dealing with frozen samples of mealworm larvae (*Tenebrio molitor*) and migratory locust (*Locusta migratoria*), detected $10^7 - 10^9$ CFU.g^{-1} of aerobic bacteria and 10^4 CFU.g^{-1} of aerobic spores **(EFSA, 2015)**.

Another microorganisms evaluated in this study were the enterobacteria, which were not detected in any sample except for the lesser mealworm (*Alphitobius diaperinus*). In lesser mealworm (*Alphitobius diaperinus*) the detected value was 1.6×10^6 CFU.g^{-1}. This value is higher than in other samples because of breeding conditions and specific species. Although there has been a general reduction in the number of enterobacteria after freezing, a certain number of these bacteria were detected after this treatment. However, after drying, the number of enterobacteria was reduced below the detection limit. There is a statistically significant difference (p <0.05) between samples just after killing and after drying.

The available literature provides similar results after heat-treatment. **Klunder et al. (2012)** detected less than 10 CFU.g^{-1} of *Enterobacteriaceae* in boiled samples of the mealworm *(Tenebrio molitor)* and house cricket *(Acheta domestica)*. Values presented by this work comply with the mentioned references. Due to killing the specimens with boiling water the samples were heat treated for a short time, which, in accordance with the literature, effectively eliminated the enterobacteria.

Lactic acid bacteria (LAB) are widespread in nature, important considering the foodstuff and biotechnological point of view **(Tančinová et al., 2008)**, and have a positive impact on human health. Bacteria *Lactobacillus* spp. produce enzymes, that allow to decompose more complex substances in the foodstuff into simpler substances. Therefore LAB have beneficial effects mainly on the intestinal microbiota - improve the peristalsis of the intestines and prevent the growth of harmful bacteria. They also affect the immune system, enable the production of vitamins and help in the absorption of iron, calcium and other substances **(Agerholm-Larsen et al., 2000; Adams et Moss, 2002)**.

Due to the widespread availability of lactic acid bacteria in the wild, a relatively high amount of these bacteria was detected in freshly killed insect. **Vandeweyer et al. (2015)** evaluated freshly killed insect and reported $2.5 \times 10^7 - 1.6 \times 10^8$ CFU.g^{-1} in mealworm larvae (*Tenebrio molitor*) and 2.0×10^7-7.9×10^7 CFU.g^{-1} in the house cricket (*Acheta domestica*). **Stoops et al. (2016)** detected $4.0 \times 10^7 - 3.2 \times 10^8$ CFU.g^{-1} of LAB in freshly killed migratory locust nymphs (*Locusta migratoria*) and $1.0 \times 10^7 - 4.0 \times 10^8$ CFU.g^{-1} in mealworm (*Tenebrio monitor*). LAB detected in this work were from $<10^2$ CFU.g^{-1} in the field cricket *(Gryllus assimilis)* (adults 2014) to 2.8×10^6 CFU.g^{-1} in the lesser mealworm (*Alphitobius diaperinus*). In this sample the content of LAB significantly higher than in the rest of the samples. It is supposed, that in the rest of the samples the water activity dropped and stayed low during the whole storage period, which prevent microbial changes and subsequent foodstuff spoiling. Content of LAB in long-time stored

samples varied from 1.8×10^3 CFU.g^{-1} in migratory locust to 5.0×10^5 CFU.g^{-1} in field cricket nymphs. This proves that the heat treatment outside the optimal temperature range reduces the amount of LAB. When comparing the number of LABs between *Tenebrio molitor* larvae and *Gryllus assimilis* nymphs, a statistically significant difference (p >0.05) was not detected in the frozen samples, neither between the dried samples. On the other hand, there was a statistically significant difference (p <0.05) in freshly killed insects.

Yeasts and microscopic fibrous fungi, which produce mycotoxins with common influence on the human health, are also the indicators of foodstuff spoiling and environmental pollution **(Tančinová et al., 2008)**. Content of yeasts and moulds in the samples varied between 7×10^2 CFU.g^{-1} in the field cricket *(Gryllus assimilis)* (adult 2014) and 2.6×10^6 CFU.g^{-1} in the lesser mealworm (*Alphitobius diaperinus*). This sample contained a much higher amount of yeasts and moulds than other samples. Despite the heat treatment used for samples analysed in our work, which should eliminate yeasts and moulds, the subsequent storage took place in room temperature that is suitable for their growth. Results show that, yeasts and microscopic fibrous fungi are resistant to low temperatures and can prosper even in these conditions – their numbers grew. Other authors declare the content of yeasts and microscopic fibrous fungi $10^5 – 10^6$ CFU.g^{-1}. This work proves that drying is an effective way of reducing the numbers of yeasts and moulds in edible insects. In general, samples dried under laboratory conditions contained *Aspergillus* spp. *and Penicillium* spp. **(EFSA, 2015)**. Furthermore, *Fusarium* spp.*, Chaetomium* spp.*, Mucor* spp.*, Mucorales* spp.*, Alternaria* spp.*, Drechslera* spp. and *Phoma* spp. were detected in insects **(Spiegel, 2013)**.

Toxins produced by microscopic fibrous fungi *Aspergillus* spp.*, Penicillium* spp. and *Fusarium* spp. can be produced not only in the digestive track of insect, but can also come from the breeding substrate. Mycotoxins are considered toxic **(Tančinováet al., 2014)**, thus the concentration of these toxins may influence the mortality of bred specimens **(EFSA, 2015)**.

Mentioned facts lead to the conclusion, that the most suitable for long-term storage is the treatment chosen by us - killing with boiling water at 100 °C and then drying for 12 hours at 103 °C and storing in hermetically sealed containers. Microbial characteristics of individual insects in the freshly killed state are reported in the available literature. The amount of microorganisms in edible insects after various treatments (e.g. freshly killed, frozen, dried) depends not only on the species, feed or breeding conditions, but also on the specific conditions of its processing and storage. However, the conditions for the processing and storage of edible insect products are not yet legislatively established.

CONCLUSION

This work evaluated microbiological characteristic of selected edible insect species with regards to different storage period, and it was declared as safe for human consumption. The only exception was lesser mealworm (*Alphitobius diaperinus*), whose samples exceeded the limit. Regarding the microbiological characteristics during long-term storage, the best sample seems to be the adult field cricket (*Gryllus assimilis*) stored in 2014, which had the lowest microbial contamination.

Mentioned studies and research suggest that it is not good to consume insect in the freshly killed state, and heat treatment along with proper storage conditions is necessary. It is one of possible steps to eliminate alimentary diseases originating from edible insect. Our own results and references lead to the conclusion that, for long-term storage, the most appropriate procedure is to kill the insect with boiling water, dry it at 103 °C for 12 hours and subsequently hermetically pack it.

REFERENCES

Adams, M., Moss, M. O. 2002. *Food microbiology*. Cambrige: The royal society of chemistry. ISBN-10: 0-85404-611-9. https://doi.org/10.1038/sj.ejcn.1600937 PMid:10745279

Agerholm-Larsen, L., Raben, A., Haulrik, N., Hansen, AS., Manders, M., Astrup, A. 2000. Effect of 8 week intake of probiotic milk products on risk factors of cardiovascular dideases. *European Journal of Clinical Nutrition*, vol. 54, no. 4, p. 288-297.

ČSN 56 9609:2008. *Pravidla správné hygienické a výrobní praxe – Mikrobiologická pravidla pro potraviny. Princip stanovení a aplikace*. (*Rules of Good Hygienic and Production Practice - Microbiological Rules for Food. Principle of determination and application.*)

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

Grabowski, N. T., Nowak B., Klein G. 2008. Proximate chemical composition of long-horned and short-homed grasshoppers (*Acheta domesticus*, *Schistocerca gregaria* and *Phymateus saxosus*) available commercially in Germany. *Archiv für Lebensmittelhygiene*, vol. 59, no. 6, p. 204-208. https://doi.org/10.1016/j.ijfoodmicro.2016.11.005 PMid:27903420

Grabowski, N. T., Klein, G. 2017. Microbiology of processed edible insect products – Results of a preliminary survey. *International Journal of Food Microbiology*, vol. 243, p. 103-107.

Hanboonsog, Y., Durst, P. 2014. *Edible insects in Lao PDR: building on tradition to enhance food security*, Bangkok (Thailand): FAO 2014, 55 p. ISBN 978-92-5-108307-9.

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

ISO 4832:2006. *Microbiology of food and animal feeding stuffs—horizontal method for the enumeration of coliforms—colony-count technique*.

ISO 4833-2:2013. *Microbiology of the food chain—horizontal method for the enumeration of microorganisms—part 2: colony count at 30 degrees C by the surface plating technique*.

ISO 15214:1998. *Microbiology of food and animal feeding stuffs—horizontal method for the enumeration of mesophilic lactic acid bacteria—colony-count technique at 30 °C*.

ISO 21527-1:2008. *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 1: Colony count technique in products with water activity greater than 0,95*.

Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., Nout, M. J. R. 2012. Microbiological aspects of processing and storage of edible insects. *Food Control*, vol. 26, p. 628-631. https://doi.org/10.1016/j.foodcont.2012.02.013

Regulation (EC) 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338/1, 22.12.2005, p. 1-26.

van der Spiegel, M., Noordam, M., van der Fels-Klerx, J. 2013. Safety of Novel Protein Sources (Insects, Microalgae, Seaweed, Duckweed, and Rapeseed) and Legislative Aspects for Their Application in Food and Feed Production. *Comprehensive Reviews in Food Science and Food Safety,* vol. 12, p. 662-678, https://doi.org/10.1111/1541-4337.12032

Ssepuuya, G., Mukisa, I. M., Nakimbugwe, D. 2017. Nutritional composition, quality, and shelf stability of processed *Ruspolia nitidula* (edible grasshoppers). *Food Science & Nutrition*, vol. 5, no. 1, p. 103-112. https://doi.org/10.1002/fsn3.369

Stoops, J., Crauwels, S., Waud, M., Claes, J. 2016. Microbial community assessment of mealworm larvae (*Tenebrio molitor*) and grasshoppers (*Locusta migratoria migratorioides*) sold for human consumtion, *Food Microbiology*, vol. 53, part B, p. 122-127. https://doi.org/10.1016/j.fm.2015.09.010

Suchánková, J. 2016. *Mikrobiální kvalita vybraných druhů jedlého hmyzu* (*Microbial quality of selected species of edible insect*): diplomat theses. Zlín, Czech republic : Tomas Bata University in Zlin. 90 p.

Tančinová, D., Maková, J., Felšociová, S., Kačániová, M., Kmeť, V. 2008. *Mikrobiológia potravín*. (*Food microbiology*) Nitra: Slovak University of Agriculture in Nitra. 144 p.

Tančinová, D., Mokrý, M., Barboráková, Z., Mašková, Z. 2014. Mycobiota of spices and aromatic herbs. *Potravinarstvo*, vol. 8, no. 1, 2014, p. 172-177. http://dx.doi.org/10.5219/375

Vandeweyer, D., Lievens, B., Van-Campenhout, L. 2015. Microbial quality of edible insects reared on industrial scale in Belgium and the Netherlands. Conference Paper. In *nnovations in Food Packaging, Shelf Life and Food Safety. Munich, Germany, 15 - 17 September 2015, Abstract No. P1.35.*

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CHARACTERISTICS OF PAPRIKA SAMPLES OF DIFFERENT GEOGRAPHICAL ORIGIN

Václav Štursa, Pavel Diviš, Jaromír Pořízka

ABSTRACT

This study investigated 11 different kinds of ground paprika of different geographical origin and tried to find some correlations between their measured chemical composition and country of origin. The parameters examined in ground paprika were as follows: sample moisture, total content of ash, total content of lipids, total content of nitrogen, content of saccharides (glucose, fructose, sucrose), elemental analysis (selected elements were Ca, K, Mg, Na, Cu, Fe, P a Zn), ASTA color value and pH value of water extract. Average content of moisture in paprika was 10.7 ±1.7 %. Average content of ash in the paprika samples was 5.8 ±0.6. Average total lipid content in paprika was 10.6 ±3.3 %. Total content of nitrogen in paprika was 1.93 ±0.17 % in average. Content of fructose (316 ±92 $mg \cdot g^{-1}$), glucose (215 ±119 $mg \cdot g^{-1}$) and sucrose (92 ±41 $mg \cdot g^{-1}$) in ground paprika was measured by HPLC-ELSD. Elemental analysis has been performed by ICP-OES. Average content of individual elements was: Ca 27 ±7 $mg \cdot g^{-1}$, K 198 ±23 $mg \cdot g^{-1}$, Mg 23 ±4 $mg \cdot g^{-1}$, Na 20 ±4 $mg \cdot g^{-1}$, Cu 0.155 ±0.015 $mg \cdot g^{-1}$, Fe 1.2 ±0.4 $mg \cdot g^{-1}$, P 33 ±6 $mg \cdot g^{-1}$ and Zn 0.17 ±0.04 $mg \cdot g^{-1}$. Average ASTA color value of paprika samples was 119 ±31 ASTA. The pH value of paprika water extract was 5.13 ±0.12 in average. Obtained data were statistically processed with Analysis of Variance (ANOVA) on p <0.05 and with Principal Component Analysis (PCA). Statistical analysis of the data confirmed, that samples from more distant regions (Hungary, Spain, Turkey, Bulgaria) can be differentiated according to their different chemical composition, while samples from similar regions (Hungary, Slovakia, Romania) is more difficult to differentiate.

Keywords: paprika; *Capsicum anuum*; chemical analysis; geographical origin; PCA.

INTRODUCTION

Paprika as a spice are considered dried and ground fruits of certain plant varieties of *Capsicum anuum* var. *longum* L. Paprika *Capsicum* comes originally from Central America. It got to Europe thanks to Spanish travelers and was one of the first crops brought from America to Europe **(Peter et al., 2012)**. Today growing of paprika is spread all over the world. Paprika fruits after the harvest undergo some technological treatments which lead to spice product in kitchen used as a sweet paprika. Paprika is in cuisine mostly used for giving to meals taste and color **(Klimešová et al., 2015)**.

Paprika is a good source of many sensory and nutritionally significant compounds, such as compounds forming color pigment (capsanthin, capsorubin, cryptoxanthin, zeaxanthin etc.) **(Peter et al., 2012)**, flavor, pungent taste (capsaicin, dihydrocapsaicin) **(Popelka et al., 2017)**, antioxidant properties (ascorbic acid, tocopherol, polyphenols) **(Škrovánková et al., 2017)** and saccharides **(Márkus et al., 1999)**. Content of these different compounds in paprika depends mostly on geographical factors, such as geographical position, sea level, annual sum of rainfall, temperature during vegetation period, annual amount of sunlight and also composition of the soil **(Marschner, 1995)**. Other factors influencing chemical composition of paprika can be maturity of the fruits **(Peter et al., 2012)**, time of harvest **(Isidoro et al., 1995)** or ripening of the fruits after the harvest **(Kerek et al., 2015)**.

Chemical composition of ground paprika relates also to quality parameters of paprika. Quality of ground paprika, as a trade commodity, is judged also by ASTA value (from shortcut American Spice Trade Association). ASTA value is a number expressing amount of carotenoid colorants in acetone extract **(Isidoro et al., 1995)**.Content of carotenoids is important parameter which relates to quality and provenience of paprika. Other quality determining parameters are unit weight, paprika's moisture, content of ash or content of lipids. European paprika of highest quality comes from Hungary and Spain and some of them have Protected Designation of Origin (PDO) mark. Nevertheless, the market offer also ground paprika which doesn't reach the quality of the protected one. That is

reason paprika becomes a commodity, where different producers put effort on its adulteration.

As an adulteration of food we may consider any inadequacy of food product with food law or intended deception of consumer in order to reach higher financial profit. The main mechanism is composition change of the food or stating false information on product's label **(Hong et al., 2017)**. In the case of paprika we can as a fraud or adulteration consider false declaration of geographical origin, misuse of PDO mark, forming mixtures of higher and lower quality paprika's, adding of oleoresin or inorganic dashes etc. As the number of food frauds grow, need for faster and more sensitive techniques revealing the adulteration grows as well.

Proving authenticity of particular food is important for whole chain from the farmer, through producer to the final consumer. It is vital to set comprehensive rules and conditions which help consumer not to be fooled, or worse, harmed on his health **(Čížková et al., 2012)**. There are many different analytical methods to be used for authentification of paprika geographical origin or country of origin adulteration. The analytical techniques are chosen according to concrete commodity, demands of methods speed, sensitivity and type of adulteration detection. Mostly used analytical techniques to reveal food fraud are spectroscopic methods (ICP-OES/ICP-MS/Sr-IR-ICP-MS/IR spectroscopy/ Raman spectroscopy/ NMR), chromatographic methods (LC/HPLC or GC/GC-MS), methods using analysis of DNA (RAPD-PCR/HRM-PCR), immune-chemical methods (ELISA, Biosensors) or electrochemical methods (CE/ FZCE) **(Hong et al., 2017, Doyle et al., 2017)**.

Hand by hand with analytical techniques go also statistical analysis methods and forming of statistical models describing particular commodity. The most important is having enough parameters basing the similarity or difference of particular products and its specificity. Among mostly used statistical methods belong Cluster and Hierarchic Cluster Analysis (CA, HCA), Discriminatory Analysis (DA, DPLS. PLS-DA), Linear Discriminatory Analysis (LDA), Artificial Neural Net (ANN), Soft Independent Modeling Class Analogy (SIMCA) and Support Vector Machines (SVM) **(Doyle et al., 2017)**.

Scientific hypothesis

Aim of this study was to test hypothesis, whether chemical composition of ground paprika can be affected by geographical origin of the paprika plant.

MATERIAL AND METHODOLOGY

Total of 11 samples (Table 1) of ground paprika with different proveniences have been chosen for the analysis. Five of the samples were provided with a mark of Protected Designation of Origin (PDO). All of the samples were obtained from market chains in Czech Republic.

Sample preparation

Samples used for determination of total nitrogen content were mineralized in Kjeldahl digestion unit (Kjeldaterm, C.Gerhardt GMBH, Germany). Total of 1 g of sample was mixed with 2 g of Weiniger catalyst (Lachema a.s., Czech Republic) and was digested for 24 hours.

For determination of saccharides 1 g of sample was extracted with 10 mL of extraction solution (ultrapure water and ethanol mixed in ration 4:1) in a 50 mL centrifugation tube placed on vertical shake table (GFL, Germany). After 1 h of extraction, samples were centrifuged for 4 min at 6000 rpm in centrifuge (EBA 21, Hettich, Germany); supernatant was filtered using filter with 0.45 μm pore size (Labicom, Czech Republic) and filled up to 50 mL in a volumetric flask with ultrapure water.

Sample for elemental analysis was prepared using wet ashing method in a microwave oven (Milestone 1200, Milestone, Italy). Total of 0.25 g sample matrix was decomposed in a mixture of nitric acid (6 mL) (Analytika Praha spol. s.r.o., Czech Republic) and hydrochloric acid (2 mL) (Analytika Praha spol. s.r.o., Czech Republic). After the decomposition sample was filtered using filter with 0.45 μm pore size and filled up to 25 mL in a volumetric flask with ultrapure water.

For determination of ASTA value 0.1 g samples were extracted by 20 mL of acetone (VWR International S.A.S, France) on vertical shake table for 3 hours. All the samples were diluted by acetone in volume ratio 1:5.

Chemical analysis

Moisture, ash and total lipid content was determined according to methods specified in ISO method (**ČSN ISO 7540, 2010**). Total nitrogen content was determined according to Kjeldahl method (**ČSN ISO 1871, 2009**). An Agilent Infinity 1260 liquid chromatograph (Agilent Technologies, USA) equipped with ELSD detector was used for determination of saccharides. As a stationary phase for analysis was used Prevail Carbohydrates ES column (250/4.6 mm). Mobile phase was formed by acetonitrile mixed with water in volume ratio 75:25. An elemental analysis was performed using ICP-OES (Ultima 2, Horiba Scientific, France) according to procedure described by **Diviš et al. (2015)**. ASTA value was determined according to ISO method (**ČSN ISO 7541, 1989**), using spectrophotometer Helios Gamma (Spectronic Unicam, USA). The pH value was measured using pH meter with combined electrodes (WTW, Germany).

Statistical analysis

All experimental data were statistically processed using software XLstat (Addinsoft, USA). Obtained data were pretreated by using Analysis of Variance (ANOVA) to find statistical significant differences between geographical groups. Tukey's comparative test on the level of importance $p < 0.05$ has been performed for individual parameters among paprika samples.

The pretreated data were used as input parameters in Principal Component Analysis (PCA) to find correlation between the chemical composition of different samples and their geographical origin.

Table 1 Name of the samples, PDO mark, country of origin and producer.

Sample name	Sample description		
	PDO mark	Country of Origin	Producer
Pimentón de la Vera dulce	YES	Spain	Orencio Hoyo S.L.
Pimentón de la Vera picante	YES	Spain	Orencio Hoyo S.L.
Žitavská paprika sladká mletá	YES	Slovakia	Mäspoma spol. s.r.o.
Sweet paprika organic	NO	Bulgaria	Family Farm Tsar
Szegedi Paprika	YES	Hungary	Szegedi Paprika ZRt.
Kalocsai Édes	YES	Hungary	Édes ZRt.
Kirmizi Pulbiber	NO	Turkey	Karden Baharat Ltd.
Paprika sladká maďarská	NO	Hungary	Goldenway, s.r.o.
Paprika sladká španělská	NO	Spain	Goldenway, s.r.o.
Paprika sladká	NO	Romania	Opal a.s.
Magyar paprika	NO	Hungary	Thymosspol s.r.o.

RESULTS AND DISCUSSION

Moisture of ground paprika is a vital parameter which impacts stability of carotenoid dyes and microbial stability of the product. Low moisture enhances oxidation of nutritionally significant compounds (ascorbic acid, tocopherol, dyes). On the other hand, moisture content above 15 % helps to develop molds and other undesirable micro flora and breaking the safety of the food **(Chetti et al., 2014)**. High content of moisture also raises total weight of the product and helps producer to sell less product with higher profit. For ensuring the food safety and setting same conditions for all the producers all spice suppliers are obliged to comply demands on maximum moisture content in ground paprika, which is specified in Decree No. 162/2016. Czech legislation permits maximum moisture content in ground paprika to be 11 %.This condition has not been met at 3 samples: Kirmizi Pulbiber (Turkey), Szegedi Paprika (Hungary) and Sweet Paprika Organic (Bulgaria). Moisture content among samples varied between 8.7 ±0.1 % and 15.0 ±0.1 % **(Table 2)**. Lowest moisture content was measured in Hungarian paprika Kalocsai füszerpaprika örlemeny, the highest in Sweet Paprika Organic from Bulgaria. Average moisture content in analyzed paprika samples was10.7 ±1.7 %. Obtained results were compared to food databases and literature. American database **USDA (2015)** states moisture content in ground paprika to be 11.24 %, which is in the interval of the results obtained in this study, while Czech database **Nutridatabáze (2014)** states much lower moisture content in paprika, such as 7.9 %. Obtained results of moisture content comply with the results of **Duman et al. (2010)**, who measured paprika moisture content during different storage conditions. Results of **Duman et al. (2010)** varied from 9.68 ±0.31 % to 12.38 ±0.19 %.Moisture in paprika had been also investigated by **Zaki et al. (2013)**.Their average sample moisture was 9.5 ±0.9 %.

The ash content in sample determines amount of inorganic compounds in food. In the case of ground paprika the information of higher ash content can reveal mixing ground paprika with some inorganic dash **(Čížková et al., 2012)**. Maximum permitted ash content according to Czech legislation Decree No. 162/2016 is

Table 2 Content of moisture, ash, total lipids, total nitrogen and measured ASTA value at paprika samples.

Sample name	Parameters				
	Moisture (% ±SD)	Ash (% ±SD)	∑ Lipids (% ±SD)	∑ Nitrogen (% ±SD)	ASTA (- ±SD)
Pimentón de la Vera dulce	9.87 ± 0.04^{cd}	4.96 ± 0.02^{e}	11.5 ± 0.2^{cd}	2.09 ± 0.02^{ab}	115 ± 1^{g}
Pimentón de la Vera picante	8.49 ± 0.04^{a}	5.0 ± 0.2^{e}	15.5 ± 0.3^{a}	2.04 ± 0.04^{bc}	111 ± 1^{g}
Žitavská paprika sladká mletá	10.45 ± 0.04^{de}	5.5 ± 0.2^{d}	10.47 ± 0.4^{de}	2.18 ± 0.03^{a}	94 ± 1^{g}
Sweet paprika organic	15.0 ± 0.1^{a}	5.8 ± 0.2^{bcd}	2.38 ± 0.03^{g}	1.59 ± 0.04^{f}	105 ± 2^{f}
Szegedi Paprika	11.09 ± 0.01^{c}	5.5 ± 0.6^{d}	7.3 ± 0.1^{f}	2.07 ± 0.01^{abc}	127 ± 1^{e}
Kalocsai Édes	8.7 ± 0.1^{h}	7.04 ± 0.03^{a}	10.6 ± 0.3^{de}	1.76 ± 0.03^{e}	84 ± 1^{h}
Kirmizi Pul Biber	11.5 ± 0.2^{b}	6.3 ± 0.3^{b}	13.4 ± 0.3^{b}	1.69 ± 0.05^{ef}	82 ± 1^{h}
Paprika sladká maďarská	10.2 ± 0.1^{e}	6.2 ± 0.2^{bc}	12.3 ± 0.3^{bc}	1.96 ± 0.07^{cd}	172 ± 4^{a}
Paprika sladká španělská	9.9 ± 0.2^{f}	6.2 ± 0.4^{bc}	9.9 ± 0.2^{e}	1.98 ± 0.04^{bcd}	148 ± 2^{c}
Paprika sladká	9.2 ± 0.2^{g}	5.8 ± 0.2^{bcd}	9.94 ± 0.04^{de}	1.9 ± 0.1^{d}	136 ± 4^{d}
Magyar paprika	8.97 ± 0.01^{gh}	5.56 ± 0.04^{d}	12.6 ± 0.1^{bc}	1.99 ± 0.02^{bcd}	153 ± 1^{b}

Note: *All samples were made in triplicates. **Values in the same column with different letters are significantly different at $p < 0.05$.

Table 3 Content of saccharides and pH value of water extract of paprika samples.

Sample name	Parameters				
	Fructose ($mg.g^{-1}$ ±SD)	Glucose ($mg.g^{-1}$ ±SD)	Sucrose ($mg.g^{-1}$ ±SD)	∑ Saccharides ($mg.g^{-1}$ ±SD)	pH (- ±SD)
Pimentón de la Vera dulce	214 ±18^{c}	185 ±20^{bc}	45 ±4^{f}	444 ±74	4.94 ±0.05^{h}
Pimentón de la Vera picante	209 ±15^{c}	33 ±9^{e}	58 ±13^{def}	299 ±78	5.09 ±0.05^{ef}
Žitavská paprika sladká mletá	339 ±22^{b}	198 ±21^{bc}	127 ±11^{b}	664 ±88	5.01 ±0.05^{g}
Sweet paprika organic	565 ±24^{a}	541 ±33^{a}	177 ±9^{a}	1284 ±178	4.94 ±0.05^{h}
Szegedi Paprika	329 ±17^{b}	217 ±12^{bc}	127 ±7^{b}	673 ±83	5.10 ±0.05^{ef}
Kalocsai Édes	293 ±23^{b}	141 ±31^{cd}	52 ±4^{ef}	486 ±100	5.09 ±0.05^{f}
Kirmizi Pul Biber	209 ±16^{c}	74 ±25de	45 ±6^{f}	329 ±72	5.35 ±0.05^{a}
Paprika sladká maďarská	292 ±27^{b}	240 ±17^{d}	79 ±8^{cde}	611 ±91	5.14 ±0.05^{cd}
Paprika sladká španělská	307 ±18^{b}	236 ±21^{b}	87 ±9^{cd}	630 ±91	5.30 ±0.05^{b}
Paprika sladká	320 ±21^{b}	216 ±22^{bc}	66 ±7^{def}	603 ±104	5.13 ±0.05^{de}
Magyar paprika	361 ±12^{b}	258 ±26^{b}	139 ±8^{bc}	759 ±91	5.10 ±0.05^{ef}

Note: *All samples were made in triplicates. **Values in the same column with different letters are significantly different at p <0.05.

7.0 %. This condition has been met at all of the analyzed samples except Hungarian sample Kalocsai füszerpaprika örlemeny. The ash content varied from 5.5 ±0.2 % to 7.04 ±0.03 % **(Table 2)**. The highest content has been determined at sample Kalocsai füszerpaprika örlemeny, the lowest at samples Žitavská paprika (Slovakia) and Szegedi paprika (Hungary). Average content of ash of paprika samples was 5.8 ±0.6 %. Obtained results were compared with Czech food database and literature. Czech database **Nutridatabáze (2014)** states ash content in ground paprika to be 6.4 % hm. **Lee et al. (2017)** determined average ash content in paprika samples to be 5.14 %, **Zaki et al. (2013)** published average paprika ash content as to be 6.5 ±0.4 %.Results obtained in this study were in compliance with literature

Lipid content of ground paprika may help in revealing other type of food fraud. Higher content of total lipids in paprika might discover added lipophilic compounds (mostly oleoresins), which might help to rise ASTA value of the product **(Minguez-Mosquera et al., 1993)**. Lowest content of total lipids was determined at sample Sweet paprika organic from Bulgaria (2.38 ±0.03 %). The highest content of total lipids was determined at the sample Pimentón de la Vera picante from Spain (15.5 ±0.3 %). Total lipid content of each paprika sample is summarized in Table 2. Average content of total lipids was 10.6 ±3.3 %. Obtained data were compared with food databases and published literature. American database **USDA (2015)** determines total lipid content in paprika to be 12.89 % and Czech database **Nutridatabáze (2014)** determines total lipid content in paprika to be 13.8 %, which is close to the higher edge of results obtained in this study. **Zaki et al. (2013)** published total lipid content in paprika 8.4 ±2.6 %, which is in the range of results obtained in this study.

Kjeldahl method helps to get information about total nitrogen in sample, which can be recalculated as crude protein contained in food sample. Content of nitrogen depend on paprika variety, used agriculture technique and geographical origin **(Minguez-Mosquera et al., 1993)**.

Table 4 Content of macroelements in paprika samples.

Sample name	Macroelements			
	Ca ($mg.g^{-1}$ ±SD)	K ($mg.g^{-1}$ ±SD)	Na ($mg.g^{-1}$ ±SD)	Mg ($mg.g^{-1}$ ±SD)
Pimentón de la Vera dulce	29 ±3^{bc}	206 ±16^{b}	22±0.4^{f}	3.9 ±0.2^{g}
Pimentón de la Vera picante	29 ±2^{bc}	202 ±15^{bc}	25 ±0.4^{d}	5.2 ±0.3^{f}
Žitavská paprika sladká mletá	28 ±3^{bc}	178 ±13de	24 ±0.4^{e}	8.0 ±0.2^{c}
Sweet paprika organic	17 ±2^{d}	182 ±13de	17 ±0.3^{g}	3.4 ±0.4^{h}
Szegedi Paprika	37 ±3^{a}	189 ±10^{cd}	21 ±0.4^{f}	6.0 ±0.2^{e}
Kalocsai Édes	36 ±2^{a}	212 ±12^{b}	29 ±0.4^{a}	9.0 ±0.4^{b}
Kirmizi Pul Biber	30 ±3^{ab}	264 ±16^{a}	16 ±0.3^{h}	161 ±0.9^{a}
Paprika sladká maďarská	21 ±3^{cd}	186 ±13de	24 ±0.4^{e}	9.1 ±0.4^{b}
Paprika sladká španělská	26 ±3^{bc}	208 ±13^{b}	28 ±0.4^{b}	7.3 ±0.3^{d}
Paprika sladká	32 ±2^{ab}	175 ±17^{e}	27 ±0.4^{c}	7.5 ±0.3^{cd}
Magyar paprika	16 ±2^{d}	185 ±11de	17 ±0.3^{g}	3.4 ±0.2^{h}

Note: *All samples were made in triplicates. **Values in the same column with different letters are significantly different at p <0.05.

Table 5 Content of microelements in paprika samples.

Sample name	Microelements			
	Cu ($mg.g^{-1}$ ±SD)	Fe ($mg.g^{-1}$ ±SD)	P ($mg.g^{-1}$ ±SD)	Zn ($mg.g^{-1}$ ±SD)
Pimentón de la Vera dulce	0.15 ±0.05^{d}	0.97 ±0.02^{f}	36 ±2^{ab}	0.22 ±0.02^{a}
Pimentón de la Vera picante	0.15 ±0.05^{d}	1.69 ±0.03^{b}	38 ±2^{a}	0.20 ±0.02^{b}
Žitavská paprika sladká mletá	0.15 ±0.06^{d}	0.99 ±0.02^{f}	38 ±2^{a}	0.23 ±0.03^{a}
Sweet paprika organic	0.14 ±0.02^{e}	0.59 ±0.01^{i}	26 ±1^{d}	0.18 ±0.02^{c}
Szegedi Paprika	0.18 ±0.06^{b}	0.90 ±0.02^{g}	37 ±2^{a}	0.17 ±0.01^{cd}
Kalocsai Édes	0.15 ±0.05^{d}	1.68 ±0.04^{b}	37 ±2^{a}	0.13 ±0.01^{e}
Kirmizi Pul Biber	0.15 ±0.05^{d}	0.69 ±0.02^{h}	18 ±2^{e}	0.14 ±0.03^{e}
Paprika sladká maďarská	0.15 ±0.06^{d}	1.21 ±0.03^{d}	34 ±2^{c}	0.13 ±0.02^{e}
Paprika sladká španělská	0.04 ±0.03^{e}	1.32 ±0.03^{c}	37 ±2^{a}	0.10 ±0.02^{f}
Paprika sladká	0.16 ±0.02^{c}	1.82 ±0.04^{a}	35 ±2^{bc}	0.16 ±0.01^{d}
Magyar paprika	0.19 ±0.06^{a}	1.05 ±0.03^{e}	37 ±2^{a}	0.16 ±0.01^{d}

Note: *All samples were made in triplicates. **Values in the same column with different letters are significantly different at p <0.05.

Content of nitrogen varied among samples between 1.59 ±0.04 and 2.18 ±0.03 % **(Table 2)**. The lowest content of nitrogen was found in Bulgarian sample Sweet Paprika Organic, while the highest content of nitrogen contained Slovakian sample Žitavská paprika. Average content of nitrogen in samples was 1.93 ±0.17 %. Results were compared with food databases and with results of other authors. Czech database **Nutridatabáze (2014)** states total nitrogen content in ground paprika to be 2.4 %. **Giuffrida et al., (2013)** during investigation of different kinds of paprika came to similar results (1.91 ±0.14 %).

Saccharides impact the taste of paprika, but they are also important during pollen development **(Shaked et al., 2004)** and help seed to withstand stress from desiccation **(Demir et al., 2008)**. Observing amount of saccharides in paprika can describe development of ripening processes in paprika **(Asnin et al., 2014)**. The most abundant carbohydrate in paprika samples was fructose. Fructose content varied between 209 ±15 and 565 ±54 $mg \cdot g^{-1}$ **(Table 3)**.The lowest concentration has been measure at Spanish sample Pimentón de la Vera picante and highest at sample Sweet paprika organic from Bulgaria. Average content of fructose was 316 ±92 $mg \cdot g^{-1}$. Second most abundant carbohydrate in paprika samples was glucose. Glucose content varied from 33 ±9 to 541 ±33 $mg \cdot g^{-1}$ **(Table 3)**.The lowest concentration was measured in Spanish sample Pimentón de la Vera picante and the highest in sample Sweet paprika organic from Bulgaria. Average glucose content was 215 ±119 $mg \cdot g^{-1}$.The least abundant saccharide was sucrose. Average content of sucrose was 92 ±41 $mg \cdot g^{-1}$. The lowest concentration was measured at sample Kirmizi Pulbiber from Turkey (45 ±6 $mg \cdot g^{-1}$).The highest concentration of sucrose was determined at sample Sweet paprika organic from Bulgaria (177 ±9 $mg \cdot g^{-1}$). Obtained data **(Table 3)** were compared with Czech food database **Nutridatabáze (2014)**, which states content of fructose to be 770 $mg \cdot g^{-1}$, glucose 300 $mg \cdot g^{-1}$ and sucrose 70 $mg \cdot g^{-1}$.Results measured in this study are in compliance with data published in database **Nutridatabáze (2014).**

Determination the mineral content of the sample is one of effective tools to consider origin of the paprika sample. Content of mineral compounds complies not only with the plant variety, but also with the soil and geographical location, where the paprika plant grows **(Brunner et al., 2014)**. Contents of calcium, potassium, magnesium, sodium, copper, iron, phosphorus and zinc were measured in this study (**Table 4, Table 5**). From all investigated elements most abundant was potassium with average concentration of 199 ±17 $mg \cdot g^{-1}$. The least abundant elements were copper and zinc. Average concentration of copper was 0.155 ±0.006 $mg \cdot g^{-1}$ and average concentration of zinc was 0.165 ±0.015 $mg \cdot g^{-1}$. The highest content of minerals has been found at Turkish sample Kirmizi Pulbiber. On the other hand the lowest content of minerals was found in sample Sweet Paprika Organic from Bulgaria. All obtained data are summarized in Table 4 and Table 5.

ASTA value is the basest qualitative parameter of ground paprika and describes content of carotenoid dyes. The content of carotenoid dyes depends on quality of the breed, freshness, storage conditions and other factors **(Peter et al., 2012)**. ASTA value varied in different samples between 82 ±1 and 172 ±4 ASTA **(Table 2)**. Average ASTA value in paprika samples was 119 ±31 ASTA. The highest ASTA value was measured at samples Paprika sladká maďarská (Hungary), Paprika sladká španělská (Spain) and Magyar paprika sladká (Hungary). Their average ASTA value was 158 ±1 ASTA. The lowest ASTA color was determined at samples Kirmizi Pulbiber (Turkey) and Kalocsai füszerpaprika örlemeny (Hungary), where average ASTA was measured to be 83 ±1 ASTA. Obtained results were in compliance with results of **Zaki et al. (2013)** and **Molnár et al. (2018)**. **Zaki et al. (2013)** measured ASTA at Moroccan paprika and resulted 125 ±12 ASTA. **Molnár et al. (2018)** determined ASTA in Peruvian paprika 140 ±35 ASTA and in Serbian paprika 101 ±28 ASTA.

The pH values of paprika samples varied in range from 4.94 ±0.05 to 5.35 ±0.05 **(Table 3)**. Average pH of all samples was 5.13 ±0.12. The lowest pH was determined at sample Sweet Paprika Organic from Bulgaria. The highest pH was determined at sample Kirmizi Pulbiber from Turkey. **Zaki et al. (2013)** and **Lee et al. (2017)**

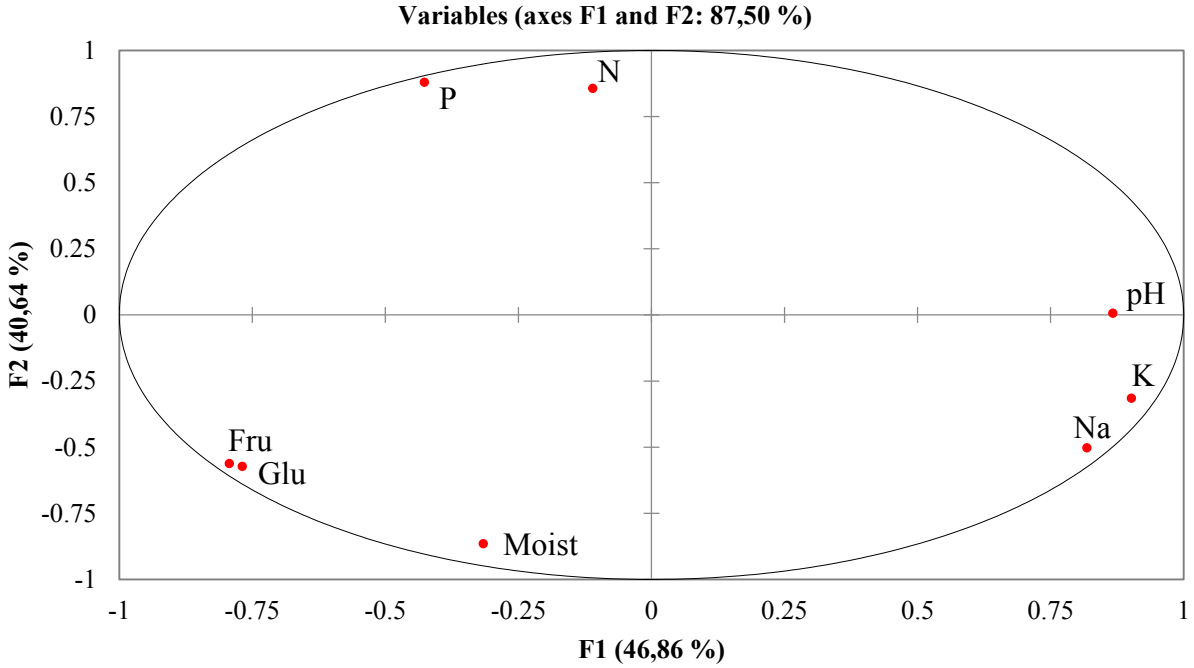

Figure 1 Projection of variables into the PCA factor plane of principal components F1 and F2 (Correlations between variables and factors).

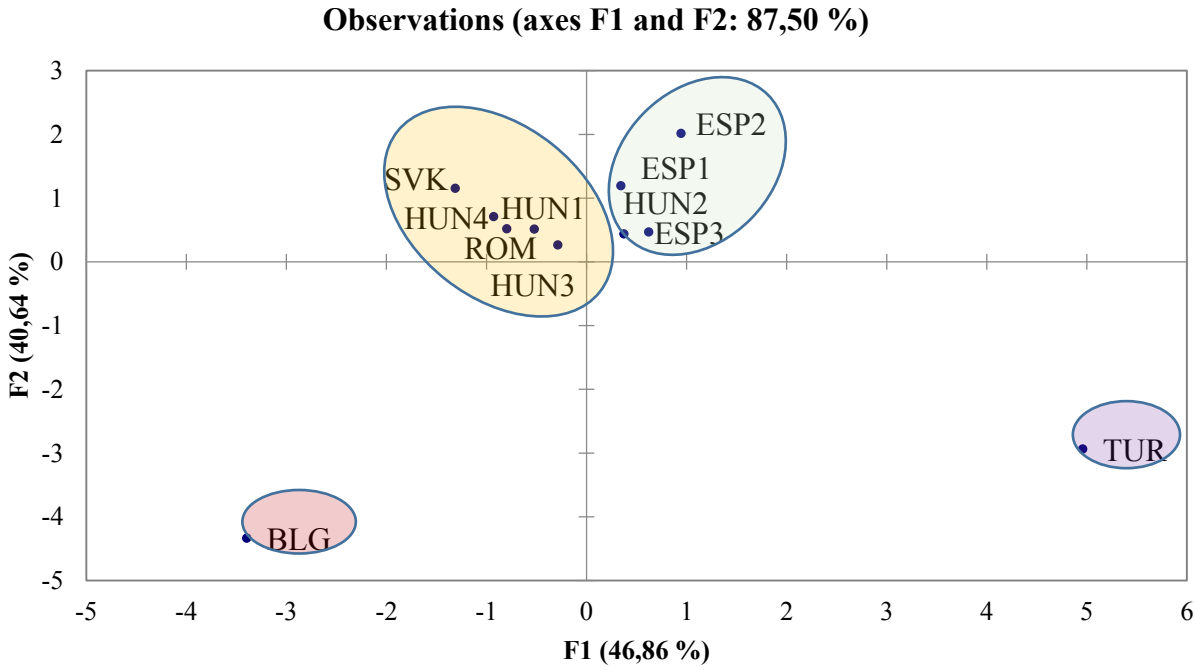

Figure 2 Projection of the PCA score of paprika geographical origin into the 2D factor plane of principal components F1 and F2. BLG – Bulgaria, ESP – Spain, HUN – Hungary, SVK – Slovakia, TUR – Turkey.

determined pH of paprika samples in the same range. **Zaki et al. (2013)** measured average pH of paprika samples to be 5.5 ±0.4, while **Lee et al. (2017)** determined average pH to be 5.05 ±0.02.

The data were processed by ANOVA and Tukey comparative test on the significance level 0.05. ANOVA was used for pretreatment of the data to find variables which exhibit statistical significant differences between the geographical groups of paprika. Statistical significant variables were sample moisture ($F = 5.6537$, $p = 0.0401$), concentration of fructose ($F = 9.6446$, $p = 0.0132$), potassium ($F = 11.4762$, $p = 0.0090$), sodium ($F = 782.9995$, $p < 0.0001$) and phosphorus concentration ($F = 43.1197$, $p = 0.0004$). Other 3 variables, which were bordering with the significance level 0.05, were total content of nitrogen ($F = 4.7834$, $p = 0.0555$), concentration of glucose ($F = 4.5750$, $p = 0.0603$) and pH value ($F = 4.5087$, $p = 0.0620$).

After ANOVA pre-treatment of the data 8 input parameters have been selected into PCA. Obtained 8 input

parameters have been reduced into 2 principal components with eigenvalue >1. According to the Kaiser criterion, components with eigenvalue less than one were excluded (F3, F4, F5, F6, F7, F8). Selected principal components F1 and F2 carried together 87.50 % of the variability of the original data set. Principal components were between each other more or less negatively or positively correlated with input variables **(Figure 1)**. Component F1 was strongly positively correlated with concentration of sodium and potassium and pH value. At the same time component F1 was strongly negatively correlated by concentration of glucose and fructose. Component F2 was strongly positively correlated by concentration of phosphorus and nitrogen. Strong negative correlation with component F2 have been observed with sample moisture and less negative correlation have been observed with amount of sodium, glucose and fructose.

The variables correlated also between each other. **(Figure 1)**. Intervariable correlations have been observed between sample moisture and concentration of glucose and fructose. Glucose and fructose are both monosaccharides participating in glycolysis. Correlation between moisture content and concentration of saccharides in ground paprika might depend on moisture, because stability of organic compounds in ground paprika depends (except other factors) also on sample moisture **(Chetti et al., 2014)**. Sample moisture showed a weak negative correlation with nitrogen content. On the other hand nitrogen content strongly positively correlated with content of phosphorus. Both of these elements belong among biogenic elements very abundant in living organisms. Weak negative correlation has been observed between nitrogen content and the rest of the parameters. As it was mentioned above, glucose and fructose content strongly positively correlated with each other and also with sample moisture. Fructose content showed strong negative correlation with pH value. Other parameters showed weak negative correlation with fructose and glucose. Another strong positive correlation has been observed between sodium and potassium content. These two elements form in living organisms' sodium-potassium pump. Content of these two elements strongly positively correlated with pH value. On the other hand has been observed strong negative correlation between sodium and potassium with phosphorus. Weak negative correlation has been observed with sodium and potassium in case of nitrogen. Phosphorous concentration had strong positive correlation with nitrogen. Strong negative correlation has been observed between concentration of phosphorus and concentration of sodium and potassium as well as sample moisture. Last observed parameter was pH value of the sample, which strongly positively correlated with sodium and potassium, but strongly negatively correlated with fructose content. Other parameters showed weak negative correlation with pH value.

Best possible graphical characterization of relations between paprika samples is dispersion of observations into the 2D factor plane of principal components F1 and F2 **(Figure 2)**. From the planar projection can be observed, with one exception, that samples have been divided into 2 clusters depending on their geographical origin. The first cluster includes samples from Hungary and geographically contiguous regions (Slovakia, Romania). The cluster is positioned in the first quadrant, which means positive correlation with component F2 and negative correlation with component F1. On the other hand the second cluster includes samples from Spain (with one exception, which is Hungarian sample from Kalocsai region). These samples forming the second cluster are positioned in second quadrant, which means positive correlation with both components F1 and F2. Sweet Paprika Organic (Bulgaria) and Kirmizi Pulbiber (Turkey) were projected separately from other samples forming clusters. Sweet Paprika Organic can be seen in third quadrant and Kirmizi Pulbiber in fourth quadrant.

Specifics of different combinations of observations, sample parameters and their variables can be visualized in 2D planar projection **(Figure 1 and Figure 2)**. Samples of paprika forming cluster in the first quadrant (samples from Hungary, Slovakia and Romania) have shown higher concentrations of phosphorous, nitrogen and saccharides. On the other hand samples forming the second cluster positioned in second quadrant (samples from Spain and Kalocsai sample) have shown lower content of saccharides, but higher content of sodium and potassium. Entirely different was sample Sweet Paprika Organic from Bulgaria, which in comparison to other samples had the highest content of saccharides, which led to projection this sample in the third quadrant. Similar observation was at Turkish sample Kirmizi Pulbiber, which differed from the other samples by the highest content of sodium and potassium, which led to projection this sample in the fourth quadrant.

CONCLUSION

Statistical analysis of obtained data confirmed hypothesis, that chemical content of paprika is influenced by geographical origin of the paprika plant. Samples from more distant regions (Hungary, Spain, Turkey, and Bulgaria) were, according to chemical analyses, successful to differentiate, while samples of paprika from similar regions (Hungary, Slovakia, Romania) were more difficult to differentiate. To separate samples of ground paprika only by their geographical origin, more complex analysis using other analytical method and obtaining more input data for multivariate analysis would be needed.

REFERENCES

Asnin, L., Park, S. W. 2014. Isolation and Analysis of Bioactive Compounds in Capsicum Peppers. *Critical Reviews in Food Science and Nutrition*, vol. 55, no. 2, p. 254-289. https://doi.org/10.1080/10408398.2011.652316

Brunner, M., Katona, R., Stefánka, Z., Prohaska, T. 2010. Determination of the geographical origin of processed spice using multielement and isotopic pattern on the example of Szegedi paprika. *European Food Research and Technology*, vol. 231, no. 4, p. 623-634.

Čížková, H., Ševčík, R., Rajchl, A., Pivoňka, J., Voldřich, M. 2012. Trendy v autenticitě potravin a v přístupech k detekci falšování. *Chemické listy*, vol. 106, no. 10, p. 903-910.

ČSN EN ISO 7540. 2010. *Ground paprika (Capsicum annuum* L.*) – Specification.*

ČSN EN ISO 1871. 2009. *Food and feed products – General guidelines for the determination of nitrogen by the Kjeldahl method.*

ČSN ISO 7541. 1989. *Ground (powdered) paprika – Determination of total natural colouring matter content.*

Demir, I., Teknin, A., Okmen, Z., Okcu, G. 2008 Seed Quality, and Fatty Acid and Sugar Contents of Pepper Seeds (*Capsicum annuum* L.) in Relation to Seed Development and Drying Temperatures. *Turkish Journal of Agriculture and Forestry*. vol. 32, p. 529-536.

Divis, P., Porizka, J., Vespalcova, M., Matejicek, A., Kaplan, J. 2015. Elemental composition of fruits from different black elder (*Sambucus nigra* L.) cultivars grown in the Czech Republic. *J. Elementol.*, vol. 20, p.549-557.

Doyle, N., Swain, D., Roberts, J. J., Cozzolino, D. 2017. The Use of Qualitative Analysis in Food Research and Technology: Considerations and Reflections from an Applied Point of View. *Food Analytical Methods*, vol. 10, p. 964-969. https://doi.org/10.1007/s12161-016-0654-8

Duman, A. D. 2010. Storage of red chili pepper under hermetically sealed or vacuum conditions for preservative of its quality and prevention of mycotoxine occurrence. *Journal of Stored Products Research*, vol. 46, p. 155-160. https://doi.org/10.1016/j.jspr.2010.02.002

Giuffrida, D., Dugo, P., Torre, G., Bignardi, C., Cavazza, A., Corradini, C., Dugo, G. 2013. Characterization of 12 Capsicum varieties by evaluation of their carotenoid profile and pungency determination. *Food Chemistry*, vol. 140, p. 794-802. https://doi.org/10.1016/j.foodchem.2012.09.060 PMid:23692768

Hong, E., Lee, S. Y., Jeong, J. Y., Park, J. M., Kim, B. H., Kwon, K., Chun, H. S. 2017. Modern analytical methods for the detection of food fraud and adulteration by food category. *Journal of the Science of Food and Agriculture*, vol. 97, p. 3877-3896. https://doi.org/10.1002/jsfa.8364 PMid:28397254

Chetti, M. B., Deepa, G. T., Antony, R. T., Khetagoudar, M. C., Uppar D. S., Navalgatti, C. M. 2014. Influence of vacuum packaging and long term storage on quality of whole chilli (*Capsicum annuum* L.). *Journal of Food Science and Technology*, vol. 51, p. 2827-2832. https://doi.org/10.1007/s13197-012-0763-3

Isidoro, E., Cotter, D. J., Fernandez, G. C. J., Southward, G. M. 1995. Color Retention in Red Chile Powder as Related to Delayed Harvest. *Journal of Food Science*, vol. 60, p. 1075-1077. https://doi.org/10.1111/j.1365-2621.1995.tb06295.x

Kerek, M., Ugróczy-Nagy, K., Mihály, M., Slezák, K., Jezdinský, A. 2015. The Effect of Postharvest Ripening Duration on Spice Pepper Powder Pigment and Sugar Content. *Acta Horticulturae*, vol. 1079, p. 619-625. https://doi.org/10.17660/ActaHortic.2015.1079.84

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, p. 154-159. https://doi.org/10.5219/440

Lee, H. S., HONG, J. Y., CHOI, Y. J., Lee, Y. S., Seo, S. J., Shin, S. R. 2017. Characteristics in the components of the paprika by drying methods. *Korean Journal of Food Preservation*, vol. 24, 497-504. https://doi.org/10.11002/kjfp.2017.24.4.497

Márkus, F., Daood, H. G., Kapitány, J., Biacs, P. A. 1999. Change in the Carotenoid and Antioxidant Content of Spice Red Pepper (Paprika) as a Function of Ripening and Some Technological Factors. *Journal of Agricultural and Food Chemistry*, vol. 47, p. 100-107. https://doi.org/10.1021/jf980485z PMid:10563856

Marschner, H. 1995. *Mineral nutrition of higher plants*. 2nd ed. London, UK : AcademicPress, 889 p. ISBN 9780124735439.

Minguez-Mosquera, M. I., Hornero-Mendez, D. 1993. Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.), paprika, and oleoresin by reversed-phase HPLC. *Journal of Agricultural and Food Chemistry*, vol. 41, p. 1616-1620. https://doi.org/10.1021/jf00034a018

Molnár, H., Kónya, E., Zalán, Z., Bata-Vidács, I., Tömösközi-Farkas, R., Székács, A., Adányi, N. 2018. Chemical characteristics of spice paprika of different origins. *Food Control*, vol. 83, p. 54-60. https://doi.org/10.1016/j.foodcont.2017.04.028

Nutridatabáze, 2014. [online database], Available at: http://www.nutridatabaze.cz/potraviny/?id = 262.

Peter, K. V., 2012. *Handbook of herbs and spices*. 2nd ed. Oxford, UK: Woodhead. Woodhead Publishing series in food science, technology and nutrition. ISBN 978-0-85709-039-3.

Popelka, P., Jevinová, P., Šmejkal, K., Roba, P. 2017. Antibacterial activity of Capsicum extract against selected strains of bacteria and micromycetes. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, p. 223-229. https://doi.org/10.5219/731

Shaked, R., Rosenfeld, K., Pressman, E. 2004. The effect of low night temperatures on carbohydrates metabolism in developing pollen grains of pepper in relation to their number and functioning. *Scientia Horticulturae*, vol. 102, p. 29-36. https://doi.org/10.1016/j.scienta.2003.12.007

Škrovánková, S., Mlček, J., Orsavová, J., Juríková, T., Dřímalová, P. 2017. Polyphenols content and antioxidant activity of paprika and pepper spices. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, p. 52-57. https://doi.org/10.5219/695

USDA: United States Department of Agriculture, 2015. [online database], [cit. 2017-03-20]. Available at: https://ndb.nal.usda.gov/ndb/foods/show/278?manu = &fgcd = &ds = .

Zaki, N., Hakmaoui, A., Ouatmane, A., Fernandez-Trujillo, J. P. 2013. Quality characteristics of Moroccan sweet paprika (Capsicum annuum L.) at different sampling times. *Food Science and Technology (Campinas)*, vol. 33, p. 577-585. https://doi.org/10.1590/S0101-20612013005000072

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STUDY ON THE MEAT ISOTOPIC COMPOSITION FOR ORIGIN IDENTIFICATION

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ABSTRACT

Russian consumer and governmental authorities are equally concerned to know where food products come from. This requires more accurate and specialized methods for the evaluation of geographical location. The following methods are used: chemometrics, histological and histochemical, genomic and proteomic, microbiological, immunochemical and mass spectrometric. Method of stable isotope analysis is becoming increasingly promising nowadays for the identification of meat and meat products‘ place of origin. The isotope ratios of the four elements - carbon, nitrogen, oxygen and hydrogen, are mainly determined. The method is successfully used to identify a country of origin of wines, juices and water. The aim of the research was to study the stable isotope ratios for pork and beef samples purchased in Moscow supermarkets (Russian Federation). The country of production of meat samples was determined according to specifications and/or labels. The geography of countries of meat samples origin includes Europe, both America continents and Australia. Databases collected by the All-Russian Scientific Research Institute of the Brewing, Non-Alcoholic and Wine Industrywere used for the analysis and interpretation of the results. Values of $^{13}C/^{12}C$, $\delta^{13}C$, $^{18}O/^{16}O$, $\delta^{18}O$, $^{2}H/^{1}H$, $\delta^{2}H$ for 30 pork and beef samples from 13 countries were obtained. Differences in stable isotope ratios were found depending on place of origin. The data correlated with the oxygen isotope characteristics for wine, which were in the range from 2.5 to 4.5 ppm. According to the $^{13}C/^{12}C$, $\delta^{13}C$ results, the assumption was made about a false indication of the region for the beef sample. Despite the fact that beef was labeled as a product of Lithuania, the region of origin was most probably defined as Germany. The studies carried out showed the possibility to identify the region of raw meat origin by the stable isotope ratio.

Keywords: region of origin; food; authenticity; stableisotope ratio; meat

INTRODUCTION

Nowadays, intentional fraud and falsificationof foods cause concerns of the governments in many countries of the world as they can create a threat to the health safety of the population and lead to a certain economic losses. To undertake effective corrective actions against food fraud, precise methods of analysis that ensure appropriate repeatability and reproducibility of the research results are necessary.

Many countries have legislative requirements that oblige manufacturers to indicate a region or country of food origin on a label or in accompanying documents. The information about the country of origin of a product or raw materials can be especially important in case of severe restrictions associated with safety, for example, in disease outbreaks or when producing products of protected designation of origin (PDO). Modern analytical physico-chemical and microbiological methods of investigation allow establishing food origin with a certain precision. As a rule, these methods are directed towards revealing a relationship between the analyzed samples and the indicators that characterize one or another region. These indicators include the quantity or ratio of micro-, macro-elements, heavy metals, radioelements, stable isotopes, comparison of DNA **(Lo and Shaw, 2018; Kawaguchi et al., 2018)**, microflora of a sample **(Nguen et al., 2008)** and a reference. The differences in the content of ^{90}Sr in cheeses produced in different European countries were found. Radioisotope analysis allows establishing the $^{234}U/^{238}U$ ratio, which can be used in origin identification **(Peres et al. 2007)**. The geological and technogenic factors affect distribution of toxic, micro- and macro-elements. The difference between green tea leaves from China, India and Japan was found **(Brzezicha-Cirocka, Grembecka and Szefer, 2016)**. The similar research was performed earlier on fresh fruit from Europe, Asia, Africa and America regarding the content of Ca, Mg, K, Na, P, Co, Mn, Fe, Cr, Ni, Zn, Cu **(Grembecka and Szefer, 2013)**.

As for animal-derived products, it should be noted that the microelement content in animal meat depends on different factors, such as feed consumption, drinking water, soil polution and composition, which, in turn, depends on the geographical origin **(Ballin, 2010)**. The content of different elements was determined in the

poultry meat and dried beef samples **(Franke et al., 2008)**, milk and cheese **(Osorioet al., 2015)**.

Tretyakov et al. (2012) found it possible to use the fingerprinting technique based on the data of mass-spectrometric analysis to identify geographical origin falsification for food products such as meat and red caviar as the ratio of macro- and micro-components is unique.

The main elemental constituents (H, C, N, O и S) of bio-organic material have different stable isotopes (^{2}H, ^{1}H, ^{13}C, ^{12}C, ^{15}N, ^{14}N, ^{18}O, ^{17}O, ^{16}O, ^{36}S, ^{34}S, ^{33}S, ^{32}S and others). The isotope ratio can be measured precisely using the isotope ratio mass spectrometry (IRMS) **(Camin et al., 2016)**. The directions of analysis of the stable isotope composition used for foods can be the following:

- isotope ratio for one element in more than one substance (fraction) of a product (intermolecular isotope correlation);
- analysis of the prevalence of isotopes of one element in a molecule (intramolecular isotopic heterogeneity **(Zyakun, 2015)**;
- determination of the isotope ratios for more than one element in a certain substance (multi-element stable isotope analysis);
- combination of theabove-mentionedmethods **(Rossmann, 2001)**.

Schellenberg et al. (2010) applied IRMS to determine the geographical origin of honey based on the stable isotope ratios of carbon, nitrogen, hydrogen and sulphur; potato, for which it was established that $\delta^{18}O$ was the most discriminating variable in three geographical groups of samples **(Longobardi et al., 2011)**; sesame seeds and sesame oil **(Horacek, et al., 2015)**, butter **(Rossmann et al., 2000)**, seafood **(Ortea and Gallardo, 2015)**.

The investigations of the isotopic composition of milk were carried out with the aim to identify and reveal variations by the region of origin **(Camin et al., 2016 Brescia et al., 2005)**. It was concluded that milk from the regions, where pastures prevailed, usually showed relatively negative $\delta^{13}C$ values, while in the regions where agriculture dominated, the $\delta^{13}C$ values were more positive; the $\delta^{15}N$ values, as was noted earlier, depend on the soil conditions, intensity of the agricultural use and climate **(Kornexl, Werner, Roßmann, and Schmidt, 1997)**. In the milk samples from Australia, the high content of ^{18}O and ^{34}S was found compared to the known values of dairy products from the European countries **(Crittendenet al., 2007)**.

Analysis of the stable isotopes of light elements is actively used to control quality of grapes and wines **(Rossmann, 2001; Christoph N., Rossmann A., Schlicht C., and Voerkelius, 2006; Zyakun, 2013)**, as well as juices. As a result of the investigations, **Rummelet al. (2010)** differentiated the orange production regions depending on the geographical, climatic and lithological differences.

The aim of this study was to test the method for determination of the C, O and H stable isotopes, as well as their ratios in raw meat and to detect the prospects of its use for identification of regional origin.

Scientific hypothesis

Geographical, climatic, botanical, zoological and zootechnical factors influence the ratio of stable isotopes, which in turn are incorporated into the animal tissue via eating, drinking, breathing and exchange with the environment. A possibility to ascertain a region of origin by the stable isotopic composition can become a reliable hurdle for falsifications in the conditions of global trade.

MATERIAL AND METHODOLOGY

Thirty pork and beef samples originated from 13 countries were purchased in Moscow supermarkets irrespective of the muscle type based on the data of **Harrison et al. (2010)**, who demonstrated that the carbon isotope ratio in different muscles of a carcass differs insignificantly.

The country of origin was determined by the label. Databases of water and wine isotopes collected at theAll-Russian Scientific Research Institute of the Brewing, Non-Alcoholic and Wine Industry were used to correlate the results obtained.

To determine the carbon isotope ratio, the meat sample was weighed, put into the aluminum capsule for combustion in the elemental analyzer at 1000 °C with following purification. Then CO_2 enters the isotope ratio mass spectrometer via the feeding devices, where the analysis of the isotopic composition takes place.

To determine the oxygen and hydrogen isotope ratios, the meat sample was homogenized; the aqueous fraction was separated and filtrated. The obtained sample of the meat aqueous fraction was transferred to the vial, which was placed into the GasBench II sample preparation device to carry out isotopic equilibration and the following measurement of the isotopic characteristics of oxygen and hydrogen of the aqueous component.

The tool base for getting data on the isotopic composition characteristics was a mass spectrometric complex Delta V Advantage of Thermo Fisher Scientific company (USA), providing precise analysis of the prevalence ratio. The isotopic characteristics were measured and compared against the international sample V-PDB.

Method is based on the determination of stable carbon isotope composition in the analyzed sample as compared to the international VPDB standard ($\delta^{13}C_{VPDB}$), by mass spectrometry.

$\delta^{13}C_{VPDB}$ value was calculated by the formula:

$$\delta^{13}C_{VPDB} = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample} - \left(\frac{^{13}C}{^{12}C}\right)_{VPDB}}{\left(\frac{^{13}C}{^{12}C}\right)_{VPDB}} \cdot 1000,$$

where $\left(\frac{^{13}C}{^{12}C}\right)_{VPDB}$ – carbon isotope ratio in the international standard sample, ‰;

$\left(\frac{^{13}C}{^{12}C}\right)_{sample}$ - carbon isotope ratio in the a test sample, ‰.

Stable oxygen and hydrogen isotope ratios, $\delta^{18}O_{VSMOW2}$ and δD_{VSMOW2}, were determined using the research complex of Delta V mass-spectrometer, sampling system GasBench II, and computer system with Isodat 3.0. programme. Method is based on oxygen isotope balance in the aqueous component of the analyzed sample and oxygen in CO_2 within CO_2- He.

$\delta^{18}O_{VSMOW2}$ value was calculated by the formula

$$\delta^{18}O_{VSMOW2} = \frac{\left(\frac{^{18}O}{^{16}O}\right)_{sample} - \left(\frac{^{18}O}{^{16}O}\right)_{VSMOW2}}{\left(\frac{^{18}O}{^{16}O}\right)_{VSMOW2}} \cdot 1000, \quad (2)$$

where $\left(\frac{^{18}O}{^{16}O}\right)_{VSMOW\,2}$ - oxygen isotope ratio in the international standard sample, ‰;

$\left(\frac{^{18}O}{^{16}O}\right)_{sample}$ - oxygen isotope ratio in a test sample, ‰.

Method is based on hydrogen isotope balance in the aqueous component of the analyzed sample and hydrogen within H_2- He.

δD_{VSMOW2} value was calculated by the formula:

$$\delta D_{VSMOW2} = \frac{\left(\frac{^{2}H}{^{1}H}\right)_{sample} - \left(\frac{^{2}H}{^{1}H}\right)_{VSMOW2}}{\left(\frac{^{2}H}{^{1}H}\right)_{VSMOW2}} \cdot 1000,$$

where $\left(\frac{^{2}H}{^{1}H}\right)_{VSMOW2}$ - hydrogen isotope ratio in the international standard sample, ‰;

$\left(\frac{^{2}H}{^{1}H}\right)_{sample}$ - hydrogen isotope ratio in a test sample, ‰.

International IAEA standards,VSMOW2; SLAP2; GISP were used as reference.

Statisic analysis

Statistical analyses results are expressed as mean of triplicate trials. Data were analyzed by one - way analysis of variance (ANOVA) on the means of values (p <0.05). STATISTICA 10.0 software was used in this study for the statistical analyses.

RESULTS AND DISCUSSION

Natural and technogenic particular qualities cause existing differences in the isotope content in geographical areas. Today in the world practice, stable isotope ratios of four elements - carbon, nitrogen, oxygen and hydrogen, in meat and meat products are used to identify a place where an animal was raised. Carbon and nitrogen isotope characteristics are used to determine the feed diet, and oxygen and hydrogen isotopes - to determine the geographical location of a product **(Rossmann, 2001; Kelly, Heaton and Hoogewerff, 2005; Camin et al., 2007)**.

Multi-element (C, O, H) stable isotope ratio analysis proved **(Table 1)** that pork and beef from the same geographical region showed different $\delta^{13}C$ values. For example, meat samples from the same species, but from different countries of production, differ in $\delta^{13}C$ value. Pork the the farms in the European part of Russia, regardless of the breeding type, shows close $\delta^{13}C$ value, which reflect close breeding and feeding conditions. The data obtained correspond with those of **Schmidt et al. (2005)** and **Camin et al. (2016)**.

Table 1 Beef and pork $\delta^{13}C$ values.

Meat	Country of origin (as in the label)	$^{13}C/^{12}C$, $\delta^{13}C$, ‰ (‰ ±SD)
Pork, sample 1	Russia	(-23.17) ±0.1
Pork, sample 2	Russia	(-23.16) ±0.1
Pork, sample 3	Russia	(-23.17) ±0.1
Pork, sample 4	Russia	(-23.15) ±0.05
Pork, sample 5	Canada	(-18.67) ±0.05
Pork, sample 6	Denmark	(-26.18) ±0.1
Pork, sample 7	Brasil	(-18.18) ±0.1
Pork, sample 8	Spain	(-21.40) ±0.1
Pork, sample 9	Czech Republic	(-24.14) ±0.1
Beef, sample 1	Lithuania	(-16.57) ±0.05
Beef, sample 2	Lithuania	(-19.58) ±0.1
Beef, sample 3	Lithuania	(3) (-26.33) ±0.1
Beef, sample 4	Germany	(-26.52) ±0.1
Beef, sample 5	Germany	(-26.21) ±0.05
Beef, sample 6	Mexico	(-16.47) ±0.1
Beef, sample 7	Russia	(-17.60) ±0.1
Beef, sample 8	USA	(-10.96) ±0.1
Beef, sample 8	Czech Republic	(-19.00) ±0.05

Table 2 Stable biophilic isotope (carbon, oxygen, hydrogen) ratios in beef and water samples from different geographical areas.

Meat (Country of origin)	$^{13}C/^{12}C$, $\delta^{13}C$, ‰	$^{18}O/^{16}O$, $\delta^{18}O$, ‰	$^{2}H/^{1}H$, $\delta^{2}H$, ‰
Beef (Italy)	(-17.26)	(-7.80)	(-47.73)
Beef (Brazil)	(-10.90)	0.94	23.4
Beef (Nicaragua)	(-15.63)	(-2.51)	(-4.94)
Beef (Australia)	(- 22.68)	2.63	13.71
Beef (Czech Republic)	(-19.00)	(-5.78)	(-32.75)

In the cow diet in Latin America **(Table 2)**, C4-type plantspredominate – maize and sugarcane. Jahren et al. **(2008)** described similar correlations: $\delta^{13}C$ ranges for corn (C4-plant) from -14 to -11‰. Alfa-alfa, wheat, sunflower (C3–plants) give $\delta^{13}C$ ranges from -28 to -22‰. The statistical processing of the results presented in **Table 2** shows that the results were significantly different (p <0.05) by all three groups of stable isotope ratios.

Carbon isotope ratioin beef from Australia and Russia indicate the predominance of C3-type plants in feeds. Plants of C3 type are, for example, most small seeded

cereal crops (wheat, barley, rye), most trees and lawn grasses and also oat; soybean, sugar beets, potato and other plants widely used to feed animals. While in Italy and the Czech Republic apparently mixed feeds which contain plants both C3 and C4 types are mainly used. For example, if to compare data for beef and pork from the Czech Republic, the differences in $\delta^{13}C$ are evident: -19.00‰ for beef and -24.14‰ for pork. But if to compare water stable isotope ratio $^{18}O/^{16}O$, $\delta^{18}O$, no difference is noted. The ratio is in the range (-5.70… − 5.78)‰ for both types of meat. Cows and pigs in this country have different feeds but drink the same water. Earlier, one of the authors showed that the oxygen isotope composition of the Czech natural waters falls within the range from -5.5 to -6.5 per mil **(Zyakun et al., 2013, 2015).**

The results of our research correspond to those of **Horacek and Min (2010)** and **Nakashita et al. (2008)** who showed similar results while comparing the beef carbon, nitrogen and hydrogen isotope ratios from South Korea, USA, Mexico, Australia and New Zealand and showed the main differences in the $\delta^{13}C$ and $\delta^{2}H$ values which reflect the plant and water regional specificity.

The most "heavy" in the isotope ratio appeared to be the values for meat which came from Australia.T hese data are correlated with the data on the water component of Australian wine. The oxygen value in meat, $\delta^{18}O$, was 2.63 ‰ and in wine, the isotope characteristics of oxygen were in the range from 2.5 to 4.5‰. Similar data of significant differences between countries in the $\delta^{18}O$ values were obtained by **Franke et al. (2008).**

Interestingly, according to the label, beef 1, 2 and 3 were beef samples originated from Lithuania. But the differences between samples 1,2 and beef sample 3 were significant (p <0.01). The carbon isotope content of beef 3 ((-26.33) ±0.1‰) was much closer to those of beef 4 and 5, which labeled as coming from Germany. The differences between beef sample 3 from Lithuania and beef samples 4, 5 from Germany were not significant. For pork from the European part of Russia $\delta^{13}C$ values are about -23,1‰. The differences in the results obtained show a possible misinformation in the lable of beef tested. Further tests should be done and a database reflecting the isotope characteristics of the regions should be developed and constantly updated.

CONCLUSION

Stable isotope ratio analysis was tested for its suitability as a means for geographical location assignment for beef and pork and have shown significant correlation. **Camin et al. (2016)** defined possibility to identify the place of origin for poultry, milk, butter, cheese, fish, and shellfish via isotope ratios of bioelements.

It is necessary to emphasize the importance of the study on the stable isotopes of oxygen, hydrogen and carbon in conjunction, which is justified by the peculiarities of the animal diet and the isotopic composition of local drinking water. The obtained results were positive and indicated that the comparison of the stable isotope ratios of carbon, hydrogen and oxygen is applicable as a potential tool for identifying the origin of meat, produced not only in different countries, but in different regions of Russia as well.

It is evident that for the practical application of this method, it is necessary to create a database on the content of the stable isotopes in different agricultural raw materials and water by world regions. When this database is available, the method can be used to prevent regional fraudulence in the food industry. The existence of an effective method for determining the region of origin for raw meat will fight unscrupulous companies, reduce the likelihood of the implementation of hazardous factors and maintain the system to protect brand and regional labels.

REFERENCES

Ballin, N. Z. 2010. Review Authentication of meat and meat products. *Meat Science*, vol. 86, no. 3, p. 577-587. http://doi.org/10.1016/j.meatsci.2010.06.001

Brescia, M. A., Monfreda, M., Buccolieri, A., Carrino, C. 2005. Characterisation of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determinations. *Food Chemistry*, vol. 89, no. 1 p. 139-147. https://doi.org/10.1016/j.foodchem.2004.02.016

Brzezicha-Cirocka, J., Grembecka, M., Szefer, P. 2016. Monitoring of essential and heavy metals in green tea from different geographical origins. *Environmental monitoring and assessment*, vol. 188, no. 3, p. 1-11. https://doi.org/10.1007/s10661-016-5157-y

Camin, F., Bontempo, L., Heinrich, K., Horacek, M., Kelly, S.D., Schlicht, C., Thomas, F., Monahan, F., Hoogewerff, J., Rossmann, A. 2007. Multi-element (H,C,N,S) stable isotope characteristics of lamb meat from different European regions. *Analytical and Bioanalytical Chemistry*, vol. 389, no. 1, p. 309-320. https://doi.org/10.1007/s00216-007-1302-3

Camin, F., Bontempo, L., Perini, M., Piasen, E. 2016. Stable Isotope Ratio Analysis for Assessing the Authenticity of Food of Animal Origin. *Comprehensive Reviews in Food Science and Food Safety*, vol. 15, no. 5, p. 868-877. https://doi.org/10.1111/1541-4337.12219

Christoph, N., Rossmann, A., Schlicht, C., Voerkelius, S. 2006. Wine Authentication Using Stable Isotope Ratio Analysis: Significance of Geographic Origin, Climate, and Viticultural Parameters. *ACS Symposium Series*, vol. 952, p. 166-179. https://doi.org/10.1021/bk-2007-0952.ch011

Crittenden, R. G., Andrew, A. S., Le Fournour, M., Young, M. D., Middleton, H., Stockmann, R. 2007. Determining the geographic origin of milk in Australasia using multi-element stable isotope ratio analysis. *International Dairy Journal*, vol. 17, no. 5, p. 421-428. https://doi.org/10.1016/j.idairyj.2006.05.012

Franke, B., Haldimann, M., Gremaud, G., Bosset, J. O., Hadorn, R., Kreuzer, M. 2008. Element signature analysis: its validation as a tool for geographic authentication of the origin of dried beef and poultry meat. *European Food Research and Technology*, vol. 227, no. 3, p. 701-708. https://doi.org/10.1007/s00217-007-0776-8

Franke, B., Koslitz, S., Micaux, F., Piantini, U., Maury, V., Pfammatter, E., Wunderli, S., Gremaud, G., Bosset, J.O., Hadorn, R., Kreuzer, M. 2008.Tracing the geographic origin of poultry meat and dried beef with oxygen and strontium isotope ratios. *European Food Research and Technology*, vol. 226, no. 4, p. 761-769. https://doi.org/10.1007/s00217-007-0588-x

Grembecka, M., Szefer, P. 2013. Comparative assessment of essential and heavy metals in fruits from different geographical origins. *Environmental Monitoring and Assessment*, vol. 185, no. 11, p. 9139-9160. https://doi.org/10.1007/s10661-013-3242-z

Harrison, S. M., Monahan, F. J., Moloney, A. P., Kelly, S. D., Cuffe, F., Hoogewerff, J., Schmidt, O., 2010. Intra-muscular and inter-muscular variation in carbon turnover of ovine muscles as recorded by stable isotope ratios. *Food Chemistry*, vol. 123, no. 2, p. 203-209

Horacek, M., Hansel-Hohl, K., Burg, K., Soja, G., Okello-Anyanga, W., Fluch, S. 2015. Control of origin of sesame oil from various countries by stable isotope analysis and DNA based markers--a pilot study. *PLoS ONE*, vol. 10, no. 4, p. 1-12. https://doi.org/10.1371/journal.pone.0123020

Horacek, M., Min, J. S., 2010.Discrimination of Korean beef from beef of other origin by stable isotope measurements. *Food Chemistry*, vol. 121, no. 2, p. 517-520. https://doi.org/10.1016/j.foodchem.2009.12.018

Jahren, A. H., Kraft, R. A. 2008. Carbon and Nitrogen Stable Isotopes in Fast Food: Signatures of Corn and Confinement. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 46, p. 17855-17860.

Kawaguchi, F., Kitamura, Y., Nakajima, R., Takahashi, M., Goto, H., Washida, Y., Yamamoto, Y., Sasazaki, S., Mannen, H. 2018. Application of DNA markers for discrimination between Japanese and Australian Wagyu beef. *Animal Science Journal*, vol. 89, no. 1, p. 257-258. http://doi.org/10.1111/asj.12938

Kelly, S., Heaton, K., Hoogewerff, J. 2005 Tracing the geographical origin of food: the application of multi-element and multi-isotope analysis. *Trends in Food Science & technology,* vol. 16, no. 12, p. 555-567. https://doi.org/10.1016/j.tifs.2005.08.008

Kornexl, B. E., Werner, T., Roßmann, A., Schmidt, H. L. 1997. Measurement of stable isotope abundances in milk and milk ingredients — a possible tool for origin assignment and quality control. *Zeitschriftfür Lebensmitteluntersuchung und - Forschung A*, vol. 205, no. 1, p. 19-24. http://doi.org/10.1007/s002170050117

Lo, Y. T., Shaw, P. C. 2018. DNA-based techniques for authentication of processed food and food supplements. *Food Chemistry*, vol. 240, no. 1, p. 767-774. https://doi.org/10.1016/j.foodchem.2017.08.022

Longobardi, F., Casiello, G., Sacco, D., Tedone, L, Sacco, A. 2011.Characterisation of the geographical origin of Italian potatoes, based on stable isotope and volatile compound analyses. *Food Chemistry*, vol. 124, no. 4, p. 1708-1713. https://doi.org/10.1016/j.foodchem.2010.07.092

Nakashita, R., Suzuki, Y., Akamatsu, F., Iizumi, Y., Korenaga, T., Chikaraishi, Y. 2008. Stable carbon, nitrogen, and oxygen isotope analysis as a potential tool for verifying geographical origin of beef. *Analytica Chimica Acta*, vol. 617, no. 1-2, p. 148-152. https://doi.org/10.1016/j.aca.2008.03.048

Nguyen, D. D. L., Gemrot, E., Loiseau, G., Monet, D. 2008. Determination of citrus fruit origin by using 16S rDNA fingerprinting of bacterial communities by PCR- DGGE: an application to clementine from Morocco and Spain. *Fruits*, vol. 63, no. 2, p. 75-84. https://doi:org/10.1051/fruits:2007049

Ortea, I., Gallardo, J. M. 2015. Investigation of production method, geographical origin and species authentication in commercially relevant shrimps using stable isotope ratio and/or multi-element analyses combined with chemometrics: an exploratory analysis. *Food Chemistry*, vol. 170, no. 1, p. 145-153. https://doi.org/10.1016/j.foodchem.2014.08.049

Osorio, M. T., Koidis, A., Papademas, P. 2015. Major and trace elements in milk and Halloumi chesse as markers for authentication of goat feeding regimes and geographical origin. *International Journal of Dairy Technology*, vol. 68, no. 4, p. 573-581. https://doi.org/10.1111/1471-0307.12213

Peres, B., Barlet, N., Loiseau, G., Montet, D., 2007. Review of the current methods of analytical traceability allowing determination of the origin of foodstuffs. *Food control*, vol. 18, no. 3, p. 228-235. https://doi.org/10.1016/j.foodcont.2005.09.018

Rossmann, A. 2001.Determination of stable isotope ratios in food analysis. *Food Reviews International*, vol. 17, no. 3, p. 347-381. http://dx.doi.org/10.1081/FRI-100104704

Rossmann, A., Haberhauer, G., Hölzl, S., Horn, P., Pichlmayer, F., Voerkelius, S. 2000. The potential of multielement stable isotope analysis for regional origin assignment of butter. *European Food Research and Technology*, vol. 211, no. 1, p. 32-40. https://doi.org/10.1007/s002170050585

Rummel, S., Hölzl, S., Horn, P., Roßmann, A., Schlicht, C. 2010. The combination of stable isotope abundance ratiosof H, C, N and S with 87Sr/86Sr for geographical origin assignment of orange juices. *Food Chemistry*, vol. 118, no. 4, p. 890-900. https://doi.org/10.1016/j.foodchem.2008.05.115

Schellenberg, A., Chmielus, S., Schlicht, C., Camin, F., Perini, M., Bontempo, L., Heinrich, K., Kelly, S. D., Rossmann, A., Thomas, F., Jamin, E., Horacek, M. 2010. Multielement Stable Isotope Ratios (H, C, N, S) of honey from different European regions. *Food Chemistry*, vol. 121, no. 3, p. 770-777. https://doi.org/10.1016/j.foodchem.2009.12.082

Schmidt, O., Quilter, J. M., Bahar, B., Moloney, A. P., Scrimgeour, C., Begley, I. S., Monahan, F. J. 2005. Inferring the origin and dietary history of beef from C, N and S stable isotope ratio analysis. *Food Chemistry*, vol. 91, no. 3, p. 545-549. http://doi.org/10.1016/j.foodchem.2004.08.036

Tretyakov A. V., Abramenkova O. I., Podcolozin I. V., Solovyev A.I. 2012. Identification of the geografic origin meat and caviar using chemical fingerprinting teqniques.*Veterinary today*, vol. 2, p. 39-46.

Zyakun, A. M., Oganesyants, L. A., Panasyuk, A. L., Kuz'mina, E. I., Shilkin, A. A., Baskunov, B. P., Zakharchenko, V. N., Peshenko, V. P. 2015. Site-specific 13C/12C isotope abundance ratios in dicarboxylic oxyacids as characteristics of their origin. *Rapid Communications in Mass Spectrometry*, vol. 29, no. 21, p. 2026-2030. http://doi.org/10.1002/rcm.7307

Zyakun, A. M., Oganesyants, L. A., Panasyuk, A. L., Kuz'mina, E. I., Shilkin, A. A., Baskunov, B. P., Zakharchenko, V. N., Peshenko, V. P. 2013. Mass spectrometric analysis of the 13C/12C abundance ratios in vine plants and wines depending on regional climate factors (Krasnodar Territory and Rostov Region, Russia). *Journal of Analytical Chemistry*, vol. 68, no. 13, p. 1136-1141. https://doi.org/10.1134/S106193481313011X

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POLYPHENOLS AND ANTIOXIDANT CAPACITY IN DIFFERENT TYPES OF GARLIC

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ABSTRACT

Garlic contains important biologically active compounds such as phytoncides, antioxidants and others. There belong organosulfur compounds, allyl thiosulfinates and phenolic compounds (phenolic acids, flavonoids), vitamins (E and C) and some minerals, that all have several positive effects on human health. In the work five types of raw garlic (*Allium sativum*) of Czech, Chinese and Spanish origin, and bear's garlic (*Allium ursinum*), and two dried garlic products, were evaluated for polyphenols content and antioxidant capacity. The highest values of total polyphenols (TP), analyzed by spectrometric method with Folin-Ciocalteu reagent, had fresh samples of bear's and Spanish garlic; the lowest ones were evaluated for Czech garlic bulbs (92.2 – 119.6 mg GAE.100g^{-1} fw) and dry garlic products (70.1 – 84.5 mg GAE.100g^{-1}). The total antioxidant capacity (TAC) was determined by spectrometric methods with DPPH and ABTS reagents. To types of fresh garlic with the best values of antioxidant capacity, evaluated by both methods, belong bear's and Spanish garlic, followed by Chinese and Czech garlic samples. These values are in agreement with polyphenols content in garlic bulbs. Dry garlic products had the highest values of TAC. Content of polyphenols and antioxidant capacity values were positively correlated, higher correlation value was detected for TP and TAC-ABTS (0.973) than for TP and TAC-DPPH (0.873). Bear's garlic and garlic belong to the vegetable types with high amount of biologically active compounds such as antioxidants.

Keywords: garlic; polyphenol; antioxidant capacity; DPPH; ABTS

INTRODUCTION

Garlic (*Allium sativum*) belongs to very popular old cultural crop with usage as seasoning for culinary and industrial utilization to improve sensory quality (taste, aroma) of foods and meals **(Pardo et al., 2007)**. It is known for over 6000 years. It is cultivated worldwide with China as the biggest producer (about 9 million tons per year), next India (0.5 million ton per year), followed by the USA, Thailand, Egypt, South Korea, Spain and Turkey **(Mayer et al., 2003)**.

Garlic attracts great attention around the world by consumers due to its sensory impact, utilization in folk medicine and also by researchers because of its widespread health properties in prevention and healing of some illnesses such as prevention of cardiovascular diseases, cancers, or stimulation of the immune system, anti-tumor and antimicrobial effects, and benefit on high blood glucose concentration **(Bayan et al., 2014)** and other biological activities.

Garlic belongs to the botanical family Allium that contains about 700 species such as onion, shallot, garlic, leek, chives. In present there is known about 300 types of garlic, 90 of them is registered in European Union. To the most used types belong *Allium sativum* that means "cultivated garlic", and *Allium ursinum*, bear's garlic **(Hanen et al., 2012; Mayer et al., 2003)**.

Allium sativum has been shown to have many positive health benefits such as antimicrobial activity, cardiovascular effects (antihypertensive, antithrombotic, antiatherosclerotic), hypocholesterolaemic, antihyperlipidemic, and hypo triacylglyceride activities, anti-inflammatory and anticancer effect **(Mikaili et al., 2013)**.

Allicin (diallyl thiosulfinate) is the principal bioactive compound present in raw garlic extract. It is produced when the garlic is chopped or crushed with allinase enzyme from alliin that is present in intact garlic. Other important compounds present in garlic are diallyl disulphide, ajoene, S-allylcysteine and diallyl trisulfide.

Allicin and other sulfur compounds are thought to be the major compounds responsible for the antimicrobial effect of garlic both *in vitro* and *in vivo*. Garlic is effective against a number of gram-negative and gram-positive bacteria such as *Staphylococcus*, *Salmonella*, *Mycobacteria*, and *Proteus* species. Among the viruses which are sensitive to garlic extracts are the human Cytomegalovirus, influenza B virus, Herpes simplex virus type 1, Herpes simplex virus type 2, Parainfluenza virus

type 3, vaccinia virus, vesicular stomatitis virus, and human Rhinovirus type 2 **(Mikaili et al., 2013)**.

Many plant materials as vegetables **(Liu, 2003)** and their products **(Škrovánková et al., 2017)** contain such bioactive substances that individually or combined, demonstrate high antioxidant capacity. They could scavenge free radicals and thus protect foods or humans against oxidative damage.

Phytochemicals such as polyphenols, some vitamins, and sulfur substances are often responsible for their antioxidant capacity and therefore could be linked to protective, antioxidant effects of these foods and beverages with many health benefits **(Kopec et al., 2013)**.

Due to the study of **Cao et al. (1996)** garlic belong to the types of vegetable, based on the fresh weight, with the highest antioxidant activity against peroxyl radicals. It is followed by kale, spinach, Brussels sprouts, alfalfa sprouts, broccoli flowers, beets, red bell pepper, onion, corn, eggplant, cauliflower, potato, sweet potato, cabbage, leaf lettuce, string bean, carrot, yellow squash, iceberg lettuce, celery, and cucumber.

S-allylcysteine has antioxidant properties, and antihypertensive effects of garlic are associated just with their antioxidant properties. Diallyl disulfide is known for its antihyperlipidemic properties **(Rai et al., 2009)**. Diallyl disulfide, diallyl trisulfide and allicin play an important role in anti-atherosclerotic activity of garlic **(Gonen et al., 2005)**. Diallyl sulfide, diallyl disulfide and diallyl trisulfide have been shown to exhibit anticancer activities **(Lai et al., 2012)**.

A. ursinum is widely used as spice and traditional folk medicine, as antiscorbutic, fever-fighting agent, and for problems with intestines. It has also cardioprotective activity with greater effect on lowering the blood pressure of rats than regular garlic **(Preuss et al., 2001a)**, antioxidative effect **(Wu et al., 2009; Stajner et al., 2008)**, antimicrobial **(Sapunjieva et al., 2011; Ivanova et al., 2009)** and antifungal properties **(Parvu et al., 2011)**. The potential health benefits of bear's garlic have been attributed mainly to the sulfur-containing compounds. It is considered to be more beneficial than *A. sativum* in *in vivo* and *in vitro* studies **(Preuss et al., 2001b)**.

Antioxidant activity of Allium family is attributed mainly to sulfur-containing compounds and their precursors. Main garlic sulfur antioxidant compounds are allicin, allyl thiosulfinates, diallyldisulfid and diallyltrisulfid. To other antioxidant components belong polyphenolic compounds (phenolic acids, flavonoids) **(Kavalcová et al., 2014; Dalaram, 2016)** that act as hydrogen or electron donors, and have ability to stabilize radical and delocalize the unpaired electron. Also vitamin C, vitamin E, β-carotene, and selenium are effective in garlic **(Rasul et al., 2012; Queiroz et al., 2009; Singh and Singh, 2008; Park et al., 2009)**.

Scientific hypothesis

The content of polyphenols and antioxidant capacity were tested in different types of garlic to determine the differences between garlic types. We assumed the significant difference in polyphenols content and antioxidant capacity measured by two methods (DPPH and ABTS) in different garlic varieties.

MATERIAL AND METHODOLOGY

Garlic samples

There were analyzed five types of fresh garlic (*Allium sativum*) of Czech origin, 2 samples – Czech type Bjetin, (FCZB) and Polish type Harnaś (FCZH), Chinese origin (FCH), and Spanish origin (FSP) from food markets; and bear's garlic (*Allium ursinum*) (FB) grown in Moravian region **(Figure 1)**. They were stored in the fridge until extraction up to 3 days. Also two dried garlic products (powder, condiments) from food market with the origin of Czech Republic (DCZ) and China (DCH) were analyzed.

Determination of Polyphenolic Content

For the determination of total polyphenolic (TP) content modified spectrometric method of **Koncić and Jug (2011)** with Folin-Ciocalteau reagent was used. Samples from fresh material were prepared with 5 g of garlic bulbs, from dried products with 2 g. After homogenization the samples were extracted with 50 mL of HCl (5 mM; Lukes, Czech Republic) under stirring in a shaker for 1 h. The extract was filtered through a paper filter and used for the analyses of TP. To garlic extract (1 mL) Folin-Ciocalteau agent (1 mL; Penta Chemicals, Czech Republic) was added and after agitation it was left for 5 min in the dark at lab temperature, then 1 mL of 10% sodium carbonate (Penta Chemicals, Czech Republic) solution was added and mixed again. After 1 h. of standing in the dark at lab temperature absorbance of samples was measured against blank at wavelength λ = 765 nm on the spectrometer (Libra S6 Biochrom, GB). Gallic acid was used as standard and results of TP were expressed as gallic acid equivalents (GAE) in $mg.100g^{-1}$ sample. Determinations were made in triplicate.

Determination of Antioxidant Capacity

Total antioxidant capacity (TAC) was measured by modified spectrometric methods **(Boonpeng et al., 2014; Ourouadi et al., 2016)** with DPPH and ABTS reagents.
Samples for both determinations were weighted from fresh garlic to 5 g of bulbs, from dried products to 2 g. After homogenization and extraction with 20 mL of methanol (80%, v/v) the solutions were stirred in a shaker for 5 h and then 20 min. by ultrasound and filtrated through a paper filter and used for the analyses.

DPPH method: Extracts of garlic or their products reacted with free stable DPPH·radical (1,1-diphenyl-2-picrylhydrazyl) that resulted in a decrease of absorption.
To extract (0.2 mL) there was added methanolic solution (4.8 mL; 0.2 mM) of DPPH (Sigma Aldrich, Czech Republic). The reaction mixture was shaken vigorously in capped glass and left for 1 h. at lab temperature without light exposure. Absorbance of samples (A) was then measured at wavelength 515 nm against blank on the spectrometer (Libra S6 Biochrom, GB). Also absorbance of control samples (K) was measured at 515 nm against blank. Inactivation (I) was calculated from the decrease of absorbance (%) according to relation (1).

$$I = \frac{K-A}{K} . 100 \quad (1)$$

Figure 1 Pictures of garlic bulbs types (left – Czech type Bjetin (*Allium sativum*), middle – Polish type Harnaś (*Allium sativum*), and right – bear's garlic (*Allium ursinum*) **(Garlik variety Harnaś, 2018; Registered varieties of winter garlic, 2018; Wild garlic (*Allium ursinum*), 2018)**.

Results of TAC were calculated from inactivation values using trolox as standard and expressed as trolox equivalents (TE) in mg.100g^{-1} sample. Average results were obtained from three parallel determinations.

IC50 method: The IC50 values express the concentration of garlic extract that is required to scavenge 50% of DPPH free radicals, 50% inactivation. There were prepared 5 diluted methanolic garlic extract solutions in the range 50 – 500 mg.mL^{-1}. The reactive mixtures with DPPH solution were made in the same way as for TAC. The IC50 values were quantified graphically (plotting the absorbance against the used extract concentration) and afterwards calculated by linear regression.

ABTS method: To extract (0.15 mL) there was added reactive radical mixture of ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; Sigma Aldrich, Czech Republic) (12 mL; 3.5 mM) with $K_2S_2O_8$ (0.06 M; Lukes, Czech Republic) and acetic buffer (pH 4.3). The mixture was shaken vigorously and left to react for 30 min. without light exposure at lab temperature. Absorbance of samples (A) was then measured at wavelength λ = 734 nm against blank by spectrometer (Libra S6 Biochrom, GB). Also absorbance of control samples (K) was measured at 734 nm against blank. Inactivation (I) was calculated from the decrease of absorbance (%) according to relation (1). Results of TAC were calculated using trolox as standard and expressed as trolox equivalents (TE) in mg.100g^{-1} sample. Average results were obtained from three parallel determinations.

Statisic analysis

The data are reported as mean values ± standard deviation (SD). Statistic evaluation of the results was made by Statistica program, StatSoft version 9.0 (USA) by the one way analysis of variance (ANOVA) at a 5% significance level, LSD test.

RESULTS AND DISCUSSION

Content of phenolics

The contents of total polyphenols (TP) of raw and dry garlic samples, measured by spectrometric method with Folin-Ciocalteau reagent, are given in **Figure 2** and **Table 1**. For raw garlic bulbs the TP range was determined from 92.2 to 119.6 mg GAE.100g^{-1} fw (fresh weight) with the average 108.3 mg GAE.100g^{-1} fw. No significant difference could be found for the means of polyphenol contents between fresh garlic samples. The determined values represent phenolic contents from 225.4 to 416.7 mg GAE.100g^{-1} dm (dry matter) as these garlic bulbs contain dry mass for about 29 – 43% (bear's garlic to Chinese garlic bulbs). Dry garlic products had lower values of TP (about 2/3 of the highest TP content of raw sample) that could signify that these products were industrially dried probably by classic method with hot air that might lower content of polyphenols in products. There was a significant difference (LSD test) between dry garlic products (DCZ and DCH) and garlic samples (FCZB, FCZH, FCH, FSP, FB).

Polyphenol content of 43 garlic cultivars from China was evaluated by **Chen et al. (2013)**. They assessed TP values in fresh garlic bulbs from 2127 to 3396 mg GAE.100g^{-1} fw in most cultivars. They are much higher values than in our observations, and also other studies showed much less amount of polyphenols than Chinese authors. Slovak scientists **(Lenková et al., 2017)** determined in five varieties of garlic grown in Slovakia polyphenols content in the range 62 – 76 mg GAE.100g^{-1} fw. Results of TP in Brazilian study of **Queiroz et al. (2009)** were in the range 699 – 870 mg GAE.100g^{-1} dm of the extract from fresh garlic bulbs. **Bozin et al. (2008)** determined in dry extract from fresh garlic bulbs only 5 mg GAE.100g^{-1} dm, the highest value (98 mg GAE.100g^{-1}) they found in ground air-dried immature garlic plants. **Beato et al. (2011)** quantified the total phenolic content in different garlic cultivars grown in Spain from 340 to 1080 mg GAE.100g^{-1} dm. Our results are in agreement with these observations, however they are in the lower level compared to their range. That could be also due to the fact that content and presence of phenolic compounds in garlic varies due to genetic factors **(Montaño et al., 2011)**, cultivar type **(Beato et al., 2011)**, different agronomic (e.g. culture, location) **(Volk and Stern, 2009)** and environmental conditions such as soil type, sun exposure, rain frequency etc. **(Naheed et al., 2017)**.

Antioxidant capacity

For the evaluation of antioxidant potential of garlic bulbs and dry garlic products in this study the antioxidant capacity (TAC) was measured by DPPH and ABTS method. TAC values for fresh extracts from garlic bulb samples are demonstrated in **Figure 2**, in **Table 1** there are results for samples recalculated to dry matter (dm).

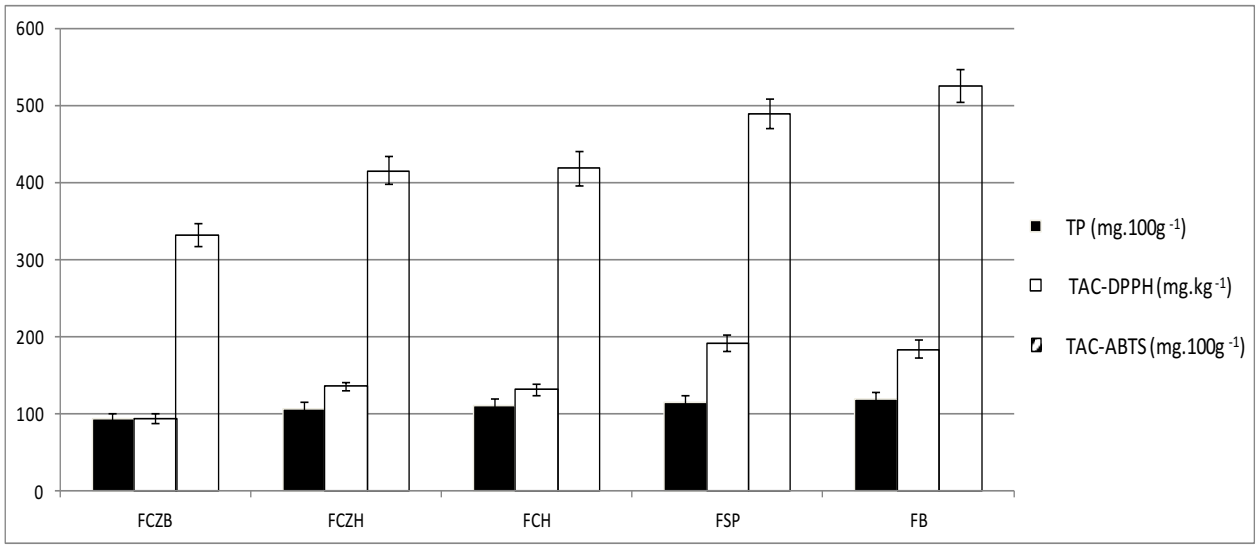

Figure 2 Polyphenols content (TP) and antioxidant capacity (TAC-DPPH, TAC-ABTS) of fresh (fw) garlic samples.

Table 1 Polyphenols content (TP) and antioxidant capacity (TAC) of garlic samples.

Garlic sample	TP (mg GAE.100g^{-1} dm±SD)	I (%)	TAC (DPPH) (mg TE.100g^{-1} dm±SD)	TAC (ABTS) (mg TE.100g^{-1} dm±SD)
FCZB	225.4 ±12.1	17.6	24.5 ±2.0	872.6 ±12.9
FCZH	277.2 ±15.7	25.5	33.0 ±1.4	1014.7 ±10.5
FCH	256.1 ±16.0	20.3	30.2 ±2.2	971.6 ±10.3
FSP	322.8 ±15.2	30.1	53.6 ±3.5	1381.2 ±15.2
FB	416.7 ±14.3	36.8	62.7 ±3.8	1809.3 ±19.6
DCZ	91.1 ±6.3	56.4	88.2 ±5.2	1825.8 ±10.1
DCH	74.9 ±4.1	61.2	100.4 ±6.7	1963.9 ±14.1

Note: dm – dry matter.

Garlic extracts from fresh bulbs of different origin exhibited similar order of garlic samples for inactivation (17.6 to 36.8%) and antioxidant capacity. The study of **Chen et al. (2013)** showed DPPH-scavenging activity of garlic bulbs that ranges from 3.6% to 45.6%, which was exceeded the range (5.1% to 11.4%) reported for the Allium genus.

The TAC values of DPPH method in our study were from 93.2 to 190.6 mg of trolox equivalents per 100 grams of fresh sample, and from 331.6 to 525.2 mg TE.100g^{-1} fw for ABTS method, respectively. Higher potency in scavenging of DPPH free radical showed Spanish and bear's garlic, Czech (Harnaś) and Chinese garlic sample had about 1/3 lower value. The lowest potency presented Czech garlic Bjetin, a half value in comparison to the highest one. There was a significant difference (p <0.05, LSD test) in the means of TAC-DPPH for garlic samples FCZB, FCZH, FCH, and samples with higher TAC values – FSP, FB and dry garlic products (DCZ and DCH).

Similar results (also for statistics) were for ABTS method, when bear's garlic showed a bit higher value than Spanish one; followed by Chinese and Czech garlic samples.

Values calculated to dry matter are analogous to results of fresh samples. The best TAC showed dry garlic products and bear's garlic, Spanish one, followed by Czech garlic (Harnaś), Chinese and Czech garlic Bjetin.

Analogous trend shows also values of IC50 in Figure 3. IC50 shows the concentration of garlic extracts needed for 50% inhibition. Lower IC50 value indicates higher antioxidant capacity. IC50 concentrations of garlic extracts from fresh bulbs were in the range 261.7 – 550.2 mg.mL^{-1}, for dry commercial products 163.4 – 177.5 mg.mL^{-1}. Dry garlic products therefore had the best TAC that could be affected by high dry matter of these samples.

To characterize the relationship between total phenolic content and antioxidant capacities the correlation

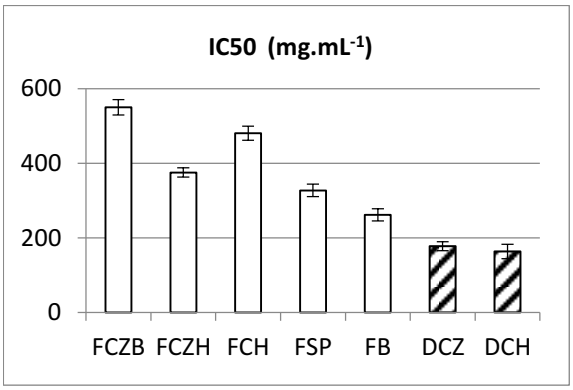

Figure 3 IC50 (mg.mL^{-1}) values of fresh (white column) and dry (hatched column) garlic samples.

coefficients were calculated. Antioxidant capacity showed a strong positive relationship comparing both assays. Antioxidant capacity detected by ABTS assay was stronger positively associated with the phenolic content (p = 0.973) than for DPPH assay (p = 0.873). Also **Queiroz et al. (2009), Lenková et al. (2017)** and **Chen et al. (2013)** found positive correlation between TP and TAC-DPPH for garlic. **Leelarungrayub et al. (2009)** proved positive correlation between TP and TAC-ABTS.

The differences may be related to the high amount of other bioactive compounds with antioxidant power besides polyphenols such as organosulfur compounds in garlic. Also methods of determination (extraction and measurement conditions) are important for acquired results **(Pisoschi and Negulescu, 2011)** therefore it could be problematic to compare exact values of TAC.

CONCLUSION

Garlic has several positive effects on human health that could be correlated with present biologically active compounds such as phenolic compounds and organosulfur compounds, vitamins and some minerals. To types of fresh garlic with the best values of antioxidant capacity, evaluated by both methods, belong bear's (62.7 and 1809.3 mg TE.100g^{-1} dm) and Spanish (53.6 and 1381.2 mg TE.100g^{-1} dm) garlic, then Chinese and Czech garlic samples. These findings are in agreement with polyphenols content in garlic bulbs (225.4 to 416.7 mg GAE.100g^{-1} dm). That was also proved by higher correlation coefficients, for TP and TAC-DPPH value p = 0.873, for TP and TAC-ABTS value p = 0.973. However, dry garlic products showed higher TAC values (88.2 and 1825.8 mg TE.100g^{-1} dm; 100.4 and 1963.9 mg TE.100g^{-1} dm, respectively). There was a significant difference (p <0.05) in the means of phenolic contents between dry garlic products (DCZ and DCH) and fresh garlic samples (FCZB, FCZH, FCH, FSP, FB) and also a significant difference for values of TAC between some garlic samples (FCZB, FCZH, FCH) and samples with higher TAC values – FSP, FB, and dry garlic products (DCZ and DCH). Some variances could be due the fact that garlic contain besides polyphenols also great amount of sulfur compounds that together contribute to overall antioxidant capacity.

REFERENCES

Bayan, L., Koulivand, P. H., Gorji, A. 2014. Garlic: a review of potential therapeutic effects. *Avicenna Journal of Phytomedicine*, vol. 4, no. 1, p. 1-14. PMid:25050296

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 Foods Hum. Nutr.*, vol. 66, no. 3, p. 218-223. https://doi.org/10.1007/s11130-011-0236-2 PMid:21667145

Boonpeng, S., Siripongvutikorn, S., Sae-wong, C., Sutthirak, P. 2014. The antioxidant and anti-cadmium toxicity properties of garlic extracts. *Food Science & Nutrition*, vol. 2, no. 6, p. 792-801. https://doi.org/10.1002/fsn3.164

Bozin, B., Mimica-Dukic, N., Samojlik, I., Anackov, G., Igic, R. 2008. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). *Food Chemistry*, vol. 111, no. 4, p. 925-929. https://doi.org/10.1016/j.foodchem.2008.04.071

Cao, G., Sofic, E., Prior, R.L. 1996. Antioxidant Capacity of Tea and Common Vegetables. *J. Agric. Food Chem.*, vol. 44, no. 11, p. 3426-3431. http://doi.org/10.1021/jf9602535

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 ONE*, vol. 8, no. 11, e79730. http://doi.org/10.1371/journal.pone.0079730

Garlik variety Harnaś (Czosnek odmiany Harnaś), 2018. [online] s.a. [cit.2018-03-07] Available at: http://www.rynek-rolny.pl/tag/czosnek-odmiany-harnas/

Dalaram, I. S. 2016. Content of Total Polyphenols and Antioxidant Activity in varieties of Onion and Garlic. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 10, no. 1, p. 444-451. https://doi.org/10.5219/658

Gonen, A., Harats, D., Rabinkov, A., Miron, T., Mirelman, D., Wilchek, M., et al. 2005. The antiatherogenic effect of allicin: possible mode of action. *Pathobiology*, vol. 72, p. 325-334. https://doi.org/10.1159/000091330 PMid:16582584

Hanen, N., Fattouch, S., Ammar, E., Neffati, M. 2012. *Scientific, Health and Social Aspects of the Food Industry: Allium Species, Ancient Health Food for the Future?* RIJEKA: InTech. 488 p. ISBN: 978-953-307-916-5.

Ivanova, A., Mikhova, B., Najdenski, H., Tsvetkova, I., Kostova, I. 2009. Chemical composition and antimicrobial activity of wild garlic *Allium ursinum* of Bulgarian origin. *Nat. Prod. Commun.*, vol. 4, p. 1059-1062. PMid:19768983

Kavalcová, P., Bystrická, J., Tomáš, J., Karovičová, J., Kuchtová, V. 2014. Evaluation and comparison of the content of total polyphenols and antioxidant activity in onion, garlic and leek. *Potravinarstvo*, vol. 8, no. 1, p. 272-276. https://doi.org/10.5219/394

Končic, Z. M., Jug, M. 2011. Antioxidant and Bioadhesive Properties of Onions (*Allium* L., Alliaceae): Processed Under Acidic Conditions. *International Journal of Food Properties*, vol. 14, no. 1, p. 92-101. https://doi.org/10.1080/10942910903147825

Kopec, A., Piatkowska, E., Leszczynska, T., Sikora, E. 2013. Healthy Properties of Garlic. *Current Nutrition & Food Science*, vol. 9, no. 1, p. 59-64.

Lai, K. C., Kuo, C. L., Ho, H. C., Yang, J. S., Ma, C. Y., Lu, H. F., Huang, H. Y., Chueh, F. S., Yu, C. C., Chung, J. G. 2012. Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells in vitro and in vivo. *Phytomedicine*, vol. 19, p. 625-630. https://doi.org/10.1016/j.phymed.2012.02.004

Leelarungrayub, N., Rattanapanone, V., Chanarat, N., Gebicki, J. M. 2006. Quantitative evaluation of the antioxidant properties of garlic and shallot preparations. *Nutrition*, vol. 22, no. 3, p. 266-274. http://doi.org/10.1016/j.nut.2005.05.010

Lenková, M., Bystrická, J., Vollmannová, A., Tóth, T., Kovarovič, J. 2017. Evaluation and comparison of the content of total polyphenols and antioxidant activity in garlic (*Allium sativum* L.). *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 65-70. https://dx.doi.org/10.5219/698

Liu, R. H. 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.*, vol. 78, no. 3, p. 517S-520S. https://doi.org/10.1093/ajcn/78.3.517S PMid:12936943

Mayer, J. G., Uehleke, B., Saum, K. 2003. *Handbuch der Klosterheilkunde (neues Wissen über die Wirkung der Heilpflanzen; vorbeugen, behandeln und heilen)*. 6th ed.

München, Germany : Sandmann. 400 p. ISBN 978-389-8830-164.

Mikaili, P., Maadirad, S., Moloudizargari, M., Aghajanshakeri, S., Sarahroodi, S. 2013. Therapeutic Uses and Pharmacological Properties of Garlic, Shallot, and Their Biologically Active Compounds. *Iranian Journal of Basic Medical Sciences*, vol. 16, no. 10, p. 1031-1048. PMid:24379960

Montaño, A., Beato, V.M., Mansilla, F., Orgaz, F. 2011. Effect of genetic characteristics and environmental factors on organosulfur compounds in garlic (*Allium sativum* L.) grown in Andalusia, Spain. *J. Agric. Food Chem*., vol. 59, p. 1301-1307. https://doi.org/10.1021/jf104494j PMid:21247176

Naheed, Z., Cheng, Z., Wu, C., Wen, Y., Ding, H. 2017. Total polyphenols, total flavonoids, allicin and antioxidant capacities in garlic scape cultivars during controlled atmosphere storage. *Postharvest Biology and Technology*, vol. 131, p. 39-45. https://doi.org/10.1016/j.postharvbio.2017.05.002

Ourouadi, S., Hasib, A., Moumene, H., El Khiraoui, A., Ouatmane, A., Boulli, A. 2016. Bioactive Constituents and Antioxidant Activity of Moroccan garlic (*Allium sativum* L). *Journal of Natural Sciences Research*, vol. 6, p. 38-43.

Pardo, J. E., Escribano, J., Gómez, R., Alvarruiz, A. 2007. Physical-chemical and sensory quality evaluation of garlic cultivars. *J. Food Quality,* vol. 30, no. 5, p. 609-622. https://doi.org/10.1111/j.1745-4557.2007.00146.x

Park, J. H., Park, Y. K., Park, E. 2009. Antioxidative and antigenotoxic effects of garlic (*Allium sativum* L.) prepared by different processing methods. *Plant Foods Hum. Nutr*. vol. 64, no. 4, p. 244-249. https://doi.org/10.1007/s11130-009-0132-1 PMid:19711184

Parvu, M., Parvu, A. E., Vlase, L., Rosca-Casian, O., Parvu, O. 2011. Antifungal properties of *Allium ursinum* L. ethanol extract. *J. Med. Plants Res*., vol. 5, p. 2041-2046.

Pisoschi, A. M., Negulescu, G. P. 2011. Methods for Total Antioxidant Activity Determination: A Review. *Biochem & Anal Biochem*. vol. 1, p. 106. http://doi.org/10.4172/2161-1009.1000106

Preuss, H. G., Clouatre, D., Mohamadi, A., Jarrells, T. 2001a. Wild garlic has a greater effect than regular garlic on blood pressure and blood chemistries of rats. *Int. Urol. Nephrol*., vol. 32, p. 525-530. https://doi.org/10.1023/A:1014417526290 PMid:11989540

Preuss, H. G., Clouatre, D., Mohamadi, A., Jarrells, T. 2001b. Wild garlic has a pharmacological investigations of *Allium ursinum* and *Allium sativum*. *Planta Med*., vol. 58, p. 1-7.

Queiroz, Y. S., Ishimoto, E. Y., Bastos, D. H. M., Sampaio, G. R., Torres, E. A. F. S. 2009. Garlic (*Allium sativum* L.) and ready-to-eat garlic products: in vitro antioxidant activity. *Food Chem*., vol. 115, p. 371-374. https://doi.org/10.1016/j.foodchem.2008.11.105

Rai, S. K., Sharma, M., Tiwari, M. 2009. Inhibitory effect of novel diallyldisulfide analogs on HMG-CoA reductase expression in hypercholesterolemic rats: CREB as a potential upstream target. *Life Sci*. vol. 85, p. 211-219. https://doi.org/10.1016/j.lfs.2009.05.020

Rasul, S. H. A., Butt, M. S., Anjum, F. M. 2012. Aqueous garlic extract and its phytochemical profile; special reference to antioxidant status. *International Journal of Food Sciences and Nutrition*, vol. 63, p. 431-439. https://doi.org/10.3109/09637486.2011.634786 PMid:22098476

Registered varieties of winter garlic (Registrované odrůdy ozimého česneku), 2018. [online] s.a. [cit.2018-03-07] Available at: https://www.ireceptar.cz/zahrada/uzitkova-zahrada/jarni-a-ozime-palicaky-a-nepalicaky-jake-odrudy-cesneku-si-vybrat/

Sapunjieva, T., Alexieva, I., Mihaylova, D., Popova, A. 2012. Antimicrobial and antioxidant activity of extracts of *Allium ursinum* L. *Journal of BioScience and Biotechnology*, vol. 1, p. 143-145.

Singh, K. V., Singh, K. D. 2008. Pharmacological Effects of Garlic (*Allium sativum* L.). *Annual Review of Biomedical Science*, vol. 10, p. 6-26. https://doi.org/10.5016/1806-8774.2008.v10p6

Škrovánková, S., Mlček, J., Orsavová, J., Juríková, T., Dřímalová, P. 2017. Polyphenols content and antioxidant activity of paprika and pepper spices. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 52-57. http://doi.org/10.5219/695

Stajner, D., Popovic, B. M., Canadanovic-Brunet, J., Stajner, M. 2008. Antioxidant and scavenger activities of *Allium ursinum*. *Fitoterapia*, vol. 79, p. 303-305. https://doi.org/10.1016/j.fitote.2007.01.008 PMid:18313233

Volk, G. M., Stern, D. 2009. Phenotypic characteristics of ten garlic cultivars grown at different North American locations. *HortScience*, vol. 44, no. 5, p. 1238-1247.

Wild garlic (*Allium ursinum*), 2018. [online] s.a. [cit.2018-03-07] Available at: https://www.amazon.co.uk/garlic-Allium-ursinum-LARGE-BULBS/dp/B071XK7HSF/ref=pd_lpo_vtph_86_tr_img_2/262-3133582-9609325?_encoding=UTF8&psc=1&refRID=N6FE8HR4JWHWR93D3FFR

Wu, H., Dushenkov, S., Ho, C. T., Sang, S. 2009. Novel acetylated flavonoid glycosides from the leaves of *Allium ursinum*. *Food Chem*., vol. 115, p. 592-595. https://doi.org/10.1016/j.foodchem.2008.12.058

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DEVELOPMENT OF RESISTANCE TO ANTIBIOTICS IN BACTERIA *STAPHYLOCOCCUS SPP.* ISOLATED FROM MILK SAMPLES IN THE SHEEP BREEDINGS ON EAST OF SLOVAKIA

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ABSTRACT

During the last three years (2015 to 2017), the frequency of occurrence of bacteria *Staphylococcus spp.* were examined in total 3466 individual and 12 pool milk samples. Experiment was carried out in two herds of breed of sheep Improved Valaska in region of East Slovakia. Were isolated and taxonomically identified 15 species of the genus *Staphylococcus spp.* (n = 444). From the coagulase positive staphylococci (CPS), *S. aureus* was isolated during the reporting period, however, most often in the first year (45). The incidence of *S. intermedius* and *S. hyicus* has been irregular. From the coagulase negative staphylococci (CNS) (n = 288), were isolated *S. epidermidis* present in 37.5% (108), *S. scheiferi* 25.69% (74) and *S. chromogenes* 23.61% (68), while other species occurred only rarely. The bacteria *S. aureus* (n = 117) showed the highest resistance to novobiocine 14.5%, to erythromycin 12.8%, lincomycin 7.69% and also 7.69% to penicillin. In the framework of the CNS sensitivity we tested 108 strains of *S. epidermidis*, from which it was 11.1% resistant to novobiocine and 8.3% to erythromycin. Statistical comparison of the incidence of resistance to penicillin and novobiocine in *S. aureus* and *S. epidermidis* was performed using Chi square test. There was statistically significant dependence of tracked characters was confirmed on significance level $\alpha = 0.05$. Also, the incidence of intermediate sensitivity of bacteria in the *Staphylococcus spp.*, indicates the unfavourable development of resistance to the most commonly used antibiotics to treat the inflammation of the udder in sheep. It is therefore recommended to regularly check the resistance to antibiotics and often isolated bacteria CNS.

Keywords: sheep milk; staphylococci; antibiotics; resistance; mastitis

INTRODUCTION

The Staphylococcus spp. forms a group of micro-organisms, which globally represents a significant proportion in the aetiology of the sheep mastitis **(Contreras et al. 2007)**.

In particular, *S. aureus* as a coagulase positive species, were intensively studied for its pathogenicity in both, human and veterinary medicine. In recent decades however, highlighted the importance of coagulase negative staphylococci and (CNS), which were initially considered as a comensals and had a minority as the mammary gland pathogens. Their significance as a pathogen of ruminants, especially in subclinical forms of mastitis, were recorded by experts in many countries **(Pyörälä and Taponen, 2009)**.

Pitkälä et al. (2004) reported up to 50% market share of CNS from total isolation of bacterial species in Finland, while the main indicator of the subclinical mastitis is increasing of somatic cell count in milk of ruminants. Prevention and control mastitis caused by CNS (controling CNS mastitis) are complex, since epidemiology is often unclear even for the fact that the group consists of more than 40 species, the characteristics of the CNS are diverse, they can be more or less virulent **(Kiossis et al., 2007; Pyörälä and Taponen, 2009).**

Also, with reference to the specific conditions of ruminants, many authors in their works referred to as the most frequently isolated: S. epidermidis, *S. chromogenes*, *S. simulans, S. xylosus, S. haemolyticus, S. warneri*, *and S. sciuri* **(Fthenakis 1994; Ergün et al., 2009)**.

Although CNS do not have a comparable range of the virulence factors, such as the S. aureus, one of the important factors of virulence is the ability to create resistance to the antibiotic, while some were described as multiresistant **(Moniri et al., 2007)**.

Hidden intramammary infection half of the udder are referred to as sources of resistant staphylococci in sheep holdings, which in practice can be confirmed only by a bacteriological examination (Ergün et al., 2009; Kiossis et al., 2007).

Zigo et al. (2014) in their work report that the coagulase-negative staphylococci were identified in 102 (65.4%) from all 156 positive isolates. The CNS and *S. aureus* caused subacute (5.1%), subclinical (3.9%) and acute (2.4%) forms of mastitis. The most frequently isolated were *S. epidermidis*, followed by *S. chromogenes* and *S.*

xylosus from ewes with subacute and subclinical mastitis. From acute and chronical forms of mastitis were predominantly isolated *S. aureus* and *S. epidermidis*.

The aim of the work was to determine the occurrence and most common types of *Staphylococcus spp*. in the investigated individual pools and sheep's milk samples and comparison of the incidence of antibiotico-resistance of the most numerous tested species *S. aureus* and *S. epidermidis.*

MATERIAL AND METHODOLOGY

Characteristics of experimental breeds of sheep

It was one of the breed of sheep with 330 Improved Valaska sheep, and another farm with 250 sheep with a program of gradual crossing with the "Lacaune" breed. Tracking the aetiology in mastitis in the findings of the pool samples was carried out during the three seasons of the machine milking, in the holdings with technological standards in Gelnica district. Have been performed a total of 12 comprehensive examination repeatedly from April to September. A significant measure in the course of the experiment was to treat all cases of clinical mastitis solely on the basis of proven susceptibility to a range of selected antibiotics.

Testing and sampling herds sheep's milk

At the beginning, and at the end of each season were carried out a clinical examination of the udder is supplemented by a Californian Mastitis Test individual sheep's milk, and bacteriological examination of samples according to the principles as stated by the authors **Fthenakis (1994); Vasil (2004); Mørk et al. (2004).**

Emphasis has been placed on aseptic sampling and transport of mixed pools samples and individual sheep's milk samples intended for bacteriological examination.

Bacteriological examination

The inoculum of each sample of milk was inoculated on the plates with 5% blood agar, incubated at 37 °C, and after 24 hours of reading from. When the growth were more than 7 colonies from one type of colony were inoculated and cultured on selective nutrient soils. Identification of *Staphylococcus* spp. bacterial cultures was carried out according to the assessment growth of suspected bacteria on nutrient agars (5% of blood Agar, N° 110, Baird-Parker agar, Brilliance^{ž} UTI Clarity Agar (OXOID Ltd., Basingstoke, Hants, UK). The pigment formation, haemolysis, catalase positivity, Gram positivity, creation of free or coupled coagulase, and other characters, were determined. The identification of each species was made by STAPHYtest 24 and evaluated by TNW ProAuto 7.0 (Erba-Lachema, Brno, CZECH REPUBLIC) with a probability of correct designations of the kind above 90%. The functionality of the set was controlled using a strain of Staphylococcus aureus CCM 7113 (CCM, Masaryk University, Brno, CZECH REPUBLIC).

Testing of the sensitivity on antibiotics of the most numerous species of Staphylococcus

Bacteria isolated from various forms of mastitis (n = 432) and pools milk samples (n = 12), were tested in vitro by a disc method (**EUCAST, 2014)** by evaluation of the zones of inhibition to grow on Mueller-Hinton agar after 24 hours incubation at 37 °C.

To the test of sensitivity of staphylococci to fourteen antibiotics (ampicilin, amoxicillin, cefoperazone, cefoxitin, cloxacilin, erythromycin, lincomycin, neomycin, penicillin, novobiocine, oxacilin, methicilin, streptomycin and tetracycline) have been use test discs (OXOID Ltd., Basingstoke, Hants, UK) as shown in table 1. The choice of antibiotics reflects the range of which is contained in a number of intramammary products to treat mastitis, which are available in Slovakia. Sensitivity or resistance of the bacteria tested were interpreted according to the reference zones in accordance with the instructions of the **EUCAST (2014)**.

In the tests were used as control the tribes *S. aureus* CCM 5973 and *S. epidermidis* 4418. In view of the abundance of the species of the CPS, or CNS was only possible for species *S. aureus* and *S. epidermidis* in practical terms, to evaluate resistance as a percentage: a negligible
(< 0.1%), very low (0.1 – 1%), low (1 - 10%), moderate (10 – 20%), high (20 – 50%) or very high (50 – 70%).

Statistical analyses

Statistical analysis we performed using software Microsoft Excel 2007. Chi square test (χ^2test) we used to compare the individual proportions (Kabrt 2013). The dependence of the individual signs was tested at a significance level $\alpha = 0.05$, with critical value $\chi^2 = 5.991$.

Table 1 Used testing discs of antibiotics (OXOID Ltd. Basingstoke, Hants, UK).

Antibiotics	**D** (µg)	**Z** (mm)	**Antibiotics**	**D** (µg)	**Z** (mm)
Ampicilin	10	28-29	Methicilin	10	9-14
Amoxicillin	25	28-29	Neomycin	10	12-17
Cefoperazone	30	14-18	Novobiocine	5	17-22
Cefoxitin	30	23-29	Oxacilin	5	10-13
Cloxacilin	5	10-13	Penicillin	10 U	28-29
Erythromycin	10	13-23	Streptomycin	10	11-15
Lincomycin	15	9-15	Tetracycline	10	14-19

Note: D – dose of antibiotics in µg, content of one disc; Z - reference zones in mm.

RESULTS AND DISCUSSION

Table 2 gives an overview of the types of the bacteria *Staphylococcus spp*., which we have been isolated from sheep's milk, during the three years on holdings in Eastern Slovakia. In the reporting period, a total of 156 coagulase positive staphylococci were isolated of which 75% (117) was *S. aureus*, in 14% (22) has been isolated *S. intermedius*, and 10.9% (17) *S. hyicus*.

S. aureus was isolated during the reporting period, but most frequently at the beginning of the reference period (45). *S. intermedius* was isolated in the first two years of tracking, however, most in the second year (19). *S. hyicus* has been isolated only in the first year of follow-up (17). During the period considered from 288 coagulase negative staphylococci S. epidermidis was isolated in 37.5% (108), *S. schleiferi* 25.69% (74), *S. chromogenes* 23.61% (68), *S. cohnii, ssp. urealyticum* 3,82%, *S. xylosus* 2.43%, and other species occurred only rarely **(Table 2)**. **Table 3** provides an overview of the incidence of resistance to 14 tested antibiotics in the four species of staphylococci (n = 367), which were the most frequently isolated from sheep's milk, during the three years of follow-up.

The table 4 is showed the occurrence of resistance to 14 antibiotics in *S. aureus* (from CPS group), and *S. epidermidis* (most commonly occurring from 12 kinds of CNS), which have been isolated from sheep's milk in the course of three years. In the evaluation of the tests of sensitivity of the two most numerous species were numerically expressed as numbers of (S) -sensitive, (IM) – intermediate, (R) – resistant as well as the values of the resistance in percentage (%).

Staphylococcus aureus (tables 3 and 4) showed the highest resistance to novobiocine 14.5%, to erythromycin 12.8%, to penicillin, 7.69%, and 7.69% to lincomycin. As negative effect we can consider the incidence of intermediate susceptibility to novobiocine (15 strains), erythromycin (12), penicillin (14), oxacilin (11) cloxacilin and neomycine (9 strains). To others antibiotics, the incidence of resistant strains of *Staphylococcus aureus,* was relatively low.

Bogdanovičová et al. (2014) reported that the antimicrobial resistance profile of the tested S. aureus strains to different antibacterial agents revealed that 17.8% (n = 11) of the strains were resistant to at least one antibiotic.

In the framework of the coagulase negative staphylococci (tables 3 and 4) was on the sensitivity tested 108 strains from these *S. epidermidis* in 11.1% was resistant to novobiocine, and 8.3% to erythromycin, moreover, can be identified with intermediate sensitivity to the adverse ampicillin (13), lincomycin (11), erythromycin (10), amoxicilin, novobiocine and penicillin (9 tribes).

At work we are comparing the incidence of following characters (S, IS, R) in two groups, the most numerous of staphylococci *S. aureus* and *S. epidermidis* using statistical method Chi-squared test. On the significance level $\alpha = 0.05$ (5%), was recorded in twelve antibiotic substances test value ($G < \chi 2$), the statistically independence of tracked characters was confirmed. The antibiotic substance penicillin and novobiocine when applied, $G > \chi^2$, in the test groups of *S. aureus* and *S. epidermidis* statistically dependence of the observed characters was confirmed, which means that the occurrence of the characters was not random.

Table 2 The types of the bacteria *Staphylococcus spp*., which have been isolated from sheep's milk, during the three years on holdings in eastern Slovakia.

Bacteria ***Staphylococcus spp.***	**2015**	**2016**	**2017**	**Total**	**%**
S.aureus	45	39	33	117	26.4
S.intermedius	3	19	-	22	5.0
S.hyicus	17	-	-	17	3.8
S.capitis	2	-	-	2	0.5
S.caprae	5	-	-	5	1.1
S.carnosus	-	-	4	4	0.9
S.cohnii spp. urealyticum	1	-	10	11	2.4
S.condimenti	-	1	-	1	0.2
S.epidermidis	15	43	50	108	24.3
S.chromogenes	30	7	31	68	15.3
S.sciuri	2	-	1	3	0.7
S.schleiferi	4	37	33	74	16.6
S.simulans	3	-	-	3	0.7
S.warneri	-	-	2	2	0.5
S.xylosus	4	3	-	7	1.6
∑	**131**	**149**	**164**	**444**	**100.0**

Table 3 Total overview of the incidence of resistance to 14 tested antibiotics in the four species of staphylococci (n = 367), which were the most frequently isolated from sheep's milk, during the three years of follow-up.

CPS	R	n	Antibiotics													
			AMP	AML	CFP	FOX	OB	E	MY	MET	N	Nv	OX	P	S	TE
S.aureus (n = 117)	I.	45	4	3	2	1	1	3	4	1	6	9	4	10	1	3
	II.	39	2	1	1	1	1	8	3	1	5	5	1	5	-	-
	III.	33	1	-	-	-	1	4	2	-	2	3	-	3	-	-
	∑	117	7	4	3	2	3	15	9	2	13	17	5	9	1	3
S. epidermidis (n = 108)	I.	15	1	-	-	-	-	2	1	-	2	4	-	2	-	-
	II.	43	1	1	1	-	2	3	2	-	2	3	1	2	11	-
	III.	50	2	-	2	-	2	4	2	-	3	5	2	3	1	-
	∑	108	4	1	3	-	4	9	5	-	7	12	3	7	2	1
S.schleiferi (n = 74)	I.	4	-	-	-	-	1	1	-	-	1	2	-	-	-	-
	II.	37	1	-	-	-	-	1	2	-	1	1	-	1	1	-
	III.	33	1	-	-	-	1	3	1	-	3	5	1	2	-	-
	∑	74	2	-	-	-	2	5	3	-	5	8	1	3	1	-
S. chromogenes (n = 68)	I.	30	-	1	-	-	-	1	1	-	2	2	1	1	-	-
	II.	7	1	-	-	-	-	-	-	-	1	1	-	-	-	1
	III.	31	2	1	2	1	1	2	1	-	1	3	1	4	1	-
	∑	68	3	2	2	1	1	3	2	-	4	6	2	5	1	1
Resist. ∑ KNS (∑n)		250	9	3	5	1	7	17	10	-	16	26	3	15	4	2

Note: (AMP) Ampicilin 10 μg; (AML) Amoxicilin 25 μg; (CFP) Cefoperazone 30 μg; (FOX) Cefoxitin 30 μg; (OB) Cloxacilin 5 μg; (E) Erythromycin 10 μg; MY) Lincomycin 15 μg; (MET) Methicilin 10 μg; (N) Neomycin 10 μg; (Nv) Novobiocine 5 μg; (OX) Oxacilin 5 μg; (P) Penicillin 10 IU; (S) Streptomycin 10 μg; (TE) Tetracycline 10 μg.

Table 4 An overview of the sensitivity and the occurrence of resistance to 14 tested antibiotics in two types: *S.aureus* as representative of the CPS, and *S.epidermidis* as most frequently isolated from 12 species of CNS, isolated from sheep's milk in the years 2015 to 2017.

Antibiotics	*S. aureus* (n = 117)				*S. epidermidis* (n = 108)				Test*
	S	IS	R	%	S	IS	R	%	G
Ampicilin	104	6	7	6.0	91	13	4	3.7	3.910
Amoxicilin	107	6	4	3.4	98	9	1	0.9	2.439
Cefoperazone	110	4	3	2.6	99	6	3	2.8	0.620
Cefoxitin	112	3	2	1.7	106	2	-	-	2.008
Cloxacilin	106	9	2	1.7	97	7	4	3.7	0.957
Erythromycin	90	12	15	12.8	89	10	9	8.3	1.330
Lincomycin	101	7	9	7.0	92	11	5	4.6	2.095
Methicilin	115	-	2	1.7	105	3	-	-	5.103
Neomycin	95	9	13	11.1	96	5	7	6.5	2.592
Novobiocine	93	7	17	14.5	77	19	12	11.1	7.559[1]
Oxacilin	100	11	5	4.3	98	7	3	2.8	1.129
Penicillin	94	14	9	7.0	98	3	7	6.5	7.102[1]
Streptomycin	109	7	1	0.8	100	6	2	1.8	0.439
Tetracycline	102	9	3	2.6	103	4	1	0.9	2.769

Note: Sensitivity (S); Intermediate sensitivity (IS); Resistance (R);% - resistance from base n; * Chi-squared test (significance level $\alpha = 0.05$ (5%); critical value $\chi^2 = 5.991$; G – testing value).

The potential pathogenity of bacteria *Staphylococcus spp*. first and foremost, in the accompanying confirmation of virulence factors such as: an increased incidence of resistance to common antibiotics and disinfectants **(Moniri et al. 2007; Vautor et al., 2009)**, the production of biofilm **(Melchior et al. 2006)**, and the production of enterotoxins in isolated strains **(Scherrer et al. 2004)**.

CONCLUSION

By bacteriological examination of samples for the analysis of individual pools and sheep's milk, during the three seasons of machine milking were gradually isolated and taxonomically identified 15 types of bacteria *Staphylococcus spp*., (n = 444). Coagulase positive staphylococci (CPS) *S. aureus* was isolated during the reporting period, however, most often in the first year (45).

The incidence of *S. intermedius* was registered the most significant in the second year (19). *S. hyicus* has been isolated only in the first year of follow-up (17). From the coagulase negative staphylococci (CNS) (n = 288), *S. epidermidis* present in 37.5% (108), *S. schleiferi* 25.69% (74) and *S. chromogenes* 23.61% (68), while the *S. cohnii ssp. urealyticum* 3.82%, *S. xylosus* 2.43% and *S. caprae* 1.7%, other types of the rarely. The bacteria *S. aureus* (n = 117) showed the highest resistance to novobiocine 14.5%, to erythromycin 12.8%, to lincomycin and penicillin 7.69%.

The incidence of intermediate sensitivity was recorded against to novobiocine (15 strains), to erythromycin (12), to penicillin (14), to oxacilin (11) to cloxacilin and neomycin (9). In the framework of the sensitivity of the test strains with CNS has tested 108 strains of *S. epidermidis*, from which it was 11.1% resistant to novobiocin and 8.3% to erythromycin. In addition, unfavourable can mark a high intermediate sensitivity to ampicillin (13), lincomycin (11), erythromycin (10), amoxicilin, novobiocine and penicillin (9 tribes). In the work aimed at testing the top representatives of the genus *Staphylococcus spp*. is credited to relatively unfavourable evolution of resistance to the most commonly used antibiotics to treat the inflammation of the udder in sheep.

REFERENCES

Bogdanovičová, K., Skočková, A., Šťástková, Z., Karpíšková, R. 2014. Occurrence and antimicrobial resistance of staphylococcus aureus in bulk tank milk and milk filters. *Potravinarstvo*, vol. 8, no. 1, p. 97-101. https://doi.org/10.5219/363

Contreras, A., Sierra, D., Sánchez, A., et al. 2007. Mastitis in small ruminants. *Small Ruminant Res.*, vol. 68, p. 145-153. https://doi.org/10.1016/j.smallrumres.2006.09.011

Ergün, Y., Aslantas, Ö. M, Dogruer, G. et al. 2009. Prevalence and etiology of subclinical mastitis in Awashi dairy ewes in southern Turkey. *Turk. J. Vet. Anim. Sci*., vol. 33, p. 477-483.

European Society of Clinical Microbiology and Infectious Diseases (EUCAST). 2015. Disk Diffusion Method for Antimicrobial Susceptibility Testing. - Version 5.0 (January). Available from internet: http://www.eucast.org.

Fthenakis, G. C. 1994. Prevalence and aetiology of subclinical mastitis in ewes of southern Greece. *Small Rumin. Res.*, vol. 13, p. 293-300. https://doi.org/10.1016/0921-4488(94)90078-7

Kabrt, M. 2013. Aplicated statistics: Test chí-square independence in contingence table. [online]. http://www.milankabrt.cz/testNezavislosti/

Kiossis, E., Brozos, C. N., Petridou, E., Boscos, C. 2007. Program for the control of subclinical mastitis in dairy Chios breed ewes during lactation. *Small Ruminant Res*., vol. 73, p. 194-199. https://doi.org/10.1016/j.smallrumres.2007.01.021

Melchior, M. B., Fink-Gremmel, S. J., Gaastra, W. 2006. Comparative assessment of the antimicrobial susceptibility of *Staphylococcus aureus* isolates from bovine mastitis in biofilm versus planktonic culture. *In. J. Vet. Med. B Infect. Dis. Vet. Public Health*, vol. 7, p. 326-332. https://doi.org/10.1111/j.1439-0450.2006.00962.x PMid:16930277

Moniri, R., Dastegholi, K., Akramian, A. 2007. Increasing Resistant CNS in Bovine Clinical Mastitis. *Pakistan J. Biolog. Sci.*, vol. 15, 10, p. 2465-2469. https://doi.org/10.3923/pjbs.2007.2465.2469

Mørk, T., Waage, S., Tollersrud, T., Kvitle, B., Sviland, S. 2007, Clinical mastitis in ewes, bacteriology, epidemiology and clinical features. *Acta Vet.,* vol. 49, no. 23, p. 1-8. https://doi.org/10.1186/1751-0147-49-23

Pitkälä, A., Haveri, M., Pyörälä S., Myllys V. and Honkanen-Buzalski T. 2004. Bovine Mastitis in Finland 2001 - prevalence, distribution of bacteria, and antimicrobial resistance. *J. Dairy Sci*., vol. 87, p. 2433-2441. https://doi.org/10.3168/jds.S0022-0302(04)73366-4

Pyörälä, S. and Taponen, S. 2009. Coagulase-negative staphylococci\; emerging mastitis pathogens. *Vet.Microbiol*., vol.134, p.3-8. https://doi.org/10.1016/j.vetmic.2008.09.015

Scherrer, D., Corti, S., Muehlherr, J. E., Zweifel, C., Stephan, R. 2004. Phenotypic and genotypic characteristics of Staphylococcus aureus isolates from raw bulk-tank milk samples of goats and sheep. *Vet. Microbiol*., vol. 101, no. 2, p. 101-107. https://doi.org/10.1016/j.vetmic.2004.03.016 PMid:15172692

Vasiľ, M. 2004. *Dairy cows milk gland inflammation (Zápaly mliečnej žľazy dojníc).* Košice, Slovakia : University of veterinary medicine in Košice, Elektronic version, 132 p. ISBN 80-8077-005-0. (In Slovak).

Vautor, E., Cockfield, J., Marechal, C., Le Loir, Y., Chevalier, M. D., Robinson, A., Thiery, R., Lindsay, J. 2009. Difference in virulence between *Staphylococcus aureus* isolates causing gangrenous mastitis versus subclinical mastitis in a dairy sheep flock. *Vet. Res*., vol. 40, no. 6, p. 40-56.

Zigo, F., Vasil, M., Elecko, J., Farkasova, Z., Lapin, M. 2014. Production of enterotoxins of *Staphylococcus spp.* isolated from samples of sheep milk. *Potravinarstvo,* vol. 8, no. 6, p. 92-96. https://doi.org/10.5219/361

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EVALUATION OF SELECTED QUALITY PARAMETERS OF REDUCED SALT FRANKFURTERS

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ABSTRACT
Higher salt content in foods brings health risks associated with hypertension and leads to an increased risk of strokes and fatal vascular diseases. For this study, a frankfurter was chosen as a frequently consumed meat product. In repetitions, four groups of products were produced with different salt contents (2.0% and 1.4%) and the kind of meat used (CPF – Control Pork Frankfurters, RPF – Reduced salt Pork Frankfurters, CBF – Control Beef Frankfurters, RBF – Reduced salt Beef Frankfurters). Basic chemical parameters (dry matter, fat and salt content), colour parameters (CIE L*, a* and b*) and basic sensory analysis were performed before (UT – untreated) and after heat treatment (HT – heat treated). The lower salt content and the type of meat used have a significant effect (p <0.05) on the colour of the products. In almost all parameters frankfurters with pork meat scored better than frankfurters with beef meat. The lower salt content and the type of meat used proved to have affected the colour of the products. After heat treatment there were found statistical significant differences in saltiness between control and salt reduced group (UT, p = 0.0098; HT p = 0.0001). Sensory results were better with pork and higher salt. A more key role than salt content in frankfurters is played by the type of meat. Debrecener´s frankfurter was selected for this study, and its formula can serve as a good example for the manufacturers that there is no need to worry about one third salt mixture reduction in the recipe on frankfurters sensory analysis.

Keywords: sensory analysis, colour, lightness, beef, pork

INTRODUCTION
At present, the salt content in foods is hot topic. Excessive intake of sodium in the diet, however, brings health risks associated with hypertension and leads to an increased risk of strokes and fatal vascular diseases **(He and MacGregor, 2010)**. Unfortunately, the Czech Republic is in the income of salt content and the occurrence of diseases with this problem associated with the leading countries. However, sodium chloride and sodium nitrite have a key role in meat production.
Based on this evidence, most of European countries, under the World Health Organization policies, have adopted strategies for dietary salt reduction towards meeting the recommended intake of 5g salt per day as around 90% of the sodium in our diets comes in this form **(WHO, 2013)**. After the bread and cereals group, the largest source of sodium (salt) in the European diet is processed meat products **(Kloss et al., 2015)**.
In Europe, North America and Australia, around 70% of consumed salt comes from processed foods, among which 20% is derived from meat products **(Ruusunen and Puolanne, 2005)**. More specifically, 77% of sodium intake is obtained from packaged and restaurant food, 12% occurs naturally in foods and 11% comes from adding salt to food while cooking or while eating at the table. In fresh foods like meat, vegetables, and fruit, salt is naturally present in small quantities, but when processed, salt levels tend to increase exponentially.
The major issue when using lower salt concentrations in processed meat products is to be able to maintain the product quality characteristics without affecting the shelf-life or the economic viability of the product **(Desmond, 2006)**.
From a culinary perspective, salt is predominantly used to enhance food flavour, making even unpalatable food taste better. However, taste and preservation are not the only reasons for the use of high levels of sodium in foods. The sodium level is generally kept high due to the additional functional roles it provides. The presence of salt (1.5% – 2.5% w/w) in meat products (i) solubilizes meat proteins, (ii) activates extraction of proteins, enhancing hydration, and water holding capacity (WHC) **(Ruusunen and Puolanne, 2005)**, (iii) increases product cooking yield and juiciness **(Desmond, 2006)**, (iv) increases the viscosity of meat batters, thereby allowing formation of heat-stable emulsions, such as frankfurters **(Terrell, 1983)** and (iv) decreases fluid loss **(Offer and Trinick, 1983)**.

A problem with low-salt meat products is that, along with saltiness, reducing sodium will also affect product texture and flavour intensity. For example, increased muscle protein (i.e. visual lean meat content) reduces the perceived saltiness in meat products. When fat content is high, lower salt additions provide a more stable structure than in low-fat products. However, lowering the salt content to 1.4% NaCl in cooked sausages and to 1.75% in lean meat products has been shown to be possible while keeping an acceptable perceived saltiness, firmness, water-binding and fat retention (**Tobin et al., 2013**). Colour and material of surface of particular packaging of visual factors influences consumers **(Géci et al., 2017)**. Meat products are a common item in our diet. In today's meat processing industry, the role of economy and quality plays a key role. The meat industry is pushed to the lowest price by the retail chain, which reduces the meat content of the products **(Fekete et al., 2016)**. It is therefore desirable to modify the recipes to improve its qualitative and nutritional value. Scientific trends are moving towards enhancing hygienic quality using antimicrobial agents **(Kročko et al., 2017)** and antioxidants **(Bobko et al., 2017).** There are no limits on salt content in foodstuffs and therefore in meat products in the Czech Republic. Nutrition claims (low sodium/salt content, very low sodium/salt content, low sodium/salt content, no sodium/salt content) have been established within the European Union.

Scientific hypothesis

The aim of this study was to examine the importance of reducing the salt content of meat products according to the type of meat used on some quality parameters. Salt content and the type of meat used have probably an effect on the colour of product and final taste. The aim was also to assess the effect of heat treatment.

MATERIAL AND METHODOLOGY

The frankfurters were produced in three repetitions according to the quality standard of ON 57 7127 (Debrecínský párek as known as Debrecener´s frankfurter, beef H3 or H4, pork V4, pork V5 or V6 according to Czech Meat Processors Association) in the pilot plant CZ 22067 (approved by the State Veterinary Administration, Czech Republic) of Mendel University in Brno. A total of four groups of products were produced with different salt contents (C – control for 2.0%, R – reduced salt group for 1.4% NaCl) and the type of meat used (CPF – Control Pork Frankfurters, RPF – Reduced salt Pork Frankfurters, CBF – Control Beef Frankfurters, RBF – Reduced salt Beef Frankfurters). A debrecener is a pork and beef sausage of uniform fine texture and reddish-orange colour, named after the Hungarian city of Debrecen. Beef contain This frankfurter is spiced with paprika and other seasonings like garlic, pepper, cumin and ginger. Usually they contain tiny pieces of pork fat as well. They are usually lightly smoked and filled in ovine intestines. Standard machines used in industrial production (cutter, filler, smoker) were used. For CPF and RPF, beef from the recipe (35%) was replaced by pork lean production meat (80% muscles: 20% fat). Raw meat was kept in 2 °C and second day was coarsely ground to obtain meat emulsion in cutter (Seydelmann, Germany) and filled (HTS 150, Germany) in ovine intestine (20/22) and treated (74 °C, 18 min) in smoker (Bastramat, Germany). The salt content was in RPF and RBF recipes reduced by a total of 0.6% NaCl.

Quality evaluation of frankfurters

For quality determination were used methods of chemical and sensory analysis. For the instrumental measurement of the surface colour, the CIE colour space was used. Frankfurters was measured and evaluated in the fifth day (approx. halftime of its shelf life) before (UT – untreated) and after heat treatment (HT – heat treated). A standard convection oven (Rational, Germany) and heating mode (80 °C, 90% humidity, 10 minutes) were used for heat treatment.

Chemical analysis

The dry matter (g.100 g^{-1}), the fat content (g.100 g^{-1}) and the salt content (g.100 g^{-1}) after homogenization of the sample (250 g) in mixer for each group (CPF, RPF, CBF and RBF) were analyzed (AOAC, 2005). All analysis was undertaken in duplicate.

Colour measurement

Colour space L*, a* and b* was determined by CM 3500d spectrophotometer (Konica Minolta, Japan). The samples were measured (D 65, 6500°K) on the surface with SCE (Specular Component Exluded) and 8 mm slot in triplicate (3 pairs and in 2 batches). Heat treated frankfurters (HT) were left to cool down (20-25 °C) and measured as well as untreated (UT). Colour variation was determined as total colour difference ΔE^*_{ab} **(Saláková, 2012)**.

Sensory analysis

Sensory analysis was evaluated by an 8-member trained panel of academic staffs (3 men, 5 women) in special room under ČSN ISO 6658 (560050) condition. Sensory analysis was undertaken at special sensory laboratory with ten chambers (Department of Food Technology). All panel´s members buy and consume frankfurters regularly. For each frankfurter, assessors were asked to indicate their score on a 100mm line scale ranging from 0 at the left to 100 at the right. Descriptors expressed as the hedonic scores, where 0 is the sign minimum and 100 is maximum of pleasure. Sensoric descriptors were ranked as follows: appearance, colour, texture, juiciness, aroma, saltiness and taste (for overall taste) and used for untreated (UT) and heat treated (HT) frankfurters as the most common forms of consumption of this product. First were analyssed untreated frankfurters (UT), then after interval (10 min) and after heat treatment were immediately offered other frankfurters (HT) to the assessors. Water and non-salted bread were used as neutralizers. Samples were identified by a four-digit code. The sample groups were offered randomly to the assessors.

Statistical analysis

Data collected from experiments were analysed by analysis of variance (one-way ANOVA) and Tukey´s test to compare the treatment and groups according to it´s salt content and used meat by programme STATISTICA 12. Samples were considered significant at 95% confidence level (p <0.05) and data were tested for normality by Shapiro–Wilk test.

RESULTS AND DISCUSSION

The experimental products of all groups did not differ much from the products from the market network. This is due to the use of high-quality recipes without meat substitutes. Frankfurters with lower salt **(Table 1)** content had a higher dry matter compared to the control group. This was significant (p <0.05) for the group with beef in recipe (RBF). The difference between the groups accounted for less than 5%, which apparently did not represent a significant loss item. The difference in fat content is given by using the type of meat. Even though pork lean meat was used as a substitute for beef in CPF and RPF variants, this difference in results is obvious **(Table 1)**. The salt content was influenced by the composition of the recipe and by the intervention of the goals of the experiment. Nevertheless, it is evident that with the higher water losses given by the higher dry matter are frankfurters with lower salt content, there is also a higher salt content in the sausage. It is obvious that the current higher losses of water given by the higher frankfurters´ dry matter are associated with it´s a lower salt content. Therefore, the salt content of the completed product is also higher in these variants (30-40% in RPF and RBF) than in control variants (5% in CPF and CBF). This was independent of the beef or pork content **(Table 1)**, because there were no significant differences (p >0.05) between CPF and CBF or CBF and RBF groups of samples.

Untreated frankfurters (UT) with less salt and only with pork (RPF) were significantly (p <0.05) the lightest (L* = 45.80) of all samples. Most darker were heat treated (HT) beef frankfurters (CBF) (L* = 41.21). Coordinates a* for red colour and b* for yellow colour had different values before and after heat treatment **(Table 2)**. The higher a* values for the red colour of untreated frankfurters (UT) was recorded in variants with beef (CBF and RBF) and after heat treatment in control groups (CPF and CBF). Yellowness (b*) were highest in untreated frankfurters (UT) and it´s variants with reduced content of salt (RPF and RBF).

The lower salt content and the type of meat used have a significant effect (p <0.05) on the colour of the products **(Table 2)**.

Frankfurters of all groups become darker (Table 2) after heat treatment. The hightest total colour difference ΔE^*_{ab} (5.96) after treatment (Table 3) was in variants with beef meat (CBF and RBF) and when comparing control variants (CF ΔE^*_{ab}) with variants with reduced salt content (RF). Differences were higher in untreated groups (UT).

Table 4 shows the sensory assessment of frankfurters and their comparison between frankfurters with pork meat and frankfurters with beef meat. There were not found statistical differences in colour between control and salt reduce group in both group of samples (with pork and beef). After heat treatment there were found statistical significant differences in saltiness between control and salt reduced group. In almost all parameters frankfurters with pork meat scored better than frankfurters with beef meat **(Table 4)**. The perception of saltiness in the meat products is influenced by other factors than simply the salt content. It is for example the proportion of fat, water content, texture etc. **(Kameník et al., 2017)**. **Aaslyng et al. (2014)** reported that salt reduction from 2.2% to 1.7% did not alter the sensory properties in sausages. **Tobin et al. (2012)** that reported salts below 1.5% had a negative effect on consumer acceptability, with 2.5% salt concentrations franks being the most preferred by consumers.

Many current innovations in the processed meat field focus on healthier reformulations, namely improving the

Table 1 Basic chemical analysis of frankfurters with different salt and meat content.

Content (g.100 g^{-1})	Group of samples			
	CPF ($\bar{x}$ ±SD)	RPF ($\bar{x}$ ±SD)	CBF ($\bar{x}$ ±SD)	RBF ($\bar{x}$ ±SD)
Dry matter	39.54 ±1.93^{a}	41.50 ±1.50^{ab}	43.09 ±0.52^{b}	44.08 ±0.19^{c}
Fat	19.38 ±0.19^{c}	20.22 ±1.73^{d}	16.45 ±0.39^{a}	18.29 ±0.36^{b}
NaCl	2.05 ±0.11^{b}	1.65 ±0.10^{a}	2.10 ±0.08^{b}	1.51 ±0.19^{a}

Note: CPF – Control Pork Frankfurters, RPF – Reduced salt Pork Frankfurters, CBF – Control Beef Frankfurters, RBF – Reduced salt Beef Frankfurters; Means with different superscripts in the same rows show significant differences (p <0.05).

Table 2 Instrumental measurement of frankfurter´s colour surface depending on different salt and meat content.

Parameter	Treatment	Group of samples			
		CPF ($\bar{x}$ ±SD)	RPF ($\bar{x}$ ±SD)	CBF ($\bar{x}$ ±SD)	RBF ($\bar{x}$ ±SD)
L* (D 65)	UT	43.82 ±0.52^{a}	45.80 ±1.16^{b}	42.97 ±0.50^{a}	45.10 ±1.08^{b}
	HT	42.48 ±0.93^{b}	43.57 ±0.78^{c}	41.21 ±0.96^{a}	42.12 ±0.53^{b}
a* (D 65)	UT	27.45 ±1.02^{b}	26.50 ±1.01^{a}	29.71 ±0.39^{d}	28.57 ±0.28^{c}
	HT	26.47 ±0.72^{b}	25.42 ±0.70^{a}	26.54 ±0.48^{b}	25.45 ±0.39^{a}
b* (D 65)	UT	32.43 ±2.18^{ab}	35.34 ±2.05^{c}	31.45 ±2.18^{a}	34.36 ±2.08^{bc}
	HT	28.43 ±1.48^{ab}	30.73 ±1.93^{c}	27.28 ±1.40^{a}	29.58 ±1.95^{bc}

Note: UT – untreated, HT – heat treated; CPF – Control Pork Frankfurters, RPF – Reduced salt Pork Frankfurters, CBF – Control Beef Frankfurters, RBF – Reduced salt Beef Frankfurters; Means with different superscripts in the same rows show significant differences (p <0.05)

Table 3 Total colour differences ΔE^*_{ab} frankfurters according to different salt and meat content.

		Treatment	
	Groups	**UT**	**HT**
ΔE^*_{ab} **(CIE1976)**	CPF RPF	3.65	2.75
	CBF RBF	3.79	2.70
	PORK BEEF	2.50	1.78
	PORK	4.78	
	BEEF	5.96	
	CF RF	3.71	2.72
	CF	4.84	
	RF	5.77	

Note: UT – untreated, HT – heat treated; CPF – Control Pork Frankfurters, RPF – Reduced salt Pork Frankfurters, CBF – Control Beef Frankfurters, RBF – Reduced salt Beef Frankfurters; Means with different superscripts in the same rows show significant differences (p <0.05).

Table 4 Sensory analysis of frankfurters with different salt and meat content.

Descriptor	**Treatment**	**Group of samples**			
		CPF ($\bar{x}$ ±SD)	**RPF** ($\bar{x}$ ±SD)	**CBF** ($\bar{x}$ ±SD)	**RBF** ($\bar{x}$ ±SD)
Appearance	UT	81.88 ±10.91	80.50 ±13.61	74.88 ±10.91	73.50 ±13.61
	HT	91.56 ±6.28^{a}	88.31 ±6.23^{ab}	84.56 ±6.28^{b}	81.31 ±6.26^{c}
Colour	UT	86.13 ±5.23^{a}	87.00 ±5.15^{a}	75.13 ±5.23^{b}	76.00 ±5.18^{b}
	HT	87.44 ±6.16^{a}	88.44 ±6.32^{a}	76.44 ±6.16^{b}	77.44 ±6.30^{b}
Texture	UT	86.31 ±8.28^{a}	85.88 ±8.47^{a}	68.31 ±8.28^{b}	67.88 ±8.45^{b}
	HT	89.50 ±5.07^{a}	84.75 ±7.98^{a}	71.50 ±5.07^{b}	66.75 ±7.91^{b}
Juiciness	UT	78.31 ±14.90^{a}	72.81 ±13.80^{a}	54.31 ±14.90^{b}	48.81 ±13.88^{b}
	HT	90.31 ±4.54^{a}	78.56 ±4.20^{b}	66.31 ±4.50^{c}	54.56 ±4.13^{d}
Aroma	UT	86.38 ±6.51	85.69 ±5.88	83.38 ±6.51	82.69 ±5.86
	HT	90.13 ±4.11^{a}	85.00 ±5.38^{b}	87.13 ±4.11^{ab}	82.00 ±3.34^{b}
Saltiness	UT	84.38 ±10.66^{a}	79.19 ±8.67^{ab}	78.38 ±10.66^{ab}	73.19 ±8.63^{b}
	HT	89.19 ±6.65^{a}	77.63 ±7.01^{b}	83.19 ±6.65^{ab}	71.63 ±7.04^{b}
Taste	UT	89.06 ±7.66	85.81 ±8.02	92.44 ±7.05	89.81 ±8.07
	HT	90.25 ±4.82^{a}	81.13 ±7.25^{b}	94.25 ±4.82^{a}	85.13 ±7.29^{ab}

Note: UT – untreated, HT – heat treated; CPF – Control Pork Frankfurters, RPF – Reduced salt Pork Frankfurters, CBF – Control Beef Frankfurters, RBF – Reduced salt Beef Frankfurters; Means with different superscripts in the same rows show significant differences (p <0.05); Descriptors expressed as the hedonic scores, where 0 is the sign minimum and 100 is maximum of pleasure.

nutritional quality and reducing adverse effects of processed meat consumption **(Shan et al., 2017)**. Given that processed meat is a significant contributor to consumers' intake of salt and saturated fat, nutrients which are consumed more than the recommended level in many developed countries, one strategy is to reduce salt and/or fat content of those processed products with particularly high salt or fat content **(Bolger et al., 2017**; **Desmond, 2006**). From this point of view should be the best variant with reduced salt content and containing beef. Our results show that, although different from other variants, this difference did not pose a complication for the manufacturers. The consumer would not have noticed the difference.

Some strategies for innovations can be done by, for instance, not only directly lowering the amount of salt and fat in the recipe, which is the first possibillity. Some authors **(Horita et al., 2016)** describe using a salt substitute (e.g. potassium chloride or herbs), or by using animal fat replacements (e.g. starch or oil from non-animal sources). This way is complicated because of the possible impact on the sensory quality of the product. Some frankfurters are made with more apparent amount and kind of spices, so potential cover-up of such substances can occur. In this experiment we used above-mentioned recipe. From this perspective, Debrecen frankfurters can potentially be used for wider innovations. Another strategy involves the incorporation of healthy ingredients (e.g. vitamins and minerals, omega 3 fatty acids, probiotics, co-enzyme Q10, and dietary fibre) into processed meat **(López-López, et al. 2009)**. These ingredients can be introduced indirectly through animal feeding or directly during processing. Other strategy involves reducing or replacing chemical-based

preservatives, such as nitrites/nitrates **(Shan et al., 2017, (Sindelar et al., 2007)**. It is true that consumers are generally interested in the content of substances in food hazardous to health. Nitrites are thus negatively perceived by different consumer groups. However, nitrite replacement is a complicated intervention in the product recipe regarding its sensory and microbiological quality.

CONCLUSION

The reduction of salt content and the substitution of meat was reflected in the quality parameters of meat products. Frankfurters without beef only with pork meat were juicier and had higher aroma and more enjoyable appearance and colour after heat treatment. The products did not differ from the usual ones that can be bought in stores.

Reducing the salt content in consumers´ of known meat products is a way to rationally reduce sodium in food. Rather than developing new recipes or making a major legislation-regulated adjustment, recommendations should be made for manufacturers. Together with an assessment of such an adjustment, could be a guideline, especially for smaller producers in the regional market. This can better meet the demands of different consumer groups and will not require legislation or major interventions in large-scale meat production.

REFERENCES

AOAC, 2005. Official methods of analysis. Washington, DC: Association of Official Analytical Chemists.

Aaslyng, M. D., Vestergaard, C., Koch, A. G. 2014. The effect of salt reduction on sensory quality and microbial growth in hotdog sausages, bacon, ham and salami. *Meat Science*, vol. 96, no. 1, p. 47-55. https://doi.org/10.1016/j.meatsci.2013.06.004

ČSN ISO 6658 (560050), 2010. Sensory Analysis - Methodology – General Condition.

Bobko, M., Haščík, P., Kročko, M., Trembecká, L., Mendelová, A., Tkáčová, J., Czako, P., Tóth, T. 2017. Effect of grape seed extract on quality of raw-cooked meat products. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 517-521. https://doi.org/10.5219/79

Bolger, Z., Brunton, N. P., Lyng, J. G., Monahan, F. J. 2017. omminuted meat products — consumption, composition, and approaches to healthier formulations (Review). *Food Reviews International*, vol. 33, no. 2, p. 143-166. https://doi.org/10.1080/87559129.2016.1149861

Desmond, E. 2006. Reducing salt: A challenge for the meat industry. *Meat Science*, vol. 74, no. 1, p. 188-196. https://doi.org/10.1016/j.meatsci.2006.04.014

Fekete, T., Šnirc, M., Belej, L., Golian, J., Zajác, P., Čapla, J. 2016. Identification of differences in chemical composition among whole stick and sliced nitran salamis trough principal component analysis. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 10, no. 1, p. 170-175. https://doi.org/10.5219/568

Géci, A., Nagyová, L., Rybanská, J. 2017. Impact of sensory marketing on consumer´s buying behaviour. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 709-717. https://doi.org/10.5219/835

He, F. J., MacGregor, G. A. 2010. Reducing population salt intake worldwide: From evidence to implementation. *Progress in Cardiovascular Diseases*, vol. 52, no. 5, p. 363-382. https://doi.org/10.1016/j.pcad.2009.12.006

Horita, C. N., Farías-Campomanes, A. M., Barbosa, T. S., Esmerino, E. A., Gomes da Cruz, A., Bolini, H. M. A., Meireles, M. A. A., Pollonio, M. A. R. 2016. The antimicrobial, antioxidant and sensory properties of garlic and its derivatives in Brazilian low-sodium frankfurters along shelf-life. *Food Research International*, vol. 84, p. 1-8, https://doi.org/10.1016/j.foodres.2016.02.006

Kameník, J., Saláková, A., Vyskočilová, V., Pechová, A., Haruštiaková, D. 2017. Salt, sodium chloride or sodium? Content and relationship with chemical, instrumental and sensory attributes in cooked meat products. *Meat Science*, vol. 131, p. 196-202. https://doi.org/10.1016/j.meatsci.2017.05.010

Kloss, L., Meyer, J. D., Graeve, J., Vetter, W. 2015. Sodium intake and its reduction by food reformulation in the European Union — A review, *NFS Journal*, vol. 1, p. 9-19. https://doi.org/10.1016/j.nfs.2015.03.001

Kročko, M., Bobko, M., Ducková, V., Čanigová, M., Haščík, P., Tkáčová, J. 2017. Effect of thyme and oregano aqueous tea infusions on the lipid oxidation and sensory characteristics of frankfurkters sausages. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 602-605. https://doi.org/10.5219/798

López-López, I., Cofrades, S., Jiménez-Colmenero, F. 2009. Low-fat frankfurters enriched with n − 3 PUFA and edible seaweed: Effects of olive oil and chilled storage on physicochemical, sensory and microbial characteristics. *Meat Science*, vol. 83, no. 1, p. 148-154. https://doi.org/10.1016/j.meatsci.2009.04.014

ON 57 7127 (Debrecínský párek). Industry standard, 1977. (In Czech)

Offer, G., Trinick, J. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. *Meat Science*, vol. 8, no. 4, p. 245-281. https://doi.org/10.1016/0309-1740(83)90013-X

Ruusunen, M., Puolanne, E. 2005. Reducing sodium intake from meat products. *Meat Science*, vol. 70, no. 3, p. 531-541. https://doi.org/10.1016/j.meatsci.2004.07.016

Saláková, A. 2012. Instrumental measurement of texture and color of meat and meat products. *Maso International*, no. 2, p. 107-117.

Shan, L. C., De Brún, A., Henchion, M., Li, Ch., Murrin, C., Wall, P. G., Monahan, F. J., 2017. Consumer evaluations of processed meat products reformulated to be healthier – A conjoint analysis study. *Meat Science*, vol. 131, p. 82-89. https://doi.org/10.1016/j.meatsci.2017.04.239

Sindelar, J. J., Cordray, J. C., Olson, D. G., Sebranek, J. G., Love, J. A. 2007. Investigating quality attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial hams, bacons, and frankfurters. *Journal of Food Science.*, vol. 72, no. 8, p. 551-559. https://doi.org/10.1111/j.1750-3841.2007.00486.x

Terrell, R. N. 1983. Reducing the sodium content of processed meats. *Food Technology*, vol. 37, no. 7, p. 66-71.

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M., Kerry, J. P. 2012. Effect of varying salt and fat levels on the sensory and physiochemical quality of frankfurters. *Meat Science*, vol. 92, no. 4, 659-666. https://doi.org/10.1016/j.meatsci.2012.06.017

Tobin, B. D., O'Sullivan, M. G., Hamill, R. M., Kerry, J. P. 2013. The impact of salt and fat level variation on the physiochemical properties and sensory quality of pork breakfast sausages. *Meat Science*, vol. 93, no. 2, p. 145-15. https://doi.org/10.1016/j.meatsci.2012.08.008

WHO 2013. Mapping salt reduction initiatives in the WHO European Region, World Health Organisation. Regional Office for Europe.

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MASTITIS PATHOGENS AND THEIR RESISTANCE AGAINST ANTIMICROBIAL AGENTS IN HERDS OF DAIRY COWS SITUATED IN MARGINAL PARTS OF SLOVAKIA

Fratišek Zigo, Milan Vasiľ, Juraj Elečko, Martina Zigová, Zuzana Farkašová

ABSTRACT

Marginal regions are relatively large part of the area Slovakia which in terms of the economy breeding ruminants can efficiently produce animal commodities only occasionally. Geographic, social and economic stability of these regions is strongly influenced by breeding of ruminants with market milk production. Mastitis is a disease complex that assumes highest clinical and economic significance in milk animals particularly medium to high yielding dairy cattle, usually in and around periparturient period. The objective of this study was to evaluate the effectiveness of different antibiotics against mastitis causing microorganisms during first month of lactation in two herds of 230 and 310 dairy cows situated in marginal parts of Slovakia. Milk samples from quarters were cultured and identified bacteria were subjected to antimicrobial susceptibility test by disc diffusion method to a large number of antibiotics. The prevalence of mastitis in the monitored herds of dairy cows was 26.1% to 17.6%, respectively. A total of 1663 milk samples from udder quarters were investigated, 446 (21.3%) samples were positive. No pathogens were isolated from 1663 (78.4%) milk samples. From all tested bacteria *Staphylococcus* spp. and *Streptococcus* spp. which were isolated from subclinical and clinical mastitis, were found amoxicillin + clavulanat and tetradelta to be most effective drug followed by ceftiofur and rifaximin. The significant difference was confirmed between the *Staph. aureus* and coagulase-negative staphylococci (CoNS) isolates with respect to their susceptibility to the various antibiotics. Antibiotic susceptibility tests should be done to determine the effectiveness of drug that can be used for successful treatment of diseases. Proper isolation and identification of the causative organism play significant role in prevention and control of the diseases.

Keywords: dairy cows; mastitis; resistance; *Staphylococcus aureus*; *Streptococcus agalactiae*

INTRODUCTION

Economic, social and geographic stability of marginal regions is strongly influenced by the existence of agriculture and especially livestock production (cows and sheep represent 75% of animal production of these areas). Products from dairy ruminants are unique, especially in the field of rational nutrition of consumers. Many of the milk products and specialties can be included among the functional foods **(Vršková et al. 2015)**.

Inflammation of the mammary gland - mastitis is the most significant disease of dairy herds, has huge effects on farm economics due to reduction in milk production and treatment costs **(Østerås 2006; Pyörälä and Taponen, 2009)**.

In Slovakia, costs of clinical mastitis is estimated about 150 – 200 € per cow/year, above the desired baseline in European dairy herds **(Tančin et al. 2006)**.

The estimated loss of milk per cow per one lactation cycle is 70% of the total losses and the cost of cows lost due to premature culling is 14%, while the cost of milk downgraded/ discarded due to mastitis is 7% and the cost incurred on medical treatment and other veterinary expenses amount to 8%, of the total losses, reported worldwide. Mastitis low prevalence herds can save up to 25% cost on losses than the high prevalence herds **(Kader et al. 2002; Shaheen et al. 2016)**.

Bovine mastitis, is predisposed by several epidemiological risk factors that play significant role in causing mammary incompetence to protect it from the invasion of infectious agents. These should receive due consideration in the course of developing an integrated mastitis control programme. The risk factors include the host factors, environmental factors and the pathogen factors **(Vasiľ et al. 2004; Pitkälä et al. 2001)**.

Mastitis, can be caused by a wide range of organisms, including gram-negative and gram-positive bacteria, mycoplasmas and algae **(Malinowsky, 2000; Malinowsky and Gajewski, 2009; Zadoks et al. 2011; Pitkälä et al. 2004)**.

Many microbial species that are common causes of bovine mastitis, such as *Escherichia coli, Streptococcus*

agalactiae, Staphylococcus aureus and *Klebsiella pneumoniae*, also occur as commensals or pathogens of humans whereas other causative species, such as *Streptococcus uberis, Streptococcus dysgalactiae subsp. dysgalactiae* or *Staphylococcus chromogenes*, are almost exclusively found in animals **(Zadoks et al. 2011; Kmeť et Bujňáková, 2018)**.

Antimicrobials are routinely used for treatment of dairy cattle affected with clinical and subclinical infections **(Aarestrup, 2005; Medveďová et al. 2009)**.

The use of antimicrobials has, over time, increased the number of antimicrobial-resistant microbes globally, and any use of these agents will to some extent benefit the development of resistant strains and also inappropriate usage of antimicrobials such as wrong dose, drug or duration may contribute the most to the increase in antimicrobial resistance without improving the outcome of treatment **(Williams, 2000; Idriss et al. 2014)**.

Scientific hypothesis

The aim of this study was to evaluate the effectiveness of different antibiotics against mastitis causing microorganisms during first phase of lactation in two dairy herds cows situated in marginal parts of Slovakia.

MATERIAL AND METHODOLOGY

Animals and milking

The study was conducted according to good veterinary practice. The practical part of study was realized in two different farms situated in marginal parts of Slovakia (Orava, Zemplín) with standard zootechnic and zoohygienic conditions. Herd size ranged from 230 (A) to 320 (B) dairy cows of Holstein breed between 2nd - 4th lactation was used, respectively. Dairy cows from both farms were kept in a free housing system with a separate calving barn and equipped with individual boxes with bedding and were allowed *ad libitum* access to water. All herds were fed total mixed ration based on grass silage, maize silage and concentrate.

All cows were milked twice daily. The cows from herd A were milked in tandem parlor DeLaval 2x5 (Tumba, Sweden). In parallel parlor Boumatic 2x12 Xpressway (Wisconsin, USA) were milked cows from herd B. The average milk yield ranged from 7300 (herd A) to 7800 l (herd B) per year. Blanket dry cow therapy was implemented in all herds.

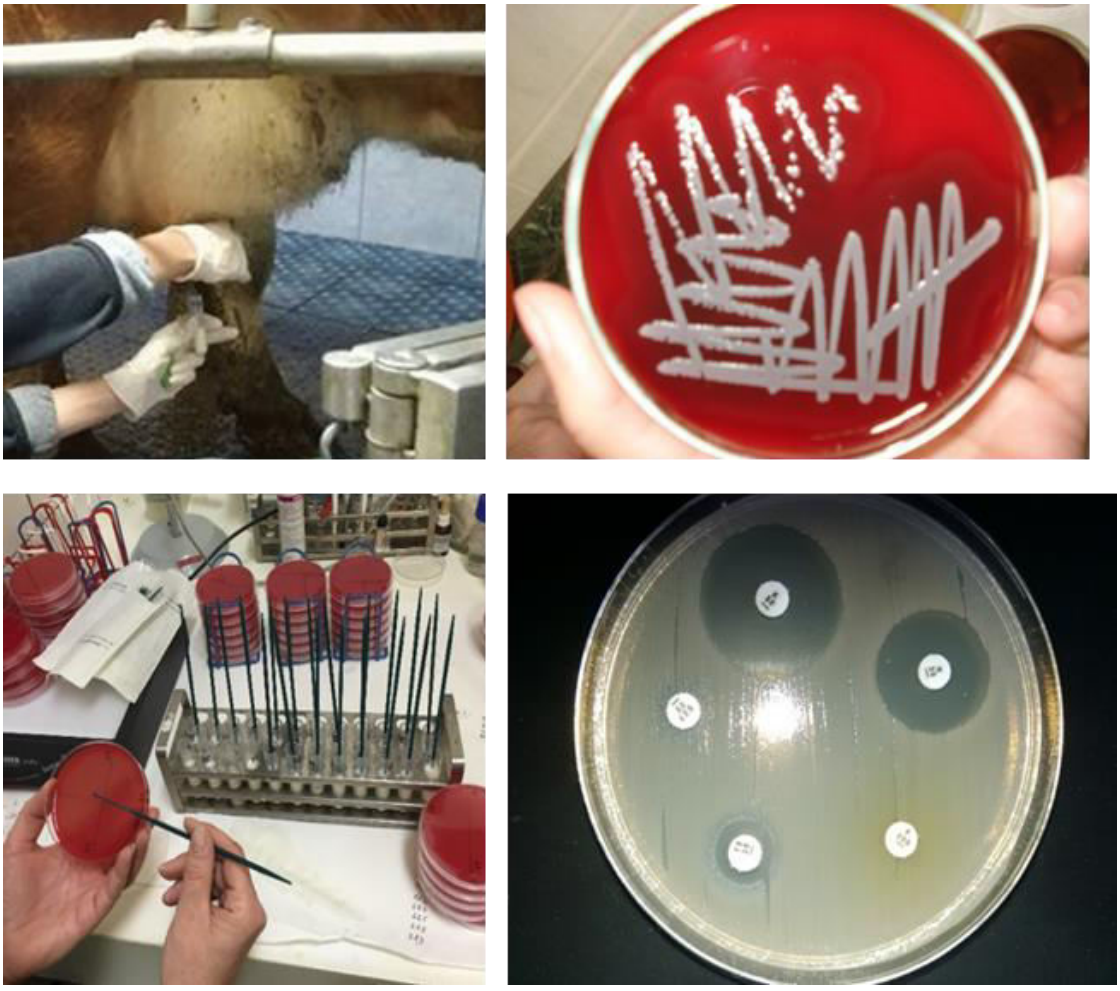

Figure 1 Quarter milk samples collection, bacteriology analysis and antimicrobial susceptibility test by disc diffusion method.

Samples collection

A complex examination of the health status of the animals included clinical examination of the mammary gland, cytological examination the first portion of milk, NK-test reaction with subsequent collecting of milk samples (quarter samples) for bacteriological examination, and subsequent cultivation and identification of pathogenic bacteria **(Figure 1)**. Quarter milk samples were collected aseptically from 2120 quarters (530 cows) a first month after calving. Eleven quarters were atrophic. Before sampling, the first streams of milk were discarded, and teat ends were disinfected. The 10 ml of the milk was collected into sterile tubes. The samples were cooled and immediately transported to the laboratory.

Laboratory analyses

Bacteriological examinations were performed according to commonly accepted rules **Malinowski and Klossowska (2002)**. Milk samples (0.05 mL) were inoculated onto blood agar (Oxoid, UK) and cultivated at 37 °C for 24 h. Based on the colony morphology and by Gram staining, bacteria *Staphylococcus* spp. were selected for the tube coagulase test (Staphylo PK, ImunaPharm, SR). Suspect colonies *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriacae* spp. were isolated on blood agar, cultivated at 37 °C for 24 h and detailed identified biochemically using the STAPHY-test, STREPTO-test, resp. ENTERO-test and identification by software TNW Pro 7.0 (Erba-Lachema, CZ).

Health udder and individual forms of mastitis (subclinical, subacute and acute) based on clinical signs, NK-test scores and bacteriological examination of milk samples were classified according to **Jackson and Cockcroft (2002)**.

Antimicrobial susceptibility test

The bacteria *Staphylococcus* spp. and *Streptococcus* spp. isolated through microbiological procedures were subjected to antimicrobial susceptibility test by disc diffusion method to identify the most effective drugs for mastitis treatment in the study area **(Hameed et al. 2008)**.

The sensitivity against penicillin, amoxicillin, amoxicillin + clavulanat acid, ceftiofur, cloxacillin, enrofloxacin, lincomycin, neomycin, nafpenzal, rifaximin, streptomycin and tetradelta were determined on Mueller Hinton agar as described by National Committee for Clinical Laboratory Standards **(NCCLS, 2002)**. The results were obtained by measuring the diameter of the growth inhibition zone around the antibiotic disc for each isolated bacterial strain and recorded as sensitive, intermediate and resistant.

Statistical analysis

Statistical analyses were performed using Graph-Pad PRISM 6.0 (GraphPad Software Inc., USA). Differences in incidence of mastitis among herds and in the distribution of the antibiotic resistant bacteria were statistically analyzed using Chi-square test. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

The prevalence of mastitis in the monitored herds of dairy cows was 26.1% to 17.6%, respectively (**Table 1**).

The economic losses are more associated with subclinical mastitis which is 40 % more prevalent than clinical mastitis **(Hortet et al. 1999)**.

However, the cost of treatment of subclinical mastitis is much low compared to that of clinical mastitis accounting for 10 – 20 times higher **(Shaheen et al. 2016)**.

Occurrence of subclinical forms in our study were from 10.4% to 7.8%, respectively. Subclinical mastitis is difficult to detect due to the absence of any visible indications, and it has major cost implications. Chronic mastitis is a rarer form of the disease but results in persistent inflammation of the mammary gland. Currently, milk quality payments are based on somatic cell counts (SCC), and elevated levels result in reduced payments. This, in addition to reduction in milk volume and treatment costs, significantly affects farm incomes **(Allore et al. 1998; Seegers et al. 2003)**.

The differences in incidence of subacute forms mastitis in examinated quarters among herds were partially statistically significant ($p < 0.05$). The incidence of subacute mastitis varied among herds from 9.0 % to 4.1 %, respectively. Occurrence of clinical mastitis were from 4.0 % to 1.9 %, respectively **(Figure 2)**.

The nutrient composition of milk is ideal medium for bacterial growth and therefore it can be considered one of the most perishable agricultural products because it can so easily be contaminated. Raw cow and sheep milk may contain microorganisms which can cause food borne disease **(Zajác et al. 2012)**.

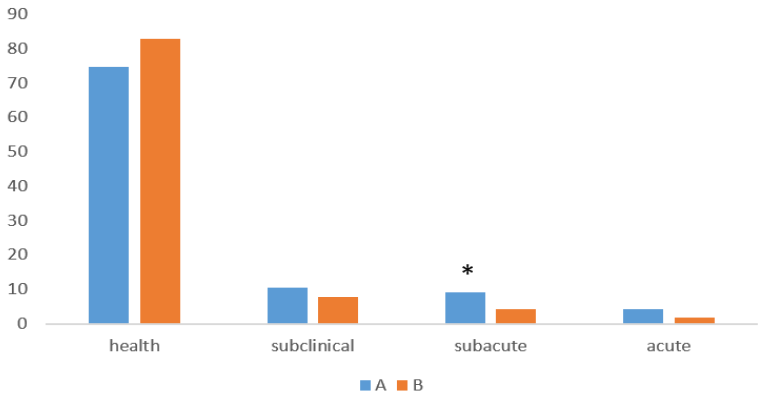

Figure 2 Comparison of individual forms of mastitis. Note: A – dairy herd with prevalence of mastitis 26.1%, B – dairy herd with prevalence of mastitis 17.6%.

Table 1 Incidence of mastitis in examinated herds.

Monitored herds	A		B		Total
	n	%	n	%	n
Healthy quarters	668	73.9	995	81.8	1663
Positive quarters	232	26.1[a]	214	17.6[b]	446
Infected quarters	207	22.8[a]	182	14.9[b]	389
Reject quarters	4	0.4	7	0.6	11
All examinated quarters	904	100	1216	100	2120
Total dairy cows in herd	226		304		530

Note: a, b – values in row with different superscript letters differ significantly at $p < 0.05$.

Table 2 Isolated microorganisms from infected quarters in monitored herds.

Isolated microorganisms	n	subclinical		subacute		acute	
		A %	B %	A %	B %	A %	B %
Staph. aureus	*49*	14.3	6.1	20.4	10.2	36.7	12.2
Str. uberis	*17*	11.8	-	41.2	-	47.5	-
Str. agalactiae	*31*	9.7	6.4	35.5	12.9	22.6	12.9
*Streptococcus spp.**	*25*	24.0	12.0	36.0	12.0	-	16.0
CNS*	*131*	16.7	34.4	12.2	9.9	5.3	3.1
CPS*	*14*	28.6	21.4	35.7	14.3	-	-
E. coli	*36*	38.9	25.0	27.8	8.3	-	-
Enterococcus spp.	*30*	16.7	50.0	16.7	20.0	-	-
Bacillus spp.	*28*	46.4	17.9	17.9	7.1	-	10.7
Enterobacter aerogenes	*17*	52.9	17.4	17.4	-	-	11.7
*Others**	*11*	54.5	27.3	18.2	-	-	-
Total	**389**	**24.1**	**18.3**	**21.3**	**9.8**	**10.3**	**5.9**

Note: n – number of isolated bacteria, Others* – *Proteus* spp., *Aerococuss* spp., CPS* – *S. hyicus, Str.* spp.* - *Streptococcus faecalis, Str. dysgalactiae, Str. suis,* CNS* – *S. haemolyticus, S. chromogenes, S. xylosus, S. epidermidis* and *S. warneri.*

A total of 1663 milk samples from udder quarters were investigated, 446 (21.3 %) samples were positive. No pathogens were isolated from 1663 (78.4 %) milk samples.

Several authors in their studies from Finland and Norway recorded, that the species of *Staphylococcus* spp. belongs to general aetiological agents of intramammary infections in ruminants (*S. aureus* in clinical and CoNS in subclinical cases) **(Pittkala et al. 2004; Pyörälä and Taponen, 2009)**.

From the CoNS are more frequently *S. xylosus, S. epidermidis* and *S. chromogenes* **(Østerås et al. 2006)**, what are also determined in our study.

It is well known that (CoNS) are the most important bacteria involved in subclinical bovine mastitis **(Pyörälä and Taponen, 2009)**, alongside *Staphylococcus aureus* **(Malinowski and Gajewski, 2009)**.

Their resistance to antimicrobial agents is common due to the high antibiotic pressure in conventional dairy farming. Usually different CoNS species from bovine milk differ significantly in their phenotypic and genotypic antimicrobial resistance profile, which is important for udder health management **(Sampimon et al. 2011)**.

Enterococci, on the other hand, have only limited clinical importance in dairy farming, but their ubiquitous nature and frequent carriage of resistance genes is a reason for concern **(Zadoks et al. 2011)**.

In addition to staphylococcus, the main mastitis pathogens are *Streptococcus agalactiae, Streptococcus dysgalactiae* and *Streptococcus uberis*. In our study, many infections caused by *Streptococcus uberis* and *Streptococcus agalactiae* were subacute and acute **(Lalrintluanga et al. 2003)**.

In recent years, reported *Streptococcus uberis* incidence has increased with 16 cases/100 cows/year reported for 2005 **(Bradley et al. 2007)**.

Bovine mastitis is the single most common cause for antibacterial use in lactating dairy cattle. Treatment of this disease is also the most common cause of illegal antibacterial residues in marketed milk **(Muhamed et al. 2012)**.

Antibacterial therapy of bacterial induced diseases in cattle has been incriminated as a catalyst for resistance in bacteria isolated from treated animals, other animals within the herd, and food derived from cattle for human consumption **(Foltys and Kirchrneová, 2005; Bengtsson et al. 2009)**.

The resistance in tested isolates are described in Table 3. From all tested bacteria *Staphylococcus* spp. and *Streptococcus* spp. were found Amoxicillin + clavulanat

Table 3 Frequency of susceptibility of *Staphylococcus* spp. and *Streptococcus* spp. to antibiotics.

Bacterial strains	***Staphylococcus aureus*** (49)			**CNS** (131)			***Streptococcus agalactiae*** (31)			***Streptococcus uberis*** (17)		
Antibiotic	**S**	**IS**	**R**	**S**	**IS**	**R**	**S**	**IS**	**R**	**S**	**IS**	**R**
Penicillin	37	4	8	112	6	13	27	2	2	15	2	0
Ampicillin	41	2	6	116	4	11	27	3	1	10	1	1
Amoxicillin	40	3	6	121	2	8	26	3	2	13	2	2
Amox. + clav.	48	0	1	126	2	3	31	0	0	16	1	0
Ceftiofur	46	0	3	123	2	6	29	2	0	17	0	0
Cloxacillin	38	4	7	121	4	6	29	0	2	14	1	2
Enrofloxacin	39	2	8	117	7	7	27	2	2	14	2	1
Lincomycin	41	3	6	118	5	8	26	2	3	13	2	2
Neomycin	38	4	7	119	4	8	25	3	3	11	3	3
Nafpenzal	42	2	5	122	2	4	30	0	1	16	1	0
Rifaximin	44	2	2	123	3	3	28	1	2	16	1	0
Streptomycin	36	3	11	116	8	7	21	4	6	10	3	4
Tetradelta	47	0	2	128	1	3	31	3	0	16	0	1

Note: R – resistant, IS – intermediate sensitive, S – sensitive.

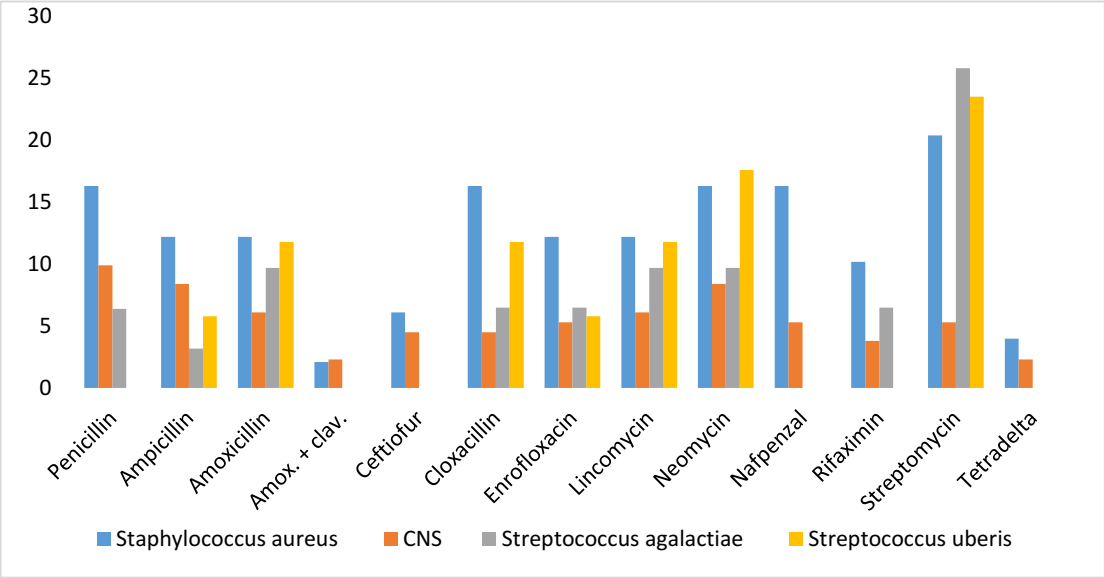

Figure 3 Difference between the pathogenic bacteria with respect to their susceptibility to the various antibiotics (%).

and tetradelta to be most effective drug followed by ceftiofur and rifaximin. On the contrary, antibiotics showing higher rate of resistance patterns were streptomycin, amoxicillin and penicillin. The significant difference (p <0.05) was confirmed between the *Staph. aureus* and CoNS isolates with respect to their susceptibility to the various antibiotics. There is no significant difference in the tested *Str. uberis* ans *Str. agalactiae* **(Figure 3)**.

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 and streptomycin. Staphylococci were mostly susceptible to antimicrobials tested but, **Muhamed et al. (2012)** found that *Staph. aureus* was resistant to penicillin and streptomycin (41.4% and 25.6% respectively).

Similar results were obtained by **Sumathi et al. (2008)** where *Staphylococcus* and *Streptococcus* spp. were resistant to streptomycin and penicillin. Those results are in accordance with our findings.

Vasil' (2009) tested isolated strains of *Streptococcus* spp. and CoNS and has found that *Strep. agalactaie* strains were sensitive to antibiotics except to penicillin, ceftiofur, while *Strep. uberis* was a complete sensitive to a combination of amoxicillin + clavulanat and ampicillin, followed by cefalotin, lincomycin, whilst it is resistant to

streptomycin, novobiocin and neomycin. Tested strains of CoNS were sensitive to a combination of amoxicillin + clavulanat and resistant to streptomycin and penicillin.

These results are in accordance with our findings that CoNS, *Strep. agalactiae*, *Strep. uberis* were sensitive to amoxicillin + clavulanat and tetradelta.

CONCLUSION

The results of our study showed that the incidence of subclinical and subacute mastitis at the start of lactation in monitored dairy herds is high. CoNS were the most frequently isolated from subclinical mastitis cases; however, clinical mastitis caused by the contagious pathogens *Staph. aureus, Str. agalactiae* and *Str. uberis* are still a problem and play an important role in dairy herds in marginal parts of Slovakia.

Antibiotic susceptibility tests should be done to determine the effectiveness of drug that can be used for successful treatment of diseases. In our study combinations of amoxicillin plus clavulanat acid, tetradelta and ceftiofur were the most effective antibiotics for control of bovine mastitis.

Antimicrobial resistance surveys in dairy production are mostly focused on udder pathogens and milk samples from drug treated animals. However, it is also important to evaluate the presence of resistant bacteria in regularly collected raw milk samples from clinically healthy animals, in order to assess the potential spread of resistant strains from raw material to dairy products.

REFERENCES

Allore, H. G., Erb, H. N., Schruben, L. W., Oltenacu, P. A., 1998. A simulation of strategies to lower tank somatic cell count below 500 000 per mililiter, *Jour. Dairy Sci*., vol 81, no. 3, p. 694-702. https://doi.org/10.3168/jds.S0022-0302(98)75625-5

Aarestrup, F. M. 2005. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. *Basic Clin. Phar. Toxicology*, vol. 96, no. 4, p. 271-281. https://doi.org/10.1111/j.1742-7843.2005.pto960401.x PMid:15755309

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. *Vet. Microbiology*, vol. 136, p. 142-149. https://doi.org/10.1016/j.vetmic.2008.10.024

Bradley, A.J., Leach, K.A., Breen, J.E. et al. 2007. Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. *Vet Rec*., vol. 160, no. 8, p. 253-257. https://doi.org/10.1136/vr.160.8.253

Foltys, V., Kirchrneová, K. 2005. Development of mastitis pathogens occurrence and their susceptibility to antibiotics in basic production of milk. *Jour. of Farm Anim. Sci*., vol. 38, p. 177-180.

Hameed, S., Arshad, M., Ashraf, M., Avais, M., Shahid, M. A. 2008. Prevalence of common mastitogens and their antibiotic susceptibility in tehsil Burewala, Pakistan. *Pakistan Journal of Agricultural Science*, vol. 45, no. 2, p.182-183.

Hortet, P., Beaudeau, F., Seegers, H. 1999. Reduction in milk yield associated with somatic cell counts up to 600 000 cells/ml in French Holstein cows without clinical mastitis. *Livest. Prod. Sci*., vol. 61, p. 33-42. https://doi.org/10.1016/S0301-6226(99)00051-2

Idriss, S. H., Foltys, V., Tančin, V., Kirchnerová, K., Tančinová, D. Zaujec, K. 2014. Mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Nitra. Slovakia. *Slovak J. Anim. Sci*. vol. 47, no. 1, p. 33-38.

Jackson, P., Cockcroft, P. 2002. Clinical Examination of Farm Animals. Oxford, UK, Blackwell Science Ltd, Wiley-Blackwell, p. 154-166. https://doi.org/10.1002/9780470752425

Kader, M. A., Samad, M. A., Saha, S., Taleb, M. A. 2002. Prevalence and etiology of mastitis with antibiotic sensitivity in milch cows of Bangladesh. *Indian Jour. Dairy Sci*., vol. 55, p. 218-223.

Malinowski, E., 2000. Mastitis prophylaxis and treatment during dry period – advantages and threats. *Med. Weter*., vol. 56, p. 759-763.

Kmeť, V., Bujňáková, D. 2018. Antimicrobial resistance escherichia coli isolated from calves, *Journal of Microbiology Biotechnology and Food Science*, vol. 7, no. 4, p. 412 – 415. https://doi.org/10.15414/jmbfs.2018.7.4.412-415

Lalrintluanga, C., Ralte, E. L., Markunga, H. 2003. Incidence of mastitis, bacteriology and antibiogram in dairy cows in Mizoram. *Indian Vet. J*., vol. 80, p. 931-932.

Malinowski, E., Gajewski, Z., 2009. Characteristics of cows mastitis caused by human foodborne pathogens. *Życie Wet*., vol. 84, p. 290-294.

Malinowski, E., Lassa, H., Kłossowska, A., Smulski, S., Markiewicz, H., Kaczmarowski, M., 2006. Etiological agents of dairy cows' mastitis in western part of Poland. *Pol. J. Vet. Sci*., vol. 9, p. 191-194. PMid:17020014

Medveďová, A., Valík, Ľ., Sirotná, Z.,Liptáková, D. 2009. Growth Characterization of Staphylococcus aureus in Milk: a Quantitative Approach. *Czech Journal of Food Science*, vol. 27, no. 6, p. 443-453.

Muhamed, H. M., Doss, A., Vijayasanthi, M., and Venkataswamy, R. 2012. Antimicrobial drug susceptibility of staphylococcus aureus from subclinical bovine mastitis in Coimbatore, Tamilnadu, *South India. Vet. World*., vol. 5 ,no. 6, p. 352-355. https://doi.org/10.5455/vetworld.2012.352-355

McDougall, S., 2003. Intramammary treatment of clinical mastitis of dairy cows with a combination of lincomycin and neomycin, or penicillin and dihydrostreptomycin. *N. Z. Vet. J*., vol. 51, no. 3, p.111-116. https://doi.org/10.1080/00480169.2003.36349

National committee for clinical laboratory standards (NCCLS). 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Approved Standard. NCCLS Document M31-A2, Wayne, 2002, PA.

Østerås, O., Sølverød, L,. Reksen, O., 2006. Milk culture results in a large Norwegian survey-effects of season, parity, days in milk, resistance, and clustering. *Jour. Dairy Sci*., vol. 89, no. 3, p. 1010-1023. https://doi.org/10.3168/jds.S0022-0302(06)72167-1

Pittkala, A., Haveris, M., Pyorala, S., Myllys, V., Buzalski, T. H. 2004. Bovine mastitis in Finland 2001 - prevalence, distribution of bacteria and antimicrobial resistance. *Journal of Dairy Science*, vol. 87, no. 8, p. 2433-42. https://doi.org/10.3168/jds.S0022-0302(04)73366-4

Pyörälä, S., Taponen, S. 2009. Coagulase-negative staphylococci; emerging mastitis pathogens. *Vet. Microbiol*., vol. 134, p. 3-8. http://dx.doi.org/10.1016/j.vetmic.2008.09.015

Shaheen, M., Tantary, H.A. and Nabi, S.U. 2016. A treatise on bovine mastitis: disease and disease economics, etiological basis, risk factors, impact on human health, therapeutic

management, prevention and control strategy. *J. Adv. Dairy Res*., vol. 4, no. 1 p. 1-10. https://doi.org/10.4172/2329-888X.1000150

Seegers, H., Fourichon, Ch., Beaudeau, F. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet. Res*., vol. 34, p. 475-491. https://doi.org/10.1051/vetres:2003027 PMid:14556691

Sampimon, O. H., Barkema, W., Berends, I., Sol, J., Lam, T. 2009. Prevalence of intramammary infection in Dutch dairy herds. *Journal of Dairy Research*, vol. 76, 2, p. 129-136. https://doi.org/10.1017/S0022029908003762

Sumathi, B. R., Veeragowda, B., Amitha, R. G. 2008. Prevalence and antibiogram profile of bacterial isolates from clinical bovine mastitis. *Vet. World*, vol. 1, no. 8, p. 237-238.

Tančin, V., Kirchnerová, K., Foltys, V., Mačuhova, L., Tančinová, D. 2006. Microbial contamination and somatic cell count of bovine milk striped and after udder preparation for milking. *Slovak Journal of Animal Science*, vol. 39, p. 214-217.

Vasiľ, M. 2009. Etiology, course and reduction of incidence of environment of dairy cows. *Slovak Journal of Animal Science*, vol. 42, 2009, p. 136-144.

Vršková, M., Tančin, V., Kirchnerová, K., Sláma, P., 2015. Evaluation of daily milk production in tsigai ewes by somatic cell count. *Potravinarstvo*, vol. 9, p. 206-210. https://doi.org/10.5219/439

Zadoks, R. N., Middleton, J. R., McDougall, S., Katholm, J., Schukken, Y. H. 2011. Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. *Journal of Mammary Gland Biology and Neoplasia*, vol. 16, no. 4, p. 357-372. https://doi.org/10.1007/s10911-011-9236-y

Williams, R. 2000. The impact of antimicrobial resistance. Acta Veterinaria Scandinavica, Suppl., vol. 93, p. 17-20.

Zajác, P.,Tomáška, M., Murárová, A.,Čapla, J.,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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BIOGENIC AMINES CONTENT IN THE FERMENTED ASIAN FOOD IN THE CZECH REPUBLIC

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ABSTRACT
The aim of this work was to study the typical fermented Asian food (miso paste, soy sauce, rice vinegar, kimchi and tempeh) to monitor their microbial quality and presence of biogenic amines in relation to time and temperature of storage. This study is focused on microbiological research in order to determinate presence of selected indicator groups of microorganisms during storage of individual products at three different temperatures, 8 °C, 23 °C, 30 °C. It was found that the highest increase of total viable counts was observed in products stored in 23 °C and 30 °C, especially in tempeh and miso paste. In soy sauce and rice vinegar were observed only very low amounts of microorganisms through the storage period. In the second part of the experiment, the biogenic amines were analyzed using high performance liquid chromatography. It was found that the levels of biogenic amines in tested products were low and does not affect human health.

Keywords: Asian fermented food, soy, microorganisms, biogenic amines, storage

INTRODUCTION
European consumption of soyfoods is similar to that of the United States, with meat and dairy alternatives comprising most of the soyfood sales. Throughout the rest of Europe, tofu is known, but it is not as popular as meat and dairy variants. As in the United States, soyfoods have become more of a mainstream food item, having crossed over from the natural products market to being widely available now in common supermarkets **(Riaz, 2006)**.

Tempeh is a traditional, fermented soyfood that is unique in its texture, flavor, and versatility. It is made from the whole soybean, which has been smashed, cracked, and boiled with added vinegar to reduce the pH. Tempeh contains about 19% protein, is richer in fiber than tofu, and is a significant source of vitamins and minerals **(Riaz, 2006)**.

Miso is a rich and flavorful paste made from either fermented and riped whole soybeans or soybeans in combination with wheat, barley, or rice. It contains enzymes and bacteria that can aid in digestion. It is high in protein, but it also contains high levels of sodium and should be consumed sparingly. Most of the miso sold today is pasteurized and refrigerated **(Fukushima, 1981; Chiou and Cheng, 2001; Riaz, 2006)**.

Soy sauce is the most well known and popular of the traditional soyfoods and is used extensively as a flavoring ingredient in most Asian dishes. When naturally processed, soy sauce is produced in a manner similar to that of miso. When made wholly of soybeans, the product is called tamari. If it is processed with a fermented wheat starter, the product is called shoyu. Much of the soy sauce sold today is not naturally fermented. Instead, it is made with hydrolyzed vegetable protein, sugar, color, and preservatives **(Riaz, 2006)**.

The soybean has long been embraced as a source of highquality protein from which a wide variety of foods can be made. The quality of soy protein is now considered as being essentially equivalent to animal protein. In fact, in 1999, the U.S. Food and Drug Administration approved a health claim for the cholesterol lowering effects of soy protein. Beyond heart disease, proposed benefits include reductions in the risk of breast and prostate cancer and osteoporosis. More speculative data suggest soyfoods may also positively affect kidney function and cognitive function and help to alleviate hot flashes in menopausal woman **(Riaz, 2006)**.

Soy protein supplies all nine essential amino acids and offers many functional benefits to food processors. Modern soy products such as soy flour, concentrates, isolates, and textured soy protein have been used for several decades as functional ingredients by the food industries in the United States and Europe. Natural fermentation process involved in the production of soy products make the process susceptible to microbial contamination from environmental sources and thus renders the process less or unhygienic. This also threatens the safety of this fermented food because of more contamination possibilities from environment containing pathogenic bacteria. Therefore, it is hard to control the quality of soya product when it is processed in an open environment **(Lee et al., 1996)**. Previous studies aiming to explore the microbiota of soy have reported the dominance of lactic acid bacteria (LAB) and species belonging to the genus *Bacillus* **(Chao et al., 2008; Sun et**

al., 2010). Lactic acid bacteria of the tofu play an important role in determining the overall flavor profile of soy food **(Liang et al., 2013; Li et al., 2014)**. The increased amount of decarboxylase positive microorganisms can accumulate production of biogenic amines **(Purevdorj et al., 2017)**. Starter cultures in fermentation of soya product are *Aspergillus oryzae* and *Saccharomyces cerevisiae* and secondary cultures that are used in soya product are *Pediococcus halophilus*, *P. cerevisiae*, *Zygosaccharomyces rouxii*, *Candida* and *Enterococcus faecalis* **(Shurtleff and Aoyagi, 1976; Farnworth, 2008)**. The addition of selected starter cultures is one of the main tools able to prevent the formation of high levels of BA in fermented meat and dairy products **(Purevdorj et al., 2017)**.

However, an increase in total biogenic amines (BA), as undesirable substances in soy product, may be harmful **(Silla Santos, 1996; EFSA, 2011)**. Excessive intake of biogenic amines from foods can lead to various physiological and toxicological problems in humans such as nausea, sweating, migraine, respiratory distress, hot flushes, bright red rash, oral burning, heart palpitation, and hyperor hypotension **(Karovicova and Kohajdova, 2005; Guan et al., 2013)**. Biogenic amines may have more serious implication as its presence in foods may lead to death in certain cases. In addition, biogenic amines have also been reported to have correlation with the spoilage of food products **(Karovicova and Kohajdova, 2005; Tian et al., 2013; Li et al., 2014)**. However, currently there are no established standards or regulation for tofu or fermented soybean products to limit biogenic amines levels. In addition, biogenic amines can also be synthesized by spontaneous chemical reactions during extended fermentation **(Beneduce et al., 2010; Liu et al., 2011)**.

The analysis of presence of biogenic amines in miso paste by HPLC was realized at the university in Korea. In most of the samples, there were detected low concentration of BA; however some samples contained histamine and tyramine in amounts exceeding levels safe for human health. Variability in BA content can be caused by addition of other materials into miso paste (rice, barley). Contamination during production is mainly caused by bacteria such as *Bacillus subtilis* and *B. amyloliquefaciens*. These bacteria are able to produce BA, especially tyramine and spermine **(Belleme and Belleme, 2011; Byun et al., 2012).**

The aim of this work was to study the typical fermented Asian food (miso paste, soy sauce, rice vinegar, kimchi and tempeh) to monitor their microbial quality and presence of biogenic amines in relation to time and temperature of storage.

MATERIAL AND METHODOLOGY

Isolation and identification of the microorganisms:

Ten grams of the fermented Asian food sample **(Figure 1)** was weighed out, aseptically removed and put into 90 ml of sterile physiological solution that was subsequently homogenised for 10 min (using a stomacher). The Asian food was then subjected to routine microbiological analysis. The first analyses were executed in May 2017, before the new norm ISO Standard No. 21528−2 (2017) was introduced. The total microorganism counts were assessed according to ISO Standard No. 4833−1 (2013), the *Enterobacteriaceae* bacteria family according to ISO Standard No. 21528−2 (2004), the yeasts and moulds according to ISO Standard No. 6611 (2004) and halotolerant microorganisms (staphylococci) on mannitol salt phenol red agar after cultivation at 37 °C for 2 days according to **Chapman (1945)**. The selected colonies were isolated into BHI broth and cultivated 24 – 48 h at 25 °C (yeasts), 37 °C (*Enterobacteriaceae*, *Staphylococcus*) or 30°C (other microorganisms). Each soya product sample was microbial analysed 3 times. Identification of the microorganisms was performed via the MALDI−TOF MS method using a Bruker Autoflex Speed (Bruker Daltonics, Bremen, Germany) and the Biotyper 3.1 database (Bruker Daltonics) after preliminary classification of isolates into individual microorganism groups. Visualisation of the protein profiles was performed via mMass 5 **(Strohalm et al., 2010)**. The individual identifications were performed in at least two independent experiments in two parallels.

Preparation:

Lyophilised soya products were used for the biogenic amines (BA) and polyamines (PA) analysis. Triple extraction of BA and PA from the lyophilised samples was carried out using a perchloric acid solution (0.6 mol.L^{-1}). Three independent extractions were performed on each soya product sample. The filtrated extract (filter porosity 0.45 μm) was then used directly for the derivatisation and determination of BA/PA content **(Dadáková et al., 2009; Buňková et al., 2013)** that followed.

Figure 1 Various types of miso paste (top left - shiro miso, top right – mugi miso, lower left - hatcho miso, lower right - genmai miso).

Biogenic amines detection by HPLC:

The concentrations of eight present biogenic amines, such as histamine (HIM), tyramine (TYM), phenylethylamine (PHE), tryptamine (TRY), putrescine (PUT), cadaverine (CAD), spermine (SPE) and spermidine (SPD), were analysed via high performance liquid chromatography (HPLC) (LabAlliance, USA and Agilent Technologies, Agilent, Santa Clara, California, USA) after derivatisation using dansylchloride. The dansylchloride sample derivatisation procedure was performed according to **Dadáková et al. (2009)**. 1,7−heptandiamine was used as the internal standard. Chromatographic separation (ZORBAX Eclipse XDB−C18, 50 9 3.0 mm, 1.8 μm; Agilent

Technologies) and detection (spectrophotometric λ = 254 nm) were performed according to **Buňková et al. (2013)**. Each extract was derivatised twice after cultivation, and each derivatised mixture was applied to the column twice. Each soya product sample was analysed 12 times (3 extractions, 2 derivatisations, 2 applications to the column). Detection limits for the individual amines were in the range 0.24 – 1.39 mg.kg^{-1}. Given the significance of biogenic amines to human health and food safety, monitoring their contents in foodstuffs is very important. Presently, HPLC based methods are the most suitable for the analysis of fermented foods. The reliability and sensitivity of these methods render them useful as important techniques to determine the concentration of all biogenic amines in fermented food **(EFSA, 2011)**.

Statistical analysis

The obtained experimental data were analysed using Statistical software Unistat 6.5 (Unistat, London, UK). The significance level of all statistical tests was set at $p < 0.05$.

The Kruskall-Wallis and Wilcoxon tests were used to evaluate the data obtained.

RESULTS AND DISCUSSION

Microbial analysis

Environment of soya products is an ideal media for the growth and survival of a variety of fungi and bacteria, particularly lactic acid bacteria.

Table 1 Viable counts (log CFU.g^{-1}) of the main microbial groups (first day) in the Asian soya food in the Czech republic.

	AGAR log CFU.g^{-1}		
Product	PCA	MSA	ENDO
K	−	3.69	−
KIM	6.83	−	2.70
MG	5.40	5.48	−
MH	4.70	−	−
MM	5.40	4.48	3.70
MS	4.70	−	3.70
TM	5.88	−	4.69
TN	3.58	−	7.05
TP	6.94	−	6.46
TS	−	−	3.69

At the beginning of the analysis, the colonies forming units of microorganisms (CFU.g^{-1}) were determined in all samples (Table 1): miso genmai (MG), miso mugi (MM), miso shiro (MS), miso hatcho (MH), tamari shoyu (TS), koikuchi shoyu (KS), komesu (K), Tempeh marinated (TM), Tempeh natural (TN), Tempeh party (TP), Usuchi shoyu (US), Shiro shoyu (SS).

Fermentation of soya is a complex process influenced by variety of factors, *e.g.* recipe and nutrient composition, temperature and fermentation time **(Chao et al., 2008; Li et al., 2014)**. The nutrient−rich environment of miso makes it an ideal media for the growth and survival of a variety of fungi and bacteria, particularly lactic acid bacteria.

The highest counts of bacteria in the "Miso shiro" samples were 7.24 log CFU.g^{-1} at 23 °C after 15 days of storage. The amount of coliform bacteria was 6.11 log CFU.g^{-1} under the same storage conditions. The amount of staphylococci was 5.34 log CFU.g^{-1} at 30 °C and after 8 days of storage. After 50 days of storage, the amount of staphylococci did not exceed 3.00 log CFU.g^{-1} at all temperatures (8, 23 and 30 °C). As for overall numbers of lactobacilli in relation to storage time, there is significant decrease from 3.00 log CFU.g^{-1} to 1.69 log CFU.g^{-1} ($p \leq 0.05$) at all storage temperatures. Yeast and moulds were only determined on the 57th day of storage at the temperature lower than 23 °C (2.17 log CFU.g^{-1}).

In "Miso mugi", total viable counts were 7.49 log CFU.g^{-1} at 23 and 30 °C and 6.10 log CFU.g^{-1} at 8 °C after 15 days of storage ($p \leq 0.05$). The highest amount of coliform bacteria was measured after 15 days of storage (7.11 log CFU.g^{-1} at temperature ≤23 °C). The highest amount of staphylococci 5.47 log CFU.g^{-1} was determined at 30 °C on the 8th day of storage. The yeast and mould growth on Chloramphenicol Yeast Glucose Agar was maximal at 23 °C on the 15th day of storage (4.84 log CFU.g^{-1}). At decreasing or increasing of the storage temperature, the amount of yeast and moulds in miso mugi dropped under 2.10 log CFU.g^{-1} ($p \leq 0.05$). Lactobacilli were not determined in this product at any of the temperatures and observed durations.

As for "Miso shiro", the highest amount of bacteria was 6.13 log CFU.g^{-1} and it was determined at 23 °C after the 15th day of storage. The highest amount of coliform bacteria (5.59 log CFU.g^{-1}) and yeast and molds (5.49 log CFU.g^{-1}) was identified at temperature 23 °C after 15th day of storage. In contrast, the highest amount of determined staphylococci was 3.78 log CFU.g^{-1} at the same temperature (23 °C) after 57th day of storage. With farther prolongation of storage time, the amount of bacteria and moulds declined logarithmically on all of the selective diagnostic agars ($p \geq 0.05$). "Miso hatcho" had log CFU.g^{-1} identical with bacteria amounts gained from miso shiro.

The maximal amount of living cells in "Miso genmai" was 7.91 log CFU.g^{-1} and the amount of coliform bacteria was 7.11 log CFU.g^{-1}; both amounts were determined at 30 °C on the 15th day of storage. The amount of identified bacteria decreased along with decrease of storage temperature ($p \leq 0.05$). The highest value of determined amount of staphylococci was 5.60 log CFU.g^{-1} at 8 °C after 8 days of storage. The highest amount of yeast and moulds was 5.18 log CFU.g^{-1} at 30 °C after 15 days. Lactobacilli were not determined in any of the storage conditions.

In "Koikuchi shoyu", the total viable counts of bacteria were 2.69 log CFU.g^{-1} at 8 °C after the 15th day of storage. Observed under the same conditions, the highest amounts of yeast and moulds were 2.00 log CFU.g^{-1}. No staphylococci or lactobacilli were detected in this sample.

"Kimchi" is a traditional product in Korean cuisine. The total viable counts of microorganisms on PCA were 9.13 log CFU.g^{-1} at 30 °C on the 2nd day of storage. The highest values for lactobacilli were 8.34 log CFU.g^{-1} after 4 days. The amounts of coliforms were the highest at 30 °C after the 4th day (4.77 log CFU.g^{-1}) and the highest amounts of styphylococci (3.93 log CFU.g^{-1}) were determined under the same conditions. he values of yeast and moulds were highest after the 5th day of storage – 3.00 log CFU.g^{-1}.

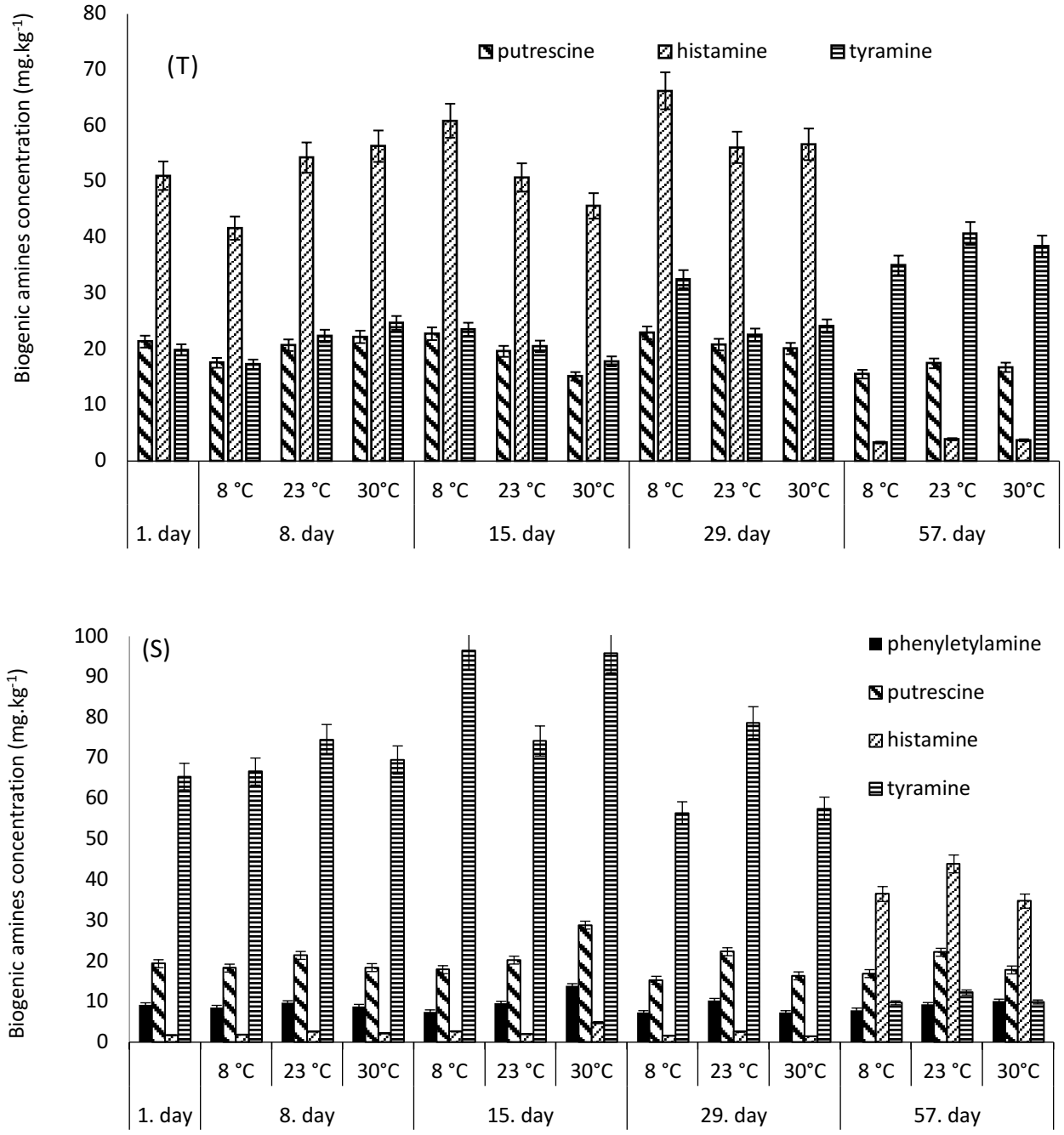

Figure 2 Biogenic amines content (histamine, tyramine, phenylethylamine, putrescine in Shoyu$^{(S)}$ and Tamari$^{(T)}$ samples (mg.kg^{-1}).

"Tempeh marinated" contained 9.00 log CFU.g^{-1} which was the highest amount of bacteria and it was determined at 30 °C after 4 days of cultivation. There were also high amounts of lactobacilli cells (8.89 log CFU.g^{-1}) in this product at 8 °C on the 5th day of storage. The highest amounts of coliform bacteria (8.00 log CFU.g^{-1}) and staphylococci (7.85 log CFU.g^{-1}) were measured at 30 °C on the 4th day of storage. As for yeast and moulds, the highest values reached 6.81 log CFU.g^{-1} and were determined at 23 °C on the 2nd day of storage. In "Tempeh natural", the highest amounts of bacteria, coliform microorganisms, yeast and moulds ranged from 9.28 to 9.48 log CFU.g^{-1} at 30 °C after the 5th day of storage. The maximal amount of lactobacilli (9.07 log CFU.g^{-1}) was determined at 8 °C after the 5th day of storage. Staphylococci values were the highest at 30 °C on the 4th day of storage (7.27 log CFU.g^{-1}). In "Tempeh party", the highest amounts of bacteria were determined at 30 °C after 4 days of storage (9.26 log CFU.g^{-1}). This product contained high amount of lactobacilli (9.12 log CFU.g^{-1}) at the storage temperature 23 °C on the 5th day. The highest amounts of coliforms (7.74 log CFU.g^{-1}) and staphylococci (7.44 log CFU.g^{-1}) were measured at 30 °C after 4 days. The highest amounts of yeast and moulds were 7.14 log CFU.g^{-1} at the temperature 23 °C on the 4th day.

"Tamari and Shoyu" contained the lowest amount of yeast and moulds (1.69 log CFU.g^{-1} at 15 °C after the 15th day of cultivation) out of all observed soy sauces.

As for "Komesu", there were identified these highest amounts: 2.00 log CFU.g^{-1} for mesophilic facultatively anaerobic bacteria and 2.30 log CFU.g^{-1} for yeast and moulds after 15 days of cultivation. There was no growth of staphylococci, lactobacilli and coliforms on selective diagnostic media.

Kim et al. (2012) reported that the aerobic plate counts of the samples varied from 3.00 to 7.59 log CFU.g^{-1}.

In the fermented foods, there is a possibility of biogenic amines presence because the number of decarboxylase positive microorganisms increases during ripening or storage **(Pleva et al., 2014)**.

Non starter isolates were chosen and identified from all of the determined numbers of bacteria and yeast by using matrix assisted laser desorption/ionization time−of−flight (MALDI): *Acinetobacter lwoffii, Aerococcus viridans, Arthrobacter oxydans, A. polychromogenes, A. scleromae, Bacillus cereus, Citrobacter koseri, Dermacoccus nishinomiyaensis, Enterobacter asburiae, E. cloacae, E. hormaechei* ssp. *hormaechei, E. kobei, Klebsiella oxytoca, Kosakonia cowanii, Micrococcus luteus, Ochrobactrum anthropi, Ochrobactrum* sp., *Ochrobactrum tritici, Paenibacillus humicus, Pantoea agglomerans, Staphylococcus warneri.*

The results of executed microbiological analysis can be summarized as follows: the highest numbers of mesophilic facultative anaerobic microorganisms, enterobacteria, moulds and yeast in miso paste were observed particularly at storage temperature 30 °C. Compared to the other types of miso paste, miso genmai contained the highest number of mesophilic facultative anaerobic microorganisms ($p \leq 0.05$), whereas miso mugi contained the highest amount of enterobacteria ($p \geq 0.05$) and the highest amount of yeast and moulds was detected in miso shiro ($p \geq 0.05$). In kimchi, the highest amount of lactobacilli was determined at storage temperature 30 °C. In soy sauce, there was only a slight increase of facultative anaerobic microorganisms, yeast and moulds observed ($p \geq 0.05$), while tamari shoyu showed greater resistance against the growth of these microorganisms. The growth of facultative anaerobic microorganisms, yeast and moulds was noticed in rice vinegar komesu but only in a very small amount. Microorganisms can exhibit the ability to produce or degrade biogenic amines *in vitro* and they could be used as microbiological indicators to prevent BAs accumulation in food **(Butor et al., 2017)**.

Biogenic amine analysis

The results of chromatographic analysis of biogenic amines can be summarized as follows: in miso paste, only tyramine was determined (0.048 − 1.765 mg.kg^{-1}); other biogenic amines were not observed**.** However, **Yamamoto et al. (1980)** reported that miso products contained 21.0 − 169.5 mg.kg^{-1} of tyramine. **Shalaby (1996)** reported that fermented soybean products (miso) contained high levels of histamine (46.2 mg.kg^{-1}), putrescine (123.4 mg.kg^{-1}), cadaverine (63.4 mg.kg^{-1}), and tyramine (356.8 mg.kg^{-1}). Variations in the contents of biogenic amines in these commercial miso products could be attributed to variability in the ratio of soybean to other seeds used, the microbiological composition, and the conditions and duration of fermentation **(Chin et al., 1983; Nout et al., 1993)**. **Kung et al. (2007)** reported that 27.0 ±43.5 mg.kg^{-1} of tryptamine, 1.2 ±3.3 mg.kg^{-1} of putrescine, 31.6 ±45.3 mg.kg^{-1} of cadaverine, 16.4 ±40.4 mg.kg^{-1} of histamine, 12.1 ±4.8 mg.kg^{-1} of tyramine, 8.0 ±31.6 mg.kg^{-1} of spermine were detected in Miso products sold in supermakets, whereas 49.0 ±52.0 mg.kg^{-1} of tryptamine, 1.1 ±2.8 mg.kg^{-1} of putrescine, 9.1 ±2.8 mg.kg^{-1} of cadaverine, 7.7 ±26.5 mg.kg^{-1} of histamine, 15.8 ±10.2 mg.kg^{-1} of tyramine, 7.2 ±25.8 mg.kg^{-1} of spermine were detected in Miso products sold in retail markets in Taiwan. **Shukla et al. (2011)** reported that 0.2 ±0.4 mg.kg^{-1} of tryptamine, 7.7 ±14.8 mg.kg^{-1} of β−phenylethylamine, 4.8 ±5.5 mg.kg^{-1} of putrescine, 1.1 ±3.1 mg.kg^{-1} of cadaverine, 16.7 ±18.9 mg.kg^{-1} of tyramine, 4.5 ±12.6 mg.kg^{-1} of spermidine were detected in Miso products sold in supermarkets in Japan.

The analysed amounts of biogenic amines (putrescine, cadaverine, histamine, tyramine and spermidine) range from 0.058 to 3.048 mg.kg^{-1} in selected tested types of tempeh. In kimchi, there were detected only very low volumes of tyramine and spermidine, with their amounts ranging from 0.161 to 0.531 mg.kg^{-1}. In tamari **(Figure 2)**, the presence of putrescine was recorded in range 15.156 − 22.922 mg.kg^{-1}, 17.287 − 40.710 mg.kg^{-1} for tyramine, 1.293 − 7.538 mg.kg^{-1} for phenylethylamine, 0.701 − 1.875 mg.kg^{-1} for cadaverine and 3.306 − 66.208 mg.kg^{-1} for histamine, which also reached the highest values. **Toro−Funees et al. (2015)** described the amount of biogenic amines in Tempeh. Tempeh showed the highest contents of spermidine and spermine with 124 and 21 mg.kg^{-1}, respectively. These results are consistent with the ones reported by **Nishimura et al. (2006)** who found similar concentrations of these polyamines in tempeh and natto samples, but lower than the levels of 250 − 475 mg.kg^{-1} of polyamines reported by **Kim et al. (2012)** in natto.

The **Figure 2** indicates obvious trend of increasing the histamine value on the 57th day of storage ($p \leq 0.05$). Production this high can be caused not only by mentioned technology of processing the soy product but also by decarboxylase activity of microorganisms. However, these histamine concentrations should not endanger human health normally. In koikuchi shoyu, there was determined phenylethylamine, putrescine, histamine, tryptamine, cadaverine in amounts ≤3.00 mg.kg^{-1}; however, tyramine was detected in range from 9.686 to 96.503 mg.kg^{-1}. The low values of putrescine and tyramine were noticed in komesu, ranging in values 0.987 − 4.858 mg.kg^{-1}. **Pachlová et al. (2017)** reported that the content of biogenic amines (such as tyramine, putrescine, histamine, and phenylethylamine) was monitored during storage of cheese. The results showed an increasement in biogenic amine concetration depending on the time of ripening in all batches of model samples.

CONCLUSION

In the first part, this research focused on fermentation process, problematics of biogenic amines in typical Asian fermented products. The best−known and the most sold fermented products (miso paste, soy sauce, tamari shoyu,

koikuchi shoyu, rice vinegar, tempeh and kimchi) were chosen for testing. The products underwent microbial analysis with goal to find out numbers of observed indicator groups of microorganisms (facultative anaerobic mesophillic microorganisms, enterobacteria, staphylococci, yeast, moulds and lactic acid bacteria). The studied samples were kept at various storage temperatures for a certain period of time, with intention to simulate conditions of environment where those products are stored in Asian countries. Lastly, the products were analysed using high-performance liquid chromatography to evaluate amounts of biogenic amines and to identify them. The most significant concentration of biogenic amines was determined in koikuchi shoyu (96.50 $mg.kg^{-1}$ of tyramine) and in tamari (66.21 $mg.kg^{-1}$ of histamine).

The achieved results of this research show that there are various representations of chosen indicator groups of microorganisms in analysed Asian fermented foods stored at temperatures 8 °C, 23 °C and 30 °C. Different numbers of colonies of observed microorganism groups and biogenic amines were probably caused by used ingredients and technological process of production. It is important to pay attention to contents of individual types of foods and to their microflora, especially in relation to their storage, and thus ensure their longevity and health harmlessness.

Modern soy products and their proteins provide all nine essential amino acids and offer many functional health benefits. In addition to an assortment of vitamins and minerals, like all plant foods, the soybean contains numerous biologically active nonnutritive substances. However it needs be emphasized that prolongation of the soy products storage increases the risk of increase of microorganism amounts or cumulation of biogenic amines respectively.

REFERENCES

Belleme J., Belleme J. 2011. *Japanese Foods that Heal Using Traditional Ingredients to Promote Health*, *Longevity*. 1st ed. New York, USA: Tuttle Pub. ISBN 14−629−0007−0.

Beneduce, L., Romano, A., Capozzi, V., Lucas, P., Barnavon, L., Bach, B. 2010. Biogenic amine in wines. *Annals of Microbiology*, vol. 60, no. 4, p. 573-578. http://doi.org/10.1007/s13213−010−0094−4

Buňková, L., Adamcová, G., Hudcová K., Velichová, H., Pachlová V., Lorencová, E., Buňka, F. 2013. Monitoring of biogenic amines in chees manufactured at small−scale farms and in fermented dairy products in the Czech Republik. *Food Chemistry*, vol. 141, no. 1, p. 548-551. https://doi.org/10.1016/j.foodchem.2013.03.036

Butor, I., Pištěková, H., Purevdorj, K., Jančová, P., Buňka, F., Buňková, L. 2017. Biogenic amines degradation by microorganisms isolated from cheese. *Potravinarstvo Slovak Journal of Food Sciences,* vol. 11, no. 1, p. 302-307. http://doi.org/10.5219/736

Byun, B. Y. and Mah, J-H. 2012. Occurrence of Biogenic Amines in Miso, Japanese Traditional Fermented Soybean Paste. *Journal of Food Science*, vol. 77, p. 216-223. http://doi.org/10.1111/j.1750−3841.2012.02983.x

Dadáková, E., Křížek, M., Pelikánová, T. 2009. Determination of biogenic amines in foods using ultra−performance liquid chromatography (UPLC). *Food Chem*istry, vol. 116, p. 365-370. http://dx.doi.org/10.1016/j.foodchem.2009.02.018

European Food Safety Authority (EFSA), 2011. Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Journal*, vol. 9, no. 10, p. 1-93. https://doi.org/10.2903/j.efsa.2011.2393

Farnworth, E., R. 2008. *Handbook of fermented functional foods*. *Functional foods & nutraceuticals series*, 2nd ed. Boca Raton, USA: CRC Press. 600 p. ISBN 978−1−4614−3480−1.

Fukushima, D. 1981. Soy proteins for foods centering around soy sauce and tofu. *Journal of the American Oil Chemists' Society*, vol. 58, no. 3-2, p. 346-354. http://doi.org/10.1007/BF02582376

Guan, R.-F., Liu, Z.-F., Zhang, J.-J., Wei, Y.-X., Wahab, S., Liu, D.-H., Ye, X.-Q. 2013. Investigation of biogenic amines in sufu (furu): A Chinese traditional fermented soybean food product. *Food Control*, vol. 31, no. 2, p. 345-352. http://doi.org/10.1016/j.foodcont.2012.10.033

Chao, S.-H., Tomii, Y., Sasamoto, M., Fujimoto, J., Tsai, Y.-C., Watanabe, K. 2008. *Lactobacillus capillatus* sp nov., a motile bacterium isolated from stinky tofu brine. *International Journal of Systematic and Evolutionary Microbiology*, vol. 58, no. 11, p. 2555-2559. http://doi.org/10.1099/ijs.0.65834−0

Chapman G. H., 1945. The significance of sodium chloride in studies of staphylococci. *Journal of Bacteriology*, vol. 50, p. 201-203.

Chin, K.-D. H., Koehler. P. E. 1983. Identification and Estimation of Histamine, Tryptamine, Phenethylamine and Tyramine in Soy Sauce by Thin−Layer Chromatography of Dansyl Derivatives. *Journal of Food Science,* vol. 48, no. 6, p. 1826-1828. http://doi.org/10.1111/j.1365−2621.1983.tb05094.x

Chiou, R. Y.-Y., Cheng. S.-L. 2001. Isoflavone Transformation during Soybean Koji Preparation and Subsequent Miso Fermentation Supplemented with Ethanol and NaCl. *Journal of Agricultural and Food Chemistry*, vol. 49, no. 8, p. 3656-3660. http://doi.org/10.1021/jf0015241

ISO 21528−2: 2004. Microbiology of Food and Animal Feeding Stuffs – Horizontal Methods for the Detection and Enumeration of Enterobacteriaceae – Part 2: Colony Count Method.

ISO 4833−1: 2013. Microbiology of the Food Chain – Horizontal Method for the Enumeration of Microorganisms – Part 1: Colony Count at 30 Degrees C by the Pour Plate Technique.

ISO 6611: 2004. Milk and Dairy Products – Enumeration of Colony−forming Units of Yeasts and/or Moulds – Colony Count Technique at 25 °C.

Karovicova, J., Kohajdova, Z. 2005. Biogenic amines in food. *ChemInform*, vol. 36, no. 34, p. 1522. http://doi.org/10.1002/chin.200534338

Kim, B., Byun B. Y., Mah, J-H. 2012. Biogenic amine formation and bacterial contribution in Natto products. *Food Chemistry*, vol. 135, no. 3, p. 2005-2011. https://doi.org/10.1016/j.foodchem.2012.06.091

Kung, H.-F., Tsai, Y.-H., Wei, Ch−I. 2007. Histamine and other biogenic amines and histamine−forming bacteria in miso products. *Food Chemistry*, vol. 101, no. 1, p. 351-356. http://doi.org/10.1016/j.foodchem.2005.12.057

Lee, S. F., Wang, C. B., Chang, P. P. 1996. Isolation and identification of protein hydrolyzing bacteria from chaw−tofu. *Food Science (Chinese)*, vol. 23, no. 1, p. 1-9.

Li, M. Y., Tian, L., Zhao, G. M., Zhang, Q. H., Gao, X. P., Huang, X. Q., Sun. L. 2014. Formation of biogenic amines and growth of spoilagerelated microorganisms in pork stored under different packaging conditions applying PCA. *Meat Science*, vol. 96, no. 2 A, p. 843-848. http://doi.org/10.1016/j.meatsci.2013.09.023

Liang, H., Deng, L., Lin, H. 2013. Distribution, functions and applications of lactic acid bacteria in traditional fermented soybean foods. *Food Science (Chinese)*, vol. 34, no. 19, p. 381-385.

Liu, Z.-F., Wei, Y.-X., Zhang, J.-J., Liu, D.-H., Hu, Y.-Q., Ye, X.-Q. 2011. Changes in biogenic amines during the conventional production of stinky tofu. *International Journal of Food Science and Technology*, vol. 46, no. 4, p. 687-694. http://doi.org/10.1111/j.1365−2621.2011.02545.x

Nishimura, K., Shiina, R., Kashiwagi, K., Igarashi, K. 2006. Decrease in Polyamines with Aging and Their Ingestion from Food and Drink. *The Journal of Biochemistry*, vol. 139, no. 1, p. 81-90. http://doi.org/10.1093/jb/mvj003

Nout, M. J. R., Ruikes, M. M. W., Bouwmeester, H. M., Beljaars, P. R. 1993. Effect of processing conditions on the formation of biogenic amines and ethyl carbamate in soybean tempe. *Journal of Food Safety*, vol. 13, no. 4, p. 293-303. http://doi.org/10.1111/j.1745−4565.1993.tb00114.x

Pachlová, V., Charousová, Z., Šopík, T. 2017. Effect of milk origin on proteolysis and accumulation of biogenic amine during ripening of Dutch−type cheese. *Potravinarstvo Slovak Journal of Food Sciences,* vol. 11, no. 1, p. 363-367. https://doi.org/10.5219/741

Pleva, P., Buňková, L., Theimrová, E., Bartošáková, V., Buňka, F., Purevdorj, K. 2014. Biogenic amines in smear and mould−ripened cheeses. *Potravinarstvo,* vol. 8, no. 1, p. 321-327. https://doi.org/10.5219/408

Purevdorj, K., Maršálková, K., Březinová, I., Žalková, A., Pleva, P., Buňková, L. 2017. Antimicrobial effect of selected lactic acid bacteria against microorganisms with decarboxylase activity. *Potravinarstvo Slovak Journal of Food Sciences,* vol. 11, no. 1, p. 230-235. http://doi.org/10.5219/740

Riaz, M. N. 2006. *Soy applications in food*. 1st ed. Boca Raton, USA: CRC Press (Taylor and Francis Group). p. 304. ISBN 9781420037951.

Shalaby, A. R. 1996. Significance of biogenic amines to food safety and human health. *Food Research International*, vol. 29, no. 7, p. 675-690. http://doi.org/10.1016/S0963−9969(96)00066−X

Shukla, S., Park, H.-K., Kim, J.-K., Kim, M. 2011. Determination of biogenic amines in Japanese miso products. *Food Science and Biotechnology*, vol. 20, no. 3, p. 851-854. http://doi.org/10.1007/s10068−011−0119−1

Shurtleff, W., Aoyagi, A. 1997. *The Book of Tempeh: Professional Edition*. 1st ed. New York, USA : Harper, p. 245. ISBN 0060140097.

Silla Santos, M. H. 1996. Biogenic amines: their importance in foods. *International Journal of Food Microbiology*, vol. 29, no. 2-3, p. 213-231. https://doi.org/10.1016/0168−1605(95)00032−1

Strohalm, M., Kavan, D., Novák, P., Volný, M., Havlíček, V. 2010. MMass 3: A Cross−Platform Software Environment for Precise Analysis of Mass Spectrometric Data. *Analytical Chemistry*, vol. 82, no. 11, p. 4648-4651. http://doi.org/10.1021/ac100818g

Sun, G. P., Zhang, X. J., Wang, Y., Wang, D., Xie, J. L. 2010. The investigation of bacteria diversity in stinky tofu brine. *Modern Food Science and Technology*, vol. 26, no. 10, p. 1087-1091.

Tian, L., Li, M., Zhao, G., Huang, X., Tian, W., Liu, Y. 2013. Correlations between biogenic amines and parameters of spoilage of modified atmosphere packaged chilled pork. *Journal of Chinese Institute of Food Science and Technology*, vol. 13, no. 8, p. 75-82.

Toro−Funes, N., Bosch−Fuste, J., Latorre−Moratalla, M. L., Veciana−Nogués, M. T., Vidal−Carou, M. C. 2015. Biologically active amines in fermented and non−fermented commercial soybean products from the Spanish market. *Food Chemistry*, vol. 173, p. 1119-1124. http://doi.org/10.1016/j.foodchem.2014.10.118

Yamamoto, S., Wakabayashi, S., Masami, M.. 1980. Gas−liquid chromatographic determination of tyramine in fermented food products. *Journal of Agricultural and Food Chemistry*, vol. 28, no. 4, p. 790-793. https://doi.org/10.1021/jf60230a028

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SAUVIGNON WINE QUALITY AS AFFECTED BY ITS PROCESSING AND STORAGE

Martina Fikselová, Peter Czako, Ján Gažo, Andrea Mendelová, Martin Mellen

ABSTRACT

The colour and limpidity are the first sensory attributes of wines that are appreciated by consumers, predisposing their acceptance or rejection. The aim of this work was to monitor the effect of harvest, processing (different clarification and treatment of must) and storage on the quality of Sauvignon wine. The wines were stored for two years in the wine cellar at 12°C and 70 % of humidity, in the bottles. The acid content, residual sugar and alcohol content among chemical parameters and sensory profile of wines were observed. Sensory quality of wines was evaluated by the aromatic profile (profile method). Based on acquired results, two years of wine storage significantly affected the total acid content of wines and alcohol content. Different treatments of must affected residual sugars, the variant with the maximum dose of the clarification preparation (highly pure cellulose, polyvinylpolypyrrolidone, gelatin and mineral adsorbents) showed statistically the highest content of residual sugars. From the sensory point of view, sensory profiles of wines were different compared to the first and second harvest of grape, sensory profiles of wines were changed also after two years of storage. The fourth variant appeared to be the best stable, treated with the addition of clarification preparation at the dose of 30 g. 100 L^{-1} must. Because from the same variety Sauvignon were produced wines of different chemical and sensory qualities, some gastronomy recommendations were done as well.

Keywords: wine; quality; sensory evaluation; harvest; clarification

INTRODUCTION

Grapevine phenology and physiology, which affect yield and fruit composition, are largely under the control of climate on a macro (regional), meso (vineyard or site) and microscale **(Šuklje et al., 2014)**. If viticultural variables remain constant, climate differences will have a major effect on fruit maturation and quality **(Mira de Orduña, 2010)**. During grape maturation, the concentration of sugars, amino acids, phenolic compounds and potassium increases, while the content of organic acids, particularly malic acid, decreases **(Adams, 2006)**. Under the term of "wine", can be understood a diversity of quality which is quite unique among the products and determined mainly by interaction among grapes, yeasts and technology. It is a natural product resulting from a number of biochemical reactions, which begin during ripening of the grapes and continue during harvesting, throughout the alcoholic fermentation, clarification and after bottling **(Torija et al., 2001)**.

During winemaking, different oenological products could be used. Generally, clarifying procedures can be achieved by centrifugation, enzymatic treatment or applying clarifying agents such as gelatin bentonite, silica sol, and polyvinyl pyrrolidone **(Chatterjee et al., 2004)**. Fining agents are commonly used to improve the most important characteristics of wine, such as colour and aroma. Clarification of wines is an important process especially from the point of view of wine color and brilliancy **(Sen et al., 2012)**. Fining agents, which are all adsorptive compounds, commonly used in winemaking are grouped according to their general nature; arths (montmorillonite, bentonite, kaolin), animal proteins (gelatin, isinglass, caseins), wood charcoal (carbons) and synthetic polymers (polyvinyl polypyrrolidone – PVPP) **(Sen et al., 2012)**. The storage temperature of must fermentation may affect final viscous behaviour of wine **(Kumbár and Votava, 2015)**.

Quality evaluation of wine is primarily based on wine tasting. Chemical analyses are however performed in order to explain some sensory changes observed. The relationship between sensory evaluation and chemical composition of wine is a critical subject of research in oenology **(Chira, 2011)**. The quality of wines is a complex property of several physico-chemical properties in their mutual synergistic combination. Individual factors affected by the

human physiological perception sensitivity are determining overall wine quality perception **(Lapčíková et al., 2017)**. Sensory analysis involves the application of human senses to the description and/or evaluation of a product for consumer use **(Blackman, 2010)**. The colour and limpidity are the first sensory attributes of wines that are appreciated by consumers, predisposing their acceptance or rejection **(González-Nevesa, 2014)**.

Scientific hypothesis

Harvest and processing of grape, storage of wine are important conditions which affect sensory and chemical quality parameters of wines important for their consumption.

MATERIAL AND METHODOLOGY

The grapes originated from Nitra wine- growing region in Slovakia (Radošinské vineyard) from year 2012. At time of harvest the sugar content 22 °NM was determined, grape was harvested on 04. 09.2012 (1st harvest). At the late harvest 11. 09. 2012 (2 nd harvest) the sugar content 24 °NM was detected **(Remeňová, 2015)**. After harvesting, the grapes were pressed and got rid of stems. Obtained must was divided into four equal homogeneous parts, of which own experimental samples were prepared. Four variants were prepared by different treatments of must:

- *variant 1 :* spontaneous fermentation without the addition of yeast, no clarification;
- *variant 2* : must with static decanting for 12 hours, without adding clarifying preparations, with the addition of active dry wine yeasts *S.cerevisae*;
- *variant 3*: must clarified by the clarification preparation at a dose of 100 g. 100 L^{-1} of must, representing the maximum dose of the clarification preparation. The preparation was applied directly to the must. Yeasts *S. cerevisae* were applied to the clarified must after the must turbidity.
- *variant 4:*must clarified by the clarification preparation at the dose of 30 g. 100 L^{-1} must, with the addition of yeasts *S. cerevisae*.

Clarification consisted of preparation of highly pure cellulose, polyvinylpolypyrrolidone, gelatin and mineral adsorbents.

The process of fermentation was performed at a standard temperature of 15 °C for 14 days. After the fermentation completion, the wine was clarified with bentonite. Then it was coiled up, filtered, and bottled.

The wines were stored for two years (from 2013 till 2015) in the wine cellar at 12°C and 70 % of humidity, in the bottles. The effect of storage on the selected parameters of wines was observed as well. For the determinations five samples of wines were taken and used for the analysis.

Methods

Alcohol content of wines was performed by electronic ebullioscopy (fi. Dujardin-Salleron, France).

Assessments of acid and residual sugar contents were determined according to the International Methods of Analysis of Wines and Musts (2010).

Total acidity of wines was performed at the device HI84502 Total Acidity Mini Titrator for Wine Analysis (Hanna Instruments, Germany) based on neutralization reaction.

Residual sugar content was detected enzymatically (glucose+ fructose) and spectrofotometrically (T80 UV-VIS spectrophotometer).

Produced wines were evaluated also by sensory profile method **(Fic et al., 2015)**. For the evaluation of the profile method were used descriptors of smell and flavour typical for Sauvignon variety. Results of the profile method are the product of intensity scales, which are compiled either for a variety of descriptors or for individual characters.

Statisical analysis

The normality of the data were analysed by the Kolmogorov-Smirnov test. Then One-Way analysis of variance (ANOVA) was used to evaluate effects of treatments on experimental data. For post-hoc tests Tukey's HSD test was applied at $\alpha = 0.05$. All means in charts were presented as vertical columns represent 95% confidence intervals for means. Analysis was conducted using software STATISTICA 10 Cz.

RESULTS AND DISCUSSION

Chemical parameters of wines

Chemical parameters of Sauvignon wines **(Figure 1 – 3)** important for their tasting were firstly observed. The content of acids in wines was statistically affected ($p = 0.000$) by the time of storage **(Figure 1)**, mean acid content determined in the year 2013 was 6. 44 $g.L^{-1}$while the mean content of acids from the year 2015 (after their storage) was only 5.61 $g.L^{-1}$.The time of harvest and the treatment of must did not show any statistical influence on the acid content of wines.

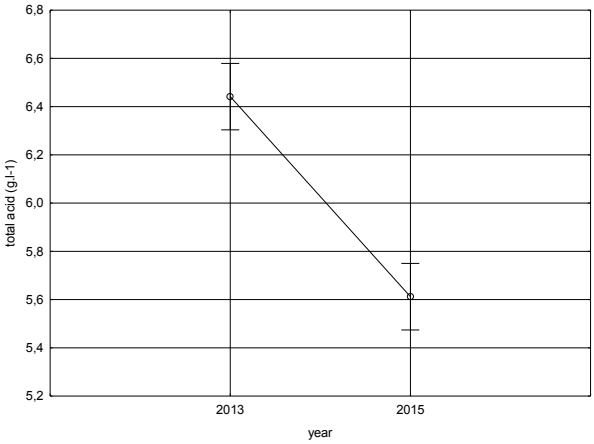


Figure 1 The effect of storage on the total acid content in the wines. Vertical columns represent 95% confidence intervals for mean.

Sugars have the capacity to mask acidity. Residual sugar content in wines important for their sweetness and harmony was significantly affected at parameters of the time of harvest and treatment of must **(Figure 2 a, b)**. It was found to be significantly higher ($p = 0.000$) from the second harvest (33.09 $g.L^{-1}$) compared to the first harvest (5.14 $g.L^{-1}$).

At different treatments of must based on Tukey's HSD test at α = 0.05, two homogenous groups were formed. The first group consisted of variants 1, 2, 4 and their residual sugar content ranged from 17.03 to 18.57 g.L^{-1}. Statistically differed just variant 3 with the maximum dose of the clarification preparation, its residual sugar content determined was 22.55 g.L^{-1}. Following this variant it seems due to the lack in nutrition of yeasts we found high residual sugar and consequently the lowest content of alcohol in this variant (12.51 %) compared the others. Clarification of must is important operation performed in winemaking, which can have major impact on the future quality of the wine. It removes components that may negatively affect hygienic and sensory quality of the wine **(Vietoris et al., 2014).**

By the **Commission regulation (EC) No 607/2009** wines can be divided into dry, semi-dry, semi-sweet or sweet by the residual sugar content. Following this classification, our wines belong to the category of semi-sweet wines, because of residual sugar content varies from 12 – 45 g.L^{-1}. Just the wines produced from the first harvest (5.14 g.L^{-1}) belong to the semi-dry wines.

Alcohol content of wines **(Figure 3 a, b)** was statistically affected by its storage and the time of harvest. Alcohol content determined in the year 2013 (at the beginning of the storage) was 13.19 %, while the mean content determined in the year 2015 (the end of storage) statistically (p = 0.000) decreased (12.08 %). Lower alcohol content (p = 0.000) from the second harvest (12.20 %) was detected compared to the first harvest (13.06 %).

Sensory quality of wines

For determination of small differences in sensory parameters of wine, methods of sensory profile evaluation can be used. They are very suitable for research and development work, for determination of similarity and correlation between taste and aroma of samples as well **(Fic et al., 2015)**. Sauvignon blanc has been described as a white wine with its characteristic varietal aroma due to relatively few volatile compounds **(Parr et al., 2013)**.

Nettle with green apple belong to the fresh plant characteristics of Sauvignon from the first harvest. The most intensive neetle flavour was recorded in the third and the first variant. Peach flavor was the most significantly detected in the third variant. Green apple was in all variants almost in balance, but the highest result achieved the second variant. Lemon/lime flavor was found to be the highest at the third variant and the least in the first variant.

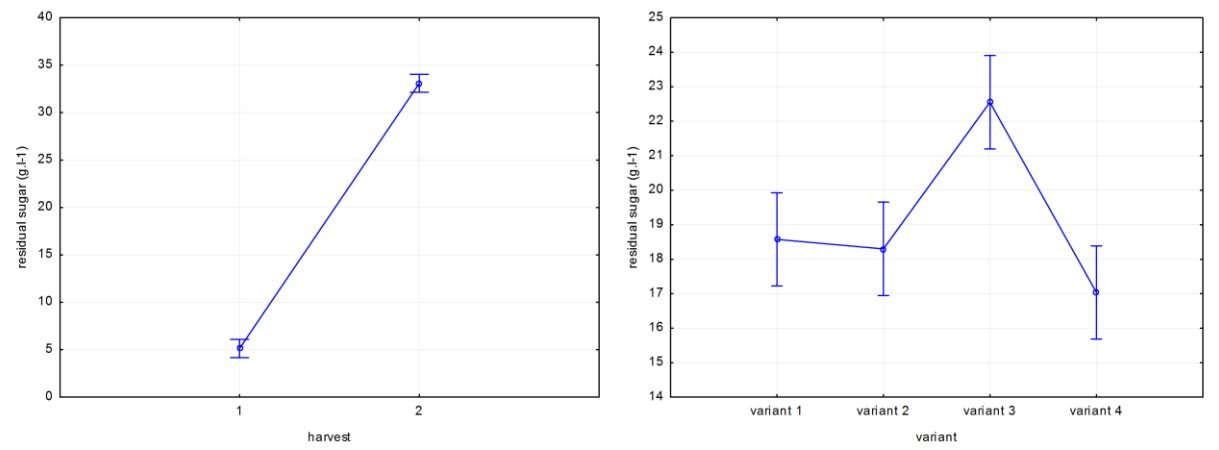


Figure 2 (a, b) The effect of harvest (a) and treatment of must (b) on the residual sugar content in the wines. Vertical columns represent 95% confidence intervals for mean.

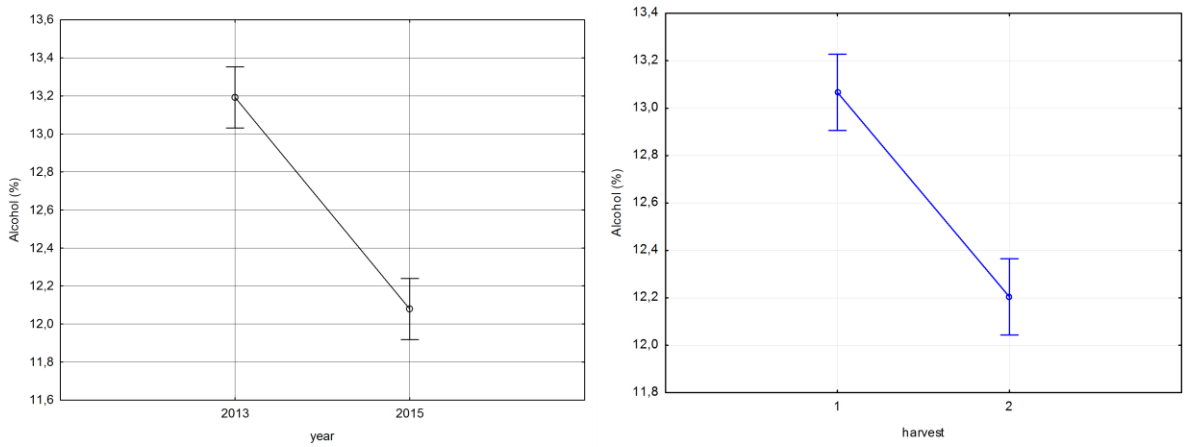
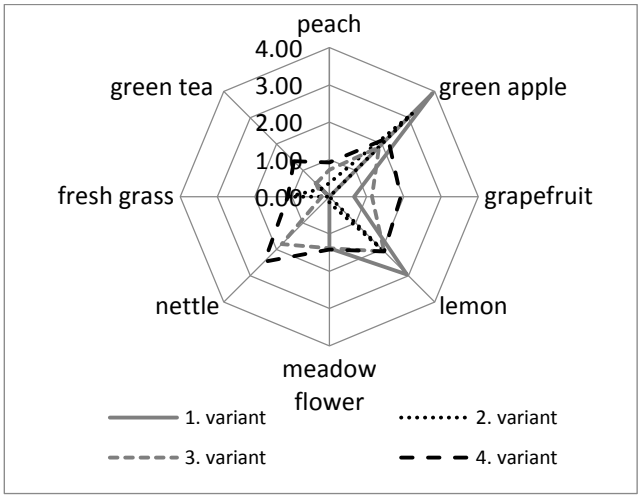
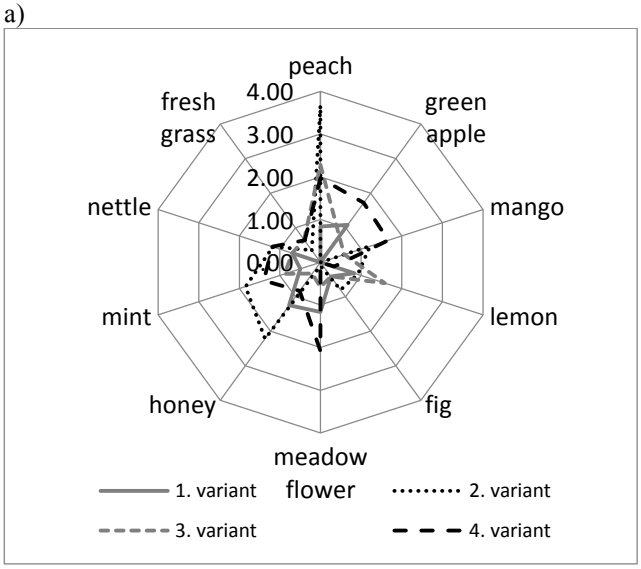


Figure 3 (a, b) The effect of storage (a) and harvest (b) on the alcohol content in the wines. Vertical columns represent 95% confidence intervals for mean.



a)



b)

Figure 4 (a, b) The sensory profile of wines from the 1.st harvest (a) and 2. nd harvest (b) after their storage

Meadow flowers were significantat at the second and fourth variant and the least in the third variant as it was published at our previus work **(Vietoris et al., 2014)**.

After the storage of wines from the early harvest several changes in sensory quality of wines were detected. Nettle at the first variant totally disappeared and remained only in the third and the fourth variant. Peach flavor decreased and green apple increased at the first variant. Grapefruit was not significantly affected, lemon increased in the first variant, nettle increased at the fourth variant. During the maturation of wine the individual flavor characteristics were transformed to the others. It can be stated that the most stable were the third and the fourth variants **(Figure 4 a)** with the addition of clarification preparations.

Assessing the sensory profile of the wines from the second harvest with higher sugar content (24 ° NM) of grape, the most intensive was detected peach flavor and generally dominated fine tropical sweet aromas. At the first, second and the third variants honey flavor was found, in high values were observed plant flavors: nettle, green tea and green apple **(Vietoris et al., 2014)**.

The aroma of wine is a unique mixture of volatile compounds originating from grapes (varietal aromas), secondary products formed during the wine fermentation (fermentative aromas) and aging (post-fermentative aromas) **(Callejona et al., 2010)**.

After the storage of wines **(Figure 4 b)** new flavors were observed: fresh grass, fig, mint and meadow flower, but peach flavour still dominated as it was found in the wine before the storage.

Produced wines in enogastronomy

Enogastronomy could be characterised as the art or science of good eating and drinking. There exist two basic principles for merger the wine with food. The first one is in mutual fusion of wine with food resulting in their harmony, and the second one uses contrast between wine and food, there is a competition and diametric difference between wine and food **(Fic et al., 2015)**. Good pairing recommendations may be crucial for the success of beverages, both in the retail and hospitality sector. Food–beverage pairings are often presented by culinary professionals such as chefs or sommeliers, however little focus is given to consumer perception **(Paulsen et al., 2015)**.

Therefore it is important to serve the right food with the right wine, e.g. in terms of actual sweetness and acidity. If the Sauvignon wine is dry, seafood with Tabasco sauce can be prepared or exotic accompaniment can be recommended. **Ailer (2016)** recommended dry Sauvignon blanc wines with combination of fruit, such as apricots, peaches, raisins with steamed fish and potato puree.

Produced semi-dry Sauvignon wines could be recommended to combine with sweet or creamy foods. Ripened cheese with nuts, lichee and pear on mustard sauce with honey and lime is one of the possibilities. Roasted beetroot with goat cheese on wine and honey can be served with parsley puree, grilled zucchini and combined with sweet Sauvignon, produced from the late harvest of grapes. Within the innovation of restaurant services, the own production, or local products can be offered. Farm visits and tastings as related with a tour of the vineyards **(Cavicchi, 2015)** can be accompanied. Interesting tasting room at a winery, such as an old cave can be qualified as an example of culinary tourism **(Lušňáková, 2012)**. One of the possibilities and ways of marketing is promotion of "regional gastronomy". Preparing food and drinks is possible to promote as science as an art, as well as a concept comprising the traditions, culture and society.

CONCLUSION

As it can be seen the quality of Sauvignon wines can be affected by different effects. The acidity of wines is significantly (p = 0.000) affected by the time of storage, it decreased during two years of its storage. Significant influence was shown also in residual sugar content at the time of later harvest (p = 0.000) and treatment of must (p = 0.022) by clarification preparation. Alcohol content by storage (p = 0.000) and the time of harvest (p = 0.000) was statistically affected as well.

With using of clarification preparation, flavor precursors formed during the ripening of wines under storage were transformed, and are responsible for the occurrence of other important flavor characteristicof the wine in the archive.

Wine testing can be connected with the farm visits or tours as a unique presentation of local products, marketing and culinary tourism opportunity. It is one of the elements

within the trend of authenticity, environmental protection and the need to have a valuable experience.

REFERENCES

Adams, D. O. 2006. Phenolics and ripening in grape berries. *American Journal of Enology and Viticulture*, vol. 57, no. 3, p. 249-256.

Ailer, Š. 2016. *Vinárstvo & somelierstvo (Winery & Sommelier proficiency)*. 1. ed. Olomouc, Czech Republic : Agriprint, 2016. 197 s. ISBN 978-80-87091-63-0. (In Czech)

Blackman, J., Rutledge, D. N, Tesic, D., Saliba, A., Geoffrey, R. S. 2010. Examination of the potential for using chemical analysis as a surrogate for sensory analysis, *Analytica Chimica Acta*, vol. 660, no. 1-2, p. 2-7. https://doi.org/10.1016/j.aca.2009.10.062

Callejona, R. M., Clavijo, A., Ortigueira, P., Troncoso, A. M., Paneque, P., Morales, M. L. 2010. Volatile and sensory profile of organic red wines produced by different selected autochthonous and commercial Saccharomyces cerevisiae strains. *Analytica Chimica Acta*, vol. 660, no. 1-2, p. 68-75. https://doi.org/10.1016/j.aca.2009.09.040

Cavicchi, A. 2015. Creativity and Innovation in Food: diversification and innovation paths in food smart specialisation areas. Smart specialisation and food: food, gastronomy and bio-economy as elements of regional innovation strategies. EXPO Milan. Available at: http://s3platform.jrc.ec.europa.eu/expo-2015

Chatterjee, S., Chatterjee, S., Chatterjee, B., P., Guha, A. K., 2004. Clarification of fruit juice with chitosan. *Process Biochemistry,* vol. 39, no. 12, p. 2229-2232. https://doi.org/10.1016/j.procbio.2003.11.024

Chira, K., Pacella, N., Jourdes, M., Teissedre, P. L. 2011. Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food Chemistry,* vol. 126, no. 4, p. 1971-1977. https://doi.org/10.1016/j.foodchem.2010.12.056

Commission regulation (EC) No 607/2009 of 14 July 2009 laying down certain detailed rules for the implementation of Council Regulation (EC) No 479/2008 as regards protected designations of origin and geographical indications, traditional terms, labelling and presentation of certain wine sector products. OJ L 193 24.7.2009, p. 60.

Fic, V. 2015. Víno: analýza, technologie, gastronomie (Wine: analysis, technology, gastronomy). Český Tešín, Czech Republic : Václav Helán-2 Theta. 299 p. ISBN 978-80-8680-77-3 (in Czech)

González-Nevesa, G., Favre, G., Gil, G. 2014. Effect of fining on the colour and pigment composition of young red wines, *Food Chemistry,* vol. 157, p. 385-392. https://doi.org/10.1016/j.foodchem.2014.02.062

Ibeas, V., Correia, A., Jordão, A., M. 2015. Wine tartrate stabilization by different levels of cation exchange resin treatments: impact on chemical composition, phenolic profile and organoleptic properties of red wines. *Food Research International,* vol. 69, p. 364-372. https://doi.org/10.1016/j.foodres.2015.01.003

Kumbár, V., Votava, J. 2015. Influence of storage temperature on the viscous behaviour of partially fermented wine must (*Pinot Gris*). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* vol. 63, no. 3, p. 781-785. https://doi.org/10.11118/actaun201563030781

Lapčíková, B., Lapčík, L., Hupková, J. 2017. Physico-chemical characterisation of Slovak wines. *Potravinarstvo,* vol. 11, no. 1, p. 216-222. https://doi.org/10.5219/727

Lušňáková, Z. 2012. Culinary tourism and Marketing Communication: Theory and the V4 Case Study. In Horská, E et al., Food Sciences &Business Studies, Nitra: SPU, p. 371-389. ISBN 978-80-552-0815-2. (In Slovak)

Mirade Orduña, R. 2010. Climate change associated effects on grape and wine quality and production. *Food Research International*, vol. 43, no. 7, p. 1844-1855. https://doi.org/10.1016/j.foodres.2010.05.001

Parr, W. V., Schlich, P., Theobald, J. C., Harsch, M. J. 2013. Association of selected viniviticultural factors with sensory and chemical characteristics of New Zealand Sauvignon blanc wines. *Food Research International*, vol. 53, no. 1, p. 464-475. https://doi.org/10.1016/j.foodres.2013.05.028

Paulsen, M. T., Rognså, G. H., Hersleth, M. 2015. Consumer perception of food–beverage pairings: the influence of unity in variety and balance. *International Journal of Gastronomy and Food Science*, vol. 2, no. 2, p. 83-92. https://doi.org/10.1016/j.ijgfs.2014.12.003

Remeňová, Z. 2015. Vplyv spracovania vín modernými technológiami na ich kvalitu počas zrenia (The impact of wine processing by modern technologies on their quality during maturation). Nitra: SPU. 92 p. (In Slovak)

Sen, K., Cabaroglub, T., Yilmazc, H. 2012. The influence of fining agents on the removal of some pesticides from white wine of *Vitis vinifera* L. *Food and Chemical Toxicology,* vol. 50, no. 11, p. 3990-3995. https://doi.org/10.1016/j.fct.2012.08.016

StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 10. www.statsoft.com.

Šuklje, K., Antalik, G., Coetzee, Z., Schmidtke, L. M., Česnik, H. B., Brandt, J., du Toit, W. J., Lisjak, K., Deloire, A. 2014. Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of *Vitis vinifera* L. cv. Sauvignon Blanc wine. *Australian Journal of Grape and Wine Research,* 20, 2, p. 223-233. https://doi.org/10.1111/ajgw.12083

Torija, N., Rozèset al. 2001. Yeast population dynamics in spontaneous fermentations: Comparison between two different wine-producing areas over a period of three years. *International Journal of General and Molecular Microbiology,* vol. 79, no. 3-4, p. 345-352.

Vietoris, V., Czako, P., Mendelová, A., Remeňová, Z., Závacký, M. 2014. Relations between must clarification and organoleptic attributes of wine varietes. *Potravinarstvo,* vol. 8, no. 1, p.155-160. https://doi.org/10.5219/359

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CHARACTERIZATION OF ESCHERICHIA COLI STRAINS ISOLATED FROM RAW VEGETABLES

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ABSTRACT

Vegetables are an important part of the human diet. Sometimes, contamination by pathogenic *Escherichia coli* can be underestimated; moreover there is a risk of antibiotic resistance spreading via the food chain. The purpose of this study was to examine the prevalence of *Escherichia coli* in fresh vegetables sold in retail market in the Czech Republic and to evaluate the risk to human health. Antibiotic resistance against 12 antibiotics, the presence of 12 virulence and 15 resistance genes were determined among 15 isolated strains. Most of tested strains belonged to B1 phylogenetic group, less frequently represented was B2 and D phylogroup. These results indicate that most strains are probably of human origin. All *E. coli* strains were resistant to at least one of twelve tested antibiotics. A multidrug resistance was observed in four strains. In this study, the presence of virulence factors *Einv* and *papC* and also genes encoding toxins (*CNF1, CNF2*) was detected. Nevertheless, none strain can be considered as STEC or EHEC. The widespread appearance of a growing trend associated with the prevalence of antibiotic resistance among enterobacterial isolates is undeniable and the possibility of transfer to humans cannot be ignored. Nevertheless, these results indicate that raw vegetables sold in the retail market can constitute a potential health risk for consumers.

Keywords: vegetables, *Escherichia coli*, virulence, resistance, safety

INTRODUCTION

Escherichia coli, a common inhabitant of intestinal tract of humans and animals, has been used as an indicator of fecal contamination in food. Fecal pollution can indicate the presence of enterobacterial pathogens. *E. coli* can be easily disseminated in different ecosystems through the food chain **(Ryu et al., 2012)**. Raw vegetables can harbour many different pathogens including *Escherichia coli* and may become contaminated through animals, people, animal manures, and contaminated irrigation water **(Harapas et al. 2010)**.

Escherichia coli strains can be assigned to one of the main phylogenetic groups: A, B1, B2 or D. These phylogroups apparently differ in their ecological niches, life-history and some characteristics, such as their ability to exploit different sugar sources, and their antibiotic-resistance profiles **(Carlos et al., 2010)**. Strains from phylogroups B2 and D often contained more virulence factors than strains from the phylogroups A and B1. The extraintestinal pathogenic strains usually belong to groups B2 and D, the commensal strains to groups A and B1, whilst the intestinal pathogenic strains may belong to groups A, B1 and D. Fecal strains isolated from birds were assigned to groups D and B1; A and B1 were isolated from non-human mammals; and A and B2 were found in humans **(Carlos et al., 2010)**. **Clermont et al. (2005)** have developed a PCR based method to characterize the phylogroups using the genetic markers chuA, yjaA and the DNA fragment TspE4.C2.

The consumption of vegetables has risen over the last decades in many countries **(Franz and van Bruggen, 2008)**. Lettuce, radish and salads are the most common causes of *E. coli* outbreaks, where vegetable was proved as a source of infection all over the world **(ICMSF, 2005)**. A large epidemic caused by shigatoxigenic *E. coli* in spring 2011 in Germany resulted in reduction of trust in the health safety of raw vegetables and sprouted seeds. It has been found that the number of outbreaks of EHEC infections associated with the consumption of contaminated vegetables has increased over the last years **(Tzschoppe et al., 2012)**. STEC and EHEC have been isolated from animal faeces, soil, water, sewage and manure and can persist in the environment over long periods of time **(Fremaux et al., 2008)**. Vegetables can become contaminated with STEC and EHEC at all stages of food production. Unlike food of an animal origin, vegetables have rarely been examined as a possible source of human infections with EHEC **(Tzschoppe et al., 2012)**.

The broad group of *E. coli* known as enterohemorrhagic *E. coli* (EHEC), including *E. coli* O157:H7, contain the LEE (locus of enterocyte effacement) encoded intestinal colonisation mechanism (*eae* gene). The *Einv* gene is responsible for encoding enteroinvasive mechanisms. The *Eagg* gene is known for its encoding enteroaggregative

mechanisms **(Kaper and O'Brien, 1998)**. Cytonecrotic factors (CNF1, CNF2) are often produced by *E. coli* strains, which were isolated from intestinal and also extraintestinal infections of humans and animals. The *iss* (increased serum survival) gene and its protein product (ISS) of avian pathogenic *E. coli* (APEC) are important characteristics of resistance to the complement system. The *iss* gene is probably located in a conserved portion of some plasmids **(Buchanan and Doyle, 1997)**. The *papC* gene encodes fimbrial adhesin. Genes encoding temperature-sensitive haemagglutinin (*tsh*), aerobactin (*iucD*), and serum resistance protein (*iss*) are known for their frequent or exclusive localization on large transmissible R plasmids in APEC **(Buchanan and Doyle, 1997)** and thus these genes are clearly associated with APEC strains **(Yu and Kaper, 1992)**.

The major virulence factors of *E. coli* are Shiga toxins encoded by Shiga toxin genes *stx1* and *stx2*. The ability to produce Stx1 and/or Stx2 is a critical determinant of whether a bacterium can cause the clinical syndrome associated with STEC. Unlike O157:H7, most non-O157 STEC strain cannot be easily distinguished from non-pathogenic *E. coli* strains. The infection of non-O157 STEC in human beings is also considered to be underestimated **(Li et al., 2016)**.

Apart from Shiga-toxin, epidemic strains can produce hydrolysing enzymes known as extended spectrum β-lactamases (ESBLs) causing resistance to several antimicrobial agents. Strains possessing ESBLs, have emerged in the last few decades as a global risk for human health and have been shown to contribute to increased morbidity and mortality **(Said et al., 2015)**. Especially, when they also possess other resistance determinants represent these strains a growing public health threat **(Tzschoppe et al., 2012)**. The high use of antibiotics not only in human medicine, but also in veterinary medicine or even in agriculture, could constitute a selective pressure for the spread of antibiotic resistant bacteria, including ESBL-Eb **(Durso and Cook, 2014)**. ESBL-Eb could persist on the surface of plants or even reach their interior, and they could be transmitted to humans or animals. It is very important to highlight the potential role of consumption of uncooked vegetables in gastrointestinal bacteria acquisition **(Hamilton-Miller and Shah, 2001)**. Future studies should be carried out to evaluate the real risk for human health of this potential transmission linked to the consumption of vegetables **(Said et al., 2015)**.

Scientific hypothesis

The study was focused on the isolation of *E. coli* strains from fresh vegetables sold in the retail market in the Czech Republic. The main objective was to evaluate the presence of antibiotic resistance and virulence factors among these *E. coli* strains and determine a potential risk of fresh vegetables to human health.

MATERIAL AND METHODOLOGY

Samples and *E. coli* isolation

A total of 108 various fresh vegetable samples were bought in the Czech retail markets and investigated for the presence of *E. coli* during year 2015 and other 108 various fresh vegetable samples were investigated during 2016.

Ten grams of sample were homogenized in 90 ml of sterile saline solution, inoculated on the plates with the selective media for *E. coli* - Endo agar or Hi-chrome agar (Oxoid Ltd., United Kingdom) and incubated at 37 °C / 24 h.

Identification of strains

Suspected colonies were identified to the species level by using commercial identification microsystem ENTEROtest24 (ErbaLachema Brno, Czech Republic). This test is designed for routine, definitive identification of important strains of family Enterobacteriaceae. The kit contains 24 biochemical tests (dehydrated substrates for biochemical reactions, e.g, IND, ONP), which are placed in microplate. Obtained data were evaluated by software TNW Lite 6.5 (ErbaLachema Brno, Czech Republic).

Bacterial isolates were cultivated on Nutrient Agar (beef extract 10 g, peptone 10 g, agar 15 g, NaCl 5 g, distilled water 1000 mL, pH 7,2) or on Mueller Hinton Agar. Subsequently, the strains were stored at −80°C in glycerol.

Phylogenetic group determination

A previously described multiplex PCR method was used for determination of phylogenetic groups according to presence of three genes (chuA, yjaA, TspE4.C2) **(Clermont et al. 2000)**. A few colonies of each strain were solved in 100 µL of 1x PCR buffer (ThermoPol Buffer, NEB, USA) and heated up to 95°C for 20 min. Supernatant after centrifugation (10.000 g/L min) was taken as DNA template for all PCR testing. The amplification products were visualized by 1% agarose gel electrophoresis and strains were assigned to the phylogenetic groups **(Carlos et al., 2010)**.

Antibiotic resistance determination

Antimicrobial susceptibility testing to twelve antibiotics was performed by disc diffusion method according to EUCAST methodology **(Matuschek et al., 2014)**. Antibiotic discs (Oxoid Ltd., United Kingdom): amoxicillin/clavulanic acid (AMC 30 µg), ampicillin (AMP 10µg), ceftazidime (CAZ 10µg), cephalotine (CEF 30µg), ciprofloxacin (CIP 5 µg), doxycycline (D 10 µg), chloramphenicol (C 30 µg), gentamicine (CN 10 µg), sulbactam/cefoperazone (SCF 10 µg), streptomycine (S 10µg), sulfamethoxazole/trimethoprim (SXT 25 µg), and tetracycline (T 10 µg) were used. The diameters of the inhibition zones were evaluated (susceptible, intermediate, resistant) according to EUCAST breakpoints.

Detection of virulence and resistance genes

Ten virulence genes (*stx1, stx2, tsh, CNF1, CNF2, papC, neuC, iss, iucD, EinV, Eagg* and *eaeA*) were assessed using PCR as was described previously **(Holko et al., 2006; Ewers et al., 2007)**. The primer sequences of virulence genes, the expected size of their products, and the cycling conditions for the determination of the presence of selected ten genes are listed in Table 1.

The genes encoding antibiotic resistance: *qnrS, tet(A), tet(B), sul1, sul3 qac, merA, qepA, int*, and the genes responsible for the ESBL phenotype (*blaTEM, blaSHV, blaOXA-1, blaOXA-7, blaPSE-4*, and *blaCTX-M-3*) were identified by polymerase chain reaction (PCR). The cycling conditions varied according to the specific gene determination **(Table 1)**.

Table 1 Oligonucleotides sequences of primers used and PCR conditions for detection of virulence and resistance genes in *E. coli* strains.

Description	Virulence gene	Oligonucleotide sequence (5´- 3´)	Annealing temperature (°C)	Size of amplified product (bp)	Reference
temperature-sensitive haemagglutinin	*tsh*	ACTATTCTCTGCAGGAAGTC CTTCCGATGTTCTGAACGT	58	824	Ewers et al. 2007
cytotoxic necrotising factor	*CNF1*	GGCGACAAATGCAGTATTGCT GACGTTGGTTGCGGTAATTTT	63	552	Holko et al. 2006
cytotoxic necrotising factor	*CNF2*	GTGAGGCTCAACGAGATTATG CCACGCTTCTTCTTCAGTTGT	63	839	
shiga toxin 1	*stx1*	TTTCCCCTCTTTTAGTCAGTCAACTG GGCAGGATTACAACAAAGTTCACAG	63	160	Holko et al. 2006
shiga toxin 2	*stx2*	CCCCCTCTCTTTTGCACTTCTTTCC TGCTCCAGCAGTACCATCTCTAACCC	63	423	
pilus associated with pyelonephritis	*papC*	TGATATCACGCAGTCAGTAGC CCGGCCATATTCACATAAC	58	501	
K1 capsular polysaccharide	*neuC*	GGTGGTACATTCCGGGATGTC AGGTGAAAAGCCTGGTAGTGT	58	670	Ewers et al. 2007
increased serum survival	*iss*	ATCACATAGGATTCTGCCG CAGCGGAGTATAGATCCCA	58	309	
aerobactin siderophore synthesis	*iucD*	ACAAAAAGTTCTATCGCTTCC CCTGATCCAGATGATCCTC	58	714	
enteroinvasive mechanisms	*EinV*	TTCTGATGCCTGATGGACCAG TGGAAAAACTGAGTGCCTCTG	63	140	
enteroaggregative mechanisms	*Eagg*	AGACTCTGGCGAAAGACTGTA ATGGCTGTCTGTAATAGATGA	63	194	Holko et al. 2006
intimin	*eaeA*	TGAGCGGCTGGCATGAGTCAT TCGATCCCCATCGTCAACAGA	63	241	
resistance genes					
Beta-lactams	bla_{TEM}	GAGTATTCAACATTTTCGT ACCAATGCTTAATCAGTGA	50	857	Maynard et al. 2004
	bla_{SHV}	TCGCCTGTGTATTATCTCCC CGCAGATAAATCACCACAATG	50	768	
	bla_{OXA-1}	GCAGCGCCAGTGCATCAAC CCGCATCAAATGCCATAAGTG	50	198	
	bla_{OXA-7}	AGTTCTCTGCCGAAGCC TCTCAACCCAACCAACCC	50	591	
	bla_{PSE-4}	CTGCTCGTATAGGTGTTTCC TCGCATCATTTCGCTCTTC	50	705	
	$bla_{CTX-M-3}$	AATCACTGCGTCAGTTCAC TTTATCCCCCACAACCCAG	50	701	

Table 1 (Continue).

Resistance genes					
plasmid-mediated quinolone resistance	*qnrS*	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	58	600	Cattoir et. al 2007
tetracycline	*tet*(A)	GGCCTCAATTTCCTGACG AAGCAGGATGTAGCCTGTGC	55	372	Guillaume et al. 2000
	tet(B)	GAGACGCAATCGAATTCGG TTTAGTGGCTATTCTTCCTGCC	55	228	
sulphonamides	*sul*1	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	63	433	Perreten, Boerlin, 2003
	*sul*3	GAGCAAGATTTTTGGAATCG CATCTGCAGCTAACCTAGGGCTTTG GA	55	880	Kerrn, 2002
quarternary ammonium compounds	*qac*	GCCCCTTCCGCCGTTGTCATAATC CGGCCTCCGCAGCGACTTCC	63	250	Johnson et al. 2012
mercury resistant genes	*merA*	GATCCGCGCCGCCCATATCGCCCAT CTG CACGCGCTCGCCGCCGTCGTTGAGT TG	60	250	
plasmid-mediated gene responsible for reduced fluoroquinolones	*qep*	GCAGGTCCAGCAGCGGGTAG CTTCCTGCCCGAGTATCGTG	60	199	Yamane et al. 2008
integron mediated antibiotic resistance	*int*	GGGTCAAGGATCTGGATTTCG	60	483	Mazel et al. 2000

Statistical analysis

The results of antibiotic resistance and virulence factors were evaluated by Pearson's correlation coefficient between the measured variables. The analysis was performed using statistical software STATISTICA CZ (StatSoft, Inc. 2007).

RESULTS AND DISCUSSION

The prevalence of *Escherichia coli* in various raw vegetables bought in Zlín region (Czech Republic) is shown in Table 2. *E. coli* was detected in 15 out of 108 samples bought in 2015 (13.9%). In contrast, among 108 fresh vegetable samples bought in 2016, there was found no *Escherichia coli* strain. Thus, the prevalence of *E. coli* strains in vegetable during two years is 15 out of 216 (6,9%). In 2013, the incidence of *E. coli* in raw vegetable in the Czech Republic was 26.4% **(Skočková et al., 2013)**. This study proves a decreasing trend in the occurrence of *E. coli* in retail market in the Czech Republic. In comparison, data from Canada reported 8.2% of *E. coli* recovered from fresh produce (lettuce, spinach, carrots, and green onions), which represents low levels of enteric pathogen contamination in vegetables sold in North America **(Bohaychuk et al., 2009)**. On the other hand, a total of 90 samples of raw salad vegetables (parsley, lettuce, radish) were collected in Lebanon and *E. coli* was present in almost half (45.5%) of samples **(Faour-Klingbeil et al., 2016)**.

All positive samples in this study came from European countries (Table 2). The most commonly contaminated vegetable samples were mung sprouts (4/12), radish (2/7), tomatoes (2/10), and spring onion (2/4). The occurrence of *E. coli* in the other types of vegetable was less frequent. **Skočková et al. (2013)** indicated leafy vegetable and sprouted seeds as the most common sources of *E. coli.*

Mukherjee et al. (2004) who analysed fresh fruits and vegetables produced by organic and conventional farmers in Minnesota have shown *E. coli* prevalence rates of 1.6 and 9.7%, respectively. **Tzschoppe et al. (2011)** have detected *E. coli* in five (12.5%) of 40 salad and sprouted seed samples. Lower prevalence of *E. coli* may also be a result of a different methodology of detection with no enrichment **(Skočková et al., 2013)**.

Phylogenetic groups

In this study, the most *E. coli* strains (73.3%) belonged to B2 phylogroup, much less of them was assigned into B1 (13.3%) and D (13.3%) phylogroup. None of the strains can be determined as A phylogroup. It is known **(Carlos et al., 2010)** that B2 phylogroup *E. coli* strains are highly probably human extraintestinal pathogenic strains. On the other hand, *E. coli* strains isolated from chicken meat mostly belong to A, less to B1 phylogroup **(Pavlíčková et al., 2015)**. This study is also in accordance with the statement that strains from phylogroups B2 and D contained more virulence factors than strains from the phylogroups A and B1 **(Carlos et al., 2010)**.

Antibiotic resistance

All 15 strains (100.0%) isolated from raw vegetables in this work were resistant to at least one antibiotic, even more 4 of these strains (26.7%) were multiresistant (3 or more). The most frequent antibiotic resistance is ampicillin resistance (93.3%) and resistance to cephalotine (53.3%) and ceftazidime (40.0%). Also, resistance against gentamicine (13.3%) and streptomycine (6.7%) were

Table 2 Prevalence of *E. coli* in raw vegetable samples sold in retail market.

Vegetable	Country of origin	Number of samples positive for *E. coli* /number of samples
mung sprouts	Czech Republic, Italy	4/12
lentils sprouts	Czech Republic	0/1
alfalfa sprouts	Czech Republic	0/1
broccoli	Spain	0/4
strawberry	Spain	0/4
carrot	Czech Republic	1/4
zucchini	Spain	1/3
spinach	Italy, Spain	0/3
ice lettuce	Italy	0/3
cucumber	Slovakia	0/6
mix of lettuces	Hungary, Italy	0/9
tomato	Morocco	2/10
eggplant	Italy	0/3
chickpeas sprouts	Italy	0/1
baby carrot	Czech Republic, Netherlands	1/3
leek	Czech Republic	0/1
radish	Czech Republic, Italy	2/7
spring onion	Germany	2/4
pepper	Poland	0/2
lettuce little gem	Netherlands	1/2
rucola	Italy	0/3
lettuce	Czech Republic	0/1
lamb's lettuce	Italy	0/1
wallflower	Netherlands	0/1
celery	Spain	0/1
champignon	Poland	0/1
parsley	Czech Republic	1/2
avocado	unknown	0/1
beet	Czech Republic	0/1
turnip	unknown	0/1
cauliflower	Czech Republic	0/1
green beans	unknown	0/1
potato	Czech Republic	0/1

present. High prevalence of resistance to aminopenicillins is also proved by presence of *bla* genes (**Table 3**).

ESBL-Eb that contaminates vegetables can be transmitted to human consumers via the food chain. In the work of **Said et al. (2015)**, ESBL-Eb were detected in 4 of 45 vegetable samples of market origin tested and in 3 of 13 markets. ESBL-Eb positive isolates were also detected among vegetables samples in the Netherlands (**Reuland et al., 2013**).

Fluoroquinolone antibiotics inhibit two bacterial enzymes, DNA gyrase and topoisomerase IV, both of which play essential roles in DNA replication. Resistance to quinolone is often linked to amino acid substitutions in the quinolone-resistance-determining regions of DNA gyrase and DNA topoisomerase IV subunits, leading to target modification (**Rezazadeh et al., 2016**). However, recent reports indicate that quinolone resistance can also be mediated by mobile genetic elements such as plasmids.

Table 3 Characterization of *E. coli* strains isolated from fresh vegetable.

Isolate	Source	Country of origin	Resistance phenotype	Resistance genes	β-lactamase genes	Virulence genes	PG
F10	mung sprouts	Italy	CAZ, AMP	-	*blaOXA-1*	*EinV*	B2
F52	mung sprouts	Czech Republic	S, CEF, AMP	*qac*	*blaTEM, blaSHV, blaOXA-1, blaPSE-4, blaCTX-M-3*	*CNF1, EinV*	B2
F53	mung sprouts	Czech Republic	CEF, AMP	*qac*	*blaTEM, blaOXA-1*	*papC, EinV*	D
F77	spring onion	Czech Republic	CAZ, AMP	*sul1*	*blaTEM*	*EinV*	B2
F78	tomato	Morocco	CAZ, AMP	-	-	*papC, EinV*	B2
F81	lettuce little gem	Italy	AMP	*tetA, int, sul1, sul3, mer*	*blaTEM*	*EinV*	B1
F84	baby carrot	Netherlands	CEF, AMP	*qnrS, qac*	*blaTEM, blaSHV, blaOXA-1, blaCTX-M-3*	*papC, CNF1, EinV*	B2
F87	mung sprouts	Czech Republic	CAZ, CEF, AMP	*qnrS, qac, mer*	*blaTEM, blaSHV, blaOXA-1, blaPSE-4, blaCTX-M-3*	*EinV*	B2
F94	tomato	Czech Republic	AMP	*sul1*	-	*CNF1, EinV*	B2
F103	parsley	Czech Republic	AMP	*qnrS, qac*	*blaOXA-1*	*EinV*	B2
F104	radish	Czech Republic	CAZ, CEF, AMP	*qnrS, qac*	*blaOXA-1*	*papC, EinV*	B2
F105	carrot	Czech Republic	AMP	*tetB*	*blaTEM, blaCTX-M-3*	*EinV*	B2
F106	radish	Czech Republic	CEF, CN	*qac*	*blaOXA-1*	*papC, CNF2, EinV*	D
F107	spring onion	Czech Republic	CAZ, CEF, AMP, CN	*tetB*	*blaTEM*	*EinV*	B1
F108	zucchini	Spain	CEF, AMP	*qac*	*blaTEM, blaSHV, blaOXA-1, blaPSE-4, blaCTX-M-3*	*papC, CNF2*	B2

Note: AMP – ampicillin; CAZ – ceftazidime; CEF – cephalotine; CN – gentamycine; S – streptomycine; PG – phylogroup.

Plasmid-mediated quinolone resistance is mediated by the genes (*qnr*) encoding proteins that protect DNA gyrase and topoisomerase IV against quinolone compounds. The three major groups of *qnr* determinants are *qnrA, qnrB*, and *qnrS*.

The first plasmid-mediated quinolone-resistance gene (*qnrA*) was identified in a clinical strain of *Klebsiella pneumoniae* isolated in Alabama in 1998. The other two determinants of qnr (qnrB and qnrS) have subsequently been observed in other enterobacteria including *E. coli*, *Enterobacter*, *Salmonella*, and *Klebsiella pneumoniae* **(Rezazadeh et al., 2016)**.

It is of interest to remark that vegetables containing ESBL-Eb of market origin are commonly eaten uncooked, and the possibility of transfer to humans cannot be ignored. Different authors also indicate the potential problems derived from these uncooked food samples containing antibiotic resistant bacteria (**Hamilton-Miller and Shah, 2001; Veldman et al., 2014)**. In fact, foodborne outbreaks due to ESBL-positive *E. coli* isolates related to vegetables have been previously reported **(Reuland et al., 2013)**. Obviously, the widespread appearance of a growing trend associated with the prevalence of antibiotic resistance among enterobacterial isolates is undeniable.

Detection of virulence factors

In this study, 15 out of 15 isolates (100.0%) possessed some virulence factor and 2 of these strains (13.3%) were even multivirulent (more than 3 factors). The most frequently found virulence factors were *Einv* gene (93.3%) and *papC* (40.0%). The *Einv* gene is responsible for encoding enteroinvasive mechanisms (EIEC) and *papC* gene encodes an adhesin. Presence of these two factors much more resemble origin in wildlife **(Pavlíčkova et al., 2017)** than in e.g. poultry meat **(Pavlíčková et al., 2015)**. Moreover, five strains were positive for cytotoxic necrotizing factors *CNF* toxins (20.0% *CNF1*; 13.3%

CNF2). These results may represent potential threat to human health. CNF1 is a major virulence factor of UPEC strains and it was found in bacteria isolated from meningitis affected children. Moreover, CNF1 is produced in some extraintestinal *E. coli* (ExPEC). There are hints that CNF1 may be involved in cancer development: CNF1 induces the expression of cyclooxigenase-2 (COX-2), activates nuclear factor-kappa B (NF-κB), increases cell motility and inhibits apoptosis. CNF2 has been demonstrated in *E. coli* isolated in calves and lambs with diarrhoea **(Knust and Schmidt, 2010)**.

Plasmid-mediated resistance is of growing clinical concern as they may transfer resistance genes to other species via horizontal gene transfer. Resistance of *eaeA*-positive STECs to fluoroquinolones constitute health threat to consumers, where resistance determinants can spread among non-pathogenic bacteria in the gastrointestinal tract due to plasmid mobility **(Khalil and Gomaa, 2016)**. Moreover, the simultaneous presence of extended-spectrum beta-lactamases (ESBLs), AmpC, and *qnr* genes on the same plasmid has been well documented and this highlights the complexity of determinants involved in plasmid-mediated resistance among the enterobacterial isolates **(Rezazadeh et al., 2016)**. It can be observed that at least four strains isolated in this study represent plasmid mediated resistance **(Table 3)**. Fortunately, neither *eaeA*-positive strains (EHEC) nor STEC strains were proved among *E. coli* strains found in the Czech raw vegetable, thus it can be summarised that vegetables sold in the Czech Republic are not enteropathogenic/shigatoxigenic.

In this work, statistically significant correlation (p <0.05) was not proved between presence of antibiotic resistance and virulence factors.

CONCLUSION

It can be concluded that presence of *Escherichia coli* on raw vegetables sold in retail market in the Czech Republic is decreasing. In 2015 it was found 13.9% positive samples and a year later there were no *E. coli* strain among 108 samples. It was observed that all 15 *E. coli* isolates were resistatnt at least to one antibiotic, especially against aminopenicillins and cephalosporines. Four strains carry resistance to three or more antibiotics. Furthermore, all strains also encode at least one of virulence factors – *Einv, papC* and even *CNF1/CNF2*, which are toxins and may represent pathogenic bacteria. In contrast, neither STEC nor EHEC were determined. There exists a possibility of antibiotic resistance transfer from enterobacteria to humans and it should not be ignored, as well as there is a small hazard of intestinal infection. These results indicate that raw vegetables sold in the retail market can represent a potential health risk for consumers.

REFERENCES

Bohaychuk, V. M., Bradbury, R. W., Dimock, R., Fehr, R. M., Gensler, G. E., King, R. K., Rieve, R., Romero Barrios, P. 2009. A Microbiological Survey of Selected Alberta-Grown Fresh Produce from Farmers' Markets in Alberta, Canada. *Journal of Food Protection*, vol. 72, no. 2, p. 415-420. https://doi.org/10.4315/0362-028X-72.2.415 PMid:19350990

Buchanan, R. L., Doyle, M. P. 1997. Foodborne Disease Significance of *Escherichia coli* O:157 and Other Enterohemorrhagic *E. coli*. *Food Technology*, vol. 51, no. 10, p. 69-76.

Carlos, C., Pires, M. M., Stoppe, N. C. C., Hachich, E. M., Sato, M. I., Gomes, T. A. T., Amaral, L. A., Ottoboni, L. M. M. 2010. Escherichia coli phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. *BMC Microbiology*, vol. 10, no. 1, p.161. https://doi.org/10.1186/1471-2180-10-161 PMid:20515490

Cattoir, V., Poirel, L., Rotimi, V., Soussy, C. J., Nordmann, P. 2007. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. *Journal of Antimicrobial Chemotherapy*, vol. 60, no. 2, p. 394-397. https://doi.org/10.1093/jac/dkm204 PMid:17561500

Clermont, O., Bonacorsi, S., Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. 2000. *Applied Environmental Microbiology,* vol. 66, no. 10, p. 4555-4558. https://doi.org/10.1128/AEM.66.10.4555-4558.2000 PMid:11010916

Durso, L. M., Cook, K. L. 2014. Impacts of antibiotic use in agriculture: what are the benefits and risks? *Current Opinion in Microbiology*, vol. 19, p. 37-44. https://doi.org/10.1016/j.mib.2014.05.019 PMid:24997398

Ewers, C., Li, G., Wilking, H., et al. 2007. Avian pathogenic, uropathogenic, and newborn meningitis-causing Escherichia coli: How closely related are they? *International Journal of Medical Microbiology*, vol. 297, no. 3, p. 163-176. https://doi.org/10.1016/j.ijmm.2007.01.003 PMid:17374506

Faour-Klingbeil, D., Murtada, M., Kuri, V., Todd, E. C. D. 2016. Understanding the routes of contamination of ready-to-eat vegetables in the Middle East. *Food Control*, vol. 62, p. 125-133. https://doi.org/10.1016/j.foodcont.2015.10.024

Franz, E., Van Bruggen, A. H. C. 2008. Ecology of *E. coli* O157:H7 and Salmonella enterica in the Primary Vegetable Production Chain. *Critical Reviews in Microbiology*, vol. 34, no. 3-4. https://doi.org/10.1080/10408410802357432 PMid:18728991

Fremaux, B. C., Prigent-Combaret, C., Vernozy-Rozand, C. 2008. Long-term survival of Shiga toxin-producing Escherichia coli in cattle effluents and environment: An updated review. *Veterinary Microbiology*, vol. 132, no. 1-2, p. 1-18. https://doi.org/10.1016/j.vetmic.2008.05.015 PMid:18586416

Guillaume, G. 2000. PCR typing of tetracycline resistance determinants (Tet A–E) in Salmonella enterica serotype Hadar and in the microbial community of activated sludges from hospital and urban wastewater treatment facilities in Belgium. *FEMS Microbiology Ecology,* vol. 32, no. 1, p. 77-85. PMid:10779622

Hamilton-Miller, J. M., Shah, S. 2001. Identity and antibiotic susceptibility of enterobacterial flora of salad vegetables. *Int. J. Antimicrob. Agents,* vol. 18, no. 1, p. 81-83. https://doi.org/10.1016/S0924-8579(01)00353-3

Harapas, D., Premier, R., Tomkins, B., Franz, P., Ajlouni, S. 2010. Persistence of Escherichia coli on injured vegetable plants. *International Journal of Food Microbiology*, vol. 138,

no. 3, p. 232-237. https://doi.org/10.1016/j.ijfoodmicro.2010.01.022

Holko, I., Bisova, T., Holkova, Z., Kmet, V. 2006. Virulence markers of Escherichia coli strains isolated from traditional cheeses made from unpasteurised sheep milk in Slovakia. *Food Control*, vol. 17, no. 5, p. 393-396. https://doi.org/10.1016/j.foodcont.2005.01.007

ICMSF. 2005. *Microbial ecology of food commodities*. 2nd ed. NEW YORK : Kluwer Academic/Plenum Publishers, c2005. ISBN 0-306-48675-x.

Johnson, T. J., Logue, C. M., Johnson, J. R., et al. 2012. Associations between multidrug resistance, plasmid content, and virulence potential among extraintestinal pathogenic and commensal Escherichia coli from humans and poultry. *Foodborne Pathogens and Disease*, vol. 9, no. 1, p. 37-46. https://doi.org/10.1089/fpd.2011.0961 PMid:21988401

Kaper, J. B., O'Brien, A. D. 1998. *Escherichia coli O157:H7 and other shiga toxin-producing E. coli strains*. WASHINGTON, DC : ASM Press, xxii, 465 p. ISBN 1555811299.

Kerrn, M. B. 2002. Susceptibility of Danish Escherichia coli strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. *Journal of Antimicrobial Chemotherapy,* vol. 50, no. 4, p. 513-516. https://doi.org/10.1093/jac/dkf164 PMid:12356795

Khalil, R. K. S., Gomaa, M. A. E. 2016. Prevalence and characterization of Shiga toxin-producing Escherichia coli (STEC) in fruits and vegetables sold at local street markets in Alexandria, Egypt. *LWT - Food Science and Technology*, vol. 74, p. 199-210. https://doi.org/10.1016/j.lwt.2016.07.041

Knust, Z., Schmidt, G. 2010. Cytotoxic Necrotizing Factors (CNFs)−A Growing Toxin Family. *Toxins*. vol. 2, no. 12, p. 116-127. https://doi.org/10.3390/toxins2010116 PMid:22069550

Matuschek, E., Brown, D. F. J., Kahlmeter, D. 2014. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clinical Microbiology and Infection*, vol. 20, no. 4, p. O255-O266. https://doi.org/10.1111/1469-0691.12373 PMid:24131428

Maynard, C., Bekal, S., Sanschgrin, F., LEVESQUE, R. Brousseau, R. C., Masson, L., Lariviere, S., Harel, J. 2004. Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal Escherichia coli isolates of animal and human origin. *Journal of Clinical Microbiology,* vol. 42, no. 12, p. 5444-5452. https://doi.org/10.1128/JCM.42.12.5444-5452.2004 PMid:15583263

Mazel, D., Dychinco, B., Webb, V. A., Davies, J. 2000. Antibiotic resistance in the ECOR collection: Integrons and identification of a novel aad gene. *Antimicrobial Agents and Chemotherapy*, vol. 44, no. 6, p. 1568-1574. https://doi.org/10.1128/AAC.44.6.1568-1574.2000 PMid:10817710

Mukherjee, A., Speh, D., Dyck, E., Diez-Gonzalez, F. 2004. Preharvest evaluation of coliforms, Escherichia coli, Salmonella, and Escherichia coli O157:H7 in organic and conventional produce grown by Minnesota farmers. *J. Food Prot.,* vol. 67, no. 5, p. 894-900. https://doi.org/10.4315/0362-028X-67.5.894 PMid:15151224

Pavlíčková, S., Doležalová, M., Holko, I. 2015. Resistance and virulence factors of Escherichia coli isolated from chicken. *Journal of Environmental Science and Health, Part B* vol. 50, no. 6, p. 417-421. https://doi.org/10.1080/03601234.2015.1011959 PMid:25844863

Pavlíčková, S., Klancik, A., Doležalová, M., Smole Možina, S., Holko, I. 2017. Antibiotic resistance, virulence factors and biofilm formation ability in Escherichia coli strains isolated from chicken meat and wildlife in the Czech Republic. *Journal of Environmental Science and Health, Part B*, vol. 52, no. 8, p. 570-576. https://doi.org/10.1080/03601234.2017.1318637 PMid:28494209

Perreten, V., Boerlin, P. 2003. A New Sulfonamide Resistance Gene (sul3) in Escherichia coli Is Widespread in the Pig Population of Switzerland. *Antimicrobial Agents and Chemotherapy*, vol. 47, no. 3, p. 1169-1172. https://doi.org/10.1128/AAC.47.3.1169-1172.2003 PMid:12604565

Reuland, E. A., Overdevest, I. T., Al Naiemi, N., et al. 2013. High prevalence of ESBL-producing Enterobacteriaceae carriage in Dutch community patients with gastrointestinal complaints. *Clinical Microbiology and Infection*, vol. 19, no. 6, p. 542-549. https://doi.org/10.1111/j.1469-0691.2012.03947.x PMid:22757622

Rezazadeh, M., Baghchesaraei, H., Peymani, A. 2016. Plasmid-mediated quinolone-resistance (qnr) genes in clinical isolates of Escherichia coli collected from several hospitals of Qazvin and Zanjan provinces, Iran. *Osong Public Health and Research Perspectives*, vol. 7, no. 5, p. 307-312. https://doi.org/10.1016/j.phrp.2016.08.003

Ryu, S. H., Park, S. G., Choi, S. M., et al. 2012. Antimicrobial resistance and resistance genes in Escherichia coli strains isolated from commercial fish and seafood. International *Journal of Food Microbiology*, vol. 152, no. 1-2, p.14-18. https://doi.org/10.1016/j.ijfoodmicro.2011.10.003 PMid:22071288

Skočková, A., Karpíšková, R., Koláčková, I., Cupáková, Š. 2013. Characteristics of Escherichia coli from raw vegetables at a retail market in the Czech Republic. *International Journal of Food Microbiology*, vol. 167, no. 2, p. 196-201. https://doi.org/10.1016/j.ijfoodmicro.2013.09.011 PMid:24135675

Tzschoppe, M., Martin, A., Beutin, L. 2012. A rapid procedure for the detection and isolation of enterohaemorrhagic Escherichia coli (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104: H4 strain from ready-to-eat vegetables. *International Journal of Food Microbiology*, vol. 152, no. 1-2, p. 19-30. https://doi.org/10.1016/j.ijfoodmicro.2011.10.009 PMid:22071287

Veldman, K., Kant, A., Dierikx, C., Van Essen-Zandbergen, A., Wit, B., Mevius, D. 2014. Enterobacteriaceae resistant to third-generation cephalosporins and quinolones in fresh culinary herbs imported from Southeast Asia. *International Journal of Food Microbiology*, vol. 177, p. 72-77. https://doi.org/10.1016/j.ijfoodmicro.2014.02.014 PMid:24607424

Yamane, K., Wachino, J., Suzuki, S., Arakawa, Y. 2008. Plasmid-mediated qepA gene among Escherichia coli clinical isolates from Japan. *Antimicrobial Agents and*

Chemotherapy, vol. 52, no. 4, p. 1564-1566. https://doi.org/10.1128/AAC.01137-07

Yu, J., Kaper, J. B. 1992. Cloning and characterization of the eae gene of enterohaemorrhagic *Escherichia coli* O157: H7. *Molecular Microbiology*, vol. 6, no. 3, p. 411-417. https://doi.org/10.1111/j.1365-2958.1992.tb01484.x

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FOOD SAFETY FROM CONSUMER PERSPECTIVE: HEALTH SAFETY

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ABSTRACT

Food industry along with agriculture constitute a major economy sector in most countries, because in addition to water intake and oxygen availability, food is another basic determinant for functioning of the human organism. For the proper functioning of human body, it is imperative that the customer chooses food so that the daily diet includes all the necessary nutrients in a reasonable proportion. At present, however, it is becoming more and more common that foodstuffs do not meet strict standards, are not properly stored, the packaging is damaged, or hygiene standards are not being adhered to, and therefore, in addition to health benefiting substances, they also contain harmful ones. According to the World Health Organization, the death of up to 2 million people a year around the world is caused by foods harmful to human health. The main objective of this report was to assess how consumers perceive the health safety of food in Slovakia and to find out whether some types of food are considered as potentially harmful to health. Primary data were obtained through a questionnaire survey conducted from October to December 2017 on a sample of 478 respondents. Respondents answered to 12 factual, and 9 classification questions, which were consequently analyzed using the Friedman test, Nemenyi test and Chi-Square test of Independence. Survey results showed that the majority of respondents had concerns about the health harming effects of food only occasionally and they trust the hygienic level of the restaurant facilities (60.5%), fast food (53%) and frozen food (49.2%) with few reservations. As the most hazardous foods are considered poultry meat, eggs and mayonnaise. If the consumer's health is endangered by food, the guilty party should be punished by ban (61.9%) or by suspension (19.5%).

Keywords: food safety; health safety; food; foodstuffs; consumer; health

INTRODUCTION

Growing concerns with the application of highly toxic pesticides and the growing world demands for food direct our attentions to the food safety as well as to the global arrangement of food production **(Dou et al., 2015)**. Population as a whole, individuals and households as self clear-cut social groups, which buy products for their personal consumption create the consumer market. Each person decides on purchase of the product range everyday. **(Kubicová and Kádeková, 2011)**. Even global consumers are nowadays more concerned about the safety of food products because of a series of food scandals that have been occurred over the last decade and because of the fact that they have not seen signs of decreasing the frequency of occurrence of food scandals **(Loc, 2006)**. Even though food products seem to be safer than ever before, from a technical point of view and due to many quality control programs, the safety perception of consumers has decreased significantly (**Trienekens and Zuurbier, 2007)**. Food production has global character; therefore the consequences of contamination can potentially be very broad and can harm human health and erode the credibility of manufacturers, regulatory bodies and also the good name of produced foodstuffs **(Golian et al., 2006)**. It is important to direct the attitudes of man and whole society towards the rational consumption and nutrition of the individual types of food, in order to make food production and consumption more efficient and reduce the threat to the health of the population by civilization diseases caused by improper nutrition **(Holienčinová, 2013)**.

Food safety includes food production hygiene, control mechanisms, food chain monitoring, and animal feed safety. In order to ensure food safety, state and state-funded institutions contribute to the creation of legislation, continuous and rigorous health safety and quality control, long-term monitoring of the occurrence of foreign matter, application of scientific knowledge to practice, informing and educating consumers in the field of food handling **(Ministry of Agriculture of the Czech Republic, 2018)**. That the foodstuff is harmless to health can only be said if

there is no nutritional, microbiological, chemical or toxicological hazard from that particular foodstuff **(Trusková, 2008)**. Food safety **(Úradníčková et al., 2007)** can be threatened by biological, chemical and physical risk factors. Biological risks include bacteria, viruses and parasites that are transmissible to humans after ingestion of food and cause disease, and also the bacteria and molds capable of producing toxins in the intestinal tract. Chemical risks cover the range of substances naturally occurring in food, foreign substances and endogenous substances that cause any acute or chronic intoxication or individual adverse reaction in humans. Physical hazards, such as sharp and hard objects of glass, ceramics, stone, metal, wood, plastic, bones, fruit stones, in the food can injure the consumer - breaking the tooth, wounding the tongue, or falling into the larynx. Pieces of plastic, peeled old paint, a piece of masonry, paper, fabric, leather in the food can disgust the consumer. Dust that adheres to the wet food, static-charged paper or plastic, settled on a kitchenware, kitchen utensils or food, can deteriorate sensory properties of food. Any foreign particulate matter or contamination of the food means the aesthetic fault of the product and at the same time may be the cause of its inappropriateness for human consumption **(Kerekréty, 2000)**.

In most developed countries, food safety regulation has focused on the imposition of standards that specify how food products should be produced and/or their final safety level **(Rouvière and Caswell, 2012)**. Food safety and consumer protection are important tasks of several international organizations, such as the Codex Alimentarius and the World Organisation for Animal health (former International Office of Epizootics) (OIE) operating under the rules of the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), the World Health Organization (WHO), and the Food and Agriculture Organization of the United Nations (FAO) **(Šinková, 2007)**. In Europe is the recent evolution of European food safety regulations characterized by the increased involvement and responsibility of private actors in food safety controls **(Fares and Rouviere, 2010)**. European Union policy **(European Commission, 2014)** is driven by three general objectives - to ensure that food and animal feed are safe and nutritious, to ensure a high level of animal health, welfare and plant protection, to ensure adequate and transparent information about the origin, content/labelling and use of food. Ministry of Agriculture of the Slovak Republic considers food safety a top priority and the system of food safety according to **Bírošová and Kačenová (2010)** is formed by three essential inseparable areas, including scientific risk assessment, legislation and official authorities control. Consumer safety is paramount when it comes to food safety regulation; however, regulators need to conduct due assessments of food safety risks on consumer, and the cost implications of enforcement strategies on industry, to mitigate costs incurred by industry, without compromising consumer safety **(Mensah and Julien, 2011)**.

Furthermore, the food system involves many factors, from e.g. seed producers, farmers, food industry to retailers, restaurants and consumers, who all have a role to play in the production and consumption of healthy and safe diets. Therefore, any consideration of future developments and challenges need to be inclusive in terms of different relevant expertise and perspectives **(Bock and Bontoux, 2017)**. 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 – during processing **(KecskesNagy, Korzenszky and Sembery, 2016)**. The quality and safety of final products depend on the constant manufacturing processes, which are followed, according to the good manufacturing practices (GMPs) and good hygiene practices (GHPs), which constitute the prerequisites of hazard analysis and critical control points (HACCP) plans in the food industry **(Wallace and Williams, 2001)**. However, final products may be further exposed to less controlled conditions along the distribution chain and in the domestic environment. In improper handling after product release and during distribution at the retail level or in households may result in significant deterioration of quality and compromise the safety of the products. Consumers tend to systematically overestimate some potential hazards related to commercially produced food, whereas optimistic biases are much greater for foodborne illnesses occurred from food prepared at home **(Verbeke, Frewer, Scholderer, & de Brabander, 2007)**. Food safety is also important in restaurants. Employees keeping their fingernails clean, employees wearing clean uniforms or protective clothing, and employees wearing gloves while handling ready-to-eat food are the key food safety aspects for casual dining restaurants **(Liu and Lee, 2018)**.

Recurrence of the issues associated with the spread of health-harming food and animal feed, food contamination incidents, the emergence of new contagious diseases in livestock and, last but not least, the contamination of ecosystems by extraneous substances leading the food chain contamination due to environmental pollution, have a significant effect on consumer behavior in the food market and have a negative effect on the credibility of food. A new consumer attitude is born. The consumer becomes an "active teammate", independent and well informed **(Skořepa, 2003).** Food consumption per capita is everyone's concern and reflects also the socio - economic conditions of people's lives **(Kubicová and Kádeková, 2012)**. Slovak consumers are increasingly perceiving and expressing interest in information on the quality of food products. Quality mark SK is one of the labels that will provide consumer with quick information that the product so labeled is of high quality, safe, and associated with either production or tradition with Slovakia **(Supeková, 2008)**.

Scientific hypotheses

In relation to questionnaire questions, we have formulated the following hypotheses:

Hypothesis 1: Respondents perceive the potential health harming effects of food differently.

Hypothesis 2: The origin of food is mainly of a concern to the respondents with higher education.

Hypothesis 3: Men and women eat at their workplaces with the same frequency.

Hypothesis 4: City dwellers visit restaurant facilities more often, as there is a larger share of restaurant facilities in urban areas than in villages.

MATERIALS AND METHODS

The main objective of this study was to assess how consumers perceive the safety of food in Slovakia and find out if they consider some types of food as potentially dangerous to their health.

Domestic and foreign literature as well as Internet sources served as a source of secondary information for processing the theoretical background on the subject. The source of primary information was a questionnaire survey, on the basis of which our theoretical observations were compared to the information obtained from consumers.

To achieve the above-mentioned goal, anonymous survey was conducted on a sample of 478 respondents from October to December 2017. Questions were divided into two groups. Respondents first answered to 12 factual questions related to food safety issues and then to 9 classification questions. Potential respondents received a questionnaire in paper form. Subsequently, all completed questionnaires were transformed into the Google Forms Internet application. The survey objects were Slovak inhabitants of all ages.

Primary information was processed using the following statistical methods: Friedman test which is a non-parametric alternative to the repeated measures ANOVA where the assumption of normality is not acceptable. Usually it is used in case of ordinal dependent variable. This occurs especially in case of questioner survey, when each respondent assesses more than two products using the same scale. In case of Friedman test applications should be met following conditions: One group that is measured on three or more different occasions.

- Group is a random sample from the population.
- Dependent variable should be measured at the ordinal or continuous level.
- Samples do not need to be normally distributed.

The non-parametric post-hoc test called Nemenyi test which is based on the Kruskal-Wallis method of ranking in a one-way classification and Chi-Square test of Independence to investigate relationship between categorical variables.

The survey included 58.3% of women and 41.7% of men of different age groups. The largest share had the age group 21-30 years old with 46.5% and the smallest was the age group of 60 and above (6.5%). Group with completed higher education represented 46.8%, secondary education 41.8%, apprenticeship 8.1% and basic education only 3.3%. The questionnaire form also included questions about the economic activity of the respondent. The sample contained 170 economically inactive persons (students, retired and unemployed). Other 308 respondents were economically active (employees, employers, self-employed). When respondents selected one of the employment options, they were also asked to specify their profession. Based on the monthly income, respondents were divided into 5 income categories: up to 330 € (8.1%), 331-500 € (8.6%), 501-660 € (21%), 661-830 € (22.1%), and € 831 and over (40.2%). One of the classification questions categorized respondents according the number of family members. Most numerous were groups with four (27.1%) and three (25.4) members. In terms of their permanent residence, respondents came from all 8 regions of the Slovak Republic, namely 47.2% from the rural areas and 52.8% from the urban areas.

RESULTS AND DISCUSSION

The first factual question asked in the questionnaire was whether the respondent had any concerns when purchasing food that it might be harmful to the health **(Figure 1)**. Only 7.8% didn't feel concerned. Majority of respondents (51.9%) worried about the health harming effects of food only occasionally and 32.1% were seldom concerned. Less than 10% of surveyed respondents feared such effects often. Thus, we can ascertain that consumers do not trust the strict legislation of the Slovak Republic and European Union, upon which the sale of the unsafe food shouldn't occur. This mistrust might also result from the fact that according to the research by **Ergönül (2013)**, up to 34 % of the consumers expressed that they had suffered from food-borne originated stomach ache, whereas the ratio was 30 % for the consumers who had suffered from food-borne diarrhea in the past. On the other hand, ratios of the consumers which suffered from nausea, pyrexia and vomiting were 37 %, 25 % and 30 %, respectively.

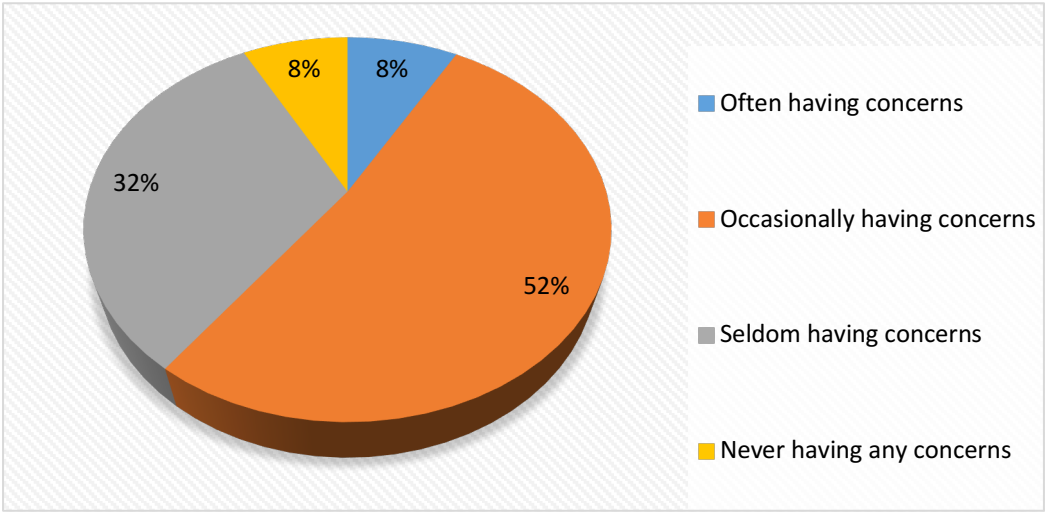

Figure 1 Frequency of being concerned about health harming effects of food.

The second question of the questionnaire **(Figure 2)** was devoted to individual types of food and should provide information on how frequently they aroused concerns about their possible health harming effects. Respondents rated 9 foods (on a scale from 1 – 4, where 1 is "often" and 4 is "never"): beef, pork, poultry meat, fish, eggs, mayonnaise, fruits, vegetables, and "others". The most unsafe foods according to Nemenyi test results were poultry meat, mayonnaise and eggs. This result can be attributed to the fact that these products are subject to rapid deterioration, especially when the appropriate thermal conditions are not met during their storage or transport. Eggs, especially those imported from abroad, are also associated with frequent food scandals when they have been contaminated by harmful chemical substances. Similarly, in the **Australian Institute of Food Safety (2016)** feature, poultry meat, eggs, and products made from them had leading positions in the top 10 chart of products causing food poisoning. Moreover, **The U.S. Department of Health and Human Services (2017)** estimates that 79,000 cases of foodborne illness and 30 deaths each year are caused by eating eggs contaminated with Salmonella, because they were not stored properly. As the safest was considered category "others", also fruits and vegetables, maybe just for the reason that with such foods the first glance can reveal the changes in their sensory properties.

In relation to this issue, Friedman test was used to determine whether there are statistically significant differences in the risk factors evaluation.

H_0: All respondents perceived potential health harming effects of food equally.
H_1: Difference existed in the perception of potential health harming effects of the individual types of food among respondents.

$$p = 0{,}0001 < \alpha = 0.05$$

To test the hypotheses, calculated p-value was compared to the estimation risk alpha. The null hypothesis was rejected and therefore the alternative hypothesis H1 was accepted. So, with 95% probability, it is possible to claim that there are significant differences among respondents in perceiving the possible health harming effects of the individual food types.

In the next question, respondents were asked to identify 5 of the 13 factors that, in their opinion, pose an actual risk to the health **(Figure 3)**. The intestinal and diarrheal diseases, which form a large group of infectious diseases with a characteristic localization of the infection process in the intestines, were considered by 264 respondents to be the foremost. Contamination may result from insufficiently heat-treated meat or eggs. Also, vegetables and fruits that have been grown in soil fertilized by human feces, hosed with contaminated surface water or contaminated by flies. Molds that can cause liver, kidney, or other chronic diseases after consumption have been identified as hazardous by 233 respondents. The same number also marked the products after expiry date. More and more consumers become aware of the fact that consuming food after its expiration is not worth the risk. However, it is worth mentioning that, according to **Retail magazine (2016)**, almost 40% of the European Union population does not know that eating expired food might be harmful to health and such food should be disposed of. Heavy metals were tagged by 230 respondents. It should be noted that some elements, otherwise considered to be toxic, are essential for human health, but only in small quantities. The problem arises when metals are bioaccumulated in the organism because they are difficult to metabolize. The fifth most relevant factor (224 respondents) was hygiene during the food processing.

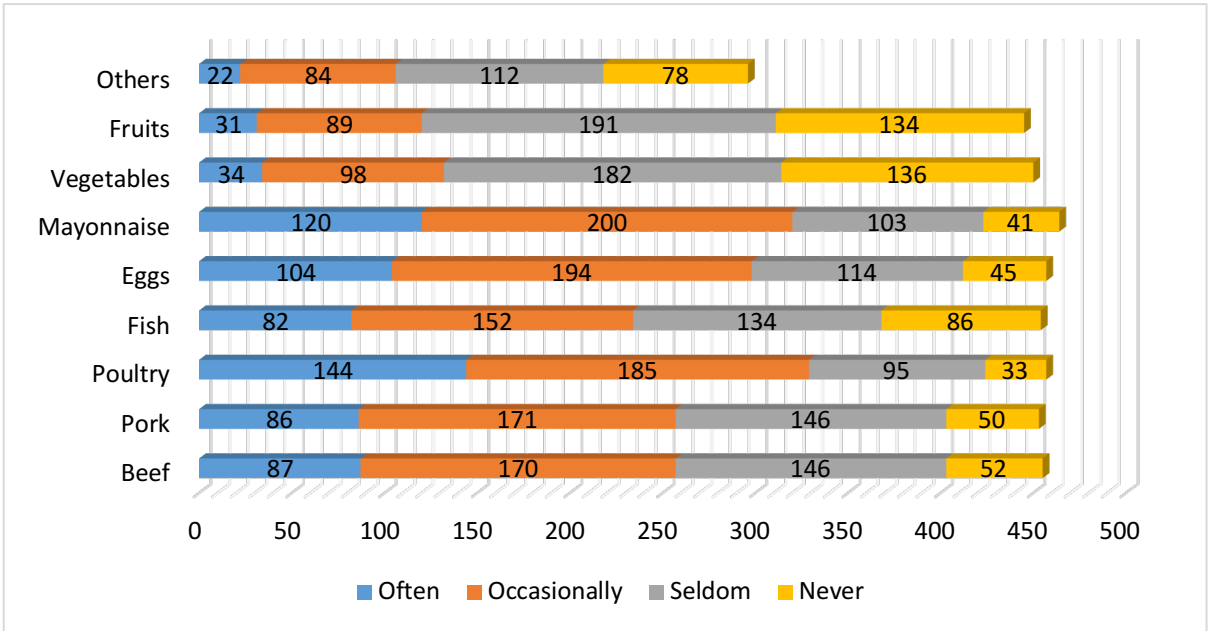

Figure 2 Frequency of being concerned about health harming effects of the individual types of food.

Always or almost always interested in origins of food was 30% of the survey sample **(Figure 4)**. Mostly reading information about country of production or distribution on packaging is 56.4% of the respondents. By contrast, only 13.5% of respondents had no interest in checking where the food products come from. They were mostly people with lower education, in bad financial situation or people from villages and small towns where they have access to local resources. Similar results have also been shown by a MasterCard study, according to which three of four Slovaks (77%) emphasize the origin of food **(SITA, 2016)**.

The answers for this question were tested by the Chi-Square test of Independence to verify whether there is a difference between expressed concern about the food origins based on the education. For this statistical test, following hypotheses were formulated:

H_0: No dependence existed between the educational attainment and degree of concern expressed about the origins of the food.
H_1: Dependence existed between the educational attainment and degree of concern expressed about the origins of the food.

$$p = 0{,}005 < \alpha = 0.05$$

According to the p-value which was less than the alpha value, alternative hypothesis H1 was accepted. So, with 5% level of significance, there is dependence between educational attainment and the fact that the person is concerned about the origin of the food. This result has confirmed the hypothesis number 2.

In their evaluation of the 5 most important factors affecting consumer decision making **(Figure 5)** when purchasing food, more than 80% of respondents considered the price to be the most influential. Similarly, **Púchlo (2011)** wrote that the Slovak consumer is really price-sensitive and still holding true is that the price plays a major role in buying decision-making. The second most influential factor (78.6%) became the flavor and aroma of the product. Customers mostly buy foods that they already know well and do not risk unnecessarily that the product would not satisfy them. The scent spreading in the store often leads to impulsive purchases, especially in case of fresh pastries or fruit and vegetables. Also, **Berčík (2017)** claims that fragrance as a means of influencing the purchase of a product or service has a long history. The composition and nutritional value of the product (74.8%) is influencing mainly the 21 – 30 years old respondents who care about healthy lifestyle and recommended daily dietary allowances. Top 5 most influential factors included also the manufacturer's brand (which is the image carrier, the guarantee of quality and health safety) and the aesthetic appearance of the product (especially in case of unpackaged foods). On the other hand, factors such as the absence of genetically modified substances, the graphic design of the packaging or the recyclability of the packaging, influenced the decision-making process the least.

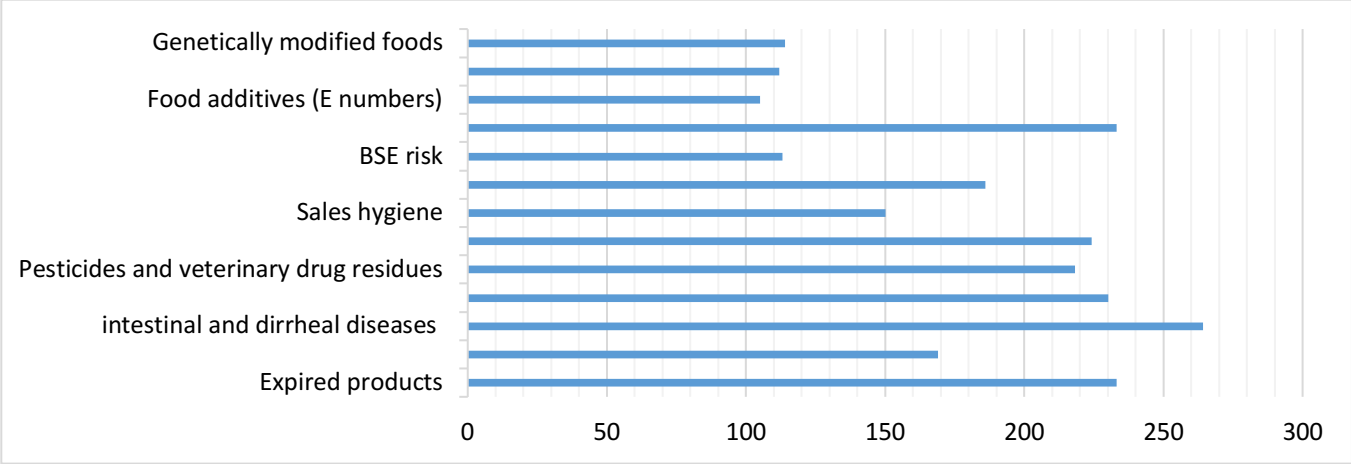

Figure 3 Factors which are representing an actual risk to the health of the respondent.

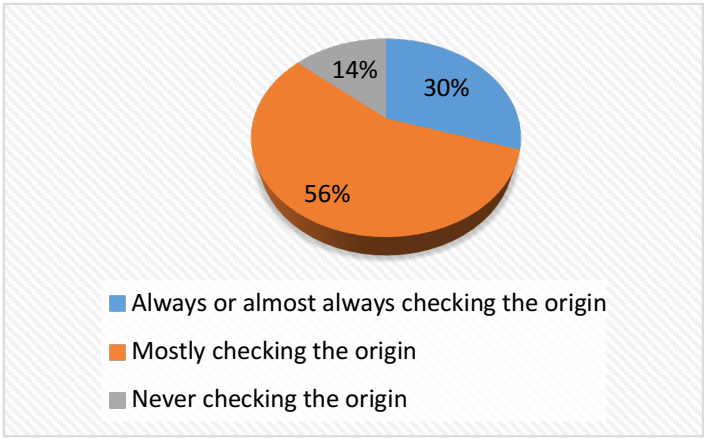

Figure 4 Frequency of checking the information about the country of origin of the food.

Fifth question of the survey asked respondents to estimate the amount of money spent monthly by their household on food in different types of stores. When estimating the amount spent in discount stores, the largest number of respondents (92) said they did not visit such stores. This can be explained by the fact that many consumers think that these stores offer lower quality food from foreign import. The hypermarkets and supermarkets received the largest variety of purchase value. Three price tiers (30 – 100 €, 101 – 200 € and over 201 €) were selected roughly by 100 respondents. In small self-service shops, consumers usually only spent up to 30 euros, as larger weekly purchases are made in bigger stores, which usually offer significant discounts on selected food items.

Next question was devoted to the matter of eating at the workplace **(Figure 6)**. Regularly eating at the workplace is 43% of respondents and 24.1% does so rarely. These respondents prefer that they do not have to cook at home and can also save money as employers often provide their employees with cheaper meals. Less than a quarter of the respondents is against the eating at the workplace. Reasons to decide not to eat at the workplace could be: improper seasoning, small portions, special dietary requirements, or insufficient hygiene when preparing or serving meals.

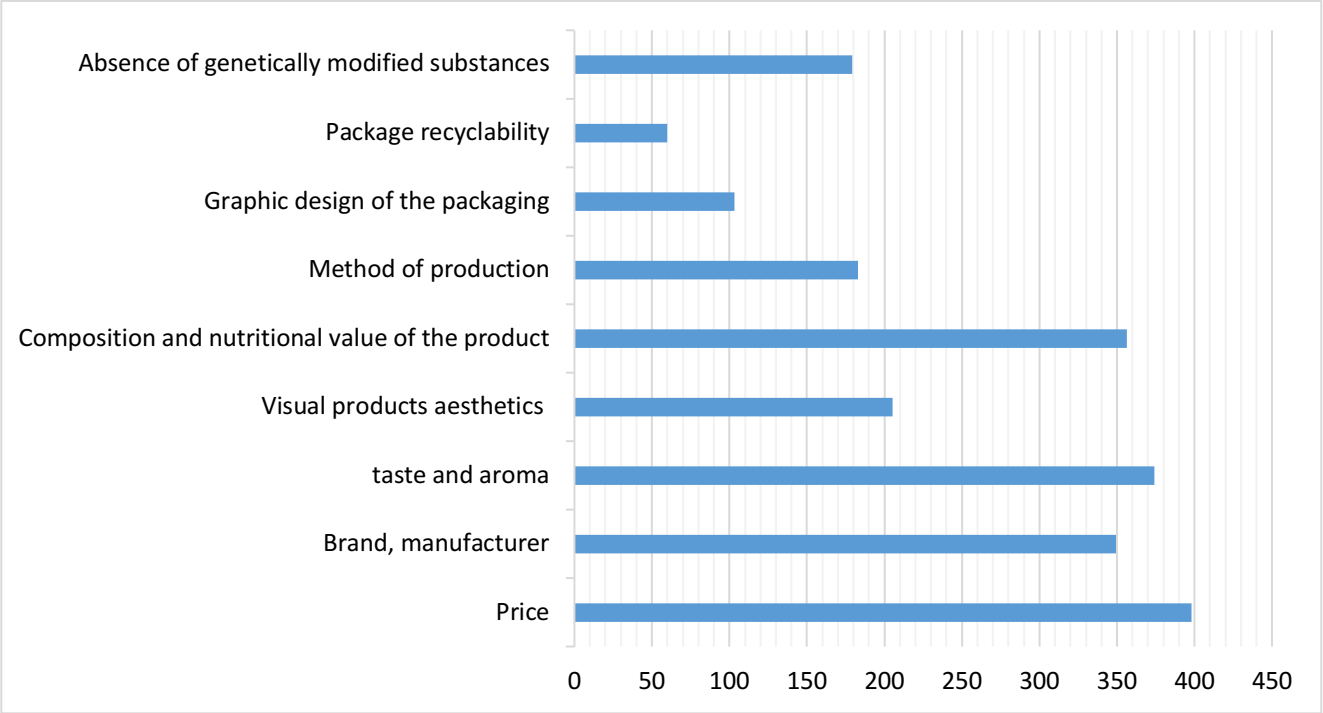

Figure 5 The most important factors influencing consumer purchase decisions when buying food.
Source: own research

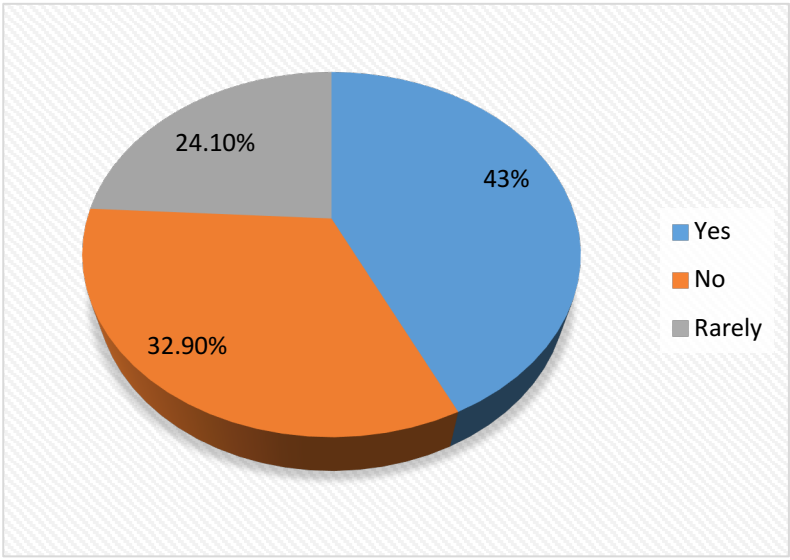

Figure 6 Frequency of eating at the workplace.

The Chi-Square test of Independence was used to discover whether there is a dependence between gender of the respondent and eating at the workplace. Hypotheses were formulated as follows:

H_0: No dependence existed between the gender and eating at the workplace.
H_1: Dependence existed between the gender and eating at the workplace.

$$p = 0{,}320 > \alpha = 0.05$$

P-value had a higher value than alpha, a null hypothesis was accepted. With 95% probability, there is no dependence between the gender of respondent and whether they are eating at the workplace. Our third hyphotesis was confirmed.

The majority of survey sample (60.5%) was trusting the restaurant facilities only with few reservations **(Figure 7)**. Trusting with no reservations was 19.3% and not trusting at all was 20.2%. The frequent inspections and strict hygiene conditions that must be met by food service providers are not sufficient enough for the consumer to guarantee health safety at food serving facilities. In addition, **Park´s (2014)** research found that 44% of foodborne illness outbreaks were tied to restaurants, compared to 24 % that occurred at home.

Most respondents (41.4%) eat only 1-2 times a month in restaurant facilities. The low frequency could be caused by the results of the previous question, which has shown that consumers do not have confidence in restaurants. Another 16.7% did not eat in gastronomical facilities at all. This alternative was selected mostly by retirees with a limited budget, for whom it is considered a luxury they cannot afford. Categories 1-2 times a week, 3-5 times a month and more than 5 times a month were selected by roughly 15% of respondents. Restaurants are especially preferred by the younger generation, as they do not have time to cook at home because of their fast lifestyle, therefore they prefer it as more convenient alternative.

In connection to this question, Chi-Square test of Independence was used to test the below-mentioned pair of statistical hypotheses.

H_0: No dependence existed between the residence of respondent and the frequency of visiting the restaurant facilities.
H_1: Dependence existed between the residence of respondent and the frequency of visiting the restaurant facilities.

$$p = 0{,}016 > \alpha = 0.05$$

After comparing the p-value and the alpha value, we found out that there was a statistically significant dependence between the respondent's residence and the frequency of visiting the restaurant facilities. The hypothesis that residents living in cities visit restaurant facilities more often because a majority of restaurant facilities is situated in cities was confirmed.

Respondents also evaluated fast food facilities **(Figure 8)**. The results were similar to those of restaurant facilities. Full confidence in fast food from street food stalls had only 10.7% and trusted with reservations 53%. Not trusting at all was 36.3% of the survey group. The paradox is that, according to **SITA (2008)**, even 10 years ago, when the street food stalls were not so numerous, half of Slovaks visited these facilities regularly.

In case they were pre-prepared frozen meals **(Figure 9)**, up to 43.5% did not trust them. With full confidence was only 7.3% and almost half of the respondents was trusting them with reservations. Considering that the overwhelming majority was not trusting frozen foods, buying such meals was only less preferable alternative for the consumer. On the contrary, respondents from research done by **Barjaktarović-Labović et al. (2018)** favored frozen foods, but only 15.6% of the study participants knew that freezing the food did not eliminate the potential hazard due to various microorganisms.

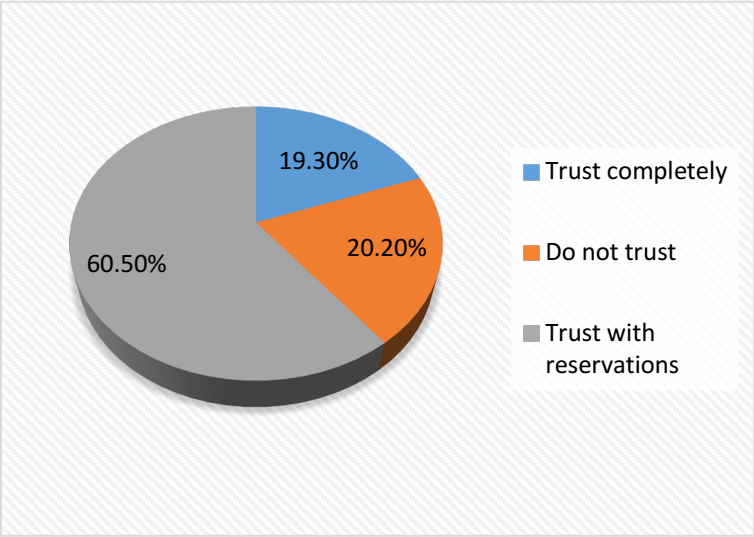

Figure 7 Confidence in satisfactory hygiene in restaurant facilities.
Source: own research

The penultimate question was focused on the form of punishment that should be given to a guilty party that threatens people's health with contaminated food. A radical solution in the form of a ban on activity was preferred by 61.9% and suspension of activity selected 19.5% of respondents. These respondents are aware that endangering the health of consumer is a serious offense, and such solution would prevent the subject from harming the health of another consumer in the future again. Another 25.6% of the respondents opted for a fine. The lowest percentage of respondents (11.6%) preferred dismissal from employment as solution. Often, however, a person who prepares food, which has caused health problems to the customer is not at fault. The failure can occur before the preparation of the food itself, so it is always necessary to identify the specific cause, time and place of contamination.

The last question in the questionnaire provided the answer as to whether it is necessary, in the opinion of the respondent, to enact the mandatory professional competence requirements for people working with food. Nearly 90% answered this question positively. It would ensure that, before a person is allowed to work with food, he / she will be adequately trained and familiar with the general professional requirements on qualification necessary to work with food, alimentary infections and food poisoning, sanitation and disinfection in the food industry and special hygienic requirements. Other 10.6% did not think professional competency is important and thought that anyone can work with food with no regard to the training. According to research by **Kendall et al. (2001)**, approximately 72% of managers would be more likely to hire workers trained in food safety, and, of those, 50% would be willing to pay a higher wage to those trained.

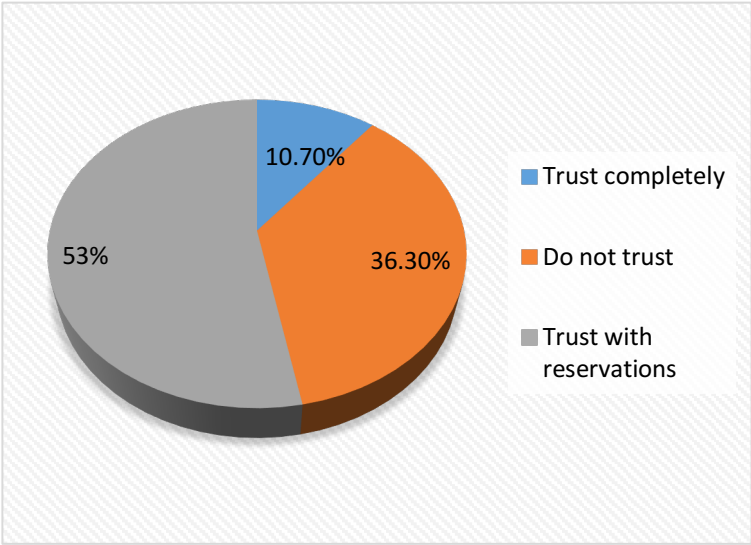

Figure 8 Confidence in health safety of foods sold in street food stalls.
Source: own research

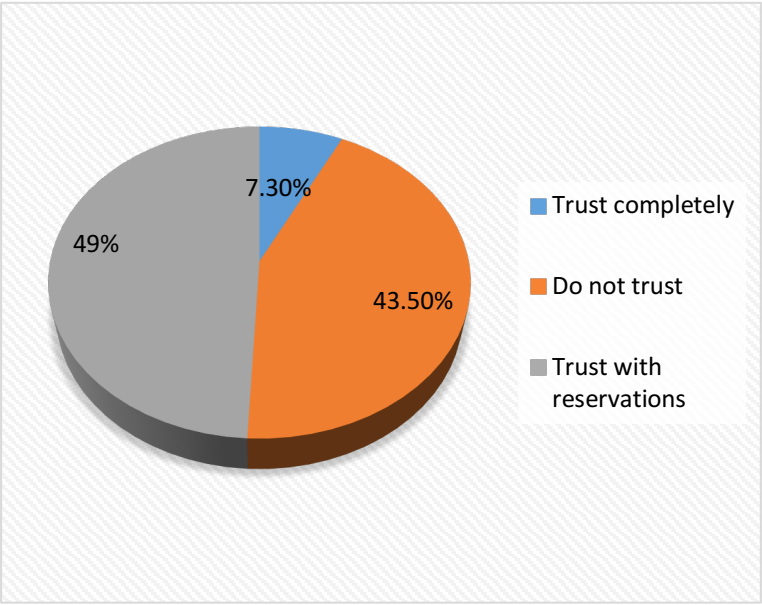

Figure 9 Confidence in health safety of pre-prepared foods sold in the frozen condition.

CONCLUSION

Based on the results of this survey, it can be concluded that the health safety of food sold on the Slovak market is insufficient from the viewpoint of consumers, as up to 8.2% of respondents are regularly concerned when buying food and 51.9% have occasional concerns. The greatest perceived risks are intestinal and diarrheal diseases, expired products, mold, heavy metals and food processing hygiene. The poultry meat, eggs and mayonnaise were evaluated as the most hazardous foods because they are subject to a rapid deterioration, especially when the appropriate thermal conditions are not met.

Majority of respondents was trusting the hygienic level of restaurant facilities, fast food and pre-prepared meals sold in frozen condition but with reservations.

An appropriate measure according to 89.4% would be enacting the mandatory professional competence requirements for food industry workers in order to avoid unnecessary failures in food processing. However, in an event that the health of the consumer would be harmed, respondents reckoned that it would be appropriate for guilty to be punished by a ban on activity (61.9%) or by financial penalties (25.6%).

Due to consumer dissatisfaction and frequent food scandals, the state authorities should tighten the legislation on food safety and increase the number of inspections in restaurant and fast food facilities.

REFERENCES

Australian Institute of Food Safety. 2016. 10 High Risk Foods More Likely to Cause Food Poisoning. Available at: https://www.foodsafety.com.au/resources/articles/10-high-risk-foods-more-likely-to-cause-food-poisoning

Barjaktarović-Labović, S. Mugoša, B., Andrejević, V., Banjari, I., Jovićević, L., Djurović, D., Martinović, A., Radojlović, J. 2018. Food hygiene awareness and practices before and after intervention in food services in Montenegro. *Food Control*, vol. 85, p. 466-471. https://doi.org/10.1016/j.foodcont.2017.10.032

Berčík, J. 2017. Is shop aromatization and scent branding a guarantee of success? (Je aromatizácia predajní a tvorba čuchovej značky zárukou úspechu?). Available at: http://www.tovarapredaj.sk/2017/12/13/jakub-bercik-je-aromatizacia-predajni-a-tvorba-cuchovej-znacky-zarukou-uspechu/

Bírošová, Z., Kačenová, D. 2010. Food safety in the Slovak Republic and cooperation with the European Food Safety Authority (Bezpečnosť potravín v Slovenskej republike a spolupráca s európskym úradom pre bezpečnosť potravín). *Food Safety and Control (Bezpečnosť a kontrola potravín)*. Nitra, Slovakia : Slovenská poľnohospodárska univerzita. 342 p. ISBN 978-80-552-0350-8 (In Slovak)

Bock, A. K., Bontoux, L. 2017. Food safety and nutrition – how to prepare for a challenging future? New approaches for using scenarios for policy-making. *European Journal of Futures Research*, vol. 5, no. 1. https://doi.org/10.1007/s40309-017-0119-3

Dou, L., Yanagishima, K., Li, X., Li, P., Nakagawa, M. 2015. Food safety regulation and ints implication on Chinese vegetable export. *Food policy Australasian marketing journal*, vol. 57, p. 128-134.

European Commission. 2014. Food safety (Bezpečnosť potravín). Brusel, Belgium : European Food Safety Authority. 16 p. ISBN 978-92-79-42455-7 (In Slovak)

Ergönül, B. 2013. Consumer awareness and perception to food safety: A consumer analysis. *Food Control*, vol. 32, no. 2, p. 461-471. https://doi.org/10.1016/j.foodcont.2013.01.018

Fares, M., Rouviere, E. 2010. The implementation mechanisms of voluntary food safety system. *Food Policy*, vol. 35, no. 5, p. 412-418. https://doi.org/10.1016/j.foodpol.2010.05.008

Golian, J., Tremlová, B., Pažout, V., Sokol, J. 2006. Food safety in the context of European research and education (Bezpečnosť potravín v kontexte európskeho výskumu a vzdelávania). *Food Safety and Control (Bezpečnosť a kontrola potravín)*. Nitra : Slovenská poľnohospodárska univerzita. 229 p. ISBN 80-8069-681-0 (In Slovak)

Holienčinová, M. 2013. Consumer behavior in the market of alcoholic beverages (Spotrebiteľské správanie na trhu alkoholických nápojov). Available at: http://mts-conference.euke.sk/zborniky/Konferencny_zbornik_MTS_2013.pdf

Kubicová, Ľ., Kádeková, Z. 2011. Comparison of the income development and the food demand elasticities of the private households in Slovakia. *Agricultural economics*, vol. 57, no. 8, p. 404-411.

Kubicová, Ľ., Kádeková, Z. 2012. Revenue impact on the demand of Slovak households for meat and meat products. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 50, no. 2, p. 503-510. https://doi.org/10.11118/actaun201260020503

Kecskes-Nagy, E., Korzenszky, P., Sembery, P. 2016. The role of color sorting machine in reducing food safety risks. *Potravinarstvo*, vol. 10, no. 1, p. 354-358. https://doi.org/10.5219/511

Kendall, P., Smith, K., Thilmany, D., Hine, S., Melcher, L., Paul, L. 2001. Value of and satisfaction with food safety training in the intermountain west. *Journal of foodservice*, vol. 13, no. 1, p. 1-15. https://doi.org/10.1111/j.1745-4506.2001.tb00026.x

Kerekréty, J. 2000. HACCP – theory and practice (HACCP – teória a prax). Bratislava, Slovakia : Potravinokonzult. 216 p. ISBN 80-968348-1-9 (In Slovak)

Liu, P., Lee, Y. M. 2018. An investigation of consumers' perception of food safety in the restaurants. *International Journal of Hospitality Management*, vol. 73, p. 29-35. https://doi.org/10.1016/j.ijhm.2018.01.018

Loc, V. T. T. 2006. Seafood supply chain quality management. Groningen, The Netherlands : University of Groningen. 248 p. ISBN 90-367-2670-0

Mensah, L. D., Julien, D. 2011. Implementation of food safety management system in the UK. *Food Control*, vol. 22, no. 8, p. 1216-1225. https://doi.org/10.1016/j.foodcont.2011.01.021

Ministry of agriculture of the Czech Republic. 2018. Food safety (Bezpečnost potravin). Available at: http://eagri.cz/public/web/mze/potraviny/ bezpecnost-potravin/?pos=50

Park, A. 2014. Which Will Make You Sicker: Four Star V. Fast Food. Available at: http://time.com/75810/ which-will-make-you-sicker-four-star-v-fast-food/

Púchlo. J. 2011. The content of shelves from the perspective of producers (Obsah regálov z pohľadu producentov). Available at: http://www.budis.sk/sk/uploads/pr/pr_clanok_instore.pdf

Retail magazin. 2016. Food quality does not change due to the expiration date (Uplynutím dátumu trvanlivosti sa kvalita potravín nemení). Available at: http://www.retailmagazin.sk/produkt/potravinarsky-

sortiment/1423-uplynutim-datumu-trvanlivosti-sa-kvalita-potravin-nemeni

Rouvière, E., Caswell, J. A. 2012. From punishment to prevention: A French case study of the introduction of co-regulation in enforcing food safety. *Food Safety*, vol. 37, no. 3, p. 246-254.

SITA. 2008. Almost a third of Slovaks eat irregularly (Takmer tretina Slovákov sa stravuje nepravidelne). Available at: https://zdravie.pravda.sk/zdrava-vyziva/clanok/13460-takmer-tretina-slovakov-sa-stravuje-nepravidelne/

SITA. 2016. 77% of Slovaks monitor the origin of food (Pôvod potravín sleduje 77 % Slovákov). Available at: https://spravy.pravda.sk/ekonomika/clanok/390471-povod-potravin-sleduje-77-slovakov/

Skořepa, L. 2004. Significant factors of consumer behavior on the regional food market (Významné faktory spotřebitelského chovaní na regionálním trhu potravín). *Auspicia Scientific Magazine (Auspicia vědecký časopis)*. České Budějovice, Czech Republic : VŠERS. 23-26 p.

Supeková, S. 2008. Food quality and "SK brand quality" from the consumer's point of view (Kvalita potravín a "značka kvality SK" z pohľadu spotrebiteľa). *Trends in food industry* (*Trendy v potravinárstve)*. Available at: http://www.vup.sk/index.php?mainID=3&navID=20

Šinková, T. 2007. Relationship between the Food Law of the European Union and the Codex Alimentarius (Vzťah medzi potravinárskou legislatívou Európskej únie a Codexu Alimentarius). *Trends in food industry* (*Trendy v potravinárstve)*. Available at: http://www.vup.sk/index.php?mainID=3&navID=20

Trienekens, J., Zuurbier, P. 2007. Quality and safety standards in the food industry, developments and challenges. *International Journal of Production Economics*, vol. 113, no. 1, p. 107-122. https://doi.org/10.1016/j.ijpe.2007.02.050

Trusková, I. 2008. Food safety and public health (Bezpečnosť potravín a verejné zdravie). Available at: http://www.forexsk.sk/domain/forex/files/clanky/bezpecnost-potravin-a-verejne-zdravie.pdf

U.S. Department of Health and Human Services. 2017. Egg Safety: What You Need to Know. Available at: https://www.fda.gov/Food/ResourcesForYou/Consumers/ucm077342.htm

Úradníčková, J., Marenčáková, J., Jurgová, O. 2007. Food and nutrition (Potraviny a výživa). Prešov, Slovakia : Polygraf print. 192 p. ISBN 978-80-10-01657-0 (In Slovak)

Verbeke, W., Frewer, L.J., Scholderer, J., De Brabander, H. 2007. Why consumers behave as they do with respect to food safety and risk information. *Analytica chimica acta*, vol. 586, no. 1-2, p. 2-7. https://doi.org/10.1016/j.aca.2006.07.065

Wallace, C., Williams, T. 2001. Pre-requisites: A help or a hindrance to HACCP? Food Control. *Food Control*, vol. 12, no. 4, p. 235-240. https://doi.org/10.1016/S0956-7135(00)00042-6

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ANTIOXIDANT ACTIVITY OF TOKAJ ESSENCE

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ABSTRACT

The aim of the presented experiment was to measure the content of total polyphenols and antioxidant potential of Tokaj essence from the years 1999, 2006, 2007, 2009, 2013, 2015. Tokaj essence is produced by fermentation of concentrated must from botrytised grape berries. The highest content of polyphenols was determined in the essence of 1999 (275.8 ±18.17 mg.L^{-1}) and the lowest in the sample of 2015 (118.8 ±12.28 mg.L^{-1}). Antioxidant activity was determined by two methods DPPH and PRAC (permanganate reducing antioxidant capacity). Antioxidant DPPH method showed that the essence of year 1999 had significantly the highest activity (63.4 ±0.81 µmol.L^{-1} Trolox) while essence of year 2015 (47.5 ±1.58 µmol.L^{-1} Trolox) featured the lowest activity. The results of method PRAC, which determine the total reducing capacity of the essence, do not correlate with the results of DPPH and total phenolic content, because of higher content of saccharides, which gave false positive test results. The method PRAC is not suitable for measuring antioxidant activity of tokaj esence. The results showed that aging of wine increased the content of polyphenols and also its antioxidant activity. High content of polyphenols and the great antioxidant activity of Tokaj wine receive their beneficial effect to human health.

Keywords: antioxidant activity; botrytised berry; essence; total polyphenols

INTRODUCTION

Grape berry are generaly used for production of wine and juice **(Jang et al., 1996; Špakovská et al., 2012)**. Only in the Tokaj region the unique Tokaj essence are made from berries. Vine growing and wine production in Tokaj region are defined by the Act on Viticulture and Winemaking 349/2015 Statue, in § 30. Vineyard hunts are situated within the cadastral areas of the following villages: Černochov, Veľká Bara, Malá Tŕňa, Veľká Tŕňa, Čerhov, Slovenské Nové Mesto, Viničky. The act allows growing of traditional varieties such as Furmint, Lipovina and Muškát žltý which ripen late, and turn into botrytised berries **(Pospišílová, 1981; Kakaš, 2005; Eftimová, 2008; Žadanský, 2009; Furdíková, & Malík 2009; Farkaš, 1998)**. Botrytised berries are only formed in dry, warm and long autumns when the berries have 19 – 20 °NM of sugar content and are infected with Botrytis cinerea (PERS.et Fries) so called Noble Rot.

Botrytis cinerea (PERS.et Fries.), perfect state Botryottinia fuckeliana (De Bary Whetzel.), is a saprophyte fungus covering infected berries with a grey coating of conidiophores **(Eftimová, 2008)**. Fungus mycelium disrupts the skin of berries and consumes acids, which leads to fructose content increase and glucose content decrease. Hypertonic conditions for fungus development are being created, the fungus does not produce conidia, water evaporates and the berries shrunken and get dry **(Kakaš, 2005; Eftimová, 2008; Furdíková & Malík, 2009)**. The fungus, due to its oxidative enzyme, decomposes flavours such as linalool, geraniol and nerol into less volatile β-pinen, α-terpineol, furan oxides. The metabolism of fungus degrades carbohydrates, acids and crude, produces surplus of glycerol, gluconic acid and sotolon, which are typical of infected berries must. Glycerol increases naturally sugar free extract and sotolon gives sweet honey and nuts like aroma to botrytis wines **(Furdíková & Malík, 2009)**.

Botrytised berries are used to make essence (nectar) and Tokaj wine selections 7,3,4,5. They are hand-picked from bunches of grapes, put into a tub with perforated base out of which Tokaj essence leaks, then. One puttonyos of botrytised berries (25 kg) provides as much as 1 to 1.5 litres of essence containing 25 to 60% of sugar. The essence ferments slowly and ripens for at least three years, out of these two in a wooden barrel **(Kováč et al., 2005)**. Tokaj essence is used to enhance Tokaj wines and selection wines **(Farkaš, 1998)**. Wine has beneficial effect of human health because of antioxidant activity **(Špakovská et al., 2012)**.

There are a lot of publications on antioxidant activity of spontaneous and variety Tokaj wines, which provide e. g. cardioprotective, antiaging, cancer chemoprotective activity. **(Staško et al., 2006; Pour Nikfardjam et al., 2003; 2006 a, b; Harmatha, 2009; Fikselová et al., 2010; Balová and Eftimová, 2015; Balová et al., 2016)**. The aim of our research was to measure the total content of

polyphenols and antioxidant potential of Tokaj essences which have not been analysed yet.

Scientific hypothesis

According to beneficial effect of tokaj essence on human health it is predicted that tokaj essence obtains high content of polyphenols with great antioxidant activity.

MATERIAL AND METHODOLOGY

Samples and chemicals

Our research included the samples of Tokaj essences of 1999, 2006, 2007, 2009, 2013, 2015 by TOKAJ & CO, Malá Tŕňa. Chemicals and standards were procured from Sigma-Aldrich (USA), the solvents were procured from Fisher (SR). The solutions were always freshly prepared and the samples of essences, until being used, were stored in dark glass containers at the temperature of 4 °C.

Figure 1 Creation of botrytised berries on Lipovina. Author: Eftimová, J.

Spectrophotometric determination of total polyphenols content

The content was determined by a standard spectrophotometric method according to **(Singleton et al., 1999)**, with the use of slightly modified Folin-Ciocalteu reagent (FC). 10 µL of sample was mixed with 200 µL of sodium carbonate (2%), 70 µL of distilled water and 20 µL of FC. The absorbance was measured after five minutes at the wave length of 750 nm with the use of spectrophotometric reader of microtiter plates Synergy 4 (BioTek, USA) and software Gen5TM (Reader Control and Data Analysis Software).

Determination of antioxidant activity

Antioxidant activity was determined with the use of radical DPPH **(Brand-Wiliams et al., 1995)** Reaction mixture of 300 µL DPPH (1 mg DPPH / 50 mL methanol) and 10 µL of Tokaj essence was measured after five minutes of incubation with the use of spectrophotometry comparison to methanol, at the wave length of 517 nm. TEAC method (Trolox equivalent antioxidant capacity) expressed the amount of known antioxidant Trolox, which is needed to reach reference sample like activity.

Determination of permanganate reducing antioxidant capacity

The method of determining total reduction ability PRAC (permanganate reducing antioxidant capacity) was employed. 18 Reaction mixture consisting of 128 µL $KMnO_4$ solution (0.006 $mol.L^{-1}$) and 60 µL H_2SO_4 (1.2 $mol.L^{-1}$) was mixed with 10 µL of our sample. The absorbance of sample was measured with the use of spectrophotometry, at the wave length of 535 nm, after 5 minutes incubation. The results are expressed as the percentage potassium permanganate reduction.

$$PRAC\ [\%] = 100 - (\frac{sample\ absorbance}{KMnO4\ absorbance} * 100)$$

Statisic analysis

GraphPad Prisma 5 (GraphPad Software, Inc., USA) was used for statistical analyses. One-way ANOVA with Dunnett's post hoc test was used to evaluate the data. All samples was measured at least 5 times in all experiments. The results are expressed as the mean ±S.E.M. Values of p <0.05 (*), p <0.01 (**), and p <0.001 (***) were considered significant.

RESULTS AND DISCUSSION

Table 1 Sugar and alcohol content in Tokaj essence.

	Sugar (g.L^{-1})	Alcohol (%)
1999	629.8	3.49
2006	615.4	4.18
2007	601.6	3.12
2009	413.48	1.5
2013	621.15	3.40
2015	562.13	0.0

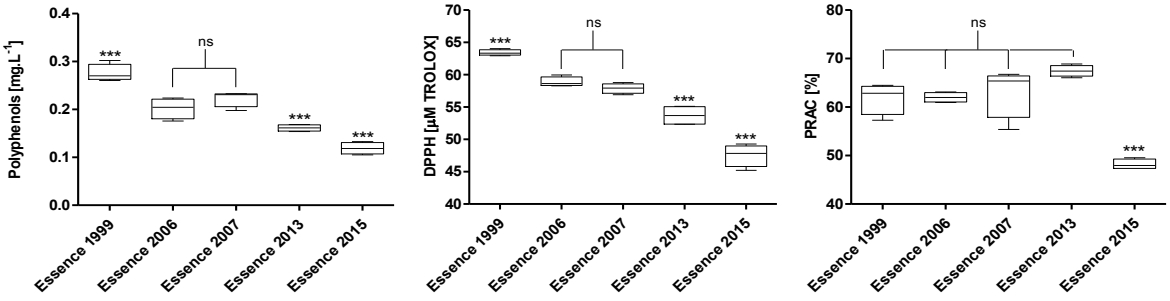

Figure 1 Concentration of total polyphenols in Tokaj essence samples expressed in mg.L^{-1}. Note: ***statistically significant, p <0.001, "ns" refers to statistically not significant values. Samples of Essence 1999 and Essence 2007 show statistical significance p < 0.01. Samples of Essence 2006 and Essence 2013 along with Essence 2013 and Essence 2015 show statistical significance p < 0.05. Antioxidant activity measured by DPPH of Tokaj essence samples converted to µM Trolox. Total reduction ability (PRAC) of Tokaj essences samples expressed as percentage of potassium permanganate reduction. ***statistically significant p <0.001, "ns" statistically not significant values.

The basis for Tokaj essence production is botrytised berries. The conditions for botrytised berries formation were only met in: 1999, 2006, 2007, 2013, and 2015. The essences of 1999, 2006, 2007, 2013, and 2015 featured the average of 562.13 to 629.8 g.L^{-1} of sugar. The essence of 2009 only had 413.48 g.L^{-1} of sugar, which is under the required limit of 450 g.L^{-1}. That is why, the company uses it to produce Tokaj essence selection wine. Significant changes in sugar formation in the berries happen in the period of transformation berries to botrytised berries **(Kakaš, 2005)**. The skin of infected berries splits, evaporation of water increases and carbohydrates get concentrated **(Kakaš, 2005; Eftimová, 2008; Furdíková & Malík, 2009)**. Alcohol content in the essences was from 0 to 4.18 volume % (Table 1). No alcohol is presented in fresh Tokaj essence, as it was determined in essence of 2015. However during maturation the content of alcohol increases. The concentration of alcohol depends on concentration of sugar, in case of essence of 2009 were detected both – low sugar content 413.48 g.L^{-1} and low alcohol content 1.5%.

Total polyphenols

Significantly highest concentration of total polyphenols (275.8 ±18.17 mg.L^{-1}, p <0.001) was detected and measured in the oldest essence sample of 1999. The essences of 2007 (223.1 ±16.84 mg.L^{-1}) and 2006 (202.2 ±21.55 mg.L^{-1}) also featured a high concentration of total polyphenols. The essences of 2006 and 2007 did not show significant mutual differences, but significantly differed from other essences. The results may be contributed to the age of essences, which is about the same in case of years 2006 and 2007. The lowest content was detected in the youngest essence sample of 2015 (118.8 ±12.28 mg.L^{-1}, p <0.001) (Figure 1).

Polyphenolic substances (anthocyanins, flavonols, flavanols, tannins, sinapinic and hydroxybenzoate acids and their derivatives) of grapes, wines and also of Tokaj essence feature antioxidant activity and many health benefits **(Caciget al., 2006)**. According to **Lianga et al. (2012)** grape contains at least 4% of polyphenols, including flavonoids up to 3.5% (quercetin-3-glucuronid, isoquercetin, hyperosid, kaempferol-3-glucoside), stilbens (resveratrol, 3.4´,5-trihydroxy-trans-stilben), condensed tannins (catechins) and phenolic acids **(Košťalová et al., 2012; Špakovská et al., 2012; Jakubcová et al., 2015)** consider grapes to be the source of mainly catechin, epicatechin and gallic acid. Resveratrol is mainly found in the berries skin. Its content depends on vine variety and the year of picking **(Pour Nikfardjam et al., 2003; 2006 b)**. Protective substances phytoalexins (resveratrol) are formed under the skin of *Botrytis cinerea* infected berry (PERS.et Fries.), however it is changed to dimers and higher oligomers **(Pour Nikfardjam et al., 2006a)**.

Antioxidant activity

DPPH assay

The highest antioxidant capacity determined by DPPH method was in the essence sample of 1999 (63.4 ±0.81 µmol.L^{-1}, p <0.001), and the lowest in the essence sample of 2015 (47.5 ±1.58 µmol.L^{-1}, p <0.001). The average antioxidant activity of Tokaj essence samples is 56.3 ±1.28 µmol.L^{-1}. The samples showed statistically significant differences p <0.001, except the essences of 2006 and 2007, due to their small age difference (Figure 1).

The measurements of Tokaj essences showed that the level of free radicals uptake determined with the use of DPPH method and polyphenols concentration correlate positively (R^2 = 0.9471). The highest level of polyphenols and antioxidant capacity were measured in the essence

sample of 1999. The lowest level of polyphenols as well as antioxidant capacity was measured in the essence sample of 2015. The results show that antioxidant activity can be attributed to polyphenols present in the essences (Figure 2). Pharmacological force of vine berries can be attributed to polyphenols, mainly flavonoids (quercetin, kaempferol, rutin) and stilbenoids **(Arnous and Mayer, 2008; Chéze et al., 2001)** which is in correlation with our observations. Author **Chlebo et al. (2011)** claims that resveratrol and its derivates are substances to be responsible for medicinal properties of Tokaj wines and essences.

Correlations between PRAC assay, DPPH assay and total content of polyphenols

Obtained results show that the level of total reduction ability does not correlate with antioxidant activity measured by DPPH method (R^2 = 0.3837), nor they correlate with concentration of total polyphenols (R^2 = 0.2696) (Figure 3).

Positive correlations were observed by **Keylor et al. (2015)** between total polyphenolics content and anthocyanins content in water extract of *Cornus mas* L., which indicates important contribution of polyphenols to result of PRAC assay. **Popović et al. (2012)** reported that

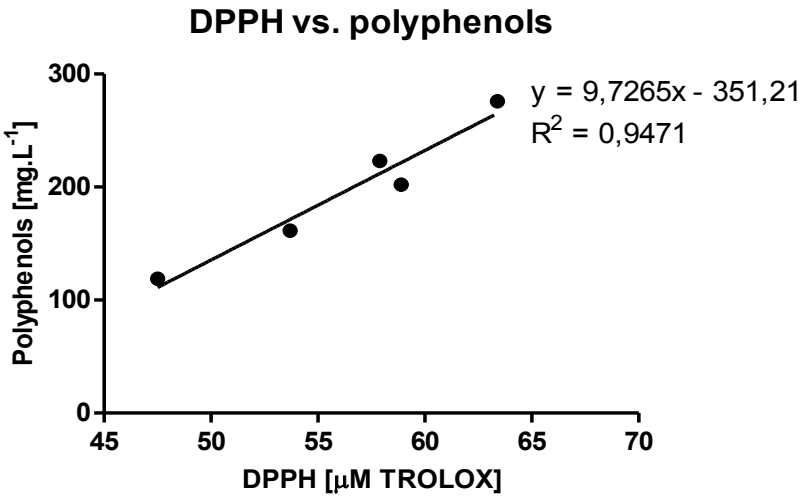

Table 2 Correlation of antioxidant activity determined with the use of DPPH method and concentration of total polyphenols.

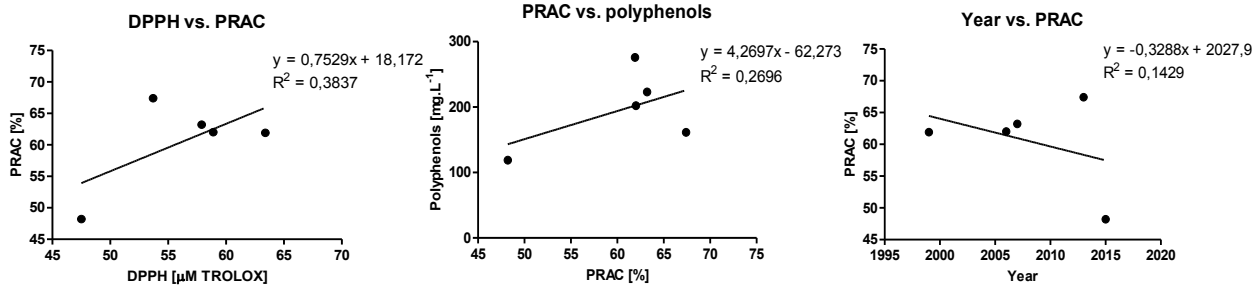

Figure 3 Correlation between antioxidant activities as determined with the use of DPPH and PRAC methods. Correlation of antioxidant activity as defined with the use of PRAC method and concentration of total polyphenols. Correlation of antioxidant activity (PRAC method) and essence age.

PRAC assay

Determined by PRAC method, essences samples of 1999, 2006 and 2007 were found to have approximately similar and statistically not significant rate of potassium permanganate reduction ability, the average being 60.6 ±3.83% (Figure 1). The highest reduction ability (67.4 ±2.58%) was measured in the essence sample of 2013, while the lowest one was measured in the essence sample of 2015, 48.2 ±1.05%, which was statistically significant in relation to other essences (p <0.001). The results, which are not in correlation with results of DPPH assay, will be discussed in part 3.3.

permanganate chemiluminescence detection is suitable to assess the antioxidant status of fruit juices, teas and other beverages. However no author tested this assay for Tokaj essence. Observed discrepancy in correlation with total polyphenols content and DPPH assay was caused by presence of sugar high levels and alcohol in the essences. The method is a disadvantageous one since besides antioxidant substances which react (polyphenols), there are also other substances (sugar) with a reduction potential present, which give false positive results. That is why the method is not suitable one to test the samples of Tokaj essences.

Relative values of antioxidant activity

Due to difficulty of absolute value comparison of Tokaj essences antioxidant activity and to make the data clearer, the obtained results were expressed as relative values. The sample with highest absolute absorbance was given the highest relative value of 1. Other values were then calculated with respect to a given sample. Relative values along with deviations are summarized in Table 2.

polyphenols was detected in the oldest sample of 1999 (275.8 ±18.17 mg.L^{-1}) (Figure 4).

Similar results were observed in case of result of DPPH assay. Figure 4 shows the correlation of essence age and antioxidant activity measured by DPPH method, reaching a high value of R^2 = 0.9181. Correlation results make it clear that the oldest essences will usually feature the highest antioxidant activity, the opposite being true for

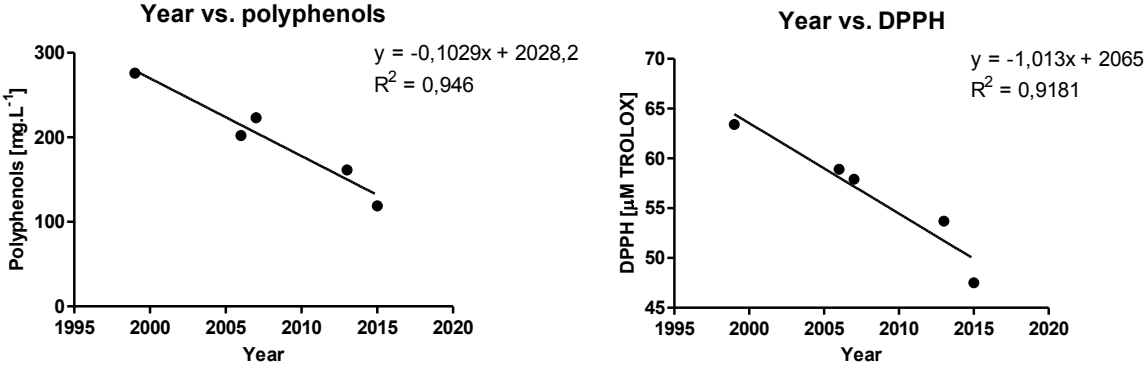

Figure 4 Correlation of sample age and concentration of polyphenols and antioxidant activity determined by DPPH assay.

Table 2 Relative values of Tokaj essences antioxidant activities determined by DPPH and PRAC methods.

Samples	DPPH	PRAC
Essence 1999	1 ±0.013	0.92 ±0.074
Essence 2006	0.92 ±0.017	0.92 ±0.023
Essence 2007	0.91 ±0.017	0.94 ±0.133
Essence 2013	0.84 ±0.028	1.00 ±0.038
Essence 2015	0.75 ±0.025	0.71 ±0.016

When determining the relative value of antioxidant activity by DPPH radical, the highest relative activity (1) was found in the essence sample of 1999, while its relative value determined by PRAC method was 0.92 ±0074. The highest relative value determined by PRAC method was found in the essence of 2013, however, its relative value determined by DPPH method was 0.84 ±0.028. The lowest relative activity was observed in the essence of 2015 (0.75 ±0.025 by DPPH method, 0.71 ±0.016 by PRAC method). Discrepancy in relative antioxidant values of both methods confirm our suggestion, that PRAC method is no suitable for detection of Tokaj essence and other samples with high content of sugar.

Aging of Tokaj essences

Riping of wine in the tuff cellars is a part of winemaking technological process (slow oxidation in wooden barrels), which significantly impacts character, taste and quality of wine **(Farkaš, 1998; Francis et al., 2010; Kakaš, 2005; Eftimová, 2008; Žadanský, 2009; Špakovská, 2012)**. Correlation dependence of the essence age on total polyphenols concentration shows their significant relationship (R2 = 0.946). The youngest essence of 2015 features the lowest polyphenols concentration (118.8 ±12.28 mg.L^{-1}), however, it grows with the essence ageing. That is why the highest concentration of young essences.

In contrast with total polyphenols concentration and antioxidant activity determined by DPPH method, there was no correlation observed (R^2 = 0.1429) between the essences age and total reduction ability (Figure 3). However, it is expectable, since PRAC assay gives false positive results.

During aging of wine, the content of reducing sugars and biogenic amines is changed and depend on the age of wine **(Vasko, 2001)**. Biogenic amines (such as histamine, tyramine and putrescine) can develop during wine ageing and also botrytization **(Souflerros et al., 2007)**. The aging of white and red wines are connected with polyphenols. Acetaldehyde, produced during oxidative aging, enhance the polymerization of anthocyanins (presented in red wines) and flavonoid tannins **(Hajos et al., 2000)**. Tartaric acid is oxidized to glyoxylic acid, which binds catechins and other phenolic compounds to long polymers **(Somers, 1986)**. Must from botrytised berries recovery decreases, more tartaric acid than malic acid is being degraded while sugar content and quality of wine increase **(Kakaš, 2005)**. Grapes infected by Botrytis cinerea produced phytoalexins resveratrol, which is polymerized by fungus to its derivates such as δ – viniferin (resveratrol trans-dehydrodimer) **(Fulcrand et al., 1997; Harmatha and Dinan, 2003)**. Botrytis cinerea forms enzymes stilbenoxidase and laccases, which play same role in phenolic metabolism

(Bavaresco et al., 2016). Enzymes laccases serve as a catalysers of oxide reduction conversion of phenol substances which contribute to golden and brown colour of Tokaj wines.

CONCLUSION

The results obtained from Tokaj essences analysis (1999, 2006, 2007, 2013, 2015) are original ones and confirm the fact that essences are a source of polyphenols and have antioxidant activity. They can be used in prophylaxis of diseases triggered by oxidation stress. Since Tokaj essences are used to make Tokaj spontaneous selection wines, they contribute to previously known theoretical knowledge of the antioxidant potential of Tokaj wines. In a correlation comparison of the two methods of antioxidant activity and total polyphenols determination, we found out that the level of total reduction ability does not correlate with antioxidant activity, nor it correlates with the total amount of polyphenols in the essences samples. The experiment has proven that PRAC method is not a suitable one to measure antioxidant activity of Tokaj essences with high content of sugars and low alcohol content.

REFERENCES

Adrian, M., Rajaei, H., Jeandet, P., Veneau, J., Bessis, R. 1998. Resveratrol oxidation in Botrytis cinerea Conidia. *Phytopathology*, vol. 88, no. 5, p. 472-476. https://doi.org/10.1094/PHYTO.1998.88.5.472 PMid:18944929

Arnous, A., Meyer, A. S. 2008. Comparison of methods for compositional characterization of grape (*Vitis vinifera* L.) and apple (*Malus domestica*) skins. *Food and bioproducts processin*, vol. 86, no. 2, p. 79-86.

Ballová, Ľ., Eftimová, J. 2015. Polyphenols and antioxidant activity of Tokaj wines made from botrytized grepes. In *Modern aspects of preserving human health*. Uzgorod : UNU, p. 210-215. ISBN 978-611-01-0716-7.

Ballová, Ľ., Eftimová, Z., Kurhajec, S., Eftimová, J. 2016. Tokaj wines as source of polyphenols with positive effects to health. In *Investigations of medicinal plants – theoretical and practical aspects*. Kharkiv : National University of Pharmacy, p. 4-5.

Bavaresco, L., Lucini, L., Busconi, M., Flamini, R., De Rosso, M. 2016. Wine resveratrol: From the ground up. *Nutrients*, vol. 8, no. 4, p. 222. https://doi.org/10.3390/nu8040222 PMid:27089363

Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft & Technologie*, vol. 28, no. 1, p. 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Cacig, S. I., Szabo-Raluca, M. I., Lupea Alfa, X. D. 2006. Spectrophotometric method for the study of the antioxidant activity applied on Ziziphus jujuba and Hydrangea paniculata aqueous extracts. *Matica Srpske Journal of Natural Sciences*, no. 111, p. 87-93.

Eftimová, J. 2008. *Integrated protection of grape vines (Vitis vinifera L.) (Integrovaná ochrana viniča hroznorodého (Vitis vinifera L.))*. Nitra, Slovakia : Agroinštitút. 94 p. ISBN 978-80-89088-66-9. (In Slovak)

Fakaš, J. 1998. *Everything about wine (Všetko o víne)*. Martin, Slovakia : Neografia a.s. 171 p. ISBN 80-88892-16-3.

Fikselová, M., Kačániová, M., Mellen, M. 2010. Antioxidant (antiradical) and antimicrobial (antifungal) efects of Slovak Tokaj wines. *Acta Alimentaria*, vol. 39, no. 3, p. 256-264. https://doi.org/10.1556/AAlim.39.2010.3.2

Francis P. S., Costin, J. W., Conlan, X. A., Bellomarino, S. A., Barnett, J. A., Barnett, N. W. 2010. A rapid antioxidant assay based on acidic potassium permanganate chemiluminescence. *Food Chemistry*, vol. 122, no. 3, p. 926-929. https://doi.org/10.1016/j.foodchem.2010.02.050

Fulcrand, H., Cheynier, V., Oszmianski, J., Moutounet, M. 1997. An oxidized tartaric acid residue as a new bridge potentially competing with acetaldehyde in flavan-3-ol condensation. *Phytochemistry*, vol. 46, no. 2, p. 223-227. https://doi.org/10.1016/S0031-9422(97)00276-8

Furdíková, K., Malík, F. 2009. *Botrytis cinerea in enology (Botrytis cinerea v enológii)*. *Vinařský obzor*, vol. 102, no. 9, p. 416.

Hajos, G., Sass-Kiss, A., Szerdahelyi, E., Bardocz, S. 2000. Changes in biogenic amine content of Tokaj grapes, wines, and aszu-wines. *Journal of Food Science*, vol. 65, no. 7, p. 1142-1144. https://doi.org/10.1111/j.1365-2621.2000.tb10254.x

Harmatha, J. 2009. Quality *of wine from the perspective of chemist and somelier (Kvalita vína z pohledu chemika a someliera)*. In *Víno jako multikultúrny fenomén*. Olomouc, Czech Republic : Univerzita Palackého v Olomouci, p. 69-75. ISBN 978-80-87273-01-2.

Harmatha, J., Dinan, L. 2003. Biological activities of lignans and stilbenoids associated with plant-insect chemical interactions. *Phytochemistry*, vol. 2, no. 3, p. 321-330. https://doi.org/10.1023/B:PHYT.0000045494.98645.a3

Chéze, C., Vercauteren, J., Verpoorte, R. 2001. *Polyfenols, Wine and Health*. Netherlands : Springer, 219 p. ISBN 978-90-481-5622-1.

Chlebo, P., Gažarová, M., Dianiška, J. 2011. *Alcohol and Nutrition (Alkohol a výživa)*. In Zdravie a výživa ľudí. Bratislava, Slovakia : NIKA, s.r.o. 1037 p. ISBN 978-80-88-969-57-0. (In Slovak)

Jakubcová, Z., Horky P., Dostálová, L. Sochor, J., Tomasková, L., Baron, M., Kalhotka, L. Zeman, L. 2015. Study of antioxidant and antimicrobila propertie of grapevine seeds, grape and rosehip pressing. *Potravinarstvo*, vol. 9, no. 1, p. 382-387. https://doi.org/10.5219/503

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W.; Moon, R. C. 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, vol. 275, no. 5297, p. 218-220. https://doi.org/10.1126/science.275.5297.218 PMid:8985016

Kakaš, M. 2005. *The influence of biochemical processes on the creation of Tokaj wine (Vplyv biochemických procesov na tvorbu tokajského charakteru vína)*. In *Tokajské vinohradníctvo a vinárstvo na Slovensku,* Nitra, Slovakia : SPU. 163 p. ISBN 80-8069-063-4.

Keylor, M. H., Matsuura, B. S., Stephenson, C. R. J. 2015. Chemistry and Biology of Resveratrol-Derived Natural Products. *Chemical Reviews*, vol. 115, no. 17, p. 8976-9027. https://doi.org/10.1021/cr500689b PMid:25835567

Košťálová, D., Fialová, S., Račková, L. 2012. *Phytotherapy in contemporary medicine (Fytoterapia v súčasnej medicíne)*. Martin, Slovakia : Osveta. 384 p. ISBN 978-80-8063-384-4.

Kováč, K., Pintér, E., Hrenyo, L., Eftimová, J., Stehlo, P., Macák, M. 2005. *Assessment of the sustainability of selected production and environmental parameters of grape vines cultivation in the Tokaj region (Hodnotenie udržateľnosti vybraných produkčných a environmentálnych parametrov pestovania viniča hroznorodého v tokajskej oblasti)*. In

Tokajské vinohradníctvo a vinárstvo na Slovensku. Nitra, Slovakia : SPU, p. 71-75. ISBN 80-8069-737-X.

Lianga, Z., Yang, Y., Chenga, L., Zhong, G. Y. 2012. Polyphenolic composition and content in the ripe berries of wild Vitis species. *Food Chemistry*, vol. 132, no. 2, p. 730-738. https://doi.org/10.1016/j.foodchem.2011.11.009

Popović, B. M., Štajner, D., Slavko, K., Sandra, B. 2012. Antioxidant capacity of cornelian cherry (*Cornus mas* L.) – Comparison between permanganate reducing antioxidant capacity and other antioxidant methods. *Food chemistry*, vol. 134, no. 2, p. 734-741. https://doi.org/10.1016/j.foodchem.2012.02.170 PMid:23107685

Pospišílová, D. 1981. *Ampelografia ČSSR*. Bratislava, Slovakia : Príroda. 352 p. (In Slovak)

Pour Nikfardjam, M. S., László, G., Dietrich, H. 2003. Polyphenols and Antioxidative Capacity in Hungarian Tokaj Wines. *Mitteilungen Klosterneuburg Rebe und Wein, Obstbau und Früchteverwertung*, vol. 53, no. 5-6, p. 159-165.

Pour Nikfardjam, M. S., László, G., Dietrich, H. 2006. Resveratrol-derivatives and antioxidative capacity in wines made from botrytized grapes. *Food Chemistry*, vol. 96, no. 1, p. 74-79. https://doi.org/10.1016/j.foodchem.2005.01.058

Pour Nikfardjam, M. S., Márk, L., Avar, P., Figler, M., Ohmacht, R. 2006. Polyphenols, anthocyanins, and trans – resveratrol in red wines from the Hungarian Villány region. *Food Chemistry*, vol. 98, no. 3, p. 453-462. https://doi.org/10.1016/j.foodchem.2005.06.014

Singleton, V. L., Orthofer, R., Lamuela-Raventos, R M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, vol. 299, p. 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Somers, T. C. 1986. Evolution of red wines I. Ambient influences on colour composition during early maturation. *Vitis*, vol. 25, p. 31-39.

Soufleros, E. H., Bouloumpasi, E., Zotou, A., Loukou, Z. 2007. Determination of biogenic amines in Greek wines by HPLC and ultraviolet detection after dansylation and examination of factors affecting their presence and concentration. *Food Chemistry*, vol. 101, no. 2, p. 704-716. https://doi.org/10.1016/j.foodchem.2006.02.028

Staško, A., Polovka, M., Brezová, V., Biskupič, S., Malík, F. 2006. Tokay wines as scavengers of free radicals (an EPR study). *Food Chemistry*, vol. 96, no. 2, p. 185-196. https://doi.org/10.1016/j.foodchem.2005.02.022

Špaková, E., Marcinčak, S., Bača, M., Turek, P. 2012. Polyfenolic content and antioxidative activity of wines from the Sobrance wine region. *Potravinarstvo*, vol. 6, no. 3, p. 32-35. https://doi.org/10.5219/204

Tomášová, L., Sochor, J., Baroň, M. 2017. Assesment of antioxidantactivity and content of polyphenplic compounds in grapevine seed. *Potravinarstvo*, vol. 11, no. 1, p. 71-76. https://doi.org/10.5219/712

Tomášová, L., Sochor, J., Baroň, M. 2017. The study of antioxidants in grapevine seed. *Potravinarstvo*, vol. 11, no. 1, p. 132-137. https://doi.org/10.5219/711

Vasko, J. 2001. Architectural peculiarities of vineyards in the area of Tokaj (Architektonické zvláštnosti vinohradníckych objektov v oblasti Tokaj). In *Prírodné bohatstvo a kultúrne dedičstvo Tokaja*. Nitra, Slovakia : SPU, p. 36-38. ISBN 80-8069-004-9. (In Slovak)

Žadanský, J. 2009. *From the history and present of Tokaj wine-growing and winemaking (Z dejín a súčasnosti Tokajského vinohradníctva a vinárstva)*. Trebišov, Slovakia : Vlastivedné múzeum 207 p. ISBN 978-80-89412-07-5. (In Slovak)

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MINERAL COMPOSITION OF AMARANTH (*AMARANTHUS* L.) SEEDS OF VEGETABLE AND GRAIN USAGE BY ARHIVBSP SELECTION

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ABSTRACT
The questions of the practical usage of the analytical scanning electron microscope JSM 600 LA by JEOL company (Japan) with EDS system – microanalysis for the studying of the ash elemental composition of seeds 9 breeds (Vegetable and Grain application) 4 species genus *Amaranthus* L. – *A. hypochondriacus, A. cruentus, A. hybridus, A. caudatus, A. tricolor*. Plant seeds by Federal center of vegetable production selection were envisaged. We studied the concentration of 14 basic elements (in weight %) contained in the mineral part of amaranth seeds. In the amaranth seeds of vegetable forms the accumulation order of the elements is the following: Ca >K >P >Mg >Si >Se >Fe >Mo ≈ S ≈ Cl ≈ Zn >Na >Al. In the seeds of the grain forms the order is different: K >P >Ca >Si >Se >Mg >Fe >Na >Mo >Cl ≈ S ≈ Mn ≈ Zn ≈ Al. The amaranth seeds of the grain forms are rich in macro – and microelements. P, K, Cl and S in the seeds of the grain forms are accumulated on 50, 37, 15 and 5% more and Si, Fe and Al in 2.6 and 1.8 times more than in the vegetable forms seeds. The breeds with the high concentration of the elements are recommended for using in the selection process. The elevated level of the essential macro- and microelements such as Ca, K, P, Mg, Mo, S and Cl stipulates the perspective of the functional products creation on the base of the studied amaranth seeds for the enrichment of the food stuffs.

Keywords: amaranth; seeds; analytical scanning electron microscopy; Energy Dispersive X-ray Analysis (EDS); ash elements

INTRODUCTION
The unique feature of the plants is their ability to extract and metabolize the mineral elements of the soil and water mediums.

It is known that for the acrospires growth and development the following elements are necessary: macroelements N, P, S, K, Ca, Mg and Fe, microelements Cu, Mn, Mo, Zn, B. However, besides these elements, a group of useful elements was distinguished, to this group such elements as Na, Cl, Si were included, they are included in the metabolic processes and with their absence in the medium the plant cannot go through all the development cycle. All the amaranth plant organs as the primary producers are the source of the mineral elements which get into the human being organism in the form of organic molecules and complexes and in the forms of ions in the balanced concentrations. Besides the plant hereditary characters the soil and other multifactorial conditions that accompany the process of amaranth growth influence on the mineral composition of the amaranth seeds **(Jamriška, 1996; Zheleznov et. al., 1997)**.

The seeds of the amaranth engineered breeds of the species – *A. hypochondriacus*, *A. cruentus*, *A. hybridus*, *A. caudatus*, *A. tricolor* are useful for the human nutrition as they have energetic, nutritive, dietary and medical values **(Rastogi and Shukla, 2013; Bojňanská and Šmitalová, 2015)**. The high dietary value of the amaranth seeds is due to the presence of organic and inorganic biochemical components such as carbohydrates (mono- and disaccharides, pectin, dietary fibers) **(Kamysheva, 2010)**, antioxidants – phenol acids, flavonoids, vitamins, proteins, fats and so on **(Tharun et.al., 2012, Gumul et.al, 2017)**.

In the amaranth seeds the essential – nonreplaceable elements (mineral substances) are 0.7 – 1.5 %; they do not possess energetic value as proteins, fats and carbohydrates, however, the human life is impossible without them.

The research up-to-date new technologies in physiological and medical researches confirm an important role of microelements on the level of metabolic reactions and submolecular processes, the activity of which depends on the presence of the certain macro- and microelements in our daily diet **(Avtsyn et al., 1991; Peter and Gandhi, 2017, Motyleva et.al., 2017)**.

The purpose of our work was to research the special features of mineral substances accumulation in the seeds of seven amaranth breeds of vegetable and grain usage created

in Federal Research Center for Vegetable Growing (FRCVG).

Scientific hypothesis

The comparative data on the mineral composition of the seeds of the different species *Amaranthus* L. breeds, being grown in Moscow region, do not exist. We checked whether there are differences in the content of macro- and microelements in species of the genus *Amaranthus sp.* vegetable and Grain forms.

MATERIAL AND METHODOLOGY

Place and objects of research

The objects of the research are the seeds of the vegetable and grain amaranth breeds by FRCVG (Moscow region, Odintsovo) (Table. 1). The plants were grown in the open on the experimental fields of FRCVG. The seeds samples for the analysis were prepared on the stage of biological ripeness.

Soil growing

The soils of the FRCVG experimental and production base are sod-podzolic medium loamy. The agrochemical characteristics of the arable (0 – 20 cm) soil layer before sowing and planting amaranth plants were as follows: humus content according to Tyurin – 1.6 – 2.3%, reaction medium pH KCl 5.9 – 6.1, hydrolytic acidity 1.30 – 1.55 meq.100 g^{-1} of soil, the sum of the absorbed bases is 18.7 – 19.2 meq.100 g^{-1} of soil, the degree of saturation with bases is 88 – 94%, the content of mobile phosphorus is 400 – 550 mg.kg^{-1} of soil, Potassium 150 – 210 mg.kg^{-1} soil, mineral nitrogen 7.0 – 10.0 mg.kg^{-1}.

Amaranth plants were grown on clean (background) soils that are not contaminated with heavy metals (within the permitted sanitary standards adopted in Russia).

Sample preparation

The data of the quantitative elemental composition, given in the present paper, are taken in the laboratory of physiology and biochemistry of the Centre of the plants genofond and bioresources of Federal State Budgetary Scientific Institution All-Russian Horticultural Institute for Breeding, Agrotechnology and Nursery, Moscow. The researches are original and are fulfilled with the usage of the modern analytical equipment. The average seeds weighing with the mass of 10 g was mineralized in the muffle furnace Naberterm (Germany) at T = 400ºC. The received ash was dispergated by ultrasound at 18 kHz frequency for 15 minutes. The dispergate even layer was applied on the object table covered with carbonic scotch.

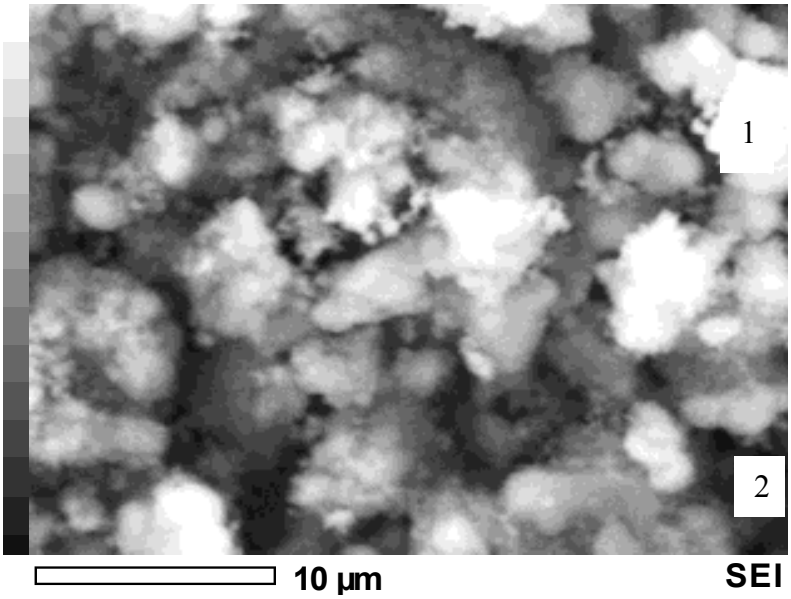
EDS - analysis

The chemical composition of the basic ash components (Na, P, S, K, Mn, Fe, Mg, Ca, Al, Si, Cl, Zn, Se, Mo) was determined by the method of energy dispersive spectrometry (ESD) on the analytical raster electron microscope JEOL JSM 6090 LA. The microscope resolution is 4 nm at accelerating voltage 20 kV (secondary electrons image), zooming is from x 10 till x 10 000. While performing the elemental analysis the working distance (WD) is 10mm. Energy-dispersive spectrometer allows to carry out the quantitative X-ray microanalysis with the desired analyzing area: in a point or areally, and to receive the maps of elements allocation.

X-ray microanalysis data are presented in the form of standard protocols which contain the microstructure picture of the sample under study, the table of the data in weighting and atomic correlation, spectra and histograms. The spectrum example is shown in Figure 1.

Table 1 Amaranth (*Amaranthus* L.) seeds of vegetable and grain application by Federal center of vegetable production selection.

Elements	Vegetable forms				Grain forms				
	Valentina	Don-Pedro	Nezhenka	Pamyati Kovasa	Kizlyarets	Krepysh	Syuita	Shuntuk	Variation coefficient (%)
Na	0.092	0.053	0.906	0.088	0.118	0.121	0.978	0.093	
K	8.991	7.804	13.774	17.673	18.768	15.574	16.598	15.471	13.78
P	9.126	8.851	7.914	13.793	14.351	14.115	13.632	12.611	6.35
Ca	17.234	20.361	14.262	13.171	10.501	11.574	10.788	13.528	10.04
Mo	2.556	2.771	2.962	3.122	3.387	3.345	2.534	2.881	0.09
Mg	6.207	6.871	4.326	6.425	6.591	5.741	4.776	5.232	0.74
S	1.770	1.728	2.054	2.071	2.021	2.098	1.718	2.242	0.03
Si	0.423	0.091	1.526	0.255	0.218	0.191	2.542	3.106	1.26
Cl	0.859	1.044	3.651	1.718	2.023	1.993	4.084	0.684	1.36
Mn	0.155	0.451	0.241	0.288	0.063	0.194	0.171	0.396	0.02
Fe	0.170	0.318	0.341	0.187	0.326	0.263	0.405	0.886	0.04
Al	0.193	0.082	0.202	0.083	0.127	0.091	0.134	0.678	0.04
Zn	0.204	0.236	0.214	0.213	0.221	0.257	0.232	0.156	0.01
Se	0.405	0.482	0.322	0.303	0.359	0.345	0.354	0.198	0.99
∑	**48.037**	**51.143**	**52.695**	**59.391**	**59.047**	**55.902**	**58.946**	**58.162**	



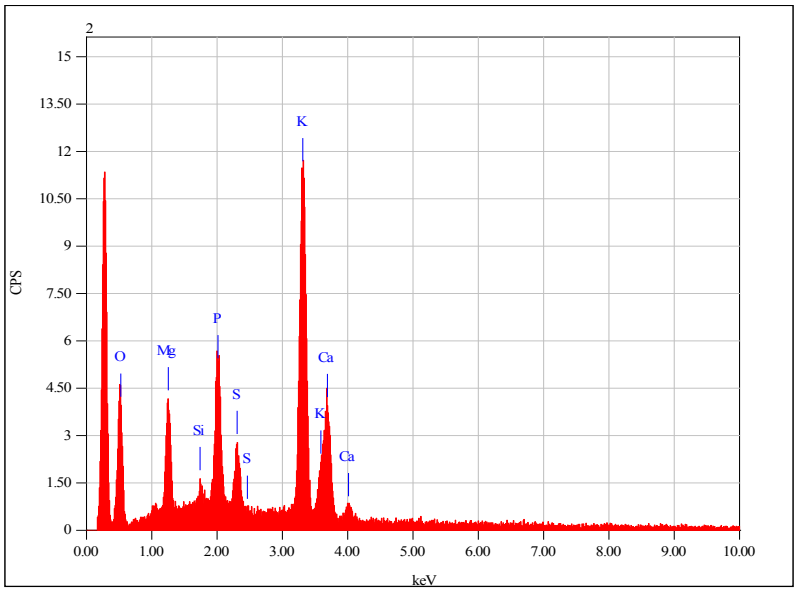


Figure 1 The microstructure picture of the sample under study (1) and the general view of the X-ray spectrum lines that show the elements presence in the analyzing area (2).

Taking into consideration the spectrum lines intensity the concentration of the desired element can be determined. The fractional accuracy of the chemical analysis is spread in the following way: at the element concentration from 1 to 5% the accuracy is less than 10%; from 5 till 10% the accuracy is less than 5%; at the element concentration more than 10% the accuracy is less than 2%. 100 ash areas of each sample were studied. The local analysis is 3 mm, the scanned area is not less than 12 µm.

Statisic analysis

For statistical evaluation were used standard metods using statisniral software Statgraphics Centurion XVII (StatPoint Inc.USA).

RESULTS AND DISCUSSION

The concentration of 14 basic elements (in mass %) contained in the amaranth seeds mineral part was studied (Table 2).

Herewith the main proportion of the ash elements in the seeds belongs to Ca. Ca takes part in the processes of living organisms growth and development, goes into the composition of coenzymes and cells nucleuses, it also takes part in the most important processes for the organism such as metabolism, immunity, regeneration and others **(Gusev, 1998; Gins and Gins, 2011)**.

The proportion of Ca in the amaranth seeds of the vegetable breeds fluctuates from 13.171 to 20.361; while in the grain cultures it varies from 10.501 to 13.598 mass %.

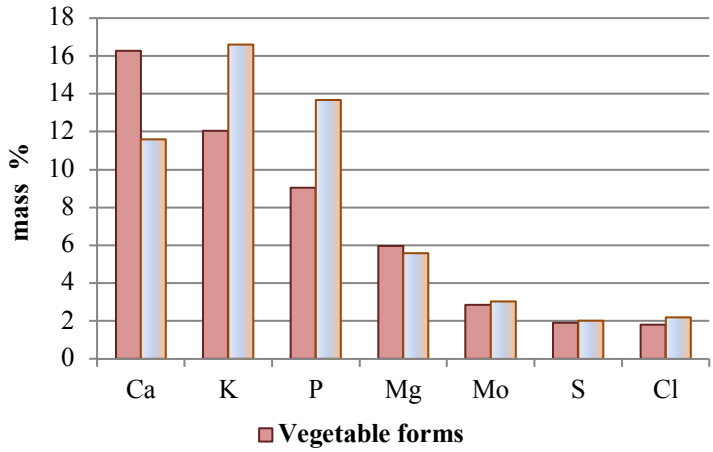


Figure 2 The comparative concentration of macroelements in the ash of the amaranth seeds of vegetable and grain forms.

Table 2 The elemental composition of amaranth seeds, mass % in the ash.

Elements	Vegetable forms					Grain forms			
	Valentina	Don-Pedro	Nezhenka	Pamyati-Kovasa	Kizlyarets	Krepysh	Syuita	Shuntuk	Variation coefficient (%)
Na	0.09 ±0.01	0.05 ±0.01	0.91 ±0.08	0.09 ±0.01	0.12 ±0.02	0.12 ±0.01	0.98 ±0.10	0.09 ±0.02	
K	8.99 ±0.60	7.80 ±1.17	13.77 ±1.67	17.67 ±1.90	18.77 ±1.50	15.57 ±1.46	16.60 ±1.47	15.47 ±1.38	13.78
P	9.13 ±1.18	8.85 ±1.49	7.91 ±1.47	13.79 ±1.18	14.35 ±1.10	14.12 ±1.66	13.63 ±1.97	12.61 ±1.34	6.35
Ca	17.23 ±1.23	20.36 ±1.77	14.26 ±1.53	13.17 ±1.15	10.50 ±1.21	11.57 ±0.84	10.79 ±1.08	13.53 ±1.50	10.04
Mo	2.56 ±0.15	2.77 ±0.20	2.96 ±0.09	3.12 ±0.17	3.39 ±0.10	3.35 ±0.19	2.53 ±0.19	2.88 ±0.24	0.09
Mg	6.20 ±0.08	6.87 ±0.18	4.33 ±0.14	6.43 ±0.06	6.59 ±0.08	5.74 ±0.15	4.78 ±0.23	5.23 ±0.06	0.74
S	1.77 ±0.04	1.73 ±0.03	2.05 ±0.19	2.07 ±0.06	2.02 ±0.03	2.10 ±0.03	1.72 ±0.02	2.24 ±0.08	0.03
Si	0.42 ±0.03	1.90 ±0.20	1.53 ±0.10	0.26 ±0.03	0.22 ±0.01	0.19 ±0.02	2.54 ±0.30	3.11 ±0.25	1.26
Cl	0.86 ±0.04	1.04 ±0.18	3.65 ±0.28	1.72 ±0.16	2.02 ±0.23	1.99 ±0.07	4.08 ±0.42	0.68 ±0.04	1.36
Mn	0.16 ±0,07	0.45 ±0.09	0.24 ±0.02	0.29 ±0.05	0.06 ±0.02	0.19 ±0.06	0.17 ±0.14	0.40 ±0.05	0.02
Fe	0.17 ±0.09	0.32 ±0.18	0.34 ±0.14	0.19 ±0.07	0.33 ±0.07	0.26 ±0.07	0.41 ±0.09	0.89 ±0.12	0.04
Al	0.19 ±0.04	0.08 ±0.01	0.20 ±0.06	0.13 ±0.01	0.13 ±0.01	0.09 ±0.01	0.13 ±0.05	0.68 ±0.04	0.04
Zn	0.20 ±0,03	0.24 ±0.02	0.21 ±0.02	0.21 ±0.01	0.22 ±0.03	0.26 ±0.03	0.23 ±0.03	0.16 ±0.03	0.01
Se	0.41 ±0.03	0.48 ±0.03	0.32 ±0.03	0.30 ±0.03	0.36 ±0.02	0.35 ±0.01	0.35 ±0.04	0.20 ±0.03	0.99
∑	**48.04**	**51.14**	**52.70**	**59.39**	**59.05**	**55.90**	**58.95**	**58.16**	

K is a macroelement that is responsible for the regulation of the majority metabolic reactions that flow in living organisms. The very special role in controlling the homeostasis belongs to K. It controls osmotic pressure transmembrane potential, charges equilibrium, cathode-anion balance, pH – everything that the homeostasis of cells and tissues consists of. In the ionic form K can be found in all the organs, tissues and cell structures in the concentrations that exceed the concentration of other ions **(Meathnis et al., 1997)**. The concentration of K in the amaranth seeds of the vegetable breeds fluctuates from 7.804 (breed Don Pedro) to 17. 673 (Pamyati Kovasa) mass % relatively. In the seeds of the grain application the fluctuations are not essential – from 15.471 to 18.768 mass % at average K contains on 37% more in the breeds of grain usage than in the seeds of vegetable breeds (Figure 2). More than 50% of P is presented in tissues in the form of inorganic P (Pin). P is a part of DNA and RNA, phospholipids, phosphate esters, nucleoside phosphates – ATP, ADP, NATPH, where it fulfills the structural function (in composition of first two types of compounds), in the rest ones – metabolic. P plays a very important role in the cell energetics. For the plants the analogue of P is phytin – Ca^{2+} – Mg^{2+} - the sol of inositephosphoric acid, essential quantities of which are accumulated in the seeds. The concentration of P in the amaranth seeds of vegetable and grain application is 9.021 and 13.677 mass % at average relatively, wherein in the ash of the amaranth seeds of grain breeds the proportion of P is on 50% more than in the seeds of vegetable forms **(Schachtman et. al., 1998)**.

Mg is necessary for the processes of regeneration and renewal of cells, tissues and organs. It activates a large number of enzymes that take part in the processes of CO^2 and N assimilation. In cytosol Mg counter-balances organic compounds (sugars groups, nucleotides, organic and amino acids). Mg is necessary for the keeping up of the cathode-anion balance and pH regulation.

In the cell wall approximately 2.5% of the general concentration of Mg can be found. In the cell wall and in the seeds membrane Mg^{2+} is coordinately connected with carboxylic groups of pectin substances and takes part in the creation of the inner physiological environment of plants. Mg, Ca and N are localized in the seed membrane. ATP, phosphoinositol (phytin) in combination with Mg are accumulated in the seeds in the form which is comfortable for storage **(Nechaev et.al., 2007)**. In the amaranth seeds of

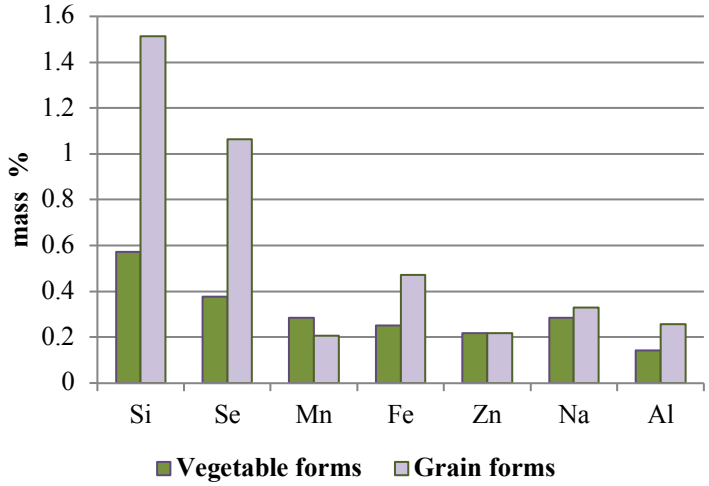


Figure 3 The comparative concentration of macroelements in the ash of the amaranth seeds of vegetable and grain forms.

vegetable and grain forms the concentration of Mg has close values 5.592 and 5.585 mass % relatively.

Mo fulfills a number of useful functions for the organism: it is a cofactor and an activator of oxidases (xanthine oxidase and serine oxidase), takes part in the amino acids synthesis, in the exchange of the vitamins C, E and B12 inside an organism **(Avtsyn et al., 1991)**. The concentration of Mo in the amaranth seeds of the breeds under study fluctuates from 2.556 till 3.387 mass %, wherein, in the seeds of grain application Mo is accumulated on 6% more than in the seeds of vegetable breeds (Figure 2).

Firstly S is necessary for the synthesis and regulation of the plant produced protein quantity and quality. S is a biogenous element as a part of proteins and glutathione, it possesses antioixidant activity, provides the process of the energy transfer in the cell by transporting electrons, takes part in the methyl groups transportation and fixation, covalent, hydrogen and mercaptide connections production, enables the genetic information transfer. In the amaranth seeds of the grain breeds S is contained on 5% more than in the seeds of the vegetable application breeds (Table 2, Figure 2).

At the present time Cl belongs to the microelements. It is the most important biogenous element of living organisms. Cl ions together with Na and K ones take part in the support of the osmotic equilibrium and salt- water exchange regulation. The transportation of Cl contributes to the realization of the following functions: electrical and Ca^{2+} – signalization, membrane potential and pH gradient control. Cl together with Ca is included in the mechanism of the stomatal movements **(Avtsyn et al., 1991)**. In the amaranth seeds of the grain breeds Cl is contained on 15% (2.196 mass %) more than in the seeds of the vegetable application breeds – 1.818 mass % (Table 1, Figure 2).

Si is an obligatory element for the plants **(Kolesnikov and Gins, 2001)**. It is accumulated in large amount in the leaves cell walls, especially in the ecderonic tissues of the scape leaves and the roots. Si is not only the base of the tissues framed element, but it also controls a number of biological and chemical processes in the living organism. It influences the seeds emergence and the seedlings growth it quickens the scapes growth and the dry biomass accumulation. Si has a protective effect toward the harmful influence of Al ions, creating alumosilicates which are included in the composition of phytoliths. Si enlarges the plants resistivity to abiogenous stresses.

The concentration of the biogenous eminent – Si in the amaranth seeds of grain breeds is in 2.6 times more than in the seeds of the vegetable forms (Table 1, Figure3).

Se is a powerful antioxidant that increases the living organism resistivity to biogenous and abiogenous stressors influence is the necessary microelement that is a part of active centers in the form of animoacid selenocysteine **(Vikhreva et al., 2001)**. The concentration of Se in the amaranth seeds is 0.314 – 0.378 mass %.

Mn is a co-factor and activator of many enzymes (pyruvate kinasa. decarboxylase. syperoxide dismutase) it takes part in the synthesis of glycoproteins and proteoglycans and possesses antioxidant activity. In the amaranth seeds the concentration of Mn is 0.206 – 0.284 mass % (Table 2, Figure 3).

Fe as a part of active centers – hemoproteins and iron-sulphur proteins determines the space structure and activity and takes part in oxidation-reduction reactions. The alternative form of Fe is the molecule of protein ferritin that may accumulate up to 4,500 atoms of Fe in soluble nontoxic form. In the amaranth seeds Fe and Cu are localized in the corcule **(Shmalko and Roslyakov, 2011)**.

Organic Fe is an essential compound for the human organism. This element is a part of catalytic centres of many oxidation-reduction enzymes. Fe as a part of active centers – hemoproteins and iron-sulphur proteins determines the space structure and activity and takes part in oxidation-reduction reactions. The alternative form of Fe is the molecule of protein ferritin that may accumulate up to 4,500 atoms of Fe in soluble nontoxic form. In the amaranth seeds Fe and Cu are localized in the corcule **(Shmalko and Roslyakov, 2011)**. Organic Fe is an essential compound for the human organism this element is a part of catalytic centres of many oxidation-reduction enzymes.

Zn stabilizes the molecules structure, plays an important role in DNA and RNA metabolism, in the protein synthesis and cells fission, in the processes of the signal transmission inside the cell **(Nechaev et al., 2007; Pedersen et al., 1987)**. Zn is also an important biogenous element, its

concentration in the amaranth seeds does not exceed 0.217 mass %.

Na is contained mostly in the intercellular fluid Na in the combination with K takes part in the membrane potential creation, the activation of enzymes and muscular contractions, supports osmosis, acid-alkaline and water balance, provides membrane transport **(Avtsyn et al., 1991)**. In the amaranth seeds of the grain forms the concentration of Na is on 11.5 % more than in the seeds of vegetable forms (Table 1, Figure 3).

Al is necessary for the growth and development of bone, cartilaginous and connective tissues and for their regeneration, the concentration of Al in the seeds of grain forms is in 1.8 times more than in the amaranth seeds of vegetable forms (Figure 3).

The average variation coefficient from 6.35 to 16.38% is typical for biologically significant elements of plum fruit and their accumulation limits are due to species peculiarities. The low variation coefficient from 0.74 to 1.36% indicates the accumulation stability of the elements by the culture and depends on the breed insufficiently. Pearson's linear correlation coefficient between 2 elemental compositions in the ash shows the significant correlations between Ca and Mg (r 0.84) and Ca and Mn (r 0.91).

CONCLUSION

Previously, we showed that the amaranth seeds are rich in ash substances – minerals, such as K, Na, Ca and Mg **(Gins and Gins, 2011)**. Using the method of the energy dispersive X-ray spectrometry the new data about the variety of the amaranth vegetable and grain forms mineral composition were received, the proportion of the elements in the ash was determined, the variation coefficients were calculated. The amaranth grain forms exceed the vegetable forms in the elements sum and the concentration of K, P, Si and Fe in 1.3 – 2 times. The peculiarity of the elements accumulation in the seeds depends on the genetic origin and the breeds and species special aspects. The predominant accumulation of Ca, K and P is typical for all the amaranth samples under study. The breeds with the high concentration of the elements are recommended for using in the selection process. The elevated level of the essential macro- and microelements such as Ca, K, P, Mg, Mo, S and Cl stipulates the perspective of the functional products creation on the base of the studied amaranth seeds for the enrichment of the food stuffs. Taking it into account, the studying of the composition and mineral elements concentration in different organs of the plant and their influence on the human life activity is an actual (global) problem, as the deficit of macro- and microelements in the industrial food stuff is extremely huge and dangerous for the human health, because the major part of food stuff is depleted in mineral substances.

REFERENCES

Avtsyn, A. P. Zhavoronkov, A. A., Rishe, A. A. Strochkova, L. S. 1991. *Microelementos of man: etiology. classification. Organopathology*. Moscow, Russia : Medicine. 496 p. ISBN 5-225-02128-X.

Bojňanská, T., Šmitalová, J. 2015. The influence of additional flours on the retention ability of dough and the technological quality of bakery products. *Potravinarstvo,* vol. 9, no. 1, p. 242-246. https://doi.org/10.5219/468

Gins, M. S., Gins, V. K. 2011. *Physiological and biochemical basis of introduction and selection of vegetable cultures*. Moscow, Russia : PFUR. 190 p. ISBN 978-5-209-03960-0.

Gusev, N. B. 1998. Intracellular Ca-connecting proteins. – Part 1. Classification and structure. – Part 2. *Structure and mechanism of functioning Sorovskiy educational journal*, vol. 5, p. 2-16.

Gumul, D., Berski, W., Ivanišová, E., Gambuš, H., Kačániová, M., Harangózo, L., Tokár, M. 2017. Characteristics of starch breads enriched with red potatoes. *Potravinarstvo Slovak Journal of Food Sciences,* vol. 11, no. 1, p. 162-166. https://doi.org/10.5219/720

Jamriška, P. 1996. Analysis of effect of selected factors on amaranth seeds yield. *Agriculture (Poľnohospodárstvo),* vol. 42, no. 5, p. 352-363.

Peter, K., Gandhi, P. 2017. Rediscovering the therapeutic potential of amaranthus species: A review. *Egyptian Journal of Basic and applied Sciences*. vol. 4, no. 3, p. 196-205. https://doi.org/10.1016/j.ejbas.2017.05.001

Kamysheva, I. M. 2010. The usage of amaranth seeds derivate products *Storage and derivation process of agricultural raw materials*, vol. 12, p.67-71.

Kolesnikov, M. P., Gins, V. K. 2001. Forms of silicon in medicinal plants. *Applied Biochemistry and Microbiology Journal*, vol. 37, no 5, p. 524-527. https://doi.org/10.1023/A:1010262527643

Meathnis, F. G. M. Ichida, A. M., Sanders, D., Schroeder, J. I. 1997. Roles of higher plant K+ channels. *Plant Physiol*ogy, vol. 114, no. 4, p. 1141-1149. https://doi.org/10.1104/pp.114.4.1141

Motyleva, S. M., Kulikov, I. M., Marchenko, L. A. 2017. EDS analysis for fruit Prunus elemental composition determination. *Material Science Forum*, vol. 888, p. 314-318. https://doi.org/10.4028/www.scientific.net/MSF.888.314

Nechaev, A. P., Trauberg, S. E., Kochetkova, A. A. 2007. *Food chemistry*. 4th ed. Russia : Gyord Publishing House. 640 p. ISBN: 5-98879-011-9.

Pedersen, B., Kalinowski, L. S, Eggum, B. O. 1987. The nutritive value of amaranth grain (*Amaranthus caudatus* L.) Protein and minerals of raw and processed grain. *Plant Foods for Human Nutrition*, vol. 36, no. 4, p. 309-324.

Rastogi, A., Shukla, S. 2013. Amaranthus: A new millennium crop of nutraceutical values. Critical reviews in food science and nutrition. *Critical Reviews in Food Science and Nutrition*, vol. 53, no. 2, p. 109-125. https://doi.org/10.1080/10408398.2010.517876

Schachtman, D. P., Reid, R. J., Ayling, S. M. 1998. Phosphorus uptake by plants: from soil to cell. *Plant Physiology,* vol. 116, no. 2, p. 447-453. https://doi.org/10.1104/pp.116.2.447

Shmalko, N. A., Roslyakov, Yu. F. 2011. *Amaranth in food industry*. Krasnodar, Russia : Prosveshchenie-South. 489 p. ISBN: 978-5-93491-395-4.

Tharun, Rao, K. N., Padhy, S. K., Dinakaran, S. K., Banji, D., Avasarala, H., Ghosh, S., Prasad, M. S. 2012. Pharmacognostic, Phytochemical, Antimicrobial and Antioxidant Activity Evaluation of *Amaranthus tricolor* Linn. Leaf. *Asian Journal of Chemistry,* vol. 24, no. 1, p. 455-460.

Vikhreva, V. A., Khryanin, V. N., Gins, V. K. Blinokhvatov, A. F. 2001. Adaptogenic role of Se in higher plants. *Reporter of Bashkirskiy University*, vol. 2, p. 65-66.

Zheleznov, A. V., Solonenko, L. P., Zheleznova, N. B. 1997. Seed protein of the wild and the cultivated *Amaranthus* species. *Euphytica*, vol. 97, no. 2, p. 177-182. https://doi.org/10.1023/A:1003073804203

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INHIBITORY EFFECT OF AQUEOUS EXTRACTS OF RAW AND ROASTED *SESAMUM INDICUM* L. SEEDS ON KEY ENZYMES LINKED TO TYPE-2 DIABETES (α-AMYLASE AND α-GLUCOSIDASE) AND ALZHEIMER'S DISEASE (ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE)

Justina Talabi, Sekinat Adeyemi, Sefunmi Awopetu, Basiru Olaitan Ajiboye, Oluwafemi Adeleke Ojo

ABSTRACT

Sesame (*Sesamum indicum* L.) seeds are nutritional food, but researches have limited knowledge about the antioxidant, antidiabetic and anticholinesterase activities of the seed. This study was conducted to determine the antioxidant activity, enzyme inhibitory potential (α-amylase and α-glucosidase) and acetylcholinesterase inhibitory property of aqueous extracts of raw and roasted sesame seeds. Antioxidant activities were analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging property, 2,2-azino-bis-(3-ethylbenthiazoline-6- sulphonic acid (ABTS) scavenging ability, iron chelating ability and ferric reducing antioxidant power (FRAP). Anti-Alzheimer's potential was determined using acetylcholinesterase and butyrylcholinesterase enzyme inhibition assay. The results showed that the total phenolic and flavonoid contents were higher in the roasted *S. indicum* sample with the values of 19.81mg/100g and 17.19 mg/100g respectively. The raw *S. indicum* sample showed higher antioxidant activity in DPPH, and iron chelation assays; while roasted *S. indicum* sample showed higher in the reducing power and ABTS scavenging activity. However, anticholinesterase activity was higher in the roasted *S. indicum* sample than in the raw *S. indicum* sample. The extracts inhibited α-amylase activity in a concentration-dependent manner (20 – 100 $\mu g.mL^{-1}$). The raw sample (16.55 ±0.89%) had higher inhibitory α-amylase activity compared to the roasted sample (15.78 ±0.48%) at 100 $\mu g.mL^{-1}$. Inhibition of α-glucosidase was higher in the roasted sample at 100 $\mu g.mL^{-1}$ (19.40 ±0.26%) compared to the raw sample at the same concentration (3.65 ±0.52%). These findings suggest that *S. indicum* L. is not only nutritious but also showed potential pharmacological properties.

Keywords: *Sesamum indicum* seeds; α-amylase; α-glucosidase; anticholinesterases; antioxidant activity; Alzheimer's disease

INTRODUCTION

Free radicals have been implicated as playing a role in the etiology of cardiovascular disease, cancer, diabetes mellitus, Alzheimer's disease and Parkinson's disease **(Enujigha et al., 2012; Ojo et al., 2017a)**. Evaluation of the antioxidant activities of natural substances has been of interest in recent years. Antioxidants scavenge free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases **(Enujigha et al., 2012)**. The antioxidant capacity of most plant food sources is usually associated with their phenolic contents. Since the well-known synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are reported to confer some degree of carcinogenicity **(Enujigha, 2010; Ojo et al., 2014)**. Current research efforts are channeled towards exploiting the antioxidant potentials of natural phenolic. Such compounds are found to be abundant in fruits, vegetables, cereal, grains, and legumes.

Sesame (*Sesamum indicum* L.) belongs to the family of Pedaliaceae and is one of the most ancient crops and oilseeds known and used by mankind. It is known as benniseed. In Nigeria, sesame seed is known and called by different vernacular names depending on locality like: '*Ridi*' (Hausa), '*Ishwa*' (Tiv), '*Yamati* or '*Eeku*' (Yoruba), '*Igorigo*' '*Igbira*' and '*Doo*' (Jukun). Fibers from sesame are used as an antidiabetic, anti-tumor, antiulcer, cancer preventive and cardio protective **(Nagendra-Prasad et al., 2012)**. In recent years, studies have implicated oxidative stress to play a crucial role in neurodegenerative diseases

such as Alzheimer's disease via lipid peroxidation of the cell membrane of the neurons. It is therefore expedient to assess the inhibitory effect of aqueous extract of sesame seeds on the key enzymes linked to type-2 diabetes and Alzheimer's disease in other to provide some possible mechanism of action by which they exert their anti-diabetic and/or anti-Alzheimer's properties.

Scientific hypothesis

Scientist have noted that natural plants product with secondary metabolites such as phenols, flavonoids, saponins, tannins have the potentials and/or ability to scavenge free radicals induced oxidative damage. Hence, the higher the polyphenolic content of an extract the higher it potency to inhibit free radical induced oxidative damage.

MATERIAL AND METHODOLOGY

Sample collection

Raw sesame seeds were procured for analysis from a local market in Ado-Ekiti. The sample preparation was done at Human Nutrition and Dietetics laboratory, Afe Babalola University Ado-Ekiti. Dirt and other foreign materials were removed from the seeds and by hand picking. The samples were divided into two; raw and roasted. For the roasted sample, the seeds were dry-heated with an open hot plate at 135 $^{\circ}$C for 25 mins. During this process, frequent agitation of seed was done for uniform roasting of seeds. The seed sample was ground into a fine powder. The raw and roasted samples were ground separately using a blender.

Aqueous Extract Preparation

The aqueous extracts of the seeds were prepared by soaking 50 g of the ground samples in 500 mL of distilled water for 48 hr according to **(Ojo et al., 2013a)**. The mixture was later filtered through Whatman filter paper, the filtrate was lyophilized and the dry extract was kept for further analysis.

In vitro antioxidant determination

DPPH radical assay

The radical scavenging ability of the extract against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was assessed as described by **(Gyamfi et al., 1999)**. 1.0 mL of various concentrations of the extracts in methanol was added to 4 mL of 0.1 mmol.L^{-1} methanol solution of DPPH. A blank probe was obtained by mixing 4 mL of 0.1 mmol.L^{-1} methanol solution of DPPH and 200 µL of deionized distilled water (H_2O). After 30 mins of incubation in the dark at room temperature, the absorbance was read at 517 nm against the prepared blank. Inhibition of free radicals by DPPH in percent was calculated using this formula:

$$\%\ inhibition = \frac{ABSsample - ABSblank}{ABScontrol} \times 100$$

Iron (Fe2+) chelating ability

Fe^{2+} chelating ability of the extract was determined using a modified method of **(Puntel et al., 2005)**. Freshly prepared 500 µM $FeSO_4$ (150 µL) was added to a reaction mixture containing 168 µL 0.1 M Tris-HCl (pH 7.4), 218 µL saline, and the extracts (0 – 25 µL). The reaction mixture was incubated for 5 min, before the addition of 13 µL 0.25% 1,10-phenanthroline (w/v). The absorbance was measured at 510 nm. The Fe (II) chelating ability was subsequently calculated.

2,2- Azinobis (3-ethylbenzo-thiazoline-6-sulfonate (ABTS) radical scavenging ability

The $ABTS^{\cdot}$ scavenging ability of the extracts was determined according to the method described by (**Re et al., 1999**). ABTS* was generated by reacting an ABTS aqueous solution (7 mmol.L^{-1}) with potassium persulfate ($K_2S_2O_8$) (2.45 mmol.L^{-1}, final concentration) in the dark for 16 h and adjusting the absorbance 734 nm. 0.2 mL of an appropriate dilution of the extract was added to 2.0 mL $ABTS^{\cdot}$ solution and the absorbance was read at 734 nm after 15mins.

Figure 1 Sesame Seed.

Ferric reducing antioxidant assay power (FRAP)

The reducing property of the *S. indicum* seeds aqueous extracts were studied by assessing the ability of the extracts to reduce ferric chloride ($FeCl_3$) solution as described by **(Pulido et al., 2000)**. A 2.5 mL aliquot was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3[Fe(CN)_6]$). Solution was incubated for 20 min at 50 °C in a water bath and then 2.5 mL of 10% trichloroacetic acid (CCl_3COOH) was added. The sample was then centrifuged at 650 g for 10 min. After that, 5 mL of the supernatant was mixed with an equal water volume and one mL, 0.1% $FeCl_3$. The above-stated process was applied to a standard ascorbic acid solution, and finally the absorbance was read at 700 nm. The reducing ability was calculated as percentage inhibition.

Quantification of Phenolic Compounds

Estimation of total phenol content

The total phenol content of the seeds extracts was assessed (as gallic acid equivalent) as described by **(McDonald et al., 2001)**. In short, 200 µL extract dissolved in 10% DMSO (240 $\mu g.mL^{-1}$) was incubated with 1.0 mL of Folin Ciocalteau chemical agent (diluted 10 times) and 800 µL of 0.7 $mol.L^{-1}$ sodium carbonate (Na_2CO_3) for 30 min at ambient temperature. Absorbance was read at 765 nm using spectrophotometer. All readings were repeated in triplicate. Results expressed as mg GAE.100 g^{-1} dry aqueous extracts.

Determination of Flavonoid Content

The flavonoid content of the seeds extracts was determined using the method reported by **(Meda et al., 2005)**. Briefly, 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 µL 10% $AlCl_3$, 50 µL 1 M potassium acetate and 1.4 mL water, and incubated at room temperature for 30 min. Absorbance of the mixture was read at 415 nm. All experiments were in triplicates. A standard curve was plotted with quercetin and the total flavonoid content of the extract was expressed as quercetin equivalent.

Enzymes assays

α-amylase inhibition activity assay

The α-amylase inhibitory activity was determined concurring to the protocol described by **(Shai et al., 2010)**. A volume of 250 µL of aqueous seeds extracts at totally different concentrations (20 – 100 $\mu g.mL^{-1}$) were incubated with 500 µL of porcine pancreatic amylase (2 $U.mL^{-1}$) in 100 $mmol.L^{-1}$ phosphate buffer (pH 6.8) at 37 °C for 20 min. Two hundred and fifty µL of 1% starch dissolved in 100 $mmol.L^{-1}$ phosphate buffer (pH 6.8) was then added to the reaction mixture and incubated at 37 °C for 1 h. One mL of DNS color chemical reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was read at 540 nm and the inhibitory activity was expressed as percentage of a control sample without inhibitors. All assays were applied in triplicate.

$$\alpha\text{-amylase inhibition (\%)} = \frac{A_{540}\text{control} - A_{540}\text{sample}}{A_{540}\text{control}} \times 100$$

α-glucosidase inhibition activity assay

The α-glucosidase inhibitory activity was assessed in line with the protocol described by **(Ademiluyi and Oboh, 2013)**, with small modifications. Briefly, 250 µL of aqueous seed extract, at different concentrations (20 – 100 $\mu g.mL^{-1}$), were incubated with 500 µL of 1.0 $U.mL^{-1}$ α-glucosidase solution in 100 $mmol.L^{-1}$phosphate buffer (pH 6.8) at 37 °C for 15 min. Thereafter, 250 µL of p-nitrophenyl-α-D-glucopyranoside (pNPG) solution (5 $mmol.L^{-1}$) in 100 $mmol.L^{-1}$ phosphate buffer (pH 6.8) was added and therefore the mixture was more incubated at 37 °C for 20 min. The absorbance of the free *p*-nitrophenol was read at 405 nm and therefore the inhibitory activity was expressed as percentage of a control sample without inhibitors.

$$\alpha\text{- glucosidase inhibition (\%)} = \frac{A_{405}\text{control} - A_{405}\text{sample}}{A_{405}\text{control}} \times 100$$

Determination of Cholinesterase Activity

Acetylcholinesterase (AChE) inhibition activity assay

Inhibitory activity of AChE was evaluated by an adapted colorimetric method as described by **(Ellman et al., 1961)**. The AChE activity was determined in a mixture containing 200 µL of a solution of AChE (0.415 $U.mL^{-1}$ in 0.1 M phosphate buffer, pH 8.0), 100 µL of a solution of 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB) (3.3 mM in 0.1 M phosphate-buffered solution, pH 7.0) containing $NaHCO_3$ (6 mM), aqueous seed extracts of *S. indicum* , and 500 µL of 4 phosphate buffer, pH 8.0. After incubation for 20 min at 25 °C, 100 µL of 0.05 mM acetylthiocholine iodide solution was added as the substrate, and AChE activity was assessed as change in absorbance at 412 nm for 3 min at 25 °C using a spectrophotometer. Inhibition of BChE was evaluated by an adjusted colorimetric method as described by **(Cunha et al., 2016)**. The BChE activity was assessed in a mixture comprising 200 µL of a solution of BChE (0.415 $U.mL^{-1}$ in 0.1 M phosphate buffer, pH 8.0), 100 µL solution of 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB) (3.3mM in 0.1 M phosphate-buffered solution, pH 7.0) containing $NaHCO_3$ (6 mM), phenolic extracts, and 500 µL of 4 phosphate buffer, pH 8.0. After incubation for 20 min at 25 °C, 100 µL of 0.05 mM butyrylthiocholine iodide solution was added as the substrate, and BChE activity was determined as change in absorbance at 412 nm for 3 min at 25 °C using a spectrophotometer. The AChE and BChE inhibitory activities were expressed as percent inhibition (%).

Statisic analysis

All samples were done in triplicates and expressed and mean ± standard error of mean (SEM). Data were obtained from the sample analysis using one-way analysis of variance (ANOVA) at 5% level of significance followed by Duncan Multiple Test, using SPSS 21.0 software package.

RESULTS AND DISCUSSION

Table 1 shows the total phenolics and flavonoids content of aqueous extract of raw and roasted sesame samples. Total phenolics and flavonoid contents were significantly (p <0.05) higher in roasted sample compared to the raw sample. Total flavonoid content of the roasted sample was 17.19 µg.g^{-1}, while in the raw sample it was 15.45 µg.g^{-1}. Total phenolic content of the roasted sample was 18.91 mg.100 g^{-1} and for the raw sample, it was 15.36 mg.100 g^{-1}. Many plants are rich sources of phytochemicals, and intake of these plant chemicals has protective potential against degenerative diseases (**Chu et al., 2002**). Phenolic compounds can protect the human body from free radicals, whose formation is associated with the convectional metabolism of aerobic cells. They are strong antioxidants capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α- tocopherol radicals and inhibit oxidases (**Marin et al., 2004**). This

Table 1 Total phenolics and flavonoids contents of aqueous extract of *Sesamum indicum L.*

SAMPLES	TOTAL PHENOLICS (mg.GAE.g^{-1})	TOTAL FLAVONOIDS (mg Quercetin. g^{-1})
RAS	15.36 ±0.68	15.45 ±0.68
RSS	18.91 ±1.39	17.91 ±1.22

Note: Mean ±SEM in triplicates (n = 3), ras: raw sesame sample, rss: roasted sesame sample.

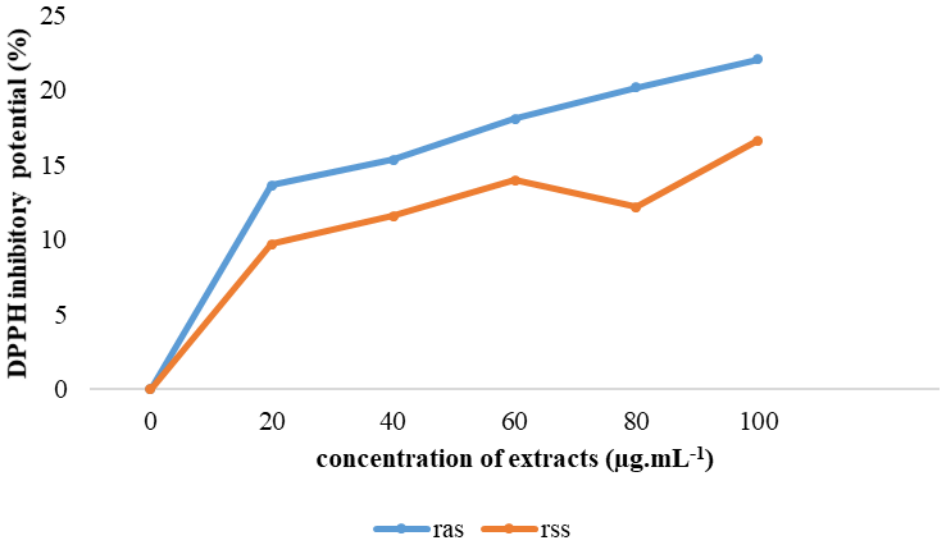

Figure 1 Inhibitory activities of aqueous extracts from sesame (*Sesamum indicum* L.*)* seeds against DPPH radical scavenging ability.
Note: Values are represented as the mean ±standard error of mean of triplicate experiments, ras: raw sesame sample, rss: roasted sesame sample.

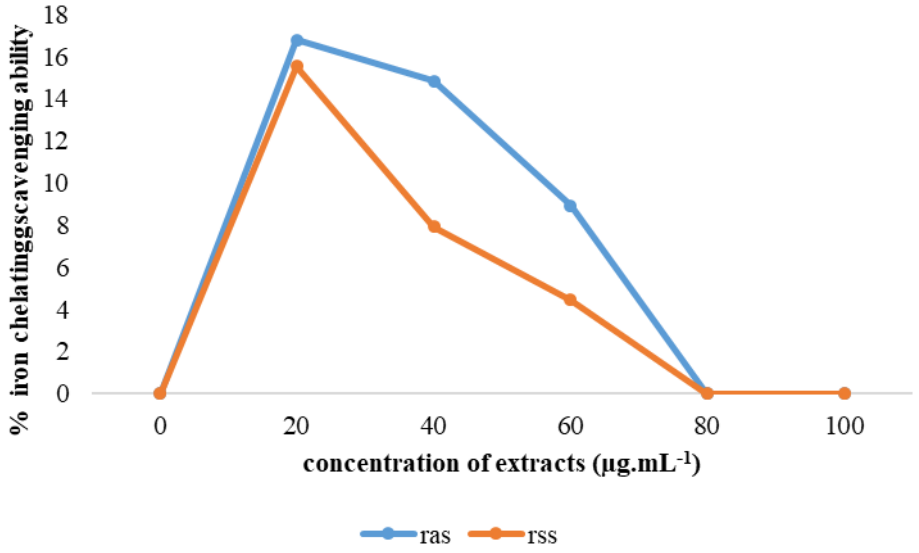

Figure 2 Inhibitory activities of aqueous extracts from sesame (*Sesamum indicum* L.) seeds against iron chelation.
Note: Values are represented as the mean ± standard error of mean of triplicate experiments, ras: raw sesame sample, rss: roasted sesame sample.

data of analysis confirms previous results reported by **(Blessing et al., 2010)** who found that sesame seeds possessed the highest amount of flavonoids, compared to other parts of *Sesame indicum*.

Figure 1 shows the DPPH radical scavenging ability of aqueous extracts of sesame (*S. indicum L.*) flour samples. The result of the raw seeds reveals a significant increase (p <0.05) at different concentrations ranging from 20 µg/mL to 100 µg.mL^{-1}. Concentration of 100 µg.mL^{-1} had the highest (22.05%) while at 20 µg.mL^{-1} was the lowest (16.78%). Similarly, the same trend was observed in the roasted sample with the highest value at 100 µg.mL^{-1} (16.62%) and the lowest at 20 µg.mL^{-1} (9.70%). Plants and plants products are known to possess excellent antioxidant properties and play a significant role in preventing the complication caused by excessive free radicals **(Sharma et al., 2014; Ojo et al., 2013b)**. The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs. This study showed that the higher the concentration of the extract, the higher the ability of the seeds extracts to scavenge free radicals. The antioxidant activity increased with increasing concentration of extract. This is in agreement with **(Enujigha et al., 2012; Oboh et al., 2007)**.

Figure 2 shows the iron chelation assay of aqueous extracts of sesame (*S. indicum L.*) flour samples. The results obtained show a significant decrease (p <0.05) at 80 and 100 µg.mL^{-1} concentrations in the raw sample. Concentration of 20 µg.mL^{-1} (16.78%) had the highest while at 100 µg.mL^{-1} there was no inhibition (0.00). The same trend was observed in the roasted sample with the highest at 20 µg.mL^{-1} (15.53%) while there was no inhibition at 100 µg.mL^{-1}. Iron has been implicated as the most important pro-oxidant of lipids. It is also known that

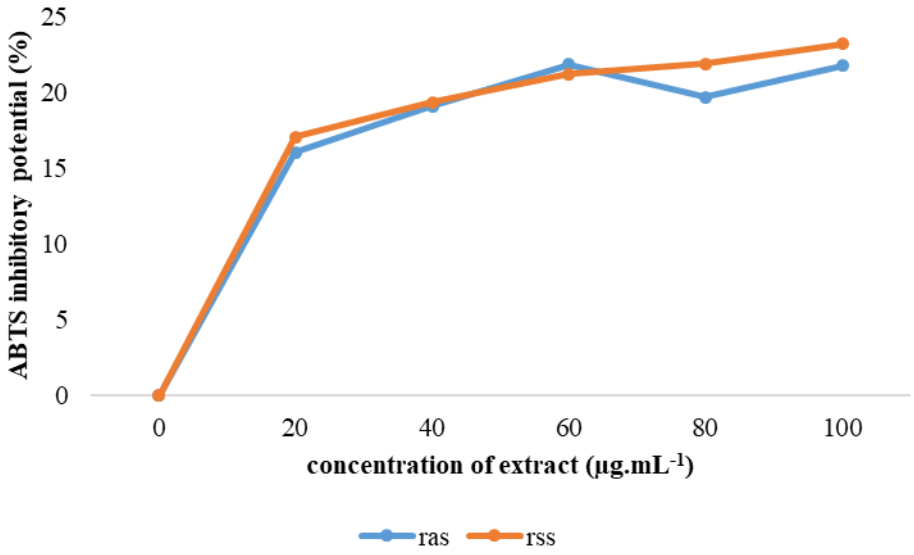

Figure 3 Inhibitory activities of aqueous extracts from sesame (*Sesamum indicum* L.*)* seeds against ABTS radical scavenging ability.
Note: Values are represented as the mean ±standard error of mean of triplicate experiments, ras: raw sesame sample, rss: roasted sesame sample.

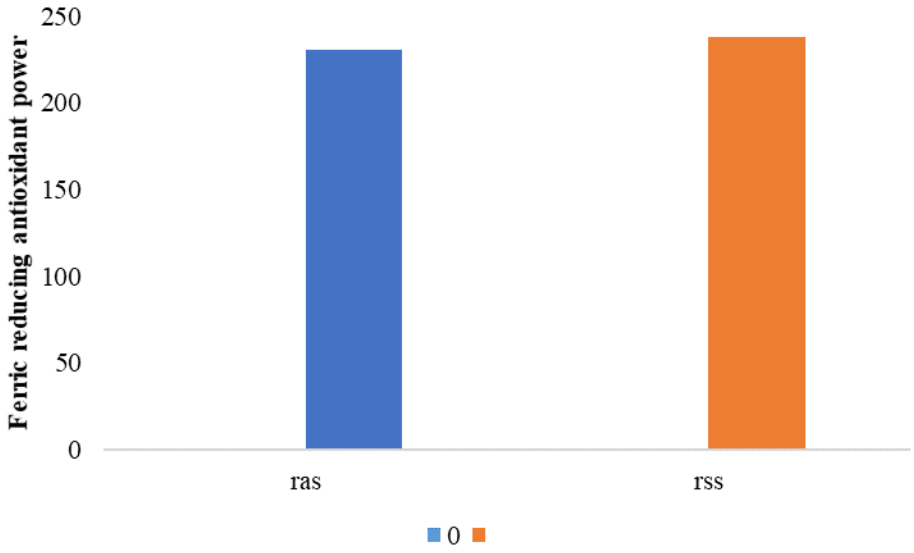

Figure 4 Ferric reducing antioxidant power of aqueous extract of raw and roasted sesame samples.
ras: raw sesame sample, rss: roasted sesame sample.

the Fe^{2+} accelerates lipid peroxidation by breaking down hydrogen and lipid peroxides formed by the Fenton free radical reaction: $Fe^{2+} + H_2O_2$, $Fe^{3+} + OH^+ OH^-$ **(Shodeinde and Oboh, 2012)**. Aqueous seed extract showed more activity in the raw sample compared to the roasted sample but the antioxidant activity decreased with increasing concentration of extract.

Figure 3 shows the ABTS scavenging capacity of aqueous extracts of sesame (*S. indicum L.)* flour samples. The result obtained in this study shows a significant increase (p <0.05) at different concentrations from (20 – 100 µg.mL^{-1}) in the raw sample. The concentration at 100 µg/mL (21.77%) had the highest while at 20 µg.mL^{-1} (16.03%) been the lowest. Similarly, the same trend was observed in the roasted sample with concentration at 100 µg.mL^{-1} (23.20%) being the highest and concentration at 20 µg.mL^{-1} (17.04%) being the lowest. The principle of ABTS involves the scavenging activity of extracts against free radicals, but ABTS salt must be generated by enzymatic or chemical reaction **(Arnao, 2000)**. In ABTS method, the roasted sample presented more activity than the raw sample.

Figure 4 shows ferric reducing antioxidant power of both raw and roasted sesame samples. FRAP activity was significantly (p <0.05) higher in the roasted sample (237.96 µg.g^{-1}) compared to the raw sample (230.48 µg.g^{-1}). Ferric reducing antioxidant power is one assay to determine the antioxidant capacity in samples which utilize single electron transfer mechanism. In this study, the Fe^{3+}-tripyridyl triazine (TPTZ) complex is reduced to the Fe^{2+}-TPTZ-complex by an antioxidant sample, this latter complex possessing an intense color in

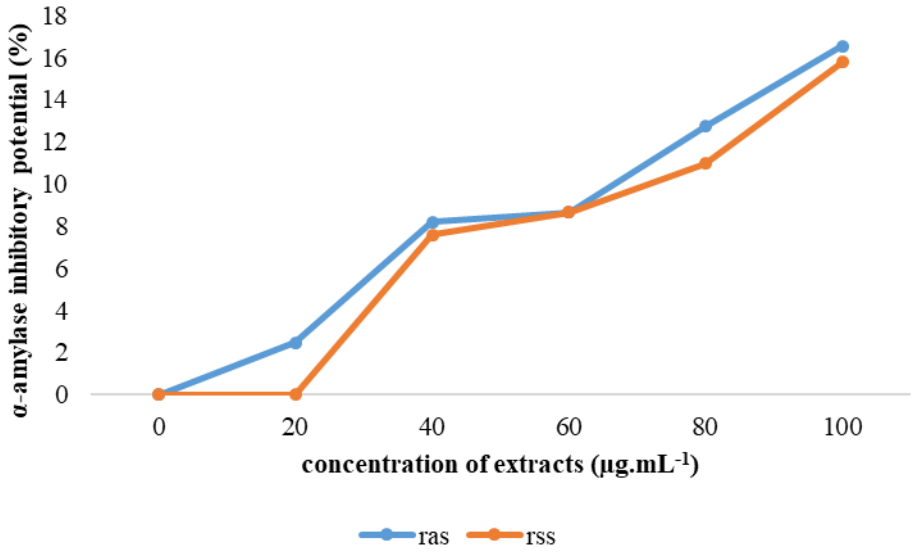

Figure 5 Inhibitory activities of aqueous extracts from sesame (*Sesamum indicum L.)* seeds against α-amylase. Values are represented as the mean ±standard error of mean of triplicate experiments, ras: raw sesame sample, rss: roasted sesame sample.

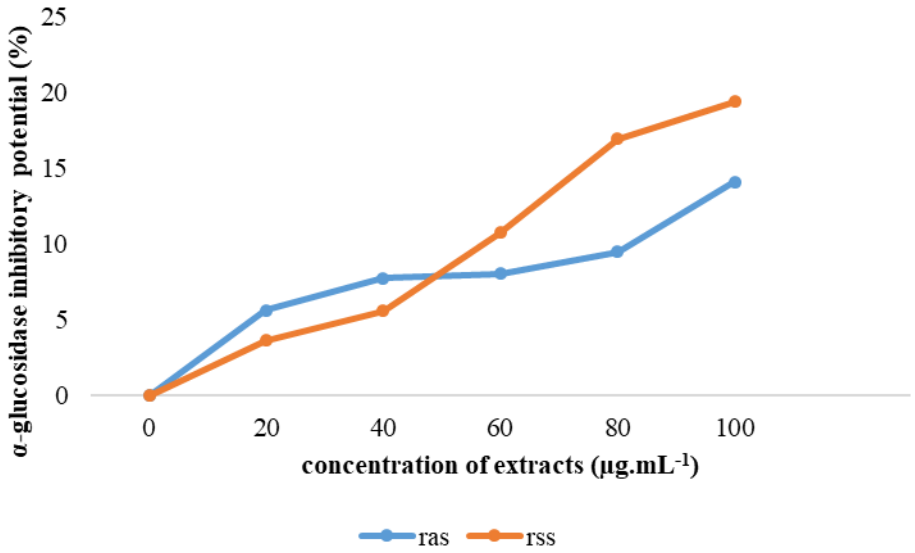

Figure 6 Inhibitory activities of aqueous extracts from sesame (*Sesamum indicum L.)* seeds against α-glucosidase. Note: Values are represented as the mean ±standard error of mean of triplicate experiments, ras: raw sesame sample, rss: roasted sesame sample.

an acidic environment **(Ali Hassan et al., 2013)**. In the present study, the FRAP activity of the roasted sample of *S. indicum* was higher than the raw sample. This finding was higher than that of a previous study by **(Siti-Hawa et al., 2013)**.

Figure 5 shows the inhibitory activity of α-amylase on aqueous extracts of sesame (*S. indicum L.)* flour samples. The results reveal a significant increase (p <0.05) at different concentrations ranging from 20 µg.mL^{-1} to 100 µg.mL^{-1} in the raw sample. The concentration at 100 µg.mL^{-1} (16.55%) had the highest while at 20 µg/mL (2.47%) been the lowest. In the roasted sample, concentration at 100 µg/mL (15.78%) had the highest, while there was no inhibition at 20 µg.mL^{-1} (0.00%) concentration. Figure 6 shows the inhibitory properties of α-glucosidase on aqueous extracts of sesame (*S. indicum L.)* flour samples. Results obtained shows a considerably increase (p <0.05) at different concentrations from 20 – 100 µg.mL^{-1} in the raw sample. The concentration at 100 µg.mL^{-1} (14.10%) had the highest while at 20 µg.mL^{-1} (5.65%) been the lowest. The same trend was observed in the roasted sample, with concentration at 100 µg.mL^{-1} (19.40%) having highest and at 20 µg.mL^{-1} (3.65%) having the lowest. Inhibition of enzymes involved in the hydrolysis of carbohydrates such as α-amylase and α-glucosidase has been exploited as a therapeutic approach for controlling postprandial hyperglycemia **(Bello et al. 2011; Ojo et al., 2016a)**. The radical scavenging abilities of the seeds could be beneficial in the management of type 2 diabetes as free radicals are involved in the development and complications of diabetes in a number of ways; the white blood cell production of reactive oxygen species mediates the autoimmune destruction of the beta cells in the islets of Langerhans in the pancreas, abnormalities in transition metal metabolism are postulated to result in the establishment of diabetes, and diabetes associated

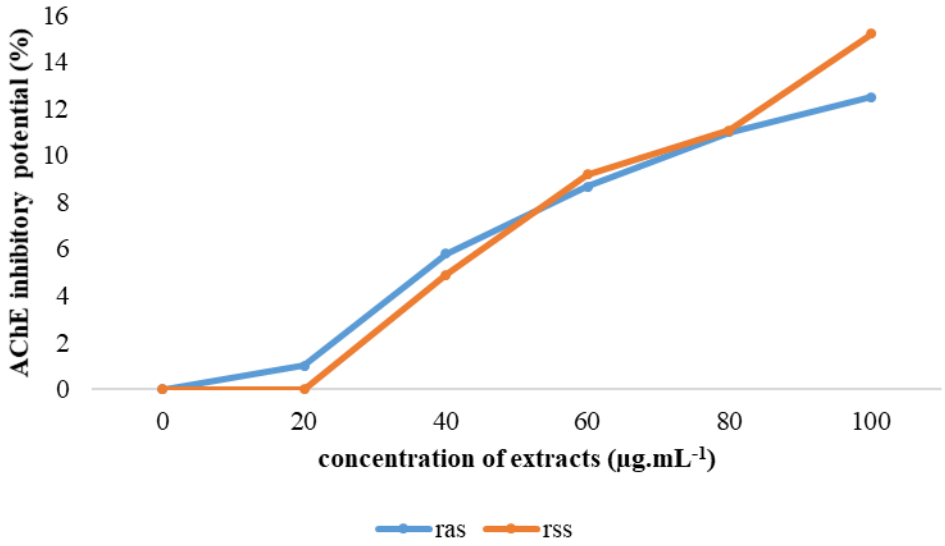

Figure 7 Inhibitory activities of aqueous extracts from sesame (*Sesamum indicum* L.) seeds against AChE.
Note: Values are represented as the mean ±standard error of mean of triplicate experiments, ras: raw sesame sample, rss: roasted sesame sample.

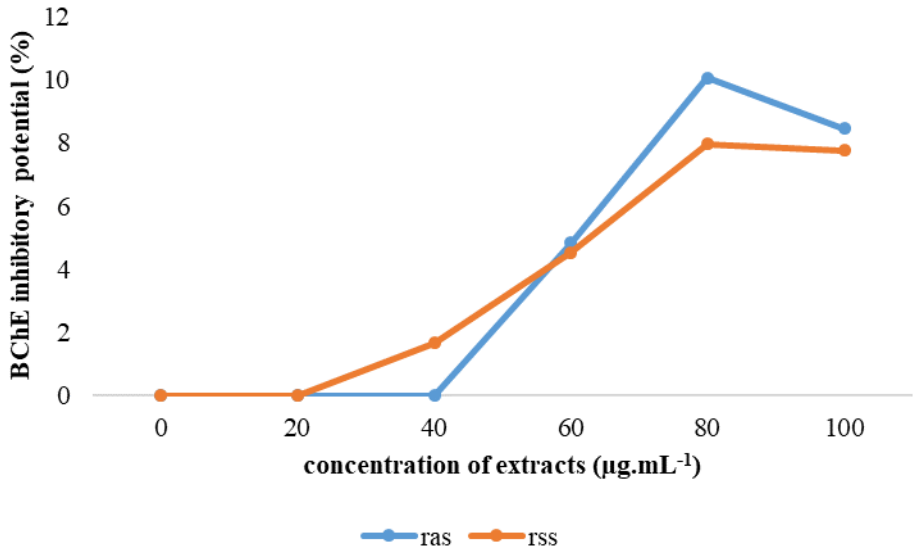

Figure 8 Inhibitory activities of aqueous extracts from sesame (*Sesamum indicum* L.) seeds against BChE.
Note: Values are represented as the mean ±standard error of mean of triplicate experiments, ras: raw sesame sample, rss: roasted sesame sample.

hyperglycemia causes intracellular oxidative stress, which contributes to vascular dysfunction **(Ademosun and Oboh, 2015)**. Pancreatic α-amylase is involved in the breakdown of starch into disaccharides and oligosaccharides before intestinal α-glucosidase catalyzes the breakdown of disaccharides to liberate glucose which is later absorbed into the blood circulation. Inhibition of these enzymes would slow down the breakdown of starch in the gastrointestinal tract, thus reducing postprandial hyperglycemia **(Kwon et al., 2007; Ojo et al., 2016b)**. The extracts inhibited α-amylase and α-glucosidase activities in a concentration-dependent manner. This indicates that both raw and roasted *Sesame indicum* L. are good choices for diabetics, and also that a large amount of the seeds is to be consumed to be effective.

Figure 7 shows the inhibitory effect of AChE on aqueous extracts of sesame (*S. indicum* L.) flour samples. The results reveal a considerably increase (p <0.05) at different concentrations ranging from 20 – 100 µg.mL^{-1} in the raw sample. The concentration at 100 µg/mL had the highest (12.51%) while at 20 µg.mL^{-1} (1.02%) been the lowest. The same trend was observed in the roasted sample with concentration at 100 µg.mL^{-1} (15.22%) having the highest and concentration at 20 µg.mL^{-1} (0.00%) having no inhibition. Figure 8 shows the inhibitory effect of BChE on aqueous extracts of sesame (*S. indicum* L.) flour samples. Results showed there was no inhibition at 20 and 40 µg/mL concentration in the raw sample, but there was a considerably increase (p <0.05) at 60 and 80 µg.mL^{-1} concentrations. Concentration of 80 µg.mL^{-1} (10.05 %) had the highest BChE activity. While in the roasted sample, there was no inhibition at 20 µg.mL^{-1} (0.00%) concentration, but there was aa appreciably increase (p <0.05) at 40, 60 and 80 µg.mL^{-1} concentrations with concentration at 80 µg.mL^{-1} (7.96%) having the highest. This study showed that both the raw and roasted *S. indicum* L. samples displayed anticholinesterase activity potential which was in a concentration dependent manner. The values obtained for AChE were lower compared to that of aqueous extract of *Glycine max* (68.4%) as reported by **(Sharififar et al., 2010)**. Inhibition of acetylcholinesterase (AChE) activity has been accepted as an effective treatment/management strategy against mild Alzheimer's disease (AD) **(Orhan et al., 2004)**. Alzheimer's disease is a form of dementia characterized by loss of central cholinergic neurons associated with a marked reduction in the content of acetylcholinesterase (AChE), the enzyme responsible for the termination of nerve impulse transmission at cholinergic synapses. Consequently, one therapeutic approach to the treatment of Alzheimer's disease is the use of plant-based anticholinesterase drugs which have little or no side effects **(Oboh et al., 2014; Ojo et al., 2017b; Ojo et al., 2018)**. Hence, inhibition of these cholinesterase's could be as a result of the important phytochemicals such as phenolics and flavonoids which have already been characterized in this extract.

CONCLUSION

In conclusion, aqueous extract of both raw and roasted sesame seed (*S. indicum* L.) is rich in phenolic compounds and exhibited antioxidant activities. Sesame seeds show potential as functional food and/or nutraceuticals in the management of diabetes mellitus and neurodegenerative diseases such as Alzheimer's disease as it exhibited inhibitory activity on key enzymes (α-amylase, α-glucosidase acetylcholinesterase and butyrylcholinesterase) linked to these diseases. Therefore, one possible mechanism through which the seeds exert their neuroprotective properties is by inhibiting cholinesterase activities as well as preventing oxidative-stress-induced neurodegeneration.

REFERENCES

Ademiluyi, A., Oboh, G. 2013. Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α-amylase and α -glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. *Experimanetal and Toxicologic Pathology*, vol. 65, p. 305-309. https://doi.org/10.1016/j.etp.2011.09.005 PMid:22005499

Ademosun, A. O., Oboh, G. 2015. Inhibition of carbohydrate hydrolyzing enzyme associated with type 2 diabetes and anti-oxidative properties of some edible seeds *in vitro*. *International Journal of Diabetes in Developing Countries*, vol. 35, no. 3, p. 516-521. https://doi.org/10.1007/s13410-015-0339-7

Ali Hassan, S. H., Fry, J. R., Mohd-Fadzelly, M., Abu-Bakar, M. F. 2013. Antioxidant Phytochemical and Anti-Cholinesterase activity of native Kembayau (*Canarium odontophyllum*) fruit of sabah, Malaysian Borneo. *Journal of Nutrition and Food Sciences*, vol. 4, no. 1, p. 1-7.

Arnao, M. B. 2000. Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. *Trends in Food Science and Technology*, vol. 11, no. 11, p. 419-421. https://doi.org/10.1016/S0924-2244(01)00027-9

Bello, A., Aliero, A. A., Saidu, Y., Muhammad, S. 2011. Phytochemical screening, polypenolic content and Alpha-gluosidase inhibitory potential of *Lepatadenia mastata* (Pers.). *Nigerian Journal of Basic and Applied Sciences*, vol. 19, no. 2, p. 181-186.

Blessing, M., Garuba, O., Auqustina, U., Oyedemi, S. 2010. Chemical composition of *Seasamum indicum* L. (sesame) grown in southeastern Nigeria and the physicochemical properties of the seed oil. *Seed Science and Biotechnology*, vol. 4, no. 1, p. 69-72.

Chu, Y., Sun, J., Wu, X., Liu, R. H. 2002. Antioxidant and antiproliferative activity of common vegetables. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 23, p. 6910-16. https://doi.org/10.1021/jf020665f PMid:12405796

Cunha, F. A. B., Waczuk, E. P., Duarte, A. E., Barrosa, L. M., Elekofehinti, O. O., Matias, E. F. F., da Costa, J. G. M., Sanmi, A. A., Boligon, A. A., da Rocha, J. B. T., Souza, D. O., Posserb, T., Coutinho, H. D. M., Franco, F. L., Kamdem, J. P. 2016. Cytotoxic and antioxidative potentials of ethanolic extract of *Eugenia uniflora* L. (*Myrtaceae*) leaves on human blood cells. *Biomedicine & Pharmacotherapy*, vol. 84, p. 614-621. https://doi.org/10.1016/j.biopha.2016.09.089 PMid:27694006

Ellman, G. L., Courtney, K. D., Andres, V., Featherstone, R. M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, vol. 7, no. 2, p. 88-95. https://doi.org/10.1016/0006-2952(61)90145-9

Enujigha, V. N., Talabi, J. Y., Malomo, S. A., Olagunju, A. I. 2012. DPPH Radical scavenging capacity of phenolic

extracts from Arican Yam bean (*Sphepostylis stenocarpa*). *Food and nutrition sciences*, vol. 3, no. 1, p. 7-13. https://doi.org/10.4236/fns.2012.31002

Enujigha, V. N. 2010. The antioxidant and free radicals scavenging capacity of phenolics from African locust bean seeds. *Advances in food sciences*, vol. 32, no. 2, p. 88-93.

Kwon, Y. I., Apostolidis, E., Kim, Y. C., Shetty, K. 2007. Health benefits of traditional corn, beans and pumpkin: In vitro studies for hyperglycemia and hypertension management. *Journal of Medicinal Foods*, vol. 10, no. 2, p. 266-275. https://doi.org/10.1089/jmf.2006.234 PMid:17651062

Marin, A., Ferreres, F., Tomas-Barberan, F. A., Gil, M. I. 2004. Characterization and Quantitation of Antioxidant Constituents of Sweet Pepper (*Capsicum annuum* L.). *Journal of Agricurtural and Food Chemistry,* vol. 52, no. 12, p. 3861-3869. https://doi.org/10.1021/jf0497915 PMid:15186108

McDonald, S., Prenzier, P. D., Autokiwich, M., Robards, K. 2001. Phenolics content and antioxidant activity of olive oil extracts. *Food Chemistry*, vol. 73, p. 73-84. https://doi.org/10.1016/S0308-8146(00)00288-0

Meda, A., Lamien, C. E., Romito, M., Millogo, J., Nacoulma, O. G. 2005. Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, vol. 91, no. 3, p. 571-577. https://doi.org/10.1016/j.foodchem.2004.10.006

Nagendra-Prasad, M. N., Sanjay, K. R., Prasad, D. S., Vijay, N., Nanjunda, S. S. 2012. Review on nutritional and nutraceutical properties of sesame. *Journal of Nutrition and Food Science*, vol. 2, no. 127, p. 996-1000.

Oboh, G., Akinyemi, J. A., Olasunkanmi, S. O., Idowu, S. O. 2014. Anticholinesterase and Antioxidative Properties of Aqueous Extract of *Cola acuminata* Seed *in Vitro*. *International Journal of Alzheimer's Disease*, vol. 2014, p. 1-8. https://doi.org/10.1155/2014/498629 PMid:25506036

Oboh, G., Puntel, R. L., Rocha, J. B. 2007. Hot pepper (*Capsicum annuum*, *Tepin* and *Capsicum Chinese, Hernero*) Prevent Fe^{2+}-induced lipid peroxidation in brain: *in vitro*. *Food Chemistry*, vol. 102, p. 178-185. https://doi.org/10.1016/j.foodchem.2006.05.048

Ojo, O. A., Oloyede, O. I., Olarewaju, O .I., Ojo, A. B., Ajiboye, B. O., Onikanni, S. A. 2013a. Toxicity Studies of the Crude Aqueous Leaves Extracts of *Ocimum gratissimum* in Albino Rats. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, vol. 6, no. 4, p. 34-39.

Ojo, O. A., Oloyede, O. I., Olarewaju, O. I., Ojo, A. B. 2013b. In Vitro Antioxidant Activity and Estimation of Total Phenolic Content in Ethyl Acetate Extract of *Ocimum gratissimum*. *PharmacologyOnline*, vol. 3, p. 37-44.

Ojo, O. A., Oloyede, O. I., Tugbobo, O. S., Olarewaju, O. I., Ojo, A. B. 2014. Antioxidant and inhibitory effect of scent leaf (*Ocimum gratissimum*) on Fe^{2+} and sodium nitroprusside induced lipid peroxidation in rat brain *in vitro*. *Advances in Biological Research*, vol. 8, no. 1, p. 8-17.

Ojo, O. A., Ojo, A. B., Ajiboye, B. O., Olayide, I., Fadaka, A. O. 2016a. *Helianthus annuus* Leaf Ameliorates Postprandial Hyperglycaemia by inhibiting carbohydrate hydrolyzing enzymes associated with Type-2 diabetes. *Iranian Journal of Toxicology,* vol. 7, no. 5, p. 17-22. https://doi.org/10.29252/arakmu.10.5.17

Ojo, O. A., Ajiboye, B. O., Olayide, I., Fadaka, A. O., Olasehinde, O. R. 2016b. Ethyl acetate fraction of bark of *Bridelia ferruginea* Benth. inhibits carbohydrate hydrolyzing enzymes associated with type 2 diabetes (*α*-glucosidase and *α*-amylase). *Advances in Bioresearch*, vol. 7, no. 3, p. 126-133.

Ojo, O. A., Ajiboye, B. O., Fadaka, A. O., Taro, P., Shariati, M. A. 2017a. Nrf2-Keap1 Activation, A Promising Strategy in the Prevention of Cancer. *Free Radicals and Antioxidants*, vol. 7, no. 1, p. 1-7. https://doi.org/10.5530/fra.2017.1.1

Ojo, O. A., Ajiboye, B. O., Ojo, A. B., Olayide, I. I., Akinyemi, A. J., Fadaka, A. O., Adedeji, E. A., Boligon, A. A., Anraku de Campos, MM. 2017b. HPLC-DAD fingerprinting analysis, antioxidant activity of phenolic extracts from *Blighia sapida* bark and its inhibition of cholinergic enzymes linked to Alzheimer's disease. *Jordan journal of Biological Sciences,* vol. 10, no. 4, p. 257-264.

Ojo, O. A., Ojo, A. B., Ajiboye, B. O., Oyinloye, B. E., Akinyemi, A. J., Okesola, M. A., Boligon, A. A., de Campos, M. M. 2018. Chromatographic fingerprint analysis, antioxidant properties, and inhibition of cholinergic enzymes (acetylcholinesterase and butyrylcholinesterase) of phenolic extracts from *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill bark. *Journal of Basic and Clinical Physiology and Pharmacology,* vol. 29, no. 2, p. 217-224. https://doi.org/10.1515/jbcpp-2017-0063

Orhan, B., Şener, M., Choudhary, I., Khalid, A. 2004. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. *Journal of Ethnopharmacology*, vol. 91, no. 1, p. 57-60. https://doi.org/10.1016/j.jep.2003.11.016 PMid:15036468

Pulido, R., Bravo, L., Saura-Calixto, F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J Agric. Food Chem.*, vol. 48, no. 8, p. 396-402. https://doi.org/10.1021/jf9913458

Puntel, R. L., Nogueira, C. W., Rocha, J. B. T. 2005. Krebs cycle intermediates modulate Thiobarbituric Acid Reactive Species (TBARS) production in rat brain *in vitro*. *Neurochemistry Research*, vol. 30, no. 2, p. 225-235. https://doi.org/10.1007/s11064-004-2445-7 PMid:15895826

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, p. 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Shai, L. J., Masoko, P., Mokgotho, M. P., Magano, S. R., Mogale, A. M., Boaduo, N., Eloff, J. N. 2010. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *South African Journal of Botany*, vol. 76, no. 3, p. 465-470. https://doi.org/10.1016/j.sajb.2010.03.002

Sharififar, F., Moshafi, M. H., Shafazand, E., Koohpayeh, A. 2012. Acetyl cholinesterase inhibitory, antioxidant and cytotoxic activity of three dietary medicinal plants. *Food Chemistry*, vol. 130, p. 20-23. https://doi.org/10.1016/j.foodchem.2011.06.034

Sharma, S., Gupta, P., Kumar, A., Ray, J., Aggarwal, B. K., Goyal, P., Sharma, A. 2014. In vitro evaluation of roots, seeds and leaves of *Sesamum indicum* L. for their potential antibacterial and antioxidant properties. *African Journal of Biotechnology*, vol. 13, no. 36, p. 3692-3701. https://doi.org/10.5897/AJB2013.12368

Shodeinde, A., Oboh, G. 2012. In vitro antioxidant activities and inhibitory effect of aqueous extracts of unripe plantain pulp (*Musa paradisiaca*) on enzymes linked with type 2

diabetes mellitus and hypertension. *Journal of Toxicology and Environmental Health Sciences*, vol. 4, no. 4, p. 65-75.

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PHYSICO-CHEMICAL STUDY OF FLAVONOIDS FROM DIFFERENT MATURENESS CORN SILK MATERIAL

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ABSTRACT

There was tested a simple extraction procedure of flavonoids separation from the original corn silk (CS) material. It was found, that the total flavonoids content differs with the extraction time and extraction temperature. There were found different flavonoids contents in extracts prepared from different maturity stages of the original corn silk material (silking stage (CS-S), milky stage (CS-M)). Extracted flavonoids content was quantified by the lutin standardization method by means of colorimetry at 510 nm wavelength. Obsreved flavonoids concentration was ranging from 2×10^{-3} mg.mL^{-1} to 7×10^{-3} mg.mL^{-1} dependent on the extraction time period and extraction temperature. The highest flavonoids concentration of 7.5×10^{-3} mg.mL^{-1} was found for CS-M after 20 minutes extraction time and 80 °C extraction temperature. There was confirmed the presence of flavonoids by fluorescence mapping experiments. There was found a typical multistep decomposition process for both CS-S and CS-M materials by TG analysis. There was found a melting temperature of flavonoids of 54.3 °C for corn silk silking stage material exhibiting 58.9 J.g^{-1} heat of fusion and 60.2 °C for corn silk milky stage material with 112.9 J.g^{-1} heat of fusion. The optimal conditions of corn silk flavonoids extraction were 40 °C, 50 minutes for CS-S, the optimal flavonoids extraction content was $(6.8\pm2.1)\times10^{-3}$ mg.mL^{-1}, 80°C, 20 minutes for CS-M and the optimal extraction content was $(7.2\pm0.3)\times10^{-3}$ mg.mL^{-1}.

Keywords: corn silk; silking stage; milky stage; flavonoids; UV-VIS

INTRODUCTION

Corn has a widespread application as a domastic animals feed, food additives and material of alcohol through fermentated or unfermentated technology **(Ivanišová et al., 2017; Michalová and Tančinová, 2017)**. Corn silk (CS) is the dried thrum and stigma of Zea mays L. (corn), cheap, high yielding and usually considered as a by-product to be abandoned, burned or used as fodder **(Zhang, 1998)**. It is one of the Chinese traditional medicine recorded in many classics. According to the Southern Yunnan Material Medicine and Chinese Medicine Dictionary, corn silk is non-poisonous, also diuretic, cholagogic and resolutive. Corn silk could be used to cure many kinds of diseases clinically, such as diabetes, nephritis and hypertension etc **(Jin, 1980)**. In addition, in terms of the results from the worldwide scientists, corn silk also has the effects of anti-fatigue **(Hu et al., 2010)**, anti-depression **(Mahmoudi and Ehteshami, 2010)**, anti-free radical, anti-cancer **(Ebrahimzadeh, Pourmorad and Hafezi, 2008; Maksimović and Kovačević, 2003)** and anti-radiation **(Bai et al., 2010)**. Native American Indians usually use corn silk to cure urinary tract infection, malaria and heart disease **(Hasanudin et al., 2012)**. In many countries, corn silk is applied to sell in markets as tea and weight-losing products for its good effect of cooling blood, purging heat and removing the damp and heat in human body.

The previous researches have successfully applied the fermentated corn fodder to improve the nutrition quality of chicken meat **(Angelovicová and Semivanová, 2013; Macanga et al., 2017; Štenclová et al., 2016)**. In addition, corn was also used to improve the sensory quality of crackers **(Kuchtová et al., 2016)**. The corn fermentated alcohol has a broad usage in food and chemistry industry, veterinary, pharmaceutical and manufacturing industry for its nutritional value and anti-oxidant properties **(Krejzová et al., 2017; Süli, Hamarová, and Sobeková, 2017)**.

Corn silk contains many sorts of nutritional and functional ingredients, including sterols, polysaccharides, alkaloids, flavones, cryptoxanthins, polyphenols, organic acids, vitamins and allantoins etc. **(Li and Lapcik, 2018)**

Corn silk flavonoids (CSF) are one of the most important sorts of nutrients in corn silk, which is not only a role of pigment but also the cause of the corn silk extracts antioxidant activity conditions **(Li and Lapcik, 2018)**. The total flavonoids extractive technology includes hot water, alkaline water or alkaline dilute alcohol and organic solvent extraction. Additionally, microwave, ultrasonic extraction, supercritical fluid extraction, enzyme, aqueous two-phase,

semi bionic extraction, membrane separation, thermal fluid extraction and high pressure liquid extraction **(Jing et al., 2016; Ko, Kwon and Chung, 2016; Shan et al., 2012; Wang et al., 2016; Wei et al., 2013; Weiz et al., 2016; Xie et al., 2017; Yang et al., 2017; Zhang, Shan and Gao, 2011; Zhou et al., 2011)**. **Peng et al. (2016)** applied 80 ℃ hot water extraction, the parity of CSF was 10.45% **(Peng, Zhang and Zhou, 2016)**; **Liu et al. (2005)** applied 50 ℃ 95% ethanol to extract CSF, the highest total CSF content was 69.4 ±5.1 μg RE.g^{-1} DCS (RE = rutin equivalents; DCS = dry mass of corn silk) **(Liu et al. 2011)**; **Liu et al. (2011)** used supercritical fluid to extract CSF and used BBD response surface methodology to analyse and optimize the extractive conditions. The maximal yield of CSF was approximately 4.24 mg.g^{-1}, the optimal conditions were 50.88 ℃, 41.80 MPa, 2.488 mL.g^{-1} water content in ethanol co-solvent, 120 min extractive time, 0.4 mm particle sizes and 20% aqueous ethanol as the co-solvent **(Liu et al., 2011)**.

UV-VIS method has been used to measure the content and determine the kinds of flavonoids in plants **(Liu et al., 2011)** but the research of extraction conditions optimization, flavonoids properties comparison and analysis and the determination of the flavonoids kind in different mature stages of corn silk is rarely reported. As found in the studies mentioned above applied extraction procedure has an effect on the total flavonoids content of corn silk material. We expect, that the maturity stage of the corn silk used as a source material for extraction will affect the total flavonoids content obtained as well. That is why, this research focuses on the evaluation of the extraction procedures, kinetics and stage of the corn maturity effects on the final composition of the extracted materials, allowing in more detail knowledge for the on going development of the novel types of nutrition or health care products both for human and veterinary applications.

Scientific hypothesis

In the current research, the content and sorts of flavonoids in plants have been measured and determined by UV-VIS and fluorescence techniques. However, the extraction conditions optimization, flavonoids properties comparison and analysis and the determination of the flavonoids kind in different mature stages of corn silk are rarely reported. We expect the maturity stage of the corn silk will be a significant effective factor for the total extracted flavonoids content. That is why, this research is focused on the quantification of the total flavonoids content extracted from different maturity stages of the corn silk at defined extraction conditions such as extraction time and temperature.

MATERIAL AND METHODOLOGY

Corn silk samples were collected from the corn kernels type dent produced in a field in Southern Moravia agricultural region (Uherské Hradiště County, Czech Republic). Fresh corn silk fibers were first 14 days dryied on air in the shade and then final drying was done in a thermostatic hot air-drying oven (Hot air sterillizator Stericell 55 Standard, BMT Medical Technology, Czech Republic), pulverized and sifted through a 80mesh sieve (Analysette 3, Fritsch, Germany) to obtain the final product powder samples. There were collected two types of corn silk materials, dependent on the growth stage. The first one was silking stage (assigned as CS-S), the second one was the milky stage (assigned as CS-M) **(Rahman and Wan Rosli, 2014; Sarepoua et al., 2015)**.

All reagents and chemicals used in this research such as rutin, ethanol, sodium nitrite, aluminium nitrate and

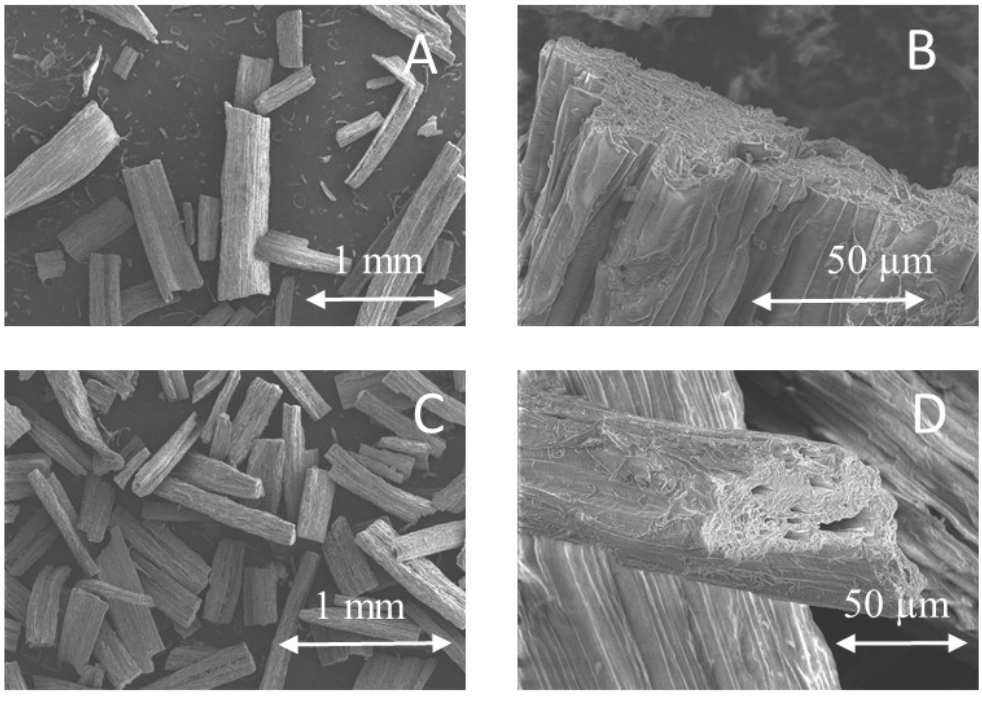

Figure 1 SEM images of the tested corn silk material: A,B – sample CS-S, C,D – sample CS-M.

sodium hydroxide were purchased from Sigma-Aldrich (USA) in an analytical reagents purity grade. As a solvent distilled water was used. Distilled water conductivity was about 0.6 $\mu S.cm^{-1}$).

UV/VIS spectrophotometer used was Lambda 25 (Perkin Elmer, MA, USA). Measurements were performed in the wavelength range from 200 to 700 nm in 1 cm quartz cells (Marques et al., 2013).

Thermogravimetry (TG) and differential thermal analysis (DTA) experiments were performed on simultaneous DTA-TG apparatus (Shimadzu DTG 60, Japan). Measurements were performed at heat flow rate of 5 °C.min^{-1} in the static nitrogen atmosphere (gas flow of 50 mL.min^{-1}) at the temperature range from 30 °C to 550 °C. The apparat was calibrated using Indium as a standard **(Liu et al., 2005; Wu et al., 2008)**.

Fluorescence excitation−emission maps of the different maturity stages corn silk extracts were measured on a FLS980 fluorescence spectrometer (Edinburgh Instruments, UK). Each experiment was repeated 10 times.

Samples were pulverized in a table top blender (Philips HR2170/40, The Netherlands).

Rutin standard curve determination procedure **(Peng et al., 2016)**: disolve 20 mg lutin into 70 v.% ethanol to 50 mL (0.4mg.mL^{-1} lutin solution); separately were brought 0, 1, 2, 4, 6, 8, 10 mL 0.4mg.mL^{-1} lutin standard solutions into 50 mL volumetric flasks, added 70% ethanol 12 mL, then added 2 mL 5 w.% $NaNO_2$, shaked up and placed for 10 min to react. Then into the solutions were added 2 mL 10 w.% $Al(NO_3)_3$, shaked up and placed for 10min to react, then diluted with 20 mL 10 w.% NaOH to the scale of volumetric flask, placed for 5 min. Each experiment was repeated 5 times. There was used 510 nm UV spectrometry to measure the absorbance of the solutions. Obtained absorbance vs. concentration dependency data were used to build up the standard curve. The numerical linear regression analysis was performed to obtain standard curve lineasr regression parameters. Each experiment was repeated 3 times.

Determination of the flavonoids content procedure **(Peng et al., 2016)**: Use the 1/10 solid-liquid ratio of cornsilk powder and 70 v.% ethanol to extract the flavonoids in temperatures of 40°C and 80°C for 20, 30, 40, 50, 60 minutes extraction time intervals. Then the flavonoids extract solutions were centrifuged on Hettich EBA 21 centrifuge (Germany) at 3000 rpm for 10 min to get the supernatant. Then there was used the same methodology as lutin standard curve to measure the flavonoids absorbance and there was used the lutin standard curve to count the given content of flavonoids. Each experiment was repeated 5 times.

STATISIC ANALYSIS

Statistical analysis of the observed data were performed by using one way analysis of variance (ANOVA) method (Microsoft Excel, USA). This analysis allowed to detect the significance of the effect of extraction time, temperature and the maturity stage on extracted amounts of flavonoids. Five extraction times, two maturity stages and two extraction temperatures were considered in this study. Each experiment was replicated 5 times. Differences were considered significant at $p \leq 0.05$. Additionally, the mean values and standard errors were calculated from all measurements by application of the SigmaPlot 8.0 software (SPSS, USA). Differencies between obtained emission peaks located at 450 nm were analyzed by one-way analysis of variance (ANOVA) method (Origin 8.5.0 software was used (OriginLab, USA)). Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

In Figure 1 there are shown SEM images of the tested corn silk powders. They are characteristic with the rectangular shape of individual particles exhibiting complex

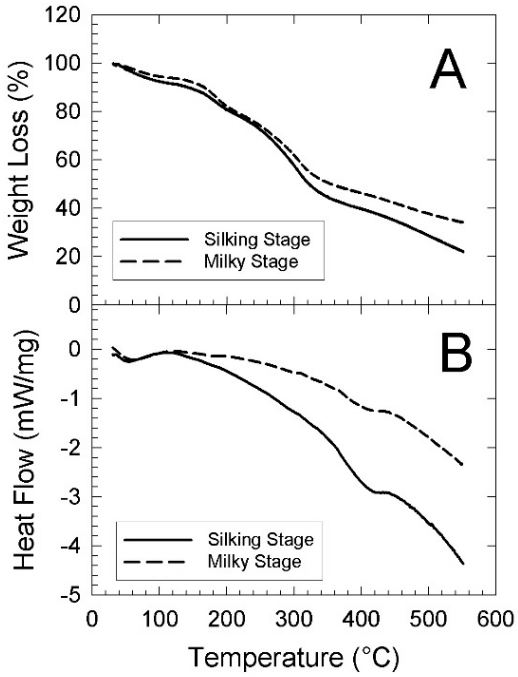

Figure 2 Results of the thermal analysis of the tested corn silk samples: A – Thermogravimetry (TG), B – differential thermal analysis (DTA) data.

microporous structure on the intersection. Such structures are typical for plants cellulose-based materials.

Prior to the extraction, the moisture content and thermoal analysis of the samples was performed. Results of TG and DTA analysis are shown in Figure 2. These are typical of multistep decomposition process for both CS-S and CS-M samples as shown in Figure 2A. The first step decomposition for sample CS-S was found in the temperature range of 30 to 120 °C with observed weight loss being 8.3% attributed to the moisture content. Total decomposition step was about 77.45% in the temperature range of 30 to 550 °C. Similarly, for the sample CS-M TG

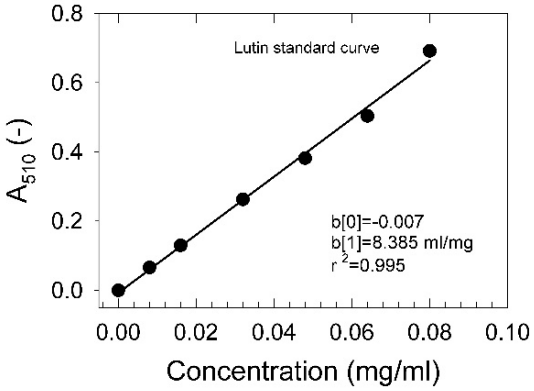

Figure 3 Lutin standard curve. Inset: Linear regression standard curve parameters.

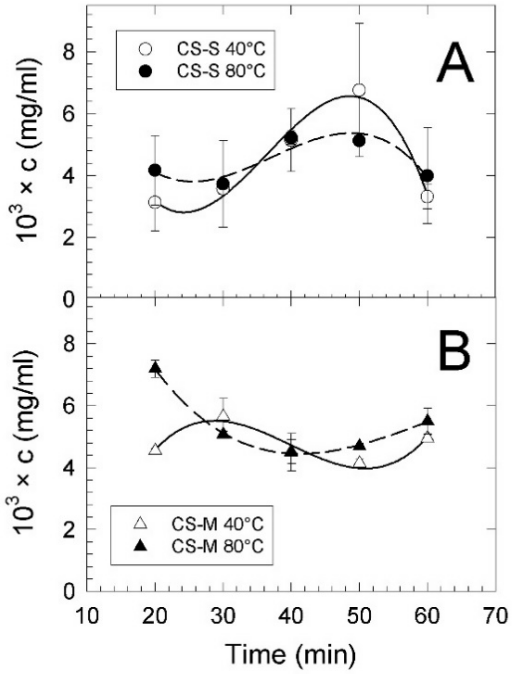

Figure 4 Flavonoids extraction kinetics: A – corn silk silking stage, B – corn silk milky stage. Values for CS-S 40 °C, CS-M 40 °C and CS-M 80 °C were considered significantly different ($p \leq 0.05$). Values for CS-S 80 °C were considered not significantly different ($p \geq 0.3$).

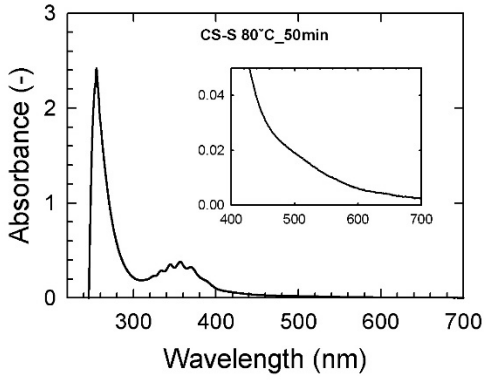

Figure 5 UV VIS spectrum of the CS-S sample extracted at 80 °C temperature after 50 min extraction time. Inset: expanded 400 nm to 700 nm region.

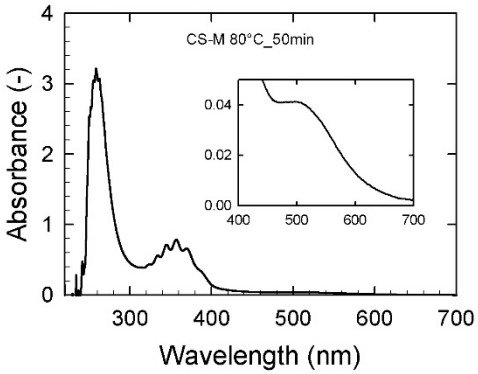

Figure 6 UV VIS spectrum of the CS-M sample extracted at 80 °C temperature after 50 min extraction time. Inset: expanded 400 nm to 700 nm region.

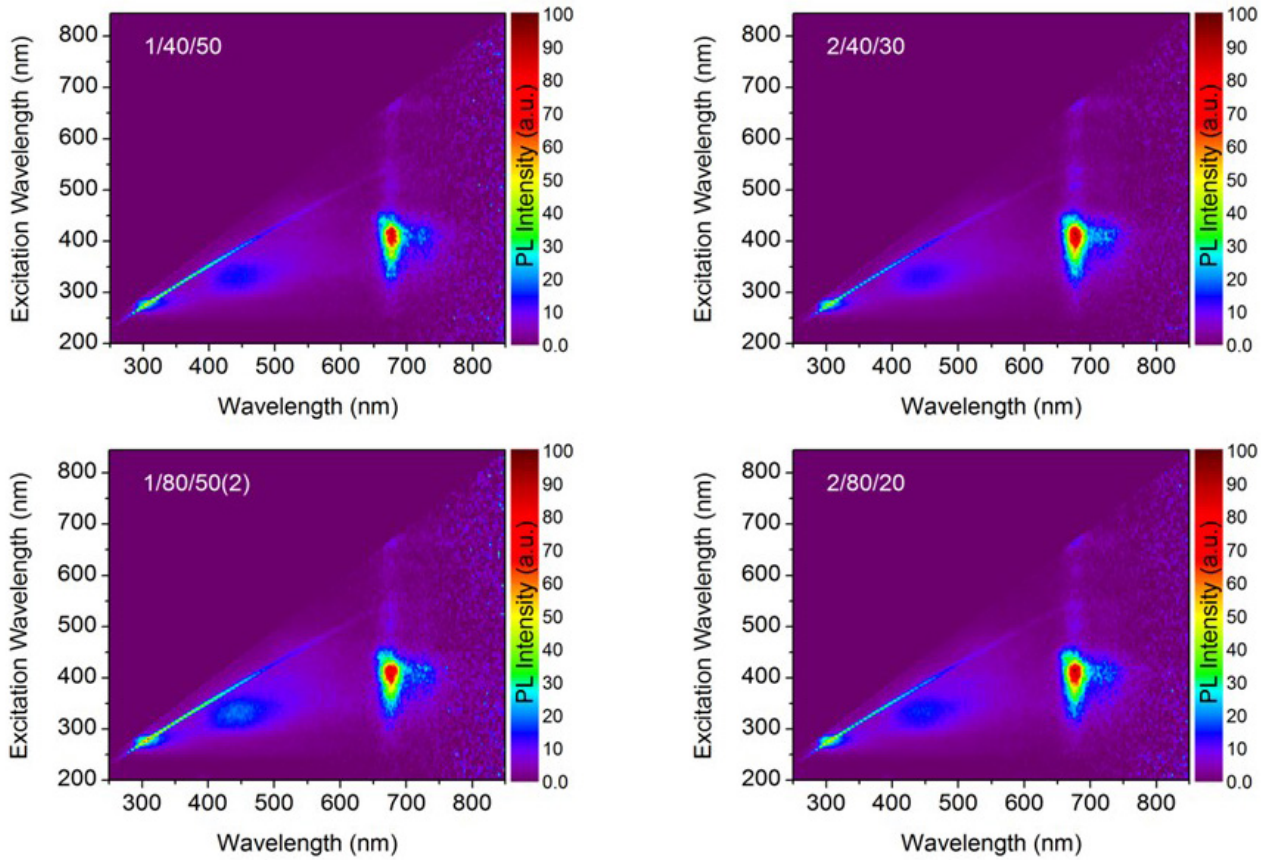

Figure 7 Results of the fluorescence excitation-emmision mapping of the studied corn silk extracts.
Note: Inset legend X/Y/Z: X=1 corresponds to CS-S sample, X=2 corresponds to CS-M sample, Y is the extraction temperature (40 °C or 80 °C), Z is extraction time in minutes (20, 30 or 50 minutes).

data exhibited the first step decomposition of 5.9% attribute to the moisture content in the same temperature range of 30 to 120 °C followed by the total weight loss step of 65.5% in the temperature ramge of 30 to 550 °C indicating, that the CS-S contains more thermally labile substances in comparison to CS-M samples. In Figure 2B there are shown two endothermic peaks. The first one located in the temperature of 54.3° for CS-S and of 60.2 °C for CS-M attributed to the melting point of flavonoids (Miziara et al. 2017). The second endothermic peaks observed at 415.1 °C (CS-M) and 419.7 °C (CS-S) were attributed to the total thermal decomposition with formation of a low quantity carbonaceous residues respectively. The heat of fusion corresponding to the first endothermic peak was of 58.9 $J.g^{-1}$ for CS-S and of 112.9 $J.g^{-1}$ for CS-M samples.

In Figure 3 there is shown a typical lutin standard curve as obtained according to the standard procedure described in detail in the materials and methodology section. Obtained regression parameters are given as the Figure 3 inset as well. Obtained data were of a high correlation as indicated by the correlation coefficient being 0.995.

Effects of the extraction time and of the extraction temperature are summarized in Figure 4, where kinetics data of the extraction of CS-S and CS-M samples are plotted

in flavonoids concentration vs. extraction time coordinates. Each extraction was performed at two different temperature, the first one was of 40 °C and the second one at 80 °C. Both, the CS-S as well as the CS-M dependencies were of a complex non-linear character, modeled as a third order polynomial dependency. However, in the case of CS-S, for both extraction temperatures the same characteristic sinusoidal pattern was found exhibiting the maximum extraction efficiency at 50 °C. Observed concentrations were of about 6.5×10^{-3} mg.mL^{-1} for CS-S extracted at 40 °C and of about 5×10^{-3} mg.mL^{-1} for CS-M extracted at 80 °C indicating, that the extracted flavonoids substances are sensitive on thermal history and are undergoing thermal decomposition similarly as reported by **Chaaban et al. (2017)**. In the latter paper, the linear degaradtion pattern was found for rutin at 70 °C degradation temperature. With increasing degradation temperature up to 130 °C the exponential decay pattern was found. In the case of CS-M, the maximum extraction efficiency was found at 30 minutes extraction time being of 5.8×10^{-3} mg.mL^{-1} at 40 °C extraction temperature. However, at higher temperature, the degradation of the flavoinoids content was observed, and only the exponential decay pattenr was found as indicated in Figure 4B. That is why, the maximum extraction was found at 20 minutes extraction time at 80 °C extraction temperature. It was found, that the highest extracted flavonoids content was $(7.2 \pm 0.3) \times 10^{-3}$ mg.mL^{-1} for CS-M 80°C sample. However for the CS-S the highest content was found of $(6.8 \pm 2.1) \times 10^{-3}$ mg.mL^{-1} for CS-S 40 °C sample. To characterize obtained extracts of flavonoids, the UV VIS as well as fluorescence spectra were measured as shown in Figures 5 to 7. Prior to the fluorescence mapping analysis of the studied corn silk extracts, the UV VIS spectra were recorded.

These were typical with three major absorption regions at 260 nm and 360 nm (near ultraviolet region), and at visible light region of 490 nm. The absorption of electromagnetic radiation in the near ultraviolet region is typical for poly-unsaturated and aromatic compounds such as flavonoids. Both CS-S as well as CS-M exhibited similar UV VIS spectra except the visible range region, where a 480 nm shoulder peak occurred for CS-M sample.

Results of the fluorescence excitation-emmision mapping of the studied extracts are shown in Figure 7. These are characteristic similarly as the UV VIS absorption spectra with the three distinct fluorescence enission regions at 320 nm, 450 nm and 680 nm. Emmision region located at 450 nm was ascribed to the flavonoids compounds similarly as observed by **Shan et al. (2017)**, who found, that the flavonols characteristic excitation/emission spectral range is 365 – 390 nm/450 – 470 nm. The excitation/emission spectral range of 480 – 500 nm/510 – 520 nm was ascribed to flavanols.

There were found three distinct excitation wavelengths regions at about 275 nm, 350 nm and 420 nm. Obtained results indicate, that the major difference between CS-S and CS-M is in the fluorescence emission centered at the 450 nm region, where the intensity of the fluorescence emission was highest for CS-S extracted at 80 °C for 50 minutes. Furthermore, there was found that the fluorescence emmision intensity region located at 450 nm region was of higher intensity for CS-S in comparison to CS-M at 80 °C extraction temperature. Observedresults were considered as statistically significant ($p \leq 0.05$). These results are in an excellent correspondence with the TG analysis, where the first step decomposition weight loss was found higher for CS-S in comparison to CS-M. However, there was not found any major difference between fluorescence emmission intensities located at 320 nm region for all studied materials.

CONCLUSION

There was confirmed in this study, that it is possible to obtain flavonoids from the studied corn silk material by a simple extraction procedure. It was found, that the total flavonoids content differs with the extraction time and extraction temperature. There were found different flavonoids contents in extracts prepared from different maturity stages of the original corn silk material (silking stage, milky stage). Extracted flavonoids content was quantified by the lutin standardization method by means of colorimetry measurements at 510 nm wavelength. Obsreved flavonoids concentrations were ranging from 2×10^{-3} mg.mL^{-1} to 7.5×10^{-3} mg.mL^{-1} dependent on the extraction time period and extraction temperature. The highest concentration of flavonoids of 7.2×10^{-3} mg.mL^{-1} was found for CS-S after 50 minutes extraction time and 40°C extraction temperature. By fluorescence mapping experiments, there was confirmed the presence of the flavonoids by the appearance of the characteristic fluorescence emission region at 450 nm. There was found a typical multistep thermal decomposition process for both CS-S and CS-M materials.

REFERENCES

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. https://doi.org/10.5219/297

Chaaban, H., Ioannou, I., Chebil, L., Slimane, M.,Gérardin, Ch., Paris, C., Charbonel, C., Chekir, L., Ghould, M. 2017. Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. *Journal of Food Processing and Preservation*, vol. 41, no. 5, p. 1-12. https://doi.org/10.1111/jfpp.13203

Ebrahimzadeh, M. A., Pourmorad, F., Hafezi, S. 2008. Antioxidant activities of iranian corn silk. *Turkish Journal of Biology*, vol. 32, no. 1, p. 43-49.

Hu, Q. L, Zhang, L. J, Li, Y. N, Ding, Y. J, Li, F. L. 2010. Purification and anti-fatigue activity of flavonoids from corn silk. *International Journal of Physical Science*, vol. 5, no. 4, p. 321-326.

Ivanišová, E., Rajtar, M., Francáková, H., Tokár, M., Dráb, Š, Kluz, M., Kacániová, M. 2017. Characterization of bioactive compounds from monascus purpureus fermented different cereal substrates. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 183-189. https://doi.org/10.5219/722

Jin, Y. Y. 1980. Chinese Medicine Dictionary. Jiangsu : People's Medical Publishing House, Jiangsu New Medical College, p. 555.

Jing, S., Wang, S., Li, Q., Zheng, L., Yue, L., Fan, S., Tao, G. 2016. Dynamic high pressure microfluidization-assisted extraction and bioactivities of cyperus esculentus (*C. esculentus* L.) leaves flavonoids. *Food Chemistry*, vol. 192, p.

319-327. https://doi.org/10.1016/j.foodchem.2015.06.097 PMid:26304354

Ko, M., Kwon, H., Chung, M. 2016. Pilot-scale subcritical water extraction of flavonoids from satsuma mandarin (citrus unshiu markovich) peel. *Innovative Food Science and Emerging Technologies*, vol. 38, p. 175-181. https://doi.org/10.1016/j.ifset.2016.10.008

Krejzová, E., Bittová, M., Krácmar, S., Vojtíšková, P., Kubán, V., Golian, J. 2017. Effect of thermal treatment on rutin content in selected buckwheat products using calcium as an internal tracer. *Potravinsrstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 679-684. https://doi.org/10.5219/853

Kuchtová, V., Minarovicová, L., Kohajdová, Z., Karovicová, J. 2016. Effect of wheat and corn germs addition on the physical properties and sensory qualityof crackers. *Potravinsrstvo Slovak Journal of Food Science*, vol. 10, no. 1, p. 543-549. https://doi.org/10.5219/598

Li, P., Lapcik, L. 2018. The Research of Functional Ingredients from Corn Silk (in Czech). *Chemické listy*, vol. 112, no. 2, p. 1-5.

Liu, J., Lin, S., Wang, Z., Wang, C., Wang, E., Zhang, Y., Liu, J. 2011. Supercritical fluid extraction of flavonoids from maydis stigma and its nitrite-scavenging ability. *Food and Bioproducts Processing*, vol. 89, no. 4, p. 333-339. https://doi.org/10.1016/j.fbp.2010.08.004

Liu, J., Wang, C., Wang, Z., Zhang, C., Lu, S., Liu, J. 2011. The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (*Zea mays* L.) and related flavone glycosides. *Food Chemistry*, vol. 126, no. 1, p. 261-269. https://doi.org/10.1016/j.foodchem.2010.11.014

Liu, Q., Lv, C., Yang, Y., He, F., Ling, L. 2005. Study on the pyrolysis of wood-derived rayon fiber by thermogravimetry – mass spectrometry. *Journaol of moleular Structure*, vol. 733, no. 1-3, p. 193-202.

Macanga, J., Popelka, P., Koréneková, B., Maskalová, I., Klempová, T., Feckaninová, A, Marcincák, S. 2017. Effect of feeding of prefermented bioproduct containing gamma-linolenic acid and beta-carotene on selected parameters of broiler chicken meat quality. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 466-471. https://doi.org/10.5219/781

Mahmoudi, M., Ehteshami, S. 2010. P.2.d.002 antidepressant activity of iranian corn silk. European Neuropsychophrmacology, vol. 20, suppl. no. 3, p. 159-676. https://doi.org/10.1016/S0924-977X(10)70556-3

Maksimović, Z. A., Kovačević, N. 2003. Preliminary assay on the antioxidative activity of maydis stigma extracts. *Fitoterapia*, vol. 74, no. 1-2, p. 144-147. https://doi.org/10.1016/S0367-326X(02)00311-8

Marques, G. S., Leão, W. F., Lyra, M. A. M., Peixoto, M. S., Monteiro, R. P. M., Rolim, L. A., Soares, L. A. L. 2013. Comparative evaluation of UV/VIS and HPLC analytical methodologies applied for quantification of flavonoids from leaves of bauhinia forficata. *Revista Brasileira de Farmacognosia*, vol. 23, no. 1, p. 51-57. https://doi.org/10.1590/S0102-695X2012005000143

Michalová, V., Tančinová, D. 2017. Growth of microorganisms in the pre-fermentation tanks in the production of ethanol. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 529-534. https://doi.org/10.5219/771

Miziara, L., Ferreira, B., Kobelnik, M., Regasini, L. O., Dutra, L. A., Bolzani da Silva, V., Ribeiro, C. A. 2017. Synthesis and evaluation of the thermal behavior of flavonoids. Thermal decomposition offlavone and 6-hydroxyflavone. *Journal of Thermal Analysis and Calorimetry*, vol. 127, no. 2, p. 1605-1610. https://doi.org/10.1007/s10973-016-5896-6

Peng, K., Zhang, S., Zhou, H. 2016. Toxicological evaluation of the flavonoid-rich extract from maydis stigma: Subchronic toxicity and genotoxicity studies in mice. *Journal of Ethnopharmacology*, vol. 192, p. 161-169. https://doi.org/10.1016/j.jep.2016.07.012 PMid:27396347

Rahman, N. A., Wan Rosli, W. I. 2014. Nutritional compositions and antioxidative capacity of the silk obtained from immature and mature corn. *Journal of King Saud University – Science*, vol. 26, no. 2, p. 119-127.

Sarepoua, E., Tangwongchai, R., Suriharn, B., Lertrat, K. 2015. Influence of variety and harvest maturity on phytochemical content in corn silk. *Food Chemistry*, vol. 169, p. 424-429. https://doi.org/10.1016/j.foodchem.2014.07.136 PMid:25236247

Shan, J. J., Wang, X., Russel, M., Zhao, J. 2017. Rapid determination of flavonoids in green tea by synchronous fluorescence spectra copled with chemometrics. *Spectroscopy Letters*, vol. 50, no. 9, p. 501-506. https://doi.org/10.1080/00387010.2017.1368028

Shan, B., Xie, J., Zhu, J., Peng, Y. 2012. Ethanol modified supercritical carbon dioxide extraction of flavonoids from momordica charantia L. and its antioxidant activity. *Food and Bioproducts Processing*, vol. 90, no. 3, p. 579-587. https://doi.org/10.1016/j.fbp.2011.09.004

Štenclová, H., Karásek, F., Štastník, O., Zeman, L., Mrkvicová, E., Pavlata, L. 2016. The effect of reduced zinc levels on performance parameters of broiler chickens. *Potravinarstvo*, vol. 10, no. 1, p. 272-275. https://doi.org/10.5219/580

Süli, J., Hamarová, I., Sobeková, A. 2017. Possible consequences of the sucrose replacement by a fructose-glucose syrup. P*otravinarstvo*, vol. 11, no. 1, p. 425-430. https://doi.org/10.5219/772

Wang, R., Wu, G., Du, L., Shao, J., Liu, F., Yang, Z, Wei, Y. 2016. Semi-bionic extraction of compound turmeric protects against dextran sulfate sodium-induced acute enteritis in rats. *Journal of Ethnopharmacology*, vol. 190, p. 288-300. https://doi.org/10.1016/j.jep.2016.05.054 PMid:27286916

Wei, M., Yang, Y., Chiu, H., Hong, S. 2013. Development of a hyphenated procedure of heat-reflux and ultrasound-assisted extraction followed by RP-HPLC separation for the determination of three flavonoids content in scutellaria barbata D. don. *Journal of Chromatography B*, vol. 940, p. 126-134. https://doi.org/10.1016/j.jchromb.2013.09.015

Weiz, G., Braun, L., Lopez, R., de María, P. D., Breccia, J. D. 2016. Enzymatic deglycosylation of flavonoids in deep eutectic solvents-aqueous mixtures: Paving the way for sustainable flavonoid chemistry. *Journal of Molecular Catalysis B: Enzymatic*, vol. 130, p. 70-73. https://doi.org/10.1016/j.molcatb.2016.04.010

Wu, Q., Pan, N., Deng, K., Pan, D. 2008. Thermogravimetry-mass spectrometry on the pyrolysis process of lyocell fibers with and without catalyst. *Carbohydrate Polymers*, vol. 72, no. 2, p. 222-228. https://doi.org/10.1016/j.carbpol.2007.08.005

Xie, X., Zhu, D., Zhang, W., Huai, W., Wang, K., Huang, X, Fan, H. 2017. Microwave-assisted aqueous two-phase extraction coupled with high performance liquid chromatography for simultaneous extraction and determination of four flavonoids in crotalaria sessiliflora L. *Industrial Crops and Products*, vol. 95, p. 632-642. https://doi.org/10.1016/j.indcrop.2016.11.032

Yang, R., Geng, L., Lu, H., Fan, X. 2017. Ultrasound-synergized electrostatic field extraction of total flavonoids from hemerocallis citrina baroni. *Ultrasonics Sonochemistry*, vol. 34, p. 571-579. https://doi.org/10.1016/j.ultsonch.2016.06.037 PMid:27773282

Zhang, L. T. 1998. Industrial application of Corn. *Starch and Starch Sugar*, vol. 2, p. 1-3.

Zhang, Y., Shan, X., Gao, X. 2011. Development of a molecularly imprinted membrane for selective separation of flavonoids. *Separation and Purification Technology*, vol. 76, no. 3, p. 337-344. https://doi.org/10.1016/j.seppur.2010.10.024

Zhou, T., Xiao, X., Li, G., & Cai, Z. 2011. Study of polyethylene glycol as a green solvent in the microwave-assisted extraction of flavone and coumarin compounds from medicinal plants. *Journal of Chromatography A*, vol. 1218, no. 23, p. 3608-3615. https://doi.org/10.1016/j.chroma.2011.04.031 PMid:21531418

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FOOD SAFETY FROM A CONSUMERS´POINT OF VIEW: FOOD QUALITY

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ABSTRACT

Food safety is a topic that is currently very much and often discussed. This may be a debate among political representatives, representatives of the food industry, but also among consumers, ie. general public. The issue of food safety and quality is very important in view of the growing globalization of economy, whose mission is to encourage food businesses to improve the production process as a whole and competitiveness. As in every sector, the food market faces many problems arising from market opening, business environment diversity or non-compliance with legislative requirements. The effects of these market imperfections are ultimately borne by the consumer. It is, therefore, appropriate to ensure that consumers are adequately informed about the food they consume. Food production is carried out according to European and world standards. A Slovak customer purchases food imported from abroad. This fact can be caused by the pricing of individual foods but also by the lack of Slovak producers. Foreign competition liquidates the quality Slovak producers. The principle of customers should be to support the economy of the state and to buy domestic food. The submitted document deals with issues of food safety, control and quality of food. The aim of the work was to examine the attitudes of consumers to food safety based on the acquired knowledge and research results, to identify their interest in food safety. It also involved identifying global food safety issues and analyzing consumers' views on the problem under consideration and its impact on their purchasing behavior. Primary data was obtained from a survey that was performed on a sample of 478 respondents. Based on the survey, it was confirmed that 85% of respondents perceive the different quality of the food sold on the Slovak market. Nearly two-thirds of the respondents said they were paying attention to the quality of the groceries. More than half of respondents expressed satisfaction with hygienic sales conditions. Almost 80% think that high-quality food is commonly available. Statistical testing has confirmed the significantly lower quality of food produced abroad. Other assumptions were formulated for more detailed analysis and their relationships were verified by using the statistical methods (Friedman Test, Chi-Square Test of Independence, Wilcoxon Signed-Rank Test).

Keywords: food; food safety; food quality; food risk; consumer

INTRODUCTION

The basic need of every person is food intake, and therefore, this term is included in personal security, which also includes food safety and food control. **Act 215/2004 Coll. Section 6 on the Protection of Classified Information and the Amendment of Certain Laws** defines personnel security as a system of measures relating to the selection, identification and control of persons who may, to a certain extent, be informed of classified information. Food control is an integral part of today's rapidly evolving world **(Suchánek, Richter, Králová, 2017)**. Recently, the consumer is increasingly confronted with the term quality. Through the quality is determined and selected the food that customers consume. If the basic hygiene rules are not observed, either in production, storage, transport or in the actual purchase and processing of food, their non-compliance can lead to very serious consequences for human health **(Zhang et al., 2017; Yu et al., 2017)**.

Food quality is associated with a set of all the important features that are sensory sensed but also with features that the consumer does not need to register at all before consuming or consuming food. Individual attributes of food quality are laid down by legislation. However, a number of other properties and criteria of food quality are specified by the manufacturer and are given in the specification of the food product concerned. Food safety criteria are the most important subgroup for food quality assessment. However, the quality and safety of food are two terms that can not be confused **(Trade Union of the Slovak Republic)**.

Foods are a set of substances needed to grow, restore and maintain the body's functions. Regular feeding is very important for the population because it supplies the organism with all necessary nutrients **(Michealidou and Hassan, 2007)**.

Food consumption pertains to all inhabitants, reflecting the socio - economic conditions of people's lives, traditions, culture and overall living standards. It is important to direct the attitudes of man and the whole society towards the rational consumption and nutrition of individual types of food, in order to make food production and consumption more efficient and to reduce the threat to the health of the population **(Holienčinová, 2013, Gorris, 2005)**. One of the most important food quality requirements is their safety, i. food should not endanger human health **(Shang and Tonsor, 2017; Stecova and Popelka, 2005)**. Safety, in this case, means the health and hygiene of food safety **(Nijage et al., 2018; Golian and Sokol, 2005)**. Hygienic harmlessness is understood to mean a food whose production is adhered to and approved, and hygiene standards, that is, that it is suitable for human consumption **(Kind et al., 2017; Pavelková and Bobková, 2008)**. Healthy food refers to foods that do not contain pathogens. Food substances should not exceed a dose that could cause disease in humans **(Boeck et al., 2018)**. Conversely, pathogenic foods are those that contain inappropriate chemical composition, inappropriate properties (debilitation, unknown origin) and also contain poisonous and harmful substances. Such foods pose a tremendous risk to human health **(Trienekens and Zuurbier, 2008; Shi and Zhu, 2009)**. Food shall not be placed on the market unless it is safe for health. Health security is also very closely related to the concept of food quality **(Marotta, Simeone and Nazzaro, 2014)**. Quality is nowadays very often inflated. This was due in particular to a different quality of food of the same brand. These foods are exported from abroad all over the world. The Food Code of the Slovak Republic defines quality as a summary of certain binding properties and product features that are primarily intended to meet the specific needs of individual consumers. In the food market, we are experiencing greater sensitivity of consumers to the required quality of products **(Kubicová and Kádeková, 2012)**. However, quality is a much broader term that includes other qualitative criteria and characters **(Pable, Lu and Auerbach, 2010)**. These features can be determined by the manufacturer himself. According to **Loureiro and Umberger (2007)**, the quality is a sum of properties, characteristics and features that contribute to a greater degree of satisfaction of identified or anticipated needs **(Grunert and Aachmann, 2016)**. Quality includes the composition of foods that are determined by their nutritional value **(Chrysochou, Krystallis and Giraud, 2012)**. These benefits affect the overall value of the product that has become the basis for determining the market price as well as the sensory characteristics of the food in question. We can say that the better the composition of food, the higher the price **(Rompay, Deterink and Fenko, 2016)**. The relationship of quality and price is then reflected in a marketing strategy that the manufacturer or seller has to identify clearly **(Kubicová and Kádeková, 2017)**.

Another level of quality characterizes the already mentioned sensory properties of foods that contribute to re-purchase **(Waldman and Kerr, 2018)**. Sensory properties serve to enable the consumer to process perceived information **(Duncan, 2011; Loutfi et al., 2015)**. Consumers choose and evaluate the foods they consume through these features **(Krishna, 2013; Géci, Nagyová and Rybanská, 2017)**. The evaluation is based on the use of individual sensory organs **(McFadden and Huffman, 2017)**. Sensory organs mean five basic senses: vision, smell, taste, hearing and touch **(Lindstrom, 2006)**. The term consumer includes everyone who buys products or services for their needs **(Lee and Yun, 2015)**. Its behavior may be affected by a number of factors such as: a mark, price or packaging **(Magnier, Schoormans and Mugge, 2016; Feldmann and Hamm, 2015)**. Consumer behavior is characterized as a course of behavior of organizations, groups and individuals in the market of products and services **(Kozelová et al., 2011)**. Where the main goal is to meet the needs of high-quality, hygienic and health-friendly products **(Nagyová, Berčík and Horská, 2014)**. The market is showing more and more often that the customer becomes demanding and is willing to pay a higher price for quality food **(Kubicová and Kádeková, 2011)**.

MATERIAL AND METHODOLOGY

The aim of the paper is to examine the attitudes of consumers to the health safety of food produced and sold in the Slovak market. In order to meet the stated objective, a questionnaire survey was conducted in the territory of the Slovak Republic in the months October - December 2017, involving 478 respondents of different age categories. Secondary information has become available to the public as well as scientific and professional publications of domestic and foreign authors dealing with the issues addressed. Questions in the questionnaire were divided into two groups - nine classification questions and 12 questions related to the food quality. Potential respondents received a questionnaire in a paper form. After completing, all correctly filled questionnaires were transformed into the Google Forms internet application.

Primarily, the information obtained through the questionnaire survey was processed by statistical methods – Friedman test which is a non-parametric alternative to the repeated measures ANOVA where the assumption of normality is not acceptable. Usually it is used in case of ordinal dependent variable. This occurs especially in case of questioner survey, when each respondent assesses more than two products using the same scale. In case of Friedman test applications should be met following conditions: One group that is measured on three or more different occasions – group is a random sample from the population, dependent variable should be measured at the ordinal or continuous level and samples do not need to be normally distributed. The non-parametric post-hoc test called Nemenyi test which is based on the Kruskal-Wallis method of ranking in a one-way classification and Chi-Square test of Independence to investigate relationship between categorical variables.

The established hypotheses were verified by appropriate mathematical – statistical methods that enabled the hypothesis to be confirmed or rejected.

The probability level is determinedon the base of alpha ($\alpha = 0.05$), which is compared with the significance level (*p*-value). Based on alpha (α), we can evaluate the hypothesis with the p-value comparison. If p-value is lower than alpha (α), H_0 will berejected. If p-value is higher than alpha (α), H_0 will be accepted.

Table 1: Characteristics of Respondents

Category of Respondents	**%**
Male	41
Female	59
Place of Residence	**%**
City	47
Village	53
Age Structure	**%**
Less than 20 years	6
21 – 30 years	46
31 – 40 years	16
41 – 50 years	17
51 – 60 years	10
61 years and more	5
Net Family Income	**%**
Up to 330 €	8
331 – 500 €	9
501 – 660 €	21
661 – 830 €	22
831 € and more	40

Scientific Hypothesis

Hypothesis No. 1: We assume, there does not exist a difference between the quality of food produced abroad and in Slovakia.

Hypothesis No. 2: We assume, there does not exist a difference between the health safety of food produced abroad and in Slovakia.

Hypothesis No. 3: We assume, there does not exist a difference between the quality and safety of food produced in the Slovak market.

Hypothesis No. 4: We assume, there does not exist a difference between health food safety control and food quality control.

RESULTS AND DISCUSSION

Of the total 478 respondents, the majority was represented by women (58%). The most respondents (46%) were aged 21 to 30years, followed by the interval from 41 to 50 years (17%) and further from 31 to 40 years (16%).

The highest achieved education was higher education, which was reported by up to 47% of the respondents. On the question related to the residence of respondents, 47% of them said they live in the village, remaining 53% of the respondents live in the city.

The monthly family income of the respondents ranged from 300 € to more than 831 € **(Figure 1)**. The obtained results were anticipated because the survey was aimed at the general public and all age groups. As could be seen in **Figure 1**, the most respondents reported a monthly family income 831 € and more. Another, the most-ranked group, was an income from 661 € to 830 € (22%). The smallest income range - up to 330 € - was marked by only 8% of the respondents.

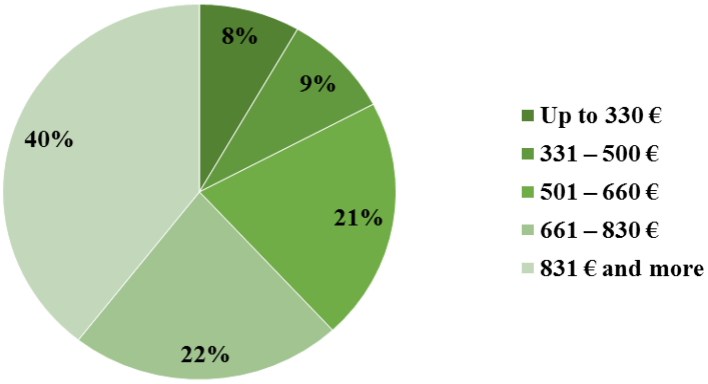

Figure 1 Monthly Family Income.

Responding to the first question in the questionnaire, the respondents pointed out whether they perceive the different quality of the food sold in the Slovak market. On this question, up to 85% of respondents answered positively, so they think the quality of the food sold in the Slovak market is different. After all, we can not be surprised because food problems are constantly appearing. Whether we refer to milk, sugar, butter or dual food quality recently promoted by media **(Aarseth and Olsen, 2006)**. A minority of respondents (15%) believe that the quality of food sold on the Slovak market does not differ from other foods offered in the market.

The following questions of the questionnaire survey focused on the food safety and quality of the food products. The respondents were asked how they perceive the safety of food produced in Slovakia, produced abroad and sold in Slovakia. In addition, they should express their opinion on the quality of food produced and sold in Slovakia and produced abroad and sold in Slovakia **(Figure 2)**.

The **Figure 2** shows that significant differences between individual responses can be observed for issues related to the safety and quality of food produced and sold on the Slovak market. For these questions, most respondents expressed their "rather high" response. The results of the survey therefore show that the respondents have positive experience, whether from a health or quality point of view, on the food market of the Slovak market. This fact is clearly caused by the informational interest of the respondents, ie. consumers are becoming more and more demanding and therefore require basic information about purchased food. However, as regards the safety and quality of food produced abroad and sold on the Slovak market, in these answers, **Figure 2** also shows the dissatisfaction of respondents with the quality of imported food. While the safety of food produced abroad and marketed in Slovakia reached the highest possible "rather high" range, the quality of food produced abroad and sold in Slovakia was "rather low". This decision could have been affected by the recent scandal over the dual quality of food. Since it has been found that products produced under one brand have a different qualitative composition in the individual countries of the European Union.

The results of research in Slovakia confirmed that Slovaks are most often dissatisfied with the freshness and quality of food in shops. This also results from a long-term review of the Staffino assessment system and the consumer portal Gazduj.sk, which involved 1070 customers purchasing food in all chains in Slovakia. Of these, 24% voiced dissatisfaction with freshness and quality of food **(Staffino and Gazduj.sk, 2017)**.

In connection with the solution of the pre-identified problems, the hypothesis was statistically verified to prove weather there does not exist a difference between the quality of food produced abroad and in Slovakia.

H_0: There does not exist a difference between the quality of food produced abroad and in Slovakia.

H_1: There exist a difference between the quality of food produced abroad and in Slovakia.

To verify the hypothesis, the Wilcoxon signed rank test and significance level were used:

$$p = 0.0001 < \alpha = 0{,}05$$

Based on the test results, we accept the hypothesis H_1 and claim that with 95% reliability there exist a difference between the quality of food produced abroad and in Slovakia. Based on the results of the Wilcoxon signed rank test, the H_0 hypothesis is rejected.

According to data obtained from the questionnaire survey, consumers pay attention to the quality of the purchased food products. This was answered by 83% of the respondents.

At present, people have greater opportunities than ever before, say the authors **Bloch, (2008) and Hultén, (2012)**. Most of them already buy on the basis of the visual sense, or when they buy food, they also use olfactie.

Figure 3 shows the results on the question regarding the feedback weather the respondents felt some change in food production in the Slovak. This change was related to food safety after 2010.

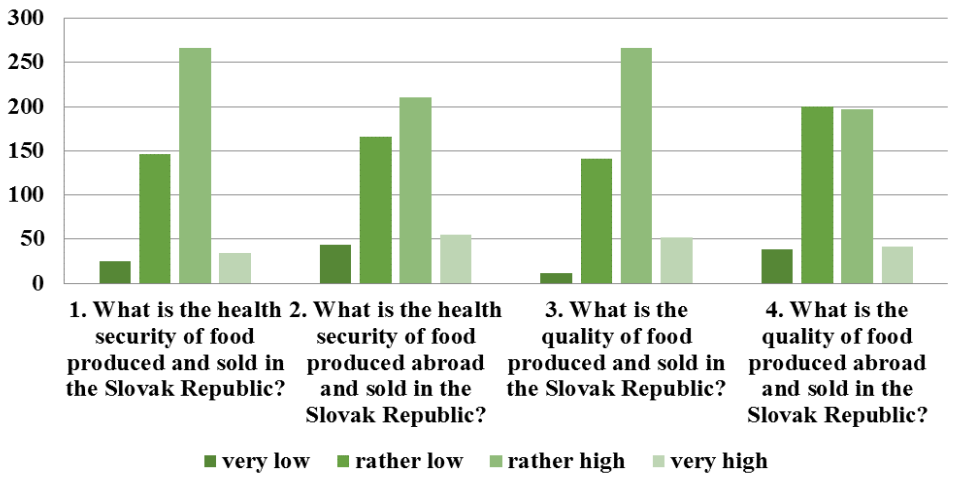

Figure 2 Food Safety and Quality.

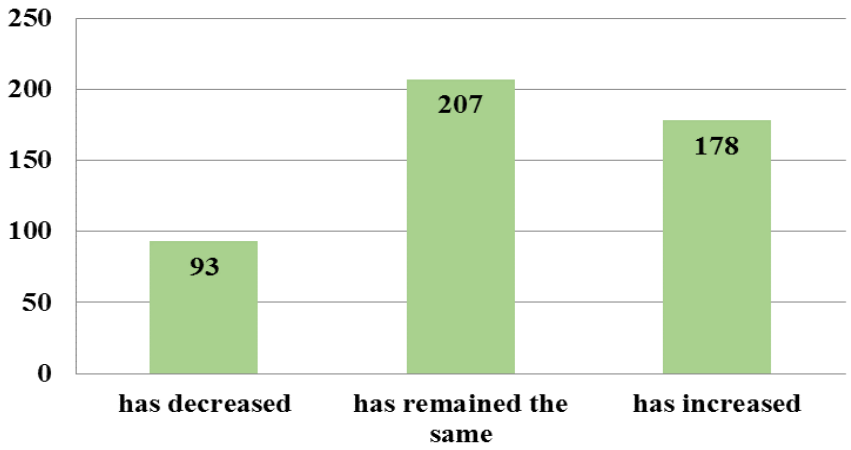

Figure 3 Change related to Food Safety after 2010.

The most respondents did not notice any change in the food safety after 2010. This unchanging feeling was marked by 43% of the respondents. In the second place was the option "increased" (37%). This could have been due to the introduction of various indicators of quality and health safety of purchased foods. 93 of the respondents felt that the safety of Slovak foodstuffs had diminished. Their negative assessment is accompanied by national awareness of individual types of food and also by various scandals of Slovak products. As an example, the meat content and the substitution of pepper for another dye in Spišské sausages. **(Trienekens and Zuurbier, 2007)**.

Following the obtained answers took place the statistical testing of hypothesis No. 2 – there does not exist a difference between the health security of food produced abroad and in Slovakia.

H_0: There does not exist a difference between the health safety of food produced abroad and in Slovakia.

H_1: There existsa difference between the health safety of food produced abroad and in Slovakia.

Statistical testing was performed by using the Wilcoxon signed rank test and significance levels:

$$p = 0.0819 > \alpha = 0.05$$

Based on the test results, we accept a zero hypothesis and claim that with 95% reliability there does not exist a difference between the health security of food produced abroad and in Slovakia. Based on the results of the Wilcoxon signed rank test, we accept the H_0 hypothesis.

In response to various food scandals, the respondnets had been asked the question related to the health safety of food sold in Slovakia **(Figure 4)**.

Only more than half of respondents (56%) consider health food safety control in the Slovak market to be reasonable. However, 17% of the total reported that food safety control is considered to be very good, and 18 questioned found this control to be inadequate. Questions of this type are based rather on the subjective opinion of the respondent **(Jarvis, 2017)**. Not everyone can have only positive or negative experience. In this case are important also the recommendations or potentially negative experiences that have been seen or directly noticed at the grocery store. Negative views are even deepened by various media scandals. The fact is that a dissatisfied consumer shares his negative experience with seven other consumers. However, he shares a positive experience with only two other consumers **(Tsui, Nifadkar and Ou, 2007)**.

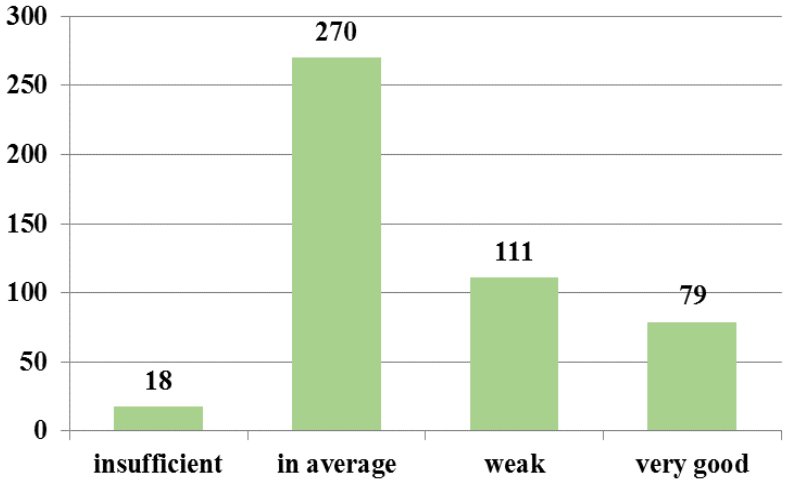

Figure 4 Control of Health Food Safety.

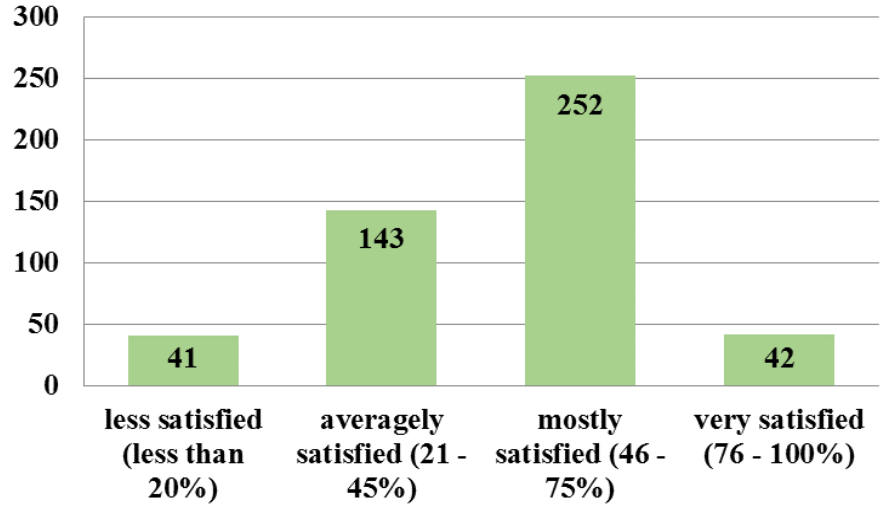

Figure 5 Satisfaction with Hygiene Conditions of Food Sales.

In view of the previous answers of respondents involved in the questionnaire survey took place the statistical testing of hypothesis No. 3 – there does not exist a difference between the quality and safety of food produced in the Slovak market.

H_0: There does not exist a difference between the quality and safety of food produced in the Slovak market.

H_1: There exists a difference between the quality and safety of food produced in the Slovak market.

To verify hypothesis No. 3, the Chi-square test of independence and the level of significance were used:

$$p = 0.3946 > \alpha = 0.05$$

Based on testing, we accept a zero hypothesis and claim that with 95% reliability there is no difference between the quality and safety of food produced on the Slovak market.

Another question was devoted to the issue of hygienic conditions of food sales in food stores operating in Slovakia **(Figure 5)**.

From the answers to the question "Are you satisfied with the hygiene conditions of food sales?", almost 9% of respondents were very satisfied and up to 53% expressed their satisfaction with hygienic conditions in the food stores. Their satisfaction stems not only from the cleanliness and lighting of the store, but also from the use of various protective equipment available in the food stores in those departments where is required their use. Relatively good results were also achieved in the second group of respondents (30%) who presented an average satisfaction with the hygienic conditions observed in the sale of fresh food in Slovakia. It is obvious that this group of people has already experienced some negative food hygiene experience (eg: non-compliance with the use of sanitary protection equipment for storing, especially fresh food).

In a research by **Paden et al. (2017)**, 35% of respondents had negative hygienic experience, which was mainly associated with non-compliance with hygiene rules by buyers in handling food without the use of protective equipment. The least respondents (more than 8%) presented their satisfaction as "not very satisfied". This satisfaction was clearly caused by a negative experience in which the hygiene conditions were seriously violated (eg spoiled meat). Based on a survey conducted by **the Retail Magazine (2016)**, it was found out that nearly 40% of the population has no knowledge about harmfulness of food after the date of consumption.

The conclusion of the questionnaire survey resulted in the testing of hypothesis No. 4 – there does not exist a difference between health food safety control and food quality control.

H_0: There does not exist a difference between health food safety control and food quality control.

H_1: There exists a difference between health food safety control and food quality control.

To test the hypothesis No. 4, the Friedman test and the level of significance were used:

$$p = 0.1605 > \alpha = 0.05$$

Based on testing, we can say that we accept a zero hypothesis and claim that with 95% reliability, there does not exist a difference between health food safety control and food quality control.

The last question was focused on the availability of quality food in stores. The most respondents (78%) replied that quality food is already available in many chain of stores in the Slovak market. The availability of high-quality food within the European Union confirmed the survey by **Sirri (2017)** and 86% of its respondents. 22% of respondents reported problematic availability of quality food. This problem can be caused by the fact that up to 47% of the respondents live in a village where the food stores are managed by the small businessemen or tradesmen. Their primary goal is to generate the profit.

CONCLUSION

Based on the research, it was confirmed that the vast majority of consumers perceive the different quality of sold food in the Slovak market. The submitted paper

showed that up to 406 respondents (85%) felt different quality of purchased food in the Slovak market. The quality of food produced abroad and sold on the Slovak market is perceived by consumers as low. Therefore, the respondents perceive the difference between the quality of food produced abroad and the quality of food produced in Slovakia. The lower quality of food produced abroad was confirmed by statistical testing. The low quality of foreign food was marked by 42% of respondents, which is a relatively high number.

Customers encounter different levels of hygiene in the food stores. Over half of respondents (53%) said they were satisfied with hygienic sales conditions. Despite the fact that more than half of respondents have expressed their satisfaction with hygienic conditions, there are still many of those respondents who more or less expressed their dissatisfaction with problems related to hygiene in the food stores. Failure to adhere to proper hygiene conditions can have serious consequences for human health.

Results of the questionnaire survey confirmed that up to 78% of respondents reported the regular availability of quality food in the stores. Consumers are gradually becoming more rational, especially in terms of information literacy. As there is a wide range of food products in store, many respondents try to choose the food according to their own mind. For these reasons, they seek to obtain the necessary information by studying, reading information on product labels, and in many cases also via Internet. This fact was also confirmed by the results of the survey, when up to 83% of respondents said the food quality was considered to be one of the most important factors influencing their choice and purchase of food. One of the reasons for this is the negative information presented in different media, but also the disclosure of various unfair practices and misleading media campaigns. However, there still remains the question, until when will be the consumers willing to listen from various sources about poor food in the Slovak market. The basic question of each consumer must be to be informed about the individual food produced in Slovakia and imported from abroad. Customers should also be aware of the uniqueness and quality of the food produced in the domestic market, the purchasing of which is necessity for domestic producers and, last but not least, the economy of the state.

REFERENCES

Aarseth, H., Olsen, B. M. 2006. Food and masculinity in dual-career couples. *Journal of Gender Studies*, vol. 17, no. 4, p. 277-287. https://doi.org/10.1080/09589230802419922

Bloch, M. 2008. Truth and sight: generalizing without universalizing. *Journal of the Royal Anthropological institute*, vol. 14, no. 1, p. 522-532. http://onlinelibrary.wiley.com/doi/10.1111/j.1467-9655.2008.00490.x/full

Boeck, E. D., Jacxsens, L., Mortier, V. A., Vlerick, P. 2018. Quantitative study of food safety climate in Belgian food processing companies in view of their organizational characteristics. *Food Control*, vol. 88, p. 15-27. https://doi.org/10.1016/j.foodcont.2017.12.037

DNES24. 2017. Slovaks in stores are most concerned about the freshness and quality of food: they also have long advices. (Slovákov v obchodoch najviac trápi čerstvosť a kvalita potravín: hnevajú aj dlhé rady). Available at: http://www.dnes24.sk/slovakov-v-obchodoch-najviac-trapi-cerstvost-a-kvalita-potravin-hnevaju-aj-dlhe-rady-277813

Duncan, T. V. 2011. Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. *Journal of Colloid and Interface Science*, vol. 363, no. 1, p. 1-24. https://doi.org/10.1016/j.jcis.2011.07.017

Feldmann, C., Hamm, U. 2015. Consumers' perceptions and preferences for local food: A review. *Food Quality and Preference*, vol. 40, part. A, p. 152-164. https://doi.org/10.1016/j.foodqual.2014.09.014

Géci, A., Nagyová, Ľ., Rybanská, J. 2017. Impact of sensory marketing on consumer´s buying behaviour. *Potravinárstvo*, vol. 11, no. 1, p. 709-717. https://dx.doi.org/10.5219/835

Golian, J., Sokol, J. 2005. Education and research for food safety. (Vzdelávanie a výskum pre bezpečnosť potravín). *Food Safety and Control: Proceedings of the International Scientific Conference. (Bezpečnosť a kontrola potravín : Zborník prác v medzinárodnej vedeckejkonferencie).* Nitra, Slovakia : Slovak University of Agriculture in Nitra, 2005, s. 53. ISBN 80-8069-503-2 (In Slovak)

Gorris, L. 2005. Food safety objective: An integral part of food chain management. *Food Control*, vol. 16, no. 9. https://doi.org/10.1016/j.foodcont.2004.10.020

Grunert, K. G., Aachmann, K. 2016. Consumer reactions to the use of EU quality labels on food products: A review of the literature. *Food Control*, vol. 59, p. 178-187. https://doi.org/10.1016/j.foodcont.2015.05.021

Hinks, D., Shamey, R. 2011. Review of retail store lighting: implications for colour control of products. *Coloration Technology*, vol. 127, no. 2, p. 121-128. http://doi.org/10.1111/j.1478-4408.2011.00286.x

Holienčinová, M. 2013. Consumer behavior in the market for alcoholic beverages. (Spotrebiteľské správanie na trhu alkoholických nápojov). *Marketing management, business and social aspects of business. (Marketing manažment, obchod a sociálne aspekty podnikania),* p. 170-079.

Hultén, B. 2012. Sensory cues and shoppers' touching behaviour: the case of IKEA. *International Journal of Retail & Distribution Management*, vol. 40, no. 4, p. 273-289. https://doi.org/10.1108/09590551211211774

Chrysochou, P., Krystallis, A., Giraud, G. 2012. Quality assurance labels as drivers of customer loyalty in the case of traditional food products. *Food Quality and Preference*, vol. 25, no. 2, p. 156-162. https://doi.org/10.1016/j.foodqual.2012.02.013

Jarvis, S. 2017. The story on global health security. *Veterinary Record,* vol. 181, no. 5, p. 100. http://doi.org/10.1136/vr.j3596

King, T., Cole, M., Farber, M. J., Eisenbrand, G., Zabaras, D., Fox, M. E., Hill, P. J. 2017. Food safety for food security: Relationship between global megatrends and developments in food safety. *Trends in Food Science & Technology*, vol. 68, p. 160-175. https://doi.org/10.1016/j.tifs.2017.08.014

Kozelová, D. Mura, L., Matejková, E., Lopašovský, Ľ., Vietoris, V., Mendelová, A., Bezáková, M., Chreneková, M. 2011. Organic products, consumer behavior on market and european organic product market situation. *Potravinarstvo*, vol. 5, no. 3, p. 20-26. https://doi.org/10.5219/96

Krishna, A. 2013. Introduction: What Is Sensory Marketing? In *Customer Sense,* New York, USA : Palgrave Macmillan. p. 1-18. ISBN: 978-1-349-34442-0 https://doi.org/10.1057/9781137346056_1

Kubicová, Ľ., Kádeková, Z. 2017. *Strategic marketing*. 1st edition. Nitra, Slovakia : Slovak University of Agriculture. 143 p. ISBN 978-80-552-1622-5

Kubicová, Ľ., Kádeková, Z. 2012. Revenue impact on the demand of slovak households for meat and meat products. *Acta UniversitatisAgriculturae et Silviculturae Mendelianae Brunensis,* vol. 50, no. 2, p. 503-510.

Kubicová, Ľ., Kádeková, Z. 2011. Comparison of the income development and the food demand elasticities of the private households in Slovakia. *Agricultural economics*, vol. 57, no. 8, p. 404-411.

Food quality and safety. (Kvalita a bezpečnosť potravín). 2018. Available at: http://www.biznis.sk/kvalita-a-bezpecnost-potravin/

Lee, H.-J., Yun, Z.-S. 2015. Consumers' perceptions of organic food attributes and cognitive and affective attitudes as determinants of their purchase intentions toward organic food. *Food Quality and Preference*, vol. 39, p. 259-267. https://doi.org/10.1016/j.foodqual.2014.06.002

Lindstrom, M. 2006. Brand Sense: How to Build Powerful Brands Through Touch, Taste, Smell, Sight and Sound. *Strategic Direction*., vol. 22 no. 2. https://doi.org/10.1108/sd.2006.05622bae.001

Loureiro, M. L., Umberger, W. J. 2007. A choice experiment model for beef: What US consumer responses tell us about relative preferences for food safety, country-of-origin labeling and traceability. *Food Policy*, vol. 32, no. 4, p. 496-514. https://doi.org/10.1016/j.foodpol.2006.11.006

Loutfi, A., Coradeschi, S., Mani, K. G., Shankar, P., Rayappan, B. B. J. 2015. Electronic noses for food quality: A review. *Journal of Food Engineerign*, vol. 144, p. 103-111. https://doi.org/10.1016/j.jfoodeng.2014.07.019

Magnier, L., Schoormans, J., Mugge, R. 2016. Judging a product by its cover: Packaging sustainability and perceptions of quality in food products. *Food Quality and Preference*, vol. 53, p. 132-142. https://doi.org/10.1016/j.foodqual.2016.06.006

Maratto, G., Simeone, M., Nazzaro, C. 2014. Product reformulation in the food system to improve food safety. Evaluation of policy intervention. *Appetite*, vol. 74, p. 107-115. https://doi.org/10.1016/j.appet.2013.12.003

McFadden, J. R., Huffman, W. E. 2017. Consumer valuation of information about food safety achieved using biotechnology: Evidence from new potato products. *Food Policy*, vol. 69, p. 82-96. https://doi.org/10.1016/j.foodpol.2017.03.002

Michaelidou, N., Hassan, L. M. 2007. The role of health consciousness, food safety concern and ethical identity on attitudes and intentions towards organic food. *International Journal of Consumer Studies*, vol. 32, no. 2, p. 163-170. https://doi.org/10.1111/j.1470-6431.2007.00619.x

Nagyová, Ľ., Berčík, J., Horská, E. 2014. The efficiency, energy intensity and visual impact of the accent light in the retail grocery stores. *Potravinarstvo*, vol. 8, no. 1, p. 296-305. https://doi.org/10.5219/398

Njage, K. M. P., Opiyo, B., Wangoh, J., Wambui, J. 2018. Scale of production and implementation of food safety programs influence the performance of current food safety management systems: Case of dairy processors. *Food Control*, vol. 85, p. 85-97. https://doi.org/10.1016/j.foodcont.2017.09.015

Pable, A., Lu, S., Auerbach, J. 2010. Integrated qualitative/quantitative techniques for food product quality planning. *Journal of Food Quality*, vol. 33, no. 1, p. 112-129. https://doi.org/10.1111/j.1745-4557.2009.00287.x

Paden, H., Hatsu, I., Lustberg, M., Grenade, C., Kane, K., Mo, K., Ilic, S. 2017. Fastors Associated with Food Safety Behaviors in Cancer Patients Seeking Treatment. *The Faseb Journal,* vol. 31, no. 1.

Pavelková, A., Bobková, A. 2008. Quality policy - labeling of agricultural products and foodstuffs. (Politika kvality – označovanie poľnohospodárskych výrobkov a potravín). *Food Safety and Control: Proceedings of the International Scientific Conference. (Bezpečnosť a kontrola potravín : Zborník prác v medzinárodnej vedeckej konferencie)*. Nitra : SPU, 2008, p. 20. ISBN 978-80-552-0350-8.

Food Code of the Slovak Republic. (Potravinový kódex Slovenskej republiky). 2017. Part Two - General Requirements. (Druhá časť – všeobecné požiadavky). Available at: http://www.svps.sk/legislativa/legislativa_kodex.asp

Retail magazin. 2016. *Due to the expiration date, food quality does not change. (Uplynutím dátumu trvanlivosti sa kvalita potravín nemení).* Available at: http://www.retailmagazin.sk/produkt/potravinarsky-sortiment/1423-uplynutim-datumu-trvanlivosti-sa-kvalita-potravin-nemeni

Rompay, van J. L. T., Deterink, F., Fenko, A. 2016. Healthy package, healthy product? Effects of packaging design as a function of purchase setting. *Food Quality and Preference,* vol. 53, p. 84-89. https://doi.org/10.1016/j.foodqual.2016.06.001

Shang, X., Tonsor, G. T. 2017. Food safety recall effects across meat products and regions. *Food Policy*, vol. 69, p. 145-153. https://doi.org/10.1016/j.foodpol.2017.04.002

Shi, X., Zhu, X. 2009. Biofilm formation and food safety in food industries. *Trends in Food Science & Technology*, vol. 20, no. 9, p. 407-413. https://doi.org/10.1016/j.tifs.2009.01.054

Sirri, F., Petracci, M., Zampiga, M., Meluzzi, A. 2017. Effect of EU electrical stunning conditions on breast meat quality of broiler chickens. *Poultry Science*, vol. 96, no. 8, p. 3000-3004. https://doi.org/10.3382/ps/pex048

Stecová, E., Popelka, P. 2005. Hygiene of food. (*Hygiena potravín)*. 1. vyd. Bratislava, Slovakia : Proxima press. 2005. p. 287. ISBN 80-85454-94-7.

Suchánek, P., Richter, J., Králová, M. 2017. Customer Satisfaction with Quality of Products of Food Business. *Prague Economic Papers*, vol. 2017, no. 1, p. 19-35. https://doi.org/10.18267/j.pep.595

Trienekens, J., Zuurbier, P. 2008. Quality and safety standards in the food industry, developments and challenges. *International Journal of Production Economics*, vol. 113, no. 1, p. 107-122. https://doi.org/10.1016/j.ijpe.2007.02.050

Tsui, A. S., Nifadkar, S. N., Ou, A. Y. 2007. Cross-National, Cross-Cultural Organizational Behavior Research: Advances, Gaps, and Recommendations. *Journal of Management*, vol. 33, no. 3, p. 426-478. https://doi.org/10.1177/0149206307300818

Waldman, K. B., Kerr, J. M. 2018. Does safety information influence consumers' preferences for controversial food products? *Food Quality and Preference*, vol. 64, p. 56-65. https://doi.org/10.1016/j.foodqual.2017.10.013

Yu, H., Gibson, E. K., Wright, G. K., Neal, A. J., Sirsat, A. S. 2017. Food safety and food quality perceptions of farmers' market consumers in the United States. *Food Control*, vol. 79, p. 266-271. https://doi.org/10.1016/j.foodcont.2017.04.010

Act no. 215/2004 Coll. 2004 of The Slovak Republic. (Zákon č. 215/2004 Z. z. 2004). *The Act on the Protection of Classified Information and on Amendments to Certain Acts. (Zákon o ochrane utajovaných skutočností a o zmene a doplnení niektorých zákonov)*.

Zhang, M., Jin, Y., Qiao, H., Zheng, F. 2017. Product quality asymmetry and food safety: Investigating the "one farm household, two production systems" of fruit and vegetable farmers in China. *China Economic Review*, vol. 45, p. 232-243. https://doi.org/10.1016/j.chieco.2017.07.009

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COMPARING THE QUALITY OF HONEY FROM BEEKEEPERS AND HONEY FROM THE MARKET CHAIN

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ABSTRACT
Honey is a valuable food for its beneficial nutritional and dietetic effects. The quality of honey fluctuates considerably according to various criteria, the adulteration of honey with cheaper substitutes is not negligible. The quality of honey in the market chain with honey taken from beekeepers was compared in this study. A total of 10 samples from each group were tested for basic qualitative markers and compared with legislative criteria. The samples were analysed for fructose and glucose content, water content, titratable acidity and two tests for illegal sugar additions. The results revealed the addition of 25% of the technical syrup in one sample of honey from the market chain, one sample had the sum of fructose and glucose 56,3%, it is below the required limit 60% (differed by 6,3%). In other parameters the samples complied with the valid legislation. All tested parameters in honey from beekeepers met the criteria of the legislation, only 1 sample of blossom honey had the sum of fructose and glucose just below the required limit. The sum of fructose and glucose in this sample was 58.3 %, it differed by 2.9% from the required content of 60%. Sensory analysis was used to assess four samples of honey from beekeepers collected by different techniques. Results have not shown significant difference in sensory properties between manually pressed honey and honey obtained after whirling. The responses characterizing the favourable sensory properties of the examined honey samples were prevailing. The difference between the perception of honey after whirling and honey harvested by press manually was not demonstrated in sensory properties.

Keywords: blossom honey; honeydew honey; adulteration; beekeeper; market chain

INTRODUCTION
Honey is a sweet primary product of bees (*Apies* family) used from history as a food source, as a sweetener, but also for other purposes, including therapeutic use. Blossom honey is obtained from the nectar of flowers, honeydew honey from secretions of aphids plant sucking insects **(Alvarez-Suarez et al., 2014)**. Composition of honey depends on many parameters and may be different according type (blossom or honeydew), pollen collection locality, season, variety of flora and also according to the method of honey harvesting and post-harvest handling, including storage. Honey contain many nutritionally important chemical components, such as sugars, oligosaccharides, organic acids, enzymes, minerals, polyphenols, vitamins and aminoacids with important functions in human nutrition, or treatment and prevention of various diseases. Most of them are associated with antioxidant, anti-inflammatory, antibacterial and antiviral functiones, antihypercholesterolemic, antiulcerous, vasodilatative, hypotensive and even antitumor functions **(Bogdanov, 2011; Viuda et al., 2008; Hadagali and Chua, 2014)**.

The main components of honey are sugars. Monosaccharides, fructose and glucose forms about 70 % of sugar content, disaccharides, trisaccharides and oligosaccharides forms the rest, about 10 % of carbohydrates of honey **(Miguel et al., 2017)**. Table 1 showes the chemical composition of honey according to **Santos-Buelga and González-Paramás (2017)**.

Table 1 Chemical composition of honey **(Santos-Buelga and González-Paramás. In: Alvarez-Suarez, 2017)**.

Major constituents (%)	Mean	SD	Range
Water	17.90	3.16	13.21 – 26.50
Fructose	39.44	2.11	37.07 – 42.65
Glucose	28.15	5.74	18.20 – 32.10
Sucrose	3.19	3.81	0.36 – 16.57
Other sugars	8.5		0.1 16.0
Minor constituents (%)			
Minerals	0.36	0.18	0.11 – 0.72
Total protein	1.13	1.22	0.22 – 2.93
Acids (as gluconic acid)			0.17 – 1.17
Vitamins, enzymes, aromas	<0.1		
Phenolic compounds	0.1		0.02 – 0.2

Proteins in honey are present in enzymes diastase, amylase, invertase, glucose oxidase, etc. as well as

individual amino acids, where proline is the most important **(Miguel et al, 2017)**.

The content of vitamins (vitamins of group B, ascorbic acid) is very low in honey. Although, it contains a negligible amount of vitamins, honey is a significant factor in both, prevention and support in treatment of diseases, probably through the combination of vitamins with other biologically active substances.

Many scientific studies show the positive effects of honey on human health. Major positive effect on living organisms, is antioxidant activity of honey, proven *in vitro* and *in vivo* studies. *In vitro* study shows, that honey is able to scavenge free radicals, can reduce ferric cations and inhibit lipid peroxidation. *In vivo*, honey is able to stimulate the antioxidative status of mice and rats, especially glutathione defence system **(Erejuwa, 2012)**. Other important role of honey is immunomodulatory activity in wound healing properties. The process of wound healing has four stages – haemostases, inflammation, proliferation and remodelling **(Song and Salcido, 2011)**. This effect depends on immunostimulatory and inflammatory action and suppression of reactive oxygen intermediates which correlates with the floral origin of honey. Honey has been also used in treatment of skin disorders, such as dermatitis, eczemas, burns, and ulcers, Fournier gangrene with positive effect on this healing processes **(Song and Salcido, 2011, McLoone et al., 2016)**.

Most of in vitro studies of anticancer activity of honey of various kinds to several types of human cancer cell lines were reported **(Tsiapara et al., 2009)**. Honey was reported as an apoptotic induce factor and has antiproliferative activity by affecting cell cycle and blocking the cell cycle of cancer cell lines **(Erujawa et al., 2014)**.

Cardiovascular disease are the most common cause of death worldwide, published by World Health Organization **(WHO, 2017)**. Cardiovascular diseases are associated with chronic inflammation. Presence of inflammation manifests with increasing high sensitivity of C-reactive protein (CRP). Some authors reported (**González-Gil et al., 2016)** decreasing of CRP levels in European children when they consume regularly medium intake of honey at breakfast. The positive effects are probably connected with other beneficial elements in the diet (**González-Gil et al., 2016)**. Other studies reported positive effect of honey in the prevention of cardiovascular diseases. According to **Yaghoobi et al.** (**2008**), natural honey decreases all risk markers in blood in both groups, healthy people and patients with high cardiovascular risk factors. Honey reduced total cholesterol (TCH), low-density lipoproteins (LDL), triacylglycerols (TG), levels of fasting blood glucose, and CRP in this study. On the other hand, honey increased high-density lipoproteins (HDL).

Honey is a beneficial in the diet for proper function of the intestinal microbiota due to the unique composition of honey containing sufficient nutrients particularly oligosaccharides (fructooligosaccharides) required for growth of intestinal microbiota such as *Lactobacillus acidophilus, Bifidobacterium* spp. **(Roberfroid, 2000)**.

In addition to these positive effects, honey is recommended for antiviral effects as a support tool in the fight against viral infection.

It is not easy to prove the antitumor effects of honey and effectiveness in preventing or improving the treatment of oncological diseases. Honey has proved the supporting effect on the body's defense against infections, the improvements in the healing of hard healing wounds, and chronic diseases. For further research on the prevention of metabolic and chronic diseases, the prevention of cardiovascular diseases, including the treatment of inflammatory skin diseases, studies of its effects have a great importance. For these properties, it is very important to maintain high quality honey in the market chain and to support the production of quality honey from local beekeepers.

Some changes in composition can occur due to the storage of honey. These changes are reflected by a change in sensory properties and reduce the quality of honey **(Kňazovická et al., 2015)**. Some biochemical processes, such as fermentation, oxidation, hydratation or dehydratation and the other reactions lead to changes in acidic content and the formation **c**ompounds, like 5-hydroxymethylfurfural (5-HMF). This compound in honey is undesirable and there is established the limit value for HMF in honey. This compound can be used also as a marker of illegal honey interferences, such as heating to a temperature above 50 °C **(Alvarez-Suarez, 2017)**.

Honey is a food that could be easily adulterated. Therefore, its quality must be regularly checked and monitored. The situation in the Czech Republic (CR) concerning honey in the market chain and the situation in beekeeping is summarized in the Situational Outlook Report of the Czech Ministry of Agriculture **(MZe, 2015)**. The consumption of honey in the Czech Republic is low, only about 0.7 kg /person/year, but it has slightly rising tendency. **Guziy et al. (2017)** compared the consumption of honey in Slovakia and Russia. According to this study the consumption of honey in both countries is higher than in the CR. From 316 respondents in Slovakia, approximately 50 % of them consume about 2 -5 kg of honey/person/year. Honey supply in the market network in the CR is sufficient, the market network is complemented by increasing import of honey not only from the EU countries.

To maintain biological and nutritional value, quality and safety properties of honey, clear criteria for honey handling during its formation and subsequent harvest and storage are given. According to the quality requirements, no component of honey must be removed (except filtration), no substances should be added **(**in the CR according to the **Decree 76/2003 Coll., Council Directive 2001/110 EU)**. The criteria are given in Table 2.

Honey quality criteria were specified in the European directive in the **EU Proposal for a directive of the European Council relating to honey (1996),** respectively **Council Directive 2001/110/EC (2001)** and in the **Codex Alimentarius (1994)**.

According to the **Council Directive 2001/110/EC (2001)** honey is natural bee product to which nothing can be added and from which nothing can be removed. International honey standards are specified in the collaborative work of the International Honey Commission **(Bogdanov et al., 1999)**. Criteria are very strict, there is not allowed to add anything (additives or substitutes) to honey and change its composition. The quality of honey in the market chain is threatened by the problem of adulteration and unauthorized interference. Honey, as well as other food, is often falsified by illegal additions and substitutes.

In the Czech Republic the quality of honeys in the market chain is monitored by the Czech Agriculture Food Inspection Authority (CAFIA). The State Veterinary Administration (SVA) supervises breeding and cooperate with CAFIA. The State Veterinary Administration carried out checks on beekeepers in 2016 and the quality of the honey was very satisfactory. But **CAFIA (2017)** revealed during inspections in 2016 in market chain a total 31 % of the samples with unsatisfactory quality and approximately 40 % of the samples did not comply in the long term period of monitoring.

Table 2 Physical and chemical requirements for honey **(Decree 76/2003 Coll.).**

Requirement	Honey type		
	flower	honeydew	bakery (industrial)
the sum of fructose and glucose contents (wt.% of at least)	60.0	45.0	-
sucrose content (wt.% maximum)	5.0	5.0	-
water content (wt.% maximum)	20.0	20.0	23.0
acidity ($meq.kg^{-1}$ maximum)	50.0	50.0	80
hydroxymethylfurfural ($mg.kg^{-1}$ maximum)	40.0	40.0	-
water insoluble matter content (% by weight)	0.10	0.10	-
electrical conductivity ($mS.\ m^{-1}$)	max. 80.0	min. 80.0	-
diastase activity (degree according Schade – at least)	8.0	8.0	-

Scientific hypothesis

The aim of our study was to determine the basic parameters of honey quality in the market chain and compare them with samples of honey from beekeepers with the expected difference between the main parameters of honey from commercial suppliers and samples from beekeepers and reveal adulteration honey by the addition of substitutes. Sensory analysis was used to identify the difference betweenthe honey harvested by the hand press from honey harvested by whirling.

MATERIAL AND METHODOLOGY

Samples

Two groups of samples for the analyses of the basic parameters of honey quality were taken in 2016. All these samples were harvested in 2016. Each sample group contained 10 samples of honey. The samples of group A were collected in market chain from commercial suppliers. The samples group B were collected directly from beekeepers from Bohemian-Moravian Highlands.

Samples for sensory analysis

Samples group C for sensory and chemical analysis were taken from harvesting honey in 2015 and assessed in this year. Two samples of honey (1 sample blossom honey, 1 sample honeydew honey) were harvested manually by pressing, two other samples of honey (1 sample blossom honey, 1sample honeydew honey) were harvested by classical methods of honey extraction by whirling.

Sensory analysis of samples

Sensory analysis of samples group C was carried out at the sensory analytical laboratory under the conditions of ISO 8589:2007. The panel comprised 10 panellists selected, trained, and monitored according to **Piana et al. (2004)**. Sensory quality was assessed using category ordinal 12-point scale. The evaluated descriptors were colour, consistency, smell and taste. Samples were coded using four-digit, randomly generated numbers and served according to the ISO 6658:2017. Drinking tap water was given as a neutraliser to the panellists between the samples.

Chemical analysis of samples

Conductivity. Determination of electrical conductivity is based on the principle of measuring electrical resistance by conductivity using the instrument (LWT-03-ATC, Voltcraft). Samples (20% honey solution) were tempered at 20 °C for 30 minutes, and then the conductivity was measured 3 times, and arithmetic mean was calculated.

Water content. Water content was determined refractometrically using the RF10 refractometer (Conrad Electronic) apparatus by applying 1 drop of sample to the surface of the refractometer prism glass. Each sample was determined 3 times and the arithmetic mean was calculated.

Sugar content. The content of fructose, glucose and sucrose were analysed by HPLC (Varian 9010, U.S.A) in both groups of samples (samples group A and B), after clarification by solutions Carrez I and Carrez II under these conditions: Agilent Hi-Plex Ca column, 7.7 x 300 mm, 8 µm (p/n PL1170-6810), mobile phase 100% deionised H_2O, flow rate 0.6 $ml.min^{-1}$, injection volume 20µl, temperature 85 °C, detector RI, using glucose, fructose and sucrose standards p.a. (Sigma-Aldrich) at a concentration of 10 $mg.mL^{-1}$, calibration in the range of 1-100 $mg.mL^{-1}$. From the recorded and evaluated areas of the peaks of each standard, the values were extrapolated and used to evaluate the calculation of the sugar content of each sample. Injection of each sample and standards was performed twice and the values were expressed as the arithmetic mean of the peak areas from which the concentration of each sugar was expressed.

Hydroxymethylfurfural (HMF) was analysed in the accredited Testing Laboratory of the Bee Research Institute in Dol, in the CR, using the HPLC method (Agilent, USA), UV detector, C18 reverse phase chromatographic column (Zorbax Eclipse XDB C18 150x4.6 mm, particle size 5µm), detection at 285 nm wavelength, column temperature 35 °C, 20µl injection volume. Mobile phase was mixture of water/methanol 90:10, flow rate 1 $ml.min^{-1}$. Calibration of the method was performed using the HMF standard p.a. (Sigma-Aldrich) in the concentration range 1-500 $mg.L^{-1}$.

Titratable acidity was determined by titration of honey with a solution of 1M NaOH using phenolphthalein as the indicator. Each sample weighing 20.0 g of honey was quantitatively transferred into a 100 ml volumetric flask and filled to the mark with distilled water. Titration of each

sample was carried out 2 times and the results were averaged **(Vorlová et al., 2002)**.

Fiehe test with tannin for the detection of adulteration of honey by starch sugar, syrup and malt extracts, was performed according to the methodology **ČSN (1974)** and **Vorlová et al. (2002)**.

Honey violation by technical syrup was performed with liquid samples of honey by pouring into a beaker with water. The test is evaluated immediately, in the case of falsification, the honey violation by technical syrup is reflected by typical landfilling **(Vorlová et al., 2002)**.

The samples group C were used for chemical analysis, the content of sugars, water, HMF, conductivity, starch and presence of unauthorized addition of caramel. All these parameters were analysed by the Testing laboratory of the Bee Research Institute in Dol, Czech Republic.

Statistical analysis

Statistical analysis was performed using software Statistica 12 (StatSoft, Inc., Texas, USA). Shapiro-Wilk test of normality was used at the level of significance $\alpha = 0.05$. Two-way ANOVA with interactions at the 95% probability level was used to detect statistically significant differences between samples and two-tailed t-test with test of equal and unequal variance. All measured parameters were performed in triplicates and results are expressed as mean with standard deviation (SD). Differences at $p \leq 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The group A honey samples from market chain consists of 7 samples of blossom honey and 3 samples of honeydew honey. Results of chemical composition honey from market chain are given in Table 3.

The measured parameters of samples group A were compared with the values given by the **Decree 76/2003 Coll. (2003)**, and the **Council Directive 2001/110/EC (2001)** respectively. According to these regulations, only 1 sample of blossom honey from market had lower sum of fructose and glucose content. Other samples have sufficient content of sugars, for blossom honeys more than 60% and for honeydew more than 45%.

Other parameters, such as the water content (limit less than 20 %) and titratable acidity (less than 50 mekv.kg^{-1}) were according to these regulations. No positive test on presence of adulteration of honey by starch sugar, syrup and malt extracts were detected. But in the group A, the addition of 25% of technical syrup in 1 honey sample was proven.

The group B honey samples from beekeepers consists of 5 samples of blossom honey and 5 samples of honeydew honey. Results of chemical composition honey from beekeepers are given in Table 4.

The measured parameters of samples group B were compared with the values given by the **Decree 76/2003 Coll**. and the **Council Directive 2001/110/EC (2001**), respectively. According to these regulations only 1 sample of blossom honey from beekeepers had the sum of fructose and glucose content 58.3%, which was just below the required limit 60%, see Table 2. Other samples have sufficient content of sugars, for blossom honeys more than 60% and for honeydew more than 45%.

Other tested parameters, water content less than 20% and titratable acidity less than 50 meq.kg^{-1}, were according to these regulations. No positive test on the presence of adulteration of honey by starch sugar, syrup and malt extracts, was detected. No sample from this group B was positively tested for adulteration by technical syrup.

To compare the difference between handpressed honey and honey harvested by whirling (4 samples of group C obtained from the beekeeper) the chemical analysis of samples, which were primarily designed for sensory analysis, was pereformed, too. These samples of honey were analysed for

Table 3 Chemical composition honey from market chain (group A).

Parameter	Fru+Glu blossom (%)	Fru+Glu honeydew (%)	Sucrose %	Water %	Acidity meq.kg^{-1}	Fiehe test	Test adulter.
min	56.3	46.6	0.8	17.9	11	NEG	POS
max	86.9	62.0	3.7	19.6	35		
Ø ±SD	**72.9**	**55.1**	**1.6 ±0.79**	**18.6**	**19.7**		
Ø ±SD total	**65.9 ±12.1**					*10	**1

Note: *Fiehe test, number of negative findings, ** test adulteration by technical syrup, number of positive findings, NEG – negative proof, POS – positive proof.

Table 4 Chemical composition honey from beekeepers (group B).

Parameter	Fru+Glu blossom (%)	Fru+Glu honeydew (%)	Sucrose %	Water %	Acidity meq.kg^{-1}	Fiehe test	Test adulter.
min	58.3	46.2	0.2	17.9	6	NEG	NEG
max	83.7	72.6	2.3	18.7	24		
Ø ±SD	**70.8**	**52.6**	**0.9± 0.57**	**18.3**	**14.3**		
Ø ±SD total	**61.7 ±10.7**					*10	**10

Note: * Fieho test, number of negative findings, ** test adulteration by technical syrup, number of negative findings, NEG – negative proof.

Table 5 Chemical composition of samples from beekeepers (group C).

Sample Group C	water %	Fru %	Glu %	Suc %	Fru+Glu %	HMF mg.kg^{-1}	conductivity mS.m^{-1}	starch	caramel
1.blossom whirling	17.3	34.0	30.0	0.2	64.0	<1.8	69.8	NEG	NEG
2.blossom pressed	18.0	33.5	30.5	0.2	64.0	<1.8	65.0	NEG	NEG
3.honeydew whirling	15.7	31.0	27.0	0.7	57.0	<1.8	83.3	NEG	NEG
4.honeydew pressed	14.8	26.5	22.7	1.1	49.2	<1.8	92.5	NEG	NEG

Note: Testing Laboratory of the Bee Research Institute in Dol,CR. Water – water content, Fru – fructose , Glu – glucose, Suc – sucrose, Fru+Glu sum fructose and glucose, HMF – fydroxymethylfurfural, NEG – negative proof.

their main chemical composition and results are given in Table 5.

According to the results of measured parameters, there was no difference between the whirling samples of honey and the pressed honey in group C. The difference was evident in the sugary content between honeydew and blossom honey. Honeydew honey shows lower levels of sum fructose and glucose in comparison to blossom honey. This difference was expected and corresponds with our results in Table 3 and Table 4. The median value of honeydew from the market chain (group A) had a sum of fructose and glucose 55.1%, and for blossom honey 72.9%. The expected difference was also confirmed in samples from beekeepers (group B). Honeydew samples had a mean value of the sum of fructose and glucose 52.6%, unlike blossom honeys 70.8%. A lower total average of 61.7 ±10.7% of the sum of fructose and glucose in samples from beekeepers is due to the higher number of samples honeydews in group B (5 samples honeydew´s honeys with naturally lower content of sugars) unlike the samples of group A (only 3 samples honeydew´s honeys) with a total average of 65. 9 ±12.1%. This statistical comparison of samples in group C was not done due to a small number of samples in this group. The difference in the chemical composition of honey harvested by different techniques was not proved. All samples of group C honeys listed in Table 5 comply with the **Decree 76/2003 Coll**. **(2003)**, **Council Directive 2001/110/EC (2001)** respectively, in all measured parameters, including conductivity, where samples of honeydew had higher conductivity than minimum required by the standard (minimum 80 mS.m^{-1}). On the other hand, for blossom honey samples the values were lower than the allowed maximum 80 mS.m^{-1}. The results corresponds with the balanced composition and quality of honey from beekeeper.

The water content in the individual samples of honey in both, groups A and B (Table 3, Table 4) and in the other 4 samples group C (Table 5) did not exceed the water content requirement of 20% (in **Decree 76/2003 Coll**., **2001/110/EC**). This limit is set to determine of honey to ferment. According to **Titěra (2006)**, the optimal average water content for blossom honey is 17.2% and for honeydew 16.3%. The water content when honey begins to ferment is above 21%.

The detection of illegal honey interferences such as heat treatment, to re-liquefy the crystallized honey by heating to a temperature higher than the allowed temperature (maximum 50 °C) or detection of additions of illegal ingredients and adulteration of honey by adding cheaper substitutes (e.g. technical syrup and others), are possible due to the specific markers. HMF is used as a marker of honey heat treatment and its value increases with the heating temperature. To detect adulteration of honey with unauthorized additives there are assays which show even low levels of substitutes, such as Fiehe test (it already detects addition of 1% starch, 2% addition is clearly demonstrable) and the test of addition of technical syrups **(Vorlová et al., 2006)**.

In this study we analysed all collected samples of honey group A and group B for both markers by the Fiehe test and by the second test to prove the adulteration of honey by technical syrup. In all the samples group A and group B, there was only 1 positive sample in group A for about 25% presence of addition of technical syrup (proof was repeated three times). Both these tests, for the presence of illegal substitutes to honey (starch or syrup) are easy to perform, but do not replace the analytical methods. However, they serve for quick detection of unauthorized interferences in honey. In contrast, HMF analysis is quantitative, expensive and requires instrumentation.

The presence of undesirable impurities, such as residues of pesticides or presence of toxic elements has not been tested in this study. But, the presence of toxic elements as zinc, copper, lead, arsenic and cadmium in honey samples from some areas has been reported in some studies **(Roman and Popiela, 2011)**. This study demonstrated that the most problematic element of honey in small area in Poland was lead. This fact can be probably caused by higher human activities in this locality. But our study was focused only on the main parameters of honey.

The results of chemical analyses of all, group A and group B honey samples, sum of fructose and glucose, water content and titration acidity were assessed in accordance with the **Decree 76/2003 Coll**. **(2003)**, and the **Council Directive 2001/110/EC (2001),** respectively. For all analysed samples, the given limits have been met, with the exception of 1 sample of honey from the market chain

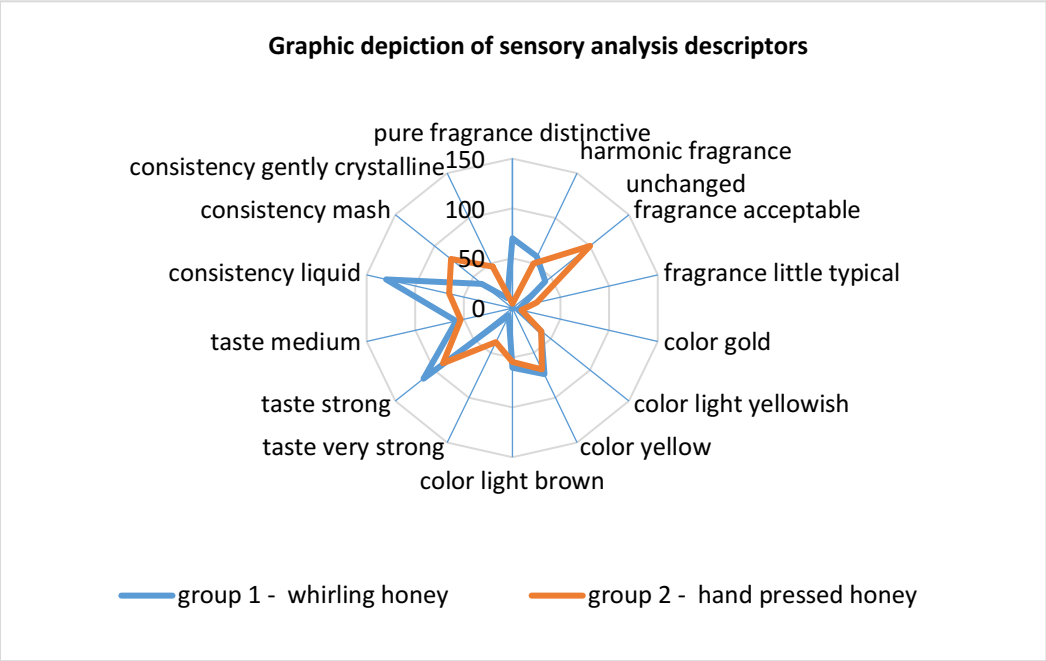

Figure 1 Sensory analysis honey from beekeepers harvested by different technics.

(group A), where we have proven 25% addition of technical syrup.

For 1 sample of honey from beekeepers (group B), the sum of fructose and glucose was just below the required value, the difference was 2.9%.

According to the Czech Agriculture Food Inspection Authority **(CAFIA, 2017**), approximately 40% of honey samples in the market chain do not meet the quality parameters over the long term. However, the results of the CAFIA summarized the data by 2016. In the study of **Cwiková et al. (2015)** only 4 from 21 honey samples complied with the requirements for content of sugars according to the **Council Directive 2001/110/EC (2001)** relating to honey. In comparison with the results of the Czech Agriculture Food Inspection Authority, our results showed a better quality of honey in the market chain and from beekeepers, too. From a total of 20 samples only 2 samples did not meet legislative requirements. The current tightening of control seems to be the positive effect to improve quality of offered honey.

The main parameters of honey samples results were evaluated statistically. The Shapiro-Wilk's test confirmed the normal distribution (p >0.05, normality was not rejected) in the measured parameters: water content, titratable acidity and sum of glucose and fructose.

Statistically significant differences for water content (p = 0.0224), titratable acidity (p = 0.0400) and sum of fructose and glucose (p = 0.0009) between honeydew honey and blossom honey were found in 20 samples of honey. For the statistical evaluation of the measured parameters between groups A (market) and B (beekeepers) a two-tailed t-test with equal variance (F<F crit) for acidity and water content, two-tailed test t-test with unequal variance (F>F crit) for sum fructose and glucose were used. A two-tailed t-test showed a statistically significant difference (p = 0.039) for titratable acidity between samples from the market chain (group A) and samples from beekeepers (group B). In the other parameters, as water content (p = 0.1435) and sum of fructose and glucose (p = 0.1883) the difference were statistically insignificant.

The diference between the sum of glucose and fructose in honeydew honey and blossom honey is expected and as in line with the **Council Directive2001/110/EC (2001**) giving different requirements for content of sugars in honey from different origin. Statistically signifiant difference in acidity, little higher in group B, honey samples from market chain, can be explained by the time of storage. We assume that samples obtain from beekeepers were fresh, and time of storage was shorter than in case of honey samples from market chain. According to the study **Kňazovická et al. (2015**), the authors recorded small increase of acidity honey samples after half year of storage.

Sensory analysis of honey samples group C was focused on 4 samples only from beekeepers harvested by different techniques. Some of differences were found in consistency and smell but generally there were not big differences in sensory profiles between manually pressed and whirling samples. This indicate that harvesting technique has no significant impact on the composition and properties of honey (Figure 1).

There are not many scientific studies documenting the proven therapeutic effects of honey for human health. But its preventive effect is observed and monitored for years. Data on the health status of the population published by the **WHO (2017)** are alarming. Cardiovascular diseases worldwide are the most common cause of death, over the past 15 years the number of deaths for cardiovascular disease has increased. Ischemic heart disease and stroke are the world's biggest killers. In 2000, worldwide, 5.41 million people died of stroke; in 2015, it was 6.24 million people. The number is even higher in deaths for coronary heart disease. In 2000, worldwide 6.88 million of people died on ischemic heart disease, in 2015 it was 8.76 million of people. For diabetes and its complications were reported in 2000, fewer than 1 million deaths worldwide, but in 2015 it was 1.59 million deaths **(WHO, 2017)**.

If the honey has proven anti-inflammatory effect, it may play an important role in the diet with other nutritional important elements, beneficial to health, as a whole grain foods and fresh fruit and vegetables, effective in prevention of cardiovascular diseases **(Gonzáles, et al., 2016)**. Studies on the health effects of honey are highly desirable. The basic premise is accessible, safe and quality honey for consumers.

CONCLUSION

Our results proved in all honey samples taken from beekeepers and samples from the market, with only one exception and with one small difference, the compliance of tested quality parameters with the values required by legislative criteria for honey. Statistically significant difference in the content of sum fructose and glucose, water content and titratable acidity were found between honeydew honey and blossom honey. Statistically significant difference between honey samples from market chain and honey samples from beekeepers was found only in titratable acidity. Sensory analysis showed no differences between sensory properties of honey obtained by whirling and hand pressed honey. Because of biologically important ingredients, easy accessibility and easy use honey is considered as an important ingredient in nutrition and could be recommended in the prevention of various diseases.

REFERENCES

Alvarez-Suarez, J. M., Gasparrini, M., Forbes-Hernández, T. Y., Mazzoni, L., Giampieri, F. 2014. The composition and biological activity of honey: a focus on Manuka honey. *Foods*. vol. 3, no. 3, p. 420-432. http://doi.org/10.3390/foods3030420

Alvarez-Suarez, J. M. 2017. *Bee Products - Chemical and Biological Properties*. Springer International Publishing AG, p. 306. https://doi.org/10.1007/978-3-319-59689-1

Bogdanov, S., Lüllmann, C., Martin, P., von der Ohe, W., Russmann, H., Vorwohl, G., Oddo, L. P., Sabatini, A. G., Marcazzan, G. L., Piro, R., Flamini, Ch., Morlot, M., Lhéritier, J., Borneck, R., Marioleas, P., Tsigouri, A., Kerkvliet, J., Ortiz, A., Ivanov, T., D'Arcy, B., Mossel, B, Vit, P. 1999. Honey quality and international regulatory standards: review by the International Honey Commission. *Bee World*. vol. 80, no. 2, p. 61-69. https://doi.org/10.1080/0005772X.1999.11099428

Bogdanov, S. 2011. Functional and biological properties of the bee products: a review. Available at: www.bee-hexagon.net. *Bee Product Science*. Published 1. February 2011.

CAFIA. 2017: CAFIA Activity Report for 2016. Available at: http://www.szpi.gov.cz/clanek/zprava-o-cinnosti-szpi-za-rok-2016.aspx

Codex Alimentarius. 1994. *Codex Standard for Honey, Codex Stan 12-1981*. Rev.1 (1987), vol. 11. FAO, Rome, Italy.

Council Directive 2001/110 EC of 20 December 2001 relating to honey.

Cwiková, O., Pavlíková, H., Ansorgová, A. 2015. Detection of honey adulteration using HPLC method. P*otravinarstvo*. vol. 9, no. 1, p. 388-392. https://doi.org/10.5219/499

ČSN 57 0190, 1974. *Testing methods for bee honey*. Prague, Czech Republic : Vydavatelství Úřadu pro normalizaci a měření. p. 28.

Decree of the Ministry of Agriculture of the Czech Republic No.76 / 2003 Coll. as amended by Decree No. 43/2005 Coll., laying down requirements for natural sweeteners, honey, confectionery, cocoa powder and mixtures of cocoa with sugar, chocolate and chocolate bonbons. Collection of Laws, 2003.

Erejuwa, O. O., Sulaiman, S. A., Wahab, M. S. 2012. Honey: a novel antioxidant. *Molecules*. vol. 17, p. 4400-4423. https://doi.org/10.3390/molecules17044400

Erejuwa, O. O., Sulaiman, S. A., Wahab, M. S. 2014. Effects of honey and its mechanisms of action on the development and progression of cancer. *Molecules*. vol. 19, p. 2497-2522. https://doi.org/10.3390/molecules19022497

EU.1 996. *Proposal for a directive of the European council relating to honey. Document 96/0114*. Bruxelles, Belgium : EC.

González-Gil, E. M., Santabárbara, J., Russo, P., Ahrens, W., Claessens, M., Lissner, L., Börnhorst, C., Krogh, V., Iacoviello, L., Molnar, D., Siani, A., Tornaritis, M., Veidebaum, T., Moreno, L. A. 2016. Food intake and inflammation in European children: the IDEFICS study. *European Journal of Nutrition*. vol. 55, p. 2459-2468. https://doi.org/10.1007/s00394-015-1054-3

Guziy, S., Šedík, P., Horská, E. 2017. Comparative study of honey consumption in Slovakia and Russia. *Potravinarstvo*. vol. 11, no. 1, p. 472-479. https://doi.org/10.5219/784

Hadagali, M. D., Chua, L. S. 2014. The anti-inflammatory and wound healing properties of honey. *European Food Research and Technology*. vol. 239, p. 1003-1014. https://doi.org/10.1007/s00217-014-2297-6

ISO 8589. 2007. *Sensory analysis - General guidance for the design of test rooms*. Geneva, Switzerland : International Organization for Standardization. 16 p.

ISO 6658. 2017. *Sensory analysis - Methodology - General guidance*. Geneva, Switzerland : International Organization for Standardization. 26 p.

Kňazovická, V., Bačiková, A., Bányiová, R., Tkáčová, J., Čanigová, M., Haščík, P. 2015. Honey characteristics after extraction and half-yearstorage. *Potravinarstvo*. vol. 9, no. 1, p. 543-549. https://doi.org/10.5219/560

McLoone, P., Warnock, M., Fyfe, L. 2016. Honey: an immunomodulatory agent for disorders of the skin. *Food and Agricultural Immunology*. vol. 27, p. 338-349. https://doi.org/10.1080/09540105.2015.1104653

Miguel, M. G., Antunes, M. D., Faleiro, M. L. 2017. Honey as a complementary medicine. *Integrative Medicine Insights*. vol. 12, p. 1-15. https://doi.org/10.1177/1178633717702869

MZe. 2015. *Situational Outlook Report of the Czech Ministry of Agriculture. Bees. November 2015*. Available at: http://eagri.cz/public/web/file/457620/SVZ_Vcely_2015_komplet_final_20042016.pdf

Piana, L. M., Oddo, L. P., Bentabol, A., Bruneau E., Bogdanov S., Declerk, Ch. G. 2004. Sensory analysis applied to honey: state of art: *Apidologie*. vol. 35, p. S26-S37. https://doi.org/10.1051/apido:2004048

Roberfroid, M. B. 2000. Prebiotics and probiotics: are they functional foods? *American Journal of Clinical Nutrition*. vol. 71, p.1682-1687. https://doi.org/10.1093/ajcn/71.6.1682S

Santos-Buelga, C., González-Paramás, A. M. 2017. *Chemical Composition of Honey*. p. 43-82. In: Alvarez-Suarez, J. M. (eds): *Bee Products - Chemical and Biological Properties*. Springer, Cham. https://doi.org/10.1007/978-3-319-59689-1-3

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

Song, J. J., Salcido, R. 2011. Use of honey in wound care: an update. *Advances in Skin & Wound Care*. vol. 24, p. 40-44. https://doi.org/10.1097/01.ASW.0000392731.34723.06

Titěra, D. 2006: *Bee products demystified (Včelí produkty mýtů* zbavené). 1st ed. Prague, Czech Republic : Vydavatelství Brázda s.r.o., 176 p. ISBN 80-209-0347-x.

Tsiapara, A. V., Jaakkola, M., Chinou, I., Graikou, K., Tolonen, T., Virtanen, V., Moutsatsou, P. 2009. Bioactivity of Greek honey extracts on breast cancer (MCF-7), prostate cancer (PC-3) and endometrial cancer (Ishikawa) cells: profile analysis of extracts. *Food Chemistry*. vol. 116, p. 702-708. https://doi.org/10.1016/j.foodchem.2009.03.024

Viuda-Martos, M., Navajas, Y., Fernández-López, J., Pérez-Álvarez, J. A. 2008. Functional properties of honey, propolis, and royal jelly. *Journal of Food Science*. vol. 73, p. R117-R124. https://doi.org/10.1111/j.1750-3841.2008.00966.x

Vorlová L., Hálková, G., Přidal, A., et al. 2002. *Honey - Comprehensive analysis (Med – Souborná analýza)*. 1st ed. Brno, Czech Republic : Veterinární a farmaceutická univerzita Brno, p. 67. ISBN 80-7305-450-7. (In Czech)

WHO. 2017. *The top 10 causes of death. Fact sheet, January 2017*. Available at: http://www.who.int/mediacentre/factsheets/fs310/en/

Yaghoobi, N., Al-Waili, N., Ghayour-Mobarhan, M., Parizadeh, S. M., Abasalti, Z., Yaghoobi, F., Esmaeili, H., Kazemi-Bajestani, S. M., Aghasizadeh, R., Saloom, K. Y., Ferns, G. A. 2008. Natural honey and cardiovascular risk factors; effects on blood glucose, cholesterol, triacylglycerole, CRP, and body weight compared with sucrose. *Scientific World Journal*. vol. 8, p. 463-469. https://doi.org/10.1100/tsw.2008.64

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THERMAL AGING OF EDIBLE OILS: SPECTROPHOTOMETRIC STUDY

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ABSTRACT
The aim of the present study was to determine the spectrophotometric and thermal aging properties of various edible oils (olive, peanut, rapeseed, soybean and sunflower oils) which are commonly available in the Czech market. The samples were measured by UV/VIS absorption spectrometry and fluorescence spectroscopy. Detected substances of UV/VIS spectra were compared to expected oil composition; the highest absorbance values were detected in a wavelength range 300-550 nm which can be related to the presence of unsaturated fatty acids. The mixtures of oils were characterized by fluorescence spectroscopy; the individual oils were successfully distinguished according to their excitation-emission profiles. This method was also used to detect the samples of adulterated oils, i.e., the adulteration of high-quality oils with soybean oil. From a physicochemical point of view, the influence of temperature on the compounds of extra virgin olive oil was examined by thermal stress simulation. This thermal aging analysis demonstrated that the amount of oxidation products in olive oil increased during the heating whereas the chlorophyll content decreased. The results showed the ability of the techniques used, UV/VIS absorption spectrometry and fluorescence spectroscopy, to characterize the quality and composition of oils, and to distinguish individual oils in blends. UV/VIS spectrometry was also successfully employed for the evaluation of olive oil qualitative parameters according to the standard quality parameters by the "International Olive Council" (EEC 702/2007).

Keywords: fluorescence spectroscopy; UV/Vis; thermal aging; oils; quality

INTRODUCTION
The investigation of physicochemical properties of oils, their composition, the effect of heat on their stability and quality and other technological aspects are the main topic of many research articles published at the present time **(Burg et al., 2017; Munasinghe and Wanspala, 2015; Chen et al., 2015; Timilsena et al., 2017)**. Particularly, the oxidation stability of edible oils and the possibilities of its enhancement are of growing interest nowadays **(Comunian et al., 2017; Hernández Sánchez et al., 2016; Zhang et al., 2017)**.

Edible oils have their own characteristics that differ from each other. They have specific aroma, taste, color and nutritional properties. These properties may change during oil storage and thermal treatment, due to the changes in oil composition. Changes in oil components can be influenced in some ways, e.g., by proper storage, suitable packaging material, and light access. In general, oils must be kept in a dark place protected from sunlight to prevent oxidation and degradation **(Gutiérrez and Fernández, 2002)**.

During only a few months of storage, changes can occur not only on lipids and fatty acids but also on minor components of stored oil. There are significant losses of chlorophyll, carotenoids and total oil phenol content throughout the whole period **(Morelló et al., 2004; Thanh et al., 2006)**.

Autooxidation is the main cause of oil deterioration, and it is of fundamental importance in the processing of oils in the food industry **(Behlau and Widmann, 2003)**. This undesirable process depends on a number of factors such as the initial composition of the oil, its chemical structure, the presence of minor substances, the content of antioxidants (minerals, tocopherols, carotenoids, chlorophylls) and storage conditions. Some oils are prone to autooxidation because of a high content of polyunsaturated fatty acids. For that reason, antioxidants such as tocopherol are added to the oils to control oxidation during their processing **(Zuta et al., 2007; Gunstone, 2013; Arora et al., 2010)**.

On the other hand, triesters can hydrolyze and produce glycerides and free fatty acids, and non-saturated chains can react with oxygen to produce oxidative products responsible for lipids deterioration. These two phenomena are the cause of two major forms of changes in food fats, i.e., acidification and oxidation **(Burg et al., 2017)**.

During thermal treatment of oils (e.g., frying), many chemical reactions give rise to a wide range of substances. The frying oils can contain more than 400 different heat-induced reaction products, the majority of which become absorbed into the fried food. Unfortunately, many of these

compounds can be harmful to human health **(Sebastian et al., 2014)**. Triacylglycerols (TAGs) as the major constituent of oils are hydrolyzed under these conditions, and pyrolysis occurs when the so-called smoke point is exceeded. Above this limit, free fatty acids (FA) are cleaved from TAGs and acrolein is formed **(Pamies and Vilanova, 2014; Osório and de Lourdes Cardeal, 2011; Suh et al., 2017; Bastos et al., 2017)**.

Free FA increase thermal oxidation of oils, and their unsaturation rather than chain length lead to significant effects upon thermooxidative degeneration of oils. The oxidation rate of a frying oil increases as the content of unsaturated fatty acids of the oil increases. The content of linolenic acid is critical to the frying performance, the stability of oil, and the flavour quality of fried food **(Choe and Min, 2007)**.

Edible oils are also characterized by the polymorphism, i.e., by the ability to crystallize in different modifications. The formation of crystalline modifications is affected by the composition and rate of oil cooling. The gradual transition of crystalline modifications proceeds from the least stable modification γ (amorphous) to the most stable β modification of grainy crystal structure. For that reason, it is very important to keep the right temperature storing conditions **(Widlak et al., 2001)**.

For the consumer, it is important to know functional properties of an edible oil, to find whether it can be used for specific purposes such as frying or just a salad oil at normal temperatures, and whether it decomposes at higher temperatures with the subsequent release of potentially toxic products or, on the other hand, with the loss of health benefit substances. For instance, phenols and tocopherols (vitamins E) decompose in olive oil at temperatures above 180 °C **(Hammer, 2008)**.

Scientific hypothesis

There were used spectrophotometric methods of edible oil analysis for detection of their adulteration and thermal degradation. There were evaluated concentrations of oxidation products and chlorophyll pigments in studied olive oil to confirm the hypothesis that the effect of thermal stress significantly changed its chemical composition. Additionally, the study was focused on the evaluation of olive oil quality according to the procedure of the „International Olive Council“ (EEC 702/2007). The compliance of oil qualitative parameters (UV/VIS data) with the criteria defined for an extra virgin olive oil were assessed.

MATERIAL AND METHODOLOGY

Materials

Olive, peanut, rapeseed, soybean and sunflower oils were purchased in the Czech market. Olive oil was declared as oil of extra virgin quality.

In accordance with the manufacturer's recommendation, the oils were stored in a dry, dark place at temperatures up to 15 °C. The relative humidity was about 40% (vol.).

The chemical composition of untreated oils is stated in Table 1.

The chemicals used as non-polar solvents to dilute the samples of oils to a specified concentrations were delivered by the suppliers; heptane 99% (v/v) of spectrophotometric grade, by Sigma Aldrich (USA); cyclohexane 99.99% (v/v) of analytical grade, by IPL (Czech Republic).

Methods

UV/VIS absorption spectrometry

UV-VIS spectrophotometric measurements were realized by UV/VIS spectrophotometer CECIL, CE 1021, series 1000 (Germany). The cuvettes of quartz glass, type 6030-UV of light path 10 mm (Hellma Analytics, Germany) were employed in the experiments. All measurements were performed at laboratory temperature (ca. 25 °C).

The samples of oils were diluted in ratio 1:3, 1:5 and 1:10 with heptane and measured in the wavelength range 300 – 800 nm. UV/VIS spectra were recorded and the concentration dependence of absorbance on wavelength was evaluated. Based on maximum absorbance values, the chemical components in the oils were specified.

UV/VIS data were used to evaluate the quality of extra virgin olive oil using the method by the „International Olive Council“ (EEC 702/2007). The principle of this method is based on the fact that an olive oil of lower quality contains the conjugated dienes and trienes formed as a result of oxidative degradation processes in oil. The conjugated carbon-carbon double bonds absorb UV light in the wavelength range 200 – 300 nm. In contrast to this, non-conjugated double bonds, which are present in an extra virgin olive oil (e.g., unsaturated fatty acids), do not absorb light within the spectral range. Consequently, low absorption in the spectral range of 200 – 300 nm indicates high quality extra virgin olive oil and high absorption oils of lower quality **(De Caro and Schubnell, 2015)**.

The "International Olive Council" (EEC 702/2007) defined three criteria that must be valid for an extra virgin olive oil; the criteria consists in several extinction coefficients Kλ at specified wavelengths λ (232 nm, 266 nm, 270 nm and 274 nm). An extra virgin olive oil diluted

Table 1 Characteristics of edible oils by the producers.

Sample of oil	Producer	Chemical composition (g $100g^{-1}$)		
		FA saturated	FA monounsaturated	FA polyunsaturated
Olive extra virgin	Kreolis	12.8	70.5	8.3
Peanut	Topvet	11.0	28.0	52.0
Rapeseed	Lukana	7.4	-	-
Soybean	Country Life	14.0	-	-
Sunflower	Viviol	14.0	-	-

Note: The hyphen means not stated value.

with cyclohexane to the concentration 1% (v/v) must meet the following relations: K232 ≤2.7, K270 ≤0.4 and ΔK ≤0.01.

Kλ is calculated by the Eq. 1:

$$K\lambda = A\lambda /(c.L) \quad (1)$$

where Aλ is the absorbance at the wavelength λ, c – the concentration of oil in solvent used, and L – the light path of measuring cuvette.

The equation of ΔK has the following form:

$$\Delta K = K270 - ((K266 + K274)/2) \quad (2)$$

where K266 and K274 are the extinction coefficients at 266 nm and 274 nm, respectively **(De Caro and Schubnell, 2015; Commission Regulation (EC) No. 1989/2003; Commission Regulation (EC) No. 702/2007)**.

According to the standard, the extra virgin olive oil was diluted to 1% (v/v) solution in cyclohexane, and the qualitative parameters were evaluated by the procedure described above.

Fluorescence spectroscopy

Spectrophotometric measurements were realized using spectrophotometer RF-1501, Shimadzu Corporation (Japan), enabling the setting (counting) of individual nanometric units (nm). The precision cells made of quartz glass with light path 10 mm, type 6030-UV (Hellma Analytics, Germany) were used for the analysis.

The fluorescent spectra of vegetable oils were measured in the range of excitation wavelengths 220 – 400 nm, and emission wavelengths 300 – 800 nm. The samples of oils were diluted with heptane in proportions 1 : 50, 1 : 100, 1 : 500 due to the observed concentration dependences. The mixtures of oils were prepared in several tested proportions about 1 : 10 of soybean to another oil in order to distinguish the individual compounds (i.e., the specific oils) by spectrophotometric method, and by this mode to detect adulterated oils.

Thermal aging of oils

Thermal aging was simulated by heating extra virgin olive oil at 110 °C for 25 hours. Before the heating process, the samples of oil were diluted with heptane in ratio 1 : 100. Then the solutions were successively heated for a 5-h, 10-h, 15-h, 20-h and 25-h period. One sample of olive oil was used in a thermally untreated (natural) state. Each sample was measured 3 times and the mean value of fluorescence emission intensity (in arbitrary units) was calculated from the results. The samples of oil were analysed by the spectrofluorometer in excitation wavelength range 220 – 400 nm and following emission range 300 – 800 nm.

Statistical analysis

All measurements were performed three times for each type of oil. Data were analyzed by ANOVA nonparametric statistics using SigmaPlot (Systat Software, USA). Where statistical differences among the data were determined, the significance was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Samples of selected oils were investigated using UV/VIS spectrometry and fluorescent spectroscopy. The blends of an oil with soybean oil as a cheap substitute were analysed in order to find the detection limit of fluorescence spectroscopy method for the accurate distinction of individual oils in the case of their adulteration. Thermal properties of olive oil were studied by the method of thermal stress simulation to describe the influence of temperature and time on oil chemical composition.

UV/VIS absorption spectrometry

The quality of oils can be evaluated according to the content of specific substances such as tocopherols, chlorophylls and carotenoids which influence the oxidative stability of oils (Gonçalves et al., 2014). UV/VIS spectra were used to evaluate the chemical composition of oils, i.e., the maximum absorbance values were related to specific substances of oils, dependent on the wavelength recorded; the values were statistically significant ($p < 0.05$).

The highest absorbance values of all samples were detected in a wavelength range 300 – 550 nm; the peaks between 400 – 500 nm can be related to unsaturated fatty acids present in the oils. In the case of soybean oil, the most intense peak was determined around 450 nm. The maximum absorbance around 670 nm found in olive oil spectrum confirmed the presence of chlorophyll pigments in relatively high amount in this oil. The comparison of UV/VIS spectra of soybean and olive oil at various dilutions is illustrated in Figure 1.

All samples showed an intense peak at wavelength about 290 nm related to the content of tocopherols. This is in accordance with the results by **Zou et al. (2018)** who detected α- and β-tocopherols at 295 nm in wheat germ oil by HPLC method. The presence of tocopherols in olive oil in our study indicates the high quality of oil examined because tocopherols protect the oil at elevated temperatures, and their content is reduced in the course of refining process, i.e., the refined oils of lower quality are less oxidation-stable than virgin oils of higher antioxidants content **(Gharby et al., 2016)**.

The qualitative parameters of olive oil obtained by UV/VIS spectrometry were assessed by the extinction parameters Kλ which are conventionally used to evaluate the quality of olive oils in the food industry.

In Table 2, there are stated the criteria for an extra virgin olive oil according to the "International Olive Council" (EEC 702/2007) which are compared with the parameters calculated for the olive oil tested in our study.

It can be summarized from the results, that olive oil in the present study significantly meets all postulated criteria ($p < 0.05$) and can be declared as oil of the highest (extra virgin) quality.

Fluorescence spectroscopy

Using fluorescence spectroscopy, the edible oils were characterized according to the presence of specific substances (fluorophores), which provide the characteristic emission spectra to each type of oil **(Xu et al., 2016)**.

Table 2 Criteria and calculated parameters for extra virgin olive oil.

Criteria (extinction coefficients)	Values according to IOC	Values calculated for olive oil
K_{232}	≤2.7	2.05 ±0.05
K_{270}	≤0.4	0.21 ±0.02
ΔK	≤0.01	0.00 ±0.00

Note: IOC, the "International Olive Council"; ± values indicate the standard deviation. The number of replicates, 3.

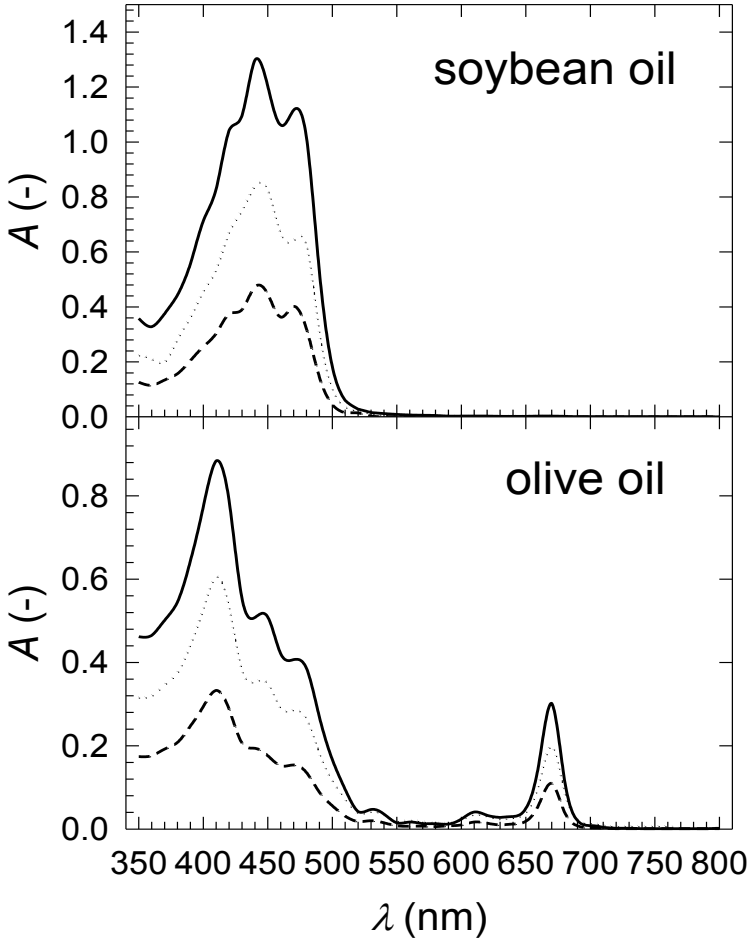

Figure 1 UV/VIS absorption spectra of soybean and olive oil diluted with heptane in the ratio 1 : 3 (full line), 1 : 5 (dotted line) and 1 : 10 (dashed line): A - absorbance, λ – wavelength.

Relatively intense signal was observed in emission range 300 – 350 nm which can be attributed to tocopherols and tocotrienols. For olive oil, fluorescence signal was distinctly intensive in emission range 660 – 700 nm related to chlorophyll pigments. Since extra virgin olive oil is not considerably technologically modified, the chlorophyll content is relatively unchanged. Thus, the intensity of fluorescence signal can indicate the quality of edible oil and the mode of its technological treatment. The signal recorded in emission range 440 – 455 nm of varying intensity for each type of oil and its concentration (dilution) correspond to the content of monounsaturated fatty acids. The most pronounced signal was detected for rapeseed oil indicating the highest amount of monounsaturated FA among all samples. As expected, the signal was increasing with rising oil concentration. On the other hand, olive oil provided signal of relatively low intensity at wavelength about 450 nm, suggesting relatively low content of monounsaturated FA. This intensity was minor in comparison to the fluorescence intensity recorded at about 670 nm (chlorophyll content) in the same oil. The results of the present study can be compared to the investigation by **Sikorska et al. (2005)** who observed the emission spectra of tocopherols and chlorophylls pigments at the same wavelengths for various types of oils.

The mixtures of oils were measured by fluorescence spectroscopy to differentiate the oils in the blends with soybean oil, i.e., to detect the adulterated oils. Because fluorescence spectroscopy is a cost-effective and non-destructive technique, it is particularly used for the quality control of oils **(Guzmán et al., 2015)**.

Based on the previous results, the characteristic excitation-emission spectra of individual oils were compared with the spectra of blends and the fluorescence intensity was assessed. It can be summarized that the peaks of all blends showed higher intensity compared to the individual oils, i.e., the addition of soybean oil to another oil was successfully detected. The data of fluorescence intensity were evaluated as statistically significant (p <0.05). The adulteration of oils was revealed at concentration of 9% (v/v) soybean oil, which is in accordance with the study by **Li et al. (2015)** who determined the detection limit of soybean oil (added

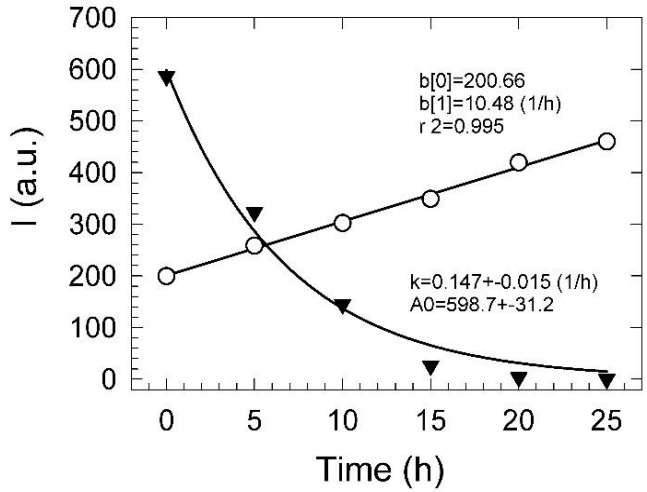

Figure 2 Dependence of fluorescence emission intensity *I* on time during thermal stress load (at 110 °C) of extra virgin olive oil detected at 450 nm (empty circles) and 667 nm (full triangles) wavelengths: a.u. – arbitrary units, h – hours. Insets: parameters of the linear regression y = b(1) * x +b(0) and 1. order formal kinetics nonlinear calculations y = A0*exp(-k*x). y = I (a.u), x = time (h).

into another oil) equal to 10% (v/v). The sensitivity of the method used seems to be adequate for the requirements of successful detection of the illegal commercial oils adulteration.

Thermal aging of oils

As investigated, the most suitable oil for frying and other thermal treatment is refined olive oil, which can be heated to 210 °C because it is stable due to the presence of a large amount of antioxidants and a lower fatty acids (FA) content. High-grade rapeseed oil shows also suitable properties for long-term processing. In opposite to these oils, soybean and sunflower oils with a relatively high content of unsaturated FA are less appropriate for longer thermal treatment **(Choe and Min, 2007)**.

For the above reasons, an extra virgin olive oil was chosen to perform the effect of a relatively long-term thermal stress on the oxidation stability of oil system. Time dependence of the formation of oxidation products and chlorophyll pigments at thermal stress was evaluated by fluorescence emission intensity. In all cases, the chlorophyll concentration was decreased at 667 nm and, on the other hand, there was an increase in concentration of oxidation products at a wavelength of 450 nm. The increasing concentration of oxidation products is consistent with the increase amount of free FA during thermal treatment of olive oils **(Gharby et al., 2016)**. With increasing time of heating procedure, the differences in fluorescence intensity indicating the concentrations of oxidation products and chlorophyll pigments became more statistically significant (p <0.05), as can be seen in Figure 2.

The results of the present study are in accordance with the results by **Guzmán et al. (2015)** who found a strong fluorescence emission band at 430 – b450 nm for oxidised olive oils which can be related to oxidation intermediates. The authors also identified a medium intensity band at 681 nm, representing a chlorophyll content, which can be compared to the emission wavelength 667 nm of chlorophylls in our study. **Gonçalves et al. (2014)** investigated the thermal aging of several oils by UV/VIS measurement. As in our study, the authors determined the increase in the concentration of oxidation intermediates during the heating procedure (by gradual increase of temperature). In the case of olive oil, they detected the change in oxidation products level already at 70 °C.

Because oil is a complicated system, there can be a correlation in the results of thermal aging with different commercial products. Moreover, the oxidation intermediates are often present in untreated oils stored at room temperature, which can be associated with using of transparent oil packaging, effect of light and other storing conditions **(Gonçalves et al., 2014)**. Therefore, the other investigation of oil thermal properties is needful and desirable.

CONCLUSION

The results of the present study demonstrate that the methods of UV/VIS absorption spectrometry and fluorescence spectroscopy can be successfully used to characterize selected oil samples with good sensitivity.

The study was performed on 5 commercial oils: olive, peanut, rapeseed, soybean and sunflower oils. Using qualitative analysis, it was possible to distinguish individual types of oils, determine the adulteration of oils in the mixture, and evaluate thermal aging of oils.

The unsaturated fatty acids in oils were detected by fluorescence spectroscopy, providing relatively high intensity in 400 – 500 nm emission area, which can be attributed to unsaturated fatty acids. For olive oil, the intensity was pronounced in the range 660 – 700 nm emission area attributable to the chlorophyll content in the oil.

Using UV/VIS absorption spectrometry, all samples examined showed an intense peak, which occurs at a wavelength of 290 nm assigned to tocopherols. Olive oil had a second peak, occurring around 670 nm, belonging to chlorophyll pigments. Moreover, the UV/VIS parameters of olive oil met the criteria defined by the "International Olive Council" (EEC 702/2007) and can be declared as oil of extra virgin quality.

The results demonstrate the ability of spectrophotometric techniques to characterize and differentiate vegetable oils. In addition, a study of the effect of thermal stress on olive oil was realized with fluorescence spectroscopy. By constant heating, the chlorophyll concentration in oil was reduced at 667 nm of emission area and, on the other hand, there was an increase in the concentration of oxidation products at emission wavelength of 450 nm. With increasing time of heating, the difference in concentrations was more significant.

The adulteration of high-quality oils by soybean oil was also studied. The results proved a sufficient sensitivity of the spectrophotometric method to distinguish individual oils; the adulteration was revealed even when added only 9% (v/v) of false (soybean) oil to the original one. The fluorescence signals were assigned to specific fluorophores according to which the individual oils can be successfully distinguished or monitored due to the oxidation changes.

REFERENCES

Arora, S., Bagoria, R., Kumar, M. 2010. Effect of alpha-tocopherol (vitamin E) on the thermal degradation behavior of edible oils. Multiple-heating rate kinetics. *Journal of Thermal Analysis and Calorimetry,* vol. 102, no. 1, p. 375-381. https://doi.org/10.1007/s10973-009-0460-2

Bastos, L. C. S., de Almeida Costa, E. A., Pereira, P. A. P. 2017. Development, validation and application of an UFLC-DAD-ESI-MS method for determination of carbonyl compounds in soybean oil during continuous heating. *Food Chemistry,* vol. 218, p. 518-524. https://doi.org/10.1016/j.foodchem.2016.09.036

Behlau, L., Widmann, G. 2003. Food. Collected Applications: Thermal Analysis. Application Handbook: Mettler-Toledo GmbH [online] s.a. [cit. 2017-12-05] Available at: https://www.mt.com/int/en/home/applications/Application_Browse_Laboratory_Analytics/Application_Browse_thermal_analysis/TA_Appl_Handb.html.

Burg, P., Mašán, V., Rutkowski, K. 2017. Evaluation of the pressing process during oil extraction from grape seeds. *Potravinarstvo Slovak Journal of Food Science,* vol. 11, no. 1, p. 1-6.

Chen, M., Zhao, Z., Lan, X., Chen, Y., Zhang, L., Ji, R., Wang, L. 2015. Determination of carbendazim and metiram pesticides residues in rapeseed and peanut oils by fluorescence spectrophotometry. *Measurement,* vol. 73, p. 313-317. https://doi.org/10.1016/j.measurement.2015.05.006

Choe, E., Min, D. B. 2007. Chemistry of Deep-Fat Frying Oils. *Journal of Food Science,* vol. 72, no. 5, p. 1-10. https://doi.org/10.1111/j.1750-3841.2007.00352.x PMid:17995742

Commission Regulation (EC) No 1989/2003 of 6 November 2003 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-pomace oil and on the relevant methods of analysis. *Official Journal of the European Union* L, vol. 295, 6.11.2003, p. 57-66.

Commission Regulation (EC) No 702/2007 of 21 June 2007 amending Commission Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Official Journal of the European Union* L, vol. 161, 22.6.2007, p. 11-27.

Comunian, T. A., Ravanfar, R., de Castro, I. A., Dando, R., Favaro-Trindade, C. S., Abbaspourrad, A. 2017. Improving oxidative stability of echium oil emulsions fabricated by Microfluidics: Effect of ionic gelation and phenolic compounds. *Food Chemistry,* vol. 233, p. 125-134. ISBN 0308-8146.

De Caro, C. A., Schubnell, M. 2015. Quality control of olive oil by UV/VIS spectroscopy and DSC [online] s.a. [cit. 2017-12-05] Available at: https://www.mt.com/int/en/home/products/Laboratory_Analytics_Browse/uv-vis-spectrometers/cuvette-uv-vis-spectrometers.html.

Gharby, S., Harhar, H., Matthäus, B., Bouzoubaa, Z., Charrouf, Z. 2016. The chemical parameters and oxidative resistance to heat treatment of refined and extra virgin Moroccan Picholine olive oil. *Journal of Taibah University for Science,* vol. 10, no. 1, p. 100-106. https://doi.org/10.1016/j.jtusci.2015.05.004

Gonçalves, R. P., Março, P. H., Valderrama, P. 2014. Thermal edible oil evaluation by UV–Vis spectroscopy and chemometrics. *Food Chemistry,* vol. 163, p. 83-86. https://doi.org/10.1016/j.foodchem.2014.04.109 PMid:24912699

Gunstone, F. D. 2013. Composition and Properties of Edible Oils. In Hamm, W. et al. *Edible Oil Processing.* Indianapolis, USA : John Wiley & Sons, Ltd., p. 1-39. ISBN 978-1-4443-3684-9.

Gutiérrez, F., Fernández, J. L. 2002. Determinant Parameters and Components in the Storage of Virgin Olive Oil. Prediction of Storage Time Beyond Which the Oil is No Longer of "Extra" Quality. *Journal of Agricultural and Food Chemistry,* vol. 50, no. 3, p. 571-577. https://doi.org/10.1021/jf0102158 PMid:11804531

Guzmán, E., Baeten, V., Pierna, J. A. F., García-Mesa, J. A. 2015. Evaluation of the overall quality of olive oil using fluorescence spectroscopy. *Food Chemistry,* vol. 173, p. 927-934. https://doi.org/10.1016/j.foodchem.2014.10.041 PMid:25466108

Hammer, A. 2008. The characterization of olive oils by DSC [online] s.a. [cit. 2017-12-05] Available at: https://www.mt.com/id/en/home/supportive_content/matchar_apps/MatChar_UC282.html.

Hernández Sánchez, M. D. R., Cuvelier, M., Turchiuli, C. 2016. Effect of α-tocopherol on oxidative stability of oil during spray drying and storage of dried emulsions. *Food Research International,* vol. 88, no. Part A, p. 32-41.

Li, B., Wang, H., Zhao, Q., Ouyang, J., Wu, Y. 2015. Rapid detection of authenticity and adulteration of walnut oil by FTIR and fluorescence spectroscopy: A comparative study. *Food Chemistry,* vol. 181, p. 25-30. https://doi.org/10.1016/j.foodchem.2015.02.079 PMid:25794716

Morelló, J., Motilva, M., Tovar, M., Romero, M. 2004. Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. *Food Chemistry,* vol. 85, no. 3, p. 357-364. https://doi.org/10.1016/j.foodchem.2003.07.012

Munasinghe, M., Wanspala, J. 2015. B-Carotene content of M. Longifolia seed oil in different agro-climatic zones in Sri Lanka, the effect of heat on its stability and the composition of seed cake. *Potravinárstvo,* vol. 9, no. 1, p. 474-479. https://doi.org/10.5219/502

Osório, V. M. de Lourdes Cardeal, Z. 2011. Determination of acrolein in french fries by solid-phase microextraction gas chromatography and mass spectrometry. *Journal of Chromatography A,* vol. 1218, no. 21, p. 3332-3336. https://doi.org/10.1016/j.chroma.2010.11.068 PMid:21168848

Pamies, D. Vilanova, E. 2014. Acrolein. *Encyclopedia of Toxicology (Third Edition)*, vol. 2014, p. 63-68.

Sebastian, A., Ghazani, S. M., Marangoni, A. G. 2014. Quality and safety of frying oils used in restaurants. *Food Research International,* vol. 64, p. 420-423. https://doi.org/10.1016/j.foodres.2014.07.033

Sikorska, E., Górecki, T., Khmelinskii, I. V., Sikorski, M., Kozioł, J. 2005. Classification of edible oils using synchronous scanning fluorescence spectroscopy. *Food Chemistry,* vol. 89, no. 2, p. 217-225. https://doi.org/10.1016/j.foodchem.2004.02.028

Suh, J. H., Ho, C., Wang, Y. 2017. Evaluation of carbonyl species in fish oil: An improved LC–MS/MS method. *Food Control,* vol. 78, p. 463-468. https://doi.org/10.1016/j.foodcont.2017.03.014

Thanh, T., Vergnes, M., Kaloustian, J., El-Moselhy, T. F., Amiot-Carlin, M., Portugal, H. 2006. Effect of Storage and Heating on Phytosterol Concentrations in Vegetable Oils Determined by GC/MS. *Journal of the Science of Food and Agriculture,* vol. 86, no. 2, p. 220-225. https://doi.org/10.1002/jsfa.2322

Timilsena, Y. P., Vongsvivut, J., Adhikari, R., Adhikari, B. 2017. Physicochemical and thermal characteristics of Australian chia seed oil. *Food Chemistry,* vol. 228, p. 394-402. https://doi.org/10.1016/j.foodchem.2017.02.021 PMid:28317740

Widlak, N., Hartel, W. R., Narine, S. 2001. *Crystallization and solidification properties of lipids.* Champaign, Illinois : AOCS Press. 246 p. ISBN 18-939-9721-9.

Xu, J., Liu, X., Wang, Y. 2016. A detection method of vegetable oils in edible blended oil based on three-dimensional fluorescence spectroscopy technique. *Food Chemistry,* vol. 212, p. 72-77. https://doi.org/10.1016/j.foodchem.2016.05.158 PMid:27374508

Zhang, Y., Zhang, F., Thakur, K., Ci, A., Wang, H., Zhang, J., Wei, Z. 2017. Effect of natural polyphenol on the oxidative stability of pecan oil. *Food and Chemical Toxicology,* vol. S0278-6915, no. 17, p. 30579-30583.

Zou, Y., Gao, Y., He, H., Yang, T. 2018. Effect of roasting on physico-chemical properties, antioxidant capacity, and oxidative stability of wheat germ oil. *LWT,* vol. 90, p. 246-253. https://doi.org/10.1016/j.lwt.2017.12.038

Zuta, P. C., Simpson, B. K., Zhao, X., Leclerc, L. 2007. The effect of α-tocopherol on the oxidation of mackerel oil. *Food Chemistry,* vol. 100, no. 2, p. 800-807. https://doi.org/10.1016/j.foodchem.2005.11.003

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FUNGAL DIVERSITY IN THE GRAPES-TO-WINES CHAIN WITH EMPHASIS ON *PENICILLIUM* SPECIES

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ABSTRACT

The aim of this work was the description of surface and endogenous mycobiota colonisation of grapes, fresh grape juice, grape must, and wine primarily focused to the current spectrum of the penicillium species. One sample of white grape variety Palava and one sample of blue grape variety Dornfelder were collected in Small Carpathian wine growing region of Slovakia in the year 2017. Direct plating of grapes on agar plates was used for analysis of surface mycobiota of grapes while surface sterilsed grapes were used for endogenous mycobiota analysis. Mycobiota of juice, must, and wine was analysed by plate dilution method. Overall, we isolated 148 strains belonging to 13 genera of filamentous microscopic fungi and *Mycelia sterilia* from grape variety Palava, while the most frequent was *Alternaria*. *Alternaria* was the most common genus in the surface and endogenous colonisation with an average relative density 50% and 73.6%, respectively. A total of 2 species of *Penicillium* were detected from the grapes to wine, potentially toxigenic *Penicillium expansum* and *P. chrysogenum*. A total of 39 strains belonging to 6 genera and *Mycelia sterilia* were identified from grape variety Dornfelder. The most abundant genus was also *Alternaria* (51.3%), followed by *Penicillium* (12.8%). *Alternaria* was the most common genus in the surface and endogenous colonisation and fresh grape juice with an average relative density from 20% (grape juice) to 71% (endogenous colonisation of grapes). A total of 3 species of *Penicillium* were detected from the grapes to wine, where *Penicillium expansum* were detected most commonly. In the second part of our work some selected isolates were tested to the ability to produce mycotoxins such as patulin, citrinin, and roquefortin C in *in vitro* condition by thin layer chromatography method. All tested strains of *Penicillium* species were able to produce at least one mycotoxin.

Keywords: wine grapes; must; wine; mycobiota; mycotoxin

INTRODUCTION

Wine is a significant contributor to the economies of many countries. However, the commodity can become contaminated with mycotoxins produced by certain fungi. Most information on mycotoxins in wine is from Spain, Italy and France **(Russell et al., 2017)**. Over 500 mycotoxins are currently known, and their number continues to rise. Mycotoxins are secondary metabolites produced by fungi that contaminate agricultural products as a result of fungal spoilage, and they may be produced before, during, or after harvest, or at any stage during the food chain. Some of the challenging aspects related to the pathogenesis of mycotoxins-caused illnesses are that one fungal species may generate more than one mycotoxin, and several fungal species may be concomitantly present in food products **(Stein and Bulboacă, 2017)**. The incidence of filamentous fungi and toxin levels in grapes and wines varies depending on the variety of grapes, the wine region, agricultural practices, weather conditions, the harvest and the winemaking process **(Alshannaq and Yu, 2017)**. The major fungi causing frequent and problematic grape rotting and spoilage are members of the fungal genera *Penicillium, Aspergillus, Alternaria, Botrytis, Cladosporium* and *Rhizopus* **(Marin et al., 2013)**. The genus *Penicillium* seems to be more frequent in temperate and cold climates, such as those in northern Europe whereas *Aspergillus* is more frequently associated with warmer and wetter regions **(Serra et al., 2006)**. The mycotoxins of greatest significance include aflatoxins, citrinin, patulin, ochratoxin A (OTA) and fumonisin B_2 **(Susca et al., 2010)**. Many mycotoxins are not easily eliminated during food processing because of their stability against heat, physical, and chemical treatments **(Marin et al., 2013)**. Most mycotoxins are chemically and thermally stable during storage and food processing, including cooking, boiling, baking, frying, roasting, and pasteurization. This makes it important to avoid the conditions that lead to mycotoxin formation at all levels of production, harvesting, transport and storage, which is not always possible and not always achieved in practice. It has been demonstrated that environmental stress conditions such as insect infestation, drought, cultivar susceptibility, mechanical damage, nutritional deficiencies, and unseasonable temperature, rainfall or humidity can

promote the distribution of fungal population, including those present on grapes, thereby also affecting the presence of mycotoxins or off-flavours in wine. In fact, changes in farming practices in the past few decades may result in increasing stress on plants and therefore enhance fungal invasion and mycotoxin contamination. The careful selection and proper storage of fruits are the most important factors in quality control **(Fernández-Cruz et al., 2010)**. We focused particularly on descriptions of the fungal microbiota on and in wine grapes, fresh grape juice, must and wine and species of genera *Penicillium* responsible for the production of mycotoxins and off-flavors from domestic crops in the year 2017.

Scientific hypothesis

Growth in the must and wine habitat is limited by low pH values and high ethanol concentrations. Therefore, only acid and ethanol tolerant microbial groups can grow in grape juice, must and wine, which include yeasts and fungi.

MATERIAL AND METHODOLOGY

Study area and samples

Slovak republic has 6 distinct wine-growing zones (the Small Carpathians, the Southern Slovak, the Nitra, the Central Slovak, the Eastern Slovak and the Tokaj wine regions). They spread from the west to the east of the country along its southern and south-western borders. The largest in size and the most important over the centuries has been the Small Carpathian area (around 5800 ha of vineyards) spreads in the western of Slovakia. The Small Carpathian wine region is divided to 12 subregions. The subregion is the area with the same soil and climate conditions. Wine-growing zones are defined as geographic regions with distinct climatic conditions for grape cultivation. The Small Carpathian wine-growing region has medium climates and abundant moisture. Grapes (*Vitis vinifera* cultivars *Palava* and *Dornfelder*) used in this study were provided by farms located in the Small Carpathians wine region, Vrbovsky subregion, village Vrbove. Two samples – 1 of white grape variety Palava and 1 of blue grape variety Dornfelder were mycologically analyzed. One sample of a wine grape variety was represented by three subsamples of wine grapes, which were sampled in left, middle and right part of the vineyard. Samples were collected at the end of September 2017, in the maturation stages corresponding to harvest. The berries from the vineyards sampled were generally in good condition without visible damage. Three kilograms of samples were taken to sterile plastic bags and transported to a mycological laboratory for immediate processing. Microfungi were monitored in fresh grape juice, must and wine. Fermentation was carried out in 15 litre tanks with yeast culture Excellence® FTH (Lamothe Abiet, France) for white wine and Excellence® TDS (Lamothe Abiet, France) for red wine. At least 50 ml of must were collected after stirring the tank content at each sampling. The fermentation lasted 3 weeks at 18 ±2 °C.

Mycological analysis of samples

A total of 50 berries (7 – 8 berries per bunch) from each sample were plated in Dichloran Rose Bengal Chloramphenicol agar medium (DRBC, MERCK, Germany) and incubated at 25 ±1 °C in the dark for one week. The detection of fungi in grape samples was also made by plating methods with surface disinfection. A total of 50 intact berries were surface-disinfected in 1 % NaClO for 1 min according methods of **Magnoli et al. (2003)** and 3 times rinsed by submersion in sterile distilled water (total amount 1L), dried, plated onto DRBC and incubated at 25 ±1 °C in the dark for 5 – 7 days. In this way was determined an endogenous mycobiota. Mycobiota of grape juice, must and wine was analysed by plate dilution method. From each sample were squeezed more than 250 g of randomly selected berries and 20 ml of the stum has been added to 180 ml of sterile peptone water containing 0.02% Tween 80. Prepared suspensions were shaken on a Stomacher easyMix®. Dilutions 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} were in the double surface inoculated in amount of 0.1 ml on DRBC agar plates. Cultivation lasted from 5 to 7 days in darkness at 25 ±1 °C. We used conventional identification techniques, such as macroscopic and microscopic observations, with guidelines by **Pitt and Hocking (2009)** facilitating the identification of isolated microorganisms. *Penicillium* strains were isolated and cultivated in MEA (Malt extract agar, **Samson et al., 2010**), CYA (Czapek yeast agar, **Samson et al., 2010**), CREA (Creatine-Sucrose agar, **Samson et al., 2010**) and YES (Yeast Extract agar, **Samson et al., 2010**) to obtain pure cultures and identify further species. Genus *Penicillium* was identified to species level based on morphological characters according to special mycological literature of **Pitt and Hocking (2009), Samson and Frisvad (2004)** and **Samson et al. (2002a, 2010)**.

Results evaluation

The obtained results were evaluated and expressed according to relative density (RD). The relative density (%) is defined as the percentage of isolates of the species or genus, occurring in the analyzed sample **(Gautam et al., 2009)**. These values were calculated according to **González et al. (1999)** as follows:

RD (%) = (ni / Ni) x 100

where ni – number of isolates of a species or genus; Ni – total number of isolated fungi.

Toxinogenity analysis

Toxinogenity of selected isolates was screened in *in vitro* conditions by means of thin layer chromatography (TLC) according to **Samson et al. (2002b),** modified by **Labuda and Tančinová (2006).** Extracellular metabolites – citrinin and patulin were carried out on YES agar and intracellular roquefortin C on CYA agar. A few pieces of mycelium with approximate size 5 x 5 mm were cut from colonies and placed in an Eppendorf tube with 500 µL of chloroform:methanol – 2:1 (Reachem, Slovak Republic). The content of the tubes was stirred for 5 min by Vortex Genie ® 2 (MO BIO Laboratories, Inc. – Carlsbad, CA, USA). The volume 30 µL of liquid phase of extracts along with 10 µL standards (Sigma, Germany) was applied on TLC plate (Alugram ® SIL G, Macherey – Nagel, Germany). The plate was put into TEF solvent (toluene:ethyl acetate:formic acid – 5:4:1, toluene – Mikrochem, Slovak Republic; ethyl acetate and formic acid – Slavus, Slovak Republic). After elution the plate was air-dried. Identification of the metabolites was done

by comparison with metabolite standards. Roquefortin C was visible after spraying with $Ce(SO_4)_2$ x 4 H_2O as an orange spot. Patulin by spraying with 0.5% methylbenzothiazolone hydrochloride (MBTH), (Merck, Germany) in methanol and heating at 130 °C for 8 min and then detectable as a yellow-orange spot. Directly under UV light with a wavelength of 365 nm was visualized citrinin as a yellow-green-tailed spot.

RESULTS AND DISCUSSION

The filamentous fungi identified in white grape variety Palava from surface and endogenous mycobiota of grapes, fresh grape juice, must, and wine are indicated in Table 1. A total of 148 strains belonging to 13 genera and *Mycelia sterilia* were identified. The most abundant genus was *Alternaria* (59.5%), followed *Botrytis, Mucor* (6.8%, each), *Rhizopus, Sordaria* (4%, each), *Penicillium* (2.7%), *Arthrinium, Fusarium,* and *Trichoderma* (2%, each) of all the fungi found. *Cladosporium* and *Talaromyces* were detected in more than 1 %, *Acremonium* and *Aspergillus* in less than 1% of all isolates.

Without surface disinfection, a total of 70 strains belonging to 11 genera and *Mycelia sterilia* were identified. The four most abundant genera found by descending order were *Alternaria, Mucor, Rhizopus,* and *Sordaria*. The occurrence of *Penicillium* spp. in our sample was generally low. *Arthrinium, Cladosporium, Fusarium* and *Penicillium expansum* were detected in more than 2% of the berries analyzed. The remaining genera were detected in less than 2% of all the isolates. *Alternaria* was one of the main fungal genera isolated also from Argentina grape berries **(Prendes et al., 2015)**, Tunisian grapes **(Fredj et al., 2009)**, Spanish grapes **(Bau et al., 2005, 2006; García-Cela et al., 2015)** or Slovakian grapes **(Felšöciová, 2016)**. Outbreaks of *Alternaria* bunch rot on grapevines in Slovakia occurred during unusually hot summer weather in 2007 and 2008 **(Kalíková et al., 2009)**. *Mucor* was one of the main fungal genera isolated also from French and Tunisian grapes **(Sage et al., 2002; Fredj et al., 2009)**. The relative density (RD) from surface mycobiota colonisation of grapes in the six wine growing regions of Slovakia in the years 2011 – 2013 was lower. *Mucor* and *Sordaria* were identiefied less than 1%, and *Rhizopus* higher (2.66% RD) **(Felšöciová, 2016)**. *Penicillium expansum* can cause rot in grapes **(Serra et al., 2005)**. In our study was isolated in low relative density (2.9%). Other studies have identified *P. expansum* as the species most frequently isolated from Portuguese **(Abrunhosa et al., 2001)** and French vineyards **(La Guerche et al., 2004, 2005; Bejaoui et al., 2006)**. **Felšöciová et al. (2015)** from Small Carpathian winemaking region during the years 2011 and 2013 identified 13 different *Penicillium* species from the 251 *Penicillium* strains. The most abundant were *Penicillium chrysogenum* (64%), *P. crustosum* (12%) and *P. griseofulvum* (8%) of the isolates. Isolation frequency among species was maximum for *P. chrysogenum* (36%), *P. crustosum* (29%), *P. expansum* and *P. griseofulvum* (21%, each). Fungal species capable of causing rot in grapes (*Aspergillus niger, Botrytis cinerea, Penicillium expansum, Rhizopus*) were also common inhabitants of the berries surface from Portuguese vineyards in four winemaking regions **(Serra et al., 2006)**. The most frequent *Penicillium* species were other than in our samples, namely *P. brevicompactum*, *P. thomii* and *P. glabrum*/*spinulosum* which together accounted for approximately 71 % of the strains identified in the genus.

Grape berries harbour a complex microbial community comprising yeasts, bacteria and filamentous fungi that inhabit not only the skin surface but also endosphere of the berry. A total of 72 isolates of microscopic fungi belonging to 7 genera and *Mycelia sterilia* were obtained from endogenous mycobiota. The most isolated genera by descending order were *Alternaria* (73.6%) and *Botrytis* (12.5%). *Trichoderma* and *Talaromyces purpurogenus* (previous name *Penicillium purpurogenum*) were detected

Table 1 Fungi identified from exogenous and endogenous mycobiota of grapes, grape juice, must and wine from variety Palava

Fungal taxa	grapes exo		grapes endo		juice	must		wine	Total	RD (%)
	No	RD (%)	No	RD (%)	No	No	RD (%)	No		
Acremonium	-		-		-	1	16.7	-	1	0.7
Alternaria	35	50	53	73.6	-	-		-	88	59.5
Arthrinium	2	2.9	1	1.4	-	-		-	3	2.0
Aspergillus	1	1.4	-		-	-		-	1	0.7
Botrytis	1	1.4	9	12.5	-	-		-	10	6.8
Cladosporium	2	2.9	-		-	-		-	2	1.3
Fusarium	2	2.9	-		-	1	16.7	-	3	2.0
Mucor	8	11.4	-		-	2	33.3	-	10	6.8
Penicillium **from it:**	2	2.9	-		-	2	33.3	-	4	2.7
P. expansum	2		-		-	-		-	2	
P. chrysogenum	-		-		-	2		-	2	
Rhizopus	5	7.1	1	1.4	-	-		-	6	4.0
Sordaria	5	7.1	1	1.4	-	-		-	6	4.0
Talaromyces	-		2	2.8	-	-		-	2	1.3
Trichoderma	1	1.4	2	2.8	-	-		-	3	2.0
Mycelia sterilia	6	8.6	3	4.2	-	-		-	9	6.1
Total isolates	**70**		**72**		**-**	**6**		-	**148**	

Note: No - number of isolates, RD – relative density.

in more than 2%, *Arthrinium, Rhizopus* and *Sordaria* in less than 2% of all the fungi found. Several studies have shown that grape rot, due to the association of *B. cinerea* with order, less visible, fungi (*Penicillium* spp., *Rhizopus* spp.) frequently leads to the development of organoleptic defects in grapes and wines. These compounds have been identified as 2-methylisoborneol, (-)-geosmin, 1-octen-3-one, 1-octen-3-ol, 2-octen-1-ol, and 2-heptanol **(La Guerche et al., 2006)**. This mould also induces the production of a pathogenesis-related (PR) protein causing haziness in white wines **(Girbau et al., 2004)**. **Felšöciová (2016)** described 19 genera and *Mycelia sterilia* with 1689 isolates from endogenous mycobiota of 14 wine grapes from Small Carpathians wine region, during the years 2011 and 2013. In all samples were found *Alternaria* (100%), *Cladosporium, Fusarium* (92.86%, each) and *Penicillium* (78.57%). Eight *Penicillium* species, namely *P. aurantiogriseum, P. citrinum, P. expansum, P. griseofulvum, P. chrysogenum, P. oxalicum, P. polonicum* and *P. thomii* were identified. *Penicillium chrysogenum* and *P. expansum* were the predominant in mycobiota, because they were the most frequent (42.9%, 35.7%, respectively) of the isolates with maximum relative density among species (63.6%, 22.6%, respectively).

In the grape juice filamentous fungi were surprisingly missed on DRBC agar medium. In grape juice and wine were identified only yeasts, but in must two isolates of *Mucor*, two isolates of *Penicillium chrysogenum* and one isolate of *Fusarium* were detected. **Felšöciová (2016)** described 14 genera and *Mycelia sterilia* with 2515 isolates from grape must. The highest frequency (100% FR) and relative density (91.2%) reached *Cladosporium*, followed *Alternaria* (86% FR) and *Penicillium* (64% FR). *Penicillium chrysogenum* (40.5%) and *Penicillium expansum* (29%) obtained the highest frequency from 4 *Penicillium* species. **Barboráková et al. (2011)** obtained the information about the mycobiota of Slovak origin wines during the production process in the year 2009. Altogether thirty three samples from the production process of 5 species white Slovak origin wines were mycologically analysed. The spectrum of isolated penicilia consisted of 21 species: *Penicillium aurantiogriseum, P. brevicompactum, P. citreonigrum, P. citrinum, P. corylophilum, P. crustosum, P. decumbens, P. expansum, P. funiculosum, P. glabrum, P. griseofulvum, P. implicatum, P. oxalicum, P. paneum/carneum, P. pinophilum, P. polonicum, P. purpurogenum, P. restrictum, P. roqueforti, P. rubrum* and *P. rugulosum*.

The freshly crushed must present one of the richest and most complex microbial communities, which functions as inoculum in spontaneous fermentations. The initial yeast diversity rapidly evolves in extremly stressful conditions, dominated by high sugar and low initial temperatures. In the grape juice the concentration of yeasts was 1.10^4 CFU.ml^{-1}, in must $1.7.10^8$ CFU.ml^{-1}, and at the end of the process, only a few strains survive ($7.4.10^3$ CFU.ml^{-1}). As observed in other fermentations, glucose and ethanol concentrations and must pH have a significant role in shaping the microbial population, with must acidity playing the predominant role, both in selecting the initial fungal population **(Charoenchai et al., 1998)** and in defining the fermentation properties of fungi **(Liu et al., 2015)**.

The filamentous fungi identified in blue grape variety Dornfelder from surface and endogenous mycobiota of grapes, grape juice, must and wine are indicated in Table 2. A total of 39 strains belonging to 6 genera and *Mycelia sterilia* were identified. The most abundant genus was also *Alternaria* (51.3% RD), followed *Penicillium* (12.8%), *Aspergillus* (7.7%), *Cladosporium, Fusarium* (5.1%, each) and *Botrytis* (2.6%) of all the isolates found. **Abrunhosa et al. (2001)** reported that *Alternaria* and *Cladosporium* were more often isolated from blue grape varieties than white, regardless of the vineyard in Portugal, which can not be confirmed from our study. The effect of *Cladosporium* rot was reported in delayed harvests in Chile **(Briceño et al., 2009)**. This type of rot reduced colour, aroma, and flavor in Cabernet Sauvignon and Carménère wines. Without surface disinfection, a total of 18 strains belonging to 4 genera and *Mycelia sterilia* were identified. The most abundant genera were *Alternaria* (50% RD) and *Penicillium* (22.2% RD). *Fusarium* and *Botrytis* were detected very rarely of all the fungi found. The *Penicillium* genus has long been known to grow on grapes and to be the causal agent of green mold, a secondary disease on mature berries resulting in a loss of must color and a decrease in sugar concentration. This genus is less frequently isolated from warmer and wetter vineyards than from cooler and drier vineyards **(Rousseaux et al., 2014)**. Two isolates of *Penicillium expansum*, one isolate of *P. aurantiogriseum* and one isolate of *P. brevicompactum* were detected. Berries affected by *P. expansum* have an off-flavor and even a small amount of infected berries add a mouldy taste to the wine **(König et al., 2009)**. *Penicillium brevicompactum* is a cosmopolitan species but never particularly frequent. However, **Serra et al. (2006)** isolated the species frequently from grape surfaces at 100% rate in some samples.

A total of 14 isolates of microscopic fungi belonging to 3 genera and *Mycelia sterilia* were obtained from endogenous mycobiota. The most isolated genus was *Alternaria* (71.4%). *Aspergillus* and *Cladosporium* were isolated only once. **Medina et al. (2005)** refered the diversity of filamentous fungi isolated from muscat grape varieties grown in Spain. *Cladosporium* was the most common strain isolated from two blue varieties Garnacha and Monastrell (78.2% and 92.2%, respectively) of all isolates.

In the grape juice were obtained 5 isolates of microfungi belonging to same 3 genera and *Mycelia sterilia* as from endogenous mycobiota. They were isolated only once. Filamentous fungi slowly missed. In must one isolate of *Alternaria* and one isolate of *Penicillium expansum* were detected. *Alternaria* already underwent a drastic decrease, suggesting their inability to survive, either due to the stressful environment of the fermenting must or due to competition with other species. In wine were not detected any fungi.

Yeast counts in fresh grape juice were 2.10^5 CFU.ml^{-1}, in must remained nearly stable - $5.3.10^5$ CFU.ml^{-1} and until the end of fermentation slightly decreased on $5.7.\ 10^4$ CFU.ml^{-1}.

Table 2 Fungi identified from exogenous and endogenous mycobiota of grapes, grape juice, must and wine from variety Dornfelder

Fungal taxa	grapes exo		grapes endo		juice		must		wine	Total	RD (%)
	No	RD (%)	No	RD (%)	No	RD (%)	No	RD (%)	No		
Alternaria	9	50	10	71.4	1	20	-		-	20	51.3
Aspergillus	-		1	7.1	1	20	1	50	-	3	7.7
Botrytis	1	5.5	-		-		-		-	1	2.6
Cladosporium	-		1	7.1	1	20	-		-	2	5.1
Fusarium	2	11.1	-		-		-		-	2	5.1
Penicillium **from it:**	4	22.2	-		-		1	50	-	5	12.8
P. aurantiogriseum	1		-		-		-		-	1	
P. brevicompactum	1		-		-		-		-	1	
P. expansum	2		-		-		1		-	3	
Mycelia sterilia	2	11.1	2	14.3	2	40	-		-	6	15.4
Total isolates	**18**		**14**		**5**		**2**			**39**	

Note: No – number of isolates, RD – relative density.

Accurate fungal identifications and mycotoxin detection from the fungi are important. The genus *Penicillium*, in particular, has been associated with the production of secondary metabolites (including mycotoxins) in food and fruits **(Pitt and Hocking, 2009)**. Two potentially toxigenic species were tested for their toxigenic ability (Table 3). All tested isolates of *Penicillium expansum* were able to produce roquefortin C and citrinin, but only one isolate produced patulin. The metabolite roquefortin C was also produced by *Penicillium chrysogenum*.

Table 3 Toxinogenity of selected *Penicillium* strains

Species	Isolated from	P	C	RC
P. expansum	Palava, exo	0/2	2/2	2*/2**
P. expansum	Dornfelder, endo	1/2	2/2	2/2
P. expansum	Dornfelder, must	0/1	1/1	1/1
P. chrysogenum	Palava, must			1/1

Note: * – number of isolates with ability to produce mycotoxin, ** - number of tested isolates, P – patulin, C – citrinin, RC – roquefortin C.

Patulin is a polyketide mycotoxin discovered in 1943. It is produced by certain species of *Penicillium, Aspergillus*, and *Byssochlamys* growing on fruit and vegetables, with *P. expansum* recognized as the most fungus for its production **(Drusch and Ragab, 2003)**. The temperature range for *P. expansum* growth and patulin production is 0 – 24 °C. Minimum a_w for patulin production is 0.99 **(Fernández-Cruz et al., 2010)**. While it predominantly contaminates apples, apple juice, and apple products, other fruit including grapes may also be vulnerable to patulin contamination **(Yang et al., 2014)**. Its presence in grapes has been associated with moldy berries, even if patulin is degraded to some extent during the fermentation process **(Abrunhosa et al., 2001)**. Patulin was initially studied as a potential antibiotic, but subsequent research demonstrated human toxicities **(Puel et al., 2010)**. The acute symptoms in animals include lung and brain oedema, liver, spleen and kidney damage and toxicity to the immune system. For humans, nausea, gastrointestinal disturbances, and vomiting have been reported. The chronic symptoms include genotoxic, neurotoxic, immunotoxic, immunosuppressive and teratogenic effects. The IARC has classified patulin as category 3, not classifiable regarding its carcinogenicity to humans **(Fernández-Cruz et al., 2010)**. Citrinin is a mycotoxin of moderate toxicity **(Pitt and Hocking, 2009)**. Citrinin is not degraded during alcoholic fermentation and may be present in very small amounts in wine. However, wine contamination is unlikely, due to the low abundance of citrinin producing species on grapes **(Pitt and Hocking, 2009)**. **Felšöciová et al. (2015)** tested 68 strains on roquefortine C from Small Carpathian winemaking region from exogenous mycobiota which all were positive, too. The metabolite citrinin, a characteristic yellow-lemon pigment, was also produced by all strains of *P. expansum* under laboratory conditions. **Tančinová et al. (2015)** analysed 47 samples of grapes, harvested in 2011, 2012 and 2013 from various wine-growing regions of Slovakia. The potential producers of patulin were isolated from 23 samples berries, 19 samples of surface sterilized berries and 6 samples of grape juice. Overall, the representatives of producers of patulin were detected in 32 (68.1%) samples (75 isolates). The ability to produce patulin in *in vitro* condition was detected in 82% of isolates of *Penicillium expansum*, 65 % of *Penicillium griseofulvum* and 100% of *Aspergillus clavatus*. The secondary metabolite profiles of microfungi of the genus *Penicillium* isolated from samples of grape berries collected in two different phases during two vegetative seasons in Slovakia is described by **Santini et al. (2014)**. Three Slovak vine regions have been selected for this study, based on their climatic differences and national economic importance. The species *Penicillium brevicompactum*, *P. crustosum*, *P. chrysogenum*, *P. expansum*, *P. palitans* and *P. polonicum* were identified according to growth and morphology. The related strains were found to produce a broad spectrum of fungal metabolites, including roquefortine C, chaetoglobosin A, penitrem A, cyclopeptin, cyclopenin, viridicatin, methylviridicatin, verrucofortine, secalonic acid D, cyclopiazonic acid, fumigaclavine and mycophenolic acid. Chemotaxonomy was performed using high-performance liquid chromatography (HPLC) and mass spectrometry (MS). Considering the 52 total strains examined, the 63 % of them produced patulin. The metabolite citrinin was produced by almost all strains under laboratory conditions.

Roquefortine C, a mycotoxin produced by this species, was produced by the 68.4 % of the total extracted strains.

CONCLUSION

Two grape varieties Palava and Dornfelder, from Small Carpathian wine growing region were analyzed by plating methods and grape juice, must and wine by plate dilution method. Samples were mycological analysed with focus on genera *Penicillium*. The most presented genera on and in grape variety Palava were *Alternaria* and *Botrytis*. The mycobiota changed with wine making process, microfungi were isolated only from grape must. The most abundant genus from grape variety Dornfelder was also *Alternaria*. A few isolates were detected also from grape juice and must. The results shown, that fermentation is a dynamic process with considerable variations in the composition of the mycobiota. In contrast with fungal species, the relative abundance of yeasts gradually increased over time. Potentially toxigenic *Penicillium* species isolated from surface colonisation of grapes Palava and must, surface sterilsed grapes Dornfelder and must were tested for their toxigenic ability by thin layer chromatography method. All tested strains were able to produce at least one mycotoxin. In the research, ochratoxigenic *Penicillium* species were not found in grape samples.

REFERENCES

Abrunhosa, L., Paterson, R. R. M., Kozakiewicz, Z., Lima, N., Venancio, A. 2001. Mycotoxin production from fungi isolated from grapes. *Lett Appl Microbiol*, vol. 32, no. 4, p. 240-242. https://doi.org/10.1046/j.1472765x.2001.00897.x

Alshannaq, A., Yu, J. H. 2017. Occurrence, Toxicity, and Analysis of Major Mycotoxins in Food. *Int. J. Environ. Res. Public Health*, vol. 14, no. 6, p. 632. https://doi.org/10.3390/ijerph14060632

Barboráková, Z., Tančinová, D., Lejková, J., Mašková, Z., Dovičičová, M., Labuda, R., Kačániová, M., Mokrý, M. 2011. Mycobiota of selected Slovak origin wines (production year 2009) during the vinification with focus on the *Aspergillus* and *Penicillium* genera and their potential mycotoxin production. *Potravinarstvo*, vol. 5, Special issue February 2011, p. 109-116. Available at: http://www.potravinarstvo.com/dokumenty/mc_februar_2011/pdf/2/barborakova.pdf (In Slovak)

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. https://doi.org/10.1016/j.ijfoodmicro.2004.05.015

Bau, M., Bragulat, M. R., Abarca, M. L., Minguez, S., Cabañes, F. J. 2006. *Ochratoxin A producing fungi from Spanish vineyards*. In Hocking, A. D., Pitt, J. I., Samson, R. A., Thrane, U. *Advances in Food Mycology*. Advances in Experimental Medicine and Biology. New York, USA : Springer, p. 173-179. ISBN-13: 978-0387-28385-2. https://doi.org/10.1007/0-387-28391-9

Bejaoui, H., Mathieu, F., Taillandier, P., Lebrihi, A. 2006. Black aspergilli and ochratoxin A production in French vineyards. *Int. J. Food Microbiol.*, vol. 111, no. 6, p. 46-52. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.03.004

Briceño, E. X., Latorre, B. A., Bordeu, E. 2009. Effect of *Cladosporium* rot on the composition and aromatic compounds of red wine. *SJAR*, vol. 7, no. 1, p. 119-128. https://doi.org/10.5424/sjar/2009071-404

Charoenchai, C., Fleet, G. H., Henschke, P. A. 1998. Effects of temperature, pH, and sugar concentration on the growth rates and cell biomass of wine yeasts. *Am. J. Enol. Vitic.*, vol. 49, p. 283-288.

Drusch, S., Ragab, W. 2003. Mycotoxins in fruits, fruit juices, and dried fruits. *J. Food. Prot.*, vol. 66, no. 8, p. 1514-1527. https://doi.org/10.4315/0362-028x-66.8.1514

Felšöciová, S. 2016. *Diverzita mikromycét asociovaných s Vitis vinifera L. s dôrazom na zástupcov rodu Penicillium Link (Diversity of micromycotes associated with Vitis vinifera L. with emphasis on representatives of the genus Penicillium Link.)* Habilitation work, Nitra, Slovakia : Slovak University of Agriculture in Nitra, 156 p.

Felšöciová, S., Tančinová, D., Rybárik, Ľ., Mašková, Z., Kačániová, M. 2015. Mycobiota of Slovak wine grapes with emphasis on *Aspergillus* and *Penicillium* species in the Small Carphathian area. *Potravinarstvo*, vol. 9, no. 1, p. 501-508. https://doi.org/10.5219/529

Fernández-Cruz, M. L., Mansilla, M. L., Tadeo, J. L. 2010. Mycotoxins in fruits and their processed products: Analysis, occurrence and health implications. *Journal of Advanced Research*, vol. 1, no. 2, p. 113-122. https://doi.org/10.1016/j.jare.2010.03.002

Fredj, M. B. S., Chebil, S., Mliki, A. 2009. Isolation and characterization of ochratoxin A and aflatoxin B_1 producing fungi infecting grapevines cultivated in Tunisia. *Afr. J. Microbiol. Res.*, vol. 3, no. 9, p. 523-527.

García-Cela, E., Crespo-Sempere, A., Gil-Serna, J., Porqueres, A., Marin, S. 2015. Fungal diversity, incidence and mycotoxin contamination in grapes from two agro-climatic Spanish regions with emphasis on *Aspergillus* species. *J. Sci. Food. Agric.*, vol. 95, no. 8, p. 1716-29. https://doi.org/10.1002/jsfa.6876

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. https://doi.org/10.5580/104b

Girbau, T., Stummer, B. E., Pocock, K. F., Baldock, G. A., Scott, E. S., Waters, E. J. 2004. The effect of *Uncinula necator* (powdery mildew) and *Botrytis cinerea* infection of grapes on the levels of haze-forming pathogenesis-related proteins in grape juice and wine. *Australian Journal of Grape and Wine Research*, vol. 10, no. 2, p. 125-133. https://doi.org/10.1111/j.17550238.2004.tb00015.x

González, H. H. L., Martinez, E. J., Pacin, A., Resnik, S. L. 1999. Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxinivalenol occurrence on Argentinian durum wheat. *Mycopathologia*, vol. 144, no. 2, p. 97-102. https://doi.org/10.1023/a:1007020822134

Kalíková, Ľ., Jankura, E., Šrobárová, A. 2009. First report of *Alternaria* bunch rot of grapevines in Slovakia. *Australasian Plant Disease Notes,* vol. 4, no. 1, p. 68-69.

König, H., Unden, G., Fröhlich, J. 2009. *Biology of microorganisms on grapes, in must and in wine.* Verlay

Berlin Heidelberg : Springer. 516 p. ISBN 978-3-540-85462-3. https://doi.org/10.1007/978-3-540-85463-0

La Guerche, S., Dauphin, B., Pons, M., Blancard, D., Darriet, P. 2006. Characterization of some mushroom and earthy off-odors microbially induced by the development of rot on grapes. *Journal of Agricultural and Food Chemistry*, vol. 54, no. 24, p. 9193-9200. https://doi.org/10.1021/jf0615294

La Guerche, S., Gracia, C., Darriet, P., Dubourdieu, D., Labarère, J. 2004. Characterization of *Penicillium* species isolated from grape berries by their internal transcribed spacer (ITS1) sequences and by gas chromatography-mass spectrometry analysis of geosmin production. *Curr. Microbiol*., vol. 48, no. 4, p. 405-411. https://doi.org/10.1007/s00284-003-4176-4

La Guerche, S., Chamont, S., Blancard, D., Dubourdieu, D., Darriet, P. 2005. Origin of (-)-geosmin of grapes: on the complementary action of two fungi, *Botrytis cinerea* and *Penicillium expansum*. *Antonie van Leeuwenhoek*, vol. 88, no. 2, p. 131-139. https://doi.org/10.1007/s10482-005-3872-4

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, p. 193-200.

Liu, X., Jia, B., Sun, X., Ai, J., Wang, L., Wang, C., *et al*. 2015. Effect of initial pH on growth characteristics and fermentation properties of *Saccharomyces cerevisiae*. *J. Food Sci.*, vol. 80, no. 4, p. M800-M808. https://doi.org/10.1111/1750-3841.12813

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. *Lett. Appl. Microbiol*., vol. 37, no. 2, p. 179-184. https://doi.org/10.1046/j.1472765x.2003.01376.x

Marin, S., Ramos, A. J., Cano-Sancho, G., Sanchis, V. 2013. Mycotoxins: occurrence, toxicology, and exposure assessment. *Food Chem Toxicol*., vol. 60, p. 218-237. https://doi.org/10.1016/j.fct.2013.07.047

Medina, A., Mateo, R., López-Ocaña, L. L., Valle-Algarra, F. M., Jiménez, M. 2005. Study of spanish grape mycobiota and ochratoxin A production by isolates of *Aspergillus tubingensis* and other members of *Aspergillus* section *Nigri*. *Appl. Environ. Microbiol*., vol. 71, no. 8, p. 4696-4702.

Pitt, J. I., Hocking, A. D. 2009. *Fungi and Food Spoilage*. 3rd ed. London, UK : Springer. 519 p. ISBN 978-0-387-92206-5. https://doi.org/10.1016/j.ijfoodmicro.2010.08.005

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

Puel, O., Galtier, P., Oswald, I. P. 2010. Biosynthesis and toxicological effects of patulin. *Toxins* (Basel). vol. 2, no. 12, p. 613-631. https://doi.org/10.3390/toxins2040613

Rousseaux, S., Diguta, C. F., Radoï-Matei, F., Alexandre, H., Guilloux-Bénatier, M. 2014. Non-botrytis grape-rotting fungi responsible for earthy and moldy off-flavors and mycotoxins. *Food Microbiol*., vol. 38, p. 104-121. https://doi.org/10.1016/j.fm.2013.08.013

Russell, R., Paterson, R. R. M., Armando, V., Lima, N., Guilloux-Bénatier, M., Rousseaux, S. 2017. Predominant mycotoxins, mycotoxigenic fungi and climate change related to wine. *Food Research International*, vol. 103, p. 478-491. https://doi.org/10.1016/j.foodres.2017.09.080

Sage, L., Krivoboc, S., Delbos, E., Seigle-Murandi, F., Creppy, E. E. 2002. Fungal flora and ochratoxin A production in grapes and musts from France. *Journal of Agriculture and Food Chemistry*, vol. 50, no. 5, p. 1306-1311. https://doi.org/10.1021/jf011015z

Samson, R. A., Frisvad, J. C. 2004. *Polyphasic taxonomy of Penicillium subgenus Penicillium: new taxonomic schemes and mycotoxins and other extrolites*. Utrecht, Netherlands : Centraalbureau voor Schimmelcultures. 260 p. ISBN 90-70351-53-6.

Samson, R. A., Hoekstra, E. S., Frisvad, J. C., Filtenborg, O. 2002a. *Introduction to food- and airborne fungi*. 6th revised ed. (with some corrections). Utrecht, Netherlands : Centraalbureau voor Schimmelcultures. 389 p. ISBN 90-70351-42-0.

Samson, R. A., Hoekstra, E. S., Lund, F., Filtenborg, O., Frisvad, J. C. 2002b. *Method for the detection, isolation and characterisation of food-borne fungi.* In Samson, R. A., Hoekstra, E. S., Frisvad, J. C., Filtenborg, O. *Introduction to food- and airborne fungi*. Utrecht, Netherlands : Centraalbureau voor Schimmecultures. p. 283-297. ISBN 90-70351-42-0.

Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C., Andersen, B. 2010. *Food and Indoor Fungi*. Utrecht, netherlands : CBS – KNAW Fungal Biodiversity Centre. 390 p. ISBN 978-90-70351-82-3.

Santini, A., Mikušová, P., Sulyok, M., Krska, R., Labuda, R., Šrobárová, A. 2014. *Penicillium* strains isolated from Slovak grape berries taxonomy assessment by secondary metabolite profile. *Mycotoxin Research*, vol. 30, no. 4, p. 213-220. https://doi.org/10.1007/s12550-014-0205-3

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

Serra, R., Lourenço, A., Alipio, P., Venâncio, A. 2006. Influence of the region of origin on the mycobiota of grapes with emphasis on *Aspergillus* and *Penicillium* species. *Mycol. Res*., vol. 110, no. 8, p. 971-978. https://doi.org/10.1016/j.mycres.2006.05.010

Stein, R. A., Bulboacă, A. E. 2017. Mycotoxins. In Dodd, Ch., Aldsworth, T., Stein, R. A., Cliver, D., Riemann, H. *Foodborne Diseases*. 3rd ed. London, United Kingdom : Elsevier, Academic Press, p. 407-446. ISBN 978-0-12-385007-2. https://doi.org/10.1016/B978-0-12-385007-2.00021-8

Susca, A., Proctor, R. H., Mulè, G., Stea, G., Ritieni, A., Logrieco, A., Moretti, A. 2010. Correlation of mycotoxin fumonisin B_2 production and presence of the fumonisin biosynthetic gene fum8 in *Aspergillus niger* from grape. *Journal of Agricultural and Food Chemistry*, vol. 58, no. 6, p. 9266-9272. https://doi.org/10.1021/jf101591x

Tančinová, D., Felšöciová, S., Rybárik, Ľ., Mašková, Z., Císarová, M. 2015. Colonization of grape berries and cider by potential producers of patulin. *Potravinarstvo*, vol. 9, no. 1, p. 138-142. http://doi.org/10.5219/460

Yang, J., Li, J., Jiang, Y., Duan, X., Qu, H., Yang, B., Chen, F., Sivakumar, D. 2014. Natural occurrence, analysis, and prevention of mycotoxins in fruits and their processed products. *Crit. Rev. Food Sci. Nutr*., vol. 54, no. 1, p. 64-83. https://doi.org/10.1080/10408398.2011.569860

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WHEAT FLOUR, BREAD AND BISCUITS ENRICHED BY LINSEED FIBRE – COMPARISON OF HARVEST YEAR, LINSEED VARIETY AND ADDITION LEVEL

Marie Hrušková, Ivan Švec

ABSTRACT

Wheat flour was fortified by 2.5, 5.0 or 10 wt. per cent of linseed fibre, gained from seeds of golden flax varieties Amon and Raciol and brown one Recital (granulation 500 – 700 m), prepared from 2015 and 2016 harvests. Using analytical tests, namely sedimentation according to Zeleny and Falling Number, basic technological quality of flour composites was mostly independent on all three observed factors (harvest year, linseed variety and addition). Rheological tests included the farinograph, the extensigraph and the amylograph proofs. Enhancement by brown and yellow flax fibre significantly contributed to rise of farinograph water absorption and to dough stability shortening, directed mainly by addition level. Extensigraph curves course depended on dough resting time, higher differences between wheat control and flour composites were observed after 60 min dough resting. Linseed fibre weakened dough extensibility, and energy as area under curve also partially decreased about 3, 8 and 25% in average as portion of alternative materials in dough has risen. Compared to control, suspension viscosities of tested flour composite generally increased; the strongest effect was recorded for composite samples from harvest year 2016. During dough leavening, tested samples were differentiated according to maturograph dough resistance, and interaction of all three factors was identified. Regardless to variantion in dough machinability, specific volumes of composite bread samples were similar through whole sample set – any unequivocal trend was found. Somewhat worse vaulting of bread was calculated for buns manufactured from raw materials of 2016 harvest. Reversely, linseed fibre produced in 2015 improved crumb softness, especially at 5% enhancement (about ca 50% in average). The lowest addition of linseed fibre resctricted biscuits spread during baking in the highest extent, but rising level of enhancement suppressed elevated dough elasticity. Both cereal products were considered as acceptable for common consumers. Multivariate PCA method verified changes mainly in protein visco-elastic properties, which were reflected in bread and biscuits quality in an opposite manner. Based on this statistics, quality of wheat controls was comparable in both harvest year if related to changes induced by linssed fibre. In opposite to this, technological and consumer's parameters of flour composites and manufactured cereal products were statistically dependent of harvest year of linseed. As presumed, the lowest addition level brough the smallest changes; multiplied fortification caused gradual variation in results of all conducted proofs. Owing to high dietary fibre content in linseed fibre (over 50%), the medium dosage of the alternative material (i.e. 5% addition) could be recommended for praxis.

Keywords: brown and golden linseed fibre; dough rheology; bread; biscuits; principal component analysis

INTRODUCTION

Comparably to barley rennasaince passed off a few years ago, also linseed recovery in food industry started recently. A reason for this comeback could be addressed both to nutritional benefit and to new type of commercial products, namely linseed oil and fibre as food supplements of affordable price level. At the same time, interest of consumers in nutritionally richer and innovated bakery products maintains, supporting industrial production of such food supplements.

Fruits of linseed (*Linum usitatissimum* L.) are medium-sized seeds coloured in brown or gold, which represent a raw material traded not only in food industry (e.g., also in cosmetics). Demanded component of seeds, i.e. dietary fibre, consists mainly of hetero-polysaccharides forming 7-12% of seeds weight. Their structure is based on arabinoxylans, acid complex of galacturonic acid and rhamnose **(Kaewmanee et al., 2014)**. Comparably to barley β-glucans, they dispose by high water absorption at concentration lower than 1% **(Mazza and Biliaderis,**

2006) and by gelling properties (mucilage) **(Prazdnik et al., 2016**). In relation to variety type, flow properties between from brown and golden linseed mucilage may differ **(Troshchynska et al., 2016)**. As found **Kaewmanee et al. (2014)**, mucilage viscosity has relation to the neutral sugars and proteins amounts, predefing a final usage (stabiliser, thickener).

Linseed fibre as commercial food supplement is a by-product during linseed oil extraction. From the New Zealand to the whole world, linseed dietary dibre is delivered by the company Functional Whole Foods. The company produces both golden and brown linseed fibre types, which contain ca 45% of total dietary fibre (38% insoluble and 7% soluble fraction; http://fwf.co.nz/). For significant health benefit, consumption of 13 g linseed fibre is recommended daily; in natural form, home consumption may include breakfast fruit cocktails, yogurt spreading etc. Further, it may replace a part of wheat flour when making pancake, cake or bread for family dinners or celebrations.

As in case of other non-traditional seeds, wholemeal flour is the simpliest form to be used. Addition of flax seed flour elevated water absorption and mixing tolerance index, but reduced dough stability and extensibility **(Koca and Anil, 2007; Xu et al., 2014)**. Mentioned changes were softly reflected in principal bread quality characteristics as the volume of bread. Testing influence of full fat or partially defatted flax flour, **El-Demery et al. (2015)** arrived at conclusion of comparable quality of both modified bread variants. Separating hulls only and preparing flaxseed hull flour, composite bread involving 5% of that raw material in recipe was characterised by decreased specific bread volume and higher crumb firmness **(Sęczyk et al., 2017)**.

Biscuits recipe modification in this way may be reflected in dough stickiness and overall acceptability of final product. Flaxseed flour portions 6% and 12% resulted in still acceptable sweetmeat, while is dosage at level 18% was found as unsatisfactory **(Khouryieh and Aramouni 2012)**.

Scientific hypothesis

The goal of the paper is a statistical comparison of harvest year, linseed variety and linseed fibre addition level effects on technological quality of wheat flour. Properties of prepared flour composites were described by basic analytical tests, and their rheological behaviour was determined in form of non-fermented as well as fermented dough. In term of final product, bread and biscuit baking trials were conducted including assessment of sensory quality.

MATERIAL AND METHODOLOGY

Preparation of flour composites

Semi-bright wheat flour (WF) was delivered by industrial mill Delta Prague in years 2016 and 2017, and they were characterized by protein contents 11.2 and 13.1%, Falling number 432 and 394 s and Zeleny values 39 and 45 mL, respectively. Linseed fibre was produced at laboratory conditions, treating seeds from golden varieties Amon and Raciol and brown one Recital, harvested in years 2015 and 2016. To disintegrate the seeds, mill Stephan UM/SK 5 (Stephan Machinery, Hameln, Germany) was employed. Using vibration laboratory hand sieve machine (Stavební strojírenství n.p. Brno, Czechoslovakia) with sieve openings: 1.0, 0.8, 0.71, 0.50, 0.315 and >0.315 mm, demanded fraction 0.5 – 0.7 mm was collected to ensure comparability with our previous study (**Hrušková and Švec 2017)**. In tested composites, linseed fibre replaced 2.5, 5.0 or 10 wt. per cent of wheat flour. Samples abbreviations combined simplified wheat flour sign, addition level of linseed fibre and first letter of flax variety name (2.5Am, 5Am, 10Am; 2.5Ra, 5Ra, 10Ra; 2.5Re 5.0Re, 10Re). Harvest years of linseed are differentiated as follows: 2.5 Am for the former and 2.5 Am for the latter one.

Technological quality of flour composites

Technological features of WF and flour composites are described by Zeleny test (abbreviation ZT; ISO 5529) and Falling number (abbreviation FN; ISO 3093). Non-fermented dough properties were determined with the help of farinograph and extensigraph Brabender (Germany) according to the approved norms ISO 5530-1 and ISO 5530-2. Behaviour of flour-water suspensions was recorded on the Amylograph Brabender (ICC norm 126/1). According to internal methods of the Cereal laboratory of the UCT Prague, rheological behaviour parameters of fermented dough was recorded by using of the Fermentograph SJA (Sweden), Maturograph and Oven rise recorder (OTG) Brabender (Germany). From mixed wheat-linseed fibre composites, leavened bread and cut-off biscuits were manufactured manually. Their quality assessment included also informative sensory analysis, employing three trained pannelists.

Dilution of skeleton in wheat dough by linseed proteins of non-gluten nature probably bring a partial worsening of composite dough machinability (viscoelastic properties) and conseutivelly final products quality. To these results also may contribute high water binding capacity of linseed fibre, which will influence viscosity of composite flour during mixing and heating (pasting phase). Besides, tested addition levels could be considered as a low, thus both types of cereal products may reach a sufficient sensory score.

Statisic analysis

Statistical evaluation of linseed fibre effect

Influence of harvest year, type and addition level of non-traditional material on selected dough rheological properties and final product features was evaluated by HSD test (p = 95%). Owing to the Principal Components Analysis (PCA), finding of final product quality features dependent on three observed factors is allowed. For PCA datasets, 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 were selected.

RESULTS AND DISCUSSION

Evaluation of technological quality of flour composites

Within compared harvest years, protein technological quality of both controls (*WF'15* and WF'16) were close

together (39 and 45 mL, respectively). Their partial replacement by linseed fibre caused a soft worsening as expected, and the effect was similar for composites of harvest 2015 and 2016 (ranges 40 – 30 mL vs. 45 – 29 mL; Figure 1) as well as for tested linseed varieties (controls mean 42 mL vs. averages 35, 36 and 36 mL, respectively). In this regard, the only one factor whose effect was statistically significant was the addition level (controls mean 42 mL vs. 39, 36 and 31 mL for enhancement 2.5, 5 and 10%, respectively).

In observed harvest years, Falling number test disclosed a rather reversal contribution to linseed fiber on amylases activity. For samples from crop 2015, 2.5% enrichment meant a lowering of the FN (from -11% to -16%). For flour composites of the year 2016, a verifiable increase of the FN caused the highest linseed fibre dosage (from +11% to +22%). Due to method repeatability quantified as ±10%, a majority of determined differences are insignificant in fact (variance *'a', 'ab', 'b'* only; Table 1).

Viscoelastic behaviour of flour composites

With respect to ANOVA resuls in Table 2, comparable water absorption of controls *WF'15* and WF'16 was more supported by all three linseed fibre types harvested in 2015 (means 73.3% and 70.2%). Increasing portion of the alternative raw material lead to provable increased amount of water during the farinograph test – an accrual caused by 10% LF was similar through the whole flour composites set (4.0 – 5.0 per cent points, related to 5% LF dosage). Testing 5% flaxseed wholemeal, water absorption maintained comparable to wheat control, but dough development time has been prolonged **(Koca and Anil 2007)**. Autors addressed this finding to increased fraction of fat in blend.

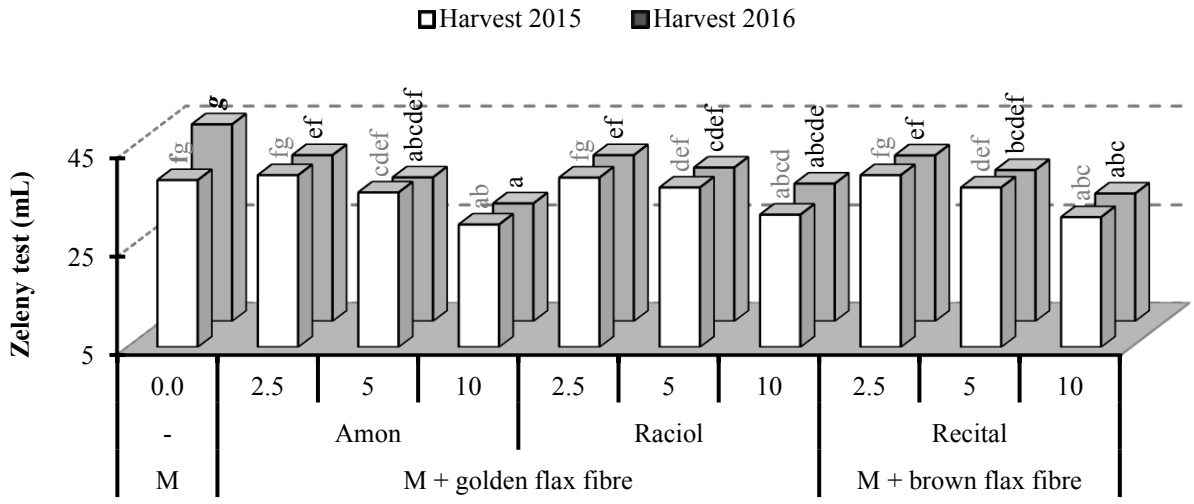

Figure 1 Zeleny test results of wheat flour (WF) and wheat-linseed fibre composites. A – f: coulmns signed by the same letter are not statistically different (p = 95%).

Table 1 Falling number of wheat flour and flour composites.

Composite flour	Harvest	Falling Number* (s)			
		Linseed fibre addition (%)			
		0	2.5	5	10
WF	2015	432b	-	-	-
	2016	394ab	-	-	-
WF+Am	2015	-	362a	387a	399ab
	2016	-	403ab	411ab	466b
WF+Ra	2015	-	386a	384a	384a
	2016	-	408ab	407ab	451b
WF+Re	2015	-	378a	364a	339a
	2016	-	390ab	434b	476b

Note: WF - wheat flour. Linseed varieties: Am - Amon, Ra - Raciol (both golden), Re - Recital (brown). * a-b: values signed by the same letter are not significantly different (p = 95%).

Combination of elevated dietary fibre content as well as water portions was reflected in dough stability shortening and degree of dough softening increase, reversely to influence of the same material from the New Zealand **(Hrušková and Švec 2016)**. The changes extent was comparable within both harvest subgroups, but twice-longer stability for *WF'15* than for WF'16 meant decrease by all three additions. Within the subset 2016, 2.5% LF addition firstly led to substantial stability prolongation (from 6.25 min up to 9.50 min); values for blends containing 5% LF were comparable to the control and only 10% of non-traditional material had a negative impact (Table 2). For the tested composites, degree of dough softening was determined in usual ranges 70 – 135 BU and

Table 2 Farinograph characteristics of non-fermented dough from wheat flour (WF) and flour composites.

Composite flour	Harvest	Water absorption* (%)		Dough stability* (min)		Dough softening degree* (FU)	
WF	2015	65.1a		11.00d		40a	
	2016		65.2a		6.75abc		35a
2.5Am	2015	70.0c		9.75cd		100ef	
	2016		67.4b		8.50bcd		55ab
5.0Am	2015	72.5d		7.00abc		95ef	
	2016		69.4c		6.00ab		80cde
10.0Am	2015	77.5f		6.00ab		70bcd	
	2016		73.4de		5.00a		110f
2.5Ra	2015	70.0c		9.25bcd		85cde	
	2016		67.8b		9.00bcd		65bc
5.0Ra	2015	72.5d		6.50abc		135g	
	2016		69.4c		7.00abc		90def
10.0Ra	2015	77.0f		4.50a		90def	
	2016		73.8e		4.75a		80cde
2.5Re	2015	70.3c		7.45abc		80cde	
	2016		67.4b		9.50cd		40a
5.0Re	2015	72.8de		6.75abc		95ef	
	2016		69.5c		7.50abc		90def
10.0Re	2015	76.8f		4.75a		100ef	
	2016		73.8e		4.50a		95ef

Note: WF - wheat flour. Golden linseed varieties: Am - Amon, Ra – Raciol, brown one: Re - Recital. Example of sample coding: W2.5A - wheat composite flour with 2.5 wt. % Amon linseed fibre. * a-f: row means described by the same letter are not significantly different (p = 95%).

Table 3 Results of extensigraph test of wheat flour (WF) and flour composites.

a) Energy, resting time 30 min

Composite flour	Harvest	Linseed fibre addition (%)			
		0		2.5 - 10	
WF	2015	105f	-	-	-
	2016		76abcdef	-	-
WF+Am	2015	-	-	91cdef – 68abcde	
	2016	-	-		73abcdef – 48a
WF+Ra	2015	-	-	98def – 65abcd	
	2016	-	-		86bcdef - 52ab
WF+Re	2015	-	-	86bcdef - 73abcdef	
	2016	-	-		71abcde – 58abc

b) Energy, resting time 60 min

Composite flour	Harvest	Linseed fibre addition (%)			
		0		2.5 - 10	
WF	2015	115de		-	-
	2016		90abcde	-	-
WF+Am	2015	-	-	118e – 80abc	
	2016	-	-		71ab – 66a
WF+Ra	2015	-	-	120e – 82abcd	
	2016	-	-		88abcde – 73ab
WF+Re	2015	-	-	112cde – 87abcde	
	2016	-	-		89abcde – 72b

Note: WF - wheat flour. Linseed varieties: Am - Amon, Ra - Raciol (both golden), Re - Recital (brown). * a-b: values signed by the same letter are not significantly different (p = 95%).

40 – 100 BU, and narrower extent of the latter 2016 composites corresponds to lower water absorptions. Main impact on dough resistance to overmixing could be noticed for 5% LF, because values of dough softening for counterparts containing 10% LF were comparable or lower.

By the extensigraph test, presumed lowering of bakery quality of non-fermented dough was confirmed. Values of energy after 30 min of dough resting were lowered by higher LF additions, for samples with 10% about 23 up to 38%. Twice longer resting time had a positive effect on the feature, depression against control was about 3 – 16% less (e.g. 105 and 83 cm^2 for *WF'15* and *WF'15-5Am* at 30 min of dough resting, and 115 and 105 cm^2 at 60 min of dough resting) (Table 3). **Koca and Anil (2007)** concluded that only 20% of flaxsedd wholemeal lowered the energy significantly (from 111.0 to 85 cm^2). Dough machinability was in majority of cases affected by variation of extensigraph elasticity and extensibility (i.e. elasticity-to-extensibility ratio) **(Inglett et al., 2013**). The lowest LF dosage lowered dough elasticity with patial support of its extensibility, while the highest dosage modified these physico-mechanical parameters in terms of prevailing decrease of extensibility (data not shown). In this regard, 5% LF addition could be consider as optimal for flour blends usage in bakery.

Pasting properties of flour composites

For all tested samples, characteristic points of amylograph curves differed mainly in parameter temperature of gelatinization beginning, which likely reflected diverse structural and physical properties of linseed polysaccharides in fibre from three tested varieties. For example, golden Amon fibre originated in the former harvest stepwise decresed this temperature; effect of the brown Recital fibre was reversal (Figure 2a). Maximal viscosity of control suspensions was analysed as statistically different; fibre from linseed harvested in 2015 modified the value 940 BU according to addition level. Fibre from the next harvest demonstrated this effect only in case of golden Amon variety; the Raciol and Recital ones reached maximum of 1000 BU (homogenous group '*m*'; Figure 2b).

Linseed fibre originated in New Zeeland also caused significant increase of viscosity during RVA test **(Hrušková and Švec 2016)**. **Inglett et al. (2013)** arrived at contrary conclusion for flaxseeds wholemeal, perhaps of testing of blends on base of barley flour.

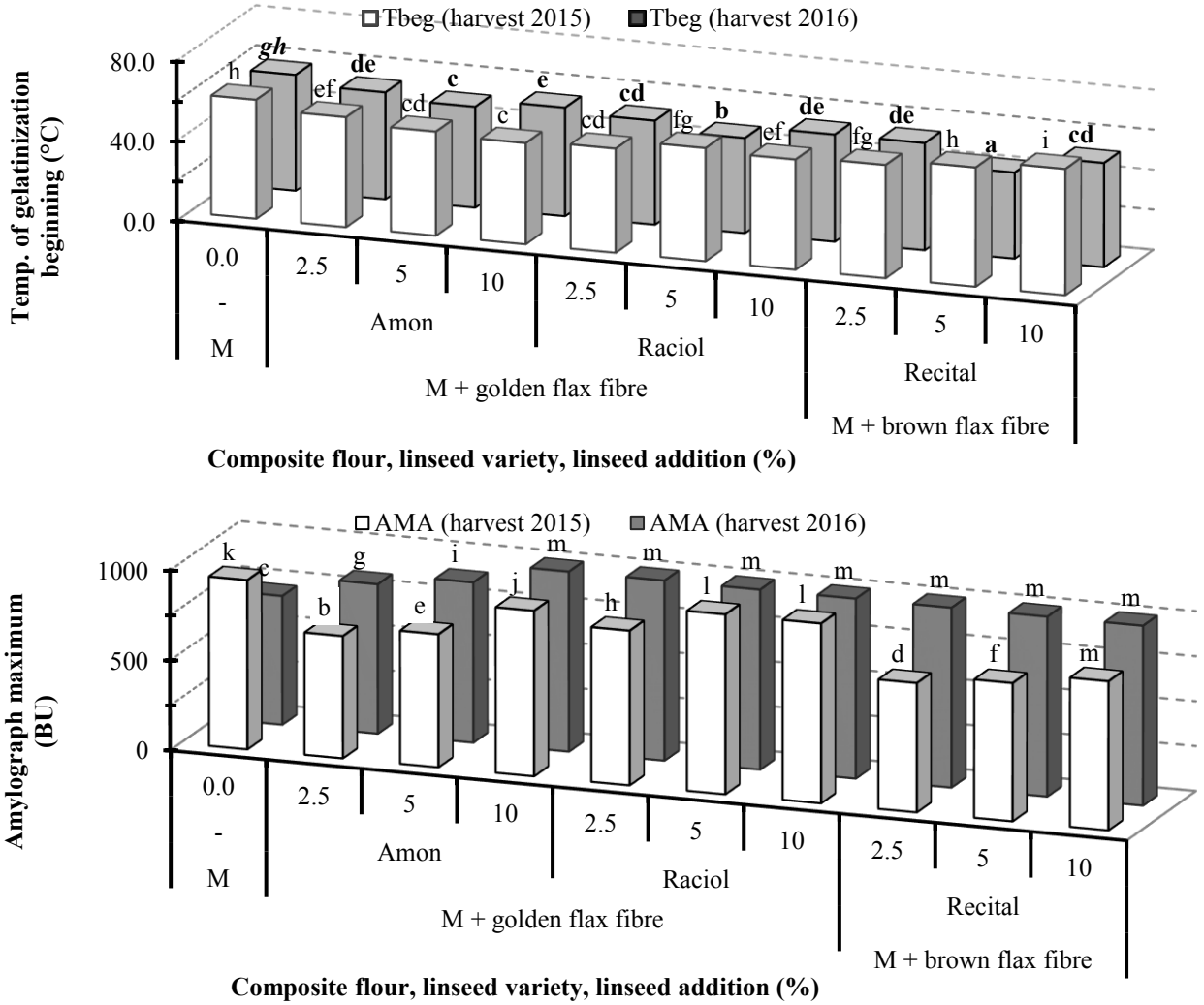

Figure 2 Pasting behaviour of wheat flour (WF) and wheat-linseed fibre composites. a) temperature of gelatinization beginning, b) amylograph viscosity maximum. For samples coding, see Table 2. a-m: columns signed by the same letter are not statistically different (p = 95%).

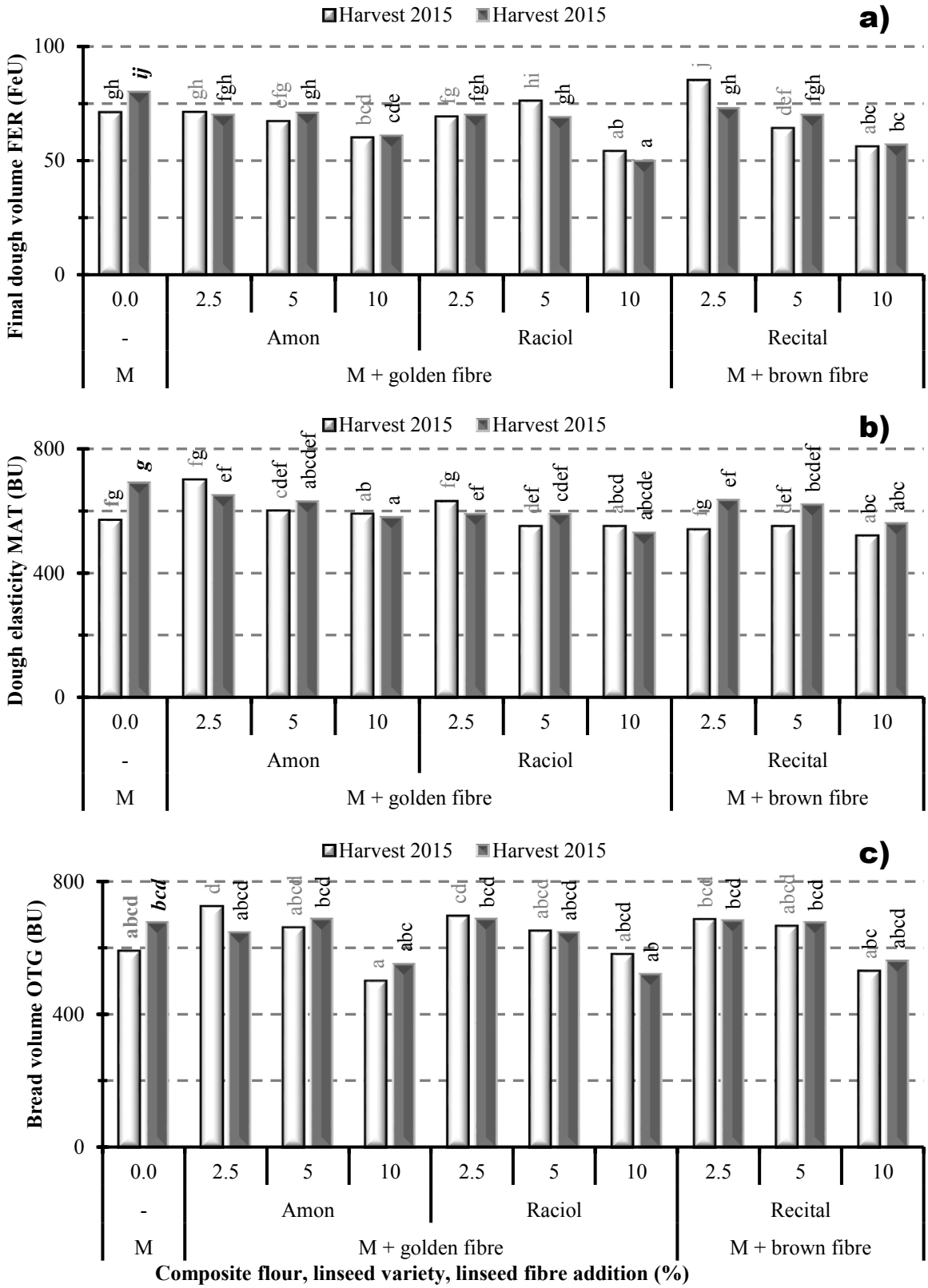

Figure 3 Rheological behaviour of leavened dough prepared from wheat flour (WF) and wheat-linseed fibre composites. FER – fermentograph, MAT – maturograph, OTG – oven rise recorder. For samples coding, see Table 2.

Evaluation of fermented dough behaviour

Within a bakery praxis, fermentation process is divided into three stages from the technological reasons – fermentation of dough mass after dough kneading, leavening of shaped dough pieces, and the first stage of baking. The fermentograph final dough volume, the maturograph dough resistance and OTG bread volume represent these operations. As illustrates Figure 3, LF affected all three technological phases of fermentation in a different extent. During the fermentograph test, identification of LF type and enhancement level could be more precise than in case of maturograph and OTG proofs

(variations '*a*'-'*f*' vs. '*a*'-'*d*'; Figure 3a, 3b, 3c). However, LF incorporation into fermented wheat dough meant lowering of values of the representative features, especially by golden Amon and brown Recital fibre. Those conclusions signified potentionally worse results of baking trial.

Table 4 Quality features of leavened bread prepared from wheat flour and flour composites.

Flour, flour composite	Harvest	Specific bread volume* (mL.100 g^{-1})	Bread shape*, ** (-)	Crumb penetration* (mm)
WF	2015	334abcd	0.61c	14.3abcd
	2016	349abcd	0.69d	23.6h
2.5Am	2015	253a	0.62c	22.6fgh
	2016	397d	0.62c	12.3abc
5.0Am	2015	354abcd	0.62c	23.1gh
	2016	255a	0.53ab	19.9defgh
10.0Am	2015	336abcd	0.57b	9.8a
	2016	341abcd	0.58b	21.5defgh
2.5Ra	2015	282abc	0.56b	22.0efgh
	2016	410d	0.58b	10.6ab
5.0Ra	2015	357abcd	0.52ab	20.1defgh
	2016	378bcd	0.56b	18.1bcdefgh
10.0Ra	2015	278ab	0.57b	20.8defgh
	2016	405d	0.67d	14.6abcde
2.5Re	2015	365bcd	0.58b	21.0defgh
	2016	308abcd	0.54ab	16.0abcdefg
5.0Re	2015	387cd	0.49a	12.1abc
	2016	385cd	0.55ab	18.2cdefgh
10.0Re	2015	338abcd	0.56b	14.8abcde
	2016	331abcd	0.51ab	15.3abcdef

Note: WF – wheat flour. Linseed varieties: A – Amon, Ra - Raciol (both golden), Re - Recital (brown). For samples coding, see Table 2. * a – g: column means described by the same letter are not significantly different (p = 95%).

Table 5 Quality features of biscuits prepared from wheat flour and flour composites.

Flour, flour composite	Harvest	Specific biscuit volume* (mL.100 g^{-1})	Spread ratio*, ** (-)	Sensory profile* (mm)
WF	2015	164.6b	4.35de	12.0a
	2016	166.7b	3.66abc	12.0a
2.5Am	2015	150.1ab	3.68abcd	12.0a
	2016	152.3ab	3.77abcd	12.0a
5.0Am	2015	142.2ab	3.55ab	12.0a
	2016	152.6ab	4.01abcd	11.0a
10.0Am	2015	163.7b	3.33a	12.0a
	2016	137.8ab	3.44a	11.5a
2.5Ra	2015	143.4ab	3.53ab	11.0a
	2016	149.8ab	3.56ab	12.0a
5.0Ra	2015	153.7ab	3.76abcd	11.0a
	2016	158.9ab	3.52ab	12.0a
10.0Ra	2015	166.9b	4.17bcde	12.0a
	2016	116.6a	3.91abcd	12.0a
2.5Re	2015	127.1ab	3.73abcd	11.0a
	2016	124.3ab	3.70abcd	11.0a
5.0Re	2015	129.5ab	4.34cde	11.0a
	2016	145.1ab	3.87abcd	11.0a
10.0Re	2015	151.0ab	4.76e	12.5a
	2016	133.9ab	3.73abcd	12.0a

Note: WF – wheat flour. Linseed varieties: Am – Amon, Ra – Raciol (both golden), Re – Recital (brown). For samples coding, see Table 2. * a – d: column means described by the same letter are not significantly different (p = 95%).

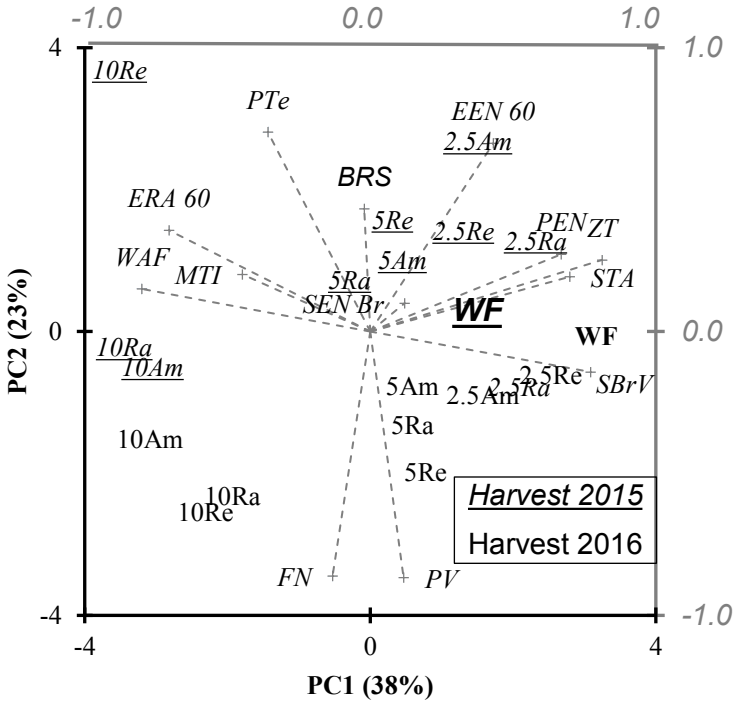

Figure 4 Principal component (PC) analysis of linseed fibre effect on dough and bread technological quality. FN – Falling number, ZT – Zeleny test; WAF – water absorption (farinograph test), STA – dough stability, MTI – mixing tolerance index (dough softening degree); ERA 60, EEN 60 – extensigraph (elasticity-to-extensibility) ratio and energy, respectively (dough resting 60 min); Tbeg – temperature of gelatinization beginning, AMA – amylograph (viscosity) maximum; SBrV – specific bread volume, BRS – bread shape (height-to-diameter ratio), PEN – crumb penetration, SEN Br – bread sensory profile. For samples coding, see Table 2.

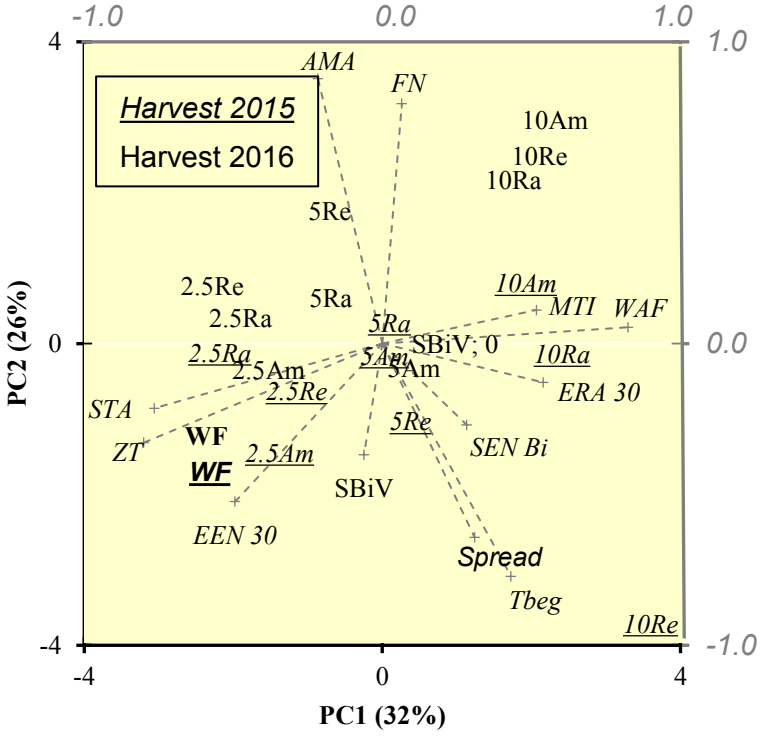

Figure 5 Principal component (PC) analysis of linseed fibre effect on dough and biscuits technological quality. For variables abbreviation FN, ZT, WAF, STA, MTI, Tbeg and AMA see Figure 4. ERA 30, EEN 30 – extensigraph (elasticity-to-extensibility) ratio and energy, respectively (dough resting 30 min); SBiV – specific biscuit volume, Spread – biscuit shape (diameter-to height ratio), SEN Bi – bread sensory profile. For samples coding, see Table 2.

Evaluation of wheat and wheat composite bread quality

Within laboratory baking test, satisfying technological potential of tested wheat-linseed fibre composites was verified. Unexpectedly, specific bread volumes from 6 composites containing 5% LF were the lowest thorough whole samples set (diminishing about 15 – 24%). In previous study, similar lowering of specific volumes caused linseed fibre produced by Walramcom company (around 33%) **(Hrušková and Švec 2016)**. For the rest of specimens, specific volumes were comparable to proper controls at least (Table 4), independently to modified dough machinability. Within both harvest years, the most together levelled volumes rendered composites WF'16-Ra and WF'16-Re (353 ±33 mL.100 g^{-1} and 351 ±24 mL.100 g^{-1}, respectively). Changed dough viscoelastic properties affected bread buns shape, their vaulting was worsened owing to gluten skeleton disruption. That effect is dominant within the harvest 2016, but together this, also samples triple *WF'15-Re* fell into this category. Increasing percentage of dietary fibre in recipe induced a worsening of crumb softness – penetration depth gradualy decreased up to about one-fourth in average. The drop by foreign linseed fibre enahncemed caused comparable drop in consumer's quality (range 5.8 – 10.4 mm vs. 14.3 mm) **(Hrušková and Švec, 2016)**, similarly to flaxseed wholemeal or flaxseed hull flour **(Conforti and Davis 2006; Sęczyk et al. 2017)**. On the other hand, our experience shown that bread with penetration 15 mm at least could be still acceptable.

From a statistical point of view, harvest year was the dominant factor directing the quality of both flour composites and leavened bread. Within the area of the first two principal componets (PC1 and PC2), all tested samples are conjoined according to this factor (Figure 4). The effect of linseed variety was important mainly for composites and bread involving 5% LF – relative shorter distance between flour composites with golden Amon and Raciol than to brown Recital ones could be noticed. In case of 10% replacement, higher dissimilarity among tested samples properties was confirmed. The plot verified protein quality-related parameters (Zeleny value ZT, dough stability STA, extensigraph energy EEN 60', and specific bread volume SBrV) as the most dependent on composition of actual flour mixture, predominating bread quality.

Evaluation of wheat and wheat composite biscuits quality

For confectionery goods, wheat flour of different technological quality than for bread is demanded. Data in Table 5 verify that premise – not specific biscuit volume, but its spread ratio (shape) is parameter the most affected by tested formula. Within both subsets, any distinct trend could not be found in specific volume of biscuits (harvests means 148 and 141 mL.100 g^{-1}). In terms of biscuits spread, an increase perhaps predominated according to rising amount of LF in the cereal products. Considering observed factors, the primary effect had linseed variety – the best results was determined for mixtures containing the Recital fibre (the lowest average specific biscuit volume 135 mL.100 g^{-1} and the highest spread 4.02). Informative results of sensory analysis indicate an acceptability of all modifications (Table 5). The non-traditional material rather lowered sweet taste, and during samples mastication, mouthful seemed to be drier and non-sticky. Similar findings published **Khouryieh and Aramouni (2012)** – up to 12% flaxseed wholemeal in recipe, biscuits had physical properties and sensory profiles close together.

PCA biplot of quality characteristics and composite samples (Figure 5) documented a primary role of harvest year on biscuits quality. In terms of further two factors, addition level partially overcame the linseed variety one. Items location along the both PC1 and PC2 is reversal related to PCA of bread, but at the same time, counterparts from harvest years 2015 and 2016 could be differentated diagonally As is mentioned supra, the best quality of biscuits is based on technological parameters water absorption WAF, degree of dough softening MTI, extensigraph ratio ERA 30, pasting profile (Tbeg) and spread factor.

CONCLUSION

Nowadays, return to local domestic plants and forgotten dishes in human nutrition intensifies. Combined with modern operation techniques, a category named novel food was established. From this category, final products render a higher nutritional benefit, either better amino- and fatty acids composition, or dietary fibre content. Flaxseed wholemeal flour or processed linseed fibre meet the pre-conditions, and their technological potential is documented in the present study. Addition of linseed fibre affected both flour analytical and dough technological properties – quality of proteins was softly weakened, but linseed polysaccharides supported water asorption during dough preparation. For bread manufacturing, linseed fibre form golden as well as brown flax seed seemed to be applicable, reaching up to 5 – 10% of flour weight in recipe.

In case of composite cut-off biscuits, all modifications were comparable to wheat control, unaffected by linseed colour type or addition level. Fibre partially depressed biscuits sweetness, but their complex sensory profile was acceptable. For biscuits preparation, linsef fibre may replaced 10% of wheat flour or likely more.

REFERENCES

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. https://doi.org/10.1111/j.1745-4603.2010.00242.x

Conforti, F. D., Davis, S. F. 2006. The effect of soya flour and flaxseed as a partial replacement for bread flour in yeast bread. *International Journal of Food Science and Technology*, vol. 41, Suppl. no. 2, p. 95-101. https://doi.org/10.1111/j.1365-2621.2006.01410.x

El-Demery, M., Mahmoud, K. F., Bareh, G. F., Albadawy, W. 2015. Effect of fortification by full fat and defatted flaxseed flour. Sensory properties of wheat bread and lipid profile laste. *International Journal of Current Microbiology and Applied Sciences*, vol. 4, no. 4, p. 581-598.

Gutiérrez, C., Rubilar, M., Jara, C., Verdugo, M., Sineiro, J., Shene, C. 2010. Flaxseed and flaxseed cake as a source of compounds for food industry. *Journal of Soil Science and Plant Nutrition*, vol. 10, no. 4, p. 454-463. https://doi.org/10.4067/S0718-95162010000200006

Hrušková, M., Švec, I. 2016. Flax – evaluation of composite flour and using in cereal products. *Potravinarstvo Slovak Journal of Food Science*, vol. 10, no. 1, p. 287-294. https://doi.org/10.5219/594

Hrušková, M., Švec, I. 2017. Linseed fibre – effect on composite flour properties and cereal products quality. *Potravinarstvo Slovak Journal of Food Science*, vol. 11, no. 4, p. 252-259. https://doi.org/10.5219/729

Inglett, G. E., Chen, D., Lee, S. 2013. Rheological properties of barley and flaxseed composites. *Food and Nutrition Sciences*, vol. 4, no. 1, p. 41-48. https://doi.org/10.4236/fns.2013.41007

Kaewmanee, T., Bagnasco, L., Benjakul, S., Lanteri S., Morelli, C. F., Speranza, G., Cosulich, M. E. 2014. Characterisation of mucilages extracted from seven Italian cultivars of flax. *Food Chemistry*, vol. 148, p. 60-69. https://doi.org/10.1016/j.foodchem.2013.10.022 PMid:24262527

Khouryieh, H., Aramouni, F. 2012. Physical and sensory characteristics of cookies prepared with flaxseed flour. *Journal of the Science of Food and Agriculture*, vol. 92, no. 11, 2366-2372. https://doi.org/10.1002/jsfa.5642 PMid:22419245

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. https://doi.org/10.1111/j.1365-2621.2010.02547.x

Koca, A. F., Munir, A. 2007. Effect of flaxseed and wheat flour blends on dough rheology and bread quality. *Journal of the Science of Food and Agriculture*, vol. 87, no. 6, p. 1172-1175. https://doi.org/10.1002/jsfa.2739

Manthey, F. A., Lee, R. E., Kegode, R. B. 2000. Quality of spaghetti containing ground flaxseed. In *Proceedings of the 58th Flax Institute of the U.S*. Fargo, ND : North Dakota State University, p. 92-99, North Dakota State University, Fargo, ND.

Manthey, F. A., Sinha, S., Wolf-Hall, Ch. E., Hall, C. A. III 2008. Effect of flaxseed flour and packaging on shelf life of refrigerated pasta. *Journal of Food Processing and Preservation*, vol. 32, no. 1, p. 75-87. https://doi.org/10.1111/j.1745-4549.2007.00166.x

Mazza, G., Biliaderis, C. G. 2006. Functional properties of flax seed mucilage. *Journal of Food Science*, vol. 54, no. 5, p. 1302-1305. https://doi.org/10.1111/j.1365-2621.1989.tb05978.x

Oomah, B. 2001. Flaxseed as a functional food source. *Journal of Science in Food and Agriculture*, vol. 81, no. 4, p. 889-894. https://doi.org/10.1002/jsfa.898

Prazdnik, W., Loeppert, R., Vierstein, H., Mueller, M. 2016. Characterization of heteropolysaccharies from seed mucilage of chia (*Salvia hispanica* L.), basil (*Ocimum basilicum* L.), and flax (*Linum usitatissimum* L.) and their healthy functionality. In *Conference on Polysaccharides – Glycoscience*. Prague, Czech Republic : Department of Carbohydrates and Cereals, p. 9-11. ISBN 978-80-86238-59-3.

Sęczyk, L., Swieca, M., Dziki, D., Anders, A., Gawlik-Dziki, U. 2017. Antioxidant, nutritional and functional characteristics of wheat bread enriched with ground flaxseed hulls. *Food Chemistry*, vol. 214, no. 1, p. 32-38. https://doi.org/10.1016/j.foodchem.2016.07.068 PMid:27507444

Troshchynska, Y., Synytsya, A., Kyselka, J., Štětina, J. 2016. Flaxseed meal mucilage polysaccharides: chemical characterization. In *Conference on Polysaccharides – Glycoscience*. Prague, Czech Republic : Department of Carbohydrates and Cereals, p. 273-277. ISBN 978-80-86238-59-3.

Xu, Y., Hall, C. A. III, 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. https://doi.org/10.5539/jfr.v3n6p83

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GLOBAL RHEOLOGICAL APPROACH TO THE QUALITY OF THE ROLLERS PUMPING OF DOUGH

Igor Stadnyk, Larysa Novak, Liudmyla Matenchuk

ABSTRACT
An analysis was made of the model approximations of three-phase medium and its behavior under deformation impacts in the rollers bulk feed, the lack of reliability of their operation was noticed and regularities and reasonable geometric parameters was determined. The influence of engineering rheology on the medium was considered and on its basis was derived an analytical mechanical model for determining the optimum value of shift deformation in order to ensure reliable operation of the rollers injection mechanism. A new design and method of determining the geometrical parameters of the pumping unit rollers were proposed and the yeast dough state was examined after cyclic action of the rollers.

Keywords: three-phase medium; roller; rheology; shift deformation; dough

INTRODUCTION
Baking and confectionery industry inherent its own specificity and variety of processes. Therefore, the engineering industry produces large range of equipment for its needs, in some cases it is metal-consuming and has complex kinematics. Presence of the rollers moving operating devices that cause mechanical impact on the medium while direct contact whit it is the specific characteristics of forming equipment (FE).

Active efforts are undertaking in Ukraine and abroad on developing a new generation of highly efficient equipment for the abovementioned industry, with the usage of different designs for rollers operating devices. The main requirement for the design of such equipment is to provide feasible designs according to the customer's specifications in a short time, at a low cost and with high reliability. Industrial technology calls for the automated systems development with computer-based programmed control, which will enable prompt respond to the present requirements for durability and quality of machines operation.

The operation mediums of these fields by its composition and nature are very diverse. They can be conventionally divided into gas, liquid, solid and those containing chemicals and surfactants.

Analysis of recent researches and publications
The literature data analysis and researches on enterprises of confectionery industry shows that the quality of the rollers operation depends on the set of parameters that can be divided into the following categories:

- form, kinematics, state and geometrical parameters of the rollers;
- physical-chemical properties of the medium;
- operation conditions: the gap between the rollers, the relative compression force of the medium, the frequency of their rotation;
- features of friction in liquid electroconductive mediums.

A large number of substances in the medium, changes in their concentrations, interactions between microorganisms and presence of the stimulators, etc., lead to the relative instability of the system. Under such conditions, there is an understanding in which way the impact of certain factors should be assessed. At once it may seem that the best case should meet the maximum satisfaction or securing at the upper level impacts. However, negative results must also be programmed, for example, values of osmotic pressure, double and triple impact of factors, production quality indicators decay and so on. Even more difficult is to evaluate the impact of compositional factors. **Gorbatov (1979)**, considers that the impact of temperature can be thoroughly traced, but there is no final point of view relatively physical pressure. However, the provisions of thermodynamics closely links pressure parameters and temperature in the gas laws, Mendeleev-Clapeyron equation, Henry's law and so on **(Naschokin, 1980)**.

Design parameters of rollers affect the structural and mechanical properties of the medium, as there is viscous friction and therefore a temperature changes **(Derkach, Stadnyk and Stadnyk, 2016)**.

Forming machines rotating rollers that operate in technological mediums (dough) with constant impact of

adhesion and friction forces can be considered as metal electrodes immersed in an electrolyte. They are charging relative to the solution which leads to the difference of potentials.

As friction is always accompanied by liquid mixing and such impacts as the rollers effect towards the dough is poorly investigated. Therefore, for the correct choice of their characteristics, operating under different temperatures, pressures and also for studding the nature of this process, determining the actual speed of the rheological process is of a great importance.

Analysis of model approximations of medium types

Formed dispersed gas phase consists of several components and is the one that consists of carbon dioxide and partly of the water vapor, spirit and other substances. Yet in this dispersed phase solid phase prevails, so further we will focus on the three-phase system.

Continuous synthesis of carbon dioxide, which takes place in full volume of the medium, is an important feature of the fermentation. This imposes differences in the structure of the medium, composition features of the gas-retaining capacity and, including its quantitative ratio in local areas. Such generation of CO_2 leads to the pronounced nonuniformity of concentration. Obviously, this must be accompanied by the same nonuniformity of energy potential **(Koval, 2016)**.

The assessment of rollers impact can be made on the basis of Archimedes' principle, which demonstration takes place in effecting on dispersed gas-phase. According to mentioned principle becomes possible to determine the total force effect on the medium for certain gas-retaining capacity. Considering though that this motive force accordingly dispersed gas-phase is distributed in dough array. Such feature of force action in full volume of medium with certain assumptions can be interpreted as stress and stressed states analogous. However, unlike the strength of materials concepts of stressed states here we have the feature that the medium is in a dynamic state with the violation of its integrity conditions.

In addition to the mentioned it is necessary to pay attention to another feature of phase interaction. It is known that holding capacity is determined by the value of generated gas flow which depends on the speed of bubbles floating of the gas phase. Absolute and relative speed is recognized at that. Just the relative speed, while the other conditions being, equal characterizes the resistance of bubble movement of the liquid phase in the deformed dough. Since the resistance is determined by the physical-chemical properties of the medium, it means that for each-one relative speed will be stabilized. This shows dependence, which determines the absolute speed of the gas phase:

$$w_{abs} = w_{rel} + w_{liq},$$

where w_{liq} – the speed of the gas phase, which it gets in the circulation circuit of the medium created between rollers.

The last depends on the geometry of the supercharger mechanism, the uniformity of flow distribution of the gas phase, transient process organization, disturbances, steady modes, etc. This is the reason to belive that the relative speed of the dispersed gas-phase for each medium is approximately constant under the conditions of bubbles size generalization. Features of physical phenomena's in gas-liquid systems, which existence is determined by the assumptions on the basis of phenomenological considerations, lead to the conclusion about the energy potential. Since, force effects of dispersed gas-phase on the liquid and the speed of points of the force factors application are known, it enables the ability to define an integral form of the power that corresponds to the kinetic energy of reverse circulation circuit of the medium phase, which is present at the rollers operation.

We give emphasize that under the deformation CO_2 self-generation changes the velocities of fermentation of the sugar-containing raw materials which are limited by microbes natural properties. Therefore fast growth of pressures in such deformation conditions is impossible. This means limited properties of the systems for creating energy impulses. Moreover, the increase in system pressure under other factors being equal leads to the decrease of gas-retaining capacity. At first, due to physical compression of the gas phase and at the second, due to the increasing of gas solubility in accordance with the Henry's law.

Demonstrations of the energy pulses in conditions of pressure sharp decrease will differ. The difference in pressures means the transition of the system to the new parameters of thermodynamic equilibrium. Transient increase of the dough gas-retaining capacity happens due to expansion of the gas phase with its amount appropriate adjunction owning to desorption of the dissolved part of gas phase. Obviously, such transient process pertains not only to the gas phase but definitely liquid one, as the volume increase of the gas phase uniquely determines the growth of the three-phase mixture and phases shift. Its dynamics is accompanied by the inertia and thereafter pressure increase in part of its general and programmed reduction. However, such inertia-dynamic load refers only to gas-liquid mixture in the dough.

Comparison of gas-liquid systems in the dynamics with elastic systems of solids with distributed masses leads to the conclusion of their equivalence and Rayleigh principle applicability for the determination of the reduced mass of liquid phase. The availability of data on the mass of the observable system and of forces that are applied means opportunity to use Lagrange–d'Alembert principle in its modeling. Definition of the driver is related to the dynamics of pressures change.

Comparison of the systems with different technologies of dispersed gas phase formation leads to the conclusion that their response on the energy pulse disturbance will be the same as far as gas phase while pressure decrease is the generators of mechanical impact.

Formulation of the problem

Significant number of components in the medium, changes in their concentrations, interactions between them and microorganisms, the stimulants presence, etc., lead to the relative instability of the system. Under such conditions, there is an understanding in which direction

assessment of the impact of certain factors should be made. At once it may seem that the best case must meet the maximum satisfaction or subsistence at all levels of impact factors. However, negative consequences must also be programmed, for example, according to osmotic pressure values, double and triple impact factors, production quality factors deterioration and so on. The impact of composition factors is even more difficult for evaluation.

However, the provisions of thermodynamics link closely pressure and temperature parameters. For instance in terms of technical availability adiabatic or polytropic process have the interest in impact on the fermented dough arrays is given by **Shynkaryk (2014)** and reasoned by **Sokolenko, Ukrainets and Yarovoi (2003)**. Due to the system compression, which is the fermented dough array, the temperature of the gas phase rises. It is clear that before the system compression temperatures of the gas phase and of the dough match. Yet, after compression the following ratio temperature is obtained:

$$T_2 = T_1\left(\frac{P_2}{P_1}\right)^{\frac{k-1}{k}}$$

for adiabatic process and

$$T_2 = T_1\left(\frac{P_2}{P_1}\right)^{\frac{m-1}{m}}$$

for polytrophic process where T_1 and T_2 – the initial and final temperature of the gas phase respectively; P_1 and P_2 – the initial and final pressure respectively; k and m – adiabatic and polytropic indices.

The energy introduced into the system at such conditions **(Naschokin, 1980)**, equals

$$E = \frac{MR}{k-1}(T_2 - T_1)$$

M corresponds to the mass of compressible gas; R – gas constant.

Energy introduced in such way should be redistributed between the dispersed gas phase in the dough and overall temperature of the system increases.

One way of changing the free energy is the presence of the surface-active substance (SAS) in medium. High tensile stresses emerge at relevant conditions in layers which are in contact under friction. These stresses will rise while the adsorption of SAS in the technological medium and at some moment can exceed critical, leading to the formation of microcracks. The adsorption of SAS molecules cause disjoining action that contributes to the boost of surfaces deformation of the medium.

Thus, the penetration of the adsorbed substances to the deformed surface layer causes its plastification and softening, facilitates its plastic flow, and sometimes even cause tearing.

Friction in the rotating rollers medium is a pulse oscillation process, resulting in possibility of fluctuation of the deformation effects values.

Problem statement

For machines with rollers operating devices the main functions are: to make the blanks of desired shapes and sizes while ensuring form-retaining ability, dosage accuracy and quality of the products, speed and duration of deformation. For the shape and quality of the products retaining machines with rollers should ensure minimal multiplicity of the masses processing and create required pressure to provide the blanks with desired shape. Rollers surface and processing mass viscosity are of great importance for bulk supply under rollers injection. Operation low reliability of the dough make-up, kneading and moulding machines with rollers caused by poorly developed surface of the rollers, which reduces the effect of their interaction with masses. From the part of operating device significant load effects while rolling out of the mass. This makes highly viscous medium to flow between two parallel surfaces of the rollers which are very close. As a result blank is compacted and partially the product loses its quality.

Based on a thorough analysis of the processes of rollers effect on the medium it has been established that during mass drag in the volume of the injection working chamber, kinetic curve has three typical parts. Each part represents a certain period of time of the process. Dispersed systems flow curve describes the degree of equilibria destruction of the structure outta the intensity of mechanical effects in all possible range of the effective viscosity change $\eta_{ef} = f(\tau)$. Numerous experimental facts confirm that there are areas on the medium flow curves where the dependence $\dot{\gamma}(\tau)$ is linear, which means that plastic viscosity is constant**. Machihin (1981)** and **Nikolaev (1976)** considered that a single formula that covers all modes of flow and does not contradict the presence of linear sections on the rheological curve does not exist. There is no formula that at finite values can provide in one range linear dependence, and in another a nonlinear.

For practical purposes there is no need to build a complete flow curve, yet as a rule, limits of typical stress and deformation speed for this process is known beforehand. In general structured dispersed systems cannot be characterized only by effective viscosity value without specifying the shift speed or stress. Any real material has all the rheological properties that varies. Wheat yeast dough, depending on the size of stress, time it is applied, deformation speed may perform flexible-elastic or plastic-viscous properties respectively **(Sokolenko, Ukrainets and Yarovoi, 2003; Guskov, 1970)**.

Complexity of obtaining form of ensuring form-retaining properties of dough and defining of rational processing modes is in close interconnection between the number of factors that effect the rollers reliability and quality of the process and the product. They are:

- presence of mechanical impact on the process by the rollers, that are out of the control measurements;
- usage of the equipment with rollers operating device that constructed without consideration of structural and mechanical properties and specific properties of raw materials (semi-finished products);
- presence of feedbacks between the properties of raw materials and equipment constructive parameters, that takes place under poor control of the process proceeding;
- impossibility to effect the structural and mechanical properties of the medium during the process.

This allows to define their regularities and calculating rational parameters of separate operations. In other words it allows to assess fully the impact of constructive parameters: the working chamber and the surface of the roller under certain angel of mass drag; constructive

parameters on the accuracy of the process flow and the dough properties after its flow out the clearance; rate of energy use, reliability and lifetime of machine with the rollers.

Scientific hypothesis

To discuss the parameters of the process of dough injection by the rollers and on their basis develop analytical mechanical model for evaluating the optimum values of deformation shift and geometric parameters of rollers in order to ensure reliable operation of the roller injection mechanism.

MATERIAL AND METHODOLOGY

During the course of the research, flour of the highest grade with 24.0% of gluten with excessively elastic quality (GDI 76 units) was used. The study was carried out as follows. By a commonly defined bagel production technology, a mixture of components with yeast has been prepared. Then dough was made. The resulting array of dough passed the initial stage of fermentation, after which it was divided on two equivalent parts; one corresponded to the experiment, and the other for control. Part of the control remained in the state of digestion, and the experimental sample was placed in the working chamber of the machine and was subjected to the effects of variable pressures in the range from 0.1 to 0.2 MPa with an exposure in time for 30 seconds. The number of cycles was n_{cycle} = 4, 7, 10, and 15. After the formation, a further 5 minutes extraction of the sample was performed, after which the resilience-elastic properties of both parts of the dough were evaluated. The results of the measurements are given in table. This part of the research has led to the conclusion about the positive role of the dispersed gas phase as an internal converter of the dough properties and dough blanks.

Numeric data table 2.5 interpreted in the form of dependency charts P = P (n_{cycle}), L = L (n_{cycle}), values of the parameters given in the table correspond to the average values obtained from ten measurements. The processing of measurements series was carried out according to the following sequence:

- arithmetic mean was determined;
- standard errors of a single measurement was determined;
- maximum possible error Δ of a separate measurement was determined and verification was made in order to ensure that there are no results in measurements that differ from the arithmetic mean for more than the maximum possible error Δ of separate measurements;
- standard errors of the arithmetic mean σ_0 was determined.

The relationship between the specific work consumption depends on the frequency of rotation of the rolls and the step of the ribs, which is described by the dependence:

$$A_{sp} = (157 \cdot S^2 - 151 \cdot S + 22) \cdot n^2 - (585 \cdot S^2 - 579 \cdot S + 83) \cdot n - 22$$

where A_{sp} – specific work; S – step of the ribs; n – frequency of the working body rotation.

The dependence of the specific work serves for the determination of the rational geometric parameters and rotation frequency of the working body, which will provide the quality parameters of the dough mass injection.

Tensile displacement (Pa) was determined by the formula:

$$\tau = z \cdot \alpha,$$

where α – indicators on the device scale; z – constant, the value of which depends on the cylinders that are used.

Dynamic viscosity (Pa·s) was determined according to the formula: $\eta = 0{,}1 \cdot (\tau/\gamma)$. For determining the dependences of the shear rate γ on the shear stress τ and the dynamic shear stress, the law of the flow of the viscoplastic medium of Shvedov, the Branopolskaya method was used.

Instruments and equipment

The research of the process of pumping and rolling of the dough was carried out on the molding machine B-58-4 of the confectionery factory (Ternopil) and the physical models were created at the department of Faculty of Engineering of Machines, Structures and Technologies (Ternopil Ivan Puluj National Technical University), it means: elastic properties of the dough were determined using the alveographer of the company «Chopin»; indicators of physical properties of the dough were determined by a rotating viscometer «Reotest-2» with two coaxial cylinders using a cylinder S2 and a full range of rotation speeds of the rotor in «AL» mode; the evaluation of tensility and strength were determined on the device for measuring the tensile dough.

RESULTS AND DISCUSSION

Dough is complex biopolymer in general and its deformation behavior under mechanical effects can be described by conventional models of rheological bodies. Thus to simplify composition of the rheological equations of biotechnological systems mechanical models (spring, sliding coupler, the cylinder with liquid and piston with holes), that emulate the basic properties just ideal bodies (elasticity, plasticity, viscosity) are used. These models can be combined, placing them in parallel, in series, in compound. The model of viscoelasticity with relaxation of the deformations (Maxwell model), viscoplastic body (Shvedov–Bingham model) and some others are the basic rheological models for dough simulation.

General mechanical deformation model of dough injection by the rollers consists of nonlinear-elastic with the elastic modulus $E_1(\sigma)$, which is ensured by the nonlinear elastic elements, the elastic with the elastic modulus E_1, viscosity η_1, which is ensured by the parallel connected linearly elastic and viscous elements. And at the same time with plastic nonlinear elastic modulus $E_2(\sigma)$, viscosity η_2 and F holder, which is ensured by consecutive connected nonlinear elastic and viscous elements with locking element attached to them. The model is mathematically described by the nonlinear rheological differential equation of the second order.

During the dough injection by the rollers when the interaction duration of rollers bodies with them is short so it does not have time to show all the features of highly elastic strain, then its modulus can be presented in the

form of elastic bodies with a certain viscosity. Additionally, the deformation in the area of injection is not always proportional to the loading, as this process is influenced by internal and external friction. This is caused by plastic deformation of structural and mechanical properties of the dough and surface structure of the rollers. However, the total deformation of the process remains at current levels. The value of the model total deformation under the load of the rollers can be presented as:

$$\varepsilon = \varepsilon_n + \varepsilon_в \text{ or } \varepsilon = \sigma / E(\sigma) + \sigma t / \eta$$

where $\varepsilon_n \cdot i \cdot \varepsilon_в$ – value of the elastic and viscous deformations; σ – value of the current load (compression), H; t – the rollers operation time, sec; E(σ) та η – complex modulus of the dough elasticity and viscosity.

The viscosity of non-newtonian fluids, which include dough, depends on the speed of deformation under the stress of rollers, related to the structure and its change while flow. In its turn, the dough fluidity depends on its nature and the physical and mechanical properties: concentration, temperature, humidity, acidity, formula. The equation of the flow of the Bingham fluid is acceptable for description of the fluidity dough between the rollers:

$$\theta = \theta_0 + \eta_{pl}\dot{\gamma}$$

Where – θ strain shift; θ_0 – fluidity limit (initial value of the shift stress); η_{pl} – plastic viscosity; $\dot{\gamma}$ – shift speed.

The most credible values of the dough reological parameters obtained on practice needed to calculate dough processing equipment with rollers. Therefore, generalized model of the deformation will be equal to the total deformations occurred in the working chamber of the force unit of the machine B-58-4.

From the Maxwell's, Bingham's, Shvedov mechanical models **(Machihin, 1981)**, and the studies conducted at the place of production the mechanical model, which includes change of the medium (dough) rheology during the process period, proposed for description of dough behavior under rollers action in force unit of the molding machine (Figure 1).

For the period of discreet effect of the rollers on dough, shear stress is constantly changing. The time of deformation forces (torsion, compression, outflow) application is so short that shear stress reached instantly. At first the dough feels viscoelastic deformation (G, η), and then, when stress exceeds the boundary shear stress (BSS), it plastically deformed and starts to flow. When rollers compress the dough partial mixing of media takes place, its partial reverse motion. Therefore, it reveals itself like Bingham's body, combining elasticity, viscosity, plasticity.

Rheological equation of the mechanical model of the dough injection by the rollers may be obtained as follows.

The total deformation of the model of process equals the sum of deformations:

$$d\gamma = d\gamma_з + d\gamma_c + d\gamma_m$$

where $d\gamma_з, d\gamma_c, d\gamma_m$ – deformations of Maxwell's, Bingham's, Shvedov bodies respectively.

Differentiation of the left and right parts of the equation gives: $\frac{d\gamma}{dt} = \frac{d\gamma_з}{dt} + \frac{d\gamma_c + d\gamma_m}{dt}$ (1)

The value $\frac{d\gamma_з}{dt}$ is determined from the equations for viscoelastic relaxing Maxwell's body that corresponds to the consecutively connected models of Hooke and Newton:

$$\frac{d\gamma_з}{dt} = \dot{\gamma} = \frac{\dot{\theta}}{G} + \frac{\theta}{\eta} \quad (2)$$

While time t goes deformation increases and asymptotically approaches to the value $\dot{\gamma} = \frac{\theta - \theta_T}{\eta_{пл}}$.

Therefore, the deformation of the Bingham's body does not develop instantly; there is delay due to the admittable conditions of the fluidity (reverse motion).

Respectively, the value of deformation at the initial flow of dough through the gap between the rollers corresponds Shvedov equation:

$$\dot{\gamma} = \frac{\theta - \theta_T}{\eta_{пл}} + \frac{\dot{\theta}}{G} \quad (3)$$

The universal equation of rheological models of the dough injection by the rollers is**:**

$$\dot{\gamma} = \frac{\dot{\theta}}{G} + \frac{\theta}{\eta} - \frac{\theta - \theta_T}{\eta_{пл}} + \frac{\theta - \theta_T}{\eta_{пл}} + \frac{\dot{\theta}}{G}$$

$$\dot{\gamma} = \frac{\dot{\theta}}{G} + \frac{\theta}{\eta} + \frac{\dot{\theta}}{G}$$

The model (Figure 1) results in instant effect of the rollers on the dough while its injection. We will consider that at the time t = 0 rollers get stress θ_0 and viscous deformation is equal to zero. Therefore, the deformation of the dough will be equal to elastic deformation only:

$$\gamma_0 = \frac{\theta_0}{G}$$

Given deformation does not change in time, so $\gamma = \gamma_0$ and $\dot{\gamma} = 0$. Therefore our equation will look like:

$$\frac{\dot{\theta}}{G} + \frac{\theta}{\eta} + \frac{\dot{\theta}}{G} = 0$$

Integration gives:

$$\frac{\dot{\theta}}{G} + \frac{\theta}{\eta} + \frac{\dot{\theta}}{G} = 0,$$

$$2\frac{\dot{\theta}}{G} + \frac{\theta}{\eta} = 0,$$

$$\frac{2}{G}\frac{d\theta}{dt} = -\frac{\theta}{\eta}; \Big| -\frac{dt}{\theta}$$

$$\frac{2}{G}\frac{d\theta}{\theta} = -\frac{1}{\eta}dt.$$

$$\frac{2}{G}\int\frac{d\theta}{\theta} = -\frac{1}{\eta}\int dt.$$

$$\frac{2}{G}\ln|\theta| = -\frac{1}{\eta}t + c, \ldots \theta(0) = \theta_0$$

$$c = \frac{2}{G}\ln|\theta_0|.$$

Thus,

$$\frac{2}{G}\ln|\theta| = -\frac{1}{\eta}t + \frac{2}{G}\ln|\theta_0|,$$

$$\frac{2}{G}(\ln|\theta| - \ln|\theta_0|) = -\frac{1}{\eta}t,$$

$$\frac{2}{G}(\ln\left|\frac{\theta}{\theta_0}\right| = -\frac{1}{\eta}t, \ldots\Big|*\frac{G}{2}$$

$$\ln\left|\frac{\theta}{\theta_0}\right| = -\frac{G}{2\eta}t,$$

$$\frac{\theta}{\theta_0} = e^{-\frac{G}{2\eta}t}.$$

Shear modulus G related with elastic modulus E by dependence $G = \frac{E}{2(1+\mu)}$,

where μ – Poisson's ratio, which is equal to 0.5 in incompressible liquid; E – elastic modulus. The value $\frac{\eta}{G} = T_з$ is a relaxation period. At t = 0, $\theta = \theta_0$ and at t $= T_{rel} = \frac{\eta}{G}$ $\theta = \frac{\theta_0}{e}$, in other words the relaxation period in the dough where the stress drop on e times.

Mechanical rheological model of the dough injection by the rollers and its mathematical formulation is required not only for the objective assessment of the dough consistence in a short process, but to evaluate its behavior at all stages. In this context we will analyze current process according to the rheological model of the dough injection by the rollers.

Geometrical parameters of the rollers medium supply unit determination

The above materials focused by most on biochemical and thermodynamic transformations related to different schemes of dough preparation with its further deformation. Initial conditions of the dough characterized by values of 28 ±2 °C, and 30 ± 2 °C in case of formation. Accurate performance of the kneading while formation is of a great importance. This partially removes carbon dioxide and other fermentation products. While this, the conditions of vital activity of yeast improves, their fermentation activity and elasticity of the medium increases. Kneading is absolutely necessary when processing strong flour and also flour with short jagged gluten.

Mentioned actions for technologies of dough blanks forming are the basis of structural and mechanical properties of elastic or plastic-viscous characteristics of dough. Last affects the stability of blanks shape during maturation and baking and also determines the volume yield of products and quality of crumb structure **(Drobot, 2002)**. Formation of the dough and products indicated properties depends on many factors, mainly on the geometry of the rollers and the ratio of flour polymers, the state of its protein complex and the dough receipt. These stipulate the products output volume and quality of their structure.

As was noted before, the dough by its properties corresponds to elasticity of solid and simultaneously, liquid as to the fluidity (spreading) characteristic. Correlation of these properties is determined by the composition and state of polymers – starch, protein, cellulose, that hydrating with the presence of water and form a colloidal system, and also by the low-molecular compounds of sugars, fats, amino acids, etc. Dough elasticity hinders volume growth, but contributes in keeping accurate form of already made blanks. Dough flexibility causes forming of the foam like structure.

The research using alveograph determined that additional dough treatment by the swaying rollers before its loading

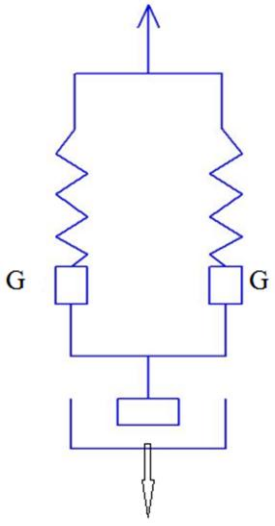

Figure 1 Rheological model of the dough injected by the rollers.

into the working chamber and the operation of the rollers unit itself effects the deformation of the dispersed phase. Under the impact of alternating pressures (compression of the medium) resilience and elasticity of the dough are improved, specific energy consumption on its deformation increases. Thereafter structural and mechanical properties of the dough that depends on the number of processing cycles improves.

The data in Table 1 indicates positive role of the dispersed gas phase as the internal converter of dough properties and also the dough blanks. Numerical data in Table 1 are interpreted in the form of dependences $P = P(n_{cycle})$, $L = L(n_{cycle})$, $P/L = P(n_{cycle})/ L(n_{cycle})$, $S = S(n_{cycle})$ and graphs (Figure 2).

Among the key requirements of the moulding machine with rollers operating devices are system performance conformity, reliability, maintaining of the nominal temperature parameters, medium flow velocity, limited foaming, etc. The rollers geometry selection focuses on some features and extra requests. Thus to the important requirements one can refer minimization of power consumption, the material quantity for rollers manufacturing, reliability of the whole process. Therefore, these factors meet equality in diameter and length of the grooves of the cylindrical roller. In this case it is obvious that the minimum consumption of the material should meet

Table 1 Resilience-elastic properties of the dough with additional mechanical processing.

Number of processing cycles	Resilience, P, mm	Tensility, L, mm	P/L	Alveogram area, S, cm^2	Specific work of the deformation, $W \cdot 10^4$, J
Control (without processing)	85	68	1,25	36.4	238.06
$n_{cycle} = 15$	105	88	1,193	48.4	316.54
$n_{cycle} = 20$	117	99	1,182	66.4	434.3
$n_{cycle} = 25$	133	120	1,108	83.4	545.4
$n_{cycle} = 30$	146	135	1,082	94.2	616.1

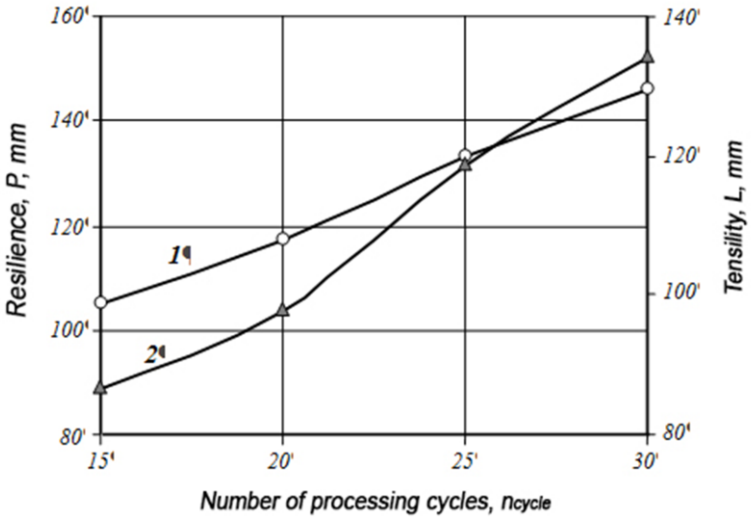

Figure 2 The graph of $P = P(n_{cycle})$ (1) and $L = L(n_{cycle})$, (2) dependences form the number of processing cycles.

Figure 3 New Spiral roller working body design.

the minimum surface area under other equal conditions while the given conditions of quality injection are kept. The mathematical correlations between the surface of the roller S and its volume V, for a cylindrical one:

$$S_в = \pi dl + \frac{\pi d^2}{4};$$

$$V_в = \frac{\pi d^2}{4} l$$

where d and l – diameter and length of the roller respectively.

In case we accept the terms of the surface minimization l = d, then inserting this into the equation and taking into account the usable area of the roller, the equation can be re-written as:

$$S_в = \pi d^2 + \frac{\pi d^2}{2} = 0{,}35\pi d^2;$$

$$V_в = \pi d^3,$$

Hence the expected conclusion is made that the rollers surface is proportional to the square of its size and the volume – to the cube of the size. Their relation can be defined as:

$$S_в / V_в = 0.35/d.$$

The analysis of the equation makes possible to establish that the specific surface area (surface area that refers to volume) dramatically decreases according to the hyperbola formula which is displayed by the following ratio:

D	0.1	0.15	0.2	0.25
$S_в / V_в$	3.5	2.3	1.75	1.4

CONCLUSION

Thus, increasing of the geometric parameters d eventually could lead to the inability to stabilize the injection, and this will change the structural and mechanical properties of the medium. Our studies confirm earlier determined geometrical parameters of the molding machines rollers with diameters of 160 – 200 mm. In dough make-up units rollers diameter may vary depending on the design of injection chamber, indexing head. Thus, the design parameters affect the structural and mechanical properties of the medium, as there is viscous friction and therefore a temperature changes. This enabled the development of the rollers new design, which will be explored in further studies.

REFERENCES

Derkach, A. V., Stadnyk, I. Y., Stadnyk, O. I. Ukraine. Declarative Patent for utility model. Dough feeding unit of the molding machine. Patent no. 111061. 2016-10-25.

Stadnyk, I. Y. Ukraine. Declarative Patent for utility model. Dough feeding unit of the molding machine. Patent no. 111058. 2016-10-25.

Drobot, V. I. 2002. *The technology of breadmaking*. Kyiv: Logos; 2002, 365 p. ISBN 966-581-363-3.

Gorbatov, A. V. 1979. *Rheology of meat and dairy products.* Moscow : Pishchevaya Promyshlennost (Food Industry), 383 p.

Guskov, K. P., Machihin Y. A., Machihin, S. A., Lunin, L. N. 1970. *Rheology of food bulk*. Moscow : Pishchevaya Promyshlennost (Food Industry), 208 p.

Karpenko, G. V. 1986. *The impact of the medium on metal strength and durability* : monography. Kyiv : Naukova Dumka. 126 p.

Machihin, Y. A. 1981. *Engineering rheology of food materials.* Moscow : Lehkaia i Pishchevaya Promyshlennost (Light and Food Industry), 216 p.

Naschokin, V. V. 1980. *Engineering Thermodynamics and Heat Transfer.* Moscow : Higher School, 469 p.

Nikolaev, B. A. 1976. *Measuring of structural and mechanical properties of flour dough*. Moscow : Pishchevaya Promyshlennost (Food Industry), 246 p.

Shynkaryk, M. M., Kravets, O. I. 2014. *Theoretical basis of thermotechnics*. handbook. Ternopil, Ukraine : Ivan Puluj National Technical University. 32 p.

Sokolenko, A. I, Ukrainets, A. I, Yarovoi, V. L., Vasylkivskyi, K. V. 2003. Under Ed. Sokolenko A. et al. *Guide for the specialist on food production*. *Thermal processes. Energy-savings*. Book 2. Kyiv, Ukraine : ArtEk. 432 p. ISBN 966-505-069-9.

Koval, O.V. 2016. *Improvement of processes and modernization of equipment fermentation* : theses. Kyiv, Ukraine : National University of Food Technologies. 22 p.

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GARLIC (*ALLIUM SATIVUM* L.) – THE CONTENT OF BIOACTIVE COMPOUNDS

Marianna Lenková, Judita Bystrická, Peter Chlebo, Ján Kovarovič

ABSTRACT

Genus *Allium* belongs to the family *Alliaceae*, which contains more than 600 species. Garlic (*Allium sativum* L.) is the most popular food ingredient widely used all over the world. During the last few decades, garlic has received tremendous attention for their wide range of therapeutic properties and great health benefits. Garlic has possessed antibacterial, antifungal, antiparasitic, antiviral, antioxidant, anticholesteremic, anti-cancerous, and vasodilator characteristics. In this work the total polyphenols content, total sulfur content and antioxidant activity was compared and evaluated in four studied varieties of garlic (Mojmír, Lukan, Záhorský and Makoi). The analyzed samples of garlic were collected at the stage of full maturity in the area Bardejov. The total polyphenols content was measured using the spectrophotometric method of Folin-Ciocalteu agents. The total polyphenols content in studied varieties of garlic were determined in the range 612.23 $mg.kg^{-1}$ (Mojmír) to 566.01 $mg.kg^{-1}$ (Lukan). The total polyphenols content in garlic can be arranged as follows: Mojmír >Makoi >Záhorský >Lukan. The determination of the total sulfur content is based on dry combustion in the presence of oxygen and allows for the quantitative conversion of sulfur to SO_2. Statistically significant highest level of total sulfur content was recorded in 0.638% (Mojmír) and the lowest level was in 0.421% (Makoi). According to determined values of total sulfur content the studied varieties of garlic can be arranged in the following order: Mojmír >Lukan >Záhorský >Makoi. Antioxidant activity was determined by the spectrophotometric method using a compound DPPH (2.2-diphenyl-1-picryhydrazyl). The highest value of antioxidant activity was measured in Mojmír (15.24%). The lowest level was observed in Makoi (11.73%). The antioxidant activity in garlic declined in the following order: Mojmír >Lukan >Záhorský >Makoi. In all studied samples of garlic was confirmed by the strong dependence of the total polyphenols content, total sulfur content and antioxidant activity.

Keywords: provide 5 words in singular form; cell; PCR; milk

INTRODUCTION

Garlic (*Allium sativum* L.) is plant belonging to genus *Allium* of the family *Alliaceae*. It is the second most widely distributed species of *Allium* after *Allium cepa* (onion). Central Asia is considered its center of origin **(Manjunathagowda et al., 2017)**. Garlic considered one of the twenty most important vegetables, with various uses throughout the world, either as a raw vegetable for culinary purposes, or as an ingredient of traditional and modern medicine **(Martins et al., 2016)**. Its medicinal effects have been used for at least 3,000 years in Chinese medicine. The Egyptians, Babylonians, Greeks, and Romans used garlic for healing purposes. In 1858, Pasteur noted garlic's antibacterial activity, and it was used as an antiseptic to prevent gangrene during World War I and World War II **(Tattelman, 2005)**.

Garlic is used in the prevention of vascular disease, and cancer of the bladder, brain, breast, colon, lungs, ovaries, and stomach. Other potential benefits include, kidney function, atherosclerosis, antibacterial, antifungal activity, cataractogenesis, immune function and prebiotic effect **(Bisen and Emerald, 2016)**. Historically, garlic has been used around the world to treat many conditions, including hypertension, infections, and some cultures have used it to ward off evil spirits. Currently, garlic is used for reducing cholesterol levels and cardiovascular risk, as well as for its antineoplastic and antimicrobial properties **(Tattelman, 2005)**. Garlic contains glycerophospholipids, lectins, saponins, glucosides, fructan, pectin, vitamins A, B_1, B_2, B_3, B_6, C, E, allixin and organoselenium compounds such as -glutamyl-Se-methylseleno-cysteine, Se-methylseleno-cysteine, "Se-alliins", Se-methionine, Se-cystine/Se-cysteine are the additional most abundant functional constituents in garlic. Sulfur compounds do not exist as such in the intact cells. They are formed as a result of enzymic reaction between allinase and volatile precursors substrate S -alk(en)yl cysteine sulfoxide and sulfonic acid.

Thiosulfinates and sulfonic acid compounds are derived when the cells are ruptured **(Bisen and Emerald, 2016)**.

Garlic has a high concentration of sulfurcontaining compounds. The thiosulfinates, including allicin, appear to be the active substances in garlic. Allicin is formed when alliin, a sulfur-containing amino acid, comes into contact with the enzyme alliinase when raw garlic is chopped, crushed, or chewed **(Tattelman, 2005)**. Allicin, which is one of the most researched therapeutic compounds of garlic, is extremely unstable and rapidly degrades with time, even at low temperatures, which causes its prompt degradation during contact with stomach acid during oral consumption **(Osman et al., 2007)**. The primary sulfur containing constituents in garlic are the S-alkyl-L-cysteine sulfoxides, such as methiin and alliin, allicin, diallyl sulfide, and diallyl disulfide. These compounds provide to garlic their characteristic odor and flavor, as well as most of their biological properties **(Bisen and Emerald, 2016)**. Garlic has numerous biological activities that are attributed to its rich content of organ sulfur compounds and other phytochemicals that work in synergy (polyphenols, antioxidant active compounds) **(Ugwu and Suru, 2016)**.

Garlic is rich sources of total phenolic compounds and has been highly ranked regarding its contribution of phenolic compounds to human diet **(Martins et al., 2016)**. Phenolic compounds can be found in all higher plants. The composition of the phenol fraction in plants depends mainly on the species, cultivars and the agronomic and climatic conditions. The main sources of polyphenols in the human diet are fruits and vegetables. The chemical structure is related to the reactivity of polyphenols, which are responsible for the color, texture, taste and appearance **(Lachowicz et al., 2016)**. Distribution of polyphenols according to the chemical structures: phenolic acids, flavonoids, polyphenolic amides, other polyphenols **(Tsao, 2010)**.

Antioxidant activity of polyphenols depends on the arrangement and the number of -OH groups in the phenolic rings and their connections with saccharides. Polyphenols may function as reducing initiators, chelating agents, inhibitors of the enzyme activity and preventers of oxidative reactions caused by active singlet oxygen **(Mitek and Gasik, 2007)**. Polyphenols, flavonoids (allixin), bioavailable water-soluble organo-sulfur compounds of garlic (S-allyl cysteine), along with stable lipid soluble allyl sulfides (diallylsulfid) and diallyl polysulfides, saponins, essential micronutrients (selenium) and macronutrients, as lectins, are known to express potent antioxidant activity **(Capasso, 2013)**. Antioxidant substances, protect our organism against oxidative stress, by scavenging free radicals. They also have a positive impact on our health and wellbeing **(Oszmianski et al., 2013)**. Garlic possess many therapeutic properties including antioxidant, antimicrobial, antiviral, antifungal, anti-protozoal, hepatoprotective, cardioprotective, anti-inflammatory, neuroprotective, anti-amnesic, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory, hypolipidemic, anti-hypertensive and anti-diabetic. These therapeutic properties are caused by the combination and biological activity of organo-sulfur compounds, polyphenols and antioxidant active compounds **(Bisen and Emerald, 2016)**.

Scientific hypothesis

In this study the effect of variety on content of total polyphenols, total sulfur content and antioxidant activity in four studied varieties of garlic (Mojmír, Lukan, Záhorský and Makoi) in area of Bardejov has been studied.

MATERIAL AND METHODOLOGY

The local climate conditions

This study was performed in area of Bardejov, Slovak Republic. She is situated on the north-eastern Slovakia. Bardejov belongs to slightly warm area in Slovakia. Bardejov has very good natural and climatic conditions for crop growth, without any adverse effects. The average annual rainfall is 700 – 750 mm and the average annual temperature is 5 – 9 ºC.

Samples of plant material

The samples of plant material – Garlic variety (Mojmír, Záhorský, Lukan and Makoi) were collected in the phase of full ripeness from area of Bardejov. For analysis was used fresh material soil samples and plant, samples were analysed by selected methodologies (determination of total polyphenols, total sulfur compounds and antioxidant activity). All samples of plant material were grown under the same conditions. The soil samples from the area, where was grown plant material, was analysed (Table 1 and Table 2). The analysis of soil samples was carried out four times in four sampling sites. Only NPK fertilization (200 g per m^2) was used for the achievement of favourable soil macroelements content.

Sample preparation

Garlic varieties were grown on 1 x 1 m^2 plots. From each parcel, we picked out from 5 random places approximately 1 kg of garlic. Garlic was divided into cloves and homogenized. 25 g of homogenized garlic were extracted in 50 ml of 80% ethanol (Sigma – Aldrich Co, USA), which were shaken (shaker GFL 3006, 125 rpm) for sixteen hours. Samples were kept at laboratory room temperature in dark conditions until the analysis. Each determination was carried out in six replications.

Determination of total polyphenols

Total polyphenols content (TPC) was determined by the method according to **Lachman et al. (2003)**. It is expressed as mg of gallic acid (Merck group, Germany) equivalent per kg of fresh matter. Total polyphenols content was determined using the Folin-Ciocalteu reagent (Merck group, Germany). 2.5 mL of Folin-Ciocalteu reagent was added to 100 μL xtract to volumetric flask. The content was mixed. After 3 minutes, 5 mL 20% solution of sodium carbonate (Merck group, Germany) was added. Then the volume was adjusted to 50 mL with distilled water. After 2 hours, the samples were centrifuged (centrifuges UNIVERSAL 320, 15000 rpm, Germany) for 10 minutes. The absorbance was measured of the spectrophotometer Shimadzu UV/VIS – 1240 (Shimadzu, Japan) at 765 nm. The concentration of polyphenols was calculated from a standard curve with known concentration of gallic acid.

Table 3 Average content of total polyphenols (mg.kg^{-1}), antioxidant activity (% inhibition) and total sulfur compounds (%).

Vegetable	Variety	TPC	AOA	TSC
Garlic	Mojmír	612.23 ±1.23^{c}	15.24 ±0.30^{d}	0.638 ±0.015^{b}
	Záhorský	577.68 ±1.16^{b}	12.36 ±0.18^{b}	0.443 ±0.011^{a}
	Lukan	566.01 ±1.14^{a}	14.20 ±0.19^{c}	0.448 ±0.012^{a}
	Makoi	612.21 ±1.17^{c}	11.73 ±0.36^{a}	0.421 ±0.013^{a}
$HD_{0.05}$		1.8122	0.4076	0.8985
$HD_{0.01}$		2.5406	0.5715	1.2597

Legend: Multiple Range Tests, Method: 95.0 percent LSD, Different letters (a, b, c and d) between the factors show statistically significant differences (p <0.05) – LSD test, TPC – total polyphenols content, AOA – antioxidant activity, TSC – total sulfur content.

Determination of total sulfur compounds

The determination of the total sulfur content is based on dry combustion in the presence of oxygen and allows for the quantitative conversion of sulfur to SO_2, the elimination of other combustion products including water and the separation of the generated gases. 50 mg of a lyophilized (Telstar Technologies LYOQUEST55, Spain) and homogenized sample is fired in a tin crucible with a V_2O_5 (Sigma – Aldrich Co, USA) catalyst in the elementar (Vario Macro Cube V 3.1.4, Elementar Analysensystem GmbH, Germany). After insertion of the crucible with the sample into the combustion tube, the oxygen stream produces a strong exothermic reaction, the temperature rises to 1250 ºC and the sample is incinerated. Combustion products are conveyed along the combustion tube where the oxidation is complete. SO_3 is reduced to SO_2. The mixture of gases flows into the chromatographic column where the separation takes place. The gases are sent to the thermal conductivity detector where the electrical signals are processed by the software and provide the % sulfur contained in the sample. Sulfanilamide (Sigma – Aldrich Co, USA) is used as the calibration standard **(Šapčanin et al., 2013)**.

Determination of antioxidant activity

Antioxidant activity (AOA) was measured according to **Brand-Williams et al. (1995)**. The method is based on using DPPH$^{\cdot}$ (2.2-diphenyl-1-picrylhydrazyl). DPPH$^{\cdot}$ (Sigma – Aldrich Co, USA) (3.9 ml) was pipetted into the cuvette and the absorbance was measured using the spectrophotometer Shimadzu UV/VIS – 1240 (Shimadzu, Japan) at 515.6 nm. The measured value corresponds to the initial concentration of DPPH$^{\cdot}$ solution at the time A_0. Then 0.1 cm^3 extract was added to start measuring dependence A = f*(t). The content of cuvette was mixed and the absorbance was measured at 1, 5 and 10 minutes in the same way as DPPH solution. The percentage of inhibition expresses how antioxidant compounds are able to remove DPPH$^{\cdot}$ radical at the given period of time.

Inhibition (%) = $(A_0 - A_t / A_0) \times 100$

Statistical analysis

Results were statistically evaluated by the Analysis of Variance. All the assays were carried out in quadruplicates 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 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. Using statistical software Statgraphics Centurion XVI.I (Statpoint Technologies, The Plains, Virginia, USA) and a correlation analysis (Microsoft Excel, Washington, USA) was used.

RESULTS AND DISCUSSION

The total polyphenols content, total sulfur content and antioxidant activity in the studied varieties (Mojmír, Záhorský, Lukan and Makoi) from area Bardejov are presented in Table 3. The total polyphenols content of the studied samples is varied from 566.01 ±1.14 mg.kg^{-1} to 612.23 ±1.23 mg.kg^{-1}. The highest level of total content of polyphenols was detected in Havran. In variety Lukan was measured the lowest value of total polyphenols content. In Mojmír average value of total polyphenols content is 1.06-times higher than in variety Lukan. The studied samples of garlic according to determined values of total polyphenols content can be arranged in following order: Mojmír >Makoi >Záhorský >Lukan. **Mahmutovic et al. (2009)** was measured the total polyphenols content in the range 488 mg.kg^{-1} to 800 mg.kg^{-1}, which is consistent with our results. The highest total polyphenols content in garlic

Table 1 Agrochemical characteristic of soil substrate in mg.kg^{-1}, content of nutrients from locality Bardejov.

Vegetable	K	Ca	Mg	P	pH_{KCl}	Humus %	Cox %
garlic	380 ±2.6	2170 ±2.1	259 ±1.1	406 ±1.6	5.50 ±0.09	3.52 ±0.03	6.15 ±0.01

Table 2 Content of heavy metals (mg.kg^{-1}) in soil substrate (extraction by aqua regia).

Vegetable	Zn	Cu	Ni	Pb	Cd
garlic	90 ±2.8	33 ±1.3	38 ±1.7	19 ±1.0	1.8 ±0.1
Limit *	150	60	50	70	0.7

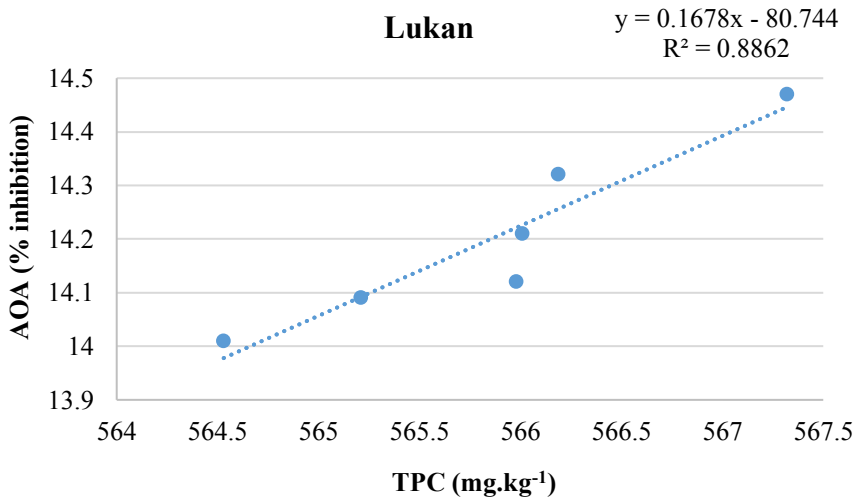

Figure 1 Relationship between TPC and AOA in Lukan.

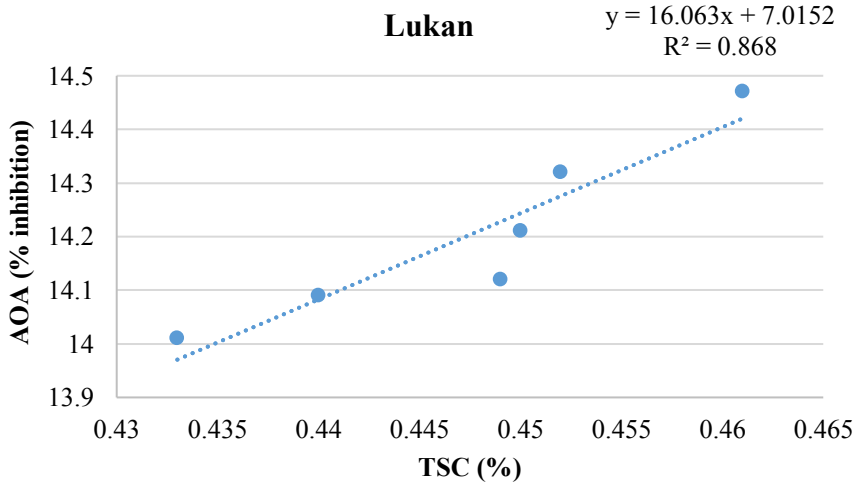

Figure 2 Relationship between TSC and AOA in Lukan.

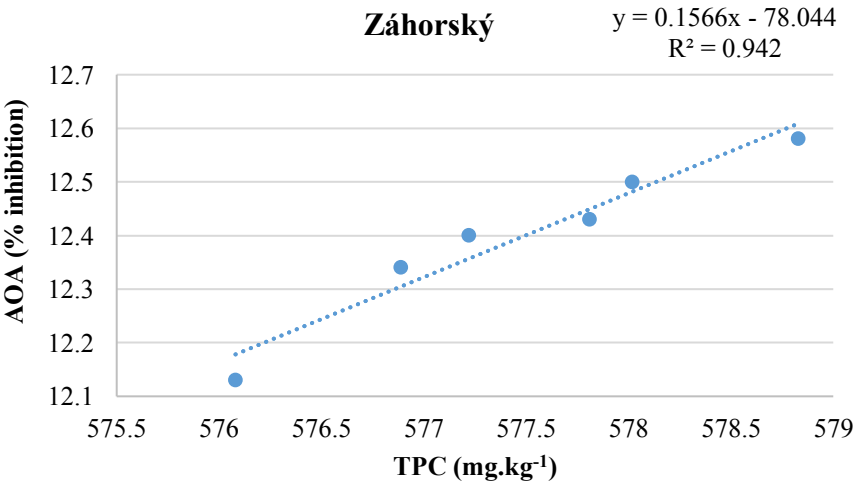

Figure 3 Relationship between TPC and AOA in Záhorský.

(741 mg.kg^{-1}) was indicated by **Bayili et al. (2011)**. The lower value of total polyphenols content in garlic was measured **by Zakarova et al.** (2014) – 450 mg.kg^{-1}, by **Benkeblia (2005)** – 490 mg.kg^{-1} and **Kavalcová et al. (2014)** – 260 mg.kg^{-1}. The content of total sulfur content in the samples ranges from 0.421 ±0.013% to 0.638 ±0.015% (Table 3). The highest value of total sulfur content was observed in Mojmír. The lowest level of total sulfur content

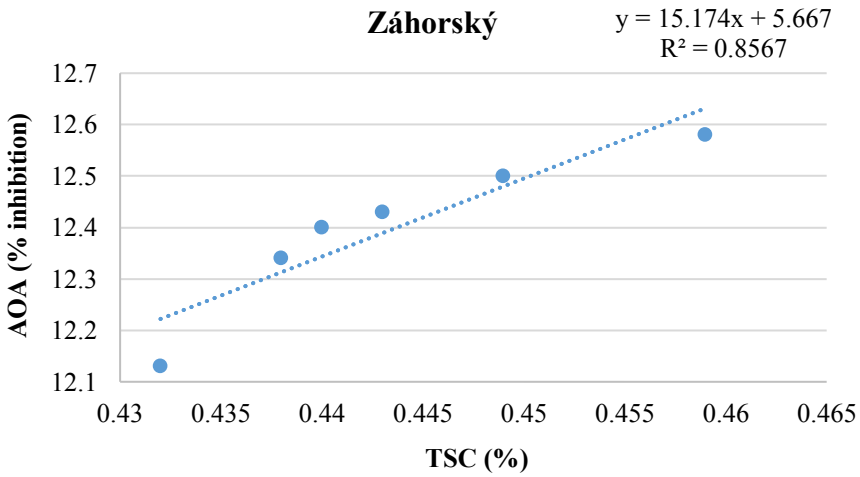

Figure 4 Relationship between TSC and AOA in Záhorský.

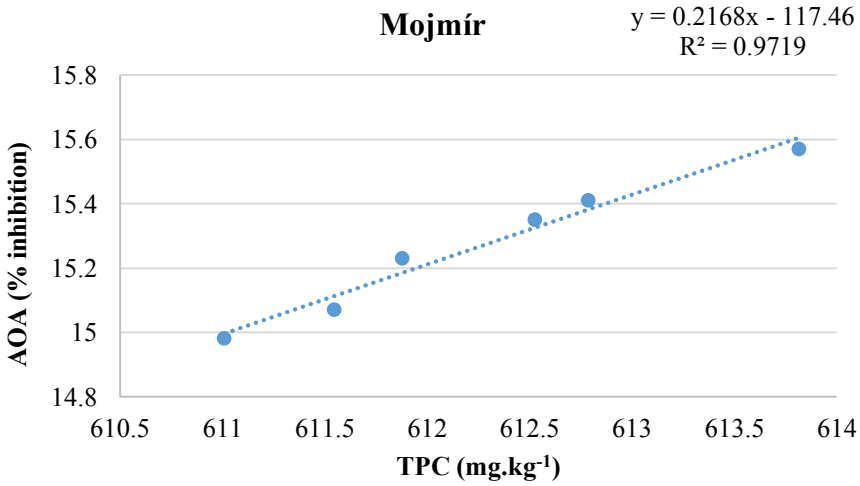

Figure 5 Relationship between TPC and AOA in Mojmír.

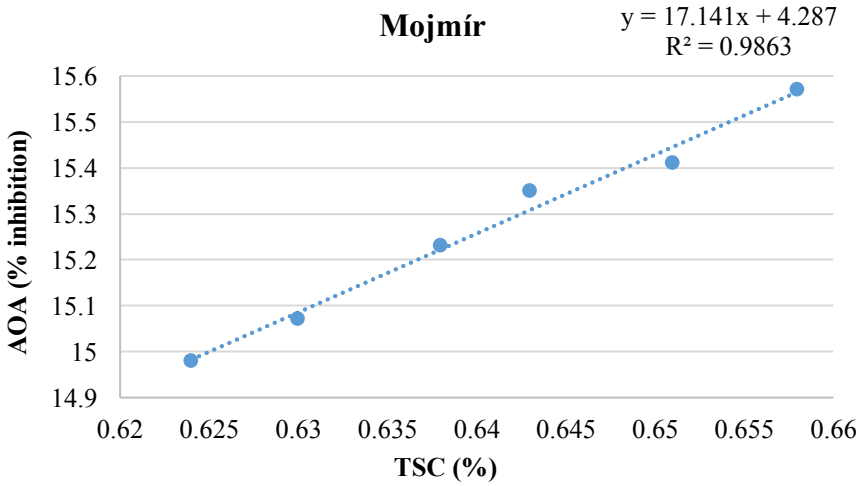

Figure 6 Relationship between TSC and AOA in Mojmír.

was measured in Makoi. In variety Mojmír average value of total sulfur is 1.52-times higher than in Makoi. The determined quantity of total sulfur content in the studied samples can be arranged in the following order: Mojmír >Lukan >Záhorský >Makoi. **Mahmutovic et al. (2009)** indicated that the value of total sulfur content of garlic move in wide range from 0.64% to 0.70%. **Schulz et al. (2004)** determined the total sulfur content with a value of up to 1%, as confirmed by the team autors of **Mills et al. (2005)**. **Benkeblia and Lanzotti (2007)** provides of total sulfur

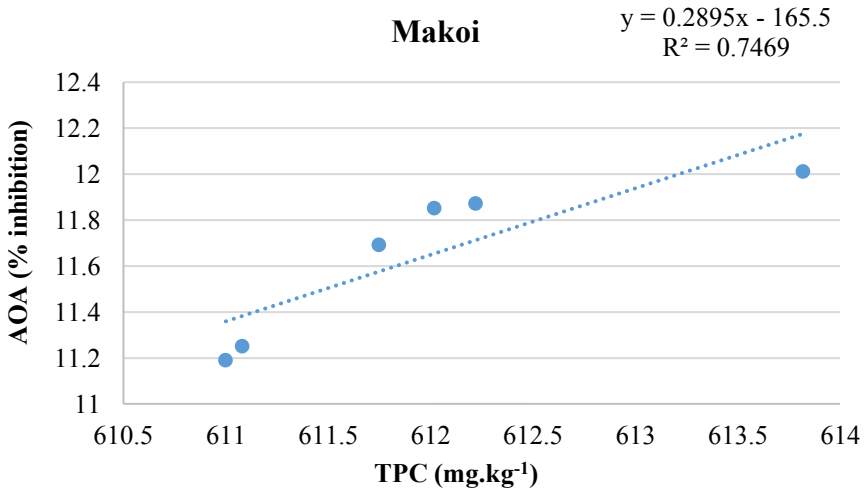

Figure 7 Relationship between TPC and AOA in Makoi.

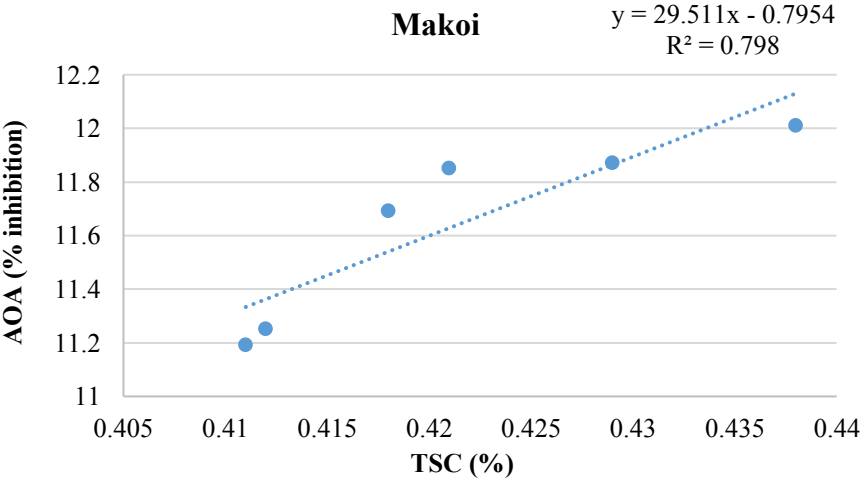

Figure 8 Relationship between TSC and AOA in Makoi.

content of garlic, which in the wide range from 0.23% to 0.56%. **Muradic et al. (2010)** indicates that the value of the total sulfur content of garlic is 0.63%, which good correlate with the results of this work. The value of antioxidant activity in the analyzed samples ranges from 11.73 ±0.36% to 15.24 ±0.30%. The statistically significant highest value of antioxidant activity was detected in variety Mojmír. The lowest level of antioxidant activity was measured in variety Makoi. The average value of antioxidant activity in variety Mojmír is 1.30-times higher than in Makoi. According to determined values of antioxidant activity the analyzed varieties of garlic can be arranged in the following order: Mojmír >Lukan >Záhorský >Makoi. **Narendhirakannan and Rajeswari (2010)** indicate that the values of antioxidant activity is 12 – 21%, which good correlate with the results of this work. **Boonpeng et al. (2014)**, **Choi et al. (2014)** reported even a lower value of antioxidant activity in garlic (7.44%, 7%). Highest content of antioxidant activity in garlic (21.5%, 16%, 28%) was measured by **Rai et al. (2015)**, **Sayin and Alkan (2015)**, **Dalaram (2016)**.

Relations among the total polyphenols content, total sulfur content and the antioxidant activity in studied varieties of garlic (Mojmír, Záhorský, Lukan and Makoi) were evaluated (Figure 1 – 8). The coefficient of correlation (r = 0.8642 – 0.9931) confirmed strong dependency between the content of polyphenols, total sulfur content and the antioxidant activity and the results are in good agreement with the findings of **Mahmutovic et al. (2014)**, **Lenková et al. (2017)** who confirmed correlations between total polyphenols content, total sulfur content and antioxidant activity in garlic. **Ramkissoon et al. (2012)**, **Chekki et al. (2014)** and **Chen et al. (2013)** indicated correlations between total polyphenols content and antioxidant activity in garlic, onion and other vegetable.

CONCLUSION

The total polyphenols content, total sulfur content and antioxidant activity in studied varieties of garlic (Mojmír, Záhorský, Lukan and Makoi), grow in locality Bardejov were comparable with literature. The statistically significant differences in the total polyphenols content, total sulfur content and antioxidant activity were detected in the studied varieties of garlic. The highest value of total polyphenols content, total sulfur content and antioxidant activity was measured in variety Mojmír. The lowest value of total polyphenols content was determined in Lukan and the lowest level of total sulfur content and antioxidant activity was measured in variety Makoi. The coefficient of

correlation confirmed strong dependency between the total content of polyphenols, total sulfur content and the antioxidant activity. The bioactive components of garlic are mainly responsible for the healing properties. The claimed health benefits of chemical constituents present in garlic that treat various disorders have been investigated in both in animals and humans.

REFERENCES

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

Bayili, R. G., Abdoul-Latif, F., Kone, O. H., Diao, M., Bassole, I. H. N., Dicko, M. H. 2011. Phenolic compounds and antioxidant activities in some fruits and vegetables from Burkina Faso. *African Journal of Biotechnology*, vol. 10, no. 62, p. 13543-13547.

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, p. 753-759. https://doi.org/10.1590/S1516-89132005000600011

Benkeblia, N., Lanzotti, V. 2007. Allium Thiosulfinates: chemistry, biological properties and their potential utilization in food preservation. *Food*, vol. 1, no. 2, p. 193-201.

Bisen, P. S., Emerald, M. 2016. Nutritional and Therapeutic Potential of Garlic and Onion (*Allium* sp.). *Current Nutrition & Food Science*, vol. 12, no. 3, p. 190-199. https://doi.org/10.2174/1573401312666160608121954

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. https://doi.org/10.1016/S0023-6438(95)80008-5

Boonpeng, S., Siripongvutikorn, S., Sae-Wong, Ch., Sutthirak, P. 2014. The antioxidant and anti-cadmium toxicity properties of garlic extracts. *Food Science & Nutrition*, vol. 2, no. 6, p. 792-801. https://doi.org/10.1002/fsn3.164 PMid:25493198

Capasso, A. 2013. Antioxidant action and therapeutic efficacy of *Allium sativum* L. *Molecule*, vol. 18, no. 1, p. 690-700. https://doi.org/10.3390/molecules18010690 PMid:23292331

Chekki, R. Z., Snoussi, A., Hamrouni, I., Bouzouita, N. 2014. Chemical composition, antibacterial and antioxidant activities of Tunisian garlic (*Allium sativum*) essential oil and ethanol extract. *Mediterranean Journal of Chemistry*, vol. 3, no. 4, p. 947-956. https://doi.org/10.13171/mjc.3.4.2014.09.07.11

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 One*, vol. 8, no. 11, p. e79730. https://doi.org/10.1371/journal.pone.0079730 PMid:24232741

Choi, S., Cha, H. S., Lee, Y. S. 2014. Physicochemical and antioxidant properties of black garlic. *Molecules*, vol. 19, no. 10, p. 16811-16823. https://doi.org/10.3390/molecules191016811 PMid:25335109

Dalaram, I. S. 2016. Content of total polyphenols and antioxidant activity in varieties of onion and garlic. *Potravinárstvo Slovak Journal of Food Science*, vol. 10, no. 1, p. 444-451. https://doi.org/10.5219/658

Kavalcová, P., Bystrická, J., Tomáš, J., Karovičová, J., Kuchtová, V. 2014. Evaluation and comparison of the content of total polyphenols and antioxidant activity in onion, garlic and leek. *Potravinarstvo Slovak Journal of Food Science*, vol. 8, no. 1, p. 272-276. https://doi.org/10.5219/394

Lachman, J., Proněk, D., Hejtmanková, A., Dudjak, J., Pivec, V., Faitová, K. 2003. Total polyphenol and main flavonoid antioxidant in different onion (*Allium cepa* L.) varieties. *Horticultural Science*, vol. 30, no. 4, p. 142-147. https://doi.org/10.17221/3876-HORTSCI

Lachowicz, S., Kolniak-Ostek, J., Oszmiański, J., Wiśniewski, R. 2016. Comparison of Phenolic Content and Antioxidant Capacity of Bear Garlic (*Allium ursinum* L.) in Different Maturity Stages. *Journal of Food Processing and Preservation*, vol. 41, no. 1, p. 95-129.

Lenková, M., Bystrická, J., Vollmannová, A., Tóth, T., Kovarovič, J. 2017. Evaluation and comparison of the content of total polyphenols and antioxidant activity in garlic (*Allium sativum* L.). *Potravinárstvo Slovak Journal of Food Science*, vol. 11, no. 1, p. 65-70.

Mahmutovic, O., Mujic, E., Toromanovic, J., Mustovic, F., Muradic, S., Huseinovic, S., Sofic, E. 2009. Comparative analysis of total phenols and sulfur content in some plant organs of ramsons and two garlic species. *Planta Medica*, vol. 75, p. 43. https://doi.org/10.1055/s-0029-1234522

Mahmutovic, O., Tahirovic, I., Copra, A., Karic, L. 2014. Correlation of Total Secondary Sulfur Compounds, Total Phenols and Antioxidant Capacity in the Ramsons and Garlic. *British Journal of Pharmaceutical Research*, vol. 4, no. 23, p. 2662-2669. https://doi.org/10.9734/BJPR/2014/13977

Manjunathagowda, D. C., Gopal, J., Archana, R., Asiya, K. R. 2017. Virus-Free Seed Production of Garlic (*Allium sativum* L): Status and Prospects. *International Journal of Current Microbiology and Applied Sciences*, vol. 6, no. 6, p. 2446-2456.

Martins, N., Petropoulos, S., Ferreira, I. C. 2016. Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review. *Food Chemistry*, vol. 15, no. 211, p. 41-50. https://doi.org/10.1016/j.foodchem.2016.05.029

Mills, S., Bone, K., Phyto, D. 2005. *The Essential Guide to Herbal Safety*. 1st ed. USA : Elsevier Churchill Livingstone, p. 704. ISBN 9870443071713.

Mitek, M., Gasik, A. 2007. Polyphenols in food. The antioxidant properties. *Przemysł Spozywczy*, vol. 9, p. 36-44.

Muradic, S., Karacic, D., Mahmutovic, O., Mutovic, F., Sofic, E., Kroyer, G. 2010. Total sulphur and organosulphur and organosulphur compounds in garlic and ramsons plant organs at the end of vegetative period. *Planta Medica*, vol. 76, p. 292. https://doi.org/10.1055/s-0030-1264590

Narendhirakannan, R. T., Rajeswari, K. 2010. In vitro antioxidant properties of three varieties of *Allium sativum* L. extracts. *Journal of Chemistry*, vol. 7, p. 573-579.

Osman, S. A. M., Ata, A. T. M., El-Hak, S. E.H. G. 2007. Morphological, germination, bolting and cytogenetical characteristics of fourteen promising garlic genotypes. *African Crop Science Society*, vol. 8, p. 2005-2012.

Oszmianski, J., Kolniak-Ostek, J., Wojdyło, A. 2013. Characterization and content of flavonol derivarives of *Allium ursinum* L. plant. *Journal of Agricultural and Food Chemistry*, vol. 61, no. 1, p. 176-184. https://doi.org/10.1021/jf304268e PMid:23249145

Rai, Ch., Bhattacharjee, S., Nandi, N., Bhattacharyya, S. 2015. Influence of blanching on antioxidant and antimicrobial activities of raw garlic (*Allium sativum*). *Indo American*

Journal of Pharmaceutical Sciences, vol. 2, no. 6, p. 1071-1076.

Ramkissoon, J. S., Mahomoodally, M. F., Ahmed, N., Subratty, A. H. 2012. Relationship between total phenolic content, antioxidant potencial, and antiglycation abilities of common culinary herbs and spices. *Journal of Medical Food*, vol. 15, no. 12, p. 1116-1123. https://doi.org/10.1089/jmf.2012.0113 PMid:23134460

Sayin, F. K., Alkan, S. B. 2015. The effect of pickling on total phenolic content and antioxidant activity of 10 vegetables. *Journal of Food and Health Science*, vol. 1, no. 3, p. 135-141. https://doi.org/10.3153/JFHS15013

Šapčanin, A., Jancan, G., Pazalja, M., Kresic, D., Pehlic, E., Uzunovic, A. 2013. Determination of total sulphur content in biological samples by using high performance ion chromatography and elemental analysis. *Bulletiin of the Chemists and Technologists of Bosnia and Herzegovina*, vol. 41, p. 11-14.

Tattelman, E. 2005. Health Effects of Garlic. *American Family Physician*, vol. 72, no. 1, p. 103-106.

Tsao, R. 2010. Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients*, vol. 2, no. 12, p. 1231-1246. https://doi.org/10.3390/nu2121231 PMid:22254006

Ugwu, C. E., Suru, S. M. 2016. The Functional Role of Garlic and Bioactive Components in Cardiovascular and Cerebrovascular Health: What We do Know. *Journal of Biosciences and Medicines*, vol. 4, no. 10, p. 28-42. https://doi.org/10.4236/jbm.2016.410004

Zakarova, A., Seo, J. Y., Kim, H. Y., Kim, J. H., Shin, J. H., Cho, K. M., Lee, Ch. H., Kim, J. S. 2014. Garlic sprouting is associated with increased antioxidant activity and concomitant changes in the metabolite profile. *Journal of Agricultural and Food Chemistry*, vol. 62, no. 8, p. 1875-1880. https://doi.org/10.1021/jf500603v PMid:24512482

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IN VITRO ANTIOXIDANT POTENTIAL AND INHIBITORY EFFECT OF HYDRO-ETHANOLIC EXTRACT FROM AFRICAN BLACK VELVET TAMARIND (*DIALIUM INDIUM*) PULP ON TYPE 2 DIABETES LINKED ENZYMES

Olakunle Bamikole Afolabi, Omotade Ibidun Oloyede, Abiodun Ayodele Ojo, Amos Adeyinka Onasanya, Shadrach Oludare Agunbiade, Bashir Olaitan Ajiboye, Johnson Jonathan, Omolara Abosede Peters

ABSTRACT

The alarming rate of diabetes mellitus (DM) globally is bothersome and has drawn the search light of researchers on naturally endowed phytonutrients being an alternative in managing the menace. Therefore, the current study was designed to investigate some antioxidant parameters embedded in the extract of *Dialium indium* (*DI*) fruit pulp and also, to elucidate its antidiabetic potentials through the inhibition of two key carbohydrate-metabolizing enzymes such as α-amylase and intestinal α-glucosidase. Hydro-ethanolic extract of *DI* fruit pulp was used for the antioxidants and enzyme inhibitory bioassays through various convectional antioxidant assay methods *in vitro*. In the results, total phenolic content of the extract had; 6.74 ±3.38 mg GAE.g^{-1}, total flavonoid contents; 0.02 ±0.01 mg QE.g^{-1} and FRAP; 0.84 ±0.47 mg AAE.g^{-1} dried sample. Also, there was a marked significant (p <0.05) difference observed in the inhibition of α-amylase and intestinal α-glucosidase by the different concentrations of the extract used in concentration-dependent manner with their different EC_{50}. The inhibition demonstrated against these two carbohydrate metabolizing enzymes possibly could be through the embedded antioxidant potentials of the fruit pulp and this if properly harnessed, it could be helpful in the management of type 2 diabetes.

Keywords: Type 2 Diabetes; α-amylase; *Dialium indium*; α-glucosidase; antioxidant

INTRODUCTION

Type 2 diabetes (T2D) is a common and chronic disorder caused by impaired cellular carbohydrate metabolism characterized by elevated serum glucose level **(Beverley and Eschwège, 2003)**. Pancreatic beta-cell damage has been major cause of this disorder, leading to failure of β-cells to appropriately secrete insulin **(Porte and Kahn, 2001)**. Extremely elevated glucose level, if not treated could induce the mitochondria and non- mitochondria generation of free radicals **(Oyedemi et al., 2017)**, that subsequently could lead to damage by the free radicals in various tissues **(Afolabi et al., 2018a; Nishikawa et al., 2000; Dave and Kalia, 2007)**. The prolonged exposure of pancreatic β-cells to these reactive oxidative species, utters insulin genetic expression and insulin production as a result of little antioxidant enzymes' capacity **(Robertson and Harmon, 2007)**. Other innately to disease factors namely; hyperinsulinemia, insulin resistance, damaged insulin production, declined insulin mediated reducing sugar absorption and metabolism have been evident as the cause of this difference between blood glucose intake and pancreatic insulin production **(Gruenwald et al., 2010)**. However, there are indications that, environmental and lifestyle factors also are of major considerations in the cause of T2D **(Zimmet et al., 2001)**. T2D is more prevalent than type 1 diabetes and rapidly growing globally with accounts for over ninety-five percent of the world diabetic population **(Inga et al., 2008)**. Regulation of postprandial hyperglycemia level is important in delaying or preventing T2D **(Madhusudhan and Kirankumar, 2015)**, however, therapeutic approach is a common strategy for decreasing postprandial hyperglycemia in T2D patients, through which there is inhibition of sugar metabolizing enzymes such as alpha-glucosidase and alpha-1,4-glucan-4-glucanohydrolases in the gastrointestinal tract (GIT) **(Krentz and Bailey, 2005; Shim et al., 2003)**. These inhibitions cause reduction in the carbohydrate digestion process and subsequently lessening the postprandial plasma glucose rise **(Rhabasa-Lhoret and Chiasson, 2004)**. The choice pharmaceuticals

African black velvet tamarind (*Dialium indium*) pulp.

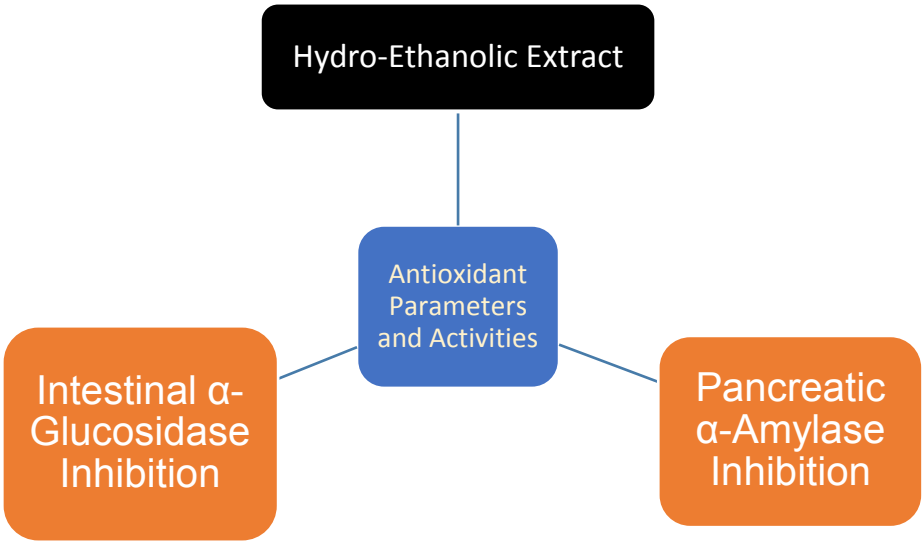


Figure 1 Schematic diagram of the manuscript (Graphical abstract).

in managing diabetes which has taken the highest trend have been reported to have side effects **(Stein et al., 2013; Singh et al., 2007; Hung et al., 2012)**, likely to be low-sugar level, diarrhea, gassiness, and bowel swelling and these have reduced their application in managing T2D. Alpha-amylases and alpha-glucosidases inhibition have been demonstrated by plants' naturally enriched secondary metabolites with promising effects than many commercially available α-glucosidase inhibitors and starch blockers (α-amylase inhibitors) used in the management of T2D **(Jalalpure et al., 2004; Hilary et al., 1998; Erasto et al., 2005)**. These plants are characteristically endowed with polyphenolic compounds, with the ability to interact with proteins, inhibit enzymatic activity, boost the immune system and causes inhibition of pathogenesis in the disease condition and as well has a terminal effect on pathogenic agents **(Afolabi and Oloyede, 2014; McCue et al., 2005; Dawra et al., 1988)**.

Dialium indium (*DI*) commonly known as black velvet tamarind (Figure 2) is among the list of plants that exhibit this natural biological response against infectious and non-infectious diseases. The current work was put together to study *in vitro* the antioxidant potentials, alpha-amylase and alpha-glucosidase inhibitory ability of combined solvent extract (Water: Ethanol, 70:30) of *DI* pulp commonly eaten in Southeastern part of Nigeria **(Nwaukwu and Ikechi, 2012)**.

Scientific hypothesis

If different mg.mL^{-1} of the same extracts is in the future subjected to the same assays as found in the current work, it could be scientifically assumed that same trend would be achieved, only if the same methods and procedures are engaged.

MATERIAL AND METHODOLOGY

Chemicals and Reagents

Chemicals and reagents used such as thiobarbituric acid (TBA), gallic acid, Folin-coicalteau's reagent, intestinal α-glucosidase (EC 3.2.1.20), pancreatic α-amylase (EC 3.2.1.1), *p*-nitrophenyl-α-D-glucopyranose (PNPG), Sodium carbonate, Aluminum chloride ($AlCl_3$), $FeCl_2$ (Iron II chloride), Sodium hydroxide (NaOH) and Potassium ferricyanide were used with 1,1-diphenyl-2-picrylhydrazyl (DPPH), Trichloroacetic acid (TCA) sourced from Sigma-Aldrich, Inc. (St Loius, MO). All these and other chemicals used were of analytical grades and prepared in all-glass apparatus using sterilized distilled water.

Sample collection and preparation

Dialium indium (*DI*) fruits were obtained from a popular place in Ikare Akoko, Ondo State, Nigeria. The voucher sample was dropped at the herbarium of the Department of Plant Science in Ekiti State University, Nigeria. where it was authenticated. Thereafter, the dried sample was blended and kept at moderate temperature.

Figure 2 Different diagrams of African black velvet tamarind (*Dialium indium*).

Preparation of 70% hydro-ethanolic of *DI* pulp

The blended *DI* fruit pulps were air-dried to a constant weight at 37 ±2 °C, about 120 g of the sample was weighed and soaked in 70% ethanol for 72 h. Thereafter, the mixture was processed and the extract concentrated at 50 ^{o}C to dryness.

Antioxidant Content assays

Total phenolic content assay (TPC)

Hydro-ethanolic extract of *DI* pulp was used to determined TPC using the method of **Singleton et al. (1999)**. Appropriate volume of the extract was added to 10% folin-ciocalteau's solution and Sodium carbonate (anhydrous). The mixture was later incubated at 45 °C for 40 min. The absorbance was read at 700 nm.

Total flavonoid content assay (TFC)

Total flavonoid content of hydro-ethanolic of *DI* pulp was determined according to the method of **Bao et al. (2005)**. Appropriate volume of the extract was added to 5% sodium nitrate at zero time. 5 min later, $AlCl_3$ was added and after 6 min, NaOH was added followed by the addition of distilled water. The absorbance of the mixture was taken at 510 nm.

Antioxidant Activity Assays

Ferric reducing antioxidant power (FRAP)

The ferric reducing power of hydro- ethanolic of *DI* pulp was carried out using **Pulido et al. (2000)**. Appropriate volume of the extract was mixed with 200 mM of sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50 ^{o}C for 20 min, thereafter 250 µL of 10% trichloroacetic acid was added and centrifuged at 2000 rpm for 10 minutes, 250 µL of one percent $FeCl_2$ was added to appropriate volume of the supernatant and the absorbance taken in the spectrophotometer at 700 nm.

1, 1 diphenyl, 2-picrylhyrazine (DPPH) radical scavenging ability assay

Inhibitory effect of hydro- ethanolic extract of *DI* pulp was carried out on DPPH by **Gyamfi et al. (1999)**. The same proportion of the extract and methanolic solution of the DPPH was left in the dark in tubes for 30 min and the absorbance taken in the spectrophotometer at 516 nm.

Hydroxyl radical scavenging assay

Hydro- ethanolic extract of *DI* pulp inhibitory potential against degradation of deoxyribose was determined by method described by **Halliwell and Gutteridge (1981)**. Appropriate dilution of the extract was added to a reaction mixture containing 20 mM deoxyribose, 0.1 M phosphate buffer (pH 7.4), 20 mM hydrogen peroxide and 500 µM $FeSO_4$ and this was made up to 800 µL with distilled water. The reaction was incubated at 37 ^{o}C for a while, and the reaction stopped by the addition of 2.8% trichloroacetic acid, then TBA solution as the colour forming agent. The reaction was boiled for 20 min and the absorbance taken in the spectrophotometer at 532 nm.

Nitric oxide radical scavenging ability

Inhibitory effect of *DI* pulp hydro-ethanolic extract on $NO^{\cdot}$ radical was carried out according to the method of **Mercocci et al. (1994)**. An appropriate volume of the extract was incubated with 25 mM Sodium nitroprusside solution and subsequently incubated for 2 h at room temperature. 500 µL of the incubated mixture was added to 300 µL of Griess solution. The absorbance of the mixture was read in the spectrophotometer at 570 nm and the results expressed as percentage radical scavenging ability.

Enzymes inhibitory Assays

Pancreatic alpha-amylase (EC 3.2.1.1) inhibitory in vitro assay

Inhibitory potential of hydro-ethanolic extract of *DI* pulp against amylase was carried out according to the method of **(Shai et al., 2010)**. Different volumes of the Hydro-alcoholic extract were added to an appropriate volume of the enzyme (2 $U.mL^{-1}$) in 0.1 M Sodium phosphate buffer (pH 6.8) and incubated at 37 °C for 20 min. 1% soluble starch in 0.1 M Sodium phosphate buffer (pH 6.8) was added to the reaction mixture and incubated at 37 °C for 1 h. 1 µL of 3.5 DNSA reagent was added to the reaction mixture boiled for 10 min. The absorbance of the test was taken in the spectrophotometer at 540 nm.

Intestinal alpha-glucosidase (EC 3.2.1.20) inhibitory in vitro assay

Inhibitory potential of hydro-ethanolic extract of *DI* pulp against glucosidase was carried out according to the

method of **Ademiluyi and Oboh (2013)**. Diluted volumes of the extract were added to alpha-glucosidase ($1U.mL^{-1}$) solution in 0.1 M Sodium phosphate buffer (pH 6.8) at 37 °C for 15 min. Thereafter, pNPG solution (0.005 M) in 0.1 M Sodium phosphate buffer (pH 6.8) was added and the mixture was kept at room temperature for 20 min. The absorbance of the *p*-nitro phenol released was measured in the spectrophotometer at 405 nm.

Determination of IC_{50} /EC_{50}.

Determination of IC_{50} / EC_{50} values was carried out from the plot of percentage inhibition caused by the various concentrations of the extract against different concentrations of the extract concentrations. The IC_{50} / EC_{50} was then calculated using a linear regression curve.

Statistical analysis

All experiments were carried out in duplicate. Data were expressed as mean ±standard deviation (SD). Differences were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple test **(Zar, 1984)**. Significance was accepted at p-value < 0.05.

RESULTS

Antioxidant Contents

Table 1. Shows the possible antioxidant contents in the hydro-ethanolic extract from *DI* pulp. The total phenolic content revealed significant (p <0.05) in value (mg $GAE.g^{-1}$ of the dried sample) higher than both the total flavonoid (mg $QE.g^{-1}$ of the dried sample).

Antioxidant Activities

Table 1 Shows ferric reducing potential antioxidant activity (mg Ascorbic acid equivalent/g of the dried sample) of the hydro-ethanolic extract from *DI* pulp.

The Figure 3. shows the DPPH inhibitory ability of hydro-ethanolic extract of *DI* pulp in various concentrations. The results indicated significant (p <0.05) difference in the various concentrations considered ranging from 30 – 150 $\mu g.mL^{-1}$ in concentration dependent manner.

The Figure 4shows inhibitory potential of hydro-ethanolic extract of *DI* pulp against deoxyribose degradation in the presence of Fe^{2+}/H_2O_2 and it reveals that, the inhibition was in dose dependent manner with significant (p <0.05) difference.

The Figure 5 shows the inhibitory ability of hydro-ethanolic extract of *DI* pulp against nitric oxide with different concentrations that were varied from 30 – 150 $\mu g.mL^{-1}$. It shows significant (p <0.05) increase in concentration dependent manner with the IC_{50} of the various concentrations shown in Table 2.

Enzymes inhibition

Figure 6 shows pancreatic α- amylase inhibitory potential of hydro-ethanolic extract of *DI* pulp considering various concentrations in $mg.mL^{-1}$. The result revealed significant (p <0.05) difference in the level of inhibition of the extract in concentration dependent manner.

Figure 7 shows the inhibitory potential of hydro-ethanolic extract of *DI* pulp against the activity of intestinal α-glucosidase in various concentrations considered in $mg.mL^{-1}$. The results revealed significant (p <0.05) difference in the level of inhibition of the extract in concentration dependent manner.

DISCUSSION

The timely Monitoring of postprandial long-term blood sugar is so important in the management of diabetes, as all forms of diabetes have very serious implications on human health **(Leszek et al., 2014)**. Recently, inhibitors of

Table 1 The results for total phenolic contents, total flavonoid and ferric reducing property (FRAP) of *DI* pulp hydro-ethanolic extract.

Total Phenolic (mg $GAE.g^{-1}$)	Total Flavonoid content (mg $QE.g^{-1}$)	Ferric reducing power (mg $AAE.g^{-1}$)
6.74 ±3.38	0.02 ±0.01	0.84 ±0.47

The results were expressed as mean values ±SD (n=2).

Table 2 The IC_{50} values in $\mu g.ml^{-1}$ (Concentration of the extracts that will cause 50 percent inhibition) of hydro-ethanolic extract of *DI* pulp were calculated from a linear regression curve of the percentage (%) inhibitions against various concentrations of the extracts.

Inhibitory concentration (IC_{50})	
DPPH Radical Scavenging	179.08 ±1.66
Nitric oxide scavenging ability (%)	96.78 ±0.01
Hydroxyl radical scavenging ability	362.05 ±0.01

Results represent mean values of duplicated sample ±SD, n = 2. Various concentrations were used for the assay to determine the $IC_{50.}$

Table 3. The **EC_{50}** values in $\mu g.ml^{-1}$ (Concentration of the extracts that will cause 50 percent inhibition of the enzyme) of hydro- ethanolic extract of *DI* pulp.

Enzyme	α-Amylase	α- glucosidase
EC_{50}	0.52 ±0.06	0.45 ±0.02

The results were expressed as mean values of duplicated sample ±SD, n = 2.

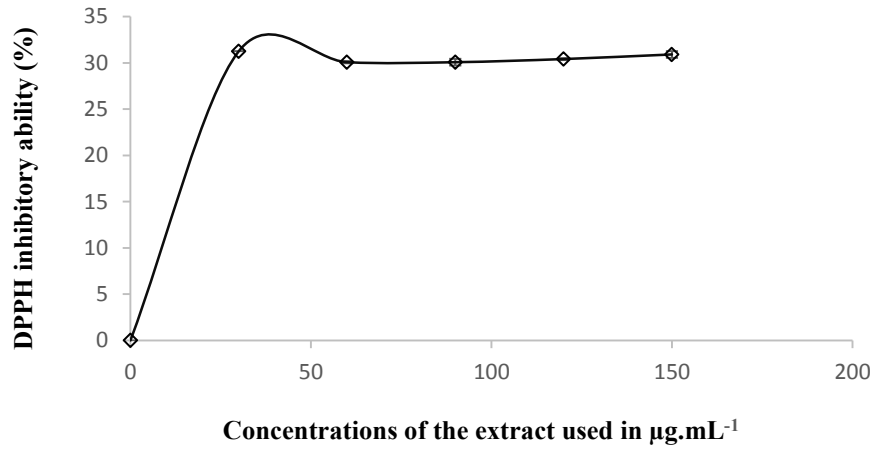


Figure 3 DPPH• radical scavenging ability of hydro-ethanolic extract of *DI* pulp.

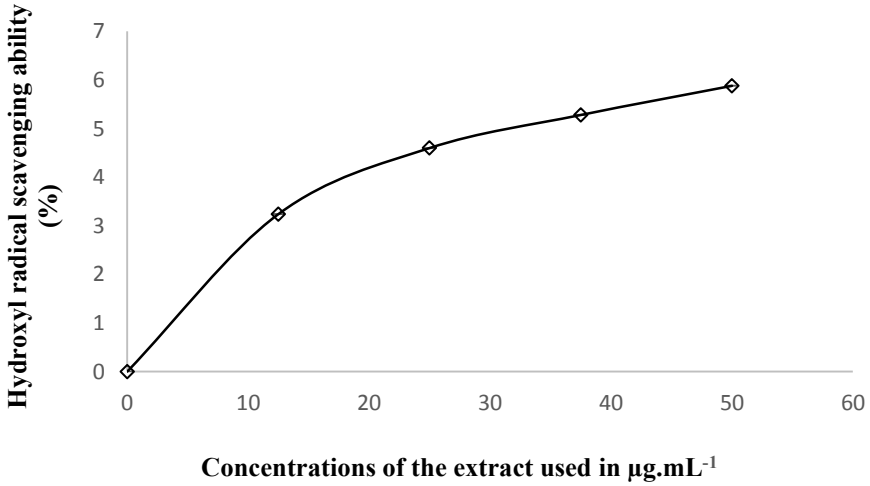
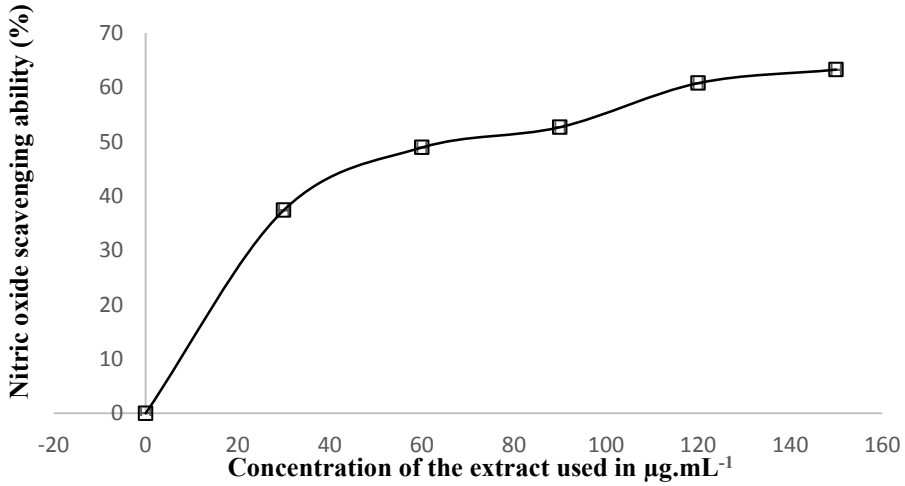


Figure 4 Hydroxyl radical scavenging ability (%) hydro-ethanolic extract of *DI* pulp.



carbohydrate-metabolizing enzymes have been considered to be important in monitoring diabetes mellitus most especially in people living with T2D **(Krentz and Bailey 2005)**. More also, the inhibitory and ameliorating potentials exhibited in the management of diabetes by plants with medicinal values have been credited to the presence naturally endowed polyphenols, which are considered to be potential antioxidants due to the reduction- oxidation properties of their hydroxyl groups **(Afolabi and Oloyede, 2014; Materska and Perucka 2005)**. These plant secondary metabolites fight against generation/proliferation of metabolically induced free radicals which are potential causative agents involve in diabetes. They are readily available in plants in isomeric forms of flavonoids and phenolic acids **(Oberley 1988; Bravo, 1998)**. Most recent studies have implicated flavonoids to have shown inhibition against ROS generation **(Jo et al., 2009)**.

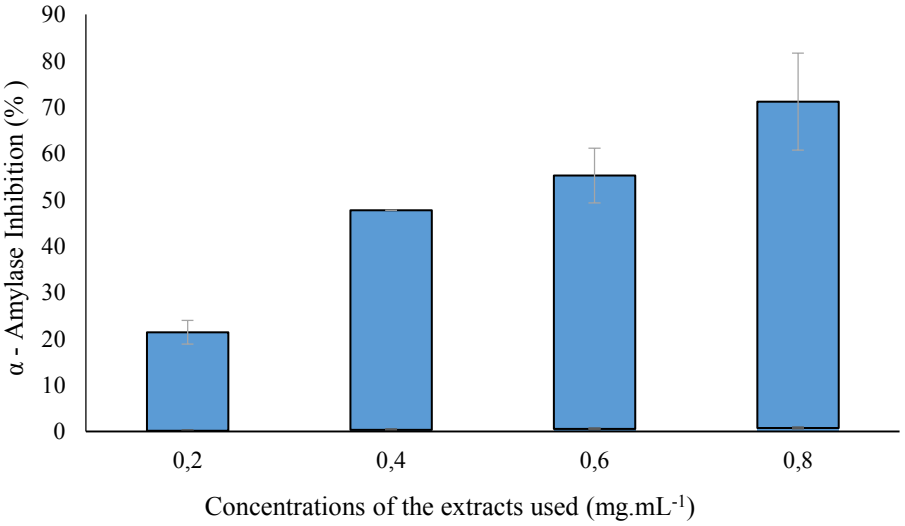


Figure 6 The inhibitory potential of hydro-ethanolic extract of *DI* pulp against the activity of Pancreatic *α*-amylase.

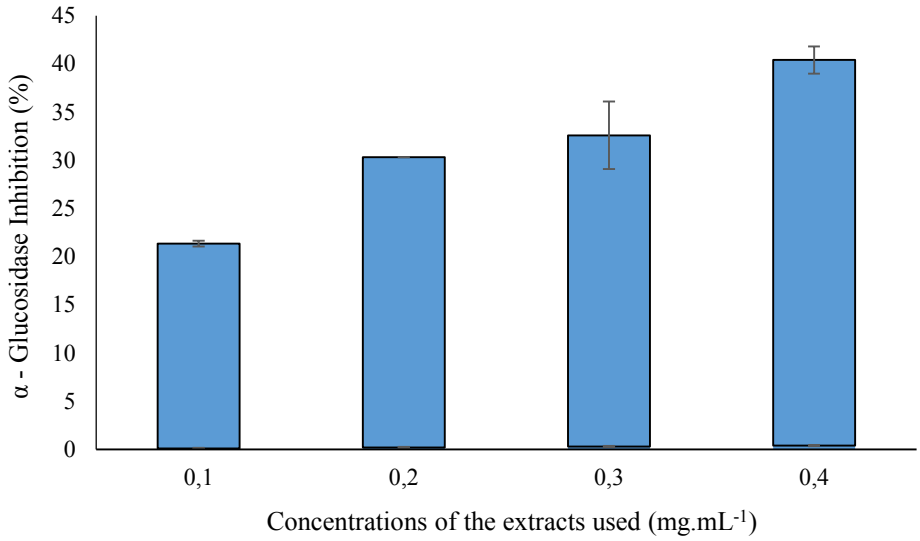


Figure 7 The inhibitory potential of hydro-ethanolic extract of *DI* pulp against the activity of intestinal *α*-glucosidase.

In the current finding, from Table 1, phytonutrients components in the hydro-ethanolic extract of *DI* pulp were revealed, total phenolic content showed predominant value
over total flavonoid and ferric reducing power (FRAP) contents, the reason behind this could not be clearly explained, but it could be attributed to the antioxidant properties embedded in the extract as shown in table 1, however, the finding was in agreement with the result of a **Inglett and Chen (2011)**, that showed high value of phenolic than flavonoid content in the pulp extract of *Synsepalum dulificum* **Ziping et al. (2009)**, their results revealed higher polyphenolic content in the extracts of different *Ziziphus jujube* cultivars. However, in Figure 3 – 5, the ability of the hydro-ethanolic extract of *DI* pulp to inhibit the generation of free radicals were clearly shown. In Figure 3 the antioxidant potential of the extract was demonstrated with the IC_{50} represented in Table 2. Several studies have shown the generation of free radicals to be underlying mechanism of action of some commonly available diabetogenic agents **(Afolabi et al., 2018)**. Antioxidant enriched extracts have been reported to play a pivotal role in the management of Diabetes by scavenging the various ROS generated in the disease. Similarly, as shown from the Figure 4, degradation of deoxyribose inhibitory ability of the hydro-alcoholic extract of *DI* pulp, in the presence of Fe^{2+}/H_2O_2 exhibited percentage inhibitory rise in concentration dependent manner, the mechanism for the inhibiton has not been reported in the current work but, it could be attributed to the antioxidant capacity of the extract as shown in Table 1. In the same vein, Figure 5, reveals the inhibitory potential of *DI* pulp against nitric oxide radical**,** the involvement of $NO^{\bullet}$ radical has been implicated in various diseases through the combination with superoxide radical ($O_2^{\bullet-}$) generated through Fenton reaction that results in the generation of peroxynitrite ($ONOO^-$), a potential cytotoxic molecule, through which oxidation can damage cellular proteins **(Obafemi et al., 2016)**. In the result, all the concentrations of the extract examined caused noticeable changes in the inhibition against the latent production of peroxynitrite. The underlying reason(s) for the several evident inhibitory

potentials of *DI* pulp against oxidant species is/are not known but it could be credited to the presence of quantifiable antioxidant contents present in the hydro-alcoholic extract of *DI* pulp mainly the phenolic acid compound as revealed in Table1.

To fully establish the antidiabetic effects of hydro-ethanolic extract of *DI* pulp, inhibitory potentials of the extract against enzymes involved carbohydrate metabolism were appraised. It has been reported that plants that are rich in secondary metabolites (i. e polyphenols) are potential inhibitors of α-amylases and α-glucosidases **(Afolabi et al., 2018b; Kwon et al., 2007)**. Therefore, pancreatic *α*- amylase and intestinal alpha-glucosidase inhibitions are shown in the Figure 6 and Figure 7 respectively. In the Figures, it was clearly shown that hydro-alcoholic extract of *DI* exhibited inhibitory potentials in all the concentrations examined against these metabolizing enzymes. Apparently, it could be deduced that, the inhibitory potentials shown against this carbohydrate-hydrolyzing enzymes could be attributed to the fact that, the extract exhibited considerable phenolic content, as revealed in the Table 1.

CONCLUSION

In the current study, inhibitory potential on the carbohydrate-hydrolyzing enzymes involved in T2D has apparently been so demonstrated by the hydro-alcoholic extract of DI pulp, this possibly could have been as a result of the embedded antioxidant contents through the free radicals scavenging ability. Howbeit, the possible mechanism (s) of most antidiabetic drugs is/are to target delay in carbohydrate break down process and to cause prolonged overall carbohydrate metabolism time frame, thereby reducing the activities of carbohydrate-metabolizing enzymes and rate of glucose absorption. Thus, the pulp could be suggested helpful as an alternative in the management of T2D when incorporated in diet.

REFERENCES

Ademiluyi, A. Oboh, G. 2013. Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (*α*-amylase and *α* -glucosidase) and hypertension (angiotensin I converting enzyme) *in vitro*. *Experimental and Toxicologic Pathology*, vol. 65, p. 305-309. https://doi.org/10.1016/j.etp.2011.09.005 PMid:22005499

Afolabi O. B, Oloyede O. I, Obajuluwa A. I, Adewale O. B, Olayide I. I, Balogun B. D, Fadugba A. 2018a. Antioxidant properties and inhibitory potentials of different solvent-fractions of *Dialium indium* pulp (African Black velvet tamarind) against Fe^{2+}- induced oxidative stress in rat tissues – *In vitro*. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 70-78. https://doi.org/10.5219/825

Afolabi, O. B., Oloyede, O. I. 2014. Antioxidant properties of the extracts of *Talinum triangulare* and its Effect on Antioxidant enzymes in tissue homogenate of swiss albino rat. *Toxicology International*, vol. 21, no. 3, p. 307-313. https://doi.org/10.4103/0971-6580.155377 PMid:25948971

Afolabi, O. B., Oloyede, O. I., Olayide, I. I., Obafemi, T. O., Awe, J. O., Afolabi, B. A., Onikani, S. A. 2015. Antioxidant enhancing ability of different solvents extractable components of *Talinum traingulare* in some selected tissue homogenates of albino rat-i*n vitro*. *Journal of Applied Pharmaceutical Science*, vol. 5, no. 9, p. 56-61. https://doi.org/10.7324/JAPS.2015.50911

Afolabi, O. B., Oloyede, O. I., Agunbiade, S. O. 2018b. Inhibitory potentials of phenolic-rich extracts from Bridelia ferruginea on two key carbohydrate-metabolizing enzymes and Fe2+-induced pancreatic oxidative stress. *Journal of Integrative Medicine*, Epub ahead of print.

Bao, J., Cai, Y., Sun, M., Wang, G., Corke, H. 2005. Anthocyanins, flavonols and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. *Journal of Agricultural and Food Chemistry*, vol. 53, no. 6, p. 2327-2332. https://doi.org/10.1021/jf048312z PMid:15769176

Beverley, B., Eschwège, E. 2003. The diagnosis and classification of diabetes and impaired glucose tolerance. In John, C. P. et al. *Textbook of Diabetes*. New Jersey : Wiley-Blackwell, p. 2.1-2.11. ISBN 978-0632059157.

Bravo, L. 1998. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, vol. 56, no. 11, p. 317-333. https://doi.org/10.1111/j.1753-4887.1998.tb01670.x PMid:9838798

Dave, G. S., Kalia, K. 2007. Hyperglycemia induced oxidative stress in type-1 and type-2 diabetic patients with and without nephropathy. *Cellular and Molecular Biology*, vol. 53, no. 5, p. 68-78. PMid:17543235

Dawra, R. K., Makkar, H. P. 1988. Singh B. Protein-binding capacity of micro quantities of tannins. *Analytical Biochemistry*, vol. 170, no. 1, p. 50-53. https://doi.org/10.1016/0003-2697(88)90088-7

Erasto, P., Adebola, P. O., Grierson, D. S., Afolayan, A. J. 2005. An ethnobotanical study of the plants used for the treatment of diabetes in the eastern Cape province, South Africa. *African Journal of Biotechnology*, vol. 4, p. 1458-1460.

Gruenwald, J., Freder, J., Armbruester, N. 2010. Cinnamon and health. *Critical Reviews in Food Science and Nutrition*, vol. 50, no. 9, p. 822-834. https://doi.org/10.1080/10408390902773052 PMid:20924865

Gyamfi, M. A., Yonamine, M., Aniya, Y. 1999. Free radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally induced liver injuries. *General Pharmacology*, vol. 32, no. 6, p. 661-667. https://doi.org/10.1016/S0306-3623(98)00238-9

Halliwell, B., Gutteridge, J. M. 1981. Formation of thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. FEBS *Letters*, vol. 128, no. 2, p. 347-352. https://doi.org/10.1016/0014-5793(81)80114-7

Hilary, K., Ronald, E. A., William, H. H. 1998. Global burden of diabetes 1995-2005. Prevalence, numerical estimates and projections. *Diabetes care*, vol. 21, no. 9, p. 141-143.

Hung, H. Y., Qian, K., Morris-Natschke, S. L., Hsu, C. S., Lee, K. H. 2012. Recent discovery of plant-derived anti-diabetic natural products. *Natural Product Reports*, vol. 29, no. 5, p. 580-606. https://doi.org/10.1039/c2np00074a PMid:22491825

Inga, P., Mark, I. M., Cecilia, M. L. 2008. Type 2 diabetes: new genes, new understanding. *Trends in Genetics*, vol. 24, no. 12, p. 613-621. https://doi.org/10.1016/j.tig.2008.09.004 PMid:18952314

Inglett, G. E., Chen, D. 2011. Contents of phenolics and flavonoids and antioxidant activities in skin, pulp, and seeds

of miracle fruit. *Journal of Food Science*, vol. 76, no. 3, p. 479-482. https://doi.org/10.1111/j.1750-3841.2011.02106.x PMid:21535817

Jalapure, S. S., Patil, M. B., Pai, A., Salahuddin, M. D. 2004. Anti-diabetics activity of *Cassia auriculata* seed in alloxan induced diabetes rats. *Nigerian Journal of Natural Products and Medicine*, vol. 8, p. 22-23.

Jo, S. H., Ka, E. H., Lee, H. S., Apostolidis, E., Jang, H. D., Kwon, Y. I. 2009. Comparison of antioxidant potential and rat intestinal α-glucosidases inhibitory activities of quercetin, rutin, and isoquercetin. *International Journal of Applied Research in Natural Products*, vol. 2, p. 52-60.

Krentz, A. J., Bailey, C. J. 2005. Oral antidiabetic agents: Current role in type 2 diabetes mellitus. *Drug*, vol. 65, no. 3, p. 385-411. https://doi.org/10.2165/00003495-200565030-00005

Kwon, Y. I., Apostolidis, E., Kim, Y. C., Shetty, K. 2007. Health benefits of traditional corn, beans and pumpkin: *In vitro* studies for hyperglycemia and hypertension management. *Journal of Medicinal Food*, vol. 10, no. 2, p. 266-275. https://doi.org/10.1089/jmf.2006.234 PMid:17651062

Leszek, C., László, B., Svetlana, B., Agata, B., Jan, B., Katarzyna C, Marek H., Andrej J., Mladen K., Nebojsa L., Emil M., Dario R., Gabriela R., Tsvetalina T., Tamás V., Bogumił W. and Nadia Z. 2014. Self-Monitoring of Blood Glucose in Diabetes: From Evidence to Clinical Reality in Central and Eastern Europe – Recommendations from the International Central-Eastern European Expert Group. *Diabetes Technology and Therapeutics*, vol. 16, no. 7, p. 460-475. https://doi.org/10.1089/dia.2013.0302 PMid:24716890

Madhusudhan, T., Kirankumar, H. 2015. In-vitro α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions. *Indian Journal of Pharmacology*, vol. 47, no. 4, p. 425-429. https://doi.org/10.4103/0253-7613.161270 PMid:26288477

Materska, M., Perucka, I. 2005. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruits (*Capsicum annuum* L.). *Journal of Agricultural and Food Chemistry,* vol. 53, no. 5, p. 1730-1758. https://doi.org/10.1021/jf035331k PMid:15740069

McCue, P., Kwon, Y. I., Shetty, K. 2005. Anti-diabetic and antihypertensive potential of sprouted and solid-state bioprocessed soybean. *Asia Pacific Journal of Clinical Nutrition*, vol. 14, no. 2, p. 145-152. PMid:15927931

Mercocci, L., Marguire, J. J., Droy-Lefaiz, M. T., Parker, L. 1994. The nitric oxide-scavenging properties of *Ginko biloba* extract EGB 761. *Biochemical and Biophysical Research Communications*, vol. 201, no. 2, p. 748-755. https://doi.org/10.1006/bbrc.1994.1764

Nishikawa, T., Edelstein, D., Du, X. L. 2000. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*, vol. 404, no. 6779, p. 787-790. https://doi.org/10.1038/35008121 PMid:10783895

Nwaukwu, I. A, Ikechi, N. C. 2012. Biochemical Changes Induced by the Effect of Six Pathogenic Fungi on *Dialium guineense*: Black Velvet Edible Fruit. *IOSR International Journal of Pharmacy and Biological Sciences*, vol. 2, no. 4, p. 20-24. https://doi.org/10.9790/3008-0242024

Obafemi, T. O., Onasanya, A., Olaoye, O. A., Afolabi, O.B., Awe, O. J, Anadozie, S. O., Ojo, O. A., Adedeji, E. 2016. *In vitro* Antioxidant Activity and High Performance Liquid Chromatography (HPLC) Analysis of methanolic extract of *Platycerium angolense. International Journal of Pharmacognosy and Phytochemical Research*, vol. 8, no.9, p. 1497-1502.

Oberley, L. W. 1988. Free radicals and diabetes. *Free Radical Biology and Medicine*, vol. 5, no. 2, p. 113-124. https://doi.org/10.1016/0891-5849(88)90036-6

Oyedemi, S. O., Oyedemi, B. O., Ijeh, I. I., Ohanyerem, P. M. E., Coopoosamy, R. M., Aiyegoro, O. A. 2017. Alpha-amylase inhibition and antioxidative Capacity of some antidiabetic plants used by the traditional healers in southeastern Nigeria. *The Scientific World Journal*, vol. 2017, p. 1-11. https://doi.org/10.1155/2017/3592491 PMid:28367491

Porte, D., Kahn, S. E. 2001. Beta-cell dysfunction and failure in type 2 diabetes: Potential mechanisms. *Diabetes,* vol. 1, p. 160-116. https://doi.org/10.2337/diabetes.50.2007.S160

Pulido, R., Bravo, L., Saura-Calixto, F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, vol. 48, no. 8, p. 3396-3402. https://doi.org/10.1021/jf9913458 PMid:10956123

Rhabasa-Lhoret, R., Chiasson, J. L. 2004. Alpha-glucosidase inhibition. In Defronzo, R. A. et al. *International textbook of diabetes mellitus*. 3rd ed. London, UK : John Wiley, 2070 p. ISBN 9780471486558.

Robertson, R. P., Harmon, J. S. 2007. Pancreatic islet β-cell and oxidative stress: the importance of glutathione peroxidase. *FEBS Letters*, vol. 581, no. 19, p. 3743-3748. https://doi.org/10.1016/j.febslet.2007.03.087 PMid:17433304

Shai, L. J., Masoko, P., Mokgotho, M. P., Magano, S. R., Mogale, A. M., Boaduo, N., Eloff, J. N. 2010. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in phalaborwa, South Africa. *South African Journal of Botany*, vol. 76, no. 3, p. 465-470. https://doi.org/10.1016/j.sajb.2010.03.002

Shim, Y. J., Doo, H. K., Ahn, S. Y., Kim, Y. S., Seong, J. K., Park, I. S., Min, B. H. 2003. Inhibitory effect of aqueous extract from the gal of *Rhuz chinesis* on alpha-glucosidase activities and postprandial blood glucose. *Journal of Ethnopharmacology*, vol. 85, no. 2-3, p. 283-287. https://doi.org/10.1016/S0378-8741(02)00370-7

Singh, S. K., Rai, P. K., Jaiswal, D., Watal, G. 2007. Evidence-based critical evaluation of glycemic potential of *Cynodon dactylon*. *Evidence-Based Complementary and Alternative Medicine*, vol. 5, no. 4, p. 415-420. https://doi.org/10.1093/ecam/nem044 PMid:18955211

Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In Packer, L. *Methods in enzymology. Oxidants and antioxidants* Part A. San Diego, California : Academic Press, p.152-178. ISBN 978-12-182200-2.

Stein, S. A., Lamos, E. M., Davis, S. N. 2013. A review of the efficacy and safety of oral antidiabetic drugs. *Expert Opinion on Drug Safety*, vol. 12, no. 2, p. 153-175. https://doi.org/10.1517/14740338.2013.752813 PMid:23241069

Zar, J. H. 1984. *Biostatistical analysis*. 2nd ed. USA : Prentice-Hall Inc, 718p. ISBN 9780130779250.

Zimmet, P., Alberti, K. G., Shaw, J. 2001. Global and societal implications of the diabetesepidemic. *Nature*, vol.

414, no. 6865, p. 782-787. https://doi.org/10.1038/414782a PMid:11742409

Ziping, X., Weihua, F., Jiankang, C., Dongdong, C., Weibo, J. 2009. Antioxidant activity and total phenolic contents in peel and pulp of Chinese *Jujube* (*Ziziphus jujuba* Mill) fruits. *Journal of Food Biochemistry*, vol. 33, no. 5, p. 613-629. https://doi.org/10.1111/j.1745-4514.2009.00241.x

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ANTIOXIDANT, COMPOSITIONAL EVALUATION AND BLOOD PRESSURE MODULATING POTENTIALS OF *BRYOPHYLLUM PINNATUM* (LAM.), *VISCUM ALBUM* (L.) AND *ARTOCARPUS ALTILIS* (PARKINSON) LEAVE EXTRACTS

Oluronke Ruth Osunlana, Muibat Olabisi Bello, Jonathan Abidemi Johnson, Olakunle Bamikole Afolabi

ABSTRACT

Bryophyllum pinnatum (Lam.), *Viscum album* (L.) and *Artocarpus altilis* (Parkinson) are medicinal plants widely used based on their ethnomedicinal properties in the regulation of blood pressure. This study was designed to evaluate the antioxidant activities and compositional constituents of these plants. The antioxidant potentials were analyzed using DPPH and FRAP assays, while Folin-Ciocalteu method was employed in the determination of the total phenolic antioxidant contents. Compositional analyses of the leave extracts were determined using Gas Chromatograghy-Mass Spectrophotometer (GC-MS). The total phenolic contents in *Bryophyllum pinnatum, Artocarpus altilis* and *Viscum album* were revealed as; 659.50 ±0.02, 1667.50 ±0.03, 1232.00 ±0.02 mg GAE.100 g^{-1} respectively. Considering the antioxidant activities, *Artocarpus altilis* leaf extract showed inhibitory activity on DPPH with IC_{50} of 2.24 ±0.26 $mg.mL^{-1}$, *Bryophyllum pinnatum* and *Viscum album* with IC_{50} values 3.63 ±0.07 and 4.65 ±0.06 $mg.mL^{-1}$ respectively.The FRAP in $mg.GAE^{-1}$ for *Artocarpus altilis*, *Bryophyllum pinnatum* and *Viscum album* revealed; 2505.20 ±0.04, 1561.80 ±0.01 and 1698.00 ±0.03 respectively. GC-MS identified some vital phenolic components and essential fatty acids in the plants. The findings therefore suggest that; the plants if properly utilized, it could serve as alternatives in regulating blood pressure.

Keywords: antioxidant activity; benzesterol; phenol-1, 3-Dodecanol; piscofuranine

INTRODUCTION

Occurrence of diseases have been increasing at a regular rate and claiming millions of lives in spite of the huge improvements in modern medicine all over the world. High blood pressure is one of such diseases. A disease of the heart and blood vessels that is defined as blood pressure persistently above 140/90 mmHg is called hypertension and persistently below 120/80 mmHg is called Hypotension. Hypertension is the most common cardiovascular disease and constitutes a major factor for several cardiovascular pathologies including atherosclerosis, coronary artery disease and renal insufficiency **(Chobanian et al., 2003; Lans, 2006)**. Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly great in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance **(Abubakar et al., 2010)**. In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs **(Borris,1996)**. Medicinal plants are resources of new drugs and many of the modern medicines are produced indirectly from plants. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons **(Hosseinzadeh et al., 2015)**.Various herbal plants have been used to combat hypertension.*Bryophylum pinnatum* (*B. pinnatum*), *Viscum album* (*V. Album*) and *Artocarpus altilis* (*A. Altilis*) are examples of the various medicinal plants basically used individually in ethno-medicine for the regulation of blood pressure and in the treatment of hypertension.

A. altilis belongs to the family, Moraceae. It is commonly referred to as breadfruit as it is similar to freshly baked bread. Breadfruit is a tropical fruit and the breadfruit tree produces fruits from March to June and from July to September **(Akanbi et al., 2009)**. *A. altilis* leaves have been reported to be used as anti-hypertensive drug **(Lans, 2006)**. The yellowing leaf is brewed into a tea and taken to reduce high blood pressure **(Orwa et al., 2009)**. *B. pinnatum* is an erect, succulent, perennial shrub that grows about 1.5 m tall and reproduces through seeds and also

Figure 1 *Bryophyllum pinnatum* (Lam.) leaf.

Figure 2 *Viscum album* (L.) leaves.

Figure 3 *Artocarpus altilis* leaves.

vegetatively from leaf bubils **(Okwu and Josiah, 2006)**. It belongs to the family, *Crassulaceae*. It has a tall hollow stems, freshly dark green leaves that are distinctively scalloped and trimmed in red and dark bell-like pendulous flowers **(Okwu and Josiah, 2006)**. *B. pinnatum* leaves have tested positive for antihypertensive activity **(Nwali et al., 2012)**. European mistletoe (V. *album*) is an evergreen, hemi-parasitic plant, normally found growing on a variety of trees, especially pine, poplar, apple trees, locust trees among others. It belongs to the family, *Moraceae*. *V. album* has been recognized for its use as anti-inflammatory, anti-diabetic and anti-hypertensive activities **(Sengul et al., 2009)**.

Despite the efficacy and wide usage of herbal plants, not all of the herbal plants reported to be useful are harmless **(Lans, 2006)**. Bioactive compounds derived from medicinal plants can be useful but might have serious dose-related side effects. Thus there is a need for compositional evaluation of herbal plants to see if their health benefit outweighs the risk of adverse effect and to detect if these plants contain other important bioactive compounds.

Scientific hypothesis

The correlation between antioxidant capacity, total phenolic content, unsaturated fatty acid content to hypertension is a little shady even though folk medicine practices uphold the correlation to be effective. From literature, 40 mg of Lisinopril, an active compound for hypertension control and regulation is the maximum allowed dose to be used, similarly, little amount of these leaves are needed to be consumed to provide the needed phenolic compounds and antioxidant compounds for blood pressure regulation and control in the estimated range of 0.024, 0.032 and 0.061 g for *A. altilis*, *V. album*, *B. pinnatum* leaves respectively for phenolic compounds and range 0.016, 0.024 and 0.026 g for *A. altilis*, *V. album*, *B. pinnatum* leaves respectively for antioxidant compounds for the regulation of blood pressure.

The aim of this research is to evaluate the plant constituent, total phenolic content and antioxidant power of the plant leaves.

MATERIAL AND METHODOLOGY

Material: All the chemicals and reagents used in this study were of analytical grade and were products of Sigma Aldrich, USA. The water used was glass distilled.

Sample collection and identification

Fresh samples of *A. altilis, B. pinnatum* and *V. album* leaves were collected from different locations in Nigeria. *A. altilis* was collected from Ajilosun area in Ado-Ekiti, Ekiti State. *B. pinnatum* was collected from sango ota, Ogun State. *Viscum album* was detached from a *Gmelina arborea* tree at Stadium area of Ogbomoso, Oyo state. The plant leaves were identified and authenticated at the Department of Biology herbarium, Ladoke Akintola University of Technology. The leaves were detached from the stem, washed with distilled water to remove dirt and other contaminants, and air dried to reduce moisture

content and then oven dried at 45 °C to constant weight. The samples were grinded and stored in an air tight container for further analysis.

Preparation of the Extracts

The grinded samples (1 g) were soaked in 50 mL of the solvents methanol for 72 h, after which the samples were filtered using whatman's filter paper. The filtrates were concentrated using water bath at 50 °C according to **Afolabi et al. (2014)**.

GC-MS Analysis

One gram of the dried samples each was soaked in 50 mL hexane for two days. The extract was concentrated in a water bath. Hexane extract of the leaves were screened by GC- MS. The GC-MS system used was GCMS-QP2010SE SHIMADZU.

Phytochemical content assay

Estimation of Total Phenolic Content

The total phenolic contents assay was carried out using the method described by **Sengul et al. (2009)**. In a 1.5 mL Eppendorf tube, 790 µL of distilled water, 10 µL of diluted sample and 50 µL of Folin-Ciocalteau reagent were added and the mixture vortexed. After 1 min, 150 µL of aqueous sodium carbonate (20%) was added and the mixture vortexed and allowed to stand in the dark at room temperature for 2 h. The absorbance was read at 750 nm using UV-visible spectrophotometer. The total polyphenol concentration was calculated from a calibration curve (100 – 500 µg.mL^{-1}) and the results were expressed as mg of gallic acid equivalents (mg GAE.g^{-1}) dry sample.

Antioxidant activities assay

Estimation of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging activities of the extracts were determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method according to **Brand-Williams et al. (1995)**. A fresh 0.002% solution of DPPH was prepared in methanol and its absorbance was recorded at 515 nm. 50 µL of pure leaf extracts was mixed with 3 mL solution of DPPH and allowed to stand in darkness for 15 min. The absorbance was again recorded at 515 nm.

Ferric reducing antioxidant power (FRAP) assay

Determination of antioxidant properties was carried out using the method of **Chan et al. (2007)**. About 1 mL appropriate dilutions of methanolic leaf extract was dispensed into different test tubes. 2.5 mL phosphate buffer was added, followed by 2.5 mL K_2FeCN solution and then incubated at 500 °C for 20 min. 2.5 mL TCA solution was added to the mixture to stop the reaction. Reaction mixture was separated into aliquots of 2.5 mL and each diluted with 2.5 mL of distilled water and allowed to stand in the dark for 30 min for color development. The absorbance was read at 700 nm against a reagent blank. Ferric reducing antioxidant power was expressed as gallic acid equivalent (mg GAE.g^{-1} sample).

Statistical analysis

The data were subjected to one-way ANOVA to analyze the significant difference in all data and Duncan's Multiple Range Test (DMRT) ($p \leq 0.05$) to analyze the significant difference between Mean values of samples using SPSS 18 software (SPSS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

The total phenolic content of the methanolic leave extracts are presented in Table 1 as 659.5, 1667.5 and 1232.0 mg GAE.g^{-1} dry extract for *B. pinnatum, A. altilis* and *V. album* respectively. *A. altilis* has the highest phenolic content followed by *V. album* and then *B. pinnatum*. Polyphenols are considered to have beneficial effects on human health and provide protection against several chronic diseases, such as cardiovascular diseases (CVD) **(Manach et al., 2005)**. All the herbal plant leaves understudy have high phenolic content. This could justify their use as anti-hypertensive herbs.

Analysis of the scavenging activities of the methanolic of three herbal plants on 1,1-diphenyl-2-picrylhydrazyl radical as reported in fig.1indicated that the *A.altilis* has the highest scavenging capacity against DPPH, with IC_{50} value of 2.24. *B. pinnatum* showed a higher scavenging power with IC_{50} value of 3.63 than *V. album* with IC_{50} value of 4.65. Higher percentage inhibition indicates better scavenging activity or antioxidant potentials. *A. altilis* showed the highest
percentage inhibition on DPPH. Therefore, *A. altilis* is the most potent out of the plant leaves studied and expected to have the highest blood pressure reducing ability, anti-aging, anti-cancer and anti-atheriosclerosis properties.

Table 1 Total phenolic contents (TPC) of the methanolic leave extracts of *A. altilis, B. pinnatum* and *V. album*.

	Concentration (mg GAE.g^{-1})
Bryophyllum pinnatum	659.5 ±0.03
Artocarpus altilis	1667.5 ±0.02
Viscum album	1232.0 ±0.02

Table 2 Ferric reducing antioxidant power (FRAP) expressed as mg.GAE^{-1} of the methanolic extract of *A. altilis, B. pinnatum* and *V. album* leaves mg GAE.g^{-1}.

Sample	**mg GAE.g^{-1}**
Bryophyllum *pinnatum*	1561.8 ±0.01
Artocarpus *altilis*	2505.2 ±0.04
Viscum *album*	1698.0 ± 0.03

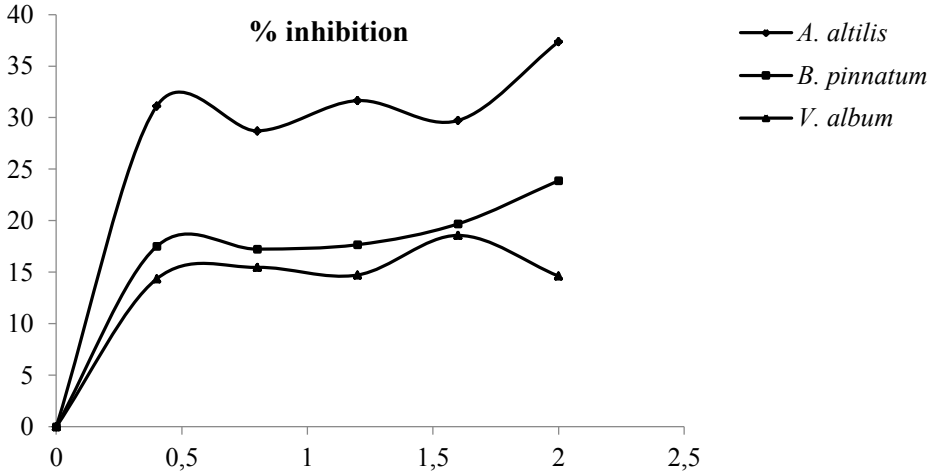


Figure 4 The Percentage (%) inhibitory potentials of *A. altilis, B. pinnatum* and *V.album* leave extracts on 1,1-diphenyl-2-picryl-hydrazyl (DPPH).
Keys: *A. altilis = Artocarpus altilis*; *B. pinnatum = Bryophyllum pinnatum; V. album = Viscum album.*

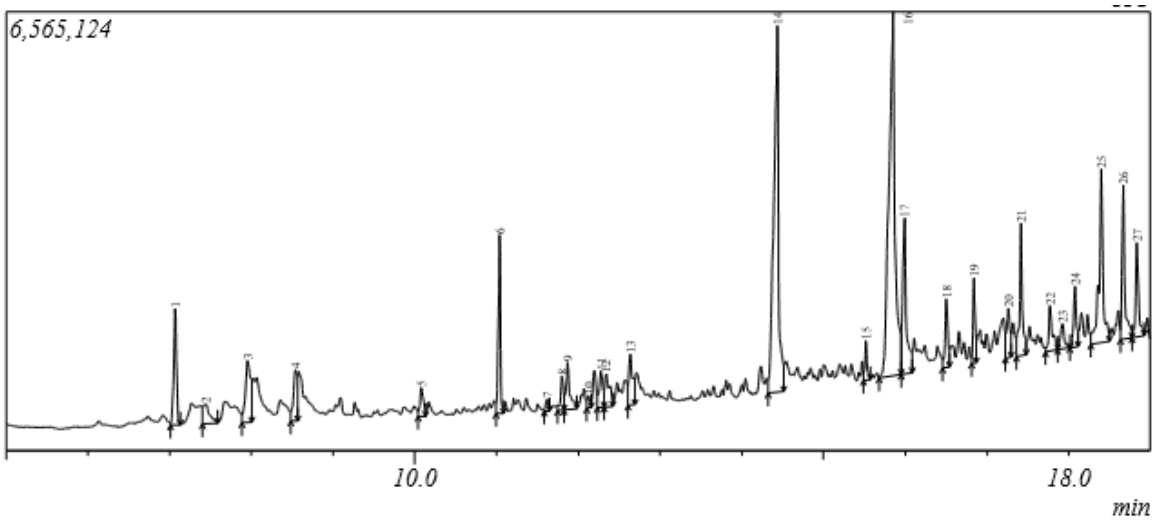


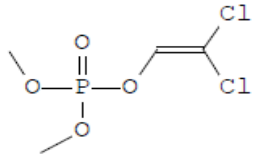
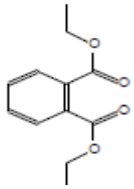
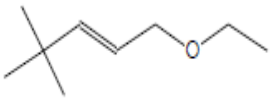
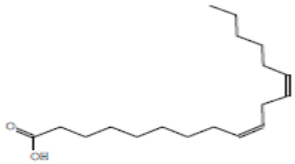
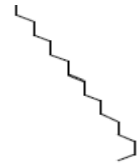
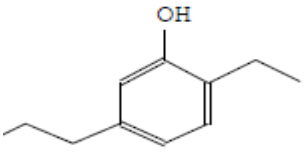
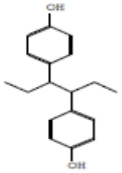
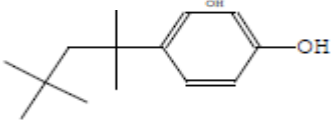
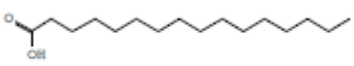
Figure 5 Chromatogram of *A. altilis* extract.

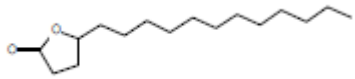
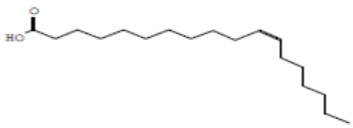
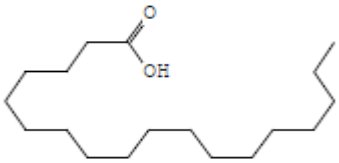
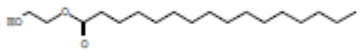
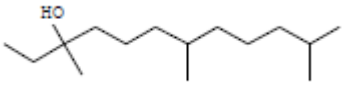
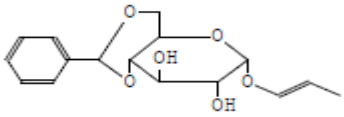
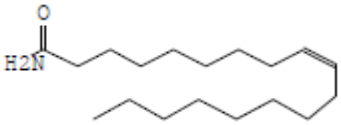
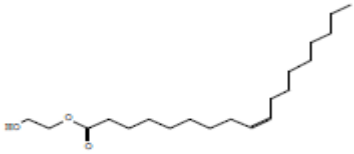
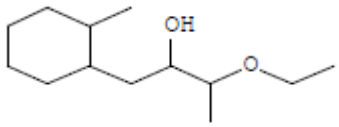
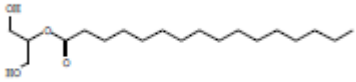
Plants with high antioxidant properties have been reported to also be efficient in treating coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, cataracts and inflammations **(Huang et al., 2005)**.

Also, Table 2 shows the reducing activities of various extracts when compared with gallic acid as the standard. The higher the absorbance of the reaction mixture, the higher would be the reducing power. At mg/ml concentration, the methanolic extract of *B. pinnatum* had the minimum reducing power of 1561.8 ±0.01 mg GAE.g^{-1}; *V. album* had 1698.0 ±0.03 mg GAE.g^{-1} while *A. altilis* showed the highest reducing ability with 12505.2 ±0.04 mg GAE.g^{-1}. This result compliments the one obtained from the DPPH assay as shown in Figure 4.

These various results indicate the highest anti-oxidant potential of *A. altilis* out of the three plants examined. This result is also justified by their phenolic compound where *A. altilis* had the highest phenolic compound (Table 1). Studies have shown that dietary phenolic compounds have a protective role against cardiovascular risk due to the numerous chemical and structural properties, and biological effects including high antioxidant capacity *in vitro* and *in vivo*, anti-inflammatory and anti-hypertensive effects, and improved endothelial functions **(AFNS, 2008)**. However, twenty five compounds were detected in the chromatogram of *A.altilis* as shown in Figure 5 and the probable structures of the components were presented in Table 3. In the results, cis vaccenic acid (22.98%) is the major fatty acid present in *A.altilis* with Palmitic acid (18.07%), stearic acid (5.77%), Linoleic acid (0.90%) also detected. The phenolic compounds detected were phenol (2.03%), 3-Dodecanol (1.88%) and Benzesterol (1.39%). These compounds could probably be responsible for the total phenolic contents shown in this plant extract (Table 1) as determined by the folin ciolcateau's method. A 2008 study at the University of Alberta suggests that vaccenic acid feeding in rats over 16 weeks resulted in lowered total cholesterol, lowered low density lipoprotein (LDL) cholesterol and lower triglyceride levels **(Stebins, 1945)**. Vaccenic acid, Alpha d glucopyranose, oleic acid amide and tetradecanoic acid content of *A. altilis* could be responsible for the observed antihypertensive activity because of the antihypertensive active sites detected in these compounds **(Wang et al., 2013)**.

Table 3 Various compounds detected in *A. altilis* extract with probable structure.

Peak	Retention time (min)	Concentration ($g.100g^{-1}$)	Name	Structures
1	7.069	3.48	Dichloros	
2	7.440	1.36	2-Nonenal, (E)-	
3	7.954	3.60	2,4-Decadienal, (E,E)-	
4	8.540	2.18	2-Undecenal	
5	10.079	1.07	2-methyltetracosane	
6	11.035	4.00	Diethyl Phthalate	
7	11.615	0.31	1-ethoxy-4,4-dimethyl-2-pentene	
8	11.801	0.90	Linoleic acid	
9	11.869	1.86	8-Heptadecene	
10	12.123	0.36	Myristic aldehyde	
11	12.277	1.39	2-Ethyl-5-n-propylphenol	
12	12.341	1.52	Hexestrol	
13	12.635	2.03	Phenol,4-(1,1,3,3-tetramethylbutyl)-phenol	
14	14.433	18.07	Palmitic acid	

15	15.518	0.93	Furanone	
16	15.848	22.98	cis-Vaccenic acid	
17	15.989	5.77	Stearic acid	
18	16.503	1.97	2-hydroxyethyl ester	
19	16.841	1.88	3-Dodecanol, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	
20	17.260	1.68	alpha.d-Glucopyranose	
21	17.416	3.96	Oleic acid amide	
22	17.771	1.69	9-Octadecenoic acid	
23	17.923	0.99	Tetradecanoic acid,	
24	18.075	1.73	3- Ethoxy-1-(2-methyl-cyclohexyl)-butan-2-ol	
25	18.400	6.99	Beta-monoglyceride	

In the chromatogram for *B. pinnatum* (Figure 6), fourteen compounds were detected and their probable structures shown in Table 4.

Oleic acid (30.41%) is the major fatty acid present in *B. pinnatum*. Palmitic acid (22.66%) and 9-Octadecenoic acid (7.78%) were also detected. Oleic acid is the major source of omega 9 fatty acid. Research has shown that omega-9 fatty acids can help to reduce the risk of cardiovascular disease and stroke. Because omega-9 fatty acids have been known to increase high density lipoprotein (HDL) good cholesterol and decrease low density lipoprotein (LDL) bad cholesterol, also, they help eliminate plaque buildup in the arteries, which causes heart attack and stroke. The stereoisomer of oleic acid is called elaidic acid or trans-9-octadecenoic acid. Elaidic acid, the most abundant trans-fatty acid in diet, appears to have an adverse effect on

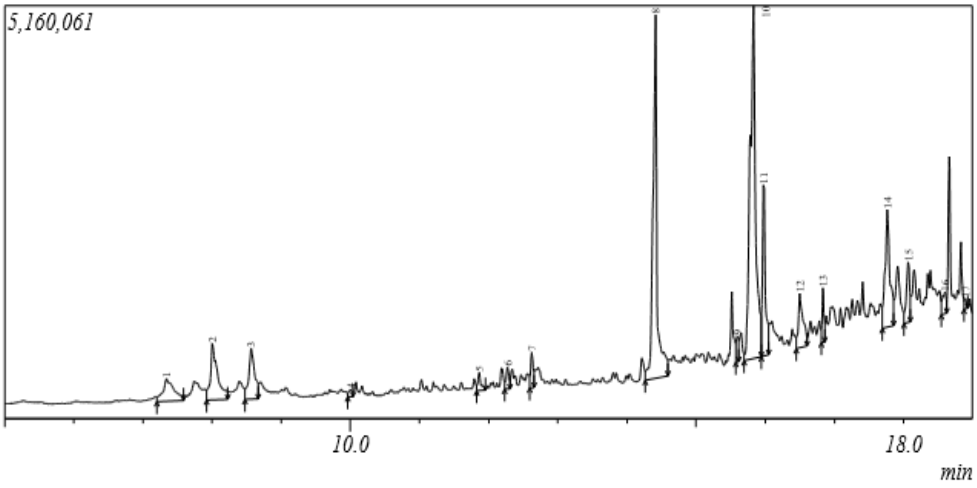
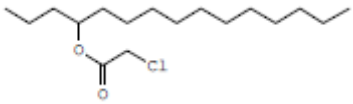
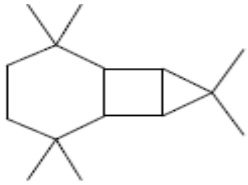
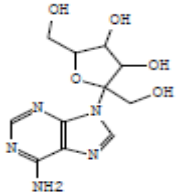
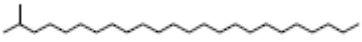


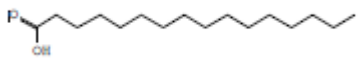
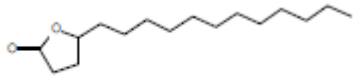
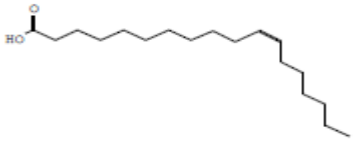
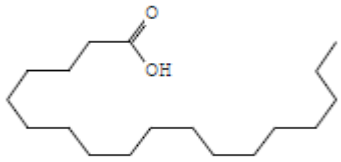
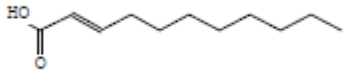
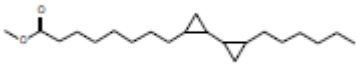
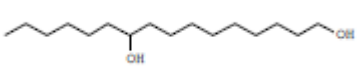
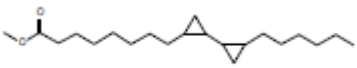
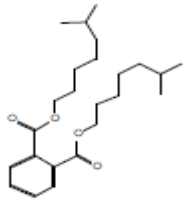
Figure 6 Chromatogram of *B. pinnatum* Extract.

health **(AFNS, 2008)**. Piscofuranine (1.41%) was the only phenolic compound present. The active site responsible for anti-hypertensive activity is the carbon of the carboxylate group for angiotensin 1 inhibitor, therefore piscofuranine, bis-ethlenetherephthalate and oleic acid content of *B. pinnatum* could be responsible for the observed antihypertensive activity.

Eighteen compounds which correspond to nineteen peaks in the chromatogram (Figure 7) were detected in the extract of *V. album* as presented in Table 5. Cis vaccenic acid (33.68%) is the major fatty acid detected in *V. album*.

Table 5 Compounds detected in *V. album* extract with probable structures.

Peak	Retention Time (min)	Concentration ($g.100g^{-1}$)	Name	Structures
1	8.012	4.59	2,4-Decadienal, (E,E)-	
2	8.565	3.83	2-Undecenal	
3	9.015	1.12	2-Nonen-1-ol, (E)-	
4	11.026	0.53	Chloroacetic acid.	
5	11.098	0.59	2-methyltetracosane	
6	12.271	0.63	Tricyclo[4.3.0.0(7,9)]nonane	
7	12.621	1.19	Psicofuranine	
8	12.915	0.25	Methyltetracosane	
9	13.844	0.26	13-Tetradecenal	

10	14.414	24.16	Palmitic acid	
11	15.592	1.65	Furanone.	
12	15.826	33.68	cis-Vaccenic acid	
13	15.975	10.66	Stearic acid	
14	16.499	2.99	trans-2-undecenoic acid	
15	16.834	1.02	[1,1'-Bicyclopropyl]-2-octanoic acid.	
16	17.762	3.73	1,10-Hexadecanediol	
17	18.067	1.77	[1,1'-Bicyclopropyl]-2-octanoic acid	
18	18.658	5.84	Diisooctyl phthalate	

However, palmitic acids (24.16%), stearic acid (10.66%) and trans-2-undecenoic acid (2.99%) were also detected. The presence of fatty acids especially Cis vaccenic acid in combination with pscofuranine, diisooctylphthalate could be responsible for the observed anti-hypertensive activity **(Sengul et al., 2009)**.

Also, stearic acid has been shown to be detrimental to human health, however, subsequently, study has also revealed that if studied in isolation, it actually contributes to a decrease in LDL levels leading to an improved overall cholesterol ratio **(Wong et al.,2006)**.

CONCLUSION

In this finding, A. altilis showed the highest anti-hypertensive potential because of the high antioxidant capacity, total phenolic content and its high blood pressure lowering fatty acids contents, while B. pinnatum showed the least anti-hypertensive potential. These antioxidants may achieve their antihypertensive effects by reducing aldehyde conjugate/AGE formation and oxidative stress by improving insulin-resistance and endothelial function, or by normalising calcium channels and peripheral vascular resistance (Grynberg, 2007; Beg et al., 2011). With this current work, it is clearly shown that the active sites involved in the process of regulating blood pressure for groups of anti-hypertensives are found in the structures of the compounds detected in these herbs, for example the structure of Lisinopril a popular anti-hypertensive has carboxylic and amine functional groups which is also present in palmitic acid, stearic acid and piscofuranine all present in the herbs. This could justify the observed antihypertensive activity of these herbs.

REFERENCES

Abubakar, M. G., Yerima, M. B., Zahriya, A. G., Ukwuani-Kwaja, A. N. 2010. Acute toxicity and antifungal studies of ethanolic leaves, stem and pulp extract of Tamarindus indica. *Research Journal of Pharmaceutical, Biological and Chemical Science*, vol. 1, no. 4, p. 104-111.

AFNS. 2008. Alberta natural trans-fat research earns global recognition [online] s.a. [cit. 20018-01-05] Available at: https://www.topcropmanager.com/news/alberta-natural-trans-fat-research-earns-global-recognition-1440.

Afolabi, O. B., Oloyede, O. I. 2014. Antioxidant properties of the extracts of Talinum triangulare and its effects on antioxidant enzymes in tissue homogenate of Swiss albino rat.

Toxicology International, vol. 21, no. 3, p. 307-313. https://doi.org/10.4103/0971-6580.155377 PMid:25948971

Akanbi, T. O., Nazamid, S., Adebowale, A. A. 2009. Functional and pasting properties of a tropical breadfruit (A. *altilis*) starch from Ile-Ife, Osun State, Nigeria. *International Food Research Journal*, vol.1, no. 16, p. 151-157.

Beg, M., Sharma, V., Akhtar, N., Mohd, J. 2011. Role of Antioxidants in Hypertension. *Journal, Indian Academy of Clinical Medicine*, vol. 12, no. 2, p. 122-127.

Borris, R. P. 1996. Natural products research: perspectives from a major pharmaceutical company. *Journal of Ethnopharmacology*, vol. 51, no. 1-3, p. 29-38. https://doi.org/10.1016/0378-8741(95)01347-4

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. https://doi.org/10.1016/S0023-6438(95)80008-5

Grynberg, A. 2007. Hypertension prevention: from nutrients to (fortified) foods to dietary patterns. Focus on fatty acids. *Journal of Human Hypertension*, vol. 3, p. 25-33.

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., Armand, R. 2015. The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of Thymus vulgaris. *International Journal of Clinical Medicine*, vol. 6, no. 9, p. 635-642. https://doi.org/10.4236/ijcm.2015.69084

Huang, D., Ou, B., Prior, R. L. 2005.The chemistry behind antioxidant capacity assays. *Journal of Agricurtural and Food Chemistry*, vol. 53, no. 6, p. 1841-1856. https://doi.org/10.1021/jf030723c PMid:15769103

Chan, E. W. C., Lim, Y. Y., Omar, M. 2007. Antioxidant and antibacterial activity of leaves of Etlingera species (*Zingiberaceae*) in Peninsular Malaysia. *Food Chemistry*, vol. 104, no. 4, p. 1586-1593. https://doi.org/10.1016/j.foodchem.2007.03.023

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izza, J L. Jr., Jones, D. W., Materson, B J., Oparil, S., Wright J. T. Jr., Roccella, E. J. 2003. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *The Journal of the American Medical Association*, vol. 289, no. 19, p. 2560-2571. https://doi.org/10.1001/jama.289.19.2560 PMid:12748199

Lans, C A. 2006. Ethnomedicine in Trinidad and Tobago for urinary problems diabetis mellitus. *Journal of Ethnobiology nd Ethnomedicine*, vol. 245, p. 157-168.

Manach, C., Mazur, A., Scalbert, A. 2005. Polyphenols and prevention of cardiovascular disease. *Current Opinion in Lipidology*, vol. 16, no. 1, p. 77-84. https://doi.org/10.1097/00041433-200502000-00013 PMid:15650567

Nwali, B. U., Okaka, A. N. C., Ibiam, U. A., Aja, P. M. 2012. Phytochemical compositon of B. pinnatum leaves. *International Journal of Advanced Biotechnology and Research*, vol. 2, no. 4, p. 614-616.

Okwu, D. E., Josiah, C. 2006. Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology*, vol. 5, no. 4, p. 357-361.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, S., Anthony, S. 2009. Agroforestree Database: A tree reference and selection guide. Version 4.0 World Agroforestry Centre, Kenya.

Sengul, M., Yildiz, H., Gungor, N., Cetin, B., Eser, Z., Ercisli, S. 2009. Total Phenolic Content, Antioxidant and Antimicrobial Activities of Some Medicinal Plants. *Park Journal of Pharmacology Science*, vol. 22, no. 1, p. 102-106.

Stebbins, G. L. 1945. The cytological analysis of species hybrids. *Botanical Review*, vol. 11, no. 9, p. 463-486. https://doi.org/10.1007/BF02861140

Wang, Y., Chun, O. K., Song, W. O. 2013. Plasma and Dietary Antioxidant Status as Cardiovascular Disease Risk Factors: A Review of Human Studies. *Nutritians*, vol. 5, no. 8, p. 2969-3004. https://doi.org/10.3390/nu5082969

Wong, K., Juli, M., Russel, L. K., Cyril, W., Azadeh, J., David, J. 2006. Colonic Health:Fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology*, vol. 40, no. 3, p. 235-243. https://doi.org/10.1097/00004836-200603000-00015 PMid:16633129

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ACCEPTABILITY AND SENSORY EVALUATION OF ENERGY BARS AND PROTEIN BARS ENRICHED WITH EDIBLE INSECT

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ABSTRACT
For consumers, one of the basic criteria of choosing a foodstuff, apart from nutritional values, is their taste and smell. In edible insect as a novel food, these criteria are not quite decisive. The main criterion in the Western countries is the acceptability of the food. This work deals with sensory evaluation of protein and energy bars, enriched with cricket flour from American and Czech producers, and their acceptability for consumers from the Czech Republic. The sensory evaluation was done using the questionnaire survey and a simple electronic nose. The survey has shown that edible insect bars are acceptable as a new type of food for consumers in the Czech Republic. Best rated by consumers were orange and pineapple flavour bars from the Czech manufacturer. Statistically significant difference was not detected between evaluation of the bars from the American and Czech manufacturers. Also, the difference between the bars of different flavours from the Czech producer was evaluated using a simple machine – a portable electronic nose. There was not a statistically significant difference between bars of different flavours from the American manufacturer. The positive contribution of the survey is that more than 80% of consumers are willing to consume food enriched with edible insect. This fact shows a change in public attitude to these foods.

Keywords: edible insect; *Acheta domestica*; sensory analysis; electronic nose; energy bars

INTRODUCTION
Sensory properties are a particularly important criterion for eating edible insect **(Borkovcová et al., 2009; Adámek et al., 2017)**. Food intake and rejection are the result of the involvement of sensory-affective functions that relate to sensory properties. The imaginary ideas of nature and origin of the food have an influence too, and there is also a concern about security that is closely linked to physical and mental harm **(Rozin and Fallon, 1987)**. In western countries, people generally link entomophagy with dirt and poverty, thus they often deny insect eating as unacceptable **(Looy et al., 2014)**. However, in other cultures, insect is commonly eaten and valued for its nutritional properties and taste **(Hanboonsong, 2010)**. According to **Regulation (EU) 2015/2283** of the European Parliament and of the Council of 25 November 2015 on novel foods, in force from January 1st 2018, edible insect became the novel food.

The visual impression is the first of indicator when assessing and choosing a food by a consumer. It depends not only on its own visual effect, but also on the psychological effect on the consumer (taste expectations as a result of past appearances and tasted flavour) **(Köster et al., 2004; Mojet et Köster, 2005)**. As with other meals, pleasant visual stimulus (nice colour) does not ensure good taste – colour serves mainly as an insect species identifier. For most species, however, mainly larvae and pupae are used for culinary treatment, and they are mostly white or colourless. Insect, as well as crustaceans, gains its final (most often attractive red) colour, through the culinary treatment. Black colour of insect may be caused by improper drying or big content of oxidized fat. After proper drying, edible insect gains golden or brown colour and can be easily crushed with fingers **(Borkovcová et al., 2009)**.

Furthermore, the consumer evaluates the shape and consistency that are not easily seen in hidden forms of eating insect. Edible insect is partly made up of an exoskeleton that causes the crispness of the insect when consumed (tactile and auditory effect), which, along with chewing, generates pleasant feelings such as the consumption of pretzels, biscuits or other durable pastry **(Ramos-Elorduy, 1998)**. Larvae after moulting are soft, because their exoskeleton has not yet hardened, thus making them more digestible **(Borkovcová et al., 2009)**. Benefit of exoskeleton is the high content of chitin, which has similar effect as fibre, but it is also an allergen **(Bednářová et al., 2010; Mlček et al., 2014)**.

Insect taste is various. It depends, among other things, on the insect environment and on the feed (fruits, vegetables,

pastry, potatoes, rice, grass ...). To enjoy taste of insect in all its richness, insect must be alive and unwashed, but this is dangerous from a health and safety point of view **(Bednářová et al., 2010)**. Therefore, **EFSA (2015)** reccomends heat treatment for security reasons, although it results in the loss of the original aroma.

In the food industry, the scent is one of the most important sensory properties in terms of consumer compliance. The laic consumer evaluates the scent only subjectively, based on his experience and preferences. The nose, as a basic olfactory organ, can be attenuated at the time of ingestion **(Carlsson and Kalinová, 2005)**. Furthermore, pheromones, often present on the exoskeleton, are removed by washing. Edible insect material is therefore de facto without scent, and it absorbs the taste and smell of the added ingredients **(Ramos Elorduy, 1998)**. To enable laic consumer to distinguish the differences between edible insect smell, the intensity of smell would have to be increased by at least 30% **(Carlsson and Kalinová, 2005)**. To better distinguish the individual smells, there is an alternative – several species of olfactometric machines, including electronic noses. As proved, even a simple electronic nose can distinguish insect species and the culinary treatment **(Adámek et al., 2017)**.

The electronic nose is usually equipped with several semiconductor gas sensors, each of which is sensitive to a particular type or group of volatile substances. More accurate devices can use combinations of different methods to measure concentrations of substances in the analysed gas. The electronic nose is used not only as a part of security systems (fire detector – detection of flammable substances hazardous for man), in the environmental segment (air pollution detector), but also in the food industry. While electronic noses cannot fully replace human smell, which is closely related to taste, they can be used to detect a firmly defined condition of a food, which is indicated by a certain odour. This can be used, for example, in monitoring food storage and determining its shelf life. **Gopal et al. (2015)** described in their study the use of electronic nose Peres to evaluate the freshness and shelf life. Also **Peris (2016)** dealt in his study with using the electronic nose in the food industry, to reveal food falsifying and evaluating food authenticity.

Scientific hypothesis

Scientific hypothesis is: Energy bars and protein bars enriched with edible insect are acceptable as novel food for consumers from the Czech Republic.

Aim of the work was to find out, if energy bars and protein bars enriched with edible insect are acceptable as novel food for consumers from the Czech Republic, if there is any consumer preference based on smell or taste, and to compare consumer evaluation with the electronic nose measurement.

MATERIAL AND METHODOLOGY

Material for sensory analysis

Protein and energy bars with cricket flour (*Acheta domestica*) produced in USA and Czech Republic were used. Samples T1 – T4 were made by Czech producer, T5 – T7 were made by American producer.

The ingredients of protein and energy bars were as follows:

T1 Protein bar Peanut Butter & Cinnamon with Cricket Flour: Peanut butter (34%) (Peanuts 100%), Cricket flour (*Acheta Domestica*) (20%), Cannabis Powder, Cocoa Butter, Agave Syrup, Beetroot, Cinnamon (1%)

T2 Protein bar Dark Chocolate & Sesame with Cricket Flour: Sesame (27%), Chocolate (21%) (Cocoa Powder 52%, Cocoa Butter 48%), Cricket Flour (*Acheta Domestica*) (20%), Cannabis Powder, Agave Syrup, Sesame Oil (4%)

T3 Energy bar Pineapple & Coconut with Cricket Flour: Pineapple (30%), Dates, Cashew Nuts, Cricket Flour (*Acheta Domestica*) (10%), Coconut (8.5%), Psyllium, Lemon Peel

T4 Energy bar Dark chocolate & Orange with Cricket Flour: Dates, Cashew Nuts, Chocolate (16%) (Cocoa Mass 69%, Cocoa Powder 31%), Cricket Flour (*Acheta Domestica*) (10%), Psyllium, Orange Peels (2%), Essential Orange Oil (0.9%)

T5 Energy bar Peanut Butter, Cherry and Cacao: Raw Honey, Brown Rice Syrup, Peanut Butter, Almonds , Pumpkin Seeds, Cherry, Cacao Nibs, Sunflower Seeds, Pistachios, Walnuts, Flax Seeds, Rolled Oats, Puffed Brown Rice, Dates, Cricket Flour, Cashews, Blueberry s & Himalayan Sea Salt

T6 Energy bar Cranberry, Blueberry and Pistachio: Brown Rice Syrup, Raw Honey, Pistachios, Almonds, Pumpkin Seeds, Cricket Flour, Cranberry, Blueberry, Cherry, Walnuts, Sunflower Seeds, Chia Seeds, Flax Seeds, Rolled Oats, Puffed Brown Rice, Apricots, Currants, Himalayan Sea Salt

T7 Energy bar Kale, Green Tea, Seaweed and Ginger: Brown Rice Syrup, Crystallized Ginger, Almonds, Pistachios, Pumpkin Seeds, Sunflower Seeds, Cricket Flour, Flax Seeds, Rolled Oats, Puffed Brown Rice, Dates, Apricots, Apple, Cashews, Green Tea Powder, Toasted Kale, Roasted Seaweed & Himalayan Sea Salt.

Nutritional values are shown in Table 1. Data for American bars were taken and recalculated for 100g from: https://www.kickstarter.com/projects/993678727/hopper-energy-bars-made-with-cricket-flour-in-aust.

Survey

Bar samples were subjected to sensory analysis and evaluated on the basis of a questionnaire survey. Respondents were presented with seven types of bars from two companies. Sticks were sliced and presented to the respondents for evaluation.

The survey was attended by 96 lay participants in September 2017. Of the total number of respondents, 18% were women and 82% men of Czech nationality, predominantly aged 20 – 29 years. Respondents only got the information that this was a sensory assessment of energy bars with the addition of cricket flour. Additionally, the bars were numbered 1 to 7 and presented for the evaluation of respondents in the form of a blind test. Respondents were asked to evaluate the smell and taste from 1 (pleasant taste or smell) to 5 (unpleasant taste or smell). The questionnaire also included questions about gender, age, interest in the consumption of insect, and the

Table 1 The nutritional composition of protein and energy bars enriched with the cricket flour.

Sample	Energy Value		Total Fat	Saturated fatty acids	Total Carbohydrate	Sugars	Fibre	Protein	Salt
	kJ	kcal	g.100 g^{-1}	g.100 g^{-1}	g.100 g^{-1}	g.100 g^{-1}	g.100 g^{-1}	g.100 g^{-1}	g.100 g^{-1}
T1	2207	530	34.2	9.8	22.0	12.3	2.7	33.3	0.14
T2	2277	535	36.5	11.3	17.2	7.3	4.8	33.3	0.19
T3	1724	412	16.4	7.0	49.2	42.6	8,4	13.0	0.09
T4	1706	408	17.4	5.8	42.0	34.4	10.6	15.4	0.08
T5	1650	395	20.4	3.4	44.0	21.4	6.1	13.5	0.21
T6	1621	388	15.4	1.6	60.4	26.5	6.6	13.4	0.23
T7	1580	378	15.9	2.0	63.1	24.5	5.8	13.6	0.24

preference of the producers. Respondents were also asked to answer questions about their willingness to eat insect in the future.

Electronic nose measurement methodology

Measurement of gas concentration (smell) was carried out on an experimental prototype of electronic nose (Figure 1), which was realized as a simple, cheap and portable device. The device was based on the Arduino Mega platform controlled by the ATmega1280 microcontroller with the ability to record data on a memory card and communicates with the web server. The measuring chamber was equipped with sensors based on the chemorezistive principle. It uses the MQ-6 sensor, which is most sensitive to propane or isobutane (300 – 10000 ppm) and less sensitive to alcohol. Furthermore, the MQ-3 sensor, which is very sensitive to alcohol (25 – 500 ppm) and the MQ-8 sensor, designed for hydrogen detection (100 – 1000 ppm) were used. As this device was meant only to compare its previous measurements with each other and accurate measurement of the absolute values of the individual gas concentrations in the measured odour was not expected, the recommended manufacturer's setting with no additional calibration was used to detect the absolute values of the individual gas concentrations. The U_S voltages [V] of the individual sensors were converted using the internal 10-bit A/D converter of the microcontroller to a digital value of d [-] (Voltage 0V and 5V corresponds to the digital level 0 and 1023). These values were further mathematically processed.

Statistic analysis

Data was evaluated using Excel 2013 (Microsoft Corporation, USA), STATISTICA Cz version 12 (StatSoft, Inc., USA) and Gnuplot 5.0: an interactive plotting program **(Williams and Kelley, 2016)**. Results were expressed by average ± standard deviation. Kruskal-Walllis test ($\alpha = 0.05$) was used to compare the taste and smell of individual samples.

RESULTS AND DISCUSSION

Sensory evaluation of protein and energy bars, enriched with cricket flour

Seven samples of energy bars, enriched with cricket flour, T1 – T7, were evaluated for sensory properties, results are in Table 2. Respondents after sensory evaluation (taste and smell) preferred samples T1 – T4 over T5 – T7. T4 had the best score (1.9) and T7 the worst (4), considering the smell. In general, samples T1 – T4, produced by Czech manufacturer, had better acceptance, than bars T5 – T7, made in the USA. This smell evaluation corresponded with taste assessment, where again bars from Czech produce had better scores than from American producers. Consumers best loved T4 (2.4), worst score was gained by T7 (3.6). The questionnaire showed that, consumers would buy insect bars because they considered the product to be healthy, and also that they prefer a Czech producer over American.

The results showed that, differences between evaluation made by men and women are not statistically significant

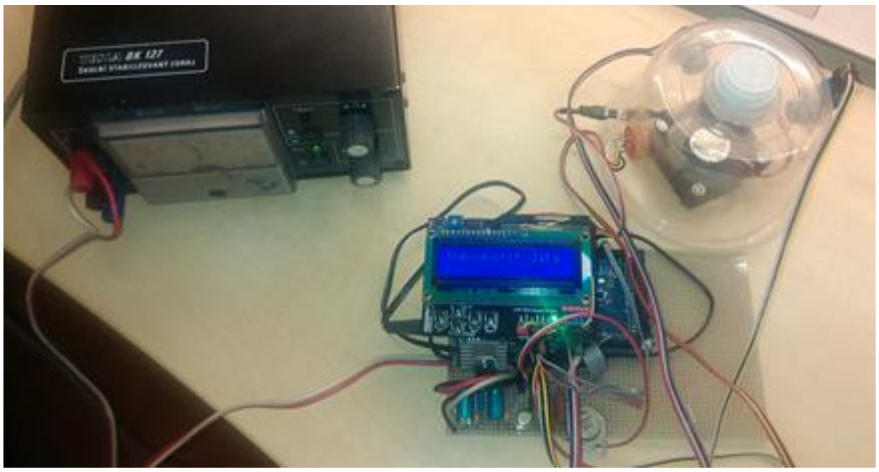

Figure 1 Experimental prototype of the electronic nose.

Table 2 Evaluation of the smell and taste of energy and protein bars enriched with the cricket flour (1 – very pleasant, 5 – unpleasant).

		Evaluation			
		Smell		Taste	
Sample	Name	M	SD	M	SD
T1	Protein bar Peanut Butter & Cinnamon with Cricket Flour	2.5	1.0	2.9	1.1
T2	Protein bar Dark Chocolate & Sesame with Cricket Flour	2.4	0.7	3.1	1.2
T3	Energy bar Pineapple & Coconut with Cricket Flour	2.4	1.2	2.5	1.4
T4	Energy bar Dark chocolate & Orange with Cricket Flour	1.9	0.9	2.4	1.3
T5	Energy bar Peanut Butter, Cherry and Cacao	2.9	0.8	3.4	0.8
T6	Energy bar Cranberry, Blueberry and Pistachio	3.0	1.0	3.1	1.0
T7	Energy bar Kale, Green Tea, Seaweed and Ginger	4.0	1.5	3.6	1.1

(p >0.05). Similar results were presented by **Adámková (2017)** while evaluating the bars enriched with cricket flour. However, **Adámková (2017)** stated that, women more often preferred bars with higher content of cocoa powder than men. This may be because women in general like food with cocoa powder, such as chocolate confectionery, more than men **(Kozelová et al., 2014)**.

Another beneficial fact is the interest of especially young people in this survey. That proves the change of Czech consumers' attitude towards edible insect as a novel food. This change was already documented by **Bednářová et al. (2013)** and **Adámková (2017)**. **Adámková (2017)**, who evaluated the acceptance of edible insect before and after the first consumption, declared the increase of the acceptance by more than 27%.

The resulting acceptance after the tasting of the bars was more than 60%. A similar result is provided on the website of the American company, producing bars T5 to T7. The manufacturer states that 58% of respondents tasted his bars without problems and most of these respondents liked them. 30% of respondents tasted a bar only after they learned about the benefits of edible insect.

The positive approach and willingness of respondents to consume these samples could be caused by the hidden form of edible insect additions. More than 80% of respondents said they were willing to consume food with the addition of edible insect in the future.

The conditions of acceptability of edible insects in the Czech Republic were also examined by **Bednářová et al. (2013)**. Based on her questionnaire survey, it was possible to divide European consumers into two groups – one group preferred consuming foodstuffs with highly visible insect, while the other group welcomed the consumption of insect in a hidden form.

Bednářová et. al. (2013) stated that in case of the field cricket, (*Gryllus assimillis*) Czech respondents were willing to consume this species in the visible form. **Capparos Megido et al. (2014)** evaluated in their study the acceptance of edible insects among Belgian consumers. The study was conducted with mealworm larvae and house cricket adults after various treatments. Although mild neophobia was revealed, people agreed to the evaluation of insect specimens and, after a hedonic test, respondents were willing to eat and cook insects in the future.

Furthermore, sample recognition test was done for samples T1-T7. Sample recognition test for various culinary treatments was carried out also by **Adámek et al. (2017)**, using an electronic nose. Available literature presents no other comparable data for this kind of edible insect evaluation.

Using the measured data for individual samples of the bars for the individual sensors MQ-8, MQ-6 and MQ-3, time-dependent curves were created. The measurement time was set to 600 s. An example of the time dependency is shown in Figure 2.

Based on the individual curves, the basic statistical variables were calculated - mean and standard deviation. Average values are listed in Table 3 and processed in a 3D graph (Figure 3**),** using the Gnuplot program.

In T1 – T4 bars, the electronic nose detected the statistically significant difference between the individual bars. It is clear from the graph on Figure 3 that the point

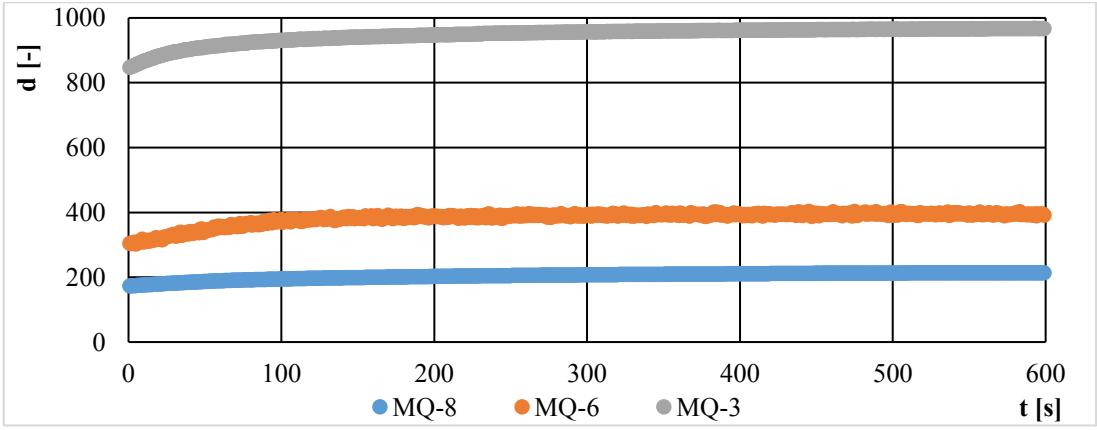

Figure 2 Example of time dependency of the measured data for sensors MQ-8, MQ-6 and MQ-3 in sample T4 (Energy bar Dark chocolate & Orange with Cricket Flour).

Table 3 Evaluation of the smell and taste of energy and protein bars enriched with the cricket flour (1 – very pleasant, 5 – unpleasant).

Sample	MQ-8		MQ-6		MQ-3	
	M	±SD	M	±SD	M	±SD
T1	158.0	2.5	263.7	4.0	768.4	13.0
	145.5	3.0	262.7	4.7	745.8	5.9
T2	163.7	1.8	313.2	6.4	830.7	13.6
	162.4	3.4	306.0	7.7	826.9	9.2
T3	181.7	7.8	317.4	15.8	868.1	15.5
	183.7	7.8	315.3	15.9	866.0	15.6
T4	204.4	9.9	383.5	20.5	947.9	23.0
	191.9	6.2	357.4	21.9	957.0	7.7
T5	105.6	1.1	243.4	4.7	753.2	5.2
	104.6	2.3	234.5	4.0	739.9	3.1
T6	104.6	1.9	242.7	7.5	748.7	9.7
	102.1	1.8	236.6	6.2	741.1	7.1
	100.0	2.6	228.8	5.2	730.4	4.9
T7	103.9	2.9	244.8	6.1	757.2	10.3
	99.8	1.1	238.6	6.5	751.8	8.9
	100.5	1.3	233.8	7.1	742.1	9.5

range for the T4 bar is further from the other points and is more easily detectable than other bars. The electronic nose did not notice any significant difference between T5 – T7 bars.

In general, the smell of food is caused by the presence of different oils, terpenes, flavonoids, etc., and different people respond to them differently. The presence of these substances can be determined by technical devices, e.g. an electronic nose. Although the electronic nose used in this work is not equipped with sensors for detecting these substances, in Figure 3 it is possible to see a certain similarity between the evaluation of the respondents and the results from the electronic nose (increasing the signal on all three sensors improves the evaluation of the individual samples).

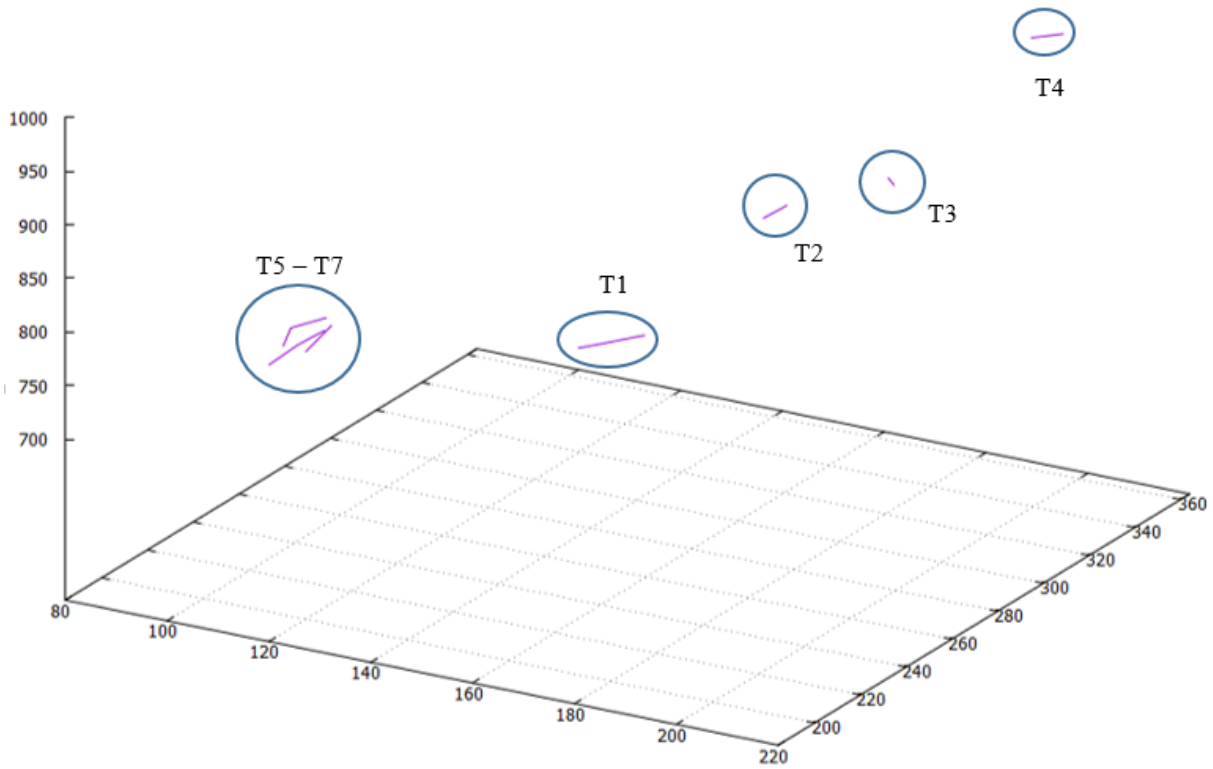

Figure 3 Electronic nose – Measured points for individual energy and protein bars enriched with the cricket flour.

CONCLUSION

Changes in public attitudes to eating edible insects were confirmed. The tasting of energy and protein bars, enriched with cricket flour by lay public, followed by a questionnaire survey confirmed that, these bars are acceptable to the Czech consumer as a novelty food under **Regulation EU 2015/2283** valid from 1st January 2018. The research also confirmed that preference is given to the Czech manufacturer's bars over the American bars. Regarding the taste and smell, the bars smelling of tropical fruit were more acceptable to consumers. Questionnaire surveys in the general public, especially young people, showed a willingness to taste samples of edible insects in the Czech Republic. The fact that respondents did not refuse the possibility of conscious consumption of edible insects in the future is positive.

Evaluated protein and energy bars are, according to the producers' advice, meant to serve as a dietary supplement for people with special needs (sportsmen), people interested in a healthy lifestyle and people with special nutrition. These bars, as the Czech producer puts it, are not a treat.

REFERENCES

Adámek, M., Adámková, A., Borkovcová, M., Mlček, J., Bednářová, M., Kouřimská, L., Skácel, J., Řezníček, M. 2017. Electronic nose in edible insects area. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 446-451. https://doi.org/10.5219/785

Adámková, A. 2017. *Nutritional analysis and optimization of rearing of selected species of edible insects under conditions of the Czech Republic with regard to human health* : dissertation theses. Prague, Czech Republic : Czech University of Life Sciences Prague. 108 p.

Bednářová, M., Borkovcová, M., Zorníková, G., Zeman, L. 2010. Insect as food in Czech republic. In *Mendelnet 2010*. Brno : Mendel University in Brno, p. 674-682. ISBN: 978-80-7375-453-2.

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://doi.org/10.11118/actaun201361030587

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.

Carlsson, M. A., Kalinová, B. 2005. Tajemství čichu poodhaleno (Disclosed mystery of olfaction). *Vesmír*, vol. 84, no. 3, p. 148-155. (In Czech)

Caparros Megido, R., Sablon, L., Geuens, M., Brostaux, Y., Alabi, T., Blecker, C., Drugmand, D., Haubruge, É., Francis, F. 2014. Edible insects acceptance by Belgian consumers: promising attitude for entomophagy development. *Journal of Sensory Studies*, vol. 29, no. 1, p. 14-20. https://doi.org/10.1111/joss.12077

EFSA, 2015. Risk profile related to production and consumption of insects as food and feed. *EFSA Journal*, vol. 13, no. 10, 60 p.

Gopal, D. 2015. Electronic Nose 'Smells' Rotting Food! In *Youngzine* [online] 2015-11-17 [cit. 2016-04-17] Available at: http://www.youngzine.org/article/electronic-nose-smells-rottingfood.

Hanboonsong, Y. 2010. Edible insects and associated food habits in Thailand. In *Forest insects as food: humans bite back, Proceedings of a workshop on Asia-Pacific resources and their potential for developmen*. Bangkok, Thailandm : FAO Regional Office for Asia and the Pacific, p. 173-182. ISBN 978-92-5-106488-7.

Kozelová, D., Matejková, E., Fikselová, M., Dékányová, J. 2014. Analysis of consumer behavior at chocolate purchase. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 8, no. 1, p. 62-66. https://doi.org/10.5219/325

Köster, M. A., Prescott, J., Köster, E. P. 2004. Incidental learning and memory for three basic tastes in food. *Chemical Senses*, vol. 29, no. 5, p. 441-453. https://doi.org/10.1093/chemse/bjh047 PMid:15201211

Looy, H., Dunkel, F. V., Wood, J. R. 2014. How then shall we eat? Insect-eating attitudes and sustainable foodways. *Agriculture and Human Values*, vol. 31, no. 1, p. 131-141. https://doi.org/10.1007/s10460-013-9450-x

Mlcek, J., Rop, O., Borkovcova, M., Bednarova, 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://doi.org/10.2478/v10222-012-0099-8

Mojet, J., Köster, E. P. 2005. Sensory memory and food texture. *Food Quality and Preference*, vol. 16, no. 3, p. 251-266. https://doi.org/10.1016/j.foodqual.2004.04.017

Peris, M., Escuder-Gilabert, L. 2016. Electronic noses and tongues to assess food authenticity and adulteration. *Trends in Food Science & Technology*, vol. 58, p. 40-54. https://doi.org/10.1016/j.tifs.2016.10.014

Ramos-Elorduy, J., Moreno, J. M. P., Prado, E. E., Perez, M. A., Otero, J. L., de Guevara, O. L. 1997. Nutritional Value of Edible Insects from the State of Oaxaca, Mexico. *Journal of Food Composition and Analysis*, vol. 10, no. 2, p. 142-157. https://doi.org/10.1006/jfca.1997.0530

Ramos-Elorduy, J. 1998. *Creepy Crawly Cuisine: The Gourmet Guide to Edible Insects*. Rochester, Vermont : Park Street Press, 160 p. ISBN-13: 978-0892817474.

Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, p. 1-22.

Rozin, P., Fallon, A. E. 1987. A perspective on disgust. *Psychological review*, vol. 94, no. 1, p. 23-41. https://doi.org/10.1037/0033-295X.94.1.23 PMid:3823304

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MICROSCOPIC FUNGI ISOLATED FROM DIFFERENT SLOVAK GRAPE VARIETIES

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ABSTRACT

The aim of this study was to isolate and identify microscopic fungi in different grape samples. We collected 13 grapes varienties samples (9 white and 4 red) from local Slovak winemakers in the end of the September 2017. Used 13 grape samples in this study: Alibernet, Irsai Oliver, Dornfelder, Blue Frankish, Feteasca regala, Green Veltliner, Pálava, Müller Thurgau, Rhinriesling, Cabernet Savignon, Pinot Blanc, Savignon Blanc and Welschriesling. Microscopic fungi in grape samples were detected on Malt extract agar by spread plate method. The number of microscopic fungi ranged from 2.85 log cfu.g^{-1} in Cabernet Savignon to 4.83 log cfu.g^{-1} in Feteasca regala. A total of 627 isolates of microscopic fungi were obtained in this study. The most abundant fungi belonged to genera *Alternaria* and *Penicillium* (100% frequency). The high frequency was also detected for *Aspergillus* (76.92%) and *Cladosporium* (76.92%) but with lesser relative density. *Alternaria* sp., *Aspergillus niger*, *Aspergillus* sp., *Botrytis cinerea*, *Cladosporium* sp., *Penicillium expansum*, *Phoma* sp., *Rhizopus* sp. and *Trichoderma* sp. species were isolated from grape berries.

Keywords: grape; microscopic fungi; *Alternaria; Penicillium*; the Lesser Carpathian region

INTRODUCTION

Grape berries are colonized by a complex microbial ecosystem, which consists of epiphytic microorganisms represented by bacteria, yeast, and filamentous fungi **(Barata et al., 2012)**. This microflora plays a major role in crop health and in winemaking process, affecting the wine quality, as reported by **Barbe et al. (2001), Nisiotou et al. (2011)** and **Verginer et al. (2010)**. The filamentous fungi and yeast of grapes has been intensevily studied due to the impact on wine quality **(Pretorius, 2000)**. Research has also covered the pathogenic fungi affecting grapes, including *Erysiphe necator* (the causal agent of grapevine powdery mildew), *Botrytis cinerea* (gray rot) and *Plamospara viticola* (downy mildew). However, saprophytic molds, like *Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp. are also responsible for grape rots. They also were involved in food poisoning by their mycotoxin production **(Martin set al., 2014)**.

During the harvest period grapes were affected by insects, yeasts, or bacteria. The most damaging form of attack is linked to gray mold, *Botrytis cinerea*, eventually associated with various fungi and typical for temperate climate **(Ribereau-Gayon et al., 1998)**.

A variety of grapevines are grown in specific wine producing regions, which results in high variability of physical and chemical characteristics of grapes, and the wines produced thereof **(Abe et al., 2007)**. The grapes may be susceptible to infection by filamentous fungi from the initial stages of maturation **(Bau et al., 2005)**. The filamentous fungi are able to produce an enzyme complex responsible for the degradation of specific substrates, production of secondary metabolites and volatile compounds **(Medina et al., 2015)**. Grapes contamination by fungi may promote the productions of mycotoxins, which may develop at pre-harvest or during the harvesting leading to vinification **(Freire et al., 2017)**.

Identification of filamentous fungi of post-harvest fruits and their storage environments, lifestyle and pathogenicity are essential to develop strategies to prevent and control thier distribution **(Narayanasam, 2006)**. This information is important to understand the fungal contamination of withered grapes. Previous investigations indicated that the fruit-drying rooms showed the diversity of several fungal groups. Notably that intraspecific variability in the ability to infect grapes under different withering conditions was found **(Lorenzini et al., 2015; Lorenzini and Zapparoli, 2014a, 2014b, 2015)**.

The aim of our study was to identify the filamentous microscopic fungi on the grape berries.

Scientific hypothesis

The scientic hypothesis of this study was that the grapes were colonized with different microscopic fungi species, which could be identified with MALDI-TOF method.

MATERIAL AND METHODOLOGY

Grape samples collection

Threeteen grape samples from 2017 year were used in this experiment. Ripe grape bunches were collected into sterile polyethylene bags and transported to laboratory for the next microbiological analysis. The grape samples were collected from the Lesser Carpathian wine region (n = 13). We investigated grape samples of the following varieties: Alibernet, Irsai Oliver, Dornfelder, Blue Frankish, Fateasca reagla, Green Veltliner, Pálava, Mūller Thurgau, Rheinriesling, Cabernet Savignon, Pinot Blanc, Savignon Blanc and Welschriesling. Eache grape was one sample.

Characterisation of „Lesser Carpathian" wine region

The Lesser Carpathian wine region is located in the southwestern part of Slovakia. Vines are grown for more than three thousand years on southern, southwestern and south-eastern slopes and plains of the Lesser Carpathians and in locality Záhorie. Geological substrate is predominantly formed by detrial cones of Lesser Carpathian rivers, soils are silty sands, medium skeletal, in the peripheral parts eventually drifting sands. Vineyards are covering 5 588 hectares within 132 specified regions. Wine from the Lesser Carpathian wine region is a product obtained exclusively by a total or partial fermentation of grapes or grape must, which originates in this region. Grapes are rich in high sugar content, wines are full bodied, with intensive taste and pleasant level of acidity, suitable for longer cellar maturation. Lesser Carpathian wine region has continental climate. The total volume of rainfall is 650 mm distributed fairly evenly throughout the year. Altitude of vineyards in the area is from 100 to 250 metres above sea level. Average air temperature from May to September ranges from 13 °C to 20 °C and is 17.5 °C in growing season. Average annual sunshine duration is 2100 hours, the sum of active air temperature during vegetation is at least 3000 °C. This area is characterized by at least 15 °C temperature difference between day and night during the vegetation, with skeletal base allowing the grapes to produce white varieties of higher acidity, while malolactic fermentation is eliminated to occur when the grapes are still on plants.

The vineyards are mainly trained on medium or high techniques of vine training system. There is up to 10 000 vines/hectare density of vineyard. Number of vine buds shall not exceed 80 000 per one hectare of vineyard for production of wine, quality wine, sparkling wine of wine region, grower's sparkling wine or liqueur wine. Maximum of 65 000 vine buds for the production of quality wines with attribute. Treatment of the white grapes varieties during processing is very fast and tactful, white wines are typically with higher acidity, wine is extracted with optimum ratio of sugars and acids. Production of white wines in the Lesser Carpathian wine region is done in reductive way, without or with only minimal access of air. Temperature is controlled during fermentation and it does not exceed 15 °C. Controlled fermentation involves possible use of indigenous or commercial preparations of isolated strains of yeast *Saccharomyces cerevisiae*. Sulfur dioxide is used as a chemical preservative.

Microbiological analyses of grape berries samples

Five gram of berries from each grape variety were diluted in 45 ml sterile physiological saline (0.85%) and stirred on a horizontal shaker for 30 minutes. The suspension was used for preparation of dilutions of 10^{-2} and 10^{-3} and 0.1 ml of each dilution (10^{-2}, 10^{-3}) was plated onto Malt extract agar (base, Oxoid, UK supplemented with bromocresol green (0.020 g/l), Centralchem®, Slovakia). Microscopic fungi were cultivated at 25 °C for five days in aerobic conditions and identified to species level according to the manuals of **Samson et al. (2002a)**, **Samson and Frisvad (2004)**, **Pitt** and **Hocking (2009)**.

The obtained results were evaluated and expressed according to isolation frequency (Fr) and relative density (RD). The isolation frequency (%) is defined as the percentage of samples within which the species or genus occurred at least once. The relative density (%) is defined as the percentage of isolates of the species or genus, occurring in the analyzed sample **(Guatam et al., 2009)**. These values were calculated according to **González et al. (1999)** as follows:

Fr (%) = (ns / N) x 100;

RD (%) = (ni / Ni) x 100

Where: ns – number of samples within a species or genus; N – total number of samples; ni – number of isolates of species or genus; Ni – total number of isolated fungi.

RESULTS AND DISCUSSION

Filamentous fungi are the main pathogens of post-harvest fruits and can cause heavy economic losses. The type of fruit, maturity stage, pre-harvest and storage conditions are known to affect the fungal contamination and growth of saprophytic microorganisms (**Narayanasam, 2006**).

Numbers of microscopic fungi in grape beries varietes isolated are shown in Table 1. The number of microscopic fungi ranged from 2.85 log cfu.g^{-1} in Cabernet Savignon to 4.83 log cfu.g^{-1} Feteasca regala.

A total of 627 isolates of microscopic fungi were obtained in this study. The most abundant moulds belonged to genera *Alternaria* and *Penicillium* and their frequency comprised 100%. The higher frequency was also detected for *Aspergillus* (76.92%) and *Cladosporium* (76.92%) but with lesser relative density. Table 2 shows the fungal isolates from grape berries.

Felsöciová et al. (2017) isolated a total of 1377 cultures of microscopic fungi and the most abundant moulds were *Alternaria, Cladosporium* and *Penicillium*. The frequency found was similar to those in our study (100%). The higher frequency was detected for *Fusarium* (100%), *Epicoccum*, *Rhizopus* (87.5%), *Botrytis*, *Aspergillus* (75%) and *Mucor* (62.5%) but with lesser relative density. Authors found different genera of fungi with higher frequency in comparison with our study.

The *Aspergillus, Botrytis* and *Penicillium* strains were identified on species level and the isolation rate for *Aspergillus* was 76.92% but the relative densiy was low (20.42%, Table 2). Figure 1 shows the isolated microscopic filamentous fungi species.

Table 1 Number of microscopic filamentous fungi in grape varieties (log cfu.g^{-1}).

Grape variety	average	SD %
Alibernet	4.70	0.12
Blue Frankish	4.69	0.17
Cabernet Savignon	2.85	0.10
Dornfelder	4.15	0.03
Feteasca regala	4.83	0.07
Green Veltliner	4.63	0.02
Irsai Oliver	4.61	0.10
Mūller Thurgau	4.28	0.21
Pálava	4.09	0.02
Pinot Blanc	4.29	0.04
Savignon Blanc	4.32	0.07
Rheinriesling	4.41	0.05
Welschriesling	4.31	0.04

Table 2 Fungi identified in Slovak grape beries.

Fungal taxa	No.	Fr	RD
Alternaria	253	100.00	40.35
Aspergillus	128	76.92	20.42
Botrytis	142	53.85	22.65
Cladosporium	22	76.92	3.51
Penicillium	35	100.00	5.58
Phoma	12	15.38	1.91
Rhizopus	15	38.46	2.39
Trichoderma	20	15.38	3.19
Total isolates	627		

Note: No. – number of isolates, Fr – isolation frequency, RD – relative density.

B. cinerea, the fungus responsible for gray mold, was the most recurrent species isolated every year on grapes containing geosmin from all samples sites. This fungus is responsible for gray mold on many fruits, and notably grapes **(La Guerche et al., 2005)**. Several species of *Penicillium* were found in association with *B. cinerea: P. expansum, P. thomii, P. purpurogenum, P. glabrum, P. brevicompactum* and *P. carneum*. This fungal genus has already been described on grapes **(Abrunhosa et al. 2001)** and is responsible for blue mold.

In our study, 8 genera and 9 species of microscopic filamentous fungi were found. The grape rotting and spoilage can be caused by a variety of fungal species, including *Penicillium, Aspergillus, Alternaria, Cladosporium* and *Rhizopus*. *Aspergillus* and *Alternaria*, followed by *Penicillium*, were the most frequently reported genera on grapes. The genus *Penicillium* was more frequently found in temperate and cold climates typical for northern Europe, whereas *Aspergillus* was more frequently associated with warmer and wetter regions **(Serra et al., 2006)**.

Mikušová et al. (2010) identified the fungi in grapes of three out of six the most important Slovakia wine making areas – Small Carpathian, Nitrian and South Slovakian in harvest year the 2008. *Cladosporium*, *Epicoccum*, *Rhizopus*, *Ulocladium*, *Trichoderma* and *Trichothecium* were identified in range of 1 – 4%. The genera *Aspergillu*s (11.4%), *Fusarium* (11.4%), *Penicillium* (29.7%) and *Alternaria alternata* (14.8%) were considered to be predominant among the toxigenic fungi. These genera were the most frequently distributed also in our study, but the incidence was significantly higher and ranged from 75% to 100%. The results of **Mikušová et al. (2010)** showed that the relative density was lower and did not exceeded 2%, while this reached the limit of 34.3% in our study.

There were comparatively few species of *Penicillium* and *Aspergillus* identified in our research compared to those recorded previously during the vineyard sampling **(Rousseaux et al., 2014; Sage et al., 2004; Serra et al., 2005)**. The lower species diversity could be an outcome of several factors, such as high osmosis, low temperature and reduced water activity during withering. This could favour the selectivity of certain species and the prevalence of *Aspergillus* species in section *Nigri* and *Penicillium* in raisins and sun-dried grapes in relation to their growth response to water activity and temperature has been documented **(Romero et al., 2007; Valero et al., 2005)**.

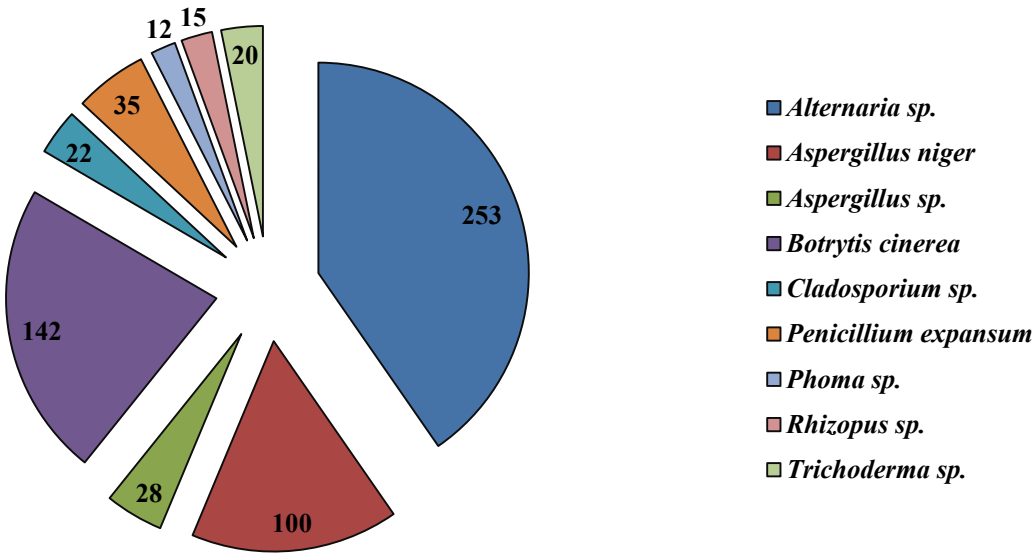

Figure 1 Number of isolated species of microscopic filamentous fungi.

Filamentous fungi were the main pathogens of withered grapes destined for passito wine production.**(Lorenzini et al., 2016)**.

The high prevalence of fungi from genera *Penicillium, Alternaria, Aspergillus* and *Botrytis* on withered grapes is the result of their generally high incidence on grapes in vineyards **(Rousseaux et al., 2014)**. The occurrence of common saprophytic fungi and their capacity to colonize berries change the fruit-drying room environment as compared with the field conditions, influencing the withering process **(Mencarelli and Tonutti, 2013)**.

Beside the most common necrotrophic-saprophytic species of *Penicillium, Aspergillus, Alternaria* and *Botrytis* species responsible for fruit rot, other identified saprobic species, e.g. *Trichoderma atroviride, Sarocladium terricola, Arthrinium arundinis* and *Diaporthe eres*, generally were not associated with post-harvest fruit diseases **(Lorenzini et al., 2016)**.

CONCLUSION

Alternaria and *Penicillium* were the most frequent genera of filamentous fungi found in the Lesser Carpathian wine region. The high frequency (100%) of those species may be attributed to the specific climatic conditions in particular wine-making region and the association with grapes on wineyards.

REFERENCES

Abe, L. T., Da Mota, R. V., Lajolo, F. M., Genovese, M. I., 2007. Compostos fenólicos ecapacidade antioxidante de cultivares de uvas *Vitis labrusca* L. e *Vitis vinifera* L. *Ciência e Tecnologia de Alimentos*, vol. 27, no. 2, p. 394-400. https://doi.org/10.1590/s0101-20612007000200032

Abrunhosa L., Paterson R. R., Kozakiewicz Z., Lima N., Venancio A. 2001. Mycotoxin production from fungi isolated from grapes. *Letters of Applied Microbiology*, vol. 32, no. 4, p. 240-242. https://doi.org/10.1046/j.1472-5x.2001.00897.x

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. https://doi.org/10.1016/j.ijfoodmicro.2004.05.015

Felšöciová, S,. Tančinová, D., Rybárik, Ľ., Mašková, Z. Mycobiota of Slovak wine grapes with emphasis on aspergillus and penicillium species in the south slovak wine region. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 496-502. https://dx.doi.org/10.5219/789

Freire, L., Passamani, F. R. F., Thomas, A. B., Nassur, R. S. M. R., Silva, L. M., Paschoal, F. N., Pereira, G. E., Prado, G., Batista, L. R. Influence of physical and chemical characteristics of wine grapes on the incidence of *Penicillium* and *Aspergillus* fungi in grapes and ochratoxin A in wines**.** *International Journal of Food Microbiology*, vol. 241, p. 181-190. https://doi.org/10.1016/j.ijfoodmicro.2016.10.027

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. https://doi.org/10.5580/104b

González, H. H. L., Martinez, E. J., Pacin, A., Resnik, S. L. 1999. Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxinivalenol occurrence on Argentinian durum wheat. *Mycopathologia*, vol. 144, no. 2, p. 97-102. https://doi.org/10.1023/A:1007020822134

La Guerche, S., Chamont, S., Blancard, D., Dubourdieu, D., Darriet, P. 2005. Origin of (-)-geosmin on grapes: on the complementary action of two fungi, botrytis cinerea and penicillium expansum. *Antonie Van Leeuwenhoek*, vol. 88, no. 2, p. 131-139. https://doi.org/10.1007/s10482-005-3872-4

Lorenzini, M., Zapparoli, G., 2014a. 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. https://doi.org/10.1016/j.ijfoodmicro.2014.06.008

Lorenzini, M., Zapparoli, G., 2014b. An isolate morphologically and phylogenetically distinct from *Botrytis cinerea* obtained from withered grapes possibly represents a new species of Botrytis. *Plant Pathology*, vol. 63, no. 6, p. 1326-1335. https://doi.org/10.1111/ppa.12216

Lorenzini, M., Zapparoli, G., 2015. Occurrence and infection of Cladosporium, Fusarium, Epicoccum and Aureobasidium in withered rotten grapes during post-harvest dehydration. *Antonie Van Leeuwenhoek*, vol. 108, no. 5, p. 1171-1180. https://doi.org/10.1007/s10482-015-0570-8

Lorenzini, M., Cappello, M. S., Zapparoli, G., 2015. Isolation of Neofusicoccum parvum from withered grapes: strain characterization, pathogenicity and its detrimental effects on passito wine aroma. *Journal of Applied Microbiology*, vol. 119, no. 5, p. 1335-1344. https://doi.org/10.1111/jam.12931

Lorenzini, M., Zapparoli, G., 2016. Description of taxonomically unde fined Sclerotiniaceae strain from withered rotten-grapes. *Antonie Van Leeuwenhoek*, vol. 109, no. 2, p. 197-205. https://doi.org/10.1007/s10482-015-0621-1

Lorenzini, M., Cappello, M. S., Logrieco, A., Zapparoli, G. 2016. Polymorphism and phylogenetic species delimitation in filamentous fungi from predominant mycobiota in withered grapes. *International Journal of Food Microbiology*, vol. 238, p. 56-62. https://doi.org/10.1016/j.ijfoodmicro.2016.08.039 PMid:27591387

Mencarelli, F., Tonutti, P. 2013. S*weet, Reinforced and Fortified Wines*. Chichester, UK : John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118569184

Medina, A., Mateo, R., López-Ocaña, L., Valle-Algarra, F. M., Jiménez, M., 2005. Study of spanish grape mycobiota and ochratoxin A production by isolates of *Aspergillus tubingensis* and other members of *Aspergillus* section Nigri. *Applied and Environmental Microbiology*, vol. 71, no. 8, p. 4696-4702. https://doi.org/10.1128/aem.71.8.4696-4702.2005

Narayanasam, P., 2006. *Postharvest Pathogens and Disease Management*. New York, NY : John Wiley & Sons, Inc., 578 p. ISBN: 9780471751984.

Pitt, J. I., Hocking, A. D. 2009. *Fungi and Food Spoilage*. 3rd ed. London, UK : Springer. 519 p. ISBN 978-0-387-92206-5. https://doi.org/10.1007/978-0-387-92207-2

Ribereau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D. 1998. *Handbook of Enology*, New York, NY : John Wiley & Sons, Ltd., p. 265-321.

Romero, S. M., Patriarca, A., Fernández Pinto, V., Vaamonde, G., 2007. Effect of water activity and temperature on growth of ochratoxigenic strains of *Aspergillus carbonarius* isolated from Argentinean dried vine fruits. *International Journal of Food Microbiology*, vol. 115, no. 2, p. 140-143. https://doi.org/10.1016/j.ijfoodmicro.2006.10.014 PMid:17161486

Rousseaux, S., Diguta, C. F., Radoï-Matei, F., Alexandre, H., Guilloux-Bénatier, M., 2014. Non-Botrytis grape-rotting fungi responsible for earthy and moldy off-fl avors and mycotoxins. *Food Microbiology*, vol. 38, p. 104-121. https://doi.org/10.1016/j.fm.2013.08.013

Samson, R. A., Frisvad, J. C. 2004. Polyphasic taxonomy of *Penicillium subgenus Penicillium:* new taxonomic schemes and mycotoxins and other extrolites. Utrecht: *Centraalbureau voor Schimmelcultures*. 260 p. ISBN 90-70351-53-6.

Samson, R. A., Hoekstra, E. S., Frisvad, J. C., Filtenborg, O. 2002a. *Introduction to food- and airborne fungi*. 6th revised ed. (with some corrections). Utrecht, Netherland : *Centraalbureau voor Schimmelcultures*. 389 p. ISBN 90-70351-42-0.

Sage, L., Garon, D., Seigle-Murandi, F., 2004. Fungal microflora and ochratoxin a risk in French vineyards. *Journal of Agricultural and Food Chemistry*, vol. 52, no. 18, p. 5764-5768. https://doi.org/10.1021/jf049497c

Serra, R., Lourenço, A., Alipio, 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. https://doi.org/10.1016/j.mycres.2006.05.010

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. https://doi.org/10.1016/j.resmic.2004.12.005

Valero, A., Marìn, S., Ramos, A. J., Sanchis, V., 2005. Ochratoxin A-producing species in grapes and sun-dried grapes and their relation to ecophysiological factors. *Letters in Applied Microbiology*, vol. 41, no. 2, p. 196-201. https://doi.org/10.1111/j.1472-765x.2005.01705.x

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THE MORPHOLOGICAL AND ANTIOXIDANT CHARACTERISTICS OF INFLORESCENCES WITHIN WILD-GROWING GENOTYPES OF ELDERBERRY (*SAMBUCUS NIGRA* L.)

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ABSTRACT
The aim of this study was to determine the basic morphological characteristics (weight, length) and antioxidant activity (using DPPH method) of elderberry (*Sambucus nigra* L.) inflorescences as well as some elderberry-derived food products prepared from fresh (honey, alcoholic extract, tea infusions) and dried inflorescences (syrup). For the study of problematic, it was used 113 wild-growing genotypes of elderberry from 56 locations in Slovakia growing at an altitude of 98.15 – 712.32 m. The weight of fresh inflorescences ranged from 0.45 to 57.59 g (75.65% coefficient of variation value), the total length of inflorescences ranged from 19.0 to 282.0 mm (22.42%), the length of inflorescence´s stems from 9.0 to 197.0 mm (31.51%), a number of petals predominated pentanumerous petals. Variability in primary and secondary branching reported a low to high degree of variability among as well as within the genotypes. Results showed significant differences in the shape of inflorescences and the colour of flowers among each genotype. Antioxidant activity by DPPH method in elderberry inflorescence water extract was between 85.12 and 89.29%. Activation of tea infusions and beverages was ensured using a mechanism Kalyxx based on galvanic effect. In beverages made from 10% diluted honey prepared from fresh inflorescences in the carbohydrate-based extract, anti-radical activity was determined in the range of 16.81 – 24.16%. In an alcoholic extract from fresh inflorescences, anti-radical activity was between 90.99 and 93.16%. In beverages acquired from the syrup of flowers, we identified antioxidant activity ranging from 37.92 (10%) to 62.82% (40%). Results indicated that elderberry inflorescences and elderberry-derived food products can be attractive to consumers and in future can increase the assortments of healthy products.

Keywords: elderberry-derived products; tea infusions; syrup; honey; beverages; DPPH radical

INTRODUCTION
Elderberry (*Sambucus nigra* L.) grows freely throughout Europe. All parts of this plant i.e. bark, roots, leaves, flowers and fruits have medicinal properties, and therefore they are economically used primarily in food, pharmaceutical and cosmetic industry **(Hejný, 2001)**. In traditional culinary practice, it used fresh and dried inflorescences to prepare tea infusions, syrups, alcohol extracts and other products **(Grieve, 1971; Miraj, 2016; Tarko et al., 2015)**. The inflorescences are commonly used as tea infusions, giving a very common refreshing drink in Northern Europe and the Balkans. In Europe, these plant parts are made into a syrup, which is diluted with water before drinking **(Jørgensen et al., 2000; Miraj, 2016)** or is possible by fermentation process prepare beverage similarly like wine. In south-western Sweden, it is traditional to make a snaps liqueur flavoured with elderflower. Inflorescences are also used in a mildly alcoholic sparkling elderflower 'champagne' (**Miraj, 2016**).

In Slovakia we can find some elderberry-derived food products particularly in health-food stores (elderberry wines, mixtures of dried flowers and berries for tea infusions, syrups, beverages, sweets, jams and jellies).

The inflorescences consist of aromatic white flowers, which are clustered in umbrella cymes **(Jančovičová, 2011)**. In elderberry upgrows rich inflorescences with flowers almost in one line at the end of twigs **(Paganová, 2001; Atkinson and Atkinson, 2002)**. **Mratinić and Fotirić (2007)** observed five selected clones from the natural populations of elderberry in Serbia and determined that average length of inflorescences ranged from 106.7 to 143.2 mm, the width from 72.8 mm to 158.8 mm. **Kabuce (2006)** also determined the average length of inflorescences 10 – 20 cm. Stems (Pedicels) of inflorescences are generally short, tomentose with two

bracteoles **(Atkinson and Atkinson, 2002)**. **Watson and Dallwitz (2007)** reported small, hermaphrodite, unfrequently diclinous flowers, radial symmetric with 3 – 5 sepals, 3 – 5 gamopetalous petals 3 – 5 mm in length, 3 – 5 free stamens and yellow anthers with 3 coupled styles, which form 3 seeds in the fruit. These authors also documented that small, white or yellow-and-white flowers collected in large, rich, multifloral and flat umbels are characterized by a specific and intense aroma. According to **Grieve (1971)**, fresh flowers have a slightly bitter taste and an unpleasant odour, but by maceration of flowers in distilled water gradually obtained a pleasant aroma. It was also reported that elderberry inflorescences are rich in biologically active compounds.

Elderflowers contain about 3% flavonoids; dominants are the following: rutin, quercetin, isoquercetin, kaempferol, astragalin **(WHO, 2002; EMEA/HMPC, 2007; Ivanišová et al., 2015; Tomašková et al., 2017)**, nicotiflorin **(Hänsel et al. 1994; Willer 1997)**, hyperoside **(Willer, 1997; Fleming, 2000; WHO, 2002)**; from phenolic acids chlorogenic acid and caffeic acid derivates are dominant (3%) **(Fleming, 2000; Wu et al., 2004; Thole et al., 2006)**. Volatile oil (0.03 – 0.14%), high share (65%) of free fatty acids, including among others palmitic acid (share 38%) **(Fleming, 2000)** and linoleic acid, 7% alcanes **(Barnes et al., 2002)** amines (choline, etylamine, isobutylamine, isoamylamine), organis acids (e.g. valeric acid, ascorbic acid, citric acid, malic acid, tartaric acid, fumaric acid, shikimic acid) **(Mikulic-Petkovsek et al., 2016)**, vitamins, mineral compounds (8%) and saccharides were also determined in elderflowers. Numerous other constituent types have been identified, including ethers and oxides, ketones, aldehydes, alcohols and esters **(Toulemonde and Richard, 1983)**.

The consumption of elderberry helps in the prevention and therapy for a number of diseases, such as diabetes **(Gray et al., 2000; Netzel et al., 2005; Fowler, 2010; Bhattacharya et al., 2013; Folmer et al., 2014; Song et al., 2014)**, obesity **(Chrubasik et al., 2008; Christensen et al., 2010; Petruţ et al., 2017)**, elderberry also exhibited antibacterial, antifungal **(Kong, 2009; Hearst et al., 2010; Krawitz et al., 2011; Kinoshita et al., 2012)**, and antitumour activity **(Thole et al., 2006; Pehlivan Karakas et al., 2012)**, immune system stimulation **(Ciocoiu et al., 2010; Groza et al., 2010; Frøkiær et al., 2012)**, protection against UV radiation (**Chen et al., 2012; Jarzycka et al., 2013)**, diuretic and laxative activity **(Beaux et al., 1999; Picon et al., 2010)**.

The aim of this study was to determine the basic morphological characteristics (weight, length) and antioxidant activity (using DPPH method) of elderberry inflorescences as well as some elderberry-derived food products prepared from fresh (honey, alcoholic extract, tea infusions) and dried inflorescences (syrup).

Scientific hypothesis

There are significant differences between various genotypes in morphometrics parameters and antioxidant activity of some products from elderberry. Inflorescences are rich in biologically active compounds with strong antioxidant activity and for this reason, can be used for preparing various kinds of products for human nutrition.

MATERIAL AND METHODOLOGY

Biological material

Sambucus nigra inflorescences were collected in 2008 – 2010 from bushes and trees growing within Slovakia localized at an altitude of 98.15 – 712.32 m above the sea level. The inflorescences were picked from bushes with stems (pedicels); the flowers were evaluated for uniformity of the colour and then transported to the laboratory for analysis. The plants were botanically identified in the Institute of Biodiversity Conservation and Biosafety of the Slovak University of Agriculture in Nitra. Samples were marked as SN (*Sambucus nigra*) in morphometric analyses, HSN (elderflower honey), TSN (tea infusion), AE (alcohol extract) in antioxidant analyses and appropriate number.

Morphometrical analysis of elderberry inflorescences

The following properties were measured by morphometrical analysis:

a) Inflorescence weight in g, n = 10 was detected for flower clusters with blossoming flowers by measuring of weight with analytical scales (Kern ADB-A01S05, Germany).
b) Inflorescence totally length in mm, n = 10 measurement was performed from the base to the apical part of the flower clusters and was measured using a ruler.
c) Inflorescence´s pedicel length in mm, n = 5 measurement was performed from the base to the apical part of the flower clusters and was measured using a ruler.
d) Number of petals, n = 5.
e) Number of primary and secondary branches in flower clusters, n = 10.
f) Shape of inflorescence.
g) Compactness of inflorescences and time of entry into the flowering phase.

Antioxidant analysis of elderberry-derived products

Chemicals

All the chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and CentralChem (Slovakia).

Preparation of elderberry inflorescences products

The tested fresh inflorescences and elderberry-derived products were made by a traditional procedure using the processing methods of native inhabitants within Slovakia.

a) Tea infusions were obtained by dried inflorescences and activation using a mechanism Kalyxx based on galvanic effect. From tea infusions, 14 variants with 4 controls were tested.
b) Honey was obtained from fresh inflorescences boiled for one hour in 1 L water, together with one lemon sliced using sucrose with ratio 1 : 1. Next day infusion strained through a gauze and boiled again. Finally, tea infusion was put into the glass and hermetically sealed. Totally 10 variants of honey with 4 controls were tested.

c) Alcoholic extract was prepared from fresh inflorescences poured 40% alcohol and put in dark place. Totally 10 variants of alcohol extracts with 4 controls were tested.

Syrup was produced from fresh inflorescences, lemon slices, 6 pieces mint´s stem, lemon balm and 2 L water mixed, allowed to stand 24 hours with the addition of sucrose with a ratio 1:1 and boiled for 30 minutes. Totally 4 samples of syrup in 4 concentrations were tested.

Activation of tea and beverages

Activation of tea and beverages was ensured using a mechanism Kalyxx – based galvanic effect. Fluid activation can be achieved by simply pouring through the mechanism. In the experiment, it was also provided multiple activations. Double activation was ensured by flushing the fluid through the mechanism device 2-fold, three times 3-fold. Activation is provided by increasing the biopoly of the fluids themselves.

Free radical scavenging activity

Free radical scavenging activity was measured by 2.2-diphenyl-1-picrylhydrazyl (DPPH˙) according to **Brand-Williams et al. (1995)**. An amount of 0.1 mL of sample of juice, wine, syrup, liqueur; sample of fruits conserved by honey, jam, compote and jelly was homogenized in a mortar and 20 g of the blended mass was extracted for 24 hours in 200 mL of distilled water, filtrated (Whatman No. 1) and after filtration the extract was used for measuring. For detection of free radical scavenging activity, the extract was mixed with 3.9 mL of DPPH radical (0.025 g was soluble in 96% ethanol and diluted as needed). Absorbance was registered at 515 nm at regular time intervals until the reaction equilibrium was reached (10 minutes) by using spectrophotometer (Genesys 20 UV-VIS, USA). The DPPH scavenging activity (% inhibition) was calculated to fresh matter (FM) by using of the following equation:

$$\% \; Inh = \frac{A_0 - A_1}{A_0} x \; 100$$

where A_0 – absorbance of control reaction and A_1 – absorbance in presence of the sample.

Statistical analysis

It was evaluated the variability of the test files in each character using descriptive statistics. For the characteristics of the files, it was used the basic descriptors of variability: average, minimum measured value, maximum measured value, the coefficient of variation (%). Data were analysed with ANOVA test and differences between means compared through the Tukey-Kramer test ($\alpha = 0.05$). The degree of variability was determined by the coefficient of variation values. The given parameter is independent of the unit of the evaluated character. Theoretically, they can acquire different values **(Stehlíková, 1998)**. Cluster dendrogram were performed in the free software for scientific data analysis PAST 2.10.

RESULTS AND DISCUSSION

Morphological analysis of elderberry inflorescences

The weight, compactness and uniformity of flowering (simultaneous flowering) of elderflower-clusters belong among considerable characteristics for practical uses in preparing of various teas. In elderberry upgrows rich inflorescences with flowers located on the top of branches and collected in corymbs **(Paganová, 2001; Atkinson and Atkinson, 2002)**.

In the collection of 113 wild-growing elderberry morphological specifics of each genotypes was confirmed and the fresh weight of inflorescences was determined: it was in the range from 0.45 (SN-77) to 57.59 g (SN-34). Genotypes with high inflorescences weight also provide their high-quality for practical uses. The average coefficient of variation was 75.65%, which shows a very high degree of variability (Table 1).

In addition to the harvesting and drying of the flowers, the length of inflorescences is also important. In the evaluated collections, it was determined the length of the flower clusters ranged from 19.0 (SN-13) to 282.0 mm (SN-51). The average coefficient of variation was 22.42%, which shows a high degree of variability.

Mratinić and Fotirić (2007) in their study of five selected clones from the natural populations of elderberry in Serbia, determined the average length of inflorescences in the range of 106.7 – 143.2 mm, the width from 72.8 to 158.8 mm. **Kabuce (2006)** determined average length of inflorescences in the range of 100 – 200 mm. Thus, the Slovak populations are more variable than the Serbian and Lithuanian ones, on this characteristic.

Flowers aggregated in inflorescences – cymes, corymb and umbels in panicles. Inflorescences are terminal, large, repeatedly branched, compound, flat-topped **(Watson and Dallwitz, 2007)**. The pedicels of inflorescences are generally short, tomentose, with two bracteoles **(Atkinson and Atkinson, 2002; Watson and Dallwitz, 2007)**.

For the evaluation the length of a pedicel, it was determined the range from 9.0 (SN-04) to 197.0 mm (SN-55). The average coefficient of variation was 31.50%, which shows a very high degree of variability.

Watson and Dallwitz (2007) reported small,

Table 1 The variability of some morphometric parameters of inflorescences *Sambucus nigra* L.

Characteristics	*Unit*	*n*	*min*	*max*	*mean*	*V%*
Inflorescences weight	g	113	0.45	57.59	9.54	75.65
Inflorescences length	mm	113	19.0	282.0	171.66	22.42
Pedicel inflorescences length	mm	113	9.0	197.0	75.11	31.51
Number of petals	pcs	113	4.0	8.0	5.07	10.36
Primary branching	pcs	64	2.0	8.0	4.95	9.58
Secondary branching	pcs	64	1.0	14.0	6.10	87.0

Note: n – the number of measurements; min, max – minimal and maximal measured values; mean – arithmetic mean; V% – coefficient of variation.

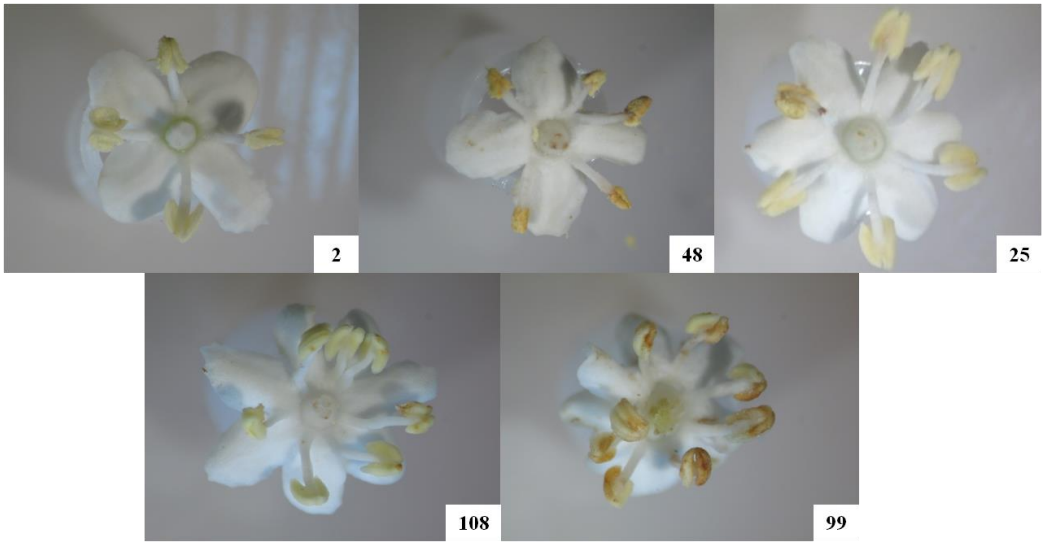

Figure 1 The variability of petal's number in collection of *Sambucus nigra* genotypes from wild-growing populations in Slovakia.

hermaphrodite, unfrequently diclinous flowers, radial symmetric with 3 – 5 sepals, 3 – 5 gamopetalous petals 3 – 5 mm in length, 3 – 5 free stamens and yellow anthers with 3 coupled styles, which form 3 seeds in the fruit **(Paganová, 2001; Atkinson and Atkinson, 2002)**. A small part of flowers is also tetramerous **(Skalická, 2001; Paganová, 2001)**.

Also, the observation on collection of genotypes indentified other aspects of investigation these plants. The average number of petals for flowers of different genotypes ranged from 4 to 8. The average coefficient of variation was 10.36%, which shows a medium degree of variability. For the great majority of flowers there were determined pentamerous flowers, less frequently hexamerous and tetramerous ones (Figure 1).

In the collection of 64 wild-growing elderberry genotypes it was evaluated primary and secondary branching. It was identified in the range from 2 to 8 branches in primary branching and from 1 to 14 branches in secondary one. It was not identified variability in the primary branching of inflorescences in 44% of genotypes. Low degree of variability (9.58%) was determined in primary branching and, conversely very high degree of variability (87.0%) in secondary ones.

The analysis of coefficient of variation showed the difference of variability of morphometrical characters between *Sambucus nigra* genotypes (Figure 2). Data showed that the most variable parameters were the following: inflorescences weight – from 11.56 to 77.32%, panicle of inflorescences length – from 7.02 to 71.96%, secondary branching – from 0.0 to 61.43%, inflorescences length – from 2.31 to 49.55%. These results indicate the promise of breeding in this way of investigations. The more stable signs are the following: primary branching – CV from 0.0 to 17.29% and a number of petals – CV from 0.0 to 23.96%.

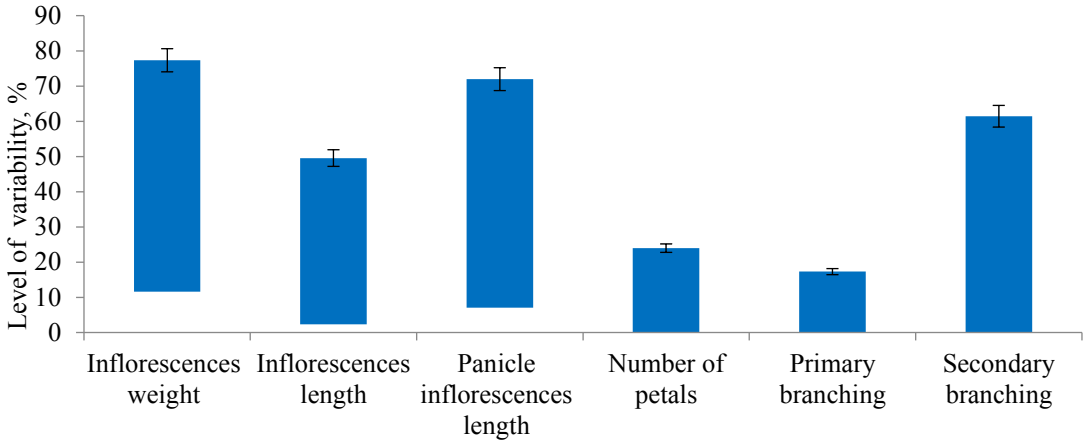

Figure 2 Level of the variability of morphometric parameters of *Sambucus nigra* L. (%).

The 6 characteristics of the 113 genotypes *Sambucus nigra* were used in clustering, and the resulting clusters are presented in Figure 3. The *Sambucus nigra* genotypes were divided into four clusters. Cluster III and cluster IV contained the largest number of genotypes. Cluster I and cluster II contained the 4 and 3 genotypes, which differ from other genotypes of collection by all parameters.

Significant differences between genotypes have been identified in the shape, size, colour of the inflorescences

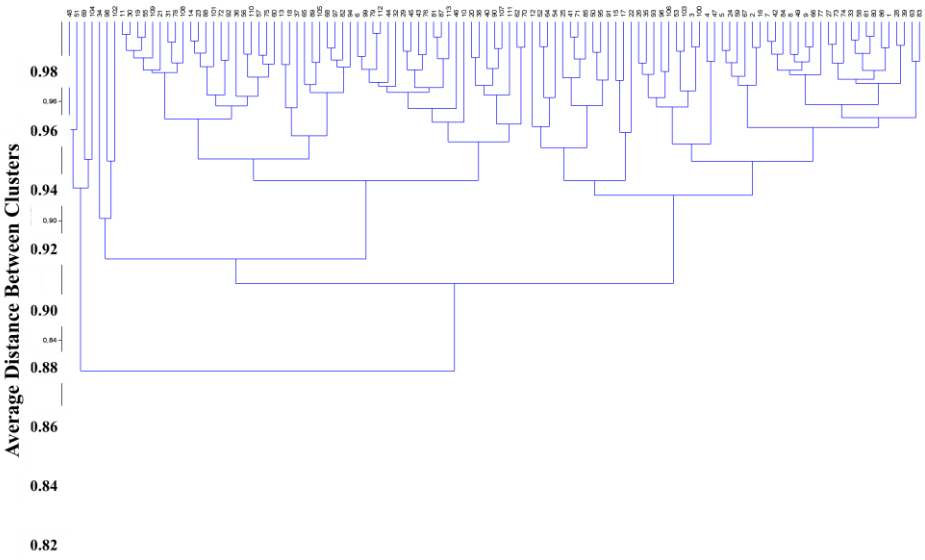

Figure 3 Cluster dendrogram based on morphometrics parameters of *Sambucus nigra* L.

and flowers. This is illustrated in Figure 4. Inflorescences are terminal, large, repeatedly branched, compound, flat-topped, umbelliform corymbs and cymes **(Watson and Dallwitz, 2007)**. They consist of aromatic white flowers, which are grouped in umbrella cymes **(Jančovičová, 2011)**. For the great majority of inflorescences there are determined creamy white flowers. The inflorescences were characterized by a more compact as well as a reduced arrangement of flowers. Our results were in accordance to **Pišťanková (2002)**, which also found medium-sized, more compact clusters with creamy white medium-sized flowers in the variety Bohatka. Accordingly, in the variety Dana, the inflorescences are characterized as big, with smaller creamy white flowers, flower clusters are semi-loose. For this reason, the cultivation of elderberry in the monoculture **(Waźbińska and Puczel, 2002)** and in the landscaping **(Waźbińska, 2000)** began to grow in many European countries.

Antioxidant activity

Antioxidant activity of tea infusions

Sambucus nigra is one of the main sources of biologically active substances for various practical uses. Elderflowers are particularly useful for the preparation of syrups and other traditional and innovative beverages. Measurement of the antioxidant activity of activated tea infusions from dried inflorescences was determined on 14 samples received from variants of dried inflorescences by pouring boiling water.

Variant TSN-01 was a control sample without activation by Kalyxx (Figure 3). Other samples were successively activated from 1 to 13 times. When assessing tea infusion samples it was determined antioxidant activity in the range

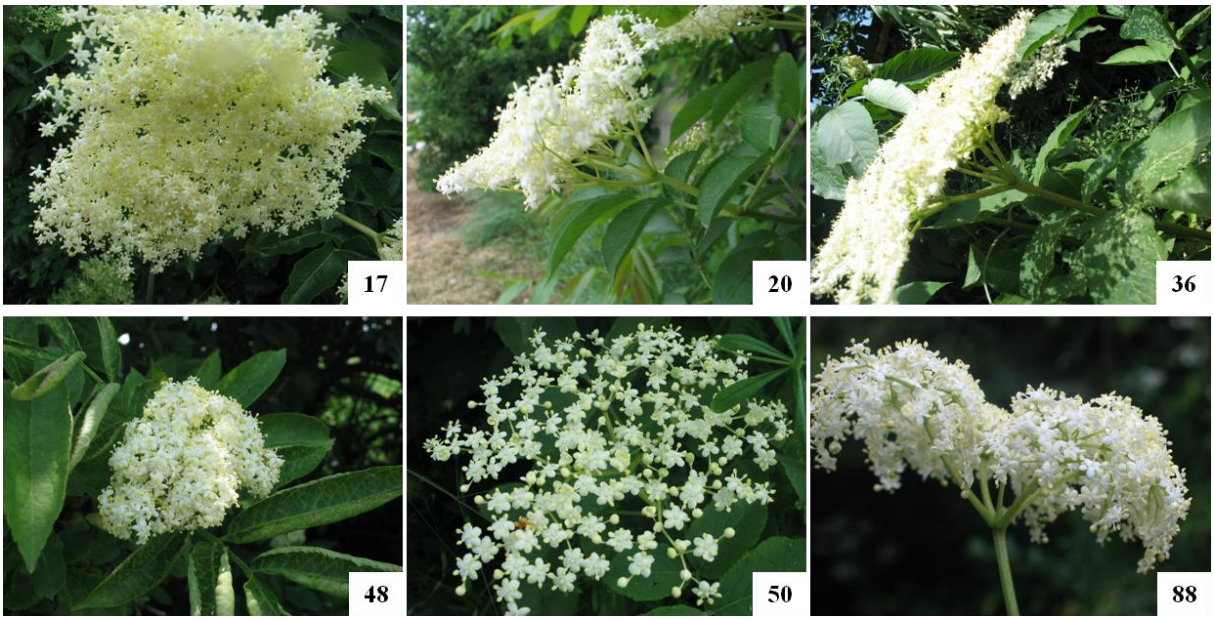

Figure 4 The comparison of selected genotypes of elderberry (*Sambucus nigra* L.) in the shape and size of inflorescences (*Sambucus nigra* L.) from wild-growing populations in Slovakia.

from 85.12 (TSN-10) to 89.29% (TSN-04). Compared results with the control variant were screened the presence or slightly reduced of anti-radical activity. It wasn't detected statistically significant differences among variants ($P < 0.05$). Tea infusion samples retained a high degree of anti-radical activity even after multiple activations (Figure 5).

Socaci et al. (2015) determined antioxidant activity from samples of fresh and dried inflorescences using DPPH radical. The capacity ranged from 28.4% (dried inflorescences) to 52.54% (fresh inflorescences) by DPPH inhibition.

Autors **Młynarczyk and Walkowiak-Tomczak (2017)** determined the antioxidant activity of fresh and dried inflorescences by spectrophotometric method using ABTS radical. The fresh inflorescences were characterized by stronger bioactive properties than dried inflorescences.

Diankov and Parlapanska (2013) reported results extraction from dried inflorescences at ambient temperature 50 % ethanol-in-water solution. The best antioxidant capacity (IC50% = 6.58) was measured for the 90 min. extract by DPPH method.

Socaci et al. (2015) determined antioxidant capacity of elder samples of fresh and dried inflorescences using DPPH method. The antioxidant capacity ranged from 28.4% (dried inflorescences) to 52.54% (fresh inflorescences) of DPPH inhibition.

Antioxidant activity of beverages prepared from elderflower honey

In the other experiment in our study it was determined antioxidant activity of activated beverages of dilute/aqueous honey prepared from fresh inflorescences in the saccharide extract. In beverages prepared from 10% honey diluted in 150 mL of water it was determined antioxidant activity in the range from 16.81% (HSN-02) to 24.16% (HSN-10). It was observed an increasing of antioxidant activity in many variants compared to the control variant HSN-K (16.18) (Figure 6). The enlarging of activation positively correlates with an increasing in antioxidant activity. That means that traditionally prepared products from inflorescences are a significant source of antioxidants.

The antioxidant activity of diluted beverages from alcohol extracts

Very high antioxidant activity was determined in the test of the multiple activated alcohol extracts of fresh

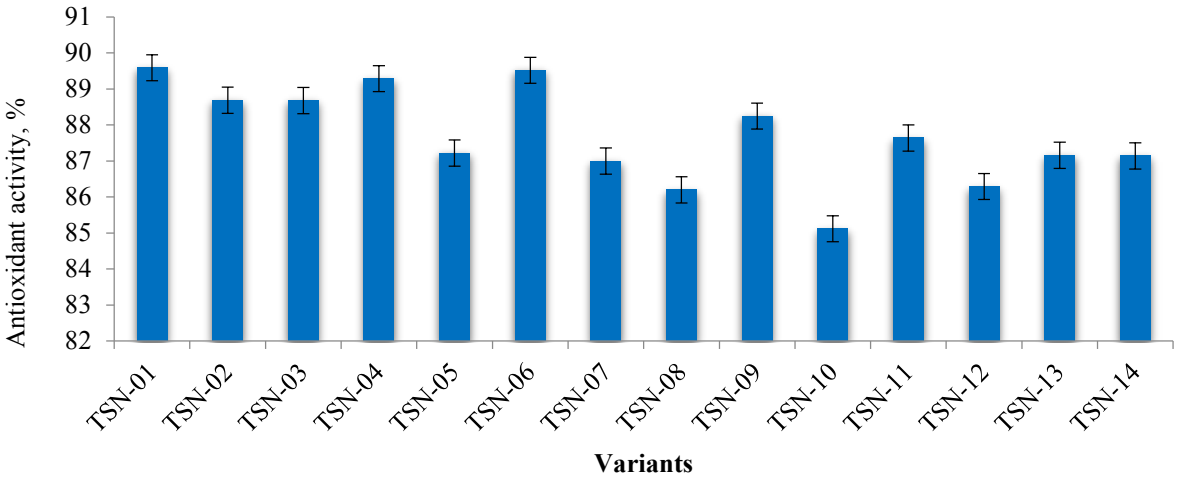

Figure 5 Antioxidant activity of tea infusions prepared from dried inflorescences of elderberry.

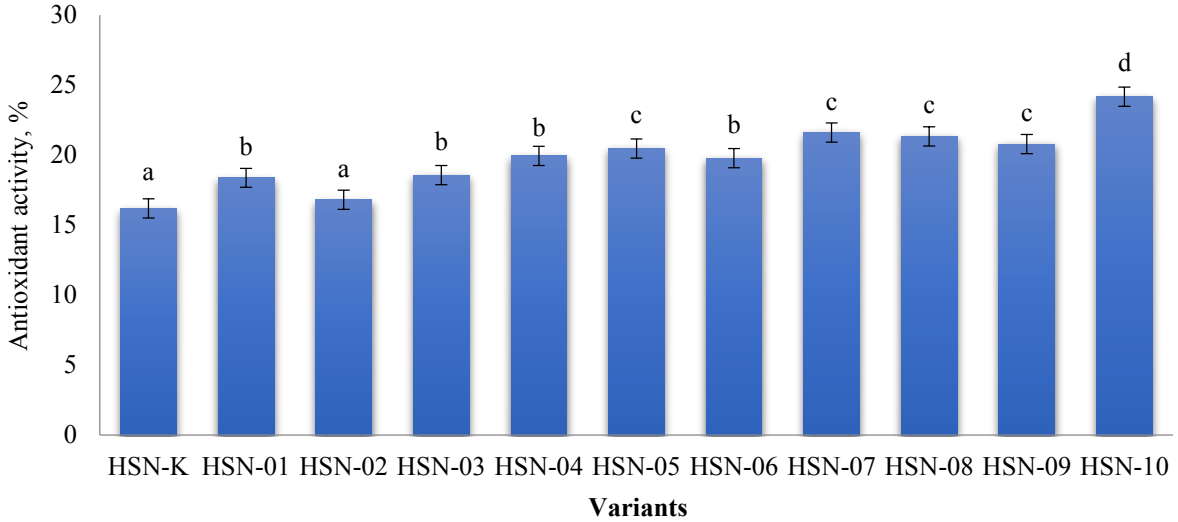

Figure 6 Antioxidant activity of the multiple activated beverages prepared from aqueous „elderflower honey" (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD)).

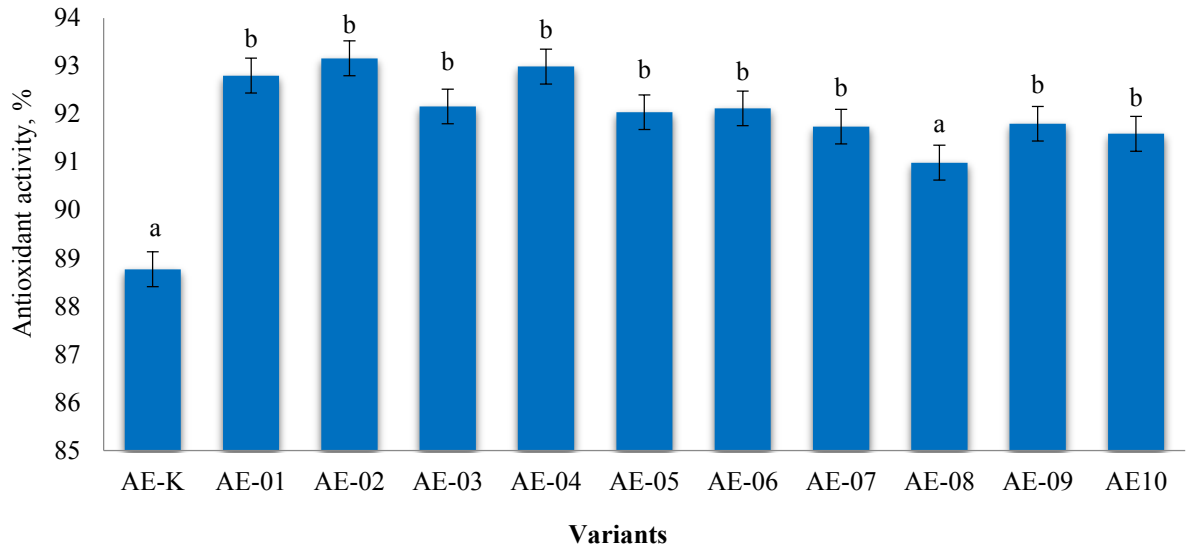

Figure 7 The variability provided antioxidant activity of the multiple diluted beverages prepared from alcohol extract (AE) of fresh inflorescences of elderberry (means in columns followed by different letters are different at $p = 0.05$. Each value represents the mean of three independent experiments (±SD)).

Table 2 Statistical characterization of variability of antioxidant activity of beverages prepared by increasing the concentration of syrup from inflorescences of elderberry in aqueous medium (SS).

Concentration %	*n*	*mean*	*V%*
10	4	37.920a	5.851
20	4	55.193b	1.911
30	4	62.643c	1.681
40	4	62.823c	6.745

Note: n – number of evaluated inflorescences; mean – average of file; V% – coefficient of variation.

inflorescences in alcohol (Figure 7). In the control variant of the alcohol extract without activation it was determined the 88.77% antioxidant activity. The antioxidant activity was increased in the range of 91.59 (AE-10) to 93.16% (AE-02) by activation, in addition to the 8-fold activated variant.

The presented results expressly demonstrate that inflorescences of elderberry are important sources of antioxidants that are clearly apparent in both aqueous and alcohol environment, in both cold and cooked beverages.

The antioxidant activity of elderflower syrup

In our study it was also determined the antioxidant activity of beverages prepared from the syrup of elderflower inflorescences. In accordance with the methodology there were prepared 4 variants of beverages from prepared syrup by extracting fresh inflorescences in the saccharide solution by increasing the syrup concentration in 100 mL of drinking water at room temperature +23°C. This method was used for preparing beverages in a concentration of 10 to 40% (Table 2). The results showed that average antioxidant activity was found in the range from 37.92 (10%) to 62.82% (40%). Simultaneously the results documented that increasing the syrup concentration in beverages also increased antioxidant activity.

Halvorsen et al. (2002) determined an antioxidant capacity of 4.31 mmoL.100g^{-1} using the ferric reducing antioxidant potential (FRAP) method. In another study it was detected scavenger activity of inflorescences extract on the two radicals in concern: DPPH and OH•, characterized the extract as a more potent scavenger with respect to OH• radicals – the IC50 value for OH• was determined to be 0.0122 µg.mL^{-1} compared to IC50 value of the 0.152 µg.mL^{-1} for DPPH **(Stoilova et al., 2007)**.

The results obtained in our study confirmed that both fresh and dried elderberry flowers recorded significant antioxidant activity and they can serve as a good source of bioactive compounds in human diet or as functional ingredients in different foods. Antioxidant activity of extracts from dried elderberry flowers is comparable to those obtained for other medicinal plants, reported in the literature **(Karcheva et al., 2010)**.

CONCLUSION

Based on the survey data and the evaluation of 113 selected genotypes from the wild-growing populations of *Sambucus nigra* within Slovakia there was determined significant variability in all evaluated morphometrical characteristics of inflorescences. The weight of inflorescences was in the range from 0.45 to 57.59 g, the length of the flower clusters ranged from 19.0 to 282.0 mm, the length of a pedicel was determined the range from 9.0 to 197.0 mm. The analysis of coefficient of variation showed the difference of variability of morphometrical characters between *Sambucus nigra* genotypes. Data showed that the most variable parameters were the

following: inflorescences weight – from 11.56 to 77.32%, panicle of inflorescences length – from 7.02 to 71.96%, secondary branching – from 0.0 to 61.43%, inflorescences length – from 2.31 to 49.55%.

These findings document that in natural populations of elderberry in Slovakia it is possible to detect genotypes with required economic characters for practical use. The findings have confirmed that both fresh and dried inflorescences used in elderberry-derived food products have high antioxidant activity and they can be used as a significant natural source of bioactive components in human diet or as functional components of various foods or nutritional supplements. Inflorescences of elderberry are also widely used for therapeutic purposes, which can be obtained by simple and traditionally proven methods and recipes in the form of natural extracts, essential oils, syrups, concentrates and other forms.

REFERENCES

Atkinson, M. D., Atkinson, E. 2002. *Sambucus nigra* L. *Journal of Ecology*, vol. 90, p. 895-923. https://doi.org/10.1046/j.1365-2745.2002.00698.x

Barnes, J., Anderson, L. A., Phillipson, D. J. 2002. *Herbal Medicines*. 2nd ed. London, UK : The Pharmaceutical Press, 187 p. ISBN 978-0-85369-623-0.

Beaux, D., Fleurentin, J., Mortier, F. 1999. Effect of extracts of *Orthosiphon stamineus* Benth, *Hieracium pilosella* L., *Sambucus* L. and *Arctostaphylos uva-ursi* (L.) Spreng. in rats. *Phytotherapy Research*, vol. 13, no. 3, p. 222-225. https://doi.org/10.1002/(SICI)1099-1573(199905)13:3<222::AID-PTR447>3.0.CO;2-P

Bhattacharya, S., Christensen, K. B., Olsen, L. C. B., Christensen, L. P., Grevsen, K., Færgeman, N. J., Kristiansen, K., Young, J. F., Oksbjerg, N. 2013. Bioactive components from flowers of *Sambucus nigra* L. increase glucose uptake in primary porcine myotube cultures and reduce fat accumulation in Caenorhabditis elegans. *Journal Agricultural Food Chemistry*, vol. 61, no. 46, p. 11033-11040. https://doi.org/10.1021/jf402838a PMid:24156563

Chen, L., Hu, J. Y., Wang, S. Q. 2012. The role of antioxidants in photoprotection: A critical review. *Journal of the American Academy of Dermatology*, vol. 67, no. 5, p. 1013-1024. https://doi.org/10.1016/j.jaad.2012.02.009 PMid:22406231

Christensen, K., Olsen, L., Kotowska, D., Bhattacharya, S., Fretté, X. C., Færgeman, N., Kristiansen, K., Oksbjerg, N., Christensen, L. 2010. Elderflowers (*Sambucus nigra* L.) have a significant impact on cellular mechanisms related to lipid storage and insulin resistance. In *58th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research*. Berlin : Germany. p. 1355 (P633).

Chrubasik, C., Maier, T., Dawid, C., Torda, T., Schieber, A., Hofmann, T., Chrubasik, S. 2008. An observation study and quantification of the actives in a supplement with *Sambucus nigra* and *Asparagus officinalis* used for weight reduction. *Phytoterapy Research*, vol. 22, no. 7, p. 913-918. https://doi.org/10.1002/ptr.2415 PMid:18350516

Ciocoiu, M., Serban, D., Bădescu, L., Tutunaru, D., Badulescu, O., Bădescu, M. 2010. The effects of *Sambucus nigra* extract in experimental arterial hypertension. *Annals of the Romanian Society for Cell Biology*, vol. 15, no. 2, p. 87-92.

Diankov, S., Parlapanska, K. 2013. Extraction from Elderberry Flowers. Examination of Antioxidant Capacity and Total Phenolic Content of the Extracts. *Journal of Chemical Technology and Metallurgy*, vol. 48, no. 5, p. 479-482.

EMEA HMPC. 2007. *Sambucus nigra* L., flos – Assessment report for the development of community monographs and for inclusion of herbal substance(s), preparation(s) or combinations thereof in the list [online] s.a. [cit. 2017-10-30] Available at: EMEA/HMPC/283170/2007/Corr.

Fleming, T. 2000. PDR for Herbal Medicines. 2nd ed. Montvale, NJ : Medical Economics Company, 287 p. ISBN-13: 9781563633614.

Folmer, F., Basavaraju, U., Jaspars, M., Hold, G., El-Omar. E., Dicato, M., Diederich, M. 2014. Anticancer effects of bioactive berry compounds. *Phytochemistry Reviews*, vol. 13, no. 1, p. 295-322. https://doi.org/10.1007/s11101-013-9319-z

Fowler, M. J. 2010. Diagnosis, classification, and lifestyle treatment of diabetes. *Clinical Diabetes*, vol. 28, no. 2, p. 79-86. https://doi.org/10.2337/diaclin.28.2.79

Frøkiær, H., Henningsen, L., Metzdorff, S. B., Weiss, G., Roller, M., Flanagan, J., Fromentin, E., Ibarra, A. 2012. Astragalus root and elderberry fruit extracts enhance the IFN-β stimulatory effects of Lactobacillus acidophilus inmurine-derived dendritic cells. *PLoS ONE*, vol. 7, no. 10, p. e47878. https://doi.org/10.1371/journal.pone.0047878 PMid:23118903

Gray, A. M., Abel-Wahab, Y. H. A., Flatt, P. R. 2000. The traditional plant treatment, *Sambucus nigra* (elder), exhibits insulin-like and insulin-releasing actions in vitro. *The Journal of Nutrition*, vol. 130, no. 1, p. 15-20. https://doi.org/10.1093/jn/130.1.15 PMid:10613759

Grieve, M. 1971. *A Modern Herbal*. London, UK : Jonathan Cape Ltd, 888 p. ISBN 9780486227993.

Groza, M., Ciocoiu, M., Bădescu, L., Bădulescu, O., Bădescu, M. 2010. The effects of *Sambucus nigra* vegetal extracts on the immune system dysfunction in the diabetes mellitus. *Annals of the Romanian Society for Cell Biology*, vol. 15, no. 1, p. 241-246.

Hänsel, R., Keller, K., Rimpler, H. 1994. *Hagers Handbuch der Pharmazeutischen Praxis*. *Drogen P-Z*. 5th ed. Band 6. Berlin, Germany : Springer Verlag, 580 p. ISBN 978-3-642-57881-6.

Hearst, C., Mccollum, G., Nelson, D., Ballard, L. M., Millar, B. C., Goldsmith, C. E., Rooney, P. J., Loughrey, A., Moore, J. E., Rao, J. R. 2010. Antibacterial activity of elder (*Sambucus nigra* L.) flower or berry against hospital pathogens. *Journal of Medicinal Plants Research*, vol. 4, no. 17, p. 1805-1809.

Hejný, S. 2001. Sambucus. In Mareček, F. et al. *Garden Dictionary Teaching 5 R-Ž (Zahradnický slovník naučný 5 R-Ž)*. Prague, Czech Republic : Ústav zemědelských a potravinářských informací. p. 174-175. ISBN 80-7271-075-3.

Ivanišová, E., Kačániová, M., Frančáková, H., Petrová, J., Hutková, J., Brovarskyi, V., Velychko, S., Adamchuk, L., Schubertová, Z., Musilová, J. 2015. Bee bread – perspective source of bioactive compounds for future *Potravinarstvo*, vol. 9, no. 1, p. 592-598. https://doi.org/10.5219/558

Jarzycka, A., Lewińska, A., Gancarz, R., Wilk, K. A. 2013. Assessment of extracts of *Helichrysum arenarium*, *Crataegus monogyna*, *Sambucus nigra* in photoprotective UVA and UVB; photostability in cosmetic emulsions. *Journal of Photochemistry and Photobiology B: Biology*, vol. 128, p. 50-

57. https://doi.org/10.1016/j.jphotobiol.2013.07.029 PMid:24007865

Kabuce, N. 2006. NOBANIS – Invasive Alien Species Fact Sheet – *Sambucus nigra*. – From: Online Database of the North European and Baltic Network on Invasive Alien Species – NOBANIS [online] s.a. [cit. 2017-10-30] Available at: http//:www.nobanis.org.

Karcheva, M., Penkina, E., Georgieva, S., Diankov, S. 2010. Comparative study of polyphenols content and antioxidant activity of Bulgarian and exotic plants, UFT Academic Publishing House. In *Proceedings of International Conference on Food Science, Engineering and Technologies*. Bulgaria, p. 375-380.

Kinoshita, E., Hayashi, K., Katayama, H., Hayashi, T. Obata, A. 2012. Anti-influenza virus effects of elderberry juice and its fractions. *Bioscience, Biotechnology and Biochemistry*, vol. 76, no. 9, p. 1633-1638. https://doi.org/10.1271/bbb.120112 PMid:22972323

Kong, F. 2009. Pilot clinical study on a proprietary elderberry extract: Efficacy in addressing influenza symptoms. *Online Journal of Pharmacology and Pharmacokinetics*, vol. 5, p. 32-43.

Krawitz, C., Mraheil, M. A., Stein, M., Imirzalioglu, C., Domann, E., Pleschka, S., Hain, T. 2011. Inhibitory activity of a standardized elderberry liquid extract against clinically-relevant human respiratory bacterial pathogens and influenza A and B viruses. *BMC Complementary and Alternative Medicine*, vol. 11, no. 16. https://doi.org/10.1186/1472-6882-11-16

Mikulic-Petkovsek, M., Samoticha, J., Eler, K., Stampar, F., Veberic, R. 2015. Traditional elderflower beverages: a rich source of phenolic compounds with high antioxidant activity. *Journal Agricultural Food Chemistry*, vol. 63, no. 5, p. 1477-1487. https://doi.org/10.1021/jf506005b PMid:25646848

Miraj, S. 2016. Chemical composition and pharmacological effects of *Sambucus nigra*. *Der Pharma Chemica*, vol. 8, no. 13, p. 231-234.

Młynarczyk, K., Walkowiak-Tomczak, D. 2017. Bioactive properties of elderflowers (*Sambucus nigra* L.). *World Scientific News,* vol. 73, no. 2, p. 115-119.

Netzel, M., Strass, G., Herbst, M., Dietrich, H., Bitsch, R., Bitsch, I., Frank, I. 2005. The excretion and biological antioxidant activity of elderberry antioxidants in healthy humans. *Food Research International*, vol. 38, no. 8-9, p. 905-910. https://doi.org/10.1016/j.foodres.2005.03.010

Paganová, V. 2001. *Fundamentals of dendrology. Autochthonous scraps of wood (Základy dendrológie. Autochtónne krovité dreviny).* Nitra, Slovakia : Slovak University of Agriculture in Nitra, 179 p. ISBN 80-7137-937-9.

Petruț, G. S., Muste, S., Mureșan, C., Păucean, A., Mureșan, A. E., Nagy, M. 2017. Chemical Profiles and Antioxidant Activity of Black Elder (*Sambucus nigra* L.) – A Review. *Bulletin UASVM Food Science and Technology*, vol. 74, no. 1, p. 9-16. https://doi.org/10.15835/buasvmcn-fst:11591

Picon, P. D., Picon, R. V., Costa, A. F., Sander, G. B., Amaral, K. M., Aboy, A. L., Henriques, A. T. 2010. Randomized clinical trial of a phytotherapic compound containing *Pimpinella anisum*, *Foeniculum vulgare*, *Sambucus nigra*, and *Cassia augustifolia* for chronic constipation. *BMC Complementary and Alternative Medicine*, vol. 10, p. 17. https://doi.org/10.15835/buasvmcn-fst:11591

Song, Y., Wu, T., Yang, Q., Chen, X., Wang, M., Wang, Y., Peng, X., Ou, S. 2014. Ferulic acid alleviates the symptoms of diabetes in obese rats. *Journal of Functional Foods*, vo. 9, p. 141-147. https://doi.org/10.1016/j.jff.2014.04.007

Stoilova, I., Wilker, M., Stoyanova, A., Krastanov, A., Stanchev, V. 2007. Antioxidant activity of extract from elder flower (*Sambucus nigra* L.). *Herba Polonica*, vol. 53, no. 1, p. 45-54.

Tarko, T., Duda-Chodak, A., Semik, D., Nycz, M. 2015. The use of fruit extracts for production of beverages with high antioxidative activity. *Potravinarstvo*, vol. 9, no. 1, p. 280-283. https://doi.org/10.5219/480

Thole, J. M., Burns Kraft, T. F., Sueiro, L. A., Kang, Y. H., Gills, J. J., Cuendet, M., Pezzuto, J. M., Seigler, D. S., Lila, M. A. 2006. A comparative evaluation of the anticancer properties of European and American elderberry fruits. *Journal of Medicinal Food*, vol. 9, no. 4, p. 498-504. https://doi.org/10.1089/jmf.2006.9.498 PMid:17201636

Tomášková, L., Sochor, J., Baroň, M. 2017. The study of antioxidants in grapevine seeds. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 132-137. https://doi.org/10.5219/711

Toulemonde, B., Richard, H. M. J. 1983. Volatile Constituents of Dry Elder (*Sambucus nigra* L.) Flowers. *Journal of Agricultural and Food Chemistry*, vol. 31, no. 2, p. 365-370. https://doi.org/10.1021/jf00116a046

Watson, L., Dallwitz, M. J. 1992. The families of flowering plants: descriptions, illustrations, identification, and information retrieval [online] s.a. [cit. 2017-10-24] Available at: delta-intkey.com/angio.

Ważbińska, J. 2000. Alternative plants in gardens and parks in north-eastern Poland (Rośliny alternatywne w uprawach ogrodowych i parkowych północno–wschodniej Polski). *Biuletyn Nauk. UWM Olsztyn*, vol. 8, p. 309-315.

Ważbińska, J., Puczel, U. 2002. Fruit characteristics of the elderbery (*Sambucus nigra* L.) grown on two different soils. *Journal of Fruit and Ornamental Plant Research*, vol. 10, p. 117-128.

Willer, B. 1997. *Untersuchungen zur Antiasthmatischen Wirkung von Sambucus nigra* : dissertation theses. München : University of Regensburg, Faculty Chemistry & Pharmacy. 25 p.

WHO – World Human Organisation. 2002. *Monographs on Selected Medicinal Plants*. Geneva, vol. 2, 269 p.

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OCCURRENCE OF SELECTED METALS IN FEED AND SHEEP´S MILK FROM AREAS WITH DIFFERENT ENVIRONMENTAL BURDEN

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ABSTRACT
The content of selected essential elements and toxic metals in feed and sheep's milk from areas with different parts of Slovak Republic was analyzed. Region of Novoť (undisturbed environment; North Slovakia) and region of Klátova Nová Ves (widely disturbed environment; Western Slovakia) were under investigation. Eleven metals have been analyzed (essential elements - calcium, zinc, selenium, iron, magnesium, copper; toxic elements – arsenic, mercury, lead, cadmium, nickel). Samples of feeds and milk were collected five-times during the year (spring and autumn season). Analyses of samples were performed by certified testing laboratory Eurofins Bel/Novamann (Nové Zámky, Slovak Republic). Analyses were performed by routine methods, according to the valid methodologies. The results showed significantly higher content of selected essential elements in feed in spring season from area with widely disturbed environment (Klátova Nová Ves). Significantly higher content of essential elements in milk was on farm of Novoť (undisturbed environment). Occurrence of toxic metals in feed from area with widely disturbed environment in spring season did not affect their content in milk. It can be concluded, that the use of milk of sheep from these areas for direct use or for dairy products processing is appropriate, safe and poses no health risk for the consumers.

Keywords: sheep's milk; toxic metal; essential element; feed; environment

INTRODUCTION
Milk and dairy products are important components of the human diet. Milk has been described as a complete food because it contains vital nutrients including proteins, essential fatty acids, lactose, vitamins and minerals in balanced proportions. However, milk and dairy products can also contain chemical hazards and contaminants which constitute a technological risk factor for dairy products, for related commercial image and, above all, for the health of the consumer **(Licata et al., 2004)**. The importance of milk in human diet is widely established and its regular consumption is recommended, especially for young children. In recent decades sheep milk has assumed an increasingly important role in human diet, not just for infants but also for adults and especially nursing mothers **(Sanz Ceballos et al., 2009; Kapila et al., 2013)**.

Sheep milk may contain various elements of nutritional or toxicological importance, and their levels can vary according to intrinsic factors (season, feeding and environment). Because the nutritional habit of these small ruminants to graze plants and grass, they may be considered such environmental bio-indicators and their milk is a good matrix to monitor the pollution status. Heavy metals, such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg) whose toxicity is well known **(Llobet et al., 2003)**, are widely dispersed in the environment and their contamination sources are various: grazing animals are exposed to their accumulation by ingestion of water, grass and feed. Studies about the presence of toxic trace elements in sheep milk have been frequently carried out in the area of Middle East, where the livestock of these species is more common **(Sanal et al, 2011; Hilali et al., 2011; Najarnezhadand and Akbarabadi, 2013; Rahimi, 2013)**. From the nutritional point of view, metals contents of milk and dairy products can be grouped into essential elements (calcium, zinc, magnesium, iron, copper, selenium) at low doses and non essential or toxic ones (cadmium, lead, nickel, mercury, arsenic). The presence of the latter, even in low concentrations, is invaluable and leads to metabolic disorders with extremely serious consequences **(Khan et al., 2008)**. Dairy animals ingest metals while grazing on the pasture and when fed on contaminated concentrate feeds.

Toxic metals such as lead and cadmium are common air pollutants and are emitted into the air as a result of various industrial activities **(WHO, 2007; Toman and Tunegová, 2017)**. Various industrial environmental contamination of soil, waters, foods and plants with these metals causes their incorporation into the food chain and imposes a great threat to human and animal health **(Bilandžić et al., 2011)**.

Lead and cadmium residues in milk and dairy products are of particular concern since they are largely consumed by infants and children. Food is the main route of lead and cadmium exposure in the general population (representing >90% of the total Cd intake in non-smokers), although inhalation can play an important role in very contaminated areas **(WHO, 2007; Chovancová et al., 2014)**. Lead and cadmium are considered potential carcinogens and are associated with etiology of a number of diseases in the cardiovascular system, kidneys, nervous system, blood and skeletal system **(Zhuang et al., 2007)**.

Micronutrient elements such as Fe, Cu, Zn, Se and macronutrients Ca, Mg, are essential for many biological functions **(Kazi et al., 2009; Lukačínová et al., 2012)**. Deficiencies of such elements contribute significantly to the global burden of disease; however, if present at higher levels, they can have a negative effect on human health. Both toxicity and necessity vary from element to element **(Kazi et al., 2009)**. The trace element contents of milk and dairy products depends on the stage of lactation, nutritional status of the animal, environmental and genetic factors, characteristic of the manufacturing practices and possible contamination from the equipment during processing **(Cashman, 2011)**.

The presence of heavy metals and trace elements in milk has been reported in different countries and regions **(Simsek et al., 2000; Maas et al., 2011; Temiz et al., 2012; Rahimi et al., 2013)**. Moreover, an additional insight into metal uptake and assessment of human risks associated with the consumption of milk are still needed.

Scientific hypothesis

Contamination of environment with heavy metals and the insidious nature of their adverse ill health effects have become a matter of growing concern. The aim of this study was to determine the content of essential elements and toxic elements in feed and milk samples collected from farms of Slovakia, to find the actual contamination of selected areas, in view of its environmental character, and to refer to the suitability of the use of milk from these areas, to other food processing.

MATERIAL AND METHODOLOGY

Monitoring areas

The monitoring of areas was realized in 2016 during spring and autumn seasons on selected farms of Slovakia. According to the Ministry of Environment of Slovak Republic (SR), regions of SR are divided into three types of environmental quality: 1st environmental quality – regions with undisturbed environment and convenient environment; 2nd environmental quality – regions with a slightly disturbed environment, areas with disturbed environment and areas with widely disturbed environment; 3rd environmental quality – regions with heavily disturbed environment (Figure 1).

For monitoring in this study, the village Klátova Nová Ves (Western Slovakia) which is characterized as area with widely disturbed of environment was selected. This region, also called Horná Nitra, is typical with contamination of soil by heavy metals (As, Hg). Contamination soil and environment of this region is caused by power station in Nováky, where the coal is burned. The ash from the burning of low quality coal contains high amounts of As.

The second monitoring area is Novoť (Northern Slovakia) with undisturbed environment. On cow's farms of Novoť (750 ewes; Tsigai breed) and Klátova Nová Ves (300 ewes; Tsigai breed) 11 compounds, including 6 essential elements (Ca, Se, Zn, Mg, Fe, Cu) and 5 toxic elements (Cd, As, Hg, Ni, Pb) were analyzed.

Samples collection

Milk

Total number samples of milk were 20. Samples of milk were obtained from sheeps at farms. About 500 mL sample of milk was collected five times during the production of milk, on spring in April (beginning of lactation) and in autumn in September (the end of lactation) on each farm. Despite the fact, that there was large number of animals on the farms, average milk samples were obtained from milk tanks. After collection, samples of milk were stored in PET bottles in deep-freezers at -18 ºC until they were analyzed.

Feed

Total number samples of feed were 20. Five average samples of feeds were obtained in spring season (April) and five average samples in autumn (September) on each farm. This feed was used for feeding the studied animals. Samples were stored in plastic bags in deep-freezers at -18 ºC until they were analyzed. Analyzed feed at sheep farm of Novoť was TMR (total mixed ration) in spring season and pasture, where the animals grazed during the autumn season. From sheep farm of Klátova Nová Ves, the analyzed feed in the both of season was pasture.

Elements analyses

Milk samples for determination of Ca, Zn, Fe, Mg, Cu, Ni, Pb, Cd were prepared by mineralization with microwave decomposition with HNO_3 and H_2O_2 (microwave oven MARS 6 240/50). Milk sample for determination of Se was prepared by mineralization with microwave oven with HNO_3 and H_2O_2 (microwave oven MARS 6 240/50), removal nitrous gases, cooling, followed addition solution of HCl, reduction from Se^{6+} to Se^{4+} by heated at 90°C. Milk samples for determination of As were prepared by dry mineralization with oxidation mixture (oxygen, oxides of nitrogen, ozone), heated at 300 – 400°C. The ash was re-diluted in solution of HCl.

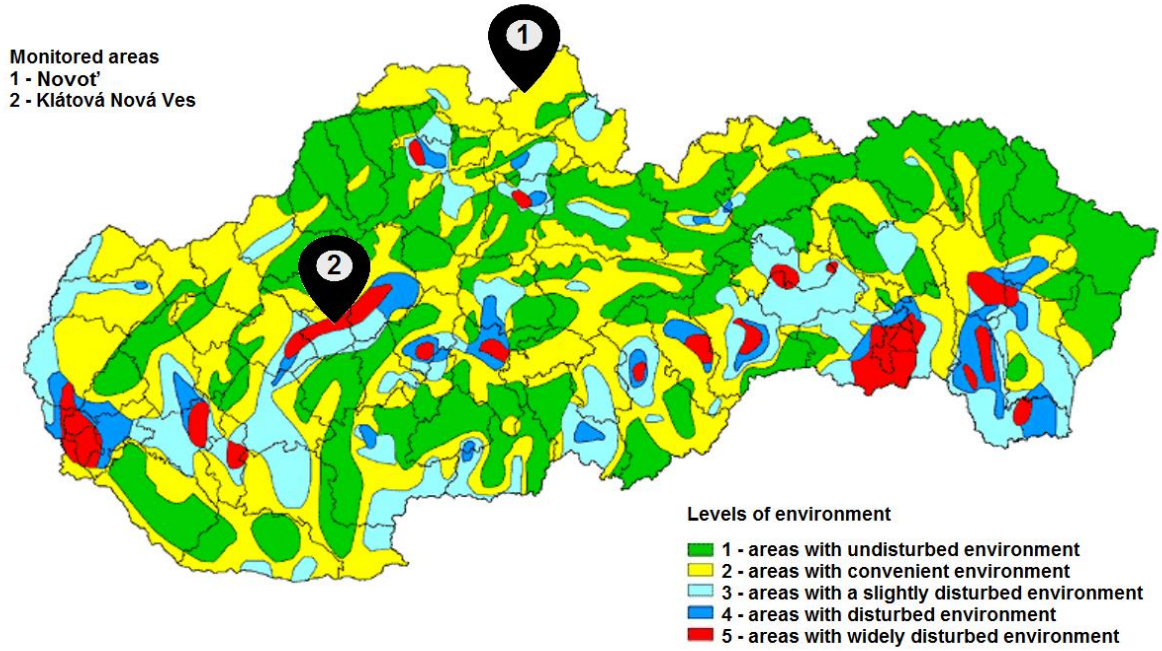

Figure 1 Environmental regionalization of Slovak Republic.

As and Se in milk and feed were analyzed using the hydride generation atomic absorption spectroscopy (HG-AAS) method with Spectr AA-220 FS (The Netherlands). Ca, Fe and Mg in milk and feed ware detected using the inductively coupled plasma-atomic emission spectrometry (ICP-AES, Varian 720-ES, USA). Cd, Pb and Ni in milk and feed were analyzed using the electro thermal atomization atomic-absorption spectrometry (ETA-AAS, Agilent DUO AA 240Z/240FS, USA). Zn and Cu in milk and feed were analyzed using the (F-AAS, DUO AA 240Z/240FS, USA). Hg in milk and feeds was analyzedusing the Advanced Mercury Analyzer and atomic-absorption spectrometry (AMA-AAS, Altec CR) without the need for chemical preparation of the sample. All analyses were conducted in certified testing laboratory Eurofins/Bel Novamann (Nové Zámky, Slovak Republic).

Statisical analysis

Statistical analysis of the data was performed using SAS 9.2 (SAS Institute Inc., USA). The results were analyzed by one-way analysis of variance (ANOVA) followed by Student's t-test. Statistical significance was set at p <0.05. All data were expressed as mean, minimum values, maximum values, standard deviation and coefficient of variation.

RESULTS AND DISCUSSION

The results of our study summarize Table 1 and Table 2. During the spring seasons, content of essential elements in feed (Ca, Zn, Fe, Mg, Cu) were significantly higher (p <0.001) on farm of Klátova Nová Ves (widely disturbed environment). Mean content of Se (0.16 mg.kg^{-1}) was higher on farm of Novoť (undisturbed environment), but this difference was not statistically significant. Content of toxic metals As, Hg, Ni, Pb, Cd in feed on farm of Klátova Nová Ves was found. Significantly higher (p <0.001) content of As and Ni was present on farm of Klátova Nová Ves. Amount of Hg, Pb and Cd in feed was below the LOQ (limit of quantification) on farm with undisturbed environment (Novoť). **Bushra et al. (2014)** studied concentrations of toxic elements in feed from rural and urban areas. They found, that the content of Cd, Ni and Pb was 0.27, 1.68, 4.11 mg.kg^{-1} in urban areas and in rural areas 0.037, 0.024, 4.52 mg.kg^{-1}, respectively. Compared to results of their study, on farm of Novoť (undisturbed environment) and Klátova Nova Ves (widely disturbed environment), content of Pb and Cd was lower and content of Ni was higher. Lower content of Ni, As, Cd, Pb in feed state **Zhou et al. (2017)** compared to our results. Higher value of Ni may be due to low soil pH, use of synthetic fertilizers and contamination of water used for irrigation **(Tahir et al., 2017)**. High quantity of Ni is known to be injurious for animal and human health. Its effects on various aspects of reproduction have been previously described. Animal studies demonstrate that nickel has negative effects on the structure and function of the testis, seminal vesicles and prostate gland; there is similar report on adverse effect on spermatozoa **(Pandey et al., 2000; Forgacs et al., 2001)**. **Lukáč et al. (2014)** reported the negative effect of nickel on spermatogenesis. The decrease in the relative volume of germinal epithelium indicates on alterations of the spermatozoa production. Cadmium causes tissue damage in humans and animals and many toxicological studies have found the functional and structural changes in the kidneys, liver, lungs, bones, ovaries and fetal effects **(Kukner et al., 2007; Massányi et al., 2007)**.

During the autumn season, in case of both farms, decrease content of the all studied toxic metals was found compared to spring season. Content of all toxic metals was below the LOQ (Table 1). Significantly higher content of microelements Zn, Fe, Cu was observed on farm of Novoť (p <0.001). Content of macroelements Ca and Mg was significantly higher (p <0.001) on farm of Klátova Nová Ves. On farm of Klátova Nová Ves, content of Se (<0.03 mg.kg^{-1}) was reported. The seasonal variations of essential trace elements in feed are presented in Table 1. In our study, a significant decrease in essential elements in feed between spring and autumn season was found in both farms. This decline could be due to the season at which the feed was taken. **Tomáš (2007)** states that the input of elements from the soil into the plant could be affected by the quality of organic matter, plant nutrition, microbial activity, the way of soil management but also fertilization, presence of other elements (synergism, antagonism), plant species and variety **(Tkáč et al., 2008)**. In ruminants, mineral deficiency can impair or even inhibit metabolic pathways required for normal body function, and produces clinical symptoms of different intensity. Severe macroelement or microelement deficiencies are manifested by symptoms corresponding to the function of the deficient element in the body, thus contributing to an accurate diagnosis of the health problem. In a minor deficiency, the symptoms are non-specific, often transient and difficult to diagnose due to low intensity. Mineral deficiency generally leads to impaired immunity, inhibited growth, reproductive disorder and lower productivity in animals **(Radwińska and Žarczyńska, 2014)**.

A positive result of our study was that content of toxic metals, which detected in feeds on both farms, did not affect on their occurrence in sheep milk. Content of all toxic elements (As, Hg, Ni, Pb, Cd) in milk was below the LOQ (Table 2) even in an area with a widely disturbed environment (Klátová Nová Ves). The content of Pb in milk in this work was significantly lower than in milk samples from Iran **(Najarnezhadand and Akbarabadi, 2013; Rahimi, 2013)** and very low in comparison to the results of **Anastasio et al. (2006)** and **Licata et al. (2012)**. **Ayar et al. (2009)** analyzed Pb concentration in dairy products and milk consumed in Turkey. According to their study, the concentrations range of Pb was reported as 0.09-0.19 mg.kg^{-1}. In other study, conducted by **Tajkarimi et al. (2008)** lead content was estimated in raw milk collected from different regions of Iran. Accordingly, the mean level of Pb content obtained from 97 samples had a range from 1.0 - 46 ng.ml^{-1}. **Issa et al. (2016)** recorded levels of Pb in dairy products from 0.018 – 7.421 mg.kg^{-1} in their study. The Cd levels found in milk in this study were significantly lower than data found in literature **(Rahimi, 2013)**, while **Coni et al. (1996)** reported levels of Cd much higher. It must be underlined that, to this day, EU Commission does not established yet a maximum admitted limit for Cd in milk. Hence, the only reference value for

Table 1 Comparison between mean content of mineral and toxic elements in feed on farm of Novoť and Klátova Nová Ves (mg.kg^{-1}).

NOVOŤ – spring season					**KLÁTOVA NOVÁ VES – spring season**			
Element	Mean ±SD	Min	Max	CV	Mean ±SD	Min	Max	CV
Ca	2450 ±1.58	2448.00	2452.00	0.065	7400 ±1.58***	7398.00	7402.00	0.02
Zn	40.2 ±0.02	40.18	40.22	0.04	42.20 ±0.16***	42.00	42.40	0.37
Se	0.16 ±0.02^{NS}	0.14	0.18	9.88	0.065 ±0.002	0.063	0.067	0.001
Fe	129 ±1.58	127.00	131.00	1.22	3330 ±1.58***	3328.00	3332.00	0.05
Mg	1180 ±1.58	1178.00	1182.00	0.13	3050 ±1.58***	3048.00	3052.00	0.05
Cu	7.30 ±0.15	7.100	7.500	2.16	11.2 ±0.16***	11.00	11.40	1.41
As	0.042 ±0.002	0.04	0.044	3.76	1.20 ±0.16***	1.00	1.40	13.1
Hg	<0.01[a]	-	-	-	0.02 ±0.01	0.04	0.009	0.001
Ni	0.16 ±0.02	0.14	0.18	9.88	6.80 ±0.16***	6.60	7.00	2.32
Pb	<0.30 [a]	-	-	-	3.80 ±0.16	3.60	4.00	4.16
Cd	<0.10 [a]	-	-	-	0.15 ±0.02	0.13	0.17	10.54
NOVOŤ – autumn season					**KLÁTOVA NOVÁ VES – autumn season**			
Ca	914 ±1.58	912.00	916.00	0.17	1140 ±1.58***	1138	1142	0.13
Zn	7.5 ±0.16***	7.30	7.70	2.11	5.30 ±0.16	5.10	5.50	2.98
Se	0.08 ± 0.002	0.079	0.083	0.00	<0.03	-	-	-
Fe	99.00 ±1.58***	97.00	101.00	1.59	72.00 ±1.58	70.00	74.00	2.19
Mg	303 ±1.58	301.00	305.00	0.52	314 ±1.58***	312.00	316.00	0.50
Cu	2.60 ±0.16***	2.40	2.80	6.08	1.60 ±0.16	1.40	1.80	9.88
As	<0.03	-	-	-	<0.03	-	-	-
Hg	<0.01	-	-	-	<0.01	-	-	-
Ni	<0.01	-	-	-	<0.1	-	-	-
Pb	<0.3	-	-	-	<0.3	-	-	-
Cd	<0.1	-	-	-	<0.1	-	-	-

Note: SD: standard deviation; min.: Minimum values; Max.: maximum values; CV: coefficient of variation; *** values in the same line present significant differences p <0.001; NS: not significant; [a] Values below LOQ (limit of quantification).

Table 2 Comparison between mean content of essential and toxic elements in sheep´s milk on farm of Novoť and Klátova Nová Ves (mg.kg^{-1}).

	NOVOŤ – spring season				**KLÁTOVA NOVÁ VES – spring season**			
Element	Mean ±SD	Min	Max	CV	Mean ±SD	Min	Max	CV
Ca	1410 ±1.58	1408.00	1412.00	0.11	2560 ±1.58***	2558.00	2562.00	0.06
Zn	4.90 ±0.16	4.70	5.10	3.22	8.00 ±0.58**	6.00	10.00	19.76
Se	0.031±0.002	0.029	0.033	5.10	<0.03 [a]	-	-	-
Fe	0.59 ±0.02	0.57	0.61	2.67	<0.50 [a]	-	-	-
Mg	107.00 ±1.58	105.00	1099.00	1.47	187.0 ±1.58***	185.00	189.00	0.084
Cu	<0.50[a]	-	-	-	<0.5[a]	-	-	-
As	<0.03[a]	-	-	-	<0.03[a]	-	-	-
Hg	<0.002[a]	-	-	-	<0.002[a]	-	-	-
Ni	<0.1[a]	-	-	-	<0.1[a]	-	-	-
Pb	<0.0[a]	-	-	-	<0.01[a]	-	-	-
Cd	<0.004[a]	-	-	-	<0.004[a]	-	-	-
NOVOŤ – autumn season					**KLÁTOVA NOVÁ VES – autumn season**			
Ca	1890 ±1.58***	1888.00	1892.00	0.08	1590 ±1.58	1588.00	1592	0.09
Zn	4.00 ±0.58*	2.00	6.00	39.53	2.20 ±1.16	2.00	2.40	7.18
Se	0.06 ±0.001	0.061	0.065	2.50	<0.03[a]	-	-	-
Fe	0.81±0.02***	0.79	0.83	1.95	0.74 ±0.02	0.72	0.76	2.13
Mg	162.00 ±1.58***	160.00	164.00	0.97	179 ±1.58	177.00	181.00	0.88
Cu	<0.50[a]	-	-	-	<0.5[a]	-	-	-
As	<0.03[a]	-	-	-	<0.03[a]	-	-	-
Hg	<0.002[a]	-	-	-	<0.002[a]	-	-	-
Ni	<0.1 [a]	-	-	-	<0.1[a]	-	-	-
Pb	<0.01[a]	-	-	-	<0.01[a]	-	-	-
Cd	<0.004 [a]	-	-	-	<0.004[a]	-	-	-

SD: standard deviation; Min.: minimum values; Max.: maximum values; CV: coefficient of variation; *** Values in the same line present significant differences (p <0.001); ** Values in the same line present significant differences (p <0.01); * Values in the same line present significant differences (p <0.05); [a] Values below LOQ (limit of quantification).

Cd in milk has been set by FAO/WHO standard **(Codex Alimentarius Commission, 2011)**, that states an authorized limit equal to 10 ng.g^{-1}. Scientific data about As contamination in ovine milk are poor, in exception of **Sanal et al. (2011)**, that reported a higher As content in sheep milk compared to our results. In spring season, content of essential elements (Ca, Zn, Mg) in sheep milk was higher on farm of Klátova Nová Ves in case of Ca (p <0.001), Zn (p <0.01), Mg (p <0.001) statistically significant too. In milk from this area, low content of Se (<0.03 mg.kg^{-1}), Fe (<0.05 mg.kg^{-1}), Cu (<0.05 mg.kg^{-1}) was found. On the other hand, statistically significant higher content of essential elements (Ca, Zn, Se, Fe, Mg) was recorded on farm from area with undisturbed environment (Novoť) compared to farm of Klátova Nová Ves. The contents of essential elements Zn, Fe, Cu in ovine milk in this study were slightly lower than values of these elements as reported **Medico et al. (2016)**.

Regarding Ca content, in the present study a low level was found in case of farm of Novoť, in comparison to our previously published results **(Tunegová et al., 2016),** that showed levels of Ca in sheep milk in spring season 1770 mg.kg^{-1} and in autumn season 2170 mg.kg^{-1}.

The concentrations of elements in raw milk are also affected by animal forage, feed and water **(Dobrzański et al., 2005; Al-Wabel, 2008)**. Animal feed with elevated levels of these elements causes also an increase of their level in milk **(Bushra et al., 2014)**. Concentrations of health-beneficial elements, e.g. Fe, Zn in milk are dependent on the animal species, feed, milk sample collection time, environmental conditions and manufacturing processes **(Herwing et al., 2011)**. Changes in composition of milk can also be affected by many genetic (breed, herd) and physiological factors (lactation, age, animal health), but also the environment (food, climate, season, method of milking) **(Komperej et al., 1999)**.

CONCLUSION

The results indicate that content of selected essential elements and toxic metals in feed and milk changes depending on the season of year. The work showed significantly higher content of selected essential elements in feed in spring season from area with widely disturbed environment (Klátova Nová Ves). Significantly higher content of essential elements in milk was found on farm of Novoť (undisturbed environment). In this work occurrence of toxic metals in feed from area with widely disturbed environment in spring season did not affect their milk content. It can be concluded, that the use of milk of sheep from these areas for direct use or for dairy products

processing is appropriate, safe and pose no health risk for the consumers.

REFERENCES

Al-Wabel, N. M. 2008. Mineral contents of milk of cattle, camels, goats and sheep in the central region of Saudi Arabia. *Asian Journal of Biochemistry*, vol. 3, no. 6, p. 373-375. https://doi.org/10.3923/ajb.2008.373.375

Anastasio, A., Caggiano, R., Macchiato, M., Catellani, P., Ragosta, M., Paino, S., Cortes, M. L. 2006. Heavy metal concentrations in dairy products from sheep milk collected in two regions of Southern Italy. *Acta Veterinaria Scandinavica*, vol. 47, no. 1, p. 69-74. https://doi.org/10.1186/1751-0147-47-69
PMid:16722307

Ayar, A., Sert, D., Akin, N. 2009. The trace metal levels in milk and dairy products consumed in middle Anatolia – Turkey. *Environmental Monitoring and Assessment*, vol. 152, no. 1-4, p. 1-12. https://doi.org/10.1007/s10661-008-0291-9
PMid:18478348

Bilandžić, N., Dokić, M., Sedak, M, Solomun, M., Varenina, I., Knežević, Z., Benić, M. 2011. Trace element levels in raw milk from northern and southern region of Croatia. *Food Chemistry*, vol. 127, no. 1, p. 63-66. https://doi.org/10.1016/j.foodchem.2010.12.084

Bushra, I., Saatea, A., Samina, S., Riaz, K. 2014. Assessment of toxic metals in dairy milk and animal feed in Peshawar, Pakistan. *British Biotechnology Journal*, vol. 4, no. 8, p. 883-893. https://doi.org/10.9734/BBJ/2014/9939

Cashman, K. D. 2011. Trace elements, nutritional significance. In Fuquay, J. W. et al. *Encyclopedia of dairy science*. 2nd ed. Mississippi, USA : Mississippi State University. 4170 p. ISBN 978-0-12-374407-4.

Codex Allimentarius Commission. 2011. Report of the 50th Session of the Codex Committee on Food Additives and Contaminants. Codex Allimentarius Commission, Hague.

Coni, E., Bocca, A., Coppolelli, P., Caroli, S., Cavallucci, C., Marinucci Trabalaza, M. 1996. Minor and trace element content in sheep and goat milk and dairy products. *Food Chemistry*, vol. 57, no. 2, p. 253-260. https://doi.org/10.1016/0308-8146(95)00216-2

Dobrzański, Z., Kolacz, R., Górecka, H., Chojnacka, K., Bartkowiak, A. 2005. The content of microelements and trace elements in raw milk from cows in the Silesian region. *Polish Journal of Environmental Studies*, vol. 14, no. 5, p. 685-689.

Forgacs, Z., Nemethy, Z., Revesz, C. S., Lazar, P. 2001. Specific amino acids moderate effects on Ni^{2+} on the testosterone production of mouse Leydig cells in vitro. *Journal of Toxicology and Environmental Health*, vol. 62, no. 5, p. 349-358. https://doi.org/10.1080/152873901300018075
PMid:11261897

Herwing, N., Stephen, K., Panne, U., Pritzkow, W., Vogl, J. 2011. Multielement screening in milk and feed by SF-ICP-MS. *Food Chemistry*, vol. 124, no. 3, p. 223-1230.

Hilali, M., El-Mayda, E., Rischkowsky, B. 2011. Characteristic and utilization of sheep and goat milk in the Middle East. *Small Ruminant Research*, vol. 101, no.1-3, p. 274-278. https://doi.org/10.1016/j.smallrumres.2011.09.029

Chovancová, H., Omelka, R., Baboňová, I., Formicki, G., Toman, R., Martiniaková, M. 2014. Bone adaptation to simultaneous cadmium and diazinon toxicity in adult male rats. *Potravinarstvo*, vol. 8, no. 1, p. 107-113. https://doi.org/10.5219/343

Issa, S. Y., Genema, D. M., Mazrouna, M. K. A., Rahman, S. M. A., Fawzi, M. M. 2016. Determination of some metals in the commonly consumed dairy products randomly collected from the market in Alexandria-Egypt, with an emphasis on toxicity, permissible limits and risk assessment. *International Journal of Pharmacology and Toxicology,* vol. 4, no. 2, p. 133-137. https://doi.org/10.14419/ijpt.v4i2.6288

Kapila, R., Kavadi, P. K., Kapila, S. 2013. Comparative evaluation of allergic sensitization to milk proteins of cow, buffalo and goat. *Small Ruminant Research*, vol. 112, no. 1-3, p. 191-198. https://doi.org/10.1016/j.smallrumres.2012.11.028

Kazi, T. G., Jalbani, N., Baig, J. A. 2009. Assessment of toxic metals in raw and processed milk samples using electrothermal atomic absorption spectrophotometer. *Food Chemical and Toxicology*, vol. 47, no. 9, p. 2163-2169. https://doi.org/10.1016/j.fct.2009.05.035
PMid:19500636

Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z., Zhu, Y. G. 2008. Health risks of heavy metals in contaminated soils of food crops irrigated with wastewater in Beijing, China. *Environmental Pollution*, vol. 152, no. 3, p. 656-692. https://doi.org/10.1016/j.envpol.2007.06.056
PMid:17720286

Komperej, A., Drobnič, M., Kompan, D. 1999. Milk yield and milk traits in Slovenian sheep breeds. *Acta Agraria Kaposváriensis*, vol. 3, no. 2, p. 97-106.

Kukner, A., Colakoglu, N., Kara, H., Oner, H., Ozogul, C., Ozan, E. 2007. Ultrastructural changes in the kidney of rats with acute exposure to cadmium and effects of exogenous metallothionein. *Journal of Biology and Trace Elements*, vol. 119, no. 2, p. 137-146. https://doi.org/10.1007/s12011-007-0049-1
PMid:17916937

Licata, P., Di Bella, G., Potorti, A. G., Lo Turco, V., Salvo, A., Dugo, G. 2012. Determination of trace elements in goat and ovine milk from Calabria (Italy) by ICP-AES. *Food Additives & Contaminants Part B*, vol. 5, no. 4, p. 268-271. https://doi.org/10.1080/19393210.2012.705335
PMid:24786408

Licata, P., Trombetta, D., Cristani, M., Giofre, F., Martino, D., Calo, M., Naccari, F. 2004. Levels of „toxic“ and „essential“ metals in samples of bovine milk from various dairy farms in Calabria, Italy. *Environment International*, vol. 30, no. 1, p. 1-6. https://doi.org/10.1016/S0160-4120(03)00139-9

Llobet, J. M., Falcó, G., Casas, C., Teixidó, A., Domingo, J. L. 2003. Concentrations of arsenic, cadmium, mercury and lead in common foods and estimated daily intake by children, adults and seniors of Catalonia, Spain. *Journal of Agricultural and Food Chemistry,* vol. 51, no. 3, p. 838-842. https://doi.org/10.1021/jf020734q
PMid:12537467

Lukáč, N., Massányi, P., Kročková, J., Toman, R., Danko, J., Stawarz, R., Formicki, G. 2014. Effect of Nickel on Male Reproduction. *Universal Journal of Agricultural Research,* vol. 2, no. 7, p. 250-252.

Lukačínová, A., Nováková, J., Lovásová, E., Cimboláková, I., Ništiar, F. 2012. Influence of lifetime exposure of sublethal doses of cadmium to selected parameters of carbohydrate metabolism. *Potravinarstvo*, vol. 6, no. 12, p. 36-40. https://doi.org/10.5219/231

Mass, S., Lucot, E., Gimbert, 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. https://doi.org/10.1016/j.foodchem.2010.09.034

Massányi, P., Lukáč, N., Uhrín, V., Toman, R., Pivko, J., Rafay, J., Forgács, Z. S., Somosy, Z. 2007. Female reproductive toxicology of cadmium. *Acta Biologica*

Hungarica, vol. 58, no. 3, p. 287-299. https://doi.org/10.1556/ABiol.58.2007.3.5 PMid:17899786

Medico, O., Tarallo, M., Pompa, C., Chiaravalle, A. E. 2016. Trace elements in sheep and goat milk samples from Apulia and Basilicata regions (Italy): Valuation by multivariate data analysis. *Small Ruminant Research*, vol. 135, p. 60-65. https://doi.org/10.1016/j.smallrumres.2015.12.019

Najarnezhad, V., Akbarabadi, M. 2013. Heavy metals in raw cow and ewe milk from north-east Iran. *Food Additives & Contaminants: Part B*, vol. 6, no. 3, p. 158-162. https://doi.org/10.1080/19393210.2013.777799 PMid:24779898

Pandey, R., Srivastava, S. P. 2000. Spermatotoxic effects on nickel in mice. *Bulletin of Environmental Contamination and Toxicology*, vol. 64, no. 2, p. 161-167. https://doi.org/10.1007/s001289910025 PMid:10656880

Radwińska, J., Żarczyńska, K. 2014. Effects of mineral deficiency on the health of young ruminants. *Journal of Elementology*, vol. 3, no. 19, p. 915-928.

Rahimi, E. 2013. Lead and cadmium concentrations in raw milk collected from different regions of Iran. *Food Chemistry*, vol. 136, no. 2, p. 389-391. https://doi.org/10.1016/j.foodchem.2012.09.016 PMid:23122075

Sanal, H., Guler, Z., Park, Y. W. 2011. Profiles of non-essential trace elements in ewe and goat milk and their yoghurt, torbayoghurt and whey. *Food Additives & Contaminants: Part B,* vol. 4, no. 4, p. 275-281. https://doi.org/10.1080/19393210.2011.617520 PMid:24786251

Sanz Ceballos, l., Ramos Morales, E., de la Torre Adarve, G., Diaz Castro, J., Perz Martinez, L., Sanz Sampelayou, M. R. 2009. Composition of goat and cow milk produced under similar conditions and analyzed by identical methodology. *Journal of Food Composition and Analysis*, vol. 22, no. 4, p. 322-329. https://doi.org/10.1016/j.jfca.2008.10.020

Simsek, O., Gültekin, R., Öksüz, O., Kurulatay, S. 2000. The effect of environmental pollution on the heavy metal content of raw milk. *Nahrung*, vol. 44, no. 5, p. 360-363. https://doi.org/10.1002/1521-3803(20001001)44:5<360::AID-FOOD360>3.0.CO;2-G

Tahir, M., Iqbal, M., Abbs, M., Tahir, M. A., Nazir, A., Iqbal, D. N., Kanwal, O., Hassan, F.,Younas, U. 2017. Comparative study of heavy metals distribution in soil, forage, blood and milk. *Acta Ecologica Sinica*, vol. 37, no. 3, p. 207-212. https://doi.org/10.1016/j.chnaes.2016.10.007

Tajkarimi M., Ahmadi F. M., Poursoltani H., Nejad, A. S., Motallebi, A. A., Mohdavi, H. 2008. Lead residue levels in raw milk from different regions of Iran. *Food Control.* vol. 19, no. 5, p. 495-498. https://doi.org/10.1016/j.foodcont.2007.05.015

Temiz, H., Soylu, A. 2012. Heavy metal concentrations in raw milk collected from different regions of Samsun, Turkey. *International Journal of Dairy Technology*, vol. 65, no. 4, p. 1-6. https://doi.org/10.1111/j.1471-0307.2012.00846.x

Tkáč, P., Kozáková, Ľ., Vaľková, M., Zeleňák, F. 2008. Heavy metals in soils in middle Spiš. *Acta Montanistica Slovaca*, vol. 13, no. 1, p. 82-86.

Toman, R., Tunegová, M. 2017. Selenium, cadmium and diazinon insecticide in tissues of rats after peroral exposure. *Potravinarstvo Slovak Journal of Food Science*, vol. 11, no. 1, p. 718-724. https://doi.org/10.5219/827

Tomáš, J., Hronec, O. 2007. *Soil and Plant Damage by Human Activities (Poškodzovanie pôd a rastlín ľudskými činnosťami)*. Nitra, Slovakia : Slovak University of Agriculture in Nitra. p. 62-76. ISBN 978-80-8069-902-4.

Tunegová, M., Toman, R., Tančin, V. 2016. Monitoring of selected essential elements and contaminants at sheep and cow farms in Eastern Slovakia. *Journal of Central European Agriculture*, vol. 17, no. 4, p. 1221-1232. https://doi.org/10.5513/JCEA01/17.4.1834

WHO – World Health Organization. 2007. Health risk of heavy metals form long-range transboundary air pollution [online] s.a [cit. 2018-02-08] Available at: http://www.euro.who.int/__data/assets/pdf_file/0007/78649/E91044.pdf.

Zhou, X., Qu, X., Zhao, S., Wang, J., Li, S., Zheng, N. 2017. Analysis of 22 Elements in Milk, Feed, and Water of Dairy Cow, Goat and Buffalo from different Regions of China. *Biology Trace Elements Research*, vol. 176, no.1, p. 120-129. https://doi.org/10.1007/s12011-016-0819-8 PMid:27550154

Zhuang, P., McBride, M. B., Xia, H., Li, N., Li, Z. 2009. Health risk of heavy metals via consumption of food crops in the vicinity of Babaoshan mine, South China. *The Science of the Total Environment*, vol. 407, no. 5, p. 1551-1561. https://doi.org/10.1016/j.scitotenv.2008.10.061 PMid:19068266

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VICIA VILLOSA PROTEIN ISOLATE: A NEW SOURCE OF PROTEIN TO MAKE A BIODEGRADABLE FILM

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ABSTRACT
Edible films from *Vicia villosa* protein isolate (VVPI) containing different contents of glycerol and sorbitol (30, 40, 50 and 60%w/w of protein) were developed. The aim of this study was to investigate the influence of type and concentration of plasticizers on the properties of edible films obtained from VVPI. Type and concentration of plasticizer significantly (p <0.05) affected the mechanical, barrier, thermal and surface properties as well as opacity of the films. As plasticizer concentration increased, tensile strength decreased concomitant with increase in elongation at break and water vapor permeability. The similar trend behavior was observed for the film solubility, which increased with increasing plasticizer concentration. Sorbitol plasticized films, showed higher film solubility compared to glycerol plasticized films. Sorbitol plasticized films provided the most tensile strength values; however, its effect on water vapor permeability was low. In contrast, glycerol plasticized films exhibited the least tensile strength values, resulting in increasing water vapor permeability. Opacity of glycerol plasticized films was lower than that of sorbitol plasticized films, and decreased with increasing plasticizer content (p <0.05). Also, a significant decrease (p <0.05) was observed in thermal features and surface hydrophobicity values with increasing in plasticizer contents. It was observed that the films plasticized with sorbitol had lower moisture content than those with glycerol.

Keywords: biodegradable film; glycerol; sorbitol; *Vicia villosa* protein isolate

INTRODUCTION
The Current global consumption of plastics is more than 300 million tonnes, with an annual grow of approximately 5% **(Bioplastics market data, 2015)**. Packaging is the biggest bazar for plastics, consuming more than 12 million tons per year. Although synthetic petrochemical-based polymers have been extensively applied in many packaging materials, they have become one of the main sources of waste after being utilized because of their weak biodegradability **(Rhim and Ng, 2007)**. Plastic materials are not inert and where direct contact between the packed commodity and the plastic container occurs, there can be transfer of sufficiently mobile or soluble substances into the product as a result of a concentration gradient. These substances may be polymer additives and/or other adventitious impurities, such as monomers, catalyst remnants, polymer breakdown products and residual polymerization solvents. This transfer may introduce the risk of toxic hazard and/or formation of off-flavours **(Mannheim and Passy, 1990)**. With the increasing demand of consumers for high quality foods and concerns on limited natural resources and the environment, the use of renewable resources to produce edible or biodegradable packaging materials that can maintain product quality and reduce waste disposal problems are being explored **(Rhim and Ng, 2007)**.

In order to prolong the postharvest shelf-life of fresh fruits and vegetables, edible films and coatings are able to supply a replacement for modified atmospheric packaging, because they could also lead to a rise in the shelf-life as in modified atmosphere storage during which the composition of the inside gas is adjusted **(Park, 1999)**. Edible films and coatings could prevent moisture transfer between components of food package that have different water activity. Furthermore, biopolymer films are very good carriers for antioxidants, antimicrobial substances, pigments and other functional substances **(Haugaard et al., 2001; Petersen et al., 1999; Rhim and Ng, 2007)**. Edible films and coatings after getting into nature decompose to water, carbon dioxide and inorganic compounds without any toxic residues in short time **(Haugaard et al., 2001; Petersen et al., 1999)**.

In general, films comprised of one ingredient have either good barrier or good mechanical properties, yet not both. In fact, the valuable properties of various substances are gathered to form composite films: polysaccharides and proteins develop polymer interactions and create a network which is liable for the mechanical properties, however, they

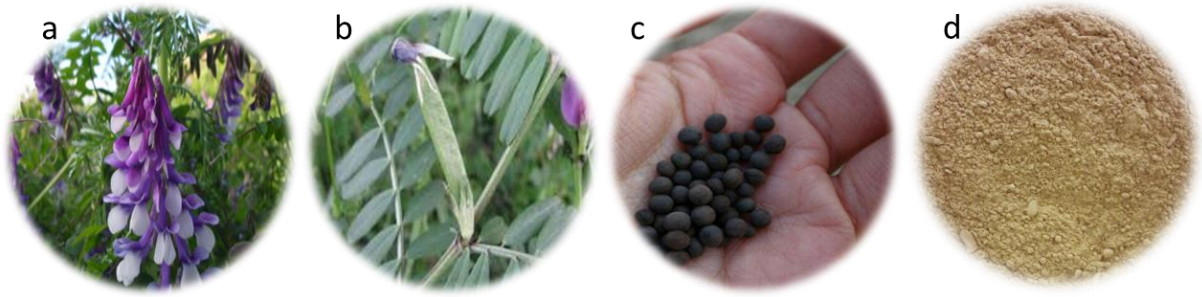

Figure 1 A pictorial view of (a) *Vicia villosa* plant, (b) cluster, (c) seeds, and (d) protein isolate.

are not appropriate water-vapor barriers due to their hydrophilic nature; in contrast, lipids cause the water-vapor barrier property of films owing to their hydrophobic nature; nevertheless, only the films made from lipids are almost brittle **(Guilbert, 1986; Kester and Fennema, 1986)**.

Protein films have been recently considered as degradable and renewable. Proteins are traditionally employed in adhesives and as edible films/coatings. However, they possess a remarkable potential as the packaging films which degrade slowly. Proteins are interesting for polymer researchers since they have a variety of chemical functionalities, and are the molecules with numerous properties in nature **(Petersen et al., 1999)**. Edible films resulting from various vegetable protein sources have been produced. These include corn zein, soy protein, wheat gluten, peanut protein, lentil protein, pea protein, faba bean protein and mung bean protein **(Bamdad et al., 2006; Bourtoom, 2008; Choi and Han, 2001; Gennadios et al., 1993a; Liu et al., 2004; Otoni et al., 2016; Saremnezhad et al., 2010; Soliman et al., 2007)**.

Vicia villosa which is locally called Mashak-Gol-Khooshei in Iran; is one of the famous genera of Legumes. It has a large amount of energy and protein (20% ca.). *V. villosa* has up to 1 m long stems. It has covered with fine long (1 – 2 mm) hairs. Its leaflets are 5 – 10 pairs, narrowly oblong to linear-lanceolate and 1 – 2.5 cm long. Flowers of *V.villosa* are 10 – 30 arranged on one side of long-peduncled racemes. Their color are reddish-purple to violet with 1.5 – 1.8 cm long. Its legume has 2 – 3 cm long (Figure 1) **(Aarssen et al., 1986; Shafiei et al., 2006; Tavili et al., 2010; Roy et al., 1968)**. The distribution of *V. villosa* is widespread throughout most of the United States, southern Canada **(Gleason and Cronquist, 1963)** and Iran **(Tavili et al., 2010)**. In Alaska, it has been recorded above 60 °N **(Hulten, 1968)**. Since this seed is an inexpensive source of protein, it has a good potential for the production of protein-based films. Despite the extensive research done in the field of edible protein films, there is no report regarding the film production of *V. villosa* protein isolate.

Scientific hypothesis

In this study, edible films from Vicia villosa protein isolate (VVPI) were prepared. Since Information on the effects of plasticizers on edible films from VVPI is poorly available at present, the aim of this investigation was to make a comparative study of different types of glycerol and sorbitol and their concentrations (30 – 60%) incorporated into edible films from VVPI. The different concentration of glycerol and sorbitol have an effect on the mechanical, barrier, thermal and surface properties as well as opacity of the films.

MATERIAL AND METHODOLOGY

Materials

Vicia villosa seeds were purchased from a local market in Shahr-e-Kord, Iran. All reagents were of high-purity grade and used as received. Glycerol (CAS-no. 56-81-5, order no. 8.18709.1000) was obtained from Merck Millipore KGaA, Darmstadt, Germany. Sorbitol (NEOSORB® 70/70, CAS-no. 50-70-4) from Roquette GmbH, Frankfurt, Germany was used.

Isolation of VVPI

V. villosa seeds were cleaned manually to remove all extra matters. *V. villosa* seed flour was defatted using hexane (1 : 5 w/v) on a stirrer (60 min) prior to being milled and sieved. The defatted flour was dried in the ventilator at 20 °C for overnight. VVPI was prepared from defatted *V. villosa* seed flour by alkali method developed by **Shahraki et al. (2013)**. Defatted flour was mixed with water at a ratio of 1 : 10 (w/v). The pH of the defatted flour suspended in water was adjusted to pH 9.9 using 1.0 M NaOH, continuously stirred with a magnetic stirrer for 1 h and centrifuged at 8000 g for 15 min. The pH of soluble phases were adjusted to pH 4.5 using 1.0 M HCl due to which proteins precipitates. The suspensions were centrifuged at 8000 g for 15 min, after which the supernatant was poured away and the precipitates were weighed and determined for protein content by the Kjeldahl method. Neutralization of precipitates was achieved by adjusting pH to 7.0 using 1.0 M NaOH, dialyzed by distilled water overnight at 4 °C and then vacuum dried and passed through 80 mesh sieve. The chemical composition of VVPI was determined according to AOAC **(Horowitz and Latimer, 1994)** standard methods.

Film preparation

Isolated *V. villosa* protein consists of 905.0 g kg^{-1} protein content, 2.08% ash content, 0.3% crude fibre, 6.86% moisture, 0.08% fat and 0.81% carbohydrates. The film forming solution was prepared by dissolving VVPI in distilled water. Glycerol and/or Sorbitol were added at different proportions as plasticizers (Table 1). pH value of the solution was adjusted to 9 with 1 N NaOH. Alkaline pH range was selected to prepare films as VVPI is insoluble at acidic pH conditions, so it is not possible to prepare films at acidic pH with casting method. The solution was heated in water bath at 90 °C for 30 minutes, strained through muslin

Table 1 Composition of VVPI film-forming solutions prior to film casting.

Film	VVPI (g)	Plasticizer (g)	Plasticizer (%)	Water (g)
10% VVPI, 30% Glycerol	10	3	30%	87
10% VVPI, 40% Glycerol	10	4	40%	86
10% VVPI, 50% Glycerol	10	5	50%	85
10% VVPI, 60% Glycerol	10	6	60%	84
10% VVPI, 30% Sorbitol	10	3	30%	87
10% VVPI, 40% Sorbitol	10	4	40%	86
10% VVPI, 50% Sorbitol	10	5	50%	85
10% VVPI, 60% Sorbitol	10	6	60%	84

cloth, degassed under vacuum for 30 minutes and cast on Teflon-coated baking trays. The films were dried at 60 °C and 50 ±2% relative humidity for 8 hrs. Finally, the obtained VVPI films were peeled from plates and conditioned at ambient temperature in desiccators containing saturated solutions of Ca $(NO_3)_2$, $6H_2O$ (50 ±2% relative humidity, RH) for at least 48 h prior to tests. The films used in the different tests were selected based on the lack of physical defects such as cracks, bubbles and holes. Film failure was designated as loss of film cohesion resulting in cracking.

Film thickness

For each film sample nine thickness measurements were taken with a manual digital micrometer (Schwyz®, Zhejiang, China). Determinations were performed in triplicate. Averaged values of thickness measurements were obtained and these values were used in all calculations.

Moisture content

The moisture content (MC) of the films was determined after drying in an oven at 105 °C for 24 h. Pieces of films (15 × 7.5 mm) were cut after adequate conditioning and placed on test tubes previously weighed before and after oven drying. MC values were determined as a fraction of the initial film weight lost during drying and reported on a wet basis, according to the ASTM D-644 **(ASTM, 1994)**. Determinations were performed in quintuplicate.

Film solubility

Film solubility in water was determined according to the method by **Gontard et al. (1994)** with modification. A piece of film sized 2.0 cm in diameter was cut, dried in an oven (DeLeo A1 SED, Porto Alegre, Brazil) at 105 °C to constant weight to obtain the initial film dry weight. Films were individually placed into 50 mL of distilled water and the mixture was shaken at a speed of 100 rpm using a shaker (Vicking, Buenos Aires, Argentina) at 25 °C for 24 h. The amount of dry matter in final samples was determined by drying at 105 °C to constant weight. The weight of solubilized dry matter was calculated by subtracting the weight of insolubilized dry matter from the initial weight of dry matter and expressed as the percentage of total weight. Film solubility represented total soluble matter dissolved in water, mainly including water-soluble proteins, glycerol and sorbitol.

Water vapor permeability

The gravimetric Modified Cup Method based on ASTM E96-92 **(McHugh et al., 1993)** was used to determine the water vapor permeability of films. The test cups were filled with 20 g of silica gel (desiccant) to produce a 0% RH below the films. A sample of VVPI film was placed in between the cup and the ring cover of each cup coated with silicone sealant (LITHELEN, Leybold System Gmbh, Germany) and held with four screws around the cup's circumference. The air gap was at approximately 1.0 cm between the film surface and desiccant. The water vapor transmission rates of each film were measured at 55 +5% RH and 25 ±2 °C. After taking initial weight of the test cup, it was placed into an environmental chamber with an air velocity rate of 106.7 $m.min^{-1}$ (Incubator, Model KBF 115). Weight gain measurements were taken by weighing the test cup to the nearest 0.0001 g with an electronic scale (Sartorious Corp.) every 3 h for 18 h. A plot of weight gained versus time was used to determine the water vapor transmission rates. The slope of the linear portion of this plot represented the steady state amount of water vapor diffusing through the film per unit time ($g.h^{-1}$). Water vapor transmission rates were expressed in gram units, per square meter, per day. Steady state over time (slope) yielded a regression coefficient of 0.99 or greater. Nine samples per treatment were tested. The water vapor permeability of film was calculated by multiplying the steady water vapor transmission rates by the film thickness and dividing that by the water vapor pressure difference across the films.

Opacity

Opacity of films was measured according to the method of **Gontard et al. (1992)**. In brief, films were cut in rectangular strips of 0.4 cm × 7.3 cm and placed in the spectrophotometer cell. A spectrum of each film was recorded using a UV-Vis spectrophotometer (Shimadzu UV-160A). The area under the absorbance curve from 400 to 800 nm was determined by Adobe Photoshop CS and defined as the film opacity.

Surface hydrophobicity

The sessile drop method, based on the optical contact angle, was used to estimate the surface hydrophobicity of the films. The contact angle (θ) was determined with a face contact angle meter OCA 20 (from Dataphysics, Filderstadt, Germany), according to **Kwok and Newman (1999)**: a 2 μL-droplet of ultrapure water was deposited on the film surface with a 500 μL precision syringe (Hamilton, Bonaduz, Switzerland), using a needle with a diameter of 0.75 mm. Ten replicated measurements of θ were obtained.

Tensile strength and Elongation at break

Tensile strength was performed with an Instron universal testing instrument (LLOYD Instrument, Model LR30K,

Hants, England) as per ASTM D882-91 Standard Method (ASTM, 1995). Fifteen samples, 2.54 cm × 10 cm, were cut from each film. Initial grip separation and cross head speed were set at 50 mm and 50 mm/min, respectively **(Ghasemlou et al., 2011)**.

Differential scanning calorimetry (DSC)

DSC measurements were performed with a Shimadzu DSC-50 calorimeter (from Shimadzu Corporation, Kyoto, Japan), equipped with STARe 6.1 Thermal Analysis System software. The instrument was calibrated with an indium standard, characterized by a T_m of 156.6 °C and a ΔH_m of 28.71 $J.g^{-1}$ (TA Instruments, New Castle DE, USA). Each sample was heated at a rate of 10 °C.min^{-1}, from -150 °C (assured with liquid nitrogen) to 250 °C, under an inert atmosphere (100 mL.min^{-1} of N_2). The glass transition temperature (T_g) was recorded as the inflexion point of the baseline, caused by the discontinuity in the specific heat of the sample **(Ghanbarzadeh and Oromiehi, 2008)**. The temperature of melting (T_m), observed as an endothermic peak, and the associated enthalpy (ΔH_m) were determined (and expressed as $J.g^{-1}$ protein) as reported by **Ryan et al. (2008)**. These experiments were performed at least in duplicate, using punctured aluminum DSC pans (Al crimp Pan C.201-52090) containing 10 mg of dry sample. The samples were weighed with an automatic electrobalance AE 200 (from Mettler, Columbus OH, USA), with a precision of ±0.01 mg. An empty pan was used as reference.

Statisic analysis

A completely randomized experimental design was used to characterize the films. Analysis of variance (ANOVA) was used to compare mean differences of the samples. If the differences in mean existed, multiple comparisons were performed using Duncan's Multiple Range Test (DMRT). The statistical analysis of the data was performed using SPSS statistical software version 18 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Normally, protein films need to be incorporated by some plasticizers to induce sufficient flexibility and to avoid cracking of the films during the drying or handling processes. The general function of plasticizers is to decrease inter and intra-molecular interactions among polymer chains, resulting in a rise in the spacing of free volume and make the molecules movements easier °C **Lieberman and Gilbert, 1973)**. Therefore, the polymeric network becomes smooth and flexible. It is so necessary that the correct type and content of plasticizer would be chosen for the preparation of a polymer film as its effect can significantly change the functional properties of the obtained products. The commercial plasticizers, namely glycerol and sorbitol, are added ranging from 0.2 to 1 g per 1 g of the film-forming agent **(Chae and Heo, 1997; Cho and Rhee, 2002; Jongjareonrak et al., 2006; Osés et al., 2009; Ryu et al., 2002; Tanaka et al., 2001)**.

The present study revealed that the edible films of coherent VVPI were prepared when these plasticizers were incorporated at the concentrations between 20% and 70% (w/w of protein). However, the films obtained from VVPI containing 20% (w/w) sorbitol were brittle and broke easily during the removal from the casting surface. In turn, the films casted from VVPI containing 70% (w/w) glycerol were found to be very adhesive and too sticky to handle. Finally, in the work, the properties of films formed from VVPI containing 30 – 60% (w/w) glycerol and sorbitol were examined.

Film thickness values at the protein concentration 10% VVPI at different content of plasticizers 30%, 40%, 50% and 60% (w/w) showed similar values in thickness from 0.060 ±0.003 to 0.069 ±0.004 mm (Table 2). The differences in thickness were not significant ($p > 0.05$). Thickness of VVPI film did not affect by the glycerol and sorbitol concentration as also observed by **Rodriguez et al. (2006)**.

Moisture content

MC of the films plasticized with different contents of glycerol and sorbitol are presented in Table 2. The films plasticized with sorbitol had lower moisture than those with glycerol ($p < 0.05$) could be due to the fact that sorbitol had ability to bind less water than glycerol thereby, provided lower MC. **Addition et al. (1993a)** reported that the rich of hydrophillicity of glycerol molecules, which is favorable to the adsorption of water molecules, could also be contribute to the increase in moisture in the films. This results show similar study of **Chick and Ustanol (1998)** who reported that casein-based films plasticized with glycerol had higher MC than films plasticized with sorbitol when the same amounts of plasticizers were used.

Water Solublity

The water solubility is also a major characteristic when selecting a film for specific applications **(Arvanitoyannis et al., 1998)**. Generally, when the water solubility of a film is high, it cannot protect food from moisture or from water loss. For some uses, including packaging wraps, the high

Table 2 Values (average ±standard deviation) of thickness, moisture content and water solubility of VVPI-based edible films, with various glycerol and sorbitol contents.

Plasticizer type	Content %(w/w)	Thickness (mm)	Moisture content (%)	Water solubility (%)
Glycerol	30	0.060 ±0.003^{a}	21.47 ±0.89^{c}	48.11 ±1.09^{d}
	40	0.062 ±0.003^{a}	27.05 ±1.62^{b}	59.14 ±0.76^{c}
	50	0.064 ±0.002^{a}	31.56 ±2.17^{ab}	68.09 ±0.91^{bc}
	60	0.066 ±0.003^{a}	36.66 ±1.51^{a}	70.65 ±1.30^{b}
Sorbitol	30	0.066 ±0.002^{a}	16.11 ±0.91^{e}	60.66 ±0.50^{c}
	40	0.066 ±0.004^{a}	16.84 ±0.77^{e}	55.87 ±0.43^{cd}
	50	0.068 ±0.002^{a}	17.28 ±1.12^{de}	71.08 ±0.19^{b}
	60	0.069 ±0.004^{a}	18.12 ±0.42^{d}	80.34 ±0.74^{a}

Note: the values with the same letter are not significantly different at the $p < 0.05$.

solubility is a biodegradability index which can be taken into account as an advantage for such **films (Stuchell and Krochta, 1994)**.

From visual observations and irrespective of plasticizer type and content, the edible films from VVPI clearly did not lose integrity after a 24 h immersion in water. Irrespective of the type, an increase in plasticizer content leads to an increase in films solubility (Table 2). It could be hastily concluded that hydrophilic plasticizers enhanced films solubility in water.

The lowest films solubility of edible films from VVPI plasticized by 30% w/w of these plasticizers were noticed, while increasing the amount of plasticizer content showed higher films solubility. Increasing glycerol concentration significantly altered the water solubility of VVPI-based film from 48.11 to 70.65% while Increasing sorbitol altered it from 60.66 to 80.34% (p <0.05) (Table 2). The highest solubility was obtained when the highest concentration of sorbitol (60%, w/w) was added. Plasticizers are capable of decreasing crosslinks among biopolymer molecules, thus increasing the film solubility **(Ghasemlou et al., 2011)**. These results were similar to those achieved by **Wittaya (2013)**, who also reported that increasing plasticizer content (glycerol and sorbitol) in mung bean protein films increased the film solubility.

Water Vapor Permeability

As a food packaging, it is normally needed that films avoid or at least decrease the moisture transfer between the food product and the surrounding medium, and water vapor permeability should be enough low **(Ma et al., 2008)**. Water vapor permeability is a proportional constant presumed to be independent of the pressure gradient of water vapor used across the films. However, hydrophilic materials, such as protein films, deviate from this ideal behavior due to the interactions of permeating water molecules with polar groups in the film's structure **(Hagenmaier and Shaw, 1990)**. Deviation from the ideal behavior can also be induced by the effects of structure on materials **(Myers et al., 1961)**. Water vapor permeability of edible films from VVPI with different type and concentration of plasticizer were examined (Figure 2). The water vapor permeability increased with increasing of plasticizer concentration. This tendency can be described by the structural changes in the protein network. The incorporation of plasticizers modified the molecular organization of the protein network, with an increase in free volume. The network becomes less dense and as a consequence more permeable **(Ashley, 1985)**. Permeability increased with plasticizer content could be related to hydrophillicity of plasticizer molecules. Introducing hydrophillic plasticizers, favorable to adsorption and desorption of water molecules, has been reported to improve the water vapor permeability of hydrocolloid-based films **(Gontard et al., 1993; McHugh et al., 1994)**. Comparing of the successive values of the water vapor permeability for each plasticized films was shown in Figure 2.

Films plasticized with sorbitol had lower water vapor permeability than those with glycerol at each plasticizer concentration (p <0.05), respectively due to the fact that sorbitol had ability to bind less water than glycerol and, thereby, provided a lower water vapor permeability **(McHugh et al., 1994)**. Chick and Ustanol **(Chick and Ustunol, 1998)** reported that casein-based films plasticized with glycerol had higher water vapor permeability values than films plasticized with sorbitol when the same amounts of plasticizers were used. The high hydrophillicity of glycerol molecules, which is favorable to the adsorption of water molecules, could also be contribute to the increase in the films water vapor permeability **(Gennadios et al., 1993b)**. The increase in water vapor permeability with increasing hydrophillicity plasticizer concentration was also common in edible films **(Cuq et al., 1997; McHugh and Krochta, 1994)**. **Sorbal et al. (2001)** reported that hydrophilicity of the plasticizers will increase the water content of the films, consequently increasing the mobility of the molecules. In addition, increasing water content could also affect permeate solubility in the films.

Film opacity

Film opacity is an important attribute in terms of food packaging, because transparency of packaging allows consumers to see the product before buying **(Gontard et al., 1992; Orliac et al., 2003)**.

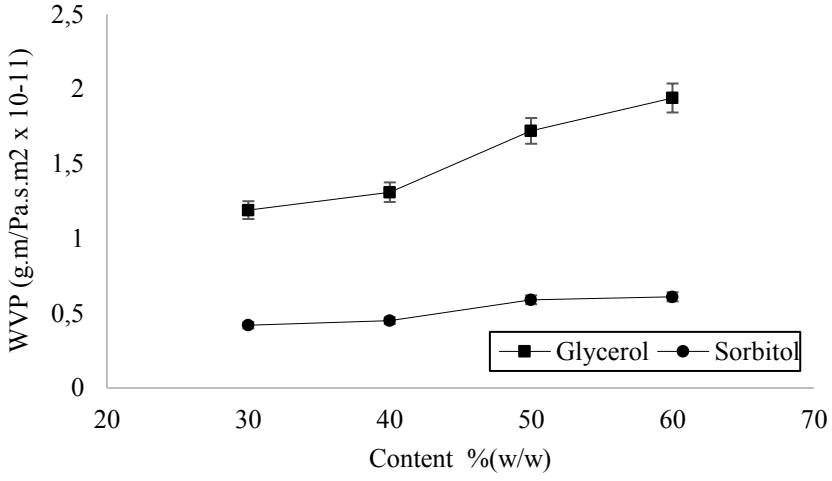

Figure 2 Water vapor permeability of 10 % (w/w) VVPI films in the presence of 30, 40, 50 and 60 % (w/w of VVPI) glycerol and sorbitol. Data represent the mean ± one standard deviation (n = 3).

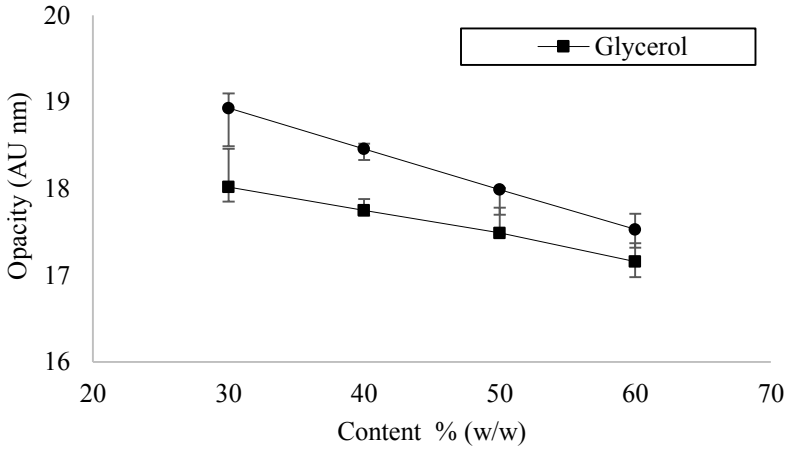

Figure 3 Opacity of 10 % (w/w) VVPI films in the presence of 30, 40, 50 and 60 % (w/w of VVPI) glycerol and sorbitol. Data represent the mean ± one standard deviation (n = 3).

Film opacity was investigated as a function of plasticizer type and concentration and presented in Figure 3. An analysis of variance found the effect of plasticizer type and concentration was significant(p <0.05), meaning that the transmission of light through the resulting films changed with plasticizer type and concentration. Overall, films prepared with glycerol were less opaque. Based on these findings, it was hypothesized that since the glycerol molecule was smaller than sorbitol, it was more homogenously dispersed. In contrast, sorbitol was more heterogeneously dispersed causing light to scatter more.

This results show similar study of **Chang and Nickerson (2014)** who reported that canola protein isolate-based films plasticized with 50% sorbitol had higher opacity than films plasticized with glycerol when 1% (w/w of CPI) genipin was used.

These findings are important since film transparency or opacity are critical properties in various film applications, particularly if the film will be used as a surface food coating or for improving product appearance **(Gontard et al., 1992)**. In many applications, an increased opacity is undesirable, although some applications need to provide protection against reactions of deterioration produced by the effect of light, offering some advantage to this type of film.

Surface hydrophobicity

Surface hydrophobicity of protein films was evaluated via measuring the contact angle of water (θ) upon the film surface by the sessile drop method. In general, films with higher (θ) values exhibit a higher surface hydrophobicity **(Tang and Jiang, 2007)**; quantitative differentiation between "hydrophobic" and "hydrophilic" surfaces is indeed based on whether θ >65 or θ <65, respectively **(Vogler, 1998)**.

From inspection of Figure 4, films containing 30% (w/w) sorbitol can be considered to have hydrophobic surfaces, since θ took values of 69.5 ±2.6° and 65.8 ±2.1°for the upper and lower surfaces, respectively. Conversely, VVPI-based film with the highest glycerol content (60%, w/w) could be considered to have the most hydrophilic surface. Furthermore, statistically significant differences (p <0.05) were recorded between films containing different content of glycerol and sorbitol Figure 4.

It is also apparent in Figure 4 that θ (for the upper and lower surfaces) of VVPI films decreased proportionally to the increase in glycerol and sorbitol; once again, such a behavior was expected due to the hygroscopic nature of glycerol and sorbitol **(Sobral et al., 2001)**.

This result is consistent with the claim by **Sobral et al. (2001)**, who reported that increasing concentrations of

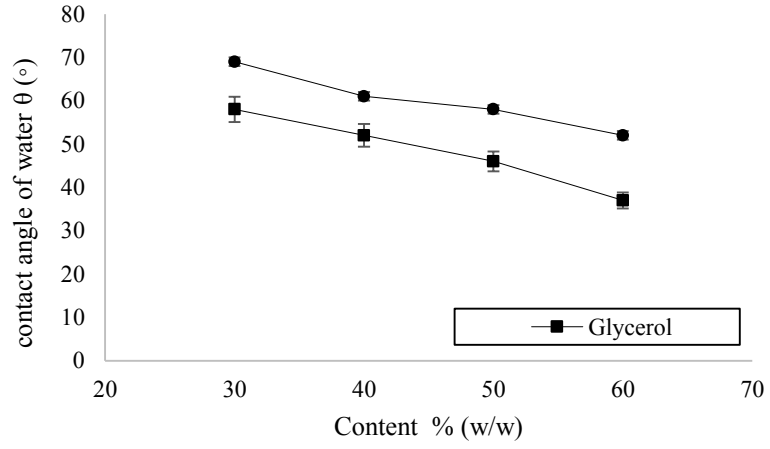

Figure 4 Water contact angle values (average ±standard deviation, n = 10) for upper and lower surface of 10 (%w/w) VVPI-edible films, with various glycerol and sorbitol contents.

glycerol facilitate water absorption and transport within the films.

Tensile Strength and Elongation at Break

Edible films may be subjected to various types of stress during use; the determination of the mechanical properties involves not only scientific but also technological and practical aspects **(Cagri et al., 2004)**. Tensile strength is the maximum tensile stress sustained by the sample during the tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated tensile strength at yield or at break respectively **(ASTM, 1995)**. Elongation at break is an indication of a film's flexibility and stretch ability (extensibility). Preliminary work demonstrated that edible films from VVPI formed without plasticizer as relatively brittle and broke easily when peeled off. Hence desirable mechanical properties of edible films were improved by using two types of plasticizer (glycerol and sorbitol) at different concentrations (30, 40, 50 and 60%). The mechanical properties of films plasticized by glycerol and sorbitol at different concentration were assessed by measuring their tensile strength and elongation at break. The results are depicted in Figure 5. It was observed that an increase in the content of these plasticizers resulted in decrease in mechanical resistance (decrease in tensile strength) and trend to increase in extensibility (increase in elongation at break). Glycerol and sorbitol are low molecular weight hydrophilic molecules that could easily fit into protein chains and establish hydrogen bonding with reactive groups of proteins. Bringing together plasticizers and proteins induced formation protein-plasticizer interactions to the detriment of protein-protein interactions. As a consequence, the density of intermolecular interaction in material decreased and the free volume between polymer chains increased **(Cuq et al., 1997)**. The changes in mechanical properties as affected by hydrophilic plasticizers were observed for various hydrocolloid-based films **(Gontard et al., 1993; Park and Chinnan, 1990)**. The mechanical properties of glycerol and sorbitol plasticized films at an equal concentration were compared (Figure 5).

The sorbitol plasticized films had significantly (p <0.05) higher tensile strength and lower elongation at break than glycerol plasticized films at all concentrations. This could be attributed to the ring molecular conformation of sorbitol molecules, which may sterically hinder insertion between the protein chains resulted in less effective in disrupting the protein-protein interruptions. McHugh and Krochta **(McHugh and Krochta, 1994)** studied whey protein isolated/sorbitol (1 : 1) and whey protein isolated/glycerol (2 : 3) films and presented similar tensile strength values. They concluded that a higher amount of sorbitol than glycerol was needed to obtain similar tensile strength

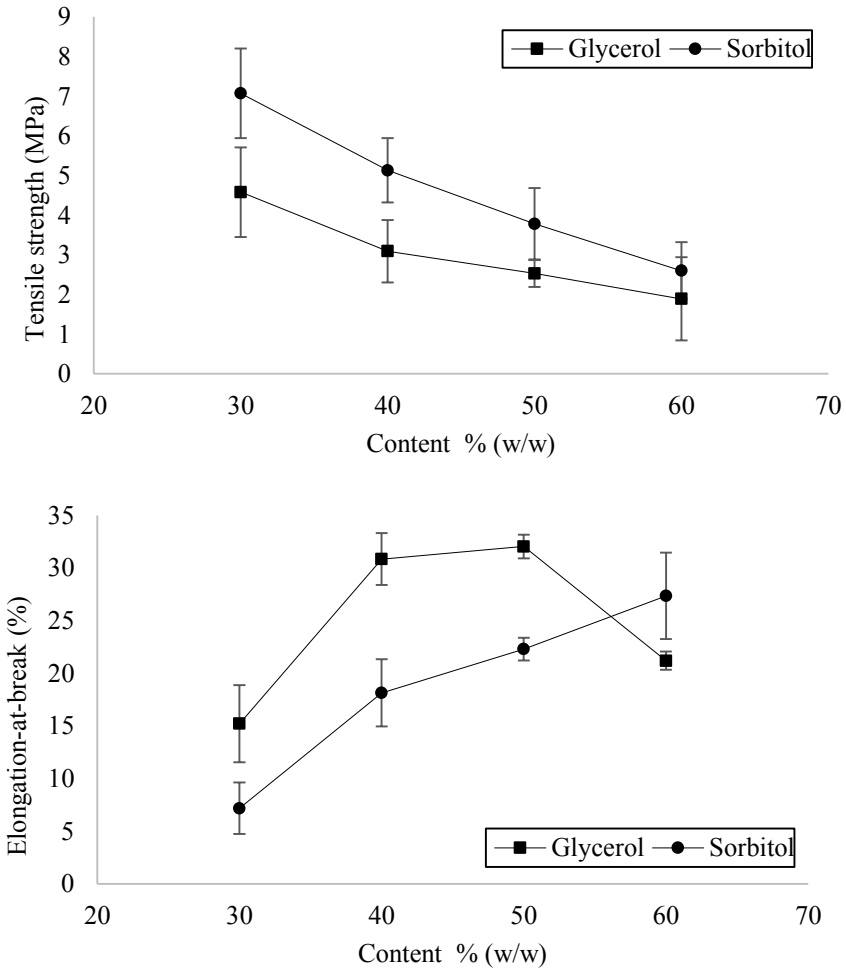

Figure 5 Tensile strength and Elongation at break of 10 % (w/w) VVPI films in the presence of 30, 40, 50 and 60 % (w/w of VVPI) glycerol and sorbitol. Data represent the mean ± one standard deviation (n = 3).

Table 3 DSC measurement results of VVPI based Films with various glycerol and sorbitol contents, in terms of glass transition temperature (T_g), melting temperature (T_m), enthalpy of melting (ΔH_m).

Plasticizer type	Content % (w/w)	ΔH_m (J.g^{-1})	T_m (°C)	T_g (°C)
Glycerol	30	193.86 ±2.13^{b}	172.85 ±1.0^{b}	41.12 ±1.56^{c}
	40	183.93 ±1.45^{bc}	168.18 ±1.28^{b}	36.43 ±1.64^{cd}
	50	180.00 ±1.12^{c}	152.91 ±1.38^{c}	30.11 ±0.97^{d}
	60	179.94 ±2.47^{c}	149.30 ±1.59^{d}	26.69 ±1.85^{e}
Sorbitol	30	209.91 ±2.42^{a}	184.55 ±1.37^{a}	53.19 ±1.54^{a}
	40	186.98 ±1.55^{bc}	161.72 ±1.0^{c}	52.61 ±0.95^{a}
	50	181.00 ±1.43^{c}	156.82 ±2.9^{cd}	50.49 ±1.09^{ab}
	60	180.94 ±2.42^{c}	155.11 ±1.61^{cd}	49.14 ±1.12^{b}

Note: the values with the same letter are not significantly different at the p <0.05.

properties. The glycerol plasticized films were more stretchable than the sorbitol plasticized films (Figure 4), suggesting that glycerol could be a more effective plasticizer in edible films than sorbitol. The effectiveness of glycerol in the edible films from VVPI are most likely due to its small size and configuration which allows it to be more readily inserted between the polymer chains, and consequently exert more influence on the mechanical properties than the larger molecule. **Donhowe and Fennema (1993)** found that plasticizer with low molecular weights such as glycerol was more effective than those with high molecular weights in methylcellulose-based films. Similarly, **McHugh and Krochta (1994)** suggested that smaller size plasticizer was more effective than larger size plasticizer in whey protein films. **Gennadios et al. (1993b)** reported that, the polar group (-OH) along plasticizer chains are believed to develop polymer-plasticizer hydrogen bonds replacing the polymer-polymer interaction in the biopolymer films. Molecular size, configuration and total number of functional hydroxide groups of the plasticizer as well as its compatibility with the polymer could affect the interactions between the plasticizer and the polymer **(Yang and Paulson, 2000)**.

Thermal properties

DSC studies of VVPI films containing different concentrations of glycerol were performed to better understand the structure and interaction between polymers and plasticizer. The glass transition temperature (T_g) is the temperature at which the material under-goes a structural transition from the glassy state to a rubbery state **(Yang and Paulson, 2000)**. Below T_g, films are rigid and brittle, whereas above it films become flexible and pliable.

The properties of VVPI films, at various levels of glycerol and sorbitol, were also analyzed in terms of thermal performance via DSC.

DSC thermograms showed two thermal transitions for VVPI films, irrespective of their content of glycerol and sorbitol; a glass transition for the amorphous fraction, and a melting transition for the crystalline one. The glass transition temperature (T_g), the melting temperature (T_m) and the melting enthalpy (ΔH_m) values are summarized in Table 3.

VVPI protein films containing sorbitol exhibited T_g, T_m and ΔH_m values significantly higher (p <0.05) than those obtained for VVPI protein films containing glycerol, at a given content of glycerol and sorbitol (Table 3), thus suggesting stronger films.

From inspection of Table 3, it is possible to conclude that T_g and T_m decreased as glycerol and sorbitol content increased from 30 to 60% (w/w) (p <0.05). This trend is a consequence of the plasticizing effect of glycerol and sorbitol molecules which typically increase the free volume of the polymer network and the segmental mobility of the polymer chains, thus decreasing both T_g and T_m **(Sobral et al., 2002, 2001)**.

Table 3 showed that ΔH_m also decreased when the glycerol and sorbitol content increased, this decrease was statistically significant (p <0.05). Such a decrease in thermal stability was affected by the presence of glycerol and sorbitol, which reduced the interaction between proteins, and thus stabilized the network structure **(Barreto et al., 2003)**; in other words, higher glycerol and sorbitol content required a lower enthalpy to disrupt inter-chain interactions.

In addition, results suggest that glycerol and sorbitol were compatible with VVPI, and confirmed the effectiveness of plasticization since only one T_g followed by an endothermic peak (T_m) was observed **(Sobral et al., 2002, 2001)**. If a polymer and the plasticizer, or two different polymers were immiscible, the mixture would in fact exhibit two T_g values, corresponding to the two pure phases **(Arvanitoyannis et al., 1997; De Carvalho and Grosso, 2004; Vanin et al., 2005)**.

This result is similar to those obtained by **Ramos et al. (2013)**. They reported that the lower T_g, T_m and ΔH_m values of plasticized whey protein isolate (WPI) and whey protein concentrate (WPC) edible films could be attributed to the reduction of intermolecular forces and increase in the mobility of polymer chains.

CONCLUSION

The results of this study pointed out that as plasticizer concentration increased, tensile strength decreased concomitant with increase in elongation at break and water vapor permeability of VVPI films. Sorbitol plasticized films provided the films with highest mechanical resistance, but the poorest film flexibility. In contrast, glycerol plasticized films exhibited flexible structure; however, the mechanical resistance was low, while inversely affecting the water vapor permeability. Increasing the plasticizer concentration from 30 to 60% resulted in higher solubility (from 48.11 to 70.65 for glycerol and from 60.66 to 80.34 for sorbitol). Sorbitol plasticized films, showed higher film solubility, opacity, thermal features and surface hydrophobicity values compared to glycerol plasticized films. Addition it was found that the films plasticized with sorbitol had lower moisture content (16.11 – 18.12%) than those with glycerol (21.47 – 36.66%). Overall, the results of present study

indicated that adding of these plasticizers seems to be useful to fabricate VVPI films with acceptable characteristics. Therefore, the results revealed substantial potential of VVPI edible films to be incorporated in food packaging applications, especially for those that require less hydrophobic films.

REFERENCES

Aarssen, L. W., Hall, I. V., Jensen, K. I. N. 1986. The biology of Canadian weeds: 76. *Vicia angustifolia* L., *V. cracca* L., *V. sativa* L., *V. tetrasperma* (L.) Schreb. and *V. villosa Roth*. *Canadian journal of plant science*, vol. 66, no. 3, p. 711-737. https://doi.org/10.4141/cjps86-092

American Society for Testing and Materials. 1994. Standard test method for moisture content of paper and paperboard by oven drying. Designation: D644-94. In ASTM, *Annual book of American Standard Testing Methods standards*. p. 1-2. Pennsylvania : West Conshohochem.

American Society for Testing and Materials. 1995. Standard test methods for tensile properties of thin plastics sheeting D882-91. In ASTM, *Annual Book of American Standard Testing Methods*. p. 182-190. Pennsylvania : West Conshohochem.

Arvanitoyannis, I. S., Nakayama, A., Aiba, S. 1998. Chitosan and gelatin based edible films: state diagrams, mechanical and permeation properties. *Carbohydrate Polymer*, vol. 37, no. 4, p. 371-382. https://doi.org/10.1016/S0144-8617(98)00083-6

Arvanitoyannis, I., Psomiadou, E., Nakayama, A., Aiba, S., Yamamoto, N. 1997. Edible films made from gelatin, soluble starch and polyols, Part 3. *Food Chemistry*, vol. 60, no. 4, p. 593-604. https://doi.org/10.1016/S0308-8146(97)00038-1

Ashley, R. J. 1985. Permeability and plastics packaging. *Polymer Permeability*, p. 269-308. https://doi.org/10.1007/978-94-009-4858-7_7

Bamdad, F., Goli, A. H., Kadivar, M. 2006. Preparation and characterization of proteinous film from lentil (*Lens culinaris*): Edible film from lentil (*Lens culinaris*). *Food Research International*, vol. 39, no. 1, p. 106-111. https://doi.org/10.1016/j.foodres.2005.06.006

Barreto, P. L. M., Pires, A. T. N., Soldi, V. 2003. Thermal degradation of edible films based on milk proteins and gelatin in inert atmosphere. *Polymer Degradation and Stability*, vol. 79, no. 1, p. 147-152. https://doi.org/10.1016/S0141-3910(02)00267-7

Bioplastics market data, 2015. European Bioplastics [online] s.a [cit 2018-02-01] Available at: http://www.european-bioplastics.org/market.

Bourtoom, T. 2008. Factors affecting the properties of edible film prepared from mung bean proteins. *International Food Research Journal*, vol. 15, no. 2, p. 167-180.

Cagri, A., Ustunol, Z., Ryser, E. T. 2004. Antimicrobial edible films and coatings. *Journal of food protection*, vol. 67, no. 4, p. 833-848. https://doi.org/10.4315/0362-028X-67.4.833 PMid:15083740

Cuq, B., Gontard, N., Cuq, J. L., Guilbert, S. 1997. Selected functional properties of fish myofibrillar protein-based films as affected by hydrophilic plasticizers. *Journal of Agricultural and Food Chemistry*, vol. 45, no. 3, p. 622-626. https://doi.org/10.1021/jf960352i

De Carvalho, R. A., Grosso, C. R. F. 2004. Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. *Food hydrocolloids*, vol. 18, no. 5, p. 717-726. https://doi.org/10.1016/j.foodhyd.2003.10.005

Donhowe, I. G., Fennema, O. 1993. The effects of plasticizers on crystallinity, permeability, and mechanical properties of methylcellulose films. *Journal of Food Processing and Preservation*, vol. 17, no. 4, p. 247-257. https://doi.org/10.1111/j.1745-4549.1993.tb00729.x

Gennadios, A., Weller, C. L., Testin, R. F. 1993a. Property modification of edible wheat, gluten-based films. *ransactions of the ASAE*, vol. 36, no. 2, p. 465-470.

Gennadios, A., Weller, C., Testin, R. F. 1993b. Temperature effect on oxygen permeability of edible protein-based films. *Journal of Food Science*, vol. 58, no. 1, p. 212-214. https://doi.org/10.1111/j.1365-2621.1993.tb03247.x

Ghanbarzadeh, B., Oromiehi, A. R. 2008. Studies on glass transition temperature of mono and bilayer protein films plasticized by glycerol and olive oil. *Journal of Applied Polymer Science*, vol. 109, no. 5, p. 2848-2854. https://doi.org/10.1002/app.28289

Ghasemlou, M., Khodaiyan, F., Oromiehie, A. 2011. Physical, mechanical, barrier, and thermal properties of polyol-plasticized biodegradable edible film made from kefiran. *Carbohydrate Polymers*, vol. 84, no. 1, p. 477-483. https://doi.org/10.1016/j.carbpol.2010.12.010

Gleason, H. A., Cronquist, A. 1963. *Manual of vascular plants of northeastern United States and adjacent Canada*. New York, USA : New York Botanical Garden Pr Dept. 910 p. ISBN-13: 978-0893273651.

Gontard, N., Duchez, C., Cuq, J., Guilbert, S. 1994. Edible composite films of wheat gluten and lipids: water vapour permeability and other physical properties. *International Journal of Food Science & Technology*, vol. 29, no. 1, p. 39-50. https://doi.org/10.1111/j.1365-2621.1994.tb02045.x

Gontard, N., Guilbert, S., Cuq, J. 1992. Edible wheat gluten films: influence of the main process variables on film properties using response surface methodology. *Journal of food science*, vol. 57, no. 1, p. 190-195. https://doi.org/10.1111/j.1365-2621.1992.tb05453.x

Gontard, N., Guilbert, S., Cuq, J. 1993. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science*, vol. 58, no. 1, p. 206-211. https://doi.org/10.1111/j.1365-2621.1993.tb03246.x

Guilbert, S. 1986. *Food packaging and preservation. Theory and Practice in Technology and Application of Edible Protective Films*. London, UK : Elsevier Applied Science Publishing Co. p. 371.

Hagenmaier, R. D., Shaw, P. E. 1990. Moisture permeability of edible films made with fatty acid and hydroxypropyl methyl cellulose. *Journal of Agricultural and Food Chemistry*, vol. 38, no. 9, p. 1799-1803. https://doi.org/10.1021/jf00099a004

Haugaard, V. K., Udsen, A., Mortensen, G., Høegh, L., Petersen, K., Monahan, F. 2001. Potential Food Applications of Biobased Materials. An EU-Concerted Action Project. *Starch-Stärke*, vol. 53, no. 5, p. 189-200. https://doi.org/10.1002/1521-379X(200105)53:5<189::AID-STAR189>3.0.CO;2-3

Horowitz, W., Latimer, G. 1994. *Official methods of AOAC International*. 18th ed. Maryland, USA.

Hulten, E. 1968. *Flora of Alaska and neighboring territories: a manual of the vascular plants*. Stanford, California : Stanford University Press. 1032 p. ISBN 9780804706438.

Chae, S. I., Heo, T. R. 1997. Production and properties of edible film using whey protein. *Biotechnology and Bioprocess Engineering*, vol. 2, no. 2, p. 122-125. https://doi.org/10.1007/BF02932339

Chang, C., Nickerson, M. T. 2014. Effect of plasticizer-type and genipin on the mechanical, optical, and water vapor barrier properties of canola protein isolate-based edible films. *European Food Research and Technology*, vol. 238, no. 1, p. 35-46. https://doi.org/10.1007/s00217-013-2075-x

Chick, J., Ustunol, Z. 1998. Mechanical and barrier properties of lactic acid and rennet precipitated casein-based edible films. *Journal of Food Science*, vol. 63, no. 6, p. 1024-1027. https://doi.org/10.1111/j.1365-2621.1998.tb15846.x

Cho, S. Y., Rhee, C. 2002. Sorption characteristics of soy protein films and their relation to mechanical properties. *LWT – Food Science and Technology*, vol. 35, no. 2, p. 151-157. https://doi.org/10.1006/fstl.2001.0829

Choi, W., Han, J. H. 2001. Physical and mechanical properties of pea-protein-based edible films. *Journal of Food Science*, vol. 66, no. 2, p. 319-322. https://doi.org/10.1111/j.1365-2621.2001.tb11339.x

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Tanaka, M. 2006. Effects of plasticizers on the properties of edible films from skin gelatin of bigeye snapper and brownstripe red snapper. *European Food Research and Technology*, vol. 222, no. 3-4, p. 229-235. https://doi.org/10.1007/s00217-005-0004-3

Kester, J. J., Fennema, O. R. 1986. Edible films and coatings: a review. *Food technology*, vol. 40, p. 47-59.

Kwok, D. Y., Neumann, A. W. 1999. Contact angle measurement and contact angle interpretation. *Advances in colloid and interface science*, vol. 81, no. 3, p. 167-249. https://doi.org/10.1016/S0001-8686(98)00087-6

Lieberman, E. R., Gilbert, S. G. 1973. Gas permeation of collagen films as affected by cross-linkage, moisture, and plasticizer content, in: *Journal of Polymer Science: Polymer Symposia*, vol. 41, no. 1, p. 33-43. https://doi.org/10.1002/polc.5070410106

Liu, C. C., Tellez-Garay, A. M., Castell-Perez, M. E. 2004. Physical and mechanical properties of peanut protein films. *LWT-Food Science and Technology*, vol. 37, no.7, p. 731-738. https://doi.org/10.1016/j.lwt.2004.02.012

Ma, X., Chang, P. R., Yu, J. 2008. Properties of biodegradable thermoplastic pea starch/carboxymethyl cellulose and pea starch/microcrystalline cellulose composites. *Carbohydrate Polymers*, vol. 72, no. 3, p. 369-375. https://doi.org/10.1016/j.carbpol.2007.09.002

Mannheim, C. H., Passy, N. 1990. Interaction between packaging materials and foods. *Packaging Technology and Science*, vol. 3, no. 3, p. 127-132. https://doi.org/10.1002/pts.2770030303

McHugh, T. H., Aujard, J., Krochta, J. M. 1994. Plasticized whey protein edible films: water vapor permeability properties. *Journal of Food Science*, vol. 59, no. 2, p. 416-419. https://doi.org/10.1111/j.1365-2621.1994.tb06980.x

McHugh, T. H., Avena-Bustillos, R., Krochta, J. M. 1993. Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. *Journal of food science*, vol. 58, no. 4, p. 899-903. https://doi.org/10.1111/j.1365-2621.1993.tb09387.x

McHugh, T. H., Krochta, J. M. 1994. Sorbitol-vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. *Journal of Agricultural and Food Chemistry*, vol. 42, no. 4, p. 841-845. https://doi.org/10.1021/jf00040a001

Myers, A. W., Meyer, J. A., Rogers, C. E., Stannett, V., Szwarc, M. 1961. Studies in the gas and vapor permeability of plastic films and coated papers. The permeation of water vapor. *Tappi Journal*, vol. 45, p. 63-77.

Orliac, O., Rouilly, A., Silvestre, F., Rigal, L. 2003. Effects of various plasticizers on the mechanical properties, water resistance and aging of thermo-moulded films made from sunflower proteins. *Industrial Crops and Products*, vol. 18, no. 2, p. 91-100. https://doi.org/10.1016/S0926-6690(03)00015-3

Osés, J., Fernández-Pan, I., Mendoza, M., Maté, J. I. 2009. Stability of the mechanical properties of edible films based on whey protein isolate during storage at different relative humidity. *Food Hydrocolloids*, vol. 23, no. 1, p. 125-131. https://doi.org/10.1016/j.foodhyd.2007.12.003

Otoni, C. G., Avena-Bustillos, R. J., Olsen, C. W., Bilbao-Sáinz, C., McHugh, T. H. 2016. Mechanical and water barrier properties of isolated soy protein composite edible films as affected by carvacrol and cinnamaldehyde micro and nanoemulsions. *Food Hydrocolloids*, vol. 57, p. 72-79. https://doi.org/10.1016/j.foodhyd.2016.01.012

Park, H. J. 1999. Development of advanced edible coatings for fruits. *Trends in Food Science & Technology*, vol. 10, no. 8, p. 254-260. https://doi.org/10.1016/S0924-2244(00)00003-0

Park, H. J., Chinnan, M. S. 1990. Properties of edible coatings for fruits and vegetables. Paper-*American Society of Agricultural Engineers*, vol. 19, p. 90-6510.

Petersen, K., Nielsen, P. V., Bertelsen, G., Lawther, M., Olsen, M. B., Nilsson, N. H., Mortensen, G. 1999. Potential of biobased materials for food packaging. *Trends in Food Science & Technology*, vol. 10, no. 2, p. 52-68. https://doi.org/10.1016/S0924-2244(99)00019-9

Ramos, Ó. L., Reinas, I., Silva, S. I., Fernandes, J. C., Cerqueira, M. A., Pereira, R. N., Vicente, A. A., Pocas, M. F., Pintado, M. E., Malcata, F. X. 2013. Effect of whey protein purity and glycerol content upon physical properties of edible films manufactured therefrom. *Food Hydrocolloids*, vol. 30, no. 1, p. 110-122. https://doi.org/10.1016/j.foodhyd.2012.05.001

Rhim, J. W., Ng, P. K. W. 2007. Natural biopolymer-based nanocomposite films for packaging applications. *Critical reviews in food science and nutrition*. vol. 47, no. 4, p. 411-433. https://doi.org/10.1080/10408390600846366 PMid:17457725

Rodríguez, M., Oses, J., Ziani, K., Mate, J. I. 2006. Combined effect of plasticizers and surfactants on the physical properties of starch based edible films. *Food Research International*, vol. 39, n. 8, p. 840-846. https://doi.org/10.1016/j.foodres.2006.04.002

Roy, L., Mulligan, Taylor, G. A. 1968. Flora of the Queen Charlotte Islands. Part 2. Cytological aspects of the vascular plants. Ottawa : Canada Department of Agriculture.

Ryan, M., McEvoy, E., Duignan, S., Crowley, C., Fenelon, M., O'Callaghan, D. M., FitzGerald, R. J. 2008. Thermal stability of soy protein isolate and hydrolysate ingredients. *Food chemistry*, vol. 108, no. 2, p. 503-510. https://doi.org/10.1016/j.foodchem.2007.11.001 PMid:26059128

Ryu, S. Y., Rhim, J. W., Roh, H. J., Kim, S. S. 2002. Preparation and physical properties of zein-coated high-amylose corn starch film. *LWT – Food Science and Technology*, vol. 35, no. 8, p. 680-686. https://doi.org/10.1006/fstl.2002.0929

Saremnezhad, S., Azizi, M. H., Barzegar, M., Abbasi, S., Ahmadi, E. 2010. Properties of a new edible film made of faba bean protein isolate. *Journal of Agricultural Science and Technology*, vol. 13, no. 2, p. 181-192.

Shafiei, A., Kazemi, E., Mozafari, S., Monapooyeh, A., Shah Moradi, A. 2006. Autecology of Vicia villosa in Kohgiloyeh-va-Boyerahmad province. Agricultural Scientific Information and Documentation Centre, Agricultural Research and Education Organization. [online] s.a [cit 2018-02-01] Available at: http://agris.fao.org/agris-search/search.do?recordID=IR2008001147.

Shahraki, F., Hadad-Khodaparast, M. H., Hesarinejad, M. A., Mortazavi, S. A., Milani, E., Hoseinzadeh, A. 2013. The quality and purity of the proteins extracted from the Lathyrus sativus seeds and optimization by the response surface methodology (RSM). In *1st International e-Conference on Novel Food Processing*. Mashhad, Iran : Ferdowsi University of Mashhad. p. 4.

Sobral, P. J. A., Menegalli, F. C., Hubinger, M. D., Roques, M. A. 2001. Mechanical, water vapor barrier and thermal properties of gelatin based edible films. *Food Hydrocolloids*, vol. 15, no. 4-6, p. 423-432. https://doi.org/10.1016/S0268-005X(01)00061-3

Sobral, P. J. A., Monterrey-Q, E. S., Habitante, A. M. Q. B. 2002. Glass transition study of Nile Tilapia myofibrillar protein films plasticized by glycerin and water. *Journal of Thermal Analysis and Calorimetry*, vol. 67, no. 2, p. 499. https://doi.org/10.1023/A:1013905400900

Soliman, E. A., Tawfik, M. S., El-Sayed, H., Moharram, Y. G. 2007. Preparation and characterization of soy protein based edible/biodegradable films. *American Journal of Food Technology*, vol. 2, no. 6, p. 462-476. https://doi.org/10.3923/ajft.2007.462.476

Stuchell, Y. M., Krochta, J. M. 1994. Enzymatic treatments and thermal effects on edible soy protein films. *Journal of Food Science*, vol. 59, no. 6, p. 1332-1337. https://doi.org/10.1111/j.1365-2621.1994.tb14709.x

Tanaka, M., Iwata, K., Sanguandeekul, R., Handa, A., Ishizaki, S. 2001. Influence of plasticizers on the properties of edible films prepared from fish water-soluble proteins. *Fisheries science*, vol. 67, no. 2, p. 346-351. https://doi.org/10.1046/j.1444-2906.2001.00237.x

Tang, C. H., Jiang, Y. 2007. Modulation of mechanical and surface hydrophobic properties of food protein films by transglutaminase treatment. *Food research international*, vol. 40, no. 4, p. 504-509. https://doi.org/10.1016/j.foodres.2006.09.010

Tavili, A., Shafiei, A., Pouzesh, H., Farajollahi, A., Saberi, M., Shahmoradi, A. A. 2010. Autecology of Vicia villosa in Kohgiluyeh and Boyerahmad Province, Iran. *Rangeland Journal*, vol. 4, no. 3, p. 422-433.

Vanin, F. M., Sobral, P. J. A., Menegalli, F. C., Carvalho, R. A., Habitante, A. 2005. Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films. *Food Hydrocolloids*, vol. 19, no. 5, p. 899-907. https://doi.org/10.1016/j.foodhyd.2004.12.003

Vogler, E. A. 1998. Structure and reactivity of water at biomaterial surfaces. *Advances in colloid and interface science*, vol. 74, no. 1-3, p. 69-117. https://doi.org/10.1016/S0001-8686(97)00040-7

Wittaya, T. 2013. Influence of type and concentration of plasticizers on the properties of edible film from mung bean proteins. *Current Applied Science and Technology Journal*, vol. 13, no. 1, p. 51-58.

Yang, L., Paulson, A. T. 2000. Mechanical and water vapour barrier properties of edible gellan films. *Food Research International*, vol. 33, no. 7, p. 563-570. https://doi.org/10.1016/S0963-9969(00)00092-2

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SOFTWARE SUPPORT FOR COST CALCULATION – APPLICATION TO THE AGRICULTURAL SECTOR

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ABSTRACT
Calculation of product costs is the source of information on the costs of selected produced products with great explanatory power. In current practice, the overhead costs on farms are monitored and calculated by species. They are allocated using an allocation base (average state of the animals, harvested area in hectares) or are converted using direct costs of the activity as an allocation base. With the current high level of overheads, this method cannot be considered effective. Only type classifications are monitored and are therefore anonymous in relation to activities. We consider high overhead costs as a good reason for implementing and using the methods of Activity Based Costing. In this paper we present a proposed model of Activity Based Costing for its use in agriculture, created in MS Excel. We create the model as a basic version, which can be more closely defined depending on the particular conditions of the business implementing the model. We complete the general model for better illustration with figures on costs. We present a comparison of model results with the traditional approach of calculating costs in agriculture. One of the biggest benefits of the ABC system is the binding of costs from accounts, activities performed and the cost of products in one system. We present a statistical comparison of model results with the traditional approach of calculating costs in agriculture.

Keywords: traditional cost calculation; Activity Based Costing method; agricultural business; Activity Based Costing model; controlling

INTRODUCTION
Information is a prerequisite for any management activity. Its value is growing continuously. Objectively, rapidly and correctly addressed information inside the company is an important basis for its effective management. One requirement is more accurate calculations of product costs. Clarification involves removing differences in the allocation of overhead costs and the replacement of a distorted image with a true picture based on the causal connections arising from real resource consumption.

Calculation of product costs is an important part of the company's information system **(Tóth et al., 2016)**. It is the source of information on the costs of selected produced products with great explanatory power **(Kubicová and Habánová, 2016)**.

At present, agriculture in the Slovak Republic mainly uses traditional methods for the calculation of production costs. Traditional calculation formulas work with overheads (as opposed to modern methods of calculations that convert non-specific, anonymous overheads into direct costs). Traditional calculations do not reflect the needs of the market environment.

The agricultural sector is characterized by a high proportion of overheads. These are costs that cannot or would not be economical to monitor by calculation unit **(Ferenczi-Vaňová et al., 2017)**. Overhead costs are therefore indirectly reflected in product costs of calculation units through an allocation base. The allocation base used is the direct costs of the different crops grown, animals raised, customer orders, work and services for others.

Managing overhead costs is complicated and therefore each business should try, in calculating production costs, to place the most costs directly on a calculated product or activity as direct costs. Therefore, it is necessary to change the content structure of individual cost items of the calculation formula. A good solution to the problems in overhead costs is non-traditional methods of calculating production costs, in particular ABC (Activity Based Costing), which brings a new perspective on overheads and turns them into direct costs.

The importance of the ABC calculation method is summarized in the following literature review.

According to **Kaplan and Anderson (2003, 2005, 2007)** Activity Based Costing is an approach to solve the problems of traditional cost management systems. These

traditional costing systems are often unable to determine accurately the actual costs of production and of the costs of related services. Consequently managers were making decisions based on inaccurate data especially where there are multiple products. Instead of using broad arbitrary percentages to allocate costs, ABC seeks to identify cause and effect relationships to objectively assign costs. Once costs of the activities have been identified, the cost of each activity is attributed to each product to the extent that the product uses the activity. In this way ABC often identifies areas of high overhead costs per unit and so directs attention to finding ways to reduce the costs or to charge more for costly products **(Chrenková, 2011; Cannavacciuolo et al., 2015; Duh et al., 2009)**. In present day manufacturing organizations, performance measurements play an important role in providing strategic directions and developing corresponding operational policies and methods. One such method is the activity-based costing (ABC) method which calculates the cost of activities and helps in making decisions on product mix and price for improving the utilization of resources and minimizing the cost of production **(Ittner et al., 2002; Quinn et al., 2017)**. Even now some manufacturing organizations employ traditional costing methods depending upon their market forces and characteristics. One of the most important decisions to be made is about the type of costing system that would be suitable for an organization **(Slangen et al., 2003; Zhang et al., 2015)**. The role of direct labour in current manufacturing environments has diminished, but at the same time the level of support services has increased. Traditional methods of cost calculation do not take into account this increased complexity and still allocate overhead costs by their diminishing labour base or even do not take into account overhead costs **(Gunasekaran, et al., 1999; Kostakis, H., 2008)**.

Veščičík (2004, 2012) explains that Activity Based Costing is a new modern method of calculating the cost of individual processes, products and customers, which eliminates the inaccuracies of traditional methods of the last century (overhead calculations, covering post).

Traditional cost accounting methods were developed at a time when direct costs of labour and material factors of production were dominant and when changes in technology and consumer demand were not so fast **(Gunasekaran et al., 1999; Kaszubski and Ebben, 2005; Kostakis, 2008)**. The problems with traditional cost accounting emerge when indirect costs (such as maintenance, insurance, production preparation, etc.) amount to significant sums or are even higher than direct costs. Activity Based Costing is a commonly used tool and has practical significance for the specific conditions of agricultural production, where it can be used to achieve the improvement of cost management **(Zakić, Borović, 2013; Kaszubski and Ebben, 2005; Khataie and Bulgak, 2013)**. Activity Based Costing represents a universal management instrument that is used not only for the purposes of cost calculations, but represents a tool enabling effective cost reduction. In addition to these advantages, the ABC method has also its restriction as it is more demanding in terms of the volume and structure of the data processing. In case of its application, it is therefore necessary to consider carefully all the benefits and costs associated with its implementation **(Popesko, 2010, 2012; Popesko et al., 2015)**.

Pokorná (2016) states that looking for factors affecting business performance is one of a central concern of business economists for several years. Activity-Based Costing (ABC) is a management tool that provides additional and more accurate information on the costs and company performance, thus contributes to better manager decision making, and thus has potential to affect the financial performance. The ABC expansion among enterprises in the Czech Republic is currently comparable with neighbouring countries, although the extent of its use is lower.

The extensive ABC use is associated with higher quality levels and greater improvements in cycle time and quality, and is indirectly associated with manufacturing cost reductions through quality and cycle time improvements **(Krumwiede and Charles, 2014; Lelkes, 2014)**. However, on average, extensive ABC use has no significant association with return on assets. Instead, weak evidence that the association between ABC and accounting profitability is contingent on the plant's operational characteristics **(Ittner et al., 2002; Dalci et al, 2010; Kuethe and Morehart, 2012)**. An Activity Based Costing system is a system that focuses attention on the costs of various activities required to produce a product or service **(Langfield-Smith et al., 1998; Jánsky et al., 2012)**. The ABC method is a progressive instrument of controlling. It enables to assign costs to products according to actually used up activities and resources. The method is designed for more accurate scheduling of indirect costs (overheads); as a schedule using the causal relationship between activities (processes) and individual performance. **(Foltínová, 2011)**. The main principle of the ABC is placing the activities among the source costs (taken over from the accountancy) and the products. One of the biggest benefits of the ABC system is the interconnection of the costs arising from the accounting, processes and costs of products into one system **(Veščičík, 2012; Greasley and Smith, 2017)**.

Cohen et al. (2005) present evidence that the possibility of future ABC adoption is related to the degree of satisfaction from the currently used cost accounting system. Companies that do not intend to adopt ABC (ABC deniers) were found to be more satisfied with their existing cost accounting system in comparison to ABC supporters. They also report the characteristics of companies that still have complete ignorance of the ABC technique (ABC unawares).

Following the above, the paper is divided into three parts. The first part presents a theoretical overview of calculation methods in agriculture, defines the specifics of agriculture and also presents an overview of opinions on the importance of the ABC method. The second part is devoted to the creation and implementation of a calculation model created by ABC method (non-traditional method for agriculture). The third part is focused on the statistic analyses and comparing the traditional calculation method with the ABC method.

Scientific hypothesis

The aim of this paper is to create a proposal for implementation of Activity Based Costing, which is also

supported by the developed software solution design in MS Excel.

MATERIAL AND METHODOLOGY

The result is the presented methodology for calculating Activity Based Costing, which is based on a model in MS Excel 2013 and takes into account the specifics of primary agricultural production. The calculation model is created in the spreadsheet Microsoft Office Excel 2013 from the company Microsoft for the operating system Microsoft Windows and Macintosh, version number 15.0.4535.1511. The presented model of implementation of ABC is designed as a framework proposal in generic agriculture companies, supplemented in the paper with real data and then calculated results.

In order to achieve the aim of this paper, we analyse the current state of costs calculated in agriculture, then we define the specifics of primary agricultural production, particularly in conditions of transition economies. In the next part of the article, we specify the main activities in the production processes of crop and livestock production as a prerequisite for the use of the Activity Based Costing method in the structure of the calculation information system. Specification of activities focuses mainly on overhead costs for major production (crop and livestock) and administrative expenses, but also on the field of auxiliary production. These activities are specified in a selected agricultural cooperative, with the fictitious name Agroprodukt. For analysis of performance in crop production, we work with the products of wheat, barley, sugar beet, corn for grain and livestock production with products of milk cows- milk, fattening beef cattle, fattening pigs, fattening chickens.

From a methodological point of view, we use the non-traditional calculation method, Activity Based Costing, which charges all the indirect costs first to activities because they cause the need for costs and then divides activities to products, because products cause the need for the implementation of activities. This new view of costs enables their cost and complexity to be assessed compared with their benefits, creating a natural pressure to eliminate activities that are not effective.

The principle of the ABC method lies in the fact that in the first step the direct costs are assigned to outputs (this method does not bring anything new) and indirect (overhead) costs are assigned to activities (this is a substantial change). In the second step, the activities are assigned to individual cost items according to their degree of load of consumption for the activities necessary for their provision. Unlike the traditional approach – "everything to everyone equally" a selective system is applied on the basis of actual causation, that is, "to each only what they really consume, or what is consumed because of them." When implementing the ABC method in the selected enterprise, we follow generally defined steps to implement this method: a preparatory phase, specification of activities, aggregation of activities, identification of activity centres, first stage of allocation – costing of activities, creating the structure of the flow of costs, goods and identification of the activity centres, specification of products, the second stage of allocation – calculation of the cost of products, evaluation of results. Attention is paid to the selection and calculation of drivers.

For the purpose of comparison, the traditional method of calculation of product costs will be used with an allocation base formulated in the analysed fictional company.

The principle of the traditional calculation method is that we use the same allocation base for all kinds of overheads. Direct costs are allocated directly to the product, as is the case with the ABC method. It is used direct labour costs or total direct costs as an allocation base. For example - the percentage assignment of overhead costs to individual products is calculated: overhead / direct labour costs.

The material for analysis is the data on the levels of cost items found in the analysed company. The starting data sources for creation of the entire model are costs by type (reported in Table 2).

Subsequently, we have established a scientific hypothesis: Cost calculation using the traditional calculation method (based on the allocation base) is less accurate, distortive compared to cost calculation by the ABC method.

-H0: Equation of the mean values for both methods.

-H1: Inequality of the mean values for both methods.

In order to perform the statistical analysis, we used the real data of 22 agricultural enterprises (Grange, Ltd., SpA.). This is partly the data of companies that have already implemented the ABC method, in part data on enterprises that use only the traditional approach and the ABC method was modelled them (5 of these enterprises we modelled on ourselves, we obtained the rest of the data from consulting companies dealing with ABC implementation in enterprises).

Since the ABC method does not bring anything new in the direct cost allocation (allocates them directly to the product as a traditional approach), we included in the statistical analysis only the amount of overheads. These calculation approaches allocate overheads differently. The aim is to confirm or refute the established hypothesis.

We perform tests of normality, we tested normality with two tests: Kolmogorov-Smirnov test and Shapiro-Wilk test (Table 7). We evaluate data statistically using a paired t-test (Paired Samples Test), we evaluate the p-value (Table 8).

Statisic analysis

In statistics, the Kolmogorov–Smirnov test (K-S test or KS test) is a nonparametric test of the equality of continuous, one-dimensional probability distributions that can be used to compare a sample with a reference probability distribution (one-sample K-S test), or to compare two samples (two-sample K-S test).

The Kolmogorov-Smirnov statistic quantifies a distance between the empirical distribution function of the sample and the cumulative distribution function of the reference distribution, or between the empirical distribution functions of two samples. The null distribution of this statistic is calculated under the null hypothesis that the sample is drawn from the reference distribution (in the one-sample case) or that the samples are drawn from the same distribution (in the two-sample case). In each case, the distributions considered under the null hypothesis are continuous distributions but are otherwise unrestricted.

The Shapiro-Wilk test is a test of normality in frequentist statistics. The test rejects the hypothesis of normality when the p-value is less than or equal to 0.05. Failing the

normality test allows you to state with 95% confidence the data does not fit the normal distribution. Passing the normality test only allows to state no significant departure from normality was found.

A t-test is appropriate for comparing means under relaxed conditions (less is assumed).

Paired tests are appropriate for comparing two samples where it is impossible to control important variables. Rather than comparing two sets, members are paired between samples so the difference between the members becomes the sample. Typicaly the mean of the differences is then compared to zero. The common example scenario for when a paired difference test is appropriate is when a single set of test subjects has something applied to them and the test is intended to check for an effect.

We established suitable null and alternative hypostheses:
Null Hypothesis H0: $\mu = \mu 0$
Alternate Hypothesis H1: $\mu > \mu 0$

We used four steps, listed below:
1. Calculate the sample mean.
2. Calculate the sample standard deviation.
3. Calculate the test statistic.
4. Calculate the probability of observing the test statistic under the null hypothesis. This value is obtained by comparing "t" to a t-distribution with (n − 1) degrees of freedom.

The test is based on the differences of the measured pair values in the compared variation ranges. We test the hypothesis that the mean value of the traditonal calculation method and non - traditional method equals (or: the difference of the mean values of the pair measurements is zero).

First, we calculate the pair differences of the sample (n - number of pairs) and calculate the arithmetic mean $\bar{x}$ and the standard deviation "s" (or the variance s^2) from the differences found.
Then we calculate test criterion (statistics) t:

$$t = \frac{|\bar{x}|}{\sqrt{\frac{s^2}{n}}}$$

The p-value (probability value) for the t-statistic is „p":

$p = 2 . Pr(T > |t|)$ (two-tailed)
$p = Pr(T > t)$ (upper-tailed)
$p = Pr(T < t)$ (lower-tailed)
determine whether the results provide sufficient evidence to reject the null hypothesis in favor of the alternative hypothesis.

Table 1 Specification of activities and their aggregation.

Activity (aggregated)	Activities included
Purchase of material (supply)	Purchase and delivery of seeds, seedlings, fertilizers, agrochemicals, feed, receiving material, control and assessment of quality, purchase of protective equipment, spare parts, maintenance material, keeping stock records, issue, inventory of stocks
Tillage of land	Tillage (shallow, medium, deep, very deep, ridge-till), ploughing in straw crops for green manure, stable manure (loading manure, taking to land, spreading manure, ploughing in manure), levelling, harrowing, cultivating, rolling and compacting soil with compactors, tilling soil with sets of tools and combinations, preparation of seedbed
Sowing	Preparation and transport of seeds, pre-sowing aeration, sowing
Cultivation	Fertilization during vegetation, chemical treatment agricultural chemicals (herbicides, fungicides, zoocides ...), mechanical treatment of crops (aerating soil dryness with a harrow, destroying weeds in crops, thinning or lightening crops), irrigation, weeder-hoeing
Harvest	Direct harvest, transportation of grain, tipping, storage, cleaning and final drying of grain, harvesting straw for animal production, stacking straw (cereals), post-harvest treatment (harvesting corn husks, harvest and silage of beet tops)
Milking	Washing the udder, massaging the udder, milking, filtering milk, cooling milk, treatment and records of collected milk, milk storage, prevention of diseases of the mammary gland, prescribed maintenance of entrusted mechanization, minor repairs
Feeding	Transport of feed and water, dosing and mixing of feed, delivery (supply of feed), operation and maintenance of lines
Treating farm animals	Veterinary surgery and treatment, individual care, disease prevention, removal of faeces, providing ventilation, lighting, proper temperature, cleaning stable buildings and paddocks, cleaning, moving animals
Sales of agricultural products	Preparing for sale, loading, delivery by road
Ancillary activities	Road maintenance, daily technical maintenance and repairs, construction activities, mowing, chemical and mechanical treatment of boundaries
Managing the cooperative	Communication with suppliers and customers, taking orders, drawing up invoices and delivery notes, quality control, directing growing and husbandry, computer processing of information – registration of receivables and payables, payments, accounting, communication with various authorities

RESULTS AND DISCUSSION

Defining the specifics of agriculture in creating a proposal for implementation of the ABC method

The level of costs for agricultural products and their calculation, as distinct from other sectors of the national economy, is influenced by other factors resulting from the character of agricultural production. Among the most important are:

Natural factors, particularly in crop production – this includes soil conditions, weather conditions and the position of the land.

High consumption of own products in the production process - in-house consumption, due to the overlap between crop and livestock production.

Fragmentation of land and its shape – negative influence on the transport costs and labour costs for mechanized work in crop production.

Cycle of current assets. Affects development costs and their reproduction with inequality during the calendar year (accounting, tax year). Crop production takes a year; in most sectors of animal production it is longer than a year.

Industry, to an ever greater extent, decides about the level of costs (range, quality of agricultural inputs).

In agriculture, there is some damage that directly or indirectly affects costs (death of animals, frost of winter crops, destruction of the plants by floods, droughts, pests, etc.).

When drawing up the proposal for implementation of the ABC method it is necessary to take these specifications into account, because of their impact on the method of calculating costs. Taking into account the overlap between crop and animal production enables the capture of the production process, regardless of the length of the production cycle and enables activity in the manufacturing process to be recorded in such a way that it is possible to attribute costs to them in causal relationship.

Besides these general specifics of agriculture, it is necessary in transition economies to consider other factors, particularly limited financial resources, poor technical equipment of the business, using the traditional system of management accounting and calculations, not least the distrust of workers towards everything new and unusual. Therefore, for an agricultural company, low financial requirements for software are important. To avoid the mistrust of workers, the simplicity of the model of Activity Based Costing and logical clarity of its individual components is important, as is the ease of use.

Proposal of implanting the model and application in MS Excel suitable for agricultural businesses

While creating the proposed model of ABC we apply the procedures described in the methodological part of the paper.

In the studied agricultural cooperative, Agroprodukt, we specified activities and linked them to the activities listed in Table 1. We selected basic activities related to production of eight products for which we are calculating the cost in the proposed model. The ABC method is intended primarily for the allocation of overhead costs. When specifying activities it is therefore necessary to maintain a balanced level of detail and not to confuse this phase of implementation with defining the technological standards and manufacturing processes. Specification of activities is a precondition for applying the ABC method.

Table 2 CostData sheet.

COSTS	Total costs in €
Consumed material	
Fuels, oils and lubricants	15 000
Protective equipment	833
Cleaning and small material for maintenance of buildings and structures for animal production	5 833
Material for maintenance of office buildings	500
Material for the daily technical maintenance and repairs	3 167
Consumption of drugs and disinfectant material	1 500
Consumed energy	2 167
Consumption of other non-inventory items	
Water	8 500
Wages and salaries	
Payroll management	17 333
Wages for the daily technical maintenance and repairs	16 333
Amortization of long-term intangible assets and depreciation of long-term tangible assets	
Harvesters and tractors, self-propelled machines, tensioning system	21 667
Office buildings and warehouses	9 000
Single-purpose buildings and facilities for animal production	19 333
Machines and mechanisms in animal production	10 667
Trucks	10 333
Cars	8 500
Equipment of office buildings	6 333
Other operating expenses	12 667
Running costs of buildings (insurance, real estate tax, cost of heat)	15 167
The tax on agricultural land	15
Cost of vehicles	6 000

Table 3 CostAllocation-1stStage sheet (Table A, Table B, Table C).

A Relationships between costs and activities

COSTS	ACTIVITIES										
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**
Consumed material											
Fuels, oils and lubricants	X	X	X	X	X		X		X	X	X
Protective equipment				X				X		X	
Cleaning and small material for maintenance of buildings and structures for animal production						X	X	X			
Material for maintenance of office buildings											X
Material for the daily technical maintenance and repairs										X	
Consumption of drugs and disinfectant material								X			
Consumed energy						X		X			X
Consumption of other non-inventory items											
Water				X		X	X				
Wages and salaries											
Payroll management											X
Wages for the daily technical maintenance and repairs										X	
Amortization of long-term intangible assets and depreciation of long-term tangible assets											
Harvesters and tractors, self-propelled machines, tensioning system		X	X	X	X					X	
Office buildings and warehouses	X										X
Single-purpose buildings and facilities for animal production						X	X	X			
Machines and mechanisms in animal production						X	X				
Trucks	X		X		X		X		X		
Cars											X
Equipment of office buildings											X
Other operating expenses											X
Running costs of buildings (insurance, real estate tax, cost of heat)	X							X			X
The tax on agricultural land		X	X	X	X						
Cost of vehicles	X								X		X

B Cost drivers of the 1st stage

COSTS	Total costs in €	Total value of driver	ACTIVITIES										
			A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**	**A10**	**A11**
Consumed material													
Fuels, oils and lubricants	15 000	100%	8	19	11	7	17		6		11	9	12
Protective equipment	833	100%				20				20		60	
Cleaning and small material for maintenance of buildings and structures for animal production	5 833	100%						33.3	33.3	33.3			
Material for maintenance of office buildings	500	100%											100
Material for the daily technical maintenance and repairs	3 167	100%										100	
Consumption of drugs and disinfectant material	1 500	100%								100			
Consumed energy	**2 167**	**100%**						**35**		**45**			**20**
Consumption of other non-inventory items													
Water	8 500	100%				30		20	50				
Wages and salaries													
Payroll management	17 333	100%											100
Wages for the daily technical maintenance and repairs	16 333	100%										100	

Amortization of long-term intangible assets and depreciation of long-term tangible assets												
Harvesters and tractors, self-propelled machines, tensioning system	21 667	100%		25	20	15	25				15	
Office buildings and warehouses	9 000	1150m2	900									250
Single-purpose buildings and facilities for animal production	19 333	100%						33,3	33,3	33,3		
Machines and mechanisms in animal production	10 667	100%						50	50			
Trucks	10 333	100%	15		10		30		25		20	
Cars	8 500	100%										100
Equipment of office buildings	6 333	100%										100
Other operating expenses	**12 667**	**100%**										**100**
Running costs of buildings (insurance, real estate tax, cost of heat)	15 167	1850m2	900							655		250
The tax on agricultural land	15	100%		25	25	25	25					
Cost of vehicles	6 000	100%	30								30	40

C Costs of activities

COSTS	Total costs in €	ACTIVITIES										
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
Consumed material												
Fuels, oils and lubricants	15 000	1 200	2 850	1 650	1 050	2 550		900		1 650	1 350	1 800
Protective equipment	833				166.5				166.5		500	
Cleaning and small material for maintenance of buildings and structures for animal production	5 833						1 944.33	1 944.33	1 944.33			
Material for maintenance of office buildings	500											500
Material for the daily technical maintenance and repairs	3 167										3 167	
Consumption of drugs and disinfectant material	1 500								1 500			
Consumed energy	**2 167**						**758.5**		**975.1**			**433.4**
Consumption of other non-inventory items												
Water	8 500				2 550		1 700	4 250				
Wages and salaries												
Payroll management	17 333											17 333
Wages for the daily technical maintenance and repairs	16 333										16 333	
Amortization of long-term intangible assets and depreciation of long-term tangible assets												
Harvesters and tractors, self-propelled machines, tensioning system	21 667		5 416.75	4 333.4	3 250	5 416.75					3 250	
Office buildings and warehouses	9 000	7 043.50										1956.5

Single-purpose buildings and facilities for animal production	19 333						6 444.33	6 444.33	6 444.33			
Machines and mechanisms in animal production	10 667						3 333.50	3 333.50				
Trucks	10 333	1 550		1 033.33		3 100		2 583.20		2 066.50		
Cars	8 500											8 500
Equipment of office buildings	6 333											6 333
Other operating expenses	**12 667**											**12 667**
Running costs of buildings (insurance, real estate tax, cost of heat)	15 167	7 562.40							5 503.70			2 1006 0
The tax on agricultural land	15		115	115	115	115						
Cost of vehicles	6 000	1 800								1 800		2 400
Total costs	**190 848**	**19 155.90**	**8 270.5**	**7 020.5**	**7 020.2**	**11 070.50**	**16 180.66**	**21 455.36**	**16 534.26**	**5 516.5**	**24 600**	**54 023.5**

Source: own software program.

After specification of activities we can proceed to the actual cost calculation, which takes place in two steps. For creating a model in Excel, it is important that the agricultural businesses have the opportunity to export the data from accounting software to MS Excel. After talking with the workers from consulting software companies, we found that at least in a basic form, this is also possible for older accounting software.

We create the proposed model in MS Excel. In the file we created individual sheets as needed for implementation methods in the agricultural firm: CostData, CostAllocation-1stLevel, CostFlowStructure, CostAllocation-2ndLevel. On each sheet there are tables needed for calculations using the ABC method. Consequently, we defined the links between cells and sheets. Models are like the basic version, which can be adjusted accordingly to the needs of the business. The general model is completed with data on costs for better

Table 4 CostFlowStructure sheet (Table A, Table B, Table C, Table D, Table E).

A Relationships between activities

COSTS	Total costs in €	ACTIVITIES										
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
Consumed material												
Fuels, oils and lubricants	15 000	1 200	2 850	1 650	1 050	2 550		900		1 650	1 350	1 800
Protective equipment	833				166.5				166.5		500	
Cleaning and small material for maintenance of buildings and structures for animal production	5 833						1 944.33	1 944.33	1 944.33			
Material for maintenance of office buildings	500											500
Material for the daily technical maintenance and repairs	3 167										3 167	
Consumption of drugs and disinfectant material	1 500								1 500			
Consumed energy	**2 167**						**758.5**		**975.1**			**433.4**
Consumption of other non-inventory items												
Water	8 500				2 550		1 700	4 250				
Wages and salaries												
Payroll management	17 333											17 333
Wages for the daily technical maintenance and repairs	16 333										16333	
Amortization of long-term intangible assets and depreciation of long-term tangible assets												
Harvesters and tractors, self-propelled machines, tensioning system	21 667		5 416.75	4 333.40	3 250	5 416.75					3 250	
Office buildings and warehouses	9 000	7 043.50										1956,5
Single-purpose buildings and facilities for animal production	19 333						6 444.33	6 444.33	6 444.33			

Machines and mechanisms in animal production	10 667						3 333.50	3 333.50				
Trucks	10 333	1 550		1 033,33		3 100		2 583.20		2 066.50		
Cars	8 500											8 500
Equipment of office buildings	6 333											6 333
Other operating expenses	**12 667**											**12 667**
Running costs of buildings (insurance, real estate tax, cost of heat)	15 167	7 562.40							5 503.70			2 100.60
The tax on agricultural land	15		115	115	115	115						
Cost of vehicles	6 000	1 800								1 800		2 400
Total costs	**190848**	**19 155.90**	**8 270.50**	**7 020.55**	**7 020.25**	**11 070.50**	**16 180.66**	**21 455.36**	**16 534.26**	**5 516.50**	**24600**	**54 023.50**

B Distribution of the costs of activity No.11

ACTIVITIES	Total costs	ACTIVITIES										
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
Total costs		19 155.90	8 270.50	7 020.55	7 020.25	11 070.50	16 180.66	21 455.36	16 534.26	5 516.50	24 600	54 023.50
A1	19 155.90											
A2	8 270.50											
A3	7 020.55											
A4	7 020.25											
A5	11 070.50											
A6	16 180.66											
A7	21 455.36											
A8	16 534.26											
A9	5 516.50											
A10	24 600.00											
A11	54 023.50	14 025	1 793	3 188.50	3 748	5 465	2 245	4 133	3 319	11 582	4 525	
Total costs	190 848	33 180.90	10 063.50	10 209.05	10 768.25	16 535.50	18 425.66	25 588.36	19 853.26	17 098.50	29 125	

C Distribution of the costs of activity No.1

ACTIVITIES	Total costs	ACTIVITIES										
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
Total costs		33 180.90	10 063.50	10 209.05	10 768.25	11 070.50	18 425.66	25 588.36	19 853.26	17 098.50	29 125	
A1	33 180.90			11 405.50				14 581			7 194.40	
A2	10 063.50											
A3	10 209.05											
A4	10 768.25											
A5	11 070.50											
A6	18 425.66											
A7	25 588.36											
A8	19 853.26											
A9	17 098.50											
A10	29 125.00											
A11												
Total costs	190 848		10 063.50	21 614.55	10 768.25	16 535.50	18 425.66	40 169.36	19 853.26	17 098.50	36 319.40	

D Distribution of the costs of activity No.10

ACTIVITIES	Total costs	ACTIVITIES										
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
Total costs			10 063.50	21 614.55	10 768.25	16 535.50	18 425.66	40 169.36	19 853.26	17 098.50	36 319.40	

A1										
A2	10 063.50									
A3	21 614.55									
A4	10 768.25									
A5	16 535.50									
A6	18 425.66									
A7	40 169.36									
A8	19 853.26									
A9	17 098.50									
A10	36 319.40	10 922	9 648	7 505	8 244,40					
A11										
Total costs	190 848	20 985.0	31 262.55	18 273.25	24 779.90	18 425.66	40 169.36	19 853.26	17 098.50	

E Distribution of the costs of activity No.2

ACTIVITIES	Total costs	ACTIVITIES									
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
Total costs			20 985.50	31 262.55	18 273.25	24 779.90	18 425.66	40 169.36	19 853.26	17 098.50		
A1												
A2	20 985.50			20 985.50								
A3	31 262.55											
A4	18 273.25											
A5	24 779.90											
A6	18 425.66											
A7	40 169.36											
A8	19 853.26											
A9	17 098.50											
A10												
A11												
Total costs	190 848			52 248.05	18 273.25	24 779.90	18 425.66	40 169.36	19 853.26	17 098.50		

Source: own software program.

illustration.

The CostData sheet (Table 2) contains items from cost accounts in their detailed analytical breakdown. They are exported from the accounts of the company. This sheet serves as the source data for further sheets of the program.

The CostAllocation-1stStage sheet (Table 3) performs the first step in the cost allocation model. It contains three tables. Table A is necessary to define relationships between costs and activities by constructing a matrix of dependency. If activity "i" causes the formation of cost "j", the cell "i.j" is filled in with "X". After recording all the relationships between the activities and costs of the analysed agricultural cooperative we can determine which activities consume various costs. In table B it is necessary to define the cost drivers of the 1st stage on the basis of correlation between the costs and activities. They are used for the first stage of allocation, i.e. allocation of costs to individual activities. In practice, it often happens that cost drivers need to be a qualified estimate. It is necessary to define the percentage of the cost of individual activities, either on the basis of technological intensity, growing share in crop production or under otherwise specified criteria. It is important that this part of created model has been subject to consultation with the workers who are directly involved in an activity or workers who have information about the technological processes and the difficulty of cultivating crops and the processes implemented. The program provides value control of division of drivers into activities. Table C contains the calculation, i.e. the result of the first stage of allocation. Tables A and B must be additionally defined in program, table C is calculated automatically. These results in costs associated with individual activities.

The CostFlowStructure sheet (Table 4) contains predefined mutual relationships between the activities; subsequently it is necessary to additionally define the distribution of the costs of some activities to other activities. Relationships are then defined in the program as fixed; specific expenses are allocated according to the source data. The base is table A, which defines relationships between activities. Here, we further characterize the nature of each activity. Activities can be production, operational support, administration or administrative activities and internal services. Thus, part of the activities has a direct relation to the products, and another part has a mediated relationship to them. The cost of activities with a mediated (indirect) relationship to the product must first be attributed to other activities to which they have a direct relationship.

This is done in the same manner used to assign costs to activities. We find out what activities are related and then express their relationship quantitatively. Thus, we divide the cost of some activities onto other activities. Again, we need to define the drivers. Between activities there is a relationship of superiority and subordination depending on the direction of allocation of costs. It is important to remember that the costs of activity A can be moved to activity B only if activity A has already taken all the costs

Table 5 CostAllocation-2ndLevel sheet (Table A, Table B, Table C).

A Relationships between activities and products

ODUCTS	ACTIVITIES						
	A3	A4	A5	A6	A7	A8	A9
Wheat	X	X	X				X
Barley	X	X	X				X
Sugar beet	X	X	X				X
Corn for grain	X	X	X				X
Milk cows- milk				X	X	X	X
Fattening beef cattle					X	X	X
Fattening pig					X	X	X
Fattening chickens					X	X	X

B Calculation of overhead costs to products

PRODUCTS	Output	Area / number	ACTIVITIES							Overhead costs of the product	Overhead costs of the unit
			A3	A4	A5	A6	A7	A8	A9		
Total costs			52 248.05	18 273.25	24 779.90	18 425.66	40 169.36	19 853.26	17 098.50	190 848	
Wheat	1890 t	420	19 082	6 674	9 050				1 682.50	36 488.50	19.31
Barley	540 t	150	6 815	2 383.25	3 232				480.6	12 910.85	23.91
Sugar beet	14586 t	330	14 993	5 244	7 110,90				12 982	40 329.90	2.76
Corn for grain	1675 t	250	11 358.05	3 972	5 387				1 490.80	22 207.85	13.26
Milk cows-milk	487.8 hl	100				18 425.66	24 463.60	15 155.10	434	58 478.36	0.12
Fattening beef cattle	15 t	50					7 339.30	3 031	13.3	10 383.60	0.69
Fattening pig	14.5 t	70					7 094.46	1 364.16	13	8 471.62	0.58
Fattening chickens	2.6 t	150					1 272	303	2.3	1 577.30	0.61

C Total costs of products

PRODUCTS	Output	Direct costs in €	Overhead costs inv €	Total costs v €	Total costs of the unit in €
Wheat	1890 t	183 017	36 488.50	219 505.50	116.14
Barley	540 t	54 439	12 910.85	67 349.85	124.72
Sugar beet	14586 t	441 220	40 329.90	481 549.90	33.02
Corn for grain	1675 t	199 585	22 207.85	221 792.90	132.41
Milk cows- milk	487.8 hl	83 811	58 478.36	142 289.40	0.29
Fattening beef cattle	15 t	11 081	10 383.60	21 464.60	1.43
Fattening pig	14.5 t	12 422	8 471.62	20 893.62	1.44
Fattening chickens	2.6 t	1 523	1 577.30	3 100.30	1.19

Source: own software program.

from its superior activities. Allocation of cost activities to other activities is therefore performed in several steps. The sheet then has as many tables as subordinate activities connected to superior activities.

The CostAllocation-2ndLevel sheet (Table 5) first in table A expresses the relations between the activities and products by constructing a matrix of dependency. When product "i" consumes activity "j", cell "i.j" is filled as "X". For proper allocation of costs of activities to products the right choice of driver is very important. We already considered this when aggregating activities, so that it would be possible to assign a driver to each for the second stage. The result of the second stage of allocation is the cost calculation for individual products in table B. In the analysed cooperative, we specifically addressed it as follows: Activity 3 – sowing is allocated to wheat, barley, sugar beet and grain maize. The driver for the allocation of costs is the area of arable land in ha for individual crops (in a ratio of 420 : 150 : 330 : 250. The total costs of Activity 3 are then allocated to the products based on this ratio. The same driver is used also for activities 4 and 5 – cultivation and harvesting the crops. For activity 6, milking, the full amount of the cost is allocated to the product of milking – milk. For allocating the cost of activity 7 – feed is the driver used, which is expressed by the ratio of live weight of livestock of each species. This driver appears to be best for the activity. There are 100 head of dairy cows. If we consider the weight of one single LSU (livestock unit = 500 kilograms), the total mass of these animals is 50 tons. So we will use the allocation ratio of 50 : 15 : 14.5 : 2.6. Another type driver can be the ratio of the number of animals of each species. When allocating the cost of activity 8 – treatment is the driver used, which represents the ratio of surface size of the buildings housing

Table 6 Data for statistical analysis.

Enterprises (Grange, Ltd., SpA.)	Overhead unit costs in € calculated by the ABC method (non-traditional)							
	Crop production				Livestock production			
	Wheat	Barley	Sugar beet	Corn for grain	Milk cows-milk	Fattening beef cattle	Fattening pigs	Fattening chickens
1.	19.31	25.49	2.76	13.26	0.12	0.69	0.58	0.61
2.	25.32	28.36	4.15	14.96	0.25	0.88	0.45	0.77
3.	45.13	75.13	*	10.56	0.13	0.75	0.17	0.55
4.	59.2	66.62	9.62	50.69	0.11	0.88	0.51	*
5.	25.64	28.8	4.16	*	0.12	0.71	0.18	0.11
6.	28.82	32.41	4.69	*	0.26	0.92	0.55	0.13
7.	27.25	30.62	4.42	23.29	0.04	0.68	0.55	0.74
8.	36.82	48.62	*	36.99	0.36	0.54	0.61	0.24
9.	76.8	73.8	12.48	65.76	0.12	0.81	0.58	0.44
10.	22.45	25.89	3.64	19.18	0.08	0.79	0.55	*
11.	35.21	39.65	5.72	30.14	0.44	0.59	0.24	*
12.	27.56	32.34	3.41	19.87	0.07	0.61	0.52	0.13
13.	33.62	37.21	5.23	29.23	0.23	0.37	0.61	0.21
14.	17.65	19.85	2.86	*	0.14	0.51	0.59	0.78
15.	33.64	39.62	5.73	29.87	0.18	0.64	0.52	0.63
16.	20.89	23.45	3.39	17.81	0.13	0.73	0.57	0.59
17.	23.22	26.12	*	19.87	0.13	0.71	0.57	0.48
18.	31.25	35.11	5.07	26.72	0.18	0.72	0.54	0.49
19.	21.62	24.38	3.77	19.86	0.19	0.69	0.68	*
20.	61.12	67.51	9.75	51.38	0.41	1.18	1.02	0.45
21.	34.42	38.73	5.59	29.45	0.31	1.25	0.58	0.63
22.	17.65	19.82	2.86	15.08	0.12	0.92	0.83	0.71

Enterprises (Grange, Ltd., SpA.)	Overhead unit cost in € calculated using the primary method (traditional)							
	Crop production				Livestock production			
	Wheat	Barley	Sugar beet	Corn for grain	Milk cows-milk	Fattening beef cattle	Fattening pigs	Fattening chickens
1.	17.7	20.49	5.91	16.72	0.15	1.19	0.55	0.11
2.	24.12	25.89	10.13	12.65	0.36	0.86	0.98	0.15
3.	46.4	66.54	*	17.88	0.14	0.81	0.33	0.32
4.	55.86	71.23	12.35	46.69	0.10	0.75	0.65	*
5.	27.65	26.69	4.26	*	0.12	0.74	0.22	0.04
6.	29.32	32.89	3.71	*	0.28	0.71	0.69	0.18
7.	26.95	31.58	4.96	22.09	0.09	1.12	0.61	0.19
8.	38.56	44.56	*	39.31	0.25	0.52	0.47	0.51
9.	72.23	78.56	15.26	62.79	0.14	0.86	0.58	0.37
10.	23.65	29.41	5.28	12.82	0.15	0.82	0.45	*
11.	37.25	31.25	9.58	32.64	0.14	0.82	0.31	*
12.	28.82	30.51	4.58	19.27	0.12	0.71	0.47	0.03
13.	30.26	39.25	5.01	30.77	0.12	0.55	0.47	0.28
14.	19.56	17.01	3.79	*	0.18	0.55	0.54	0.75
15.	35.36	37.25	8.36	27.89	0.19	1.25	0.47	0.06
16.	25.69	21.56	5.68	12.61	0.15	0.99	0.55	0.33
17.	22.12	28.32	*	18.77	0.16	0.65	0.56	0.52
18.	33.26	34.21	7.31	23.37	0.19	0.69	0.68	0.37
19.	22.36	23.89	5.02	18.36	0.15	1.01	0.40	*
20.	58.23	69.45	6.56	55.52	0.35	0.99	0.89	0.83
21.	31.56	39.85	6.18	30.6	0.29	0.78	0.69	1.01
22.	19.56	22.32	4.02	9.51	0.15	1.18	0.79	0.46

Source: own table.

different species. We decided on this driver because the activity of treatment includes, for the most part, providing light and heat as well as cleaning the buildings. Thus, the ratio is 500 : 100 : 45 : 10. The driver for allocating the cost of activity 9 – selling products, is determined depending on the weight of products, especially since it involves loading and delivering products.

Direct costs do not enter into the ABC model; they are defined right at the beginning. In the ABC model we only allocate overhead costs. The result is therefore the overheads associated with each product. To determine the total costs for products, the direct costs that were defined at the outset must be added, Table C.

We consider the benefits of the proposed model to be accurate and objective calculation of overhead costs on farms, low financial requirements of software support for the ABC method created in this way, ease of use, flexibility of the model that can be supplemented as needed. The model can be used for the calculated loads at any stage of the production process. Information should be used to deal with different decision-making tasks. The proposed model allows you to make a monthly final calculation, to constantly optimise processes and the

Table 7 Tests of normality.

Tests of normality	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
difference_Wheat	0.151	11	0.200*	0.948	11	0.612
difference _Barley	0.170	11	0.200*	0.967	11	0.857
difference _Sugar_ beet	0.165	11	0.200*	0.950	11	0.645
difference _ Corn_for_grain	0.149	11	0.200*	0.942	11	0.546
difference _ Milk_cows_milk	0.240	11	0.076	0.930	11	0.408
difference _ Fattening_beef_cattle	0.110	11	0.200*	0.975	11	0.934
difference _ Fattening_pigs	0.220	11	0.142	0.784	11	**0.006**
difference _ Fattening_chickens	0.167	11	0.200*	0.908	11	0.230

Source: own table, * – this is a lower bound of the true significance, a – Lilliefors Significance Correction.

Table 8 Output of paired t-test.

Paired Samples Test		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Wheat_ABC - Wheat	0.09	2.38	0.51	-0.97	1.14	0.17	21	0.868
Pair 2	Barley_ABC - Barley	-0.76	3.67	0.78	-2.39	0.86	-0.98	21	0.340
Pair 3	Sugar_ beet _ABC - Sugar_ beet	1.51	1.96	0.45	0.56	2.45	3.35	18	**0.004**
Pair 4	Corn_for_grain _ABC - Corn_for_grain	-0.72	3.63	0.83	-2.47	1.03	-0.87	18	0.398
Pair 5	Milk_cows_milk _ABC - Milk_cows_milk	-0.01	0.08	0.02	-0.04	0.03	-0.38	21	0.706
Pair 6	Fattening_beef_cattle _ABC - Fattening_beef_cattle	0.09	0.25	0.05	-0.02	0.20	1.69	21	0.105
Pair 7	Fattening_pigs _ABC - Fattening_pigs	0.02	0.16	0.03	-0.05	0.09	0.47	21	0.646
Pair 8	Fattening_chickens _ABC - Fattening_chickens	-0.12	0.31	0.08	-0.28	0.04	-1.60	16	0.129

Source: own table.

product portfolio; it offers information support during business negotiations.

Statistical model of comparison of traditional calculation approach with ABC calculation method

Normality for the difference of values is one of the prerequisites for the use of the pair t-test. In Table 11, we tested normality with two tests: Kolmogorov-Smirnov test and Shapiro-Wilk test. New variables have been created: Difference_Wheat etc., always in the way of Wheat_ABC-Wheat, etc. In newly created variables, the zero H0 hypothesis of normality was rejected in only one case at 5% of the test level (difference_ Fattening_pigs), the value in the Sig. column is less than 0.05.

Table 8 shows the pair t-test output. The table has the following columns: Mean – Average Difference, Std. Deviation - standard deviation, Std. Error Mean – standard error of the average, Confidence Interval of the Difference – confidence interval, t-value of the test statistic, df-number of degrees of freedom, Sig. (2-tailed) – significance (p-value).

The zero hypothesis testifies that there is no statistically significant difference between the traditional and non-traditional method of calculation. In the case of rejection H0, there is a statistically significant difference (the value in the Sig. Column is less than 0.05) – this is the p-value. We are working at 5% of the level of the test.

In this case, the difference is statistically significant only for sugar beet; the other differences are not statistically significant (the ABC method is on average 1.51 higher). However, we must be aware that we are testing the average of differences. Absolute deviations may appear large, but after averaging the effect is disturbed, or deviations occur in both directions.

In general, however, the ABC method more precisely allocates overheads to the particular product, according to the activities that generated the costs. It uses a different cost allocation key, more directly assigns product overhead. The main contribution of ABC is the "insertion" of activities between source costs (from accounting) and products. In this way, there is a logical linkage between costs by type and activity on the one hand (each cost is due to some activity) and also the relationship between the cost of the activities and the products (the cost of the product equals the sum of the parts of the costs of the activities required for its implementation - supply, production, sales, etc.).

CONCLUSION

We presented a design for the ABC model in MS Excel. We created the model in a basic version, which can be additionally defined depending on the particular conditions of the business implementing the model. For better illustration, the general model is supported with data on costs. Based on theoretical assumptions the ABC costing method provides accurate, objective information on overhead costs, eliminates the non-specific nature of overheads. One of the biggest benefits of the ABC system is the binding of costs from accounts, activities performed and the cost of products in one system. It is also very important in various stages of implementation of ABC to communicate with a middle management of the establishment, because it may happen that the people responsible for existing methods of monitoring costs do not cooperate effectively. They might be afraid of the future increase in the difficulty of their work. Without such close cooperation of the implementation team with middle management it is not possible to create a model and then implement it. The ABC method is a tool for controlling and it has been used in many sectors of the national economy. We believe that it can be used also in agriculture. We consider high overhead costs to be a good reason for the implementation and use of ABC method. It is a much more valid reason than the size of the enterprise or line of business. The ABC method enables connection of a large part of the overhead costs to products, which provides greater accuracy compared to traditional methods. Finally, we have presented a statistical data analysis where we compared both calculation methods from this point of view as well.

REFERENCES

Cannavacciuolo, L., Illario, M., Ippolito, A., Ponsiglione, C. 2015. An activity-based costing approach for detecting inefficiencies of healthcare processes. *Business Process Management Journal*, vol. 21, no. 1, p. 55-79. https://doi.org/10.1108/BPMJ-11-2013-0144

Cohen, S., Venieris, G., Kaimenaki, E. 2005. ABC: Adopters, supporters, deniers and unawares. *Managerial auditing journal*, vol. 20, no. 9, p. 981-1000. https://doi.org/10.1108/02686900510625325

Dalci, I., Tanis, V., Kosan, L. 2010. Customer profitability analysis with time-driven activity-based costing: a case study in a hotel. *International Journal of Contemporary Hospitality Management*, vol. 22, no. 5, p. 609-637. https://doi.org/10.1108/09596111011053774

Duh, R. R., Lin, T. W., Wang, W. Y., Huang, Ch. H. 2009. The design and implementation of activity-based costing: A case study of a Taiwanese textile company. *International Journal of Accounting & Information Management*, vol. 17, no. 1, p. 27-52. https://doi.org/10.1108/18347640910967726

Ferenczi-Vaňová A., Krajčírová, R., Munk, M., Košovská, I., Váryová, I. 2017. The influence of some selected variables from accounting system on profit or loss of agricultural companies in the Slovak republic. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, 2017, p. 279-287. https://doi.org/10.5219/777

Foltínová, A. et al. 2011. Cost controlling (Nákladový controlling). Bratislava, Slovakia : Wolters Kluwer. 450 p. ISBN 9788080784256.

Greasley, A., Smith, C. M. 2017. Using activity-based costing and simulation to reduce cost at a police communications centre. *Policing: An International Journal of Police Strategies & Management*, vol. 40, no. 2, p. 426-441. https://doi.org/10.1108/PIJPSM-03-2016-0044

Gunasekaran, A., Marri, H. B., Yusuf, Y. Y. 1999. Application of activity-based costing: some case experiences. *Managerial Auditing Journal*, vol. 14, no. 6, p. 286-293. https://doi.org/10.1108/02686909910280217

Chrenková, I. 2011. Controlling in the Conditions of Czech Republic. *AGRIS on-line Papers in Economics and Informatics*, vol. 3, no. 2, p. 3-14.

Ittner, C. D., Lanen, W. N., Larcker, D. F. 2002. The association between activity-based costing and manufacturing performance. *Journal of Accounting Research*, vol. 40, no. 3, p. 711-726. https://doi.org/10.1111/1475-679X.00068

Jánsky, J., Poláčková, J., Kozáková, P. 2012. Methodological Approaches to Costs Evaluation of Canned Feed. *AGRIS on-line Papers in Economics and Informatics*, vol. 4, no. 4, p. 27-34.

Kaplan, R. S., Aderson, S. R. 2003. Time-Driven Activity-Based Costing [online] s.a. [cit. 2017-10-10] Available at: http://nliah.com/Portal/microsites/Uploads/Resources/o1NEDPiVg.pdf.

Kaplan, R. S., Aderson, S. R. 2005. Rethinking Activity-Based Costing – HBS Working Knowledge [Online] s.a. [cit. 2017-10-10] Available at: http://hbswk.hbs.edu/item/4587.html.

Kaplan, R. S., Aderson, S. R. 2007. *Time – Driven Activity - Based Costing: A Simpler and More Powerful Path to Higher Profits*. Boston: Harvard Business School Press.

Kaszubski, M. A., Ebben, S. 2005. Using activity-based costing to implement behavioural cost initiatives successfully. *Journal of Facilities Management*, vol. 3, no. 2, p. 184-192. https://doi.org/10.1108/14725960510808473

Khataie, A. H., Bulgak, A. A. 2013. A cost of quality decision support model for lean manufacturing: activity-based costing application. *International Journal of Quality & Reliability Management*, vol. 30, no. 7, p. 751-764. https://doi.org/10.1108/IJQRM-Jan-2011-0016

Kostakis, H., Sarigiannidis, C., Boutsinas, B., Varvakis, K., Tampakas, V. 2008. Integrating activity-based costing with simulation and data mining. *International Journal of Accounting & Information Management*, vol. 16, no. 1, p. 25-35. https://doi.org/10.1108/18347640810887744

Krumwiede , K. R., Charles, L. S. 2014. The Use of Activity-based Costing with Competitive Strategies: Impact on Firm Performance. In Epstein, M. J. et al. *Advances in Management Accounting*. Bingley, UK : Emerald Group Publishing Limited, p. 113-148. https://doi.org/10.1108/S1474-787120140000023004

Kubicová, Ľ., Habánová, M. 2016. Development Of Milk Consumption And Marketing Analysis Of Its Demand. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 10, no. 1, p. 557-562.

Kuethe, T., Morehart, M. 2012. The Agricultural Resource Management Survey: An information system for production agriculture. *Agricultural Finance Review*, vol. 72, no. 2, p. 191-200. https://doi.org/10.1108/00021461211250429

Langfield-Smith, K., Thorne, H., Hilton, R. W. 1998. Management Accounting: An Australian Perspective. 2nd ed. Hong Kong : McGraw-Hill. 54 p. ISBN 9780074705018.

Lelkes, A. M. T. 2014. The Technical Efficiency Portrayed by Duration-Based and Activity-Based Costing Systems. In Mallina, M. A. *Advances in Management Accounting*. Bingley, UK : Emerald Group Publishing Limited, p. 61-76. ISBN 978-1-78350-632-3.

Pokorná, J. 2016. Impact of Activity-Based Costing on Financial Performance in the Czech Republic. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 64, no. 2, p. 643-652. https://doi.org/10.11118/actaun201664020643

Popesko, B. 2010. Calculation methodology of Activity-Based Costing in industrial companies (Metodika aplikace kalkulace Activity-Based Costing v průmyslových firmách). *E&M Economie and Management*, vol. 13, no. 1, p. 103-114.

Popesko, B. 2012. Process cost management methods using Activity Based – Costing (Procesní řízení nákladu s využitím metódy Activity Based – Costing). In Success - innovation and productivity in contexts (Úspěch – produktivita a inovace v souvislostech), no. 2.

Popesko, B., Papadaki, Š., Novák, P. 2015. Cost and reimbursement analysis of selected hospital diagnoses via Activity-Based costing. *E&M Economie and Management*, vol. 18, no. 3, p. 50-61.

Quinn, M., Elafi, O., Mulgrew, M. 2017. Reasons for not changing to activity-based costing: a survey of Irish firms. *PSU Research Review*, vol. 1, no. 1, p. 63-70. https://doi.org/10.1108/PRR-12-2016-0017

Slangen, L. H. G., Suchánek, P., Cornelis van Kooten, G. 2003. Trust in countries in transition: empirical evidence from agriculture. *International Journal of Social Economics*, vol. 30, no. 10, p. 1095-1109. https://doi.org/10.1108/03068290310492887

Tóth, M., Matveev, V., Boháčiková, A. 2016. Risk of agricultural production in Russian orel region. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 10, no. 1, 2016, p. 557-562.

Veščičík, M. 2004. The successful introduction of Activity Based Costing (Predpoklady úspešného zavedenia Activity Based Costing.) [Online] s.a. [2016-10-10] Available at: http://www.gradient5.sk/download/Predpoklady%20zavedenia%20ABC.pdf.

Veščičík, M. 2012. By inaccurate overheads to addressable process costs (Od nepresných réžií k adresným nákladom procesov). *Financial Management - Controlling Section (Finančný manažment – rubrika controlling)*, vol. 10, p. 1-6.

Zakić, V., Borović, N. 2013. Application Of Activity-Based Costing In Agricultural Enterprises. In *Agriculture and Rural Development – Chalanges of Transition and Integration Processes.* Belgrade-Zemun : University of Belgrade, p. 289-296. ISBN 978-86-7834-181-6.

Zhang, Y. F., Hoque , Z., Isa , Ch. R. 2015. The Effects of Organizational Culture and Structure on the Success of Activity-Based Costing Implementation. In Malina, M. A. *Advances in Management Accounting*. Bingley, UK : Emerald Group Publishing Limited, p.229-257. ISBN 978-1-78441-650-8.

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CHICORY SYRUP AS A SUBSTITUTION OF SUGAR IN FINE PASTRY

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ABSTRACT

Fine pastry is a favorite snack but contains big amount fats and sugars. Sugar consumption is a major factor in the development of diabetes and obesity. Because of the growing interest in low-calorie alternatives to sucrose, producers react with different new products which can replace sugar and do not compromise the consumer acceptability of food products. This study deals with replacement of sugar with chicory oligofructose syrup, which provides same sweetness as sugar but contain over 70 % of fiber. Chicory syrup is composed of oligo-fructose and inulin. Inulin-type fructans are beneficial for immune and cardiovascular systems and its prebiotic function can protect colon health. In this study, the influence of sucrose (7 $g.100g^{-1}$) replacement with chicory oligofructose syrup (0; 3.5; 7 $g.100g^{-1}$) on the texture, specific loaf volume and sensory acceptability of biologically leavened muffins was tested. The substitution of sugar by the chicory syrup decreased specific loaf volume from 2.15 to 2.01 $mL.g^{-1}$ while hardness, springiness and chewiness increased. There was no detrimental impact of syrup addition to cohesiveness of fine pastry muffin. It was observed that due to the syrup addition, pastry hardens faster, as showed results of hardnesss measured after 24 h. Bread crumb and crust sensory characteristics were not affected by the syrup addition. Weak impact of sugar replacement on sensory evaluation may be related to a reduced amount of the panelists involved in this study. More extensive study will, however, be performed to describe the impact of sugar replacement of fine biologically leavened muffins in more details. The results of this preliminary study shows, that the substitution of sugar by chicory syrup change textural properties and loaf volume. Despite all, based on sensory analysis the chicory syrup up to amount 7 $g.100g^{-1}$ seems to be adequate for its use as a sugar replacer in fine pastry as biologically leavened muffins.

Keywords: inulin; chicory; muffin; sugar; texture

INTRODUCTION

In Europe, fine pastry is a favorite snack or cake consumed on various occasions by all generations. However, it contains a lot of calories, fats and sugars. According to the Commodity Decree No. 333/1997 Coll. of the Czech Republic as subsequently amended, fine pastry is defined as a bakery product obtained by heat treatment of a dough containing at least 8.2 % of anhydrous fat or 5 % of sugar on the total weight of used mill products.

Excessive sugar consumption is a major factor in the development of diabetes. This disease, along with the many other health problems associated with the rising incidence of obesity in Europe, are major concerns from a public-health perspective. There is growing interest in low-calorie alternatives to refined sucrose. Synthetic sweeteners are often regarded either as having an undesirable aftertaste or as being linked to health concerns. There is strong demand for natural sweeteners as maple, date, agave or chicory syrups.

Chicory syrup is composed of oligo-fructose and inulin. The main source of oligo-fructose and inulin used in the food industry are chicory and artichoke from Jerusalem **(Franck, 2002)**. Oligo-fructose and inulin belong to the class of carbohydrates known as fructans. Inulin-type fructans have been reported to be beneficial for colon health by selectively promoting the growth of bifidobacteria and lactobacilli probiotic bacteria **(Gibson et Roberfroid, 1995)** and cardiovascular systems by decreasing cholesterol and triglyceride levels in serum **(Kaur et Gupta, 2002).** The health benefits of oligo-fructose also include increased mineral absorption **(van den Heuvel et al., 1999)** and improved immune response and while there is evidence that oligo-fructose used as prebiotics play a role in colorectal cancer prevention **(Morris et Morris, 2012)**. In addition, according to **EFSA (2015)** health claim, chicory inulin contributes to

maintenance of normal defecation by increasing stool frequency.

Oligo-fructose is more soluble than sucrose and provides about 30 to 50 % of the sweetness of table sugar. It is often used in combination with high intensity sweeteners **(Kaur et Gupta, 2002)**.

The aim of this work was to evaluate the influence of chicory syrup, as a possible substitute for sugar, on technology, textural properties and sensory quality of fine pastry, namely biologically leavened muffins. There is a deal to establish whether fine pastry sweetened by chicory syrup can be manufactured without compromising consumer acceptance.

Scientific hypothesis

We established the hypothesis that the substitution of sugar by chicory syrup does not impair textural properties, loaf volume and sensory acceptability of biologically leavened muffins.

MATERIAL AND METHODOLOGY

Chicory syrup

Chicory syrup was kindly provided by KAUMY s.r.o., Czech Republic. According to producer, the syrup energetic value is 650 kJ.100g^{-1} and contains 4.7 g.100g^{-1} of sugars and 71.3 g.100g^{-1} of fiber. The producer states that the glykemic index of the chicory syrup is GI <5, which is in comparison with sacharose GI = 65 **(Atkinson et al., 2008)** very low.

Muffins preparation

A formula for dough preparation consisted of wheat fine flour (45.5 g.100g^{-1}), water (23 g.100g^{-1}), canola oil (19 g.100g^{-1}), sweetener (sugar or chicory syrup) (7 g/100g), dry egg mélange (3.5 g.100g^{-1}), salt (1 g.100g^{-1}) and dry yeast (1 g.100g^{-1}). Three batches were baked, where the first contained only sugar **(B0Sy)**, in the other one half of the sugar was replaced with chicory syrup **(B50Sy)**, in the third one the whole amount of sugar was replaced by the syrup **(B100Sy)**. The amounts of the ingredients were related to 100 g of flour dry matter. Dry yeast was reactivated for 10 ±1 min. in a sweetener solution (35 ±1 °C). The dough ingredients were placed into the Eta Exclusive Gratus mixer bowl (Eta, a.s. CZ), mixed for 6 min and placed into a proofer for 40 min. at 35 ±1 °C and 85% relative air humidity. Then 100 g of dough was scaled into silicone muffin cups and proofed again for 20 min. at 35 ±1 °C and 85% relative air humidity. Muffins were baked for 10 min. at 200 ±5 °C in the oven MIWE cube (Pekass s.r.o. Plzeň, CZ). After baking, the mufins were stored at room temperature for 2 hours and then analyzed. Each test was performed on samples prepared at least in three replicates.

Muffin analysis

Specific loaf volume

The loaf volume was measured using plastic granulate of rape seed size. Specific loaf volume was obtained as a ratio of muffin volume and muffin weight.

Texture analysis

Textural properties of muffin crumb were measured using texture profile analysis **(TPA)** on a texture analyzer TA.XT plus (Stable Micro Systems Ltd., UK). TPA was performed on cylindrical samples obtained from the loaf crumb centre (35 mm in diameter and 15 mm in height). Three loafs from each batch were used for TPA measurement. Each loaf provided three individual samples for compression. Those samples were placed onto the analyzer base and squeezed in two cycles to 4 mm with the 75.0 mm diameter cylinder probe P/75. Test speed of probe was 1.00 mm/s. The crumb parameters (hardness, springiness, cohesiveness and chewiness) were determined using Exponent Lite software v. 4.0.13.0.

Sensory evaluation

Muffins were subjected to sensory evaluation by a panel of 10 department staff and students, both male and female between the ages of 19 – 50 years. A nine point hedonic scale was used to evaluate the characteristics of crumb and crust. Sensory score range from 1 – dislike extremely to 9 – like very much was used. For evaluating sweetness and off-flavor intensity grade 1 – no sweetness/ no off flavor and grade 9 extreme sweetness/ extreme off-flavor. An extensive sensory evaluation realized by higher number of panelists will be performed to support the preliminary results obtained in this study.

Statistic analysis

The results showed a normal distribution, therefore they were statistically analyzed using variance analysis (ANOVA). The differences were tested on $\alpha = 0.05$ significance level using Fisher LSD test. Analysis was performed using Statistica CZ9.1 software (StatSoft Ltd., Czech Republic)

RESULTS AND DISCUSSION

Specific loaf volume is largely related to the ability of gas retention of the dough during proofing and achieving crumb porosity. Lack or small pores can influent the crumb hardness and lead to reduced consumer acceptability. The substitution of sugar by the chicory syrup significantly (p <0.05) decreased specific loaf volume from 2.15 to 2.01 mL.g^{-1} (Table 1). The reduction obtained is comparable previously reported by **Wang et al. (2002)**. The possible explanation according to **Silva (1996)** is, that inulin, which is presented in chicory syrup,

Table 1 The texture characteristics and specific loaf volume of muffins with different chicory syrup/sugar ratio.

Batch	Specific loaf volume (ml.g^{-1} ±SD)	Hardness (N ±SD)	Hardness (after 24 h) (N ±SD)	Springiness (1 ±SD)	Cohesiveness (1 ±SD)	Chewiness (N ±SD)
B0Sy	2.15 ±0.06^{b}	5.3 ±0.1^{a}	5.3 ±0.1^{a}	0.84 ±0.01^{a}	0.59 ±0.01^{a}	2.62 ±0.07^{a}
B50Sy	2.01 ±0.05^{a}	6.5 ±0.2^{b}	7.3 ±0.7^{b}	0.86 ±0.01^{ab}	0.60 ±0.03^{a}	3.4 ±0.3^{b}
B100Sy	2.05 ±0.04^{a}	5.3 ±0.3^{ab}	8.1 ±0.6^{b}	0.88 ±0.02^{b}	0.63 ±0.02^{a}	3.0 ±0.3^{ab}

Note: *****values in one column with different letters are significantly different p <0.05.

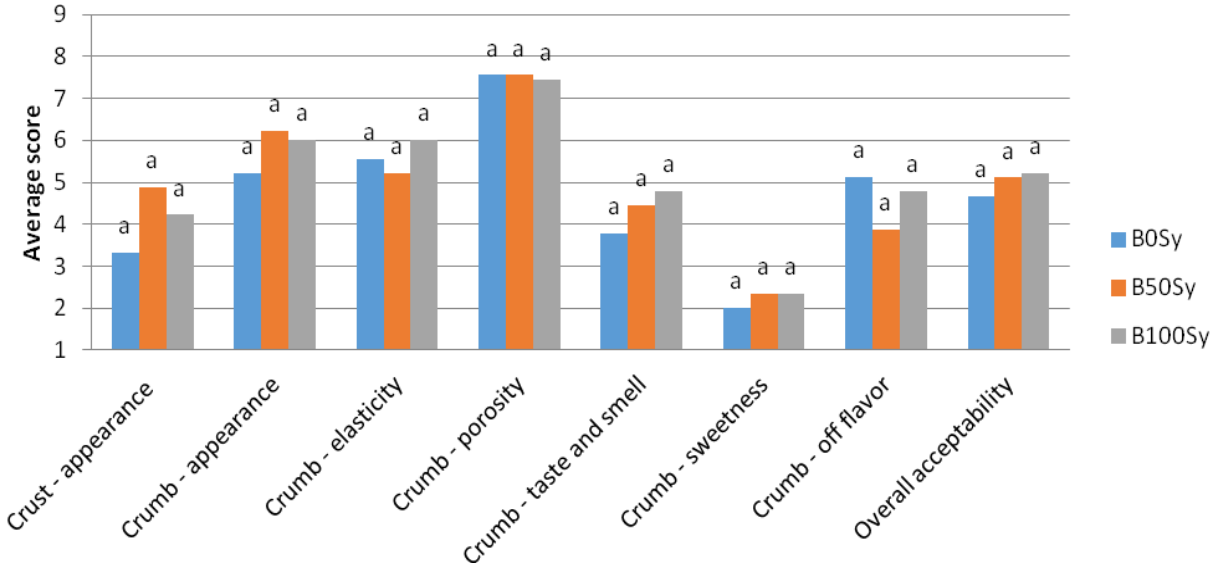

Figure 1 The sensory characteristics of muffins with different chicory syrup/sugar ratio.
Note: *the average scores of one sensory characteristic with different letters are significantly different $p < 0.05$.

increases the viscosity of the dough and hence the volume of muffins where sucrose was replaced by syrup is lower.

Crumb hardness is correlated with the perception of freshness. Crumb hardening, which is one of the most obvious manifestations of staling, is caused by starch retrogradation as well as differences in vapor pressure between crumb and crust resulting in moisture migration **(Esteller et Lannes, 2008)**. Across the results, hardness of a control sample only with sugar B0Sy is the lowest. Higher levels of syrup increased the crumb hardness. Similar observations for bread were made by other authors where the inclusion of prebiotics increased crumb hardness (**O'Brien et al., 2003; Rössle et al., 2011**). The muffins hardness was also measured after 24 hours. The results show increasing hardening with rising syrup ratio. So with a higher syrup content, pastry hardens faster. It is in agreement with previously reported results for wheat breads, where the addition of inulin resulted in higher crumb hardness as well as an increased rate of staling **(Wang et. al, 2002; Ronda et. al., 2005; Peressini et Sensidoni, 2009; Beikzadeh et al., 2017).**

Interestingly **Korus et al. (2006)** and **Ziobro et al. (2013)** have reported, that inulin can reduce the crumb hardening rate during the storage period of gluten-free bread.

Muffin springiness and chewiness both rised with substituting sugar by syrup. The positive is that there has been no significant effect on cohesiveness.

In therms of sensory characteristics there was no significant diference between control sample with sugar and samples treated by syrup (Figure 1). Despite the higher hardness of products sweetened by chicory syrup compared to sugared products, measured during TPA, consumers did not notice a significant difference in crumb taste and overall acceptability. Although differences in sensory analysis results are not statistically significant, the average score of muffins, where sugar was completely replaced by syrup, was higher in crumb taste and smell and overall acceptability. The addition of chicory syrup has also improved the appearance of the muffin crust especially in therms of color (compared to muffins without syrup), altough not statistically significant. According to **Huebner et al. (2008); Poinot et al. (2010); Drabińska et al. (2016)** and also **Süli, 2017)** during the baking the Maillard reaction occurs, in which the reducing sugars present in the fructan chains react with amino acids, producing compounds of higher molecular weight that may influence the flavor, aroma and color of the food. The intensive caramel flavor and more brown crust color is obviously appreciated by consumers.

CONCLUSION

Based on the results of this preliminary study, we can reject the hypothesis, that the substitution of sugar by chicory syrup does not impair textural properties and loaf volume. Biologically leavened muffins with chicory syrup presented lower specific loaf volume, higher hardness, springiness and chewiness than those sweetened with sugar. There was no detrimental impact of syrup to cohesiveness. The pastry was considered to be acceptable by the sensory panel, indicating that this ingredient up to amount 7g/100g is adequate for its use as a sugar replacer in studied type of bakery product.

In further research, it would be good to determine the most appropriate ratio of sucrose and chicory syrup in order to obtain the product without compromising the crumb hardness and shelf life.

REFERENCES

Atkinson, F. S., Foster-Powell K., Brand-Miller, J. C. 2008. International Tables of Glycemic Index and Glycemic Load Values: 2008. *Diabetes Care*, vol. 31, no. 12, p. 2281-2283. https://doi.org/10.2337/dc08-1239

Beikzadeh, M., Peighambardoust, S. H., Beikzadeh, S. Homayouni, A. 2017. Effect of inulin, oligofructose and oligofructose-enriched inulin on physicochemical, staling, and sensory properties of prebiotic cake. *Journal of Agricultural Science and Technology*, vol. 19, no. 2, p. 1241-1252.

Decree No. 333/1997 Coll. Of the Ministry of Agriculture, which is implemented by § 18 (a), (d), (h), (i), (j) and (k) of

Act No. 110/1997 Coll., on Food and Tobacco Products and on Amendments to Certain Related Acts for Milk Cereal Products, Pasta, Bakery Products and Confectionery Products and dough. (Vyhláška č. 333/1997 Sb. Ministerstva zemědělství, kterou se provádí §18 písm. a), d), h), i), j) a k) zákona č. 110/1997 Sb., o potravinách a tabákových výrobcích a o změně a doplnění některých souvisejících zákonů, pro mlýnské obilné výrobky, těstoviny, pekařské výrobky a cukrářské výrobky a těsta.)

Drabińska, N., Zieliński, H., Krupa-Kozak, U. 2016. Technological benefits of inulin-type fructans application in gluten-free products. *Trends in Food Science & Technology*, vol. 56, p. 149-157. https://doi.org/10.1016/j.tifs.2016.08.015

EFSA Panel on Dietetic Products, Nutrition and Allergens. 2015. Scientific Opinion on the substantiation of a health claim related to "native chicory inulin" and maintenance of normal defecation by increasing stool frequency pursuant to Article 13.5 of Regulation (EC) No 1924/2006. *EFSA Journal*, vol. 13, no. 1, p. 3951.

Esteller, M. S., Lannes, S. C. 2008. Production and characterization of sponge-dough bread using scalded rye. *Journal of Texture Studies*, vol. 39, iss 1., p. 56-67. https://doi.org/10.1111/j.1745-4603.2007.00130.x

Franck, A. 2002. Technological functionality of inulin and oligofructose. *British journal of nutrition*, vol. 87, no. S2, p. S287-S291. https://doi.org/10.1079/BJN/2002550

Gibson, G. R., Roberfroid, M. B. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. 1995. *Journal of Nutrition*, vol. 125, no. 6, p. 1401-1412. PMid:7782892

Huebner, J. Wehling, R. L., Parkhurst, A. Hutkins, R. W. 2008. Effect of processing conditions on the prebiotic activity of commercial prebiotics. *International Dairy Journal*, vol. 18. no. 3, p. 287-293. https://doi.org/10.1016/j.idairyj.2007.08.013

Kaur, N., Gupta, K. A. 2002. Applications of inulin and oligofructose in health and nutrition. *Journal of Biosciences*, vol. 27, no. 7. p. 703-713. https://doi.org/10.1007/BF02708379 PMid:12571376

Korus, J., Grzelak, K., Achremowicz, K., Sabat, R. 2006. Influence of prebiotic additions on the quality of gluten-free bread and on the content of inulin and fructooligosaccharides. *Food Science and Technology International*, vol. 12, no. 6, p. 489-495. https://doi.org/10.1177/1082013206073072

Morris, C., Morris, G. A. 2012. The effect of inulin and fructo-oligosaccharide supplementation on the textural, rheological and sensory properties of bread and their role in weight management: A review. *Food Chemistry*, vol. 133, no. 2, p. 237-248. https://doi.org/10.1016/j.foodchem.2012.01.027

O'Brien, C. M., Mueller A., Scannell A. G. M., Arendt E. K. 2003. Evaluation of the effects of fat replacers on the quality of wheat bread. *Journal of Food Engineering*, vol. 56, no. 2-3, p. 265-267. https://doi.org/10.1016/S0260-8774(02)00266-2

Peressini, D., Sensidoni, A. 2009. Effect of soluble dietary fibre addition on rheological and breadmaking properties of wheat doughs. *Journal of Cereal Science*, vol. 49, no. 2, p. 190-201. https://doi.org/10.1016/j.jcs.2008.09.007

Poinot, P., Arvisenet, G., Grua-Priol, J., Filonneau, C., Le-Bail, A., Prostl, C. 2010. Influence of inulin on bread: Kinetics and physico-chemical indicators of the formation of volatile compounds during baking. *Food Chemistry*, vol. 119, no. 4, p. 1474-1484. https://doi.org/10.1016/j.foodchem.2009.09.029

Ronda, F., Gómez, M., Blanco, C. A., Caballero, P. A. 2005. Effects of polyols and nondigestible oligosaccharides on the quality of sugar-free sponge cakes. *Food Chemistry*, vol. 90, no. 4, p. 549-555. https://doi.org/10.1016/j.foodchem.2004.05.023

Rössle, CH., Ktenioudaki, A., Gallagher, E. 2011. Inulin and oligofructose as fat and sugar substitutes in quick breads (scones): a mixture design approach. *European Food Research and Technology*, vol. 233, no. 1, p. 167-181. https://doi.org/10.1007/s00217-011-1514-9

Silva, R. F. 1996. Use of inulin as a natural texture modifier. *Cereal Foods World*, vol. 41, no. 10, p. 792-794.

Süli, J. Hamarová, I., Sobeková, A. 2017. Possible consequences of the sucrose replacement by a fructose-glucose syrup. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 425-430. https://doi.org/10.5219/772

van den Heuvel, E., Muys, T., van Dokkum, W., Schaafsma, G. 1999. Oligofructose stimulates calcium absorption in adolescents. *American Journal of Clinical Nutrition*, vol. 69, no. 3, p. 544-548. https://doi.org/10.1093/ajcn/69.3.544 PMid:10075343

Wang, J. S., Rosell, C. M., de Barber, C. B. 2002. Effect of the addition of different fibres on wheat dough performance and bread quality. *Food Chemistry*, vol. 79, no. 2, p. 221-226. https://doi.org/10.1016/S0308-8146(02)00135-8

Ziobro, R., Korus, J., Juszczak, L., Witczak, T. 2013. Influence of inulin on physical characteristics and staling rate of gluten-free bread. *Journal of Food Engineering*, vol. 116, no. 1, p. 21-27. https://doi.org/10.1016/j.jfoodeng.2012.10.049

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EFFECT OF ESSENTIAL OILS OF *LAMIACEAE* PLANTS ON THE *RHIZOPUS* SPP.

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ABSTRACT

The aim of this study was to evaluate the fungicidal effect of eleven essential oils against six isolates of the genus *Rhizopus*. Isolates were obtained from various moldy foods (chestnut, bread, strawberry, nectarine, blackberry and cherry tomatoes). The essential oils used in this study were extracts of basil (*Oscimum basilicum* L.), hyssop (*Hyssopus officinalis* L.), lavender (*Lavandula angustifolia* MILLER.), marjoram (*Origanum majorana* L.), mint (*Mentha piperita* L.), oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), summer savory (*Satureja hortensis* L.), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.). Semi-quantitative composition of the essential oil samples was determined by gas chromatography coupled with mass spectrometry (GC-MS). The GC-MS analyses of the essential oils led to identification of 139 compounds, of which 49 were presented in ≥1% amount in at least one essential oil. The antifungal activity of essential oils against the *Rhizopus* spp. was determined, using micro-atmosphere method (0.625 $\mu L.ml^{-1}$ of air), during 7 days. Seven essential oils: thyme, mint, summer savory, lavender, marjoram, oregano and wild thyme completely inhibited the growth of all isolates. Other essential oils have different effects on the growth of isolates. Basil essential oil stimulated growth of two isolates on the second day of cultivation. The growth of other isolates was, by contrast, inhibited by this essential oil in the same time of cultivation. Hyssop essential oil completely inhibited growth of two isolates, other 4 isolates were inhibited to fourth day of cultivation. In conclusion, certain essential oils are highly effective in vapour phase and can be used in another test of their antifungal activity and could be used in control of *Rhizopus* spp. or other fungal pathogens.

Keywords: essential oils; *Rhizopus* spp.; antifungal activity; vapour phase

INTRODUCTION

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 attentions **(Farzaneh, 2015)**. 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. Essential oils from plants have great potential as a new source of fungicide to control the pathogenic fungi **(Císarová et al., 2016, Nikkhah et al., 2017)**. In the past decade, due to concerns regarding safety of the chemical control measures, particular attention has been given to the potential applications of essential oils as alternative **(Nikkhah et al., 2017)**.

Essential oils are odiferous, highly volatile substances present in plants. Because of their volatility, these substances can be isolated by means of steam distillation from an aromatic plant of a single botanical species and can be detected by both smell and taste. Individual essential oils are known by the name of the plant from which they are derived and the odor is similar to that of the part of the plant from which they are obtained, although the aroma is generally more intense **(Ríos, 2016)**.

Basil (*Oscimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), mint (*Mentha piperita* L.), savory (*Satureja hortensis* L.), sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* MILLER.), marjoram (*Origanum majorana* L.), wild thyme (*Thymus serpyllum* L.), oregano (*Origanum vulgare* L.) and hyssop (*Hyssopus officinalis* L.) (*Lamiacea* plants) are herbs widely used for culinary purposes. These plants are also used for the production of essential oils.

The use of essential oils is extremely diverse depending on the source, quality, extraction procedure, ect. Essential oils have proven industrial applications in the manufacture of perfumes, cosmetics, soaps, shampoos, or cleaning gels. Another interesting aspect of these oils is their potential as therapeutic agens in aromatherapy or as active principles or excipients of medicine. Another significant application of essential oils is in the agrofood industry, both for producing beverages and for flavoring foods **(Ríos, 2016)**.

Fruit and vegetables are rich in essential vitamins, minerals, fibre, and health promoting compound, and the consumption thereof increased during the past years.

Consumers have a right to good quality produce that is safe for consumption and therefore they are increasingly interested in the nutritional value, good taste and flavour of the fruits and vegetables they consume. According to consumers, the term "quality" can be defined as a fruit with a perfect shape, size, colour, aroma, and an absence of defects such as cuts, bruises or decay **(Sivakumar and Bautista-Baños, 2014)**. Postharvest diseases are one of the major causes for the postharvest loss of horticultural fresh produce during the supply chain **(Mari et al., 2016; Sivakumar and Bautista-Baños, 2014)**.

Rhizopus stolonifer is one of the most common and fastest-growing species in the *Zygomycota* phylum. Disease caused by this fungus is known as soft rot, black mould and *Rhizopus* rot **(Bautista-Baños et al., 2014)**. *Rhizopus* rot is common on soft fruits, more abundant in warm, humid climates than in cool climate. In several fruits and crops such as strawberry, peaches, avocados, tomato, cucumber and table grapes *Rhizopus* rot causes soft rot during transport and storage **(Kassemeyer and Berkelmann-Löhnertz, 2009 Samson et al., 2010)**.

Rhizopus species are considered among the most devastating fungi during storage of various horticultural commodities **(Bautista-Baños et al., 2014**).

Scientific hypothesis

The aim of the present research was to determine the inhibitory effect of eleven essential oils to growth of different *Rhizopus* isolates.

MATERIAL AND METHODOLOGY

Fungal culture

Isolates from moldy foods were transfer on the potato dextrose agar (PDA, HIMEDIA India) and after incubation (25 ±1 °C, 7 days) were identificated to the genus according **Samson et al. (2010)**, **Pitt and Hocking (2009)**. These isolates belong to the collection of microorganisms at the Department of Microbiology of the Slovak Agricultural University in Nitra.

Plant essential oils

The essential oils used in this study were extracts of basil (*Oscimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), mint (*Mentha piperita* L.), savory (*Satureja hortensis* L.), sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* MILLER.), marjoram (*Origanum majorana* L.), wild thyme (*Thymus serpyllum* L.), oregano (*Origanum vulgare* L.) and hyssop (*Hyssopus officinalis* L.).

Chemical composition of essential oils

Semi-quantitative composition of the essential oil samples was determined by gas chromatography coupled with mass spectrometry (GC-MS) using an Agilent 7890B oven coupled with Agilent 5977A mass detector (Agilent Technologies Inc., Palo Alto, CA, USA) and CombiPal autosampler 120 (CTC Analytics AG, Zwingen, Switzerland). Prior to the analysis, essential oil samples were diluted in hexan (HPLC ≥97%, Sigma Aldrich GmbH, Germany) to a concentration of 10 $\mu L.mL^{-1}$. One microliter of diluted sample was injected in inlet operated in split mode (1 : 10; 250 °C). Separation was achieved using a ZB-WAXplusTM capillary column (10 m × 0.1 mm × 0.10 μm) (Phenomenex Inc., Torrance, CA, USA) and the following oven temperature programme: 50 °C for the first 5 minutes, increased to 240 °C at the rate of 3 °C min^{-1}, where it was kept constant for 2 minutes. Helium was used as carrier gas at the constant flow (1.2 $mL.min^{-1}$). The mass detector parameters were as follows: ionization energy of filament: 70 eV, transfer line temperature: 250 °C, MS source temperature: 230 °C, quadrupole temperature: 150 °C. The mass spectrometer was programmed under electron impact (EI) in a full scan mode at m/z 40 – 400. The identification of compounds was carried out by comparing of mass spectra (over 80% match) with a commercial database NIST® 2014 and

Rhizopus spp. were obtainted from moldy foods:

Isolate	Source
KMi 383	chestnut
KMi 392	bread
KMi 510	strawberry
KMi 511	nectarine
KMi 512	blackberry
KMi 524	cherry tomatoes

Figure 1 *Rhizopus* sp. (KMi 524) growing on the cherry tomatoes (Photo: E. Čunderlíková).

retention times of reference standards (nerol, linalool, geraniol, citral, α-pinene and β-pinene). Semi-quantitative content of determined compounds were calculated by dividing individual peak area (excluded by solvent peak area) by total area of all peaks. Peaks under 0.1% were not counted.

Antifungal activity of essential oils

The antifungal activity of selected essential oils was investigated by microatmosphere method. The test was performed in sterile plastic Petri dishes (Ø 90 mm) containing 15 mL of PDA. Evaluation by filter paper was made by the method adapted from **Guynot et al. (2003)**. Essential oils were tested in concentration 0.625 μL.cm^{-3} of air. A round sterile filter paper (Ø 9 cm) was placed in the lid of Petri dish and 50 μL of essential oil was pipetted by micropipette to the paper. Dishes were kept in inverted position. Filter paper discs impregnated with sterilized distilled water were used as a control to confirm no solvent effect of bioactivity. Each isolate was inoculated in the center of Petri dishes with needle. Dishes were tightly sealed with parafilm and incubated for seven days at 25 ±1 °C (three replicates were used for each treatment). Diameters (Ø mm) of the growing colonies were measured at the 2nd, 4th and 7th day with a digital capiler.

Inhibition of mycelial growth

According to **Cakir et al. (2005)** and **Kordali et al. (2008)** growth inhibition of treated samples (*T*) against control (*C*) was calculated by percentage of growth inhibition using the following equation:

$$\% \text{ of inhibition } = \frac{C - T}{C} \; x \; 100$$

where, C is the mean of six replicates of hyphal extension (mm) of controls and T is the mean of six replicates of hyphal extension (mm) of plates treated with either essential oil.

Statistical analysis

The size of colonies of isolates (mm) for each day of cultivation within treatment was evaluated. Also the size of colonies of isolate for each treatment to the same isolate in control group was evaluated too. The results were mathematically processed using the Microsoft Excel program and statistically evaluated by SAS/9.3 (2010). Used statistical model can be written in the following form:

yij = μ + ISOLATEi /TREATMENTj/ + eij,
yij = the measurements for size of colonies,
μ = overall mean,
ISOLATEi = the fixed effects of isolates (i = 1 to 6),
TREATMENTj = the fixed effect of treatment (j = 1 to 5),
eijk = random error, assuming eijkl ~ N(0, I σe2).

RESULTS AND DISCUSSION

According to market data, there are about 400 species, from 67 plant families, which are cultivated on a large commercial scale for production of essential oils. The most important families from this point of view are *Asteraceae* (syn. *Compositae*), *Lamiaceae* (syn. *Labiatae*), and

Table 1 Essential oils tested for the fungicidal effect and their compounds (%)* determined by gas chromatography coupled with mass spectrometry (GC-MS).

	Compound	Essential oils										
		Ros.	Thym.	Mint	Sum. sav.	Lav.	Marj.	Wild thy.	Hys.	Bas.	Sage	Oreg.
1	α-pinene	10.74	1.79	0.73	2.71	1.52	1.85	2.61	0.99	0.30	6,08	
2	β-pinene	7.43	0.17	0.89			0.46	0.18	11.07	0.34	2,34	0,31
3	(+)-4-Carene		1,02		3.76	0.04	9.28	1.27				1.15
4	Camphene	4.66	1.62		0.23	0.06		1.28			5.86	0.31
5	β-Myrcene					0.36		1.25	0.79	0.17	0.73	2.63
6	Sabinene	0.17		0.10	0.70		6.91		1.71	0.10		
7	β-Myrcene	0.92	1.48	0.09	2.49		2.04					
8	D-Limonene	2.82		2.03	0.48	0.32	2.30	1.57	1.10	0.28	1.88	0.48
9	Eucalyptol	43.17	1.64	7.01		1.01		1.16	0.43	4.10	10.84	
10	γ-Terpinene	0.52	5.67		45.09		16.85	10.43			0.94	7.91
11	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-					1,18		0.03				
12	o-Cymene	2.75				0.25	6.30		0.70		1.74	
13	Linalool	0.69	5.80			33.15		5.06	1.14	1.84		0.9
14	endo-Borneol	3.83	2.25		0.15	0.79		2.71			4.45	1.03
15	cis-sabinene hydrate						15.09					
16	cis-α-Bergamotene									2.76		
17	Thujone								0.27		22,37	
18	Thymol		40.41		20.20			12.13				61.45
19	(+)-2-Bornanone	12.80	2.36			0.46		1.47		0.30	19.65	
20	Caryophyllene	3.78	6.77	3.33	0.41	3.32		2.50	2.16	0.15	5.62	2.28

Table 1 Continue.

	Compound	Essential oils										
		Ros.	Thym.	Mint	Sum. sav.	Lav.	Marj.	Wild thy.	Hys.	Bas.	Sage	Oreg.
21	Benzene, 4-ethyl -1,2-dimethyl-		19.45					18.07				13.14
22	α-Terpineol	2.31	0.26	0.14		1.17	0.93	1.82	0.27			0.28
23	Humulene	0.47	0.12	0.05		0.08		1.60			6.92	0.35
24	isobornil acetate	1.28										
25	terpinen-4-ol	0.46	2.16							84.89	0.44	
26	Estragole	0.29							0.19			
27	terpinene-4-ol						34.52		1.22			1.1
28	β-thujone								0.25		6.58	
29	3-Hexen-1-ol, (E)-							0.15	0.12			
30	menthofuran			1.62								
31	menthone			22.51								
32	p-menthone			4.22								
33	menthol			6.30								
34	Levomenthol			44.94						0.10		
35	p-Cymene				19.64							
36	Linalyl acetate					38.06						
37	Bornyl acetate			0.16				0.70		0.20	2.29	
38	Pulegone			0.62					2.87			
39	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-				0.41	2.52		2.39				
40	Geranyl acetate					0.98		4.77				
41	(E)-beta-Famesene					2.58						
	(-)-Lavandulol					3.43						
42	Geraniol					0.49		10.74				
43	(-)-beta-Bourbonene								1.66			
44	trans-3-pinanone								25.05			
45	cis-pinane								38.39			
46	Germacrene D									1.08		
47	(-)-Myrtenol									1.63		
48	α-thujene											2.92

Legend: *listed are the components that represented min. 1% in at least one essential oil.
Ros. – rosemary, Thym. – thyme, Sum. sav. – summer savory, Lav. – lavender, Marj. – marjoram, Wild thy. – wild thyme, Hys. – hyssop, Bas. – basil, Oreg. – oregano.

Apiaceae (syn. *Umbelliferae*). Each includes more than 15 species producing essential oils on a large scale **(Bhattacharya, 2016)**. In this study, we evaluated the antifungal properties of 11 essential oils from family *Lamiaceae.* According authors **(Ben Farhat, et al., 2016; Méndez-Tovar et al., 2016; Dušková et al., 2016)**, the growing seasons, different growth stage of plants, climatic conditions of each years in terms of the essential oil content and composition were proven. Based on the above, we also focused on the composition of the essential oils used. The GC-MS analyses of the essential oils led to identification of 139 compounds, 49 from them are presented in ≥1% amount in minimal one essential oil. The identified compounds (49) are listed in Table 1. The major components according concreated essential oil were: basil – estragole (84.98%), hyssop-cis-pinane (38.39%), trans-3-pinanone (25.05%), lavender – linalyl acetate (38.06%), linalool (33.15%), marjorom – terpinene-4-ol (34.52%), γ-Terpinene (16.85%), cis-sabinene hydrate (15.09%), mint – Levomenthol (44.94%), menthone (22.51%), oregano – Thymol (61.45%), Benzene, 4-ethyl-1,2-dimethyl- (13.14%), rosemary – Eucalyptol (43.17%), (+)-2-Bornanone (12.80%), α-pinene (10.74%), sage – Thujone (22.37%), (+)-2-Bornanone (19.65%), Eucalyptol (10.84%), summer savory-γ-Terpinene (45.09%), Thymol (20.20%), p-Cyneme (19.64%), thyme – Thymol (40.41%), Benzene, 4-ethyl-1,2-dimethyl- (19.45%), wild thyme-Benzene, 4-ethyl-1,2-dimethyl- (18.07%), Thymol (12.13%), Geraniol (10.74%), γ-Terpinene (10.45%).

The antifungal activity of 11 essential oils against the *Rhizopus* spp. was determined, using micro-atmosphere method (0.625 μL.cm^{-3} of air). Seven essential oils: thyme (*Thymus vulgaris* L.), mint (*Mentha piperita* L.), summer savory (*Satureja hortensis* L.), lavender (*Lavandula angustifolia* MILLER.), marjoram (*Origanum majorana* L.), oregano (*Origanum vulgare* L.) completely inhibited the growth of all isolates. Other essential oils (Table 2, Figure 2) have different effects on the growth of *Rhizopus* isolates. Basil essential oil stimulated growth of two isolates (KMi 511and KMi 512) on the second day of cultivation. The growth of other isolates was, by contrast, inhibited by this essential oil in the same time of cultivation. Hyssop essential oil completely inhibited growth of two isolates (KMi 510 and KMi 512), other 4 isolates were inhibited to fourth day of cultivation.

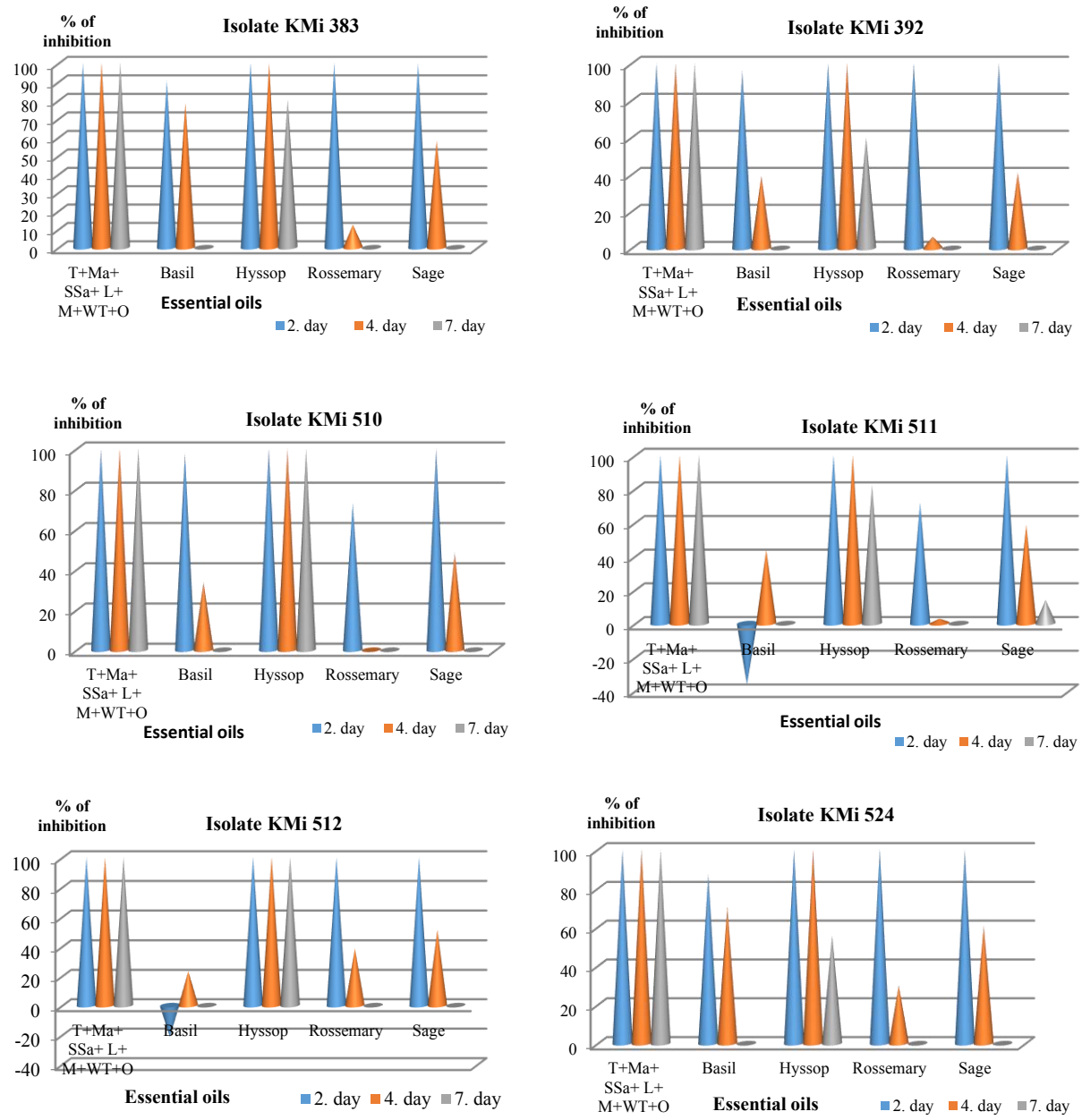

Figure 2 Inhibition of *Rhizopus* spp. growth caused by tested essential oils.
Legend: T– thyme, M – marjoram, SSa – summer savory, L – lavender, M – mint, WT – wild thyme, O – oregano.

The tested antifungal activity (against the *Rhizopus* spp.) of essential oils can be presented as: lavender = marjoram = mint = oregano = savory = thyme = wild thyme >hyssop >sage >rosemary >basil.

Strong inhibition effect (100%) of lavender, thyme and mint determined **Císarová et al. (2016b)**, against *Aspergillus flavus* and *Aspergillus parasiticus*. Inhibition of *Rhizopus stolonifer* growth by thymus essential oil recorded **Bosquez-Molina et al. (2010)**, **Plotto et al. (2003),** too. The strongest antifungal activity of savory essential oil against mycelial growth of *Rhizopus stolonifer* in the vapour phase showed **Alizadeh-Sallteh et al. (2010)**. These authors, like in our research, did not showed a significant influence of sage essential oil on the growth of *Rhizopus stolonifer*. **Farzaneh et al. (2015)** reported that *in vitro* results showed that at the maximum concentration of summer savory essential oil did not possess fungicidal effects on *Aspergillus niger* but they exhibited fungicidal activities against *Penicillium digitatum*, *Botrytis cinerea* and *Rhizopus stolonifer*. **Císarová et al. (2016a)** tested antifungal acitivity of lemon, eucalyptus, thyme, oregano,
sage and lavender essential oils against *Aspergillus niger* and *Aspergillus tubingensis* isolated from grapes. The most effective tested essential oils 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). Lavender essential oil was less active against tested strains. Antifungal activity of essential oils on *Botrytis cinerea* was tested by **Rattanapitigorn et al. (2006)**. Antifungal activity was examined *in vitro* in plastic Petri dishes containing PDA (like in our research, but essential oils were in different concentrations directly in PDA). Authors reported that lavender, rosemary, peppermint, basil, rose,

Table 2 Effect of essential oils (treatment) on the growth of *Rhizopus* isolates.

Treatment	Isolate	Days of cultivation					
		Second day		Fourth day		Seventh day	
		Lsmean	standarderr	Lsmean	standarderr	Lsmean	standarderr
Basil	**KMi 383**	2.97^{A}	0.72	19.41^{Aa}	4.18	90.00	1.49
Basil	**KMi 392**	7.23^{A}	0.72	54.80	4.18	90.00	1.49
Basil	**KMi 510**	1.97^{A}	0.72	59.82^{bd}	4.18	90.00	1.49
Basil	**KMi 511**	8.25	0.72	50.79^{A}	4.18	90.00	1.49
Basil	**KMi 512**	4.12	0.72	69.11^{bc}	4.18	90.00	1.49
Basil	**KMi 524**	4.62^{A}	0.72	26.40^{Aad}	4.58	90.00	1.49
Rosemary	**KMi 383**	0.00^{Aa}	0.72	78.92	4.18	90.00	1.49
Rosemary	**KMi 392**	0.00^{Aa}	0.72	84.39	4.18	90.00	1.49
Rosemary	**KMi 510**	8.36^{Ab}	0.72	90.00	4.18	90.00	1.49
Rosemary	**KMi 511**	8.72^{b}	0.72	87.55	4.18	90.00	1.49
Rosemary	**KMi 512**	0.00^{a}	0.72	55.30	4.18	90.00	1.49
Rosemary	**KMi 524**	0.00^{Aa}	0.72	63.01	4.18	90.00	1.49
Sage	**KMi 383**	0.00^{A}	0.72	37.66^{A}	4.18	90.00	1.49
Sage	**KMi 392**	0.00^{A}	0.72	53.00	4.18	90.00	1.49
Sage	**KMi 510**	0.00^{A}	0.72	46.46^{A}	4.18	90.00	1.49
Sage	**KMi 511**	0.00	0.72	37.33^{A}	4.18	77.50	1.49
Sage	**KMi 512**	0.00	0.72	43.93^{A}	4.18	90.00	1.49
Sage	**KMi 524**	0.00^{A}	0.72	35.23^{A}	4.18	90.00	1.49
Hyssop	**KMi 383**	0.00^{A}	0.72	0.00^{A}	4.18	17.74^{Aa}	1.49
Hyssop	**KMi 392**	0.00^{A}	0.72	0.00^{A}	4.18	36.12^{Ab}	1.49
Hyssop	**KMi 510**	0.00^{A}	0.72	0.00^{A}	4.18	0.00^{Ac}	1.49
Hyssop	**KMi 511**	0.00	0.72	0.00^{A}	4.18	16.0^{Ad}	1.49
Hyssop	**KMi 512**	0.00	0.72	0.00^{A}	4.18	0.00^{Ae}	1.49
Hyssop	**KMi 524**	0.00^{A}	0.72	0.00^{A}	4.18	39.91^{Ab}	1.49
Mint **Thyme** **Lavender** **Marjoram** **Summer savory** **Oregano** **Wild thyme**	**KMi 383** **KMi 392** **KMi 510** **KMi 512** **KMi 524**	Essential oils completely inhibited growth of isolates					
Control	**KMi 392**	**30.83^{a}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**
Control	**KMi 510**	**51.66^{b}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**
Control	**KMi 511**	**90.00^{c}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**
Control	**KMi 512**	**6.05^{d}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**
Control	**KMi 524**	**3.39^{d}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**
Control	**KMi 524**	**36.67^{a}**	**0.72**	**90.00**	**4.18**	**90.00**	**1.49**

Legend: a, b, c, d, e – different letters are significant within treatment at the level $p < 0.05$.
A – significant difference ($p < 0.05$) of the same isolate within all treatments to the same isolate in control.

ginger and thyme resulted reduction in colony diameter, but not a complete inhibition of mycelial growth. Antifungal activity of selected 16 essential oils (savory, oregano, thyme, rose, geranium, lavander, coriander, bergamot, lemon, orange, anise, tea tree, violet, basil and calmonile) tested **Stević et al. (2014)** against fungi isolates from medical plant. Among all oils tested, savory, oregano and thyme oils proved to be the best inhibitors of all tested pathogenes. These three essential oils completely inhibited the growth of *Rhizopus* isolates in our experiment as well. As in our experiment, they did not notice a significant effect of basil oil on the testing fungi. **Rattanapitigorn et at. (2006)** reported that although the antifungal activity of plant essential oils is documented, the dose response of the inhibition effect varies widely, depending on the type of essential oils, extraction method, and antifungal test

method. Influence of essential oils addition to the food tested several scientists. **Busatta et al. (2008)** tested addition of different concentrations of marjoram essential oils in sausage on the aerobic heterotrofic bacteria´s growth. A significant reduction in the number of CFU was noted during the first time step (10 days) after a 35-day storage period. At the end of the storage period it was observed that the lowest oil concentration exerted not only antimicrobial activity but also a bactericidal effect, which may as account for the extended shelf-life of certain products. **Michalczyk et al. (2012)** reported that the most signicicant benefit of applying essential oils (coriander and hyssop) to stored ground beef was to inhibit undesirable sensory changes and the growth of *Enterobacteriaceae* bacteria (by 1 – 2 log cycles), especialy at 6 ±1 ºC. Based on our results and the results of other authors, it is important to test not only different species but also different isolates of the same species of moulds to gain important insights into the antifungal properties of essential oils.

CONCLUSION

In this study, we evaluated the antifungal properties of basil (*Oscimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), mint (*Mentha piperita* L.), savory (*Satureja hortensis* L.), sage (*Salvia officinalis* L.), lavender (*Lavandula angustifolia* MILLER.), marjoram (*Origanum majorana* L.), wild thyme (*Thymus serpyllum* L.), oregano (*Origanum vulgare* L.) and hyssop (*Hyssopus officinalis* L.) essential oils. Seven essential oils: thyme, mint, summer savory, lavender, marjoram, oregano and wild thyme completely inhibited the growth of all isolates. Other essential oils demonstrated different effects on the growth of isolates. Basil essential oil stimulated growth of two isolates on the second day of cultivation. The tested antifungal activity (against the *Rhizopus* spp.) of essential oils can be presented as: lavender = marjoram = mint = oregano = savory = thyme = wild thyme >hyssop >sage >rosemary >basil.In conclusion, certain essential oils are highly effective in vapour phase and can be used in another test of their antifungal activity and could be used in control of *Rhizopus* spp. or another fungal pathogens.

REFERENCES

Alizadeh-Salteh, S., Arzani, K., Omidbeili, R., Safaie, N. 2010. Essential oils inhibit mycelial growth of Rhizopus stolonifer. *European Journal of Horticurtural Science*, vol. 75, no. 6, p. 278-282.

Bautista-Baños, S., Bosquez-Molina, E., Barrera-Necha, L. L. 2014. Rhizopus stolonifer (soft rot). In Bautista-Baños, S. *Postharvest Decay*. Amsterdam, Netherland : Elsevier, p. 1-44. ISBN 978-0-12-411552-1.

Ben Farhat, M., Jordán, M. J., Chaouch-Hamada, R., Landoulsi, A., Sotomayor, J. A. 2016. Phenophase effects on sage (*Salvia officinalis* L.) yield and composition of essential oil. *Journal of Applied Research on Medicinal and Aromatic Plants*, vol. 3, no. 3, p. 87-93. https://doi.org/10.1016/j.jarmap.2016.02.001

Bhattacharya, S. 2016. Cultivation of Essential Oils. *Essential Oils in Food Preservation, Flavor and Safety*, p. 19-29. https://doi.org/10.1016/B978-0-12-416641-7.00003-1

Bosquez-Molina, E., Jesús, E. R., Bautista-Baños, S., Verde-Calvo, J. R., Morales-López, J. 2010. Inhibitory effect of essential oils against Colletotrichum gloeosporioides and Rhizopus stolonifer in stored papaya fruit and their possible application in coatings. *Postharvest Biology and Technology*, vol. 57, no. 2, p. 132-137. https://doi.org/10.1016/j.postharvbio.2010.03.008

Busatta, C., Vidal, R. S., Popiolski, A. S., Mossi, A. J., Dariva, C., Rodrigues, M. R. A., Corazza, F. C., Corazza, M. L., Vladimir Oliveira, J., Cansian, R. L. 2008. Application of *Origanum majorana* L. essential oil as an antimicrobial agent in sausage. *Food Microbiology*, vol. 25, no. 1, p. 207-211. https://doi.org/10.1016/j.fm.2007.07.003 PMid:17993397

Cakir, A., Kordali, S., Kilic, H., Kaya, E. 2005. Antifungal properties of essential oil and crude extracts of *Hypericum linarioides Bosse*. *Biochemical Systematics and Ecology*, vol. 33, no. 3, p. 245-256. https://doi.org/10.1016/j.bse.2004.08.006

Císarová, M., Tančinová, D., Medo, J. 2016a. Antifungal activity of lemon, eucalyptus, thyme, oregano, sage and lavender essential oils against *Aspergillus niger* and *Aspergillus tubingensis* isolated from grapes. *Potravinarstvo Slovak Journal of Food Science*, vol. 10, no. 1, p. 83-88. https://doi.org/10.5219/554

Císarová, M., Tančinová, D., Medo, J., Kačániová, M. 2016b. The in vitroeffect of selected essential oils on the growth and mycotoxin production of *Aspergillus* species. *Journal of Environmental Science and Health, Part B*, vol. 51, no. 10, p. 668-674. https://doi.org/10.1080/03601234.2016.1191887 PMid:27322876

Dušková, E., Dušek, K., Indrák, P., Smékalová, K. 2016. Postharvest changes in essential oil content and quality of lavender flowers. *Industrial Crops and Products*, vol. 79, p. 225-231. https://doi.org/10.1016/j.indcrop.2015.11.007

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. https://doi.org/10.1016/j.postharvbio.2015.06.014

Guynot, M. E., Ramos, A. J., Seto, L., Purroy, P., Sanchis, V., Marin, 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. https://doi.org/10.1046/j.1365-2672.2003.01927.x PMid:12694455

Kassemeyer, H. H., Berkelmann-Löhnertz, B. 2009. Fungi of grapes. In König, H. et al. *Biology of Microorganisms on Grapes, in Must and in Wine*. Berlin Heidelberg, Germany : Springer-Verlag, p. 61-87. ISBN 978-3-540-85463-0.

Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., Mete, E. 2008. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish Origanum acutidens and its three components, carvacrol, thymol and p-cymene. *Bioresource Technology*, vol. 99, no. 18, p. 8788-8795. https://doi.org/10.1016/j.biortech.2008.04.048 PMid:18513954

Mari, M., Bautista-Baños, S., Sivakumar, D. 2016. Decay control in the postharvest system: Role of microbial and plant volatile organic compounds. *Postharvest Biology and Technology*, vol. 122, p. 70-81. https://doi.org/10.1016/j.postharvbio.2016.04.014

Méndez-Tovar, I., Novak, J., Sponza, S., Herrero, B., Asensio-S-Manzanera, M. C. 2016. Variability in essential oil composition of wild populations of Labiatae species collected in Spain. *Industrial Crops and Products*, vol. 79, p. 18-28. https://doi.org/10.1016/j.indcrop.2015.10.009

Michalczyk, M., Macura, R., Tesarowicz, I., Banaś, J. 2012. Effect of adding essential oils of coriander (*Coriandrum sativum* L.) and hyssop (*Hyssopus officinalis* L.) on the shelf life of ground beef. *Meat Science*, vol. 90, no. 3, p. 842-850. https://doi.org/10.1016/j.meatsci.2011.11.026 PMid:22153611

Nikkhah, M., Hashemi, M., Habibi Najafi, M. B., Farhoosh, R. 2017. Synergistic effects of some essential oils against fungal spoilage on pear fruit. *International Journal of Food Microbiology*, vol. 257, p. 285-294. https://doi.org/10.1016/j.ijfoodmicro.2017.06.021 PMid:28763743

Pitt, J. I., Hocking, A. D. 2009. *Fungi and food spoilage*. 3rd ed. London, New York : Springer Science & Business Media, LLC, 519 p. ISBN 978-0-387-92206-5.

Plotto, A., Roberts, D. D., Roberts, R. G. 2003. Evaluation of plant essential oils as natural postharvest disease control of tomato (*Lycopericun esculentum*). Acta Horticulturae, vol. 628, p. 737-745. https://doi.org/10.17660/ActaHortic.2003.628.93

Rattanapitigorn, P., Arakawa, M., Tsuro, M. 2006. Vanillin enhances the antifungal effect of plant essential oils against Botrytis cinerea. *International Journal of Aromatherapy*, vol. 16, no. 3-4, p. 193-198. https://doi.org/10.1016/j.ijat.2006.09.003

Ríos, J. L. 2016. Essential oils: what they are and how the terms are used and defined. *Essential Oils in Food Preservation, Flavor and Safety*, p. 3-10. https://doi.org/10.1016/B978-0-12-416641-7.00001-8

Samson, R. A., Houbraken, U., Thrane, U., Frisvad, J. C., Andersen, B. 2010. *Food and Indoor Fungi*. Utrecht, Netherlands : CBS-KNAW Fungal Biodiversity Centre, 390 p. ISBN 978-90-70351-82-3.

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Sivakumar, D., Bautista-Baños, S. 2014. A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Protection*, vol. 64, p. 27-37. https://doi.org/10.1016/j.cropro.2014.05.012

Stević, T., Berić, T., Šavikin, K., Soković, M., Gođevac, 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. https://doi.org/10.1016/j.indcrop.2014.02.011

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PROTEIN QUALITY CHICKEN MEAT AFTER FEEDING WITH ACTIVE SUBSTANCES OF CITRUS FRUITS AND DICLAZURIL AND SALINOMYCIN SODIUM

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ABSTRACT
The purpose of this study was an experimental investigation of the influence of active substances obtained mainly from citrus fruits in the experimental feed mixtures, and diclazuril and salinomycin sodium in the control feed mixtures of broiler chickens on productive efficiency and protein quality of the breast and thigh muscles. *In vivo* experiment was carried out with hybrid chickens Cobb 500. Basic feed mixtures were equal a soy cereal type for experimental and control group. Indicators of productive efficiency were measured and calculated, and protein, lysine and methionine contents in the breast and thigh muscles were measured by the method of FT IR, Nicolet 6700. Active substances obtained mainly from citrus fruits confirmed a statistically significant (p <0.05) positive effect on the body weight gain; tended to slightly increase feed intake per bird, protein, energy, lysine and methionine intake per bird; slightly decrease feed intake per 1 kg of body weight gain, protein, energy, lysine and methionine intake per 1 kg of body weight gain; slightly increase protein efficiency ratio and energy efficiency ratio. Additive substances used in the feed mixtures did not have a statistically significant effect on protein, lysine and methionine contents in the breast and thigh muscles but displayed a strong positive, statistically significant relation between lysine and methionine in them.

Keywords: chicken breast; chicken thigh; protein; lysine; methionine; additive substance

INTRODUCTION
Citrus (*Citrus* L. from Rutaceae) is one of the most favorite fruit, which contains active phytochemicals that can help to protect health. Besides, it is good sources of vitamin C, folic acid, potassium and pectin **(Anagnostopoulou et al., 2006; Guimarães et al., 2009)** and it was reported that citrus fruits, citrus fruit extracts and citrus flavonoids exhibit a wide range of potential biological efficiency due to their phenolic profile and antioxidant properties **(Montanari et al., 1998)**. Citrus fruits are highly consumed worldwide as fresh produce, juice and the peel is discarded most often as waste which contains a wide variety of secondary ingredients with important antioxidant activity in comparison with other parts of the fruit **(Manthey and Grohmann, 2001)**.

A number of studies confirmed the presence of polyphenols, vitamins, minerals, dietary fibres, essential oils and carotenoids content in citrus fruit. Presence these substances predetermines a citrus fruit prefered product **(Rafiq et al., 2016)**.

Some valuable biologically active substances occur rarely in the citrus fruits. They are oxyprenylated natural products, for example 3,3-dimethylallyloxy-(C5), geranyloxy-(C10), and the farnesyloxy-(C15) related compounds that have been recognized in the last 12 years in citrus varieties **(Munakata et al., 2012)**. Citrus fruits were also seen to be a good source of many natural compounds: prenyloxycoumarins such as auraptene, bergamottin, imperatorin, heraclenin, oxypeucedanin and many others which have been isolated from the citrus juice and peel extracts **(Epifano and Genovese, 2013; Genovese et al., 2014)**. There is a growing acceptance that phenols, amino acids, essential oils, pectin, carotenoids, flavonoids, and vitamin C present in citrus fruits exert beneficial effects **(Wang et al., 2014)**.

The purpose of this study was investigation and a statistically evaluation of the influence of experimental active substances obtained mainly from citrus fruits compared to control coccidiostatats diclazuril and salinomycin sodium in feed mixtures of broiler chickens on their productive efficiency, protein and energy efficiency, protein content and content of selected aminoacids in the breast and thigh muscles.

Scientific hypothesis
Active substances obtained from citrus fruits has an effect on the body weight gain of broiler chickens.

MATERIALS AND METHODS

The object of investigation

The object of the investigation were feed additives, on the basis of active substances obtained mainly from citrus fruits comparised to control coccidiostatats, broiler chickens, productive efficiency of the feed mixtures, protein and energy efficiency and quality of breast and thigh muscles from aspect of the protein, lysine and methionine contents.

Procedure of carrying out an experiment with broiler chickens

In vivo experiment was conducted in practical conditions on poultry experimental station with deep litter breeding system. Experiment included 100 one-day-old broiler chickens of hybride combination Cobb 500 divided into two groups, experimental and control (n = 50).

All conditions were adhered to protection of broiler chickens in the experiment **Council Directive 2007/43/EC**. Spaced deep litter box allowed broiler chickens to perform natural activities. Microclimatic conditions were equal for experimental and control group in accordance with the producent recommendations for the final type broiler chickens Cobb 500 (temperature, relative humidity, air exchange and the light mode). The broiler chickens to the age of 14 days intake a feed from plate feeders and water from the hat drinkers located on the floor. Older chickens intake feed from the tube feeders and drank water from bucket drinkers till the end of the experiment.

Experimental period was divided into four phases during which fed different feed mixtures: starter phase, starter feed mixture, from day 1 to day 10; grower phase, grower feed mixture 1, from 11 to day 20; grower phase, grower feed mixture 2, from 21 to day 35 and final phase, final feed mixture, from 36 to day 42.

Basic feed mixtures were equaled for experimental and control group. Composition of feed mixtures is given in Table 1. Additive substances used in feed mixtures were different for each group. In experimental feed mixtures was used commercial feed additive on the basis of active substances obtained mainly from citrus fruits. Experimental feed mixtures were without coccidiostatas. Control feed mixtures contained commercial feed additive of coccidiostats, which are commonly used in practical conditions. Control feed mixtures were without feed additive on the basis of active substances obtained mainly from citrus fruits.

Basic feed mixtures were a soy cereal type. Base of feed mixtures consisted of corn, wheat and soybean meal during the starter phase 90.33% (starter feed mixture), during the growth phase 90.1% (growth feed mixture 1), 93.05% (growth feed mixture 2) and during final phase of 92.61% (final feed mixture). Meat-and-bone meal it is banned as feed material and deficient amino acids in feed mixtures must be complemented with amino acids pre-mixes. We used pre-mixes L-Lysine HCl and DL-Methionine in our feed mixtures, which were source their active substance, lysine and methionine, in accordance with the physiological needs of broiler chickens.

Composition of L-Lysine HCl:
L-Lysine monohydrochloride, technically pure NH_2-$(CH_2)_4$-CH(NH_2)-COOH.HCl,
L-Lysine min 78%.

Composition of DL-Methionine:
DL-Methionine, technically pure $CH_3S(CH_2)_2$-CH(NH_2)-COOH,
DL-Methionine min 98%.

We calculated ratio between metabolizable energy and crude protein and between methionine and lysine, which are important for the productive efficiency of feed in broiler chickens, especially in terms of protein intake.

A value of metabolizable energy in kJ.kg^{-1}/crude protein in g.kg^{-1} ratio ranged from 55.91 : 1 (starter feed mixture) to 69.95 : 1 (final feed mixture). A value of methionine in g.kg^{-1}/lysine in g.kg^{-1} ratio varied from 0.42 : 1 (grower feed mixtures) to 0.43 : 1 (starter or final feed mixture).

Scheme and characteristics of feed additives used in experiment

Scheme of experiment is given in Table 2.

Experimental group

Biocitro feed additive is to be used in the drinking water 1.0 mL per 1.0 L.

Composition of Biocitro:

mixture of citrus fruit essential oils obtained by pressing of *Citrus paradisi*, *Citrus reticulata* Blanco, *Citrus aurantium bergamia* ss. and *Citrus sinensis*; the main

Table 1 Content of nutrients and metabolizable energy in basal feed mixtures.

	Feed mixtures			
	Starter (from day 1 to day 10)	**Grower 1 (from day 11 to day 20)**	**Grower 2 (from day 21 to day 35)**	**Final (from day 36 to day 42)**
Crude protein, g.kg^{-1}	220.00	207.00	197.00	188.00
ME_N, kJ.kg^{-1}	12,300	12,750	13,150	13,150
ME_N/Crude protein	55.91:1	61.59:1	66.75:1	69.95:1
Lysine, g.kg^{-1}	14.00	12.50	12.50	11.50
Methionine, g.kg^{-1}	6.00	5.20	5.20	5.00
Methionine/Lysine	0.43 :1	0.42:1	0.42:1	0.43:1
Calcium, g.kg^{-1}	9.00	8.50	8.50	8.50
Phosphorus (non-phytate), g.kg^{-1}	4.20	4.00	4.00	4.00

ME_N = apparent metabolizable energy corrected for nitrogen balance.

Table 2 Scheme of experiment.

Group	Broiler chickens Cobb 500, *n*	Feed additive	Active substances
Experimental	50	Biocitro*	obtained mainly from citrus fruits
Control	50	Clinacox 0.5%**	diclazuril
		Sacox 12%***	salinomycin sodium

*Drinking water; **Starter feed mixture; ***Grower feed mixtures 1, 2.

compounds of citrus biomass are L-ascorbic acid and flavonoids, sucha s naringin, hesperidin, quercetin and rutin, vitamin C – L-ascorbic acid, the organic acids – citric acid and fatty acids, such as *α*-linolenic acid, linoleic acid, *γ*-linolenic acid and oleic acid.

Control group

Clinacox and Sacox are premixes used in commercial feed mixtures of the control group. Clinacox 0.5% is premix with the active substance diclazuril 0.5 g per 100 g for the prevention of coccidiosis caused by *Eimeria acervulina*, *E. brunetti, E. maxima, E. mitis, E. necatrix and E. tenella.*

Composition of Clinacox 0.5%:
diclazuril 0.5 g per 100 g,
soybean meal 99.25 g per 100 g,
polyvidon K30 0.2 g per 100 g,
sodium hydroxide 0.0538 g per 100 g.
Characteristics of active substance diklazuril $C_{17}H_9C_{l3}N_4O_2$:
(±)-4-chlorofenyl[2,6-dichloro-4-(2,3,4,5-tetrahydro-3,5-dioxo-1,2,4-triazin-2-yl)fenyl]acetonitril. Number CAS: 101831-37-2.

Related impurities:
degradation compound (R064318) ≤0.1%, other related impurities (T001434, R066891, R068610, R070156, R070016) ≤0.5% individually, total impurities ≤1.5%.
Sacox 12% is premix with active substances salinomycin sodium for the prophylactic control of various kinds of coccidia, such as *Eimeria acervulina*, *E. maxima, E. necatrix, E. mivati, E. tenella and E. brunetti*. Its dose is from 50 to 70 mg per 1,000 g of feed mixture.

Composition sacox 12%:
salinomycin sodium: ≥120 *g* per 1,000 g, silicon dioxide from 10 to100 g per 1,000 g and calcium carbonate from 350 to 700 g per 1,000 g.
Characteristics of active substance salinomycin sodium $C_{42}H_{69}O_{11}Na$:
Number CAS: 53003-10-4, sodium salt of a polyether monocarboxylic acid produced by fermentation of *Streptomyces albus* (DSM 12217).

Related impurities:
<42 mg elaiophylin per kg salinomycin sodium, <40 g 17-epi-20-desoxy-salinomycin per kg salinomycin sodium.

Parameters and procedure their an investigation

Productive parameters

Body weight gain per bird

We calculated average body weight gain per bird (BWG) as difference between total final body weight of broiler chickens in group and body weight of one-day-old broiler chickens in group. We divided the result by the number of birds in group.

Each one-day-old broiler chicken under the experiment weighed 42.0 g in the experimental and control group. We weighted their body weight by scale Kern 440-43 N type with a maximum weight of 400.0 g and final body weight by ECE20K20 type with a maximum weight of 20.0 kg.

Feed intake per bird

We calculated an average feed intake per bird (FI) on the basis of total consumed a starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture in group. We divided consumed a quantity feed by number of birds in the group. Average feed intake per bird presents an average feed intake per total body weight gain and bird.

Feed intake per 1 kg of body weight gain

We calculated an average feed intake per 1 kg of body weight gain (FI per 1 kg of BWG) on the basis of total consumed a starter feed mixture, grower feed mixture 1, grower mixture 2 and final feed mixture in group. We divided consumed a quantity feed by total body weight gain of broiler chickens in group.

Protein intake per total body weight gain and bird

We calculated of average protein intake per total body weight gain and bird according to Equation 1.

$$PI = FI \times CP \quad (1)$$

where:
PI = average protein intake per total body weight gain and bird, g;
FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;
CP = crude protein content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$.

Protein intake per 1 kg of body weight gain

We calculated of average protein intake per 1 kg of body weight gain according to Equation 2.

$$PI \text{ per 1 kg of BWG} = FI \times CP/BWG \quad (2)$$

where:
PI per 1 kg of BWG = average protein intake per 1 kg of body weight gain, g;
FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;
CP = crude protein content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$;
BWG = average body weight gain per bird, kg.

Energy intake per total body weight gain per bird

We calculated of average energy intake per total body weight gain and bird according to Equation 3.

$$EI = FI \times ME_N \quad (3)$$

where:

EI = average energy intake per total body weight gain per bird, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

ME_N = metabolizable energy content per 1 kg starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $kJ.kg^{-1}$.

Energy intake per 1 kg body weight gain

We calculated of average energy intake per 1 kg of body weight gain according to Equation 4.

$$EI \text{ per 1 kg of BWG} = FI \times ME_N/BWG \quad (4)$$

where:

EI per 1 kg of BWG = average energy intake per 1 kg of body weight gain, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

ME_N = metabolizable energy content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$;

BWG = average body weight gain per bird, kg.

Lysine intake per total body weight gain per bird

We calculated of average lysine intake per total body weight gain and bird according to Equation 5.

$$LysI = FI \times Lys \quad (5)$$

where:

LysI = average lysine intake per total body weight gain and bird, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

Lys = lysine content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$.

Lysine intake per 1 kg body weight gain

We calculated of average lysine intake per 1 kg of body weight gain according to Equation 6.

$$LysI \text{ per 1 kg of BWG} = FI \times Lys/BWG \quad (6)$$

where:

LysI per 1 kg of BWG = average lysine intake per 1 kg of body weight gain, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

Lys = lysine content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$;

BWG = average body weight gain per bird, kg.

Methionine intake per total body weight gain per bird

We calculated of average methionine intake per total body weight gain and bird according to Equation 7.

$$MetI = FI \times CP \quad (7)$$

where:

MetI = average methionine intake per total body weight gain and bird, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

Met = methionine content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$.

Methionine intake per 1 kg body weight gain

We calculated of average protein intake per 1 kg of body weight gain according to Equation 8.

$$MetI \text{ per 1 kg of BWG} = FI \times CP/BWG \quad (8)$$

where:

MetI per 1 kg of BWG = average methionine intake per 1 kg of body weight gain, g;

FI = average intake of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture per bird, kg;

Met = crude protein content per 1 kg of starter feed mixture, grower feed mixture 1, grower feed mixture 2 and final feed mixture, $g.kg^{-1}$;

BWG = average body weight gain per bird, kg.

Protein and energy efficiency ratio

Protein efficiency ratio

We calculated a protein efficiency ratio (PER) according to Equation 9:

$$PER = BWG/PI \quad (9)$$

where:

PER = protein efficiency ratio;

BWG = average body weight gain per bird, g;

PI = average protein intake per total body weight gain and bird, g.

Energy eficiency ratio

We calculated a energy efficiency ratio (EER) according to Equation 10:

$$EER = BWG/EI \times 100 \quad (10)$$

where:

EER = energy efficiency ratio, %

BWG = average body weight gain per bird, g

EI = average energy intake per total body weight gain and bird, kJ.

Breast and thigh muscle quality from aspect of the protein, lysine and methionine contents

Sample analyses

We carried out a sampling and chemical analysis of breast and thigh muscles according to **Angelovičová et al. (2016)**. Broiler chickens in the number of 6 pcs from experimental and control group, above average body (live) weight of 1800.0 g, were randomly selected at the end of the experiment (day 42). A slaughtering of broiler chickens by human rapid cut of the carotid artery (*Ateria carotis communis*) was realized. Subsequently, feathers as well as internal parts of chickens were mechanically removed. Breast and thigh muscles, which have been used for chemical analysis, were separated from the carcass as well. A sample (50.0 g) of breast and thigh muscles without a skin of every chicken was measured by the method of FT IR Nicolet 6700 (the program OMNIC™

Series Software, producer Thermo Fisher Scientific, USA). A molecular spectroscopy was performed 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. Samples of breast and thigh muscles were measured for protein content, lysine content and methionine content.

Statistical analyses

The results of productive efficiency of feed mixtures and protein and energy efficiency are presented as average values. The values of these parameters were investigated jointly by groups. The results of total body weight gain, breast and thigh muscle quality from aspect protein, lysine and methionine contents were investigated individually and statistically evaluated for group as average values, standard deviation, coefficient of variation and variation range as the difference between the minimum and maximum value of the data distribution.

T-test was ued at the significance level $\alpha = 0.05$ to compare a difference between experimental and control groups. A Pearson´s correlation coefficient (r_{xy}) reflects a degree of relation between two variables of the indicators of chicken breast muscle and thigh muscle. Pearson´s correlation coefficient (r_{xy}) 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. The results of correlation coefficient are complemented by statistical significance at the significance level of $\alpha = 0.05$, $\alpha = 0.01$ and $\alpha = 0.001$. SAS statistical package (5) was used to perform statistical analyses.

RESULTS AND DISCUSSION

Productive parameters

Body weight gain per bird

Average total body weight gain per bird is given in Table 3.

Obtained the active substances from vegetable products confirmed in experiments *in vivo* effects for the secretion of gastric juice, which led to the increase of body weight of the broiler chickens. Mentioned an effect is associated with improved digestibility and absorption of nutrients from feed **Močár et al. (2012)**. A similar conclusion is shown in otther study. Important propriety which has also been observed recently in chickens is the benefit of some natural substances on the gastrointestinal enzymatic activity, most likely improving nutrient digestibility **(Jang et al., 2006)**.

Average total body weight gain of broiler chickens was found 2036.09 g in our experiment by effect of active substances obtained mainly from citrus fruits compared to average total body weight gain 1771.27 g in control group with diclazuril and salinomycin sodium. The results of SD and %CV for total body weight gain per bird by effect of active substances obtained mainly from citrus fruits varied more compared to calculated results of SD and %CV for total body weight gain per bird of control group with diclazuril and salinomycin sodium. Many research institutions focused their attention on the development of alternatives to feed antibiotics, including a control of coccidiosis, with respect to recent global trend to ensure poultry production without them **(Lillehoj and Lee, 2012)**. According to some authors **(Langhout, 2000; Manzanilla et al., 2004)**, positive results may be obtained, when essential oils extracted from different plants are enriched by the addition of the more relevant active substances. Investigated active substances obtained mainly from citrus fruits, according to our experimental results clearly demonstrate significant positive effects on total body weight gain of broiler chickens, which were confirmed by increased values compared to control group with coccidiostatats diclazuril and salinomycin sodium.

Feed intake per bird, and 1 kilogram of body weight gain, protein, energy, lysine and methionine intake per total body weight gain and bird, and 1 kg of body weight gain

Average feed intake per bird, and 1 kg of body weight gain, protein, energy, lysine and methionine intake per total body weight gain and bird, and 1 kg of body weight gain is given Table 4.

An effect of active substances obtained mainly of citrus fruits tended to slightly increase feed intake per bird in our experiment in contrast to feed intake of control group with diclazuril and salinomycin sodium (4340.0 g). Feed supplemented with active substances obtained mainly from citrus fruits is probably tastier for broiler chickens. Our results of an average feed intake per 1 kg of body weight gain had the opposite tendency as average feed intake expressed per bird. Average feed intake per 1 kg of body weight gain showed slightly lower average value 2,161.0 g in the experimental group, in which the broiler chickens consumed of feed mixtures with active substances

Table 3 Average total body weight gain per bird in g.

Group	n	M	SD	%CV	R	t-test
Control	50	1771.27	161.30	9.11	1378 – 2178	$p < 0.05$
Experimental	50	2036.09	325.16	15.97	1178 – 2678	

Note: n = number of samples; M = mean; SD = standard deviation; %CV = coefficient of variation; R = variation range as the difference between the minimum and maximum value of the data distribution; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits; $p < 0.05$ = statistically significant difference between groups.

Table 4 Average feed intake per bird, and 1 kg of body weight gain, protein, lysine and methionine intake per total body weight gain per bird, and 1 kg of body weight gain.

	Group	
	Control	**Experimental**
Feed intake per bird*, g	4,340.00	4,400.00
Feed intake per 1 kg of body weight gain, g.kg^{-1}	2,450.22	2,161.00
Protein intake per total body weight gain and bird, g	864.42	875.70
Protein intake per 1 kg of body weight gain, g.kg^{-1}	488.20	430.09
Energy intake per total body weight gain and bird, g	56,246.00	57,035.00
Energy intake per 1 kg of body weight gain, g.kg^{-1}	31,754.62	28,012.02
Lysine intake per total body weight gain and bird, g	53.66	54.35
Lysine intake per 1 kg of body weight gain, g.kg^{-1}	30.29	26.69
Methionine intake per total body weight gain and bird, g	22.70	23.00
Methionine intake per 1 kg of body weight gain, g.kg^{-1}	12.82	11.30

Note: Feed intake per bird = feed intake per total body weight and bird; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits.

obtained mainly from citrus fruits compared to 2,450.0 g in the control group with diclazuril and salinomycin sodium. Active substances obtained from citrus fruits can affect the endogenous secretion of broiler chickens **(Cross et al., 2007)**. Feed conversion ratio was found significantly better after feeding diet supplemented with 1.0% and 2.0% dried sweet orange (*Citrus sinensis*) pulp than in control group without this feed additive. Based on some observed data, dried or pelleted citrus pulp is one of the most desirable energy feeds and can be considered in feeding programs as a feed with high digestible nutrient content. It includes a mixture of citrus peel, pulp, and seed as by-product energy concentrate feed for domestic animals **(Arthington et al., 2002)**. The consumption of feed mixtures with supplements on the natural base has been investigated in other *in vivo* experiment with broiler chickens. Average consumption of feed mixtures with supplements on a natural base in other experiment, expressed as consumption of feed per 1 kg of body weight gain, was found slightly lower opposite our results, i.e. 1807.0 g, 1822.0 g and 1835.0 g and of commercial feed mixtures 2013.0 g **(Azza a Naela (2014)**. These values of feed intake per 1 kg of body weight gain are lower compared to the average feed intake per kg body weight gain of broiler chickens in our experiment. This difference in feed intake per 1 kg of body weight gain can be related to different final hybrid of broiler chickens, which was used in the experiment. These authors used the final hybrid Arbor Acres and we used in our experiment a final hybrid Cobb 500. Digestive system development of broiler chickens is faster shortly after hatching than the body development. Gastrointestinal tract begins to form already during the embryonic stage **(Moran, 1985)** and it becomes fully functional within a few days after hatching **(Iji et al., 2001a, 2001b)**. The development of the gastrointestinal tract after hatching is very important for the growth chickens and future performance through effective utilization of dietary nutrients **(Faria et al., 2005)**. Submission of high quality nutrition to young chickens at an early stage of development is very important to ensure the rapid development and growth of the gastrointestinal tract, as well as the body. Proteins in this respect the most essential nutrients, which determine the initial development of the gastrointestinal tract and muscle mass in the later stages of broiler chicken growth **(Hargis a Creger, 1980)**.

Our results of average dietary protein intake per total body weight gain of broiler chickens were found 875.70 g by effect of active substances obtained mainly from citrus fruits and slightly lower 864.42 g in control group with dikluzaril and salinomycin sodium. Average dietary protein intake per 1 kg body weight gain of broiler chickens was achieved 430.09 g by active substances obtained mainly citrus fruits opposite slightly higher protein consumption 488.02 g in control group with diclazuril and salinomycin sodium. Protein source and protein quality are very important for growth and development of broiler chickens. This fact was confirmed in different growth intensity of broiler chickens that were fed with different proportion of vegetable and animal proteins in the feed mixtures **(Hossain et al., 2012, 2013a)**. Dietary protein is the most expensive component of a broiler diet. Methionine is an amino acid of critical importance in commercial poultry diets, because it is typically the first-limiting amino acid **(Adbalqdadir and Arabi, 2014)**. One of the most expensive components of broiler chickens diets is protein or more specifically amino acids **(Dozier et al., 2009)**. A need for dietary lysine is greater for breast meat yield than for growth rate **(Sterling et al., 2006; Dozier et al., 2006)**.

Birds regulate their energy need by feed intake. If energy level increases in feed mixtures, birds satisfy their energy needs by decreasing feed intake **(Nahashon et al., 2005; Veldkamp et al., 2005)**. Our results of average dietary energy intake per bird were found slightly higher (57,035.00 kJ) in the experimental group with active substances obtained mainly from citrus fruits in contrast to the control group with diclazuril and salinomycin sodium (56,246.0 kJ). Average dietary energy intake per 1 kg of body weight gain was found 28,012.02 kJ.kg^{-1} in the experimental group by effect of active substances obtained mainly from citrus fruits, and 2,450.0 kJ.kg^{-1} in the control group with diclazuril and salinomycin sodium. According to literary knowledge is know that modern broiler chickens selected for rapid growth do not regulate voluntary feed intake to achieve energy balance. This altered ability of broiler chickens to adjust feed intake due to differences in content of dietary metabolizable energy was most likely to result from continued selection for rapid juvenile growth

rates. These animas may have altered hypothalamic mechanisms that regulate feed intake **(Bokkers and Koene, 2003)**. Other studies shown no effect of dietary metabolizable energy content on feed intake between broiler chickens fed *ad libitum* feed mixtures containing two energy levels of 13,380 and 15,000 $kJ.kg^{-1}$ **(Mbajiorgu et al., 2011)**. Feed intake may be affected by flavoring substances which today can be used in feed mixtures. We can include our investigated feed additive obtained mainly from citrus fruits these gustatory substances. Metabolizable energy intake is distributed in body into energy requirement for maintenance and energy retained in the body in the form of protein and fat **(Lopez and Leeson, 2005)**.

Because body proteins are in a dynamic state, with synthesis and degradation occur ring continuously, an adequate intake of dietary amino acid is required **(NRC, 1994)**. Broiler chickens are characterized by good storage capabilities muscle. Lysine provides the basic functions to create muscle mass. Lysine is considered to be the second most limiting amino acid after the sulfur-containing amino acids for broiler chickens fed feed mixtures based on corn and soybean meal. For these reasons lysine was chosen as the reference amino acid as the ideal protein concept, in which all other essential amino acids are formulated into the feed mixtures as a ratio to lysine **(Emmert and Baker, 1997)**. Our results of average dietary lysine intake per bird were found 54.35 g in experimental group with active substances obtained mainly from citrus fruits and slightly lower 53.66 g in control group with diclazuril and salinomycin sodium. Values of average lysine intake per 1 kg body weight gain have been demonstrated reduction in experimental group with active substances obtained mainly from citrus fruits (26.69 g) compared to control group with diclazuril and salinomycin sodium (30.29 g). In general, amino acid sufficient need for broiler chickens was determined depending on many factors, including genotype, age and sex. This potential was determined based on nitrogen balance **(Samadi and Liebert, 2007)**.

Methionine was set in the first position among amino acids limiting chicken growth **(Fancher and Jensen, 1989)**. Methionine is very important for poultry as an essential amino acid for protein synthesis, a methyl donor group for normal cellular metabolism and for normal formation of coenzyme S-adenosylmethionine, a precursor of important intermediates in metabolic pathways such as cystine or carnitine, an amino acid involved in polyamine synthesis, and also as a sulfur donor **(Bunchasak, 2009)**. Average dietary methionine intake per bird was found 23.0 g in our experiment by effect of active substances obtained mainly from citrus fruits and 22.70 g, i.e. less in control group with diclazuril and salinomycin sodium. Values of average methionine intake per 1 kg body weight gain have been demonstrated reduction in experimental group with active substances obtained mainly from citrus fruits (11.30 g) in contrast to control group with diclazuril and salinomycin sodium (12.82 g). The methionine addition in the poultry diet demonstrated a tendency to less total body fat **(Rostagno et al., 1995)**, to support growth performance **(Chavez et al., 2004)**. Feed with dietary methionine excess has been reported to impair body weight gain **(Harper et al., 1970)**.

Feed mixtures are submitted to broiler chickens with nutrient balances, including amino acids and energy. Broiler chickens have feed available *ad libitum*. They consume sufficient and balanced amount of amino acids. A proper balance feed mixture provides enough amino acids in a balanced ratio, thereby preventing a lack of amino acids or amino acid toxicity **(Zulkifli et al., 2001)**. The essential amino acid requirements for broiler chickens meet the formulation of feed mixture with addition of synthetic amino acids. Such feed mixture compositium improves the overall balance of amino acids and maintains overall performance **(Zarate et al., 2003)**. It is not easy to obtain accurate dietary amino acid needs for broiler chickens. Numerous researchs have been conducted to determine the essential amino acid requirements. These needs are influenced by factors such as growth in response to changing levels of dietary amino acids is not linear; antagonism or toxicity may occurr between two or more amino acids or anti-nutritional factors or interactions between certain amino acids and other nutrients; metabolizable variations under physiological and environmental conditions **(Oviedo-Rondon and Waldroup, 2002)**. Precise estimation of the lysine requirement is of the importance to defining the ideal ratios of other essential amino acids **(Baker, 2009)**. Lysine deficiency or fasting posthatch may decrease protein synthesis. It concerns mainly of the breast muscle **(Bigot et al., 2003)**. The lack of lysine increases proteolysis in breast muscle **(Tesseraud et al., 2009)**. Lysine and methionine are precursors of L-carnitine and assotiated to energy and lipid metabolism in broiler chickens **(Borum, 1983)**.

Protein and energy efficiency ratio

Protein and energy efficiency ratio is given in table 5.

In general, the nutritional quality of proteins depends on the balancing of amino acids that contribute to body weight gain of broiler chickens. The amount of body weight gain is very important per unit of protein intake by feed. The content of amino acids and proteins in the feeding of the feed is determined by analytical methods but information on their bioavailability is insufficient. For this reason, growth test used to compare the relative value of different proteins on the basis of qualitative growth of broiler chickens **(Rezaeipour et al., 2014)**. We used an indicator of protein efficiency ratio (PER) to evaluation the protein quality of the feed mixture quality in our experiment.Average protein efficiency ratio was found 2.36 in the broiler chickens in the broiler chickens that fed feed mixtures with active substances obtained mainly from

Table 5 Protein and energy efficiency ratio.

Group	Control	Experimental
PER	2.05	2.36
EER	3.15	3.57

Note: PER = protein efficiency ratio; EER = energy efficiency ratio; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits.

citrus fruits compared to slightly lower value of average protein efficiency ratio 2.05 in broiler chickens of control group with diclazuril and salinomycin sodium. Tendency of the higher protein efficiency ratio by effect of active substances obtained mainly from citrus fruits is related to the higher body weight gain of broiler chickens compared to protein efficiency ratio by effect of diclazuril and salinomycin sodium.

Energy efficiencies can be significantly affected by composition of feed mixtures and the purpose for which the energy is used **(Lopez and Leeson, 2008)**. Dietary energy is utilised more efficiently for body fat deposition than for body protein deposition. This variation prevents accurate prediction of nutrient requirements for broiler chickens **(Lopez and Leeson, 2005; Shantawi, 2014)**. Energy efficiencies of protein deposition can range from 0.36 to 0.70 and the energy efficiencies of fat deposition from 0.55 to 0.92 **(Sakomura et al., 2005)**. These variations may have resulted from differences in the genetic factor, feed mixture composition and environment **(Shantawi, 2014)**. The conditions in which was conducted our experiment were the same for experimental and control group except that groups differed according to each feed additive. We found higher energy efficiency ratio in these defined experimental conditions (see section Materials and methods) by effect of active substances obtained mainly from citrus fruits n contrast to the effect of control group coccidiostatats diclazuril and salinomycin natrium. **Angelovičová et al. (2016)** investigated the same feed additive Biocitro in relation to the fatty acid profile in the chicken meat. The effect this active substance tendentiously reduced fat content in breast and thigh muscles compared to control feed with mixtures coccidiostatats narasin, salinomycin sodium and nicarbasin.

Breast and thigh muscles quality from aspect of protein, lysine and methionine contents

Protein content in the breast and thigh muscles

Average protein content in the breast and thigh muscles is given in Table 6.

Chemical composition of chicken meat is affected by nutrition, age, animal genotype and other factors of environment **(Haščík et al., 2009)**. Energy value of meat is relatively low, but the protein content is relatively high compared to other types of meat **(Medved' and Angelovičová, 2010)**. The most abundant muscle protein fractions are structural proteins that participate 54-70% of total muscle protein. Myofibrillar proteins contribute to meat tenderness, determine the capacity of water retention and meat hydration, fat emulsifying and gelling capacity. They are important for technological quality, as well as the nutritional value of meat. Myofibrillar proteins contribute about 70% to the nutritional value of meat by essential amino acids contents **(Ionescu et al., 2009)**. Chicken meat contains about 16.44-3.31% protein **(Chuaynukool et al., 2007).** Our results of average protein content in breast muscle was found 24.13 g.100 g^{-1} by effect of active substances obtained mainly from citrus fruits and compared to slightly less protein content 23.87 g.100 g^{-1} in control group with diclazuril and salinomycin sodium.

The results of SD and %CV for protein content in the breast muscle by effect of active substances obtained mainly from citrus fruits varied more compared to calculated results of SD and %CV for protein content in the breast muscle of control group with diclazuril and salinomycin sodium. Feed additives used in our experiment, such as active substances obtained mainly from citrus fruits in experimental group, and coccidiostatats diclazuril and salinomycin sodium in control feed mixtures, did not have a statistically significant (p >0.05) effect on protein content in the breast

Table 6 Average protein content in the breast and thigh muscles, g per 100 g.

	n	M	SD	%CV	R	t-test
Group			**Breast muscles**			
Control	50	23.87	0.28	1.17	23.50 – 24.52	p >0.05
Experimental	50	24.13	0.35	1.45	23.56 – 24.61	
			Thigh muscles			
Control	50	22.44	0.49	2.18	22.20 – 22.83	p >0.05
Experimental	50	22.10	0.51	2.31	21.56 – 22.90	

Note: n = number of samples; M= average; SD = standard deviation; %CV = coefficient of variation; R = variation range as the difference between the minimum and maximum value of the data distribution; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits; p >0.05 = no statistically significant difference between groups.

Table 7 Average lysine content in the breast and thigh muscles, g per 100 g.

	n	M	SD	%CV	R	t- test
Group			**Breast muscles**			
Control	50	2.16	0.18	8.33	1.96 – 2.44	p >0.05
Experimental	50	2.42	0.25	10.33	2.21 – 2.81	
			Thigh muscles			
Control	50	2.11	0.15	7.11	1.92 – 2.30	p >0.05
Experimental	50	2.24	0.19	8.48	2.03 – 2.47	

Note: n = number of samples; M = average; SD = standard deviation; %CV = coefficient of variation; R = variation range as the difference between the minimum and maximum value of the data distribution; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits; p >0.05 = no statistically significant difference between groups.

Table 8 Average methionine content in the breast and thigh muscles, g per 100 g.

	n	M	SD	%CV	R	t- test
Group			**Breast muscles**			
Control	50	0.79	0.11	13.92	0.71 – 0.90	p >0.05
Experimental	50	0.89	0.11	12.36	0.79 – 0.98	
			Thigh muscles			
Control	50	0.76	0.06	7.89	0.68 – 0.82	p >0.05
Experimental	50	0.84	0.07	8.33	0.73 – 0.90	

Note: n = number of samples; M = average; SD = standard deviation; %CV = coefficient of variation; R = variation range as the difference between the minimum and maximum value of the data distribution; Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits; p >0.05 = no statistically significant difference between groups.

Table 9 Korelation relation (r_{xy}) between lysine and methionine in breast and thigh muscles of broiler chickens.

Group		Breast muscle	Thigh muscle
		Lysine	
Control	**Methionine**	0.98^{+++}	0.93^{++}
Experimental		0.95^{+}	0.82^{++}

Note: numerical value is result of correlation coefficients (rxy) between two variables;
Control = diclazuril and salinomycin sodium; Experimental = active substances obtained mainly from citrus fruits;
$^{+++}$, $^{++}$, $^{+}$ = Difference between groups means is statistically significant (p <0.001, p <0.01, p <0.05).

muscle.

Average protein content in thigh muscle was found 22.10 g.100 g^{-1} by effect of active substances obtained mainly from citrus fruits and compared to slightly higher protein content 22.44 g.100 g^{-1} in control group with diclazuril and salinomycin sodium. The results of SD and %CV for protein content in the thigh muscle by effect of active substances obtained mainly from citrus fruits varied more compared to calculated results of SD and %CV for protein content in the thigh muscle of control group with diclazuril and salinomycin sodium.

Difference between groups was not statistically significant (p >0.05). Proteins are a major component of muscle tissue, which affects their nutritional value, functional properties and sensory properties **(Lawrie, 1998)**.

Lysine content in the breast and thigh muscles

Average lysine content in the breast and thigh muscles is given in Table 7.

Average lysine content in breast muscle was found 2.42 g.100 g^{-1} by effect of active substances obtained mainly from citrus fruits and compared to slightly lower fat content 2.16 g.100 g^{-1} in control group with diclazuril and salinomycin sodium. The values of SD and %CV for lysine content in the breast muscle by effect of active substances obtained mainly from citrus fruits varied more compared to calculated results of SD and %CV for lysine content in the breast muscle of control group with diclazuril and salinomycin sodium.

Difference between groups was not statistically significant (p >0.05).

The measured lysine content in the thigh muscle was tendentiously higher 2.24 g.100 g^{-1} by effect of active substances obtained from citrus fruits in the feed mixtures versus lower lysine content 2.16 g.100 g^{-1} in the thigh musle by effect of control feed mixtures with diclazuril and salinomycin sodium.

The values of SD and %CV for lysine content in the thigh muscle by effect of active substances obtained mainly from citrus fruits varied more compared to calculated results of SD and %CV for lysine content in the thigh muscle of control group with diclazuril and salinomycin sodium.

Difference between groups was not statistically significant (p >0.05).

Protein rich in essential amino acids are the most important component of poultry meat **(Straková et al., 2002)**.

Methionine content in the breast and thigh muscles

Average methionine content in the breast and thigh muscles is given in Table 8.

Methionine has important function in the broiler chickens. It participates in the depositing of breast muscles of broiler chickens. This knowledge was confirmed in an experiment. Studied object was methionine and its impact on the improvement of the production and deposition breast muscle in broiler chickens hatched with lower body weight **(Zhai et al., 2012)**. The average methionine content in the breast and thigh muscles were 0.89 g.100 g^{-1} in the breast muscle and 0.84 g.100 g^{-1} in the thigh muscle by effect of active substances obtained from citrus fruits in the feed mixtures compared to methionine content 0.79 g.100 g^{-1} in the breast musle and 0.74 g.100 g^{-1} in the thigh muscle by effect of control feed mixtures with diclazuril and salinomycin sodium.

The values of SD and %CV for methionine content in the breast and thigh muscles varied nearly the same by effect of active substances obtained mainly from citrus fruits versus diclazuril and salinomycin sodium.

Difference between groups was not statistically significant (p >0.05). Chemical composition of breast musle differs from the chemical composition of thigh muscle **(Straková et al., 2002)**.

Correlation between lysine and methionine in breast and thigh muscles of broiler chickens

Correlation between lysine and methionine in breast and thigh muscles of broiler chickens is given in Table 9.

Methionine and lysine are probably the most studied amino acids in nutrition of broiler chickens. However, the relation between methionine and lysine has not been extensively investigated **(Cafe and Waldroup, 2006)**. The results investigation a methionine and lysine showed no interactions between these amino acids **Si et al. (2004)**. The increase of dietary lysine and methionine can reduce abdominal fat, improve feed conversion ratio, breast muscle yield, carcass efficiency (economical traits) in broiler chickens. It is suggested that amount of lysine and methionine in higher levels of NRC (1994) recommendations may result an increase of economical trait, performance in the broiler chickens **(Bouyeh and Gevorgyan, 2016)**.

In our experiment were composed the feed mixtures with same nutrient and energy contents for experimental and control group. Lysine and methionine contents as well as methionine/lysine ratio were balanced in dependence to growth phase, i.e. Starter, Grower 1, Grower 2 and Final. We focused on an investigation of correlation between lysine and methionine in the breast and thigh muscles in dependence on feed experimental active substances obtained mainly from citrus fruits and control diclazuril and salinomycin sodium. Strong positive dependence, statistically significant (p <0.001, p <0.01, p <0.05), was found between lysine and methionine in the breast muscle as well as in the thigh muscle.

CONCLUSION

Based on achieved results of our experiment with broiler chickens Cobb 500, it can be alleged that experimental active substances obtained mainly from citrus fruits and control diclazuril and salinomycin sodium had comparable influence on protein guality of chicken meat. Differences were reflected on the productive efficiency of feed and performance of broiler chickens.

Active substances obtained mainly from citrus fruits confirmed a statistically significant (p <0.05) effect on the body weight gain of broiler chickens, compared with effect of diclasuril and salinomycin sodium in control group.

An effect of active substances obtained mainly from citrus fruits tended to:

a) slightly increase feed intake per bird, protein, energy, lysine and methionine intake per total body weight gain and bird,

b) slightly decrease feed intake per 1 kg of body weight gain, protein, energy, lysine and methionine intake per 1 kg of body weight gain,

c) slightly increase protein efficiency ratio and energy efficiency ratio.

Active substances obtained mainly from citrus fruits in the feed mixtures did not have a statistically significant effect on protein, lysine and methionine contents in the breast and thigh muscles.

Active substances obtained mainly from citrus fruits and likewise also coccidiostatats diclazuril and salinomycin sodium displayed a strong positive, statistically significant relation between lysine and methionine in the breast and thigh muscles.

We can allege in conclusion that active substances obtained mainly from citrus fruits have shown a tendency to increase productive efficiency of feed mixtures in the broiler chickens, which was reflected in an increase in total body weight gain in the unaltered protein, lysine and methionine contents in breast and thigh muscles.

REFERENCES

Adbalqdadir, M. O., Arabi, S. A. 2014. The effects of different lysine and methionine levels on broiler chickens performance. *International Journal of Innovative Research,* vol. 2, no. 4, p. 46-52.

Anagnostopoulou, M. A., Kefalas, P., Papageorgiou, V. P., Assimopoulou, A. N., Boskou, D. 2006. Radical scavenging activity of various extracts and fractions of sweet orange flavedo (*Citrus sinensis*). *Food Chemistry*, vol. 94, no. 1, p. 19-25. https://doi.org/10.1016/j.foodchem.2004.09.047

Angelovičová, M., Kunová, S., Čapla, J., Zajac, P., Bučko, O., Angelovič, M. 2016. Comparison of fatty acid profile in the chicken meat after feeding with narasin, nicarbazin and salinomycin sodium and phyto-additive substances. *Journal of Environmental Science and Health*, *Part B,* vol. 51, no. 6, p. 374-382. https://doi.org/10.1080/03601234.2016.1142320 PMid:26950416

Arthington, J. D., Kunkle, W. E., Martin, A. M. 2002. Citrus pulp for cattle. *Veterinary Clinics of North America*: *Food Animal*, vol. 1, no. 2, p. 317-326. https://doi.org/10.1016/S0749-0720(02)00023-3

Azza, M. K., Naela, M. R. 2014. Effect of Dietary Supplementation of Organic Acids on Performance and Serum Biochemistry of Broiler Chicken. *Science of Nature*, vol. 12, no. 2, p. 38-45.

Baker, D. H. 2009. Advances in protein - amino acid nutrition of poultry. *Amino Acids*, vol. 37, no. 1, p. 29-41. https://doi.org/10.1007/s00726-008-0198-3 PMid:19009229

Bigot, K., Mignon-Grasteau, S., Picard, M., Tesseraud, S. 2003. Effects of delayed feed intake on body, intestine, and muscle development in neonate broilers. *Poultry Science*, vol. 82, no. 5, p. 781-788. https://doi.org/10.1093/ps/82.5.781 PMid:12762401

Bokkers, E. A. M., Koene, P. 2003. Eating behavior, and preprandial and postprandial correlations in male broiler and layer chickens. *British Journal of Applied Poultry Research*, vol. 44, no. 4, p. 538-544. https://doi.org/10.1080/00071660310001616165 PMid:14584843

Borum, P. R. 1983. Carnitine. *Annual Review of Nutrition*, vol. 3, no. 1, p. 233-259. https://doi.org/10.1146/annurev.nu.03.070183.001313 PMid:6357236

Bouyeh, M., Gevorgyan, O. K. 2016. Effects of dietary lysine and methionine in broilers. *Iranian Journal of Applied Animal Research*, vol. 6, no. 4, p. 917-923.

Bunchasak, Ch. 2009. Role of dietary methionine in poultry production. *Journal of Poultry Science*, vol. 46, no. 3, p. 169-179. https://doi.org/10.2141/jpsa.46.169

Café, M. B., Waldroup, P. W. 2006. Interactions between levels of methionine and lysine in broiler diets changed at typical industry intervals. *International Journal of Poultry Science*, vol. 5, no. 11, p. 1008-1015. https://doi.org/10.3923/ijps.2006.1008.1015

Chavez, C., Coufal, C. D., Lacey, R. E., Carey, J. B. 2004. The impact of methionine source on poultry fecal matter odor volatiles. *Poultry Science*, vol. 83, no. 10, p. 359-364. https://doi.org/10.1093/ps/83.3.359 PMid:15049487

Chuaynukool, K., Wattanachant, S., Siripongvutikorn, S. 2007. Chemical and properties of raw and cooked spent hen, broiler and Thai indigenous chicken muscles in mixed herbs

acidified soup (Tom Yum). *Journal of Food Science and Technology*, vol. 5, no. 2, p. 180-186.

Council Directive 2007/43/EC. Council Directive 2007/43/EC laying down minimum rules for the protection of chickens kept for meat production. OJ L 182, 12.7.2007, p. 19-28.

Cross, D. E., McDevitt, R. M., Hillman, K., Acamovic, T. 2007. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. *British Poultry Science*, vol. 48, no. 4, p. 496-506. https://doi.org/10.1080/00071660701463221 PMid:17701503

Dozier III, W. A., Kidd, M. T., Corzo, A., Anderson, J., Branton, S. L. 2006. Growth performance, meat yield, and economic responses of broilers provided diets varying in amino acid density from thirty-six to fifty-nine days of age. *Journal of Applied Poultry Research*, vol. 15, no. 3, p. 383-393. https://doi.org/10.1093/japr/15.3.383

Dozier III, W. A., Corzo, A., Kidd, M. T., Tillman, P. B., Branton, S. L. 2009. Digestible Lysine Requirements of Male and Female Broilers from Fourteen to Twenty-Eight Days of Age. *Poultry Science*, vol. 88, no. 8, p. 1676-1682. https://doi.org/10.3382/ps.2008-00539 PMid:19590083

Emmert, J. L., Baker, D. H. 1997. Use of the ideal protein concept for precision formulation of amino acid levels in broiler diets. *Journal of Applied Poultry Research*, vol. 6, no. 4, p. 462-470. https://doi.org/10.1093/japr/6.4.462

Epifano, E., Genovese, S. 2013. Recent acquisitions on naturally occurring oxyprenylated secondary plant metabolites. In Brahmachari, G. *Chemistry and Pharmacology of Naturally Occurring Bioactive Compounds*. Boca Raton, USA : CRC Press. p. 239-257. ISBN 9781439891674.

Fancher, B. I., Jensen, L. S. 1989. Influence on performance of three to six weeks old broilers of varying dietary protein contents with supplementation of essential amino acid requirements. *Poultry Science*, vol. 68, no. 1, p. 113-123. https://doi.org/10.3382/ps.0680113 PMid:2704667

Faria, F. D. E., Rosa, P. S., Viera, B. S., Macari, M., Furlan, R. L. 2005. Protein levels and environmental temperature effects on carcass characteristics, performance, and nitrogen excretion of broiler chickens from 7 to 21 days of age. *Brazilian Journal of Poultry Science*, vol. 7, no. 4, p. 247-253. https://doi.org/10.1590/S1516-635X2005000400009

Genovese, S., Fiorito, S., Locatelli, M., Carlucci, G., Epifano, F. 2014. Analysis of biologically active oxyprenylated ferulic acid derivatives in citrus fruits. *Plant Foods for Human Nutrition*, vol. 69, no. 3, p. 255-260. https://doi.org/10.1007/s11130-014-0427-8 PMid:24928688

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 Chemical Toxicology*, vol. 48, no. 1, p. 99-106. https://doi.org/10.1016/j.fct.2009.09.022 PMid:19770018

Hargis, P. H., Creger, C. R. 1980. Effects of varying dietary protein and energy levels on growth rate and body fat in broilers. *Poultry Science*, vol. 5, no. 7, p. 1499-1504. https://doi.org/10.3382/ps.0591499

Harper, A. E., Benevenga, N. J., Wohlhueter, R. M. 1970. Efects of ingestion of disproportionate amounts of amino acids. *Physiology Reviews*, vol. 50, no. 3, p. 428-558. https://doi.org/10.1152/physrev.1970.50.3.428 PMid:4912906

Haščík, P., Kačániová, M., Čuboň, J., Bobko, M., Nováková, I., Vavrišinová, K., Arpášová, H., Mihok, M. 2009. Aplication *of Lactobacillus fermentum* and its efect on chemical composition of ROSS PM3 chicken meat. *Acta fytotechnica et zootechnica,* vol. 12, p. 197-205.

Hossain, M. A., Islam, A. F., Iji, P. A. 2012. Energy utilization and performance of broiler chickens raised on diets with vegetable proteins or conventional feeds. *Asian Journal of Poultry Science*, vol., 6, no. 4, p. 117-128. https://doi.org/10.3923/ajpsaj.2012.117.128

Hossain, M. A., Islam, A. F., Iji, P. A. 2013. Growth responses, excreta quality, nutrient digestibility, bone development and meat yield traits of broiler chickens fed on vegetable or animal protein diets. *South African Society for Animal Science*, vol. 43, no. 2, p. 208-218. https://doi.org/10.4314/sajas.v43i2.11

Iji, P. A., Saki, A., Tivey, D. R. 2001a. Body and intestinal growth of broiler chicks on a commercial starter diet. 1. Intestinal weight and mucosal development. *British Poultry Science*, vol. 42, no. 4, p. 505-513. https://doi.org/10.1080/00071660120073151 PMid:11572627

Iji, P. A., Saki, A., Tivey, D. R. 2001b. Body and intestinal growth of broiler chicks on a commercial starter diet. 2. Development and characteristics of intestinal enzymes. *British Poultry Science*, vol. 42, no. 4, p. 514-522. https://doi.org/10.1080/00071660120073142 PMid:11572628

Ionescu, A., Aprodu, I., Alexe, P. 2009. *General Technologies – Technology and Control in Industria meat (Tehnologii generale – Tehnologie si control în industria cărnii)*. Galaţi, Turky : Galaţi University Press. p. 123.

Jang, I. S., Ko, Y. H., Kang, S. Y., Lee, C. Y. 2007. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. *Animal Feed Science and Technology*, vol. 134, no. 3-4, p. 304-315. https://doi.org/10.1016/j.anifeedsci.2006.06.009

Langhout, P. 2000. New additives for broiler chickens. *Feed Mix*, vol. 18, no. 6, p. 24-27.

Lawrie, R. A., Ledward, D. A. 2006. *Lawrie's meat science*. 7th ed. Abington Cambridge, UK : Woodhead Publishing Limited. p. 75-126. ISBN-13: 978-1845691592.

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. https://doi.org/10.1080/0007166031000085508 PMid:12964629

Lillehoj, H. S., Lee, K. W. 2012. Immune modulation of innate immunity as alternatives-to-antibiotics strategies to mitigate the use of drugs in poultry production. *Poultry Science*, vol. 91, no. 6, p. 1286-1291. https://doi.org/10.3382/ps.2012-02374 PMid:22582284

Lopez, G., Leeson, L. 2005. Utilization of metabolisable energy by young broilers and birds of intermediate growth rate. *Poultry Science*, vol. 84, no. 7, p. 1069-1076. https://doi.org/10.1093/ps/84.7.1069 PMid:16050124

Lopez, G., Leeson, S. 2008. Energy partitioning in broiler chickens: a review. *Canadian Journal of Animal Science*, vol. 8, no. 2, p. 205-212. https://doi.org/10.4141/CJAS07087

Manthey, J. A., Grohmann, K. 2001. Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *Journal of Agricultural and Food Chemistry*, vol. 49, no. 7, p. 3268-3273. https://doi.org/10.1021/jf010011r PMid:11453761

Manzanilla, E. G., Perez, J. F., Kamel, C., Baucells, F., Gasa, J. 2004. Effect of plant extracts and formic acid on the intestinal equilibrium of early-weaned pigs. *Journal of Animal Science*, vol. 82, no. Suppl. 1, p. 3210-3218.

Mbajiorgu, C. A., Ng'ambi, J. W., Norris, D. 2011. Effect of varying dietary energy to protein ratio level on growth and productivity of indigenous Venda chickens. *Asian Journal* of *Animal and Veterinary Advances*, vol. 6, no. 4, p. 344-352. https://doi.org/10.3923/ajava.2011.344.352

Medveď, J., Angelovičová, M. 2010. Protein and fat in breast muscules of broilers in application welfare principles in practical conditions. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 4, no. 3, p. 50-52. https://doi.org/10.5219/66

Močár, K., Štofan, D., Angelovičová, M., Kačániová, M. 2012. *The use of origanum aetheroleum and probiotics in the chicken meat production.* Nitra, Slovakia : Slovak University of Agriculture. p. 161.

Montanari, A., Chen, J., Widmer, W. 1998. Citrus flavonoids: a review of past biological activity against disease. In Manthey, J. A., Buslig, B. S. *Flavonoids in the Living System.* New York, USA : Plenum Press. p. 103-113. https://doi.org/10.1007/978-1-4615-5335-9_8

Moran, E. T. 1985. Digestion and absorption of carbohydrates in fowl and events through perinatal development. *Journal of Nutrition*, vol. 115, no. 5, p. 665-674. https://doi.org/10.1093/jn/115.5.665 PMid:2582103

Munakata, R., Inoue, T., Koeduka, T., Sasaki, K., Tsurumaru, Y., Sugiyama, A., Uto, Y., Hori, H., Azuma, J., Yazaki. K. 2012. Characterization of coumarin-specific prenyltransferase activities in Citrus limon peel. *Bioscience*, *Biotechnology*, *and Biochemistry*, vol. 76, no. 7, p. 1389-1393. https://doi.org/10.1271/bbb.120192 PMid:22785469

Nahashon, S. N., Adefope, N., Amenyenu, A., Wright, D. 2005. Effect of dietary metabolisable energy and crude protein concentrations on growth performance and carcass characteristics of French guinea fowl broilers. *Poultry Science*, vol. 84, no. 2, p. 337-344. https://doi.org/10.1093/ps/84.2.337 PMid:15742972

NRC. 1994. National Research Council. 1994. Nutrient requirements of poultry. 9th ed. Washington, DC., USA : Academies Press. p. 1-155. ISBN 978-0-309-04892-7.

Oviedo-Rondon, E. O., Waldroup, P. W. 2002. Models to estimate amino acid requirements for broiler chickens: A review. *International Journal of Poultry Science*, vol. 1, no. 5, p. 106-113. https://doi.org/10.3923/ijps.2002.106.113

Rafiq, S., Kaul, R., Sofi, S. A., Bashir, N., Nazir, F., Gulzar Ahmad Nayik, G. A. 2016. Citrus peel as a source of functional ingredient: a review. *Journal of the Saudi Society* of *Agricultural Sciences.* (IN PRESS) https://doi.org/10.1016/j.jssas.2016.07.006

Rezaeipour, V., Aghajan Nejad, O. A., Miri, H. I. 2014. Growth performance, blood metabolites and jejunum morphology of broiler chickens fed diets containing earthworm (*Eisenia foetida*) meal as a source of protein. *International Journal of Biomedical and Advance Research,* vol. 2, 8, p. 483-2494.

Rostagno, H. S., Pupa, J. M. R., Pack, M. 1995. Diet formulation for broilers based on total versus digestible amino acid. *Journal of Applied Poultry Research*, vol. 4, no. 3, p. 293-299. https://doi.org/10.1093/japr/4.3.293

Sakomura, N. K., Longo, F. A., Oviedo-Rondon, E. O., Boa-Viagem, C., Ferraudo, A. 2005. Modelling energy utilisation and growth parameter description for broiler chickens. *Poultry Science*, vol. 84, no. 9, p. 1363-1369. https://doi.org/10.1093/ps/84.9.1363 PMid:16206556

Samadi, F., Liebert, F. 2007. Lysine requirement of fast growing chickens: effects of age, sex, level of protein deposition and dietary lysine efficiency. *Journal of Poultry Science*, vol. 44, no. 1, p. 63-72. https://doi.org/10.2141/jpsa.44.63

Shatnawi, K. 2014. Investigation of energy partitioning in modern broiler chickens : dissertation theses in Poultry nutrition. Palmerston North, New Zealand : Massey University. p. 218.

Si, J., Kersey, J. H., Fritts, C. A., Waldroup, P. W. 2004. An evaluation of the interaction of lysine and methionine in diets for growing broilers. *International Journal of Poultry Science*, vol. 3, no. 1, p. 51-60. https://doi.org/10.3923/ijps.2004.51.60

Sterling, K. G., Pesti, G. M., Bakalli, R. I. 2006. Performance of different broiler genotypes fed diets with varying levels of dietary crude protein and lysine. *Poultry Science*, vol. 85, no. 6, p. 1045-1054. https://doi.org/10.1093/ps/85.6.1045 PMid:16776474

Straková, E., Jelínek, P., Suchý, P., Antonínová, M. 2002. Spectrum of amino acids in muscles of hybrid broilers during prolonged feeding. *Czech Journal of Animal Science*, vol. 47, no. 12, p. 519-526.

Tesseraud, S., Bouvarel, I., Collin, A., Audouin, E., Crochet, S., Seiliez, I., Leterrier, C. 2009. Daily variations in dietary lysine content alter the expression of genes related to proteolysis in chicken pectoralis major muscle. *Journal of Nutrition*, vol. 139, no. 1, p. 38-43. https://doi.org/10.3945/jn.108.095752 PMid:19056657

Veldkamp, T., Kwakkel, R. P., Ferket, P. R., Verstegen, M. W. A. 2005. Growth responses to dietary energy and lysine at high and low ambient temperature in male Turkeys. *Poultry Science*, vol. 84, no. 2, p. 273-282. https://doi.org/10.1093/ps/84.2.273 PMid:15742964

Wang, L., Wang, J., Fang, L., Zheng, Z., Dexian, Z., Wang, S., Li, S., Ho, C. T., Zhao, H. 2014. Anticancer activities of citrus peel polymethoxyflavones related to angiogenesis and others. *BioMed Research International*, vol. 2014, p. 453972. https://doi.org/10.1155/2014/453972 PMid:25250322

Zarate, A., Moran, E., Burnham, D. 2003. Reducing crude protein and increasing limiting essential amino acid levels with summer-reared, slow-and fast-feathering broilers. *Journal of Applied Poultry Research*, vol. 12, no. 2, p. 160-168. https://doi.org/10.1093/japr/12.2.160

Zhai, W., Araujo, L. F., Burgess, S. C., Cooksey, A. M., Pendarvis, K., Mercier, Y., Corzo, A. 2012. Protein expression in pectoral skeletal muscle of chickens as influenced by dietary methionine. *Poultry Science*, vol. 91, no. 10, p. 2548-2555. https://doi.org/10.3382/ps.2012-02213 PMid:22991541

Zulkifli, I., Imanrahayu, H. S., Alimon, A. R., Vidyadaran, M. K., Babjee, S. A. 2001. Responses of choice-fed red jungle fowl and commercial broiler chickens offered a complete diet, corn and soybean. *Asian Australasian Journal of Animal Sciences*, vol. 14, no. 12, p. 1758-1762. https://doi.org/10.5713/ajas.2001.1758

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MODELING OF UREASE THERMAL INACTIVATION PROCESSES IN SOYBEAN AT HIGH-TEMPERATURE MICRONIZATION

Sergey Zverev, Otari Sesikashvili

ABSTRACT

The use of soybean, in particular in forage production without preliminary heat treatment is not appropriate, and sometimes dangerous, because of the presence of antinutrients. As a marker in assessing safety of cakes and meals, there is often used urease in forage production. This paper describes the results of thermal inactivation of urease in soybean during the process of high-temperature micronization (heating of grain in the flux of infrared radiation). There have been obtained the empirical dependencies of the degree of its inactivation on time of heat treatment and energy exposure (the product of irradiation by the time of treatment). The similar dependences of urease activity on grain temperature are invariant to infrared heating (irradiation and time) regimes, but their nature is affected by the initial moisture content. The paper proposes the models of inactivation of antinutrients based on of the first-order equations of chemical kinetics with the reaction rate constant in various forms (Arrhenius and Hinshelwood, the transition state theory). The models have been tested on literature data on the inactivation of a trypsin inhibitor at a constant temperature. The models are further refined taking into account the variable (increasing) temperature and are reduced to the simplest form: $Y = k\,[\mathrm{Exp}\,(-\varepsilon_R/T) - T_0\,\mathrm{exp}\,(-\varepsilon_R/T_0)]$, where T, T_0 – are the current and initial temperatures of grain, k, ε_R – the empirical coefficients. The identification of the model coefficients was carried out based on the results of inactivation of urease during heating in the flux of infrared radiation. It has been established that the results of thermal inactivation of soybean do not depend on the IR processing regimes and are determined only by the initial moisture content of grain, and by the end heating temperature. The efficiency of inactivation is higher the higher is the used irradiation. There is a compensating effect - with the growth in one coefficient, another is also increased. The considered models can be used for the thermal degradation processes and other thermolabile substances.

Keywords: Soybean; urease; trypsin inhibitor; thermal inactivation; infrared heat treatment

INTRODUCTION

The use of native soybean, for example, in forage production, is not rational, and in some cases is dangerous because of the presence of antinutrients in it **(Zverev, Sesikashvili and Bulakh, 2013)**. Raw soybeans contain a number of antinutrients complicating the digestive process, which, however, can be inactivated by heat treatment. In particular, soyabean is characterized by high activity of inhibitors of digestive proteinases, and primarily of trypsin, which is responsible for protein digestion. In addition, among undesirable enzymes there are singled out:

- Lipoxygenase – an enzyme oxidizing the lipids. In addition, under its action, during continuous seed storage, there are created in them aldehydes and ketones, which impart soyabean a specific unpleasant odor and taste. As a result, the food benefits of soybean are thereby reduced.
- Urease — an enzyme, which performs hydrolytic cleavage of urea with the formation of ammonia and carbon dioxide. The level of its activity is important only for milk cattle breeding, when using soybean in feeds containing urea, since the interaction of urease with urea of feeds results in ammonia formation, which poisons the animal's body. In the initial soybean seeds, the share of urease may reach 6% from the amount of all proteins.

Since these undesirable components are thermolabile substances, heat treatment is the available and relatively inexpensive way to inactivate them **(Perez-Maldonado, Mannion and Farell, 2002)**. The listed substances have different degrees of heat resistance. The available data show that during the treatment of soybeans for 15 minutes, complete inactivation of lipoxygenase was observed at a temperature of 80 °C, of urease – at 90 °C, and of a trypsin inhibitor – at 100 °C. Similar results for

Table 1 Residual activity of antinutrients of soybean after the isothermical heat treatment.

Antinutrients	Heat treatment temperature, °C			
	60	65	70	75
Lipoxygenase	1	0.25	0	
Urease	1	0.8	0.53	0
Trypsin inhibitor	1	0.94	0.66	0

long (1 hour) heat treatment are given in Table 1 **(Reshetnik, 2007)**.

As can be seen, lipoxygenase is the least stable, the values of thermal stability of a trypsin inhibitor and urease are comparable and closely correlated, but the activity of urease is evaluated by much simpler method, therefore it is often used as a marker **(Erickson, 2002)**. Moreover, the activity of urease is rationed in soya cakes and meals intended for feed production **(Ruis, 2013)**.

The most commonly used model of thermal degradation is the differential equation well known in chemical kinetics in the form as follows **(Romanovsky, 2006)**:

$$Y = - K[T(t)] Y^n dt \quad (1)$$

where Y – quantitative measure of a reagent content; T(t) – the absolute temperature; t – time, K[T(t)] – reaction rate constant; n – reaction order.

We note that such an equation describes not only the processes in chemical reactions, but also, for example, the thermal inactivation of enzymes, bacteria, and so on.

The reaction rate constant in the generalized form can be represented as follows:

$$K[T(t)] = kT^m \exp(\frac{-\varepsilon}{RT}) \quad (2)$$

where k – coefficient of proportionality, c^{-1}; ε – energy of activation, J/mole; R=8.314 – universal gas constant, J/(mole K); T –temperature, K; m – coefficient.

Depending on the physical concepts of the mechanism of intermolecular interaction in the process of thermal degradation in various theories, m accepts value m = 0 (Arrhenius), m = 0.5 (kinetic theory of gases), m = 1 (transient state theory), m = -1 (Hinshelwood). The reaction rate constant in the Arrhenius form is most often used. The use of other forms somewhat changes the coefficients k and ε for their identification but does not significantly affect the accuracy of the approximation of the experimental data.

After the substitution of (2) for (1), separation of variables and integration, we obtain:

$$F(Y) = \int_{Y_0}^{Y} \frac{dY}{Y^n} = k \int_0^t T^m \exp(\frac{-\varepsilon}{RT}) dt \quad (3)$$

where Y, Y_0 – quantitative measures of the reagent content at the current and starting points in time.

In general,

$$F(Y) = \int_{Y_0}^{Y} \frac{dY}{Y^n} = Y^{n-1} - Y_0^{n-1}, \quad n \neq 1,$$

$$F(Y) = \ln(\frac{Y}{Y_0}), \quad n=1.$$

In the case of a constant temperature T = const, solving the equations (3) is not difficult:

$$F(Y) = -kT^m \exp\left(\frac{-\varepsilon}{RT}\right) t \quad (4)$$

Table 2 The values of the coefficients of a model (4) for some substances, when n = 1 and the reaction rate constant in the Arrhenius form (m = 0).

Substance	ε, kJ.mol^{-1}	k, sec^{-1}
Vitamins		
Folic acid (B_9)	70.3	2.10^{10}
Cyanocobalamine (B_{12})	17.2	10^{11}
Enzymes		
Malt amylase	177.1	10^{24}
Lipase		
– dry	104.7	10^{10}
– humid	192.6	10^{28}
Trypsin inhibitor	108.8	10^{12}

Taking the logarithm and subsequent transformations of the expression (4) lead to a nonlinear model:

$$y = k_0 + \varepsilon_R /T + \ln(t) + m \ln(T) \quad (5)$$

where $y = \ln[- F(Y)]$, $k_0 = \ln[k]$, $\varepsilon_R = - \varepsilon /R$,

When m = 0 (the kinetic coefficient is in the form of the Arrhenius), the model becomes linear and for the identification of coefficients it is possible to use the method of linear regression analysis. In this form, it describes the results of the experiments on inactivation of antinutrients of soybean **(Chen et al., 2014; Kargov et al., 2015)**.

In the contrary case, the nonlinear regression analysis methods are used, including also directly in respect to (4). By this means, the expression (4) allows us for evaluating thermal degradation of the object under isothermal conditions with the known coefficients k, m and ε. The values of the coefficients can be obtained as a result of their identification by the results of the experiments. For fixed n and m and involving the empirical data for the dependence Y (T, t = const), in the models (4) and (5), it is possible to identify the coefficients ε and k.

The models of thermal degradation of biorefinery of grain are of theoretical and practical interest. A series of evaluation of the model coefficients reported in the literature or obtained by processing the results of the described experiments, are given in Table. 2.

Table 3 The values of the parameters of a model (4), when n = 1.

Model coefficients				
m	**k**	**ε, kJ.mol^{-1}**	**ε_R, K**	**Squared multiple correlation, R^2**
1	7.327×10^{8} K^{-1} s^{-1}	103.8	12483	
0	7.754×10^{11} s^{-1}	107.0	12872	0.99
-1	8.205×10^{14} K s^{-1}	110.2	13261	

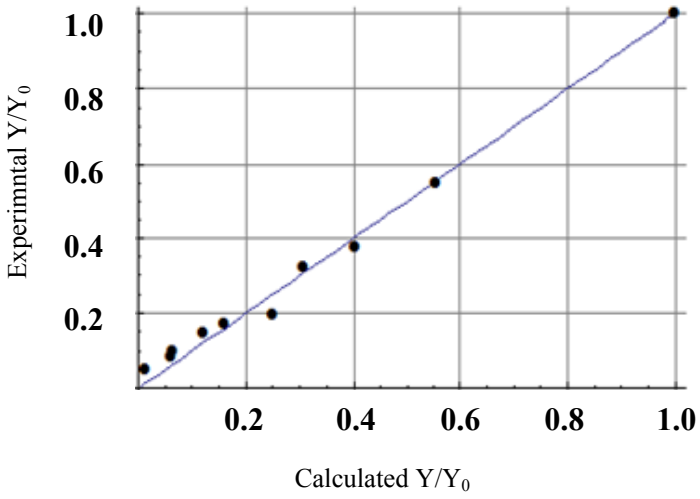

Figure 1 The results of the experiment and calculation on a model (4), when m = 1.

The values of the coefficients depend substantially on external factors, for example, with increased humidity, the parameters are also increased, as is the case with the example of lipase. Environment acidity plays an important role.

Let's consider the process of thermal degradation of a trypsin inhibitor in soybean **(Egorov et at., 1986)**. One of such methods of inactivation is autoclaving, for which, in a given paper, there are presented the experimental data on residual activity a trypsin inhibitor with variation of time, temperature and pressure within the ranges of 0 <t <900, c, 383 <T <398, K, 0.14 <P <0.23, MPa. Unfortunately, the experiment is not set up correctly, since the pressure also changed with the temperature, however, it is difficult to avoid this with this method of heat treatment. Therefore, the effect of pressure in explicit form was not taken into account, howe ver, it indirectly affects the energy of activation of the process, lowering it to some extent.

As a result of the identification, there have been obtained the values of the coefficients presented in Table 3, which,

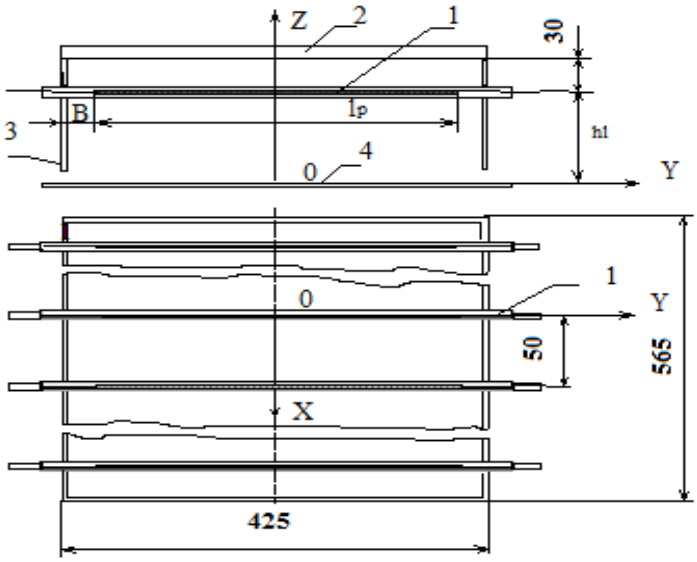

Figure 2 Diagram of the experimental-industrial block of the emitters: 1. The KGT-1000-220-type infrared source; 2 – Top reflector; 3 – Side reflector; 4 – A working surface of the processing zone.

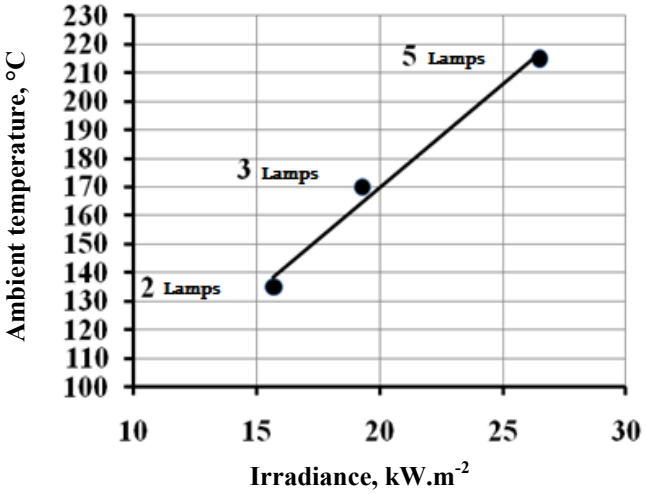

Figure 3 The dependency diagram of the ambient temperature in the treatment zone on the irradiance.

although they differ from each other, but, taking into account the activating influence of pressure, they fairly well correspond to the data of Table. 2.

Figure 1 graphically represents estimated and experimental data corresponding to the model (4).

We note that the obtained values of the coefficients are close to the values presented in Table 1 for a trypsin inhibitor, but they are applicable to the autoclaving operation, and how they correspond to other heat treatment methods, can be demonstrated only by the comparative experiments. The use of the reaction rate constants in the various forms (m = -1; 0; 1) leads to some change in the identified parameters (mostly of the coefficient of proportionality) but does not affect the accuracy of the approximation.

Scientific hypothesis

An essential part of antinutrients in soybean is a trypsin inhibitor – a substance of protein nature. At elevated temperatures, its activity decreases due to the destruction of its structure. In cakes, such inactivation is provided by toasting. Similar results can be expected for soybean grain when heating in a flux of infrared radiation. As a model of the inactivation process, it is expedient to test the modified Arrhenius equation taking into account the variable product temperature. The similar dependences on energy exposure are shown in Figure 5.

MATERIAL AND METHODOLOGY

High-temperature micronization (HTM) is the operation of heat treatment of product in the flux of infrared (IR) radiation **(Zverev, 2009)**. Heat transfer is carried out in two ways: convective from the air in the treatment zone, and radiative (IR radiation). Therefore, we can talk about the combined heat supply.

The industrial installations based on this principle of heating are used in small and medium-sized grain processing enterprises in the processes of the production of instant cereal, cereal flakes, feed ingredients, including for disinfection and inactivation of antinutrients. The heating process, as a rule, is carried out with a high thermal head and is limited by the time of the beginning of the browning of the grain surface. The temperature of product varies continuously throughout the entire treatment period, that is, the process is substantially non-isothermal.

The experiments on IR heating of soybean were carried out on a laboratory facility with the quartz halogen linear infrared emitters of the KGT-1000-220 type.

Diagram of a facility is given in Figure 2.

The experiments were carried out with a fixed height of the lamp h_l = 100 mm. Variation with irradiation was carried out by changing the number of lamps per unit area. Change in the number of lamps, that is, of the installed total power of the radiators in a fixed closed volume of the working area of a laboratory facility leads to increased ambient temperature. The graph of the interdependence of irradiance and ambient temperature in the treatment zone is given in Figure 3. The value of irradiation was estimated by a calculation according to a specially developed program, the activity of urease - according to State Standard GOST 13979.9-69 (Cakes and meals. Method – for performing measurements of urease activity), the initial moisture – according to State Standard GOST 13586.5-2015 (Grain. Method for determination of moisture content).

The soybean grain was placed in a monolayer on a pallet, which for a fixed time was placed in a heated treatment zone. Then, it was poured into a thermally insulated tank, where using a thermocouple and an electrnic thermometer, the average temperature of its mass was determined.

Statisic analysis

Nonlinear modeling was carried out by using an application software package “STADIA-6", developed at the M.V. Lomonosov Moscow State University, **(Kulaychev, 1999)**.

Scoping the adequacy of the models is a complex procedure, requiring high computational costs, which are rapidly growing with dimensions of space of external parameters. By the volume, this task may greatly exceed the task of parametric optimization of a model itself (especially in the case of a nonlinear model), that’s why for the newly-designed objects, it may not be resolved. Some indication of the adequacy of the models is provided by the Squared multiple correlation, R2. In addition,

directly in the diagrams we can see that the residual dispersion and the dispersion medium differ considerably.

RESULTS AND DISCUSSION

The independent variables for the IR heating of the soybean monolayer were the irradiance on the surface of the grain monolayer, the moisture content of the grain, and the processing time. Dependent variables are the temperature of grain and urease activity.

Figure 4 illustrates the empirical dependences of urease activity on the heat treatment time in the range of variation with the initial moisture content W0 = 8-30% for different irradiations on the surface of the monolayer of grain.

Similar dependences on the energy exposure (the product of irradiance for the processing time) are given in Figure 5.

Figure 6 illustrates the experimental data of urease activity depending on the soybean heating temperature with different initial humidity in a wide range of irradiation variation.

The dependences of the activity of urease in soya on time of heat treatment and energy exposure are invariant to its initial moisture content, but depend on the irradiation on the surface of a monolayer of grain. With the growth in the irradiation, with equal processing time or energy exposure, the degree of inactivation increases. It should be noted that during the process of the experimnet the irradiation was changing by increasing the number of IR emitters, which also led to a temperature riuse in the treatment zone.

That is, With the growth in the irradiation, the intensity of heat transfer was increased, including due to the growth in ambient temperature.

If we consider the dependence of urease activity on the temperature of product, they will be be invariant to the heating regimes, but depend on the initial humidity of grain.

As a model of the process of urease inactivation, we use the dependence (3). In the case of infrared heat treatment, the process proceeds in an unsteady period, that is, the temperature is a function of time, which, in this case, leads to the nonlinear dependencies. The identification of coefficients of such models according to experimental data is a much more complex task, although a number of packaged applications have already been developed. The reliability of the estimates is higher, the less is the number of the estimated parameters, and the more information is available. Often, the values of the parameters obtained, depend on the initial values, since the residual functions (in particular, the sum of the least squares) may have several minimum values. So, there is always the need to check the coefficient values obtained on their compliance with a common sense and with data from independent sources. For example, knowingly we have to abandon negative values if, from physical considerations, the value of the coefficient must be positive, and so on.

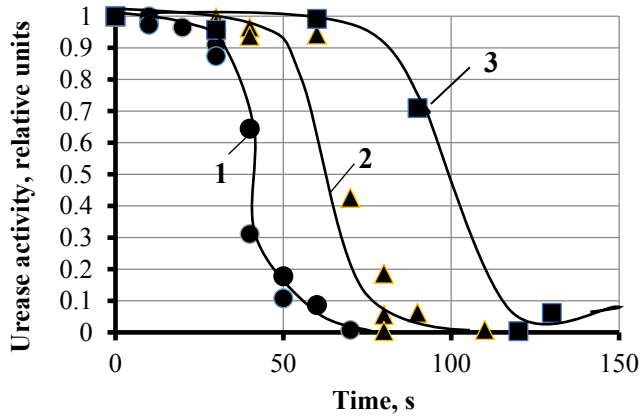

Figure 4 The dependence of urease activity in soybean (humidity 8 – 30%) on processing time with the irradiation as follows: 1 – 26.5 kW.m^{-2}; 2 – 19.3 kW.m^{-2}, 3 – 15 kW.m^{-2}.

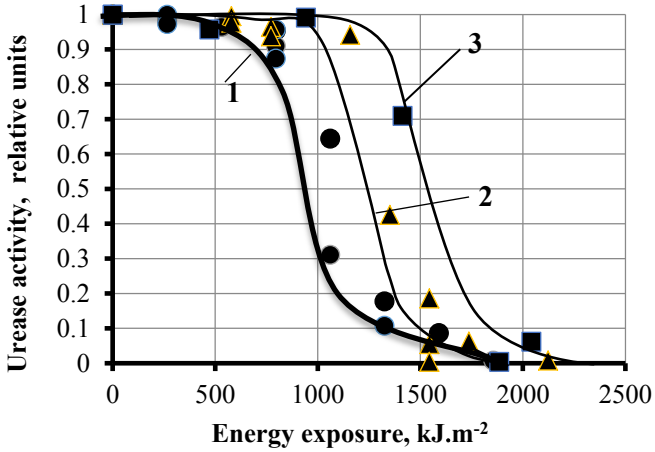

Figure 5 The dependence of urease activity in soybean (humidity 8-30%) on energy exposure with the irradiation as follows: 1 – 26.5 kW.m^{-2}; 2 – 19.3 kW.m^{-2}, 3 – 15 kW.m^{-2}.

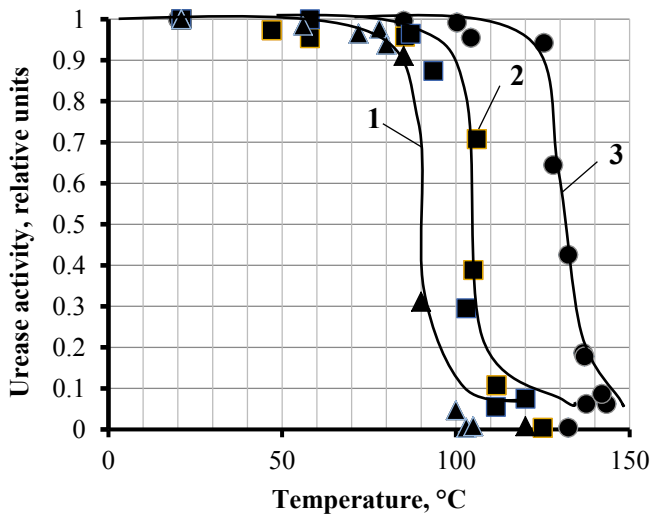

Figure 6 The dependence of urease activity in soybean on itys temperature (E = 15,7–26,5 kW.m^{-2}) with humidity: 1 – 30%; 2 – 17%, 3 – 8%.

Table 3 The values of the coefficients of the models (10) and (11), when C = ∞, n = 1.

Model	W, %	k	ε, kJ.mol^{-1}	ε_R, K	Squared multiple correlation, R^2
(10)	8	1.368×10^{16}	113	13541	0.93
	17	4.172×10^{22}	138	16603	0.99
	30	3.530×10^{86}	582	70378	0.99
(11)	8	2.460×10^{12} K^{-1}	117	14081	0.89
	17	2.384×10^{16} K^{-1}	138	16595	0.99
	30	4.966×10^{81} K^{-1}	585	70376	0.99

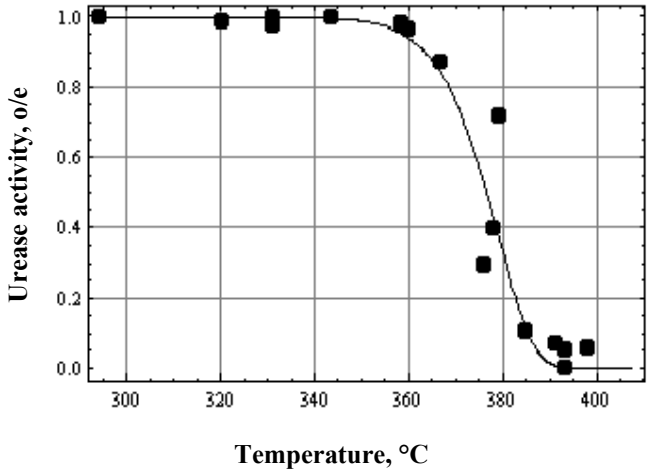

Figure 7 Experimental and calculating dependences of urease in soybean with the irradiation *E* = 15,7 – 26,5 kW.m^{-2} and humidity 17%.

The dependence of the temperature of product on time during IR heating is well described by the expression **(Egorov et al., 1986)**.

$$\Delta T(t) = \Delta T_{\infty}[1-\exp(-K_t t)], \quad (6)$$

whence it follows that $dt = dT/[K_t(C - T)]$, (7)

where t – time;

$C = T_0 + \Delta T_{\infty} > T$;

T_0 – initial temperature of grain;

ΔT_{∞}, K_t – coefficients.

Upon integrating (3), we obtain

$$Y = k\{- T \exp(-\varepsilon_R/T) - C \exp(-\varepsilon_R/C) Ei(z) + (C - \varepsilon_R) Ei(-\varepsilon_R/T), \quad m = 1 \quad (8)$$

$$Y = k \{Ei(-\varepsilon_R/T) - Ei(-\varepsilon_R/T_0) + \exp(-\varepsilon_R/C) [Ei(z_0)] - Ei(z)], \quad m = 0 \quad (9)$$

and $Y = k [Ei(z_0) - Ei(z)]$, $m = -1$ (10)

$z = \varepsilon_R(1/C - 1/T)$, $z_0 = \varepsilon_R(1/C - 1/T_0)$, $C = T_0 + \Delta T_{\infty} > T$

where Ei – an exponential integral function (Eulerian function));

k, C and ε_R – empirical coefficients.

Comparative analysis of the integrals (8, 9, 10) in a wide range of variation by the parameters ($50000 < \varepsilon_R < 1000$, K; $T_0 = 273$, K; $75 < \Delta T_{\infty} < 200$, K; $0.005 < K_t < 0.5$, c^{-1}; $0 < t < 120$, c) shows that their graphs lie well on each other with an appropriate selection of the constant factor k. Therefore, in the indicated range of variation by variables, it is advisable to use the simplest form (10) as a model, identifying the coefficients k, ε R and C from the results of the Y (T) experiments.

However, the identification of three parameters might cause difficulty. Additional information on the value of C can be obtained by assessing the parameters of the temperature dependence (4), according to the results of the experiments T (t).

If we make one more assumption, namely, C is much larger than T, i.e. $z \approx -\varepsilon_R/T$, then the expressions (10) can be simplified further, reducing them to a two-parameter model.

The Eulerian function is not always convenient for work. Let us therefore use its property for large values of the argument. In this case the following expansion is fair

$Ei(z) = [\exp(z)/z] (1 + 1/z + 1/z^2 + \ldots)$,

which, taking into account the first term, will lead (10) to the expression:

$$Y = k[T \exp(-\varepsilon_R/T) - T_0 \exp(-\varepsilon_R/T_0)] \quad (11)$$

The values of the identified coefficients are given in table 3.

Experimental data and the approximating curve for humidity W = 17% are shown in Figure 7.

CONCLUSION

Heat treatment, including in a flux of IR radiation, provides effective inactivation of urease and a trypsin inhibitor in soya when product is heated above 100°C.

The dependences of the level of urease activity on time and energy exposure are invariant to the initial moisture content of product, and the similar temperature dependences - to the initial moisture content.

The results of thermal inactivation under conditions of increasing temperature do not depend on the IR processing regimes, in particular on time, and they are determined only by the initial moisture content of grain, and by the end heating temperature. The lower is the humidity, the higher the temperature is needed for heating.

Since the energy exposure reflects the energy inputs for heating of product located on the unit of the working surface of the treatment area, the inactivation efficiency is the higher, the higher is the used irradiation. However, quick browning of the grain surface imposes restrictions on the upper limit of the intensity of heat supply.

The proposed model of inactivation of a trypsin and urease inhibitor, under conditions of unsteady temperature, describes quite well the experimental results. We can try to generalize the model by including humidity as an independent variable.

The considered models can be used for the thermal degradation processes and other thermolabile substances under the similar heating conditions.

REFERENCES

Perez-Maldonado, R. A., Mannion, P. F., Farell, D. J. 2002. Effects of heat treatment on the inhibitor activity, *British Poultry Science*, vol. 44, no. 2, p. 299-308. https://doi.org/10.1080/00071660310000854 63

Egorov, B. V, Levitsky, A. P., Sherstobitov, V. V., Chayka, I. K. 1986. Kinetics of heat treatment of soybean grains. Food Technology. *News of Higher Education Institutions*, vol. 6, p. 95-96.

Kulaychev, A. P. 1999. The complete works in three volumes, Vol. 1. In Kulaychev, A. et.al. *Methods and tools of data analysis in a Windows environment STADIA-6*. M.: Information science and computers, 344 p. ISBN 5-89-357-016-2.

Kargov, I. S, Kleimenov, S. Y., Savin, S. S., Tishkov, V. I., Alekseeva, A. A. 2015. Improvement of the soy formate dehydrogenase properties by rational design. *Protein Engineering, Design & Selection*, vol. 28, no. 6, p. 171-178. https://doi.org/10.1093/protein/gzv007

Erickson, D. R. 2002. *Guidance manual on processing and use of soybean*. Russia : Makcentr, 672 p. ISBN 5-8121-0046-2.

Reshetnik, E. Y. 2007. *The use of a soya-milk concentrate in the production of foods*. Blagoveshchensk, Russoa : Dalgau publishers, 190 p. ISBN 978-5-9642-0031-4.

Romanovsky, B. V. 2006. *Fundamentals of chemical kinetics: a textbook.* Moscow, Russia : Examination, 415 p. ISBN 5-472-01551-0.

Ruis, N. 2013. Activity of urease in soybean meal. A New Look. *Combined feed*, vol. 10, p.59-61.

Chen, Y., Xu, Z., Zhang, C., Kong, X., Hua, Y. 2014. Heat-induced inactivation mechanisms of Kunitz trypsin inhibitor and Bowman-Birk inhibitor in soymilk processing. *Food Chemistry*, vol. 154, p. 108-116. https://doi.org/10.1016/j.foodchem.2013.12.092

Zverev, S. V., Sesikashvili, O. Sh., Bulakh, Yu. G. 2013. *Soybean. Properties. Heat treatment. Using.* Kutaisi : Akaki Tsereteli State University publishers, 198 p. ISBN 978-9941-417-96-2.

Zverev, S. V. 2009. *High-temperature micronization in production of cereal products*. Moscow : DELI PRINT publishers, 222 p. ISBN 978-5-94343-202-6.

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DETERMINATION OF THE CARROT (*DAUCUS CAROTA* L.) YIELDS PARAMETERS BY VERMICOMPOST AND EARTHWORMS (*EISENIA FOETIDA*)

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ABSTRACT

The impact of different types of vermicomposts as well as different species and genera of earthworms on the quantity of the cultivated crops yield has been studied for decades. There is scarce information about the effects of these factors on the quality of plant production. One of the qualitative parameters of vegetables, to which a special attention is paid, is the content of antioxidants (vitamin C, total polyphenols and other substances). The pot experiment carried out in the vegetation cage studied: A) the influence of soil itself, soil mixed with vermicompost in a ratio of 4:1; B) the influence of earthworms number (genus *Eisenia foetida*, 10 and 20 individuals per pot) supplied to soil mixed with vermicompost in a ratio of 4:1 on the weight of radish roots and leaves, to the total chlorophylls content in leaves, to the selected qualitative parameters of the roots and leaves (vitamin C, total polyphenols content, total antioxidant activity). The results showed that the supplementation of vermicompost into soil increased the content of the total chlorophylls in leaves. The carrot roots and leaves yield has also been risen. In the roots the content of vitamin C and content of total polyphenols (TPC) was decreased and the total antioxidant activity (TAA) dropped, too. The increased content of vitamin C and TPC was detected in leaves. The inoculation of soil containing vermicompost by earthworms increased the root yield and TAA in roots. It increased the content of vitamin C and TPC in leaves. From the viewpoint of antioxidant content (vitamin C and total polyphenols) the leaves are more attractive than a root.

Keywords: carrot; antioxidant; vitamin C; total polyphenols; yield

INTRODUCTION

The positive impact of earthworms on soil and plants was monitored several centuries ago **(Kováčik, 2007)**. In spite of it, at present the research of earthworms impact on soil, environment, quantity and quality of the grown yield has been done with more attention **(Milcu et al., 2006; Amador et al., 2006; Xiang et al., 2016)**. The researchers of the whole world study the requirements of the different species of earthworms to soil parameters and substrates in order to create the optimal conditions for their growth and reproduction **(Nuutinen et al., 2001; Gunadi and Edwards, 2003; Arnold and Hodson, 2007)**. They detect which genera (species) of earthworms are optimal for the purposes of the ecological valuation of precisely defined waste produced by chemical, food processing, textile, papermaking or other type of industry **(Nurhidayati et al., 2016; Santos et al., 2017; Bhat et al., 2018)**. The researchers also test which temperature, substrate humidity, salts content, ammonia, total nitrogen, methane and ratio C:N occur in the most effective decay, the transformation of organic "waste" into the high quality vermicompost. They also study the different technological procedures of vermicompost production for its direct usage in the plant production, recultivation and production of vermiextracts **(Scheuerell, 2004; Padmavathiamma et al., 2008; Gutiérrez-Miceli et al., 2008; Kováčik et al., 2015)**.

The vermicomposts are composts in the production of which the process of biological transformation of organic substances takes part the earthworms. For these purposes predominanlty the earthworms of genera *Eisenia foetida* and *Eudrilus eugeniae* **(Lalander et al., 2015; Najjari and Ghasemi, 2018)**, or *Lumbricus terrestris and Eisenia Andrei* **(Rämert et al., 2000; Amossé et al., 2013)**, as well as others, are being used. More scientific knowledge about the impact of vermicomposts on the quantity and quality of the cultivated crops yields has been gathered than the knowledge about the impact of earthworms on the given parameters.

The positive impact of vermicomposts on the quantity of the cultivated field and garden crops, fruit trees and bushes was recorded by **Arancon et al. (2004), Manh and Wang (2014), Khan et al. (2015), Goswami et al. (2017)**.

The positive impact of earthworms (of different genera) on the plant growth, the quantity and quality of yield was recorded by **Brown et al. (2004)**, **Groenigen et al. (2014)** and others. For example, **Xiang et al. (2016)**, taking into consideration the knowledge of meta-analysis, claim that the presence of earthworms in the ecosystem increases crop yield on average by 25%, formation of aboveground phytomass by 23% and underground phytomass by 20%. Zero and negative plant responses to the presence of earthworms were recorded **(Doan et al., 2013; Nurhidayati et al., 2016; Elmer, 2016)**. **Kováčik et al. (2018)** also detected the negative impact of earthworms (*Eisenia foetida*) on the formation of radish roots, however, the positive impact on the content of vitamin C and negative impact on the content of nitrates in radish roots was also recorded.

It is evident that the impact of earthworms on the particular plants has not been explored sufficiently. This statement is related not only to the quantity of yield but also to its qualitative parameters, which are the contents of antioxidants.

Scientific hypothesis

The objective of the presented experiment is to give the answer to the question how the supplementation of vermicompost into soil and soil inoculation enriched by vermicompost of earthworms genus *Eisenia foetida* affect the yield of carrot roots and leaves, the content of total chlorophylls in leaves, the content of antioxidants (vitamin C and total polyphenols) and the total antioxidant activity in carrot roots and leaves.

MATERIAL AND METHODOLOGY

Experimental design and field management

The pot experiment was carried out in the vegetation cage located in the area of the Slovak University of Agriculture in Nitra, Slovakia. The size of the cage was 20 m × 20 m × 5 m. On its sides and ceiling there was the metal mesh with the size of a mesh 15 mm × 15 mm, which protected the experiment against birds.

The experiment was established on March 13, 2017. The weighted soil (treatment 1) and mixture of soil and vermicompost (treatments 2 – 4) were put into the cylindrical pots 35 cm high with the diameter 35 cm. In the treatment 1, twenty (20) kg of soil (Haplic Luvisol) was used and in the treatments 2, 3 and 4 was put into the pots of 20 kg of mixture of soil and vermicompost. The mixture was created by 16 kg of soil (S) and 4 kg of vermicompost (V), which was the ratio S:V = 4:1, (20% proportion of V). In the treatments 2 – 4 the same soil was used like in the treatment 1. The used soil was taken from the field located in Párovské Háje, (cadaster Nitra, Slovakia), in particular, from the upper horizon of soil 0.0 – 0.3 m. The used vermicompost was produced from cow dung (about 50%), sheep manure (about 10%), green grass (about 10%) and wood chips (about 30%). After 3 to 4 months of fermentation (composting) was prepared a mixture of the new with the old vermicompost in a ratio of 1:1. In the old vermicompost were earthworms and cocoons. Basic agrochemical parameters of soil and vermicompost used in experiment are presented in Table 1.

Ten individuals of adult earthworms (*Eisenia foetida*) were placed to the pots of the treatment number 3, and twenty individuals of earthworms were introduced to the pot of the treatment 4. The weighed out pots were placed into the dishes, which were able to keep 1,000 mL of the leaked soil solution during the period of precipitation. The leaked through solution was returned back to the pots.

The experiment was established according to the method of random arrangement of pots in four repetitions. The model crop was carrot (*Daucus carota* L. ssp. *sativus*) cultivar Nantes 3. The sowing was carried out on March 16. Subsequently, the experiment was irrigated to the level of 75% FWC (field or full water capacity). In the following eight weeks all pots were irrigated by the same dose of water containing the minimal quantity of nutrients. During the last days the treatments 2, 3 and 4 were irrigated by a higher dose of water, because the plants in these treatments evaporated more water as a result of the significantly larger leaf area. On Jun 27, 103 days after sowing, samplings of plant material were accomplished. 20 average individuals of carrot were taken from each treatment and repetition, which served for the evaluation of the root and leaf weight, the content of total chlorophylls in leaves, the content of vitamin C, total polyphenols and antioxidant activity in carrot roots and leaves.

Analysis of soil and vermicompost

Were used the following analytical methods for the indication of the agrochemicals parameters of used soil and vermicompost. $N\text{-}NH_4^+$ by Nessler's colorimetric method; $N\text{-}NO_3^-$ by colorimetric method with phenol – 2.4 disulphonic acid, where the extract from soil was achieved by using the water solution 1% K_2SO_4. N_{min}= $N\text{–}NH_4^+$ + $N\text{–}NO_3^-$. The contents of available P, K, Ca,Mg were determined by Mehlich 3 extraction procedure **(Mehlich, 1984)**. The content of P was determined by colorimetric method, K by flame photometry, Ca and Mg by atomic absorption spectrophotometry, S spectrophotometrically (in the leachate of ammonium acetate), N_t by distillation after the mineralization of strong H_2SO_4 (Kjeldahl – **Bremner, 1960**), C_{ox} spectrophotometrically after the oxidation according to Tyurin **(Dziadowiec and Gonet, 1999)**, EC by the method of specific electrical conductivity and pH/KCl (in solution of 1.0 $mol.dm^{-3}$ KCl) potentiometrically.

Determination of total chlorophylls

For the analysis of the pigment content, the last fully developed leaves were used. The segments of the youngest mature leaves were homogenized with using sea sand, $MgCO_3$ and 100% acetone and then extracted with 80% acetone. Extracts were centrifuged 2 minutes. Absorbance (A) of the solution was measured by UV-VIS spectrophotometer, at 647 nm and 663 nm, with correction for scattering at 750 nm; the measurements were done in three repetitions. The concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) in $mg.L^{-1}$ were determined by using the equations of **Lichtenthaler (1987)**:

Chl *a* = 12.25 x (A_{663} - A_{750}) - 2.79 x (A_{647} - A_{750}) x D
Chl *b* = 21.50 x (A_{647} - A_{750}) - 5.10 x (A_{663} - A_{750}) x D
Chl *a*+*b* = 7.15 x (A_{663} - A_{750}) + 18.71 x (A_{647} - A_{750}) x D
D is the optical thickness of cuvette.

Results were also recalculated in $mg.m^{-2}$ using the volume of solution and the area of leaf segments: $mg.m^{-2}$ =

V/1000 x 1/A, when V is volume of 80% acetone and A is area of leaf segments.

Determination of L-ascorbic acid

Three grams of homogenized fresh samples were stabilized with 10 mL of acid solution prepared as follows: 10% perchloric acid and 1% orthophosphoric acid in ultra pure water. The mixture was thoroughly vortexed for 1 minute. This solution was diluted to 50 mL with HPLC mobile phase. The sample was filtered with 0.45 μm filter.

L-ascorbic acid was determined by HPLC Agilent 1260 with quaternary solvent manager coupled with degasser, sample manager, column manager and DAD detector. All analyses were performed on C18 end capped column. Mobile phases consisted of methanol (B) and 0.1% H_3PO_4 (C). The isocratic elution was as follows: 0 – 6 min (20% B and 80% C) and 3 minutes post-run. The mobile phase flow was 1 $mL.min^{-1}$ and the sample injection was 20 μL. Column thermostat was set to 30 °C and the samples were kept at 4 °C the sampler manager. The detection wavelength was set at 256 nm. The spectral data were collected and processed using Agilent OpenLab ChemStation software for LC 3D Systems.

Determination of total polyphenols

Total polyphenols content (TPC) was determined by the method according to **Lachman et al. (2003)** and expressed as mg of gallic acid equivalent per kg fresh mater. Gallic acid is usually used as a standard unit for phenolic content determination because a wide spectrum of phenolic compounds. The total polyphenol content was determined using Folin-Ciocalteau reagent. 2.5 mL of 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. Then 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.

Determination of total antioxidant activity

Total antioxidant activity (TAA) was measured according to **Brand-Williams et al. (1995)**. The method is based on using DPPH (2.2-diphenyl-1-picrylhydrazyl). DPPH was pipetted into the cuvette number 1 (3.9 ml) and the absorbance was measured using the spectrophotometer at wavelength 515.6 nm. The measured value corresponds to the initial concentration of DPPH solution at the time A0. Then 0.1 cm^3 extract was added to start measuring dependence A = f x (t). The content of cuvette number 2 was mixed and the absorbance was measured at 1, 5 and 10 minutes in the same way as DPPH solution. The percentage of inhibition expresses how antioxidant compounds are able to remove $DPPH^{\cdot}$ radical at the given period of time.

Inhibition (%) = (A0 - At/A0) x 100.

Statistical analysis

The acquired results were processed by mathematical and statistical method, by analysis of variance (ANOVA) and linear regression analysing Statgraphics PC program, version 5.0. The differences between the treatments were evaluated subsequently by LSD test at the significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

Vermicompost in soil (var. 2) increased significantly the content of total chlorophylls in carrot leaves (Table 2). The increase of total chlorophylls contents was pursued via the increase of contents of chlorophyll *b*. The content of chlorophylls *a* was decreased. The increase of contents of chlorophylls *b*, which was recorded in all treatments with vermicompost (trt. 2, 3 and 4), according to **Razzaq et al. (2017)** was the consequence of higher content of mineral nitrogen in soil of the given treatments compared with the treatment 1 (Table 1). A higher statistical dependence is recorded very often between the content of chlorophylls *b* and the yield of cultivated crops than between the contents of chlorophylls *a* and yield **(Kováčik, et al., 2016)**. These data emphasize the practical significance of finding the

Table 1 Parameters of the soil substrates used in the experiment.

Subs.	N_{min}	P	K	Ca	Mg	S	N_t	C_{ox}	C:N	EC	pH_{KCl}
	($mg.kg^{-1}$)	($mg.kg^{-1}$)	($mg.kg^{-1}$)	($mg.kg^{-1}$)	($mg.kg^{-1}$)	($mg.kg^{-1}$)	(%)	(%)		($mS.cm^{-1}$)	
Soil	13.2	21.9	156	4,250	444	1.3	0.077	0.915	11.88	0.14	6.97
V	313.8	351.3	19,000	4,350	3,052	4,688	3.775	20.880	5.53	5.58	7.06

Note: Subs. – substrate, V – vermicompost.

Table 2 Impact of vermicompost and earthworms on chlorophyl *a*, chlorophyl *b* and total chlorophylls content in carrot leaves.

Treatment		Chl. *a*	Chl. *b*	Chl. *a*+*b*
number	mark	($mg.kg^{-1}$)	($mg.kg^{-1}$)	($mg.kg^{-1}$)
1	S	0.616 b	0.562 a	1.178 a
2	SV	0.608 a	0.781 b	1.389 b
3	$SV+EW_{10}$	0.607 a	0.824 b	1.431 b
4	$SV+EW_{20}$	0.605 a	0.836 b	1.441 b
$LSD_{0.05}$		0.00645	0.06525	0.06941

Note: Chl. *a* – chlorophyll a; Chl. *b* – chlorophyll b; S – soil; SV – soil + vermicompost, EW_{10} – ten individuals of earthworms; $LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$; different letter behind a numerical value respond to the statistically significant difference at the level 95.0%.

increase of contents of chlorophyll *b* in crops cultivated on soils where vermicompost was supplied.

The presence of earthworms in soil (trt. 3 and 4 versus trt. 2) had impact neither on the content of total chlorophylls nor its particular components (chorophyll *a* and chlorophyll *b*).

Weight of aboveground and underground phytomass

Vermicompost in soil (trt. 2) became evident by the considerable growth of aboveground and underground carrot phytomass (Table 3). The positive impact of vermicompost on the growth of carrot roots and leaves is the result of its agrochemical parameters (significant content of available nutrients – mineral nitrogen, P, K, Mg and S).

Soil inoculation treated by vermicompost with earthworms (trt. 3 and 4) had a positive impact on the weight of roots. The weight of leaves was not determined by the presence of earthworms in soil. The differences were significant between the root weight of the treatment without earthworms and with 10 individuals of earthworms in soil (trt. 2 versus trt.3) and also between the treatments with 10 and 20 individuals of earthworms in soil (trt. 3 versus trt. 4). This finding does not correspond with our knowledge **(Kováčik et al., 2018**) about the impact of earthworms on the growth of radish roots, where during the whole growing season the negative impact of earthworms on the roots weight was recorded. The different impact of earthworms on the root weight of carrot and radish was caused by the different reaction of these crops to the attack of earthworms at the root hair of young plants. The different reaction of carrot and radish was caused by the different duration of growing season of these crops. Carrot has a longer growing season therefore the monitored attack of earthworms at the root hair of germinated plants did not have the negative impact on the roots yield. A longer growing season of carrot created a longer space-time for the regeneration of the young plants, which had been attacked by earthworms, also for the positive impact of earthworms on physical, chemical and biological soil parameters **(Garg et al., 2006; Jouquet et al., 2010; Spurgeon et al., 2013)** and on the yield formation **(Friberg et al., 2005; Groenigen et al., 2014)**.

The positive finding is that the vermicompost and earthworms influenced more significantly the roots weight than leaves weight, because in Slovakia the consumed organ is a root. The vermicompost and earthworms in soil increased the ratio between the roots weight and leaves weight (Table 3).

Vitamin C content

20 % proportion of vermicompost in soil (trt. 2) decreased the content of vitamin C in carrot roots almost by o 40 % (Table 4). The drop was the natural consequence of the well-known negative correlation between the content N_{min} in soil and content of vitamin C in plants. The negative impact of vermicompost (rich in the available nutrients – Table 1) on the content of vitamin C in carrot roots was statistically insignificantly decreased by the soil inoculation with earthworms (trt.3 and 4 versus trt.2). Vermicompost (trt. 2 versus trt. 1) had the opposite impact on the content of vitamin C in leaves than on the content in carrot roots. The content of vitamin C rose significantly.

The impact of earthworms on the content of vitamin C in radish leaves was unlike the impact of the content of vitamin C in roots significantly positive (trt. 3 versus trt. 2), or (trt. 4 versus trt.2).

In leaves there was considerably more vitamin C than in roots (Table 4). Similarly, in leaves there was a higher content of the total polyphenols and also the total antioxidant capacity was higher in leaves than in roots, which supports the idea that the countries with the food

Table 3 Impact of vermicompost and earthworms on weight of carrot roots and leaves (fresh phytomass).

Treatment		Root	Leaf	Ratio R/L
number	mark	(g)	(g)	
1	S	4.70 a	11.64 a	0.40
2	SV	89.50 b	45.98 b	1.95
3	SV+EW_{10}	109.62 c	48.92 b	2.24
4	SV+EW_{20}	120.69 d	50.48 b	2.39
$LSD_{0.05}$		8.832	5.002	

Note: S – soil; SV – soil + vermicompost, EW_{10} – ten individuals of earthworms; $LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$; different letter behind a numerical value respond to the statistically significant difference at the level 95.0%.

Table 4 Impact of vermicompost and earthworms on content of vitamin C in carrot roots and leaves.

Treatment		Root		Leaf		Ratio L/R
number	mark	($mg.kg^{-1}$)	(rel. %)	($mg.kg^{-1}$)	(rel. %)	
1	S	245.89 b	100.00	714.88 a	100.00	2.90
2	SV	150.80 a	61.33	842.72 b	117.88	5.59
3	SV+EW_{10}	167.23 a	68.01	994.83 c	139.16	5.95
4	SV+EW_{20}	167.37 a	68.07	972.71 c	136.07	5.81
$LSD_{0.05}$		24.730		59.574		

Note: S – soil; SV – soil + vermicompost, EW_{10} – ten individuals of earthworms; $LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$; different letter behind a numerical value respond to the statistically significant difference at the level 95.0%

sufficiency could adopt some eating habits from those countries which suffer from the food shortage (Table 5 and Table 6).

Total polyphenols content (TPC) and total antioxidant activity (TAA)

The roots of carrot variety Nantes contained the content of polyphenols on average 2.5 higher than the content of polyphenols in garlic **(Lenková et al., 2018)**, 1.7 times higher than in the young green peas **(Hegedűsová et al., 2018**) and 1.3 times higher than in flesh-raw of sweet potato **(Musilová et al., 2017**). Despite the detected relatively high content of the total polyphenols in carrot roots compared with other vegetables, the content of the total polyphenols in roots was 5.11 even 11.8 times lower than in carrot leaves. The total antioxidant activity detected in leaves was 3.26 even 7.84 times higher that in carrot roots (Table 5 and Table 6). The polyphenols are known for their antioxidant and antiradical activity. They protect the animal organism from the oxidation stress. The application of vermicompost into soil (trt. 2) decreased the content of the total polyphenols in carrot roots and also the value of the total antioxidant activity, which is the negative effect, because polyphenols have the positive impact on our health **(Oszmianski et al., 2013; Ražná et al., 2018)**. The decrease of content of vitamin C, the total polyphenols and consequently also monitoring the decrease of the total antioxidant activity in carrot roots after the application of vermicompost shows the evidence that the fertilization by the fertilizers containing nitrogen, leads mostly to the growth of yield, on the other hand, the decrease of the quality of the cultivated crops.

The impact of vermicompost on TPC in carrot leaves was significantly positive and the content of TAA in carrot leaves was not influenced by vermicompost.

The soil inoculation by earthworms (trt. 3 and 4 versus trt. 2) increased the content of total polyphenols and also the total antioxidant activity of the whole carrot phytomass. However, the impact of earthworms on TPC and TAA in roots and leaves was not equally significant. In the consumed part of carrot – in roots – the increase of TPC was insignificant and TAA significant. On the contrary, in leaves the increase of TPC was significant and TAA insignificant.

CONCLUSION

The supplemantation of vermicompost into soil led to the increase of yield of carrot roots, but the content of vitamin C and total polyphenols was decreased in roots, also the total antioxidant activity dropped. The inoculation of soil containing vermicompost with earthworms inhibited the negative impact of vermicompost on the content of vitamin C and the total polyphenols in carrot roots. The impact of earthworms on the total antioxidant activity in leaves was positive.

The supplementation of vermicompost into soil increased the leaves yield and it increased the content of total chlorophylls, content of vitamin C and total polyphenols in leaves. Earthworms had impact neither on the content of total chlorophylls in leaves, leaves yield nor TAA in leaves. They had the positive impact on the content of vitamin C and TPC in leaves. Carrot leaves contain several times more vitamin C and total polyphenols than roots. Similarly, the total antioxidant activity in leaves is several times higher than in carrot roots.

Table 5 Impact of vermicompost and earthworms on content of total polyphenols of aboveground and underground carrot phytomass.

Treatment		Root		Leaf		Ratio
number	mark	(mg.kg^{-1})	(rel. %)	(mg.kg^{-1})	(rel. %)	L/R
1	S	2,385 b	100.00	12,183 a	100.00	5.11
2	SV	1,157 a	48.50	12,773 b	104.84	11.04
3	SV+EW$_{10}$	1,200 a	50.32	13,292 c	109.10	11.08
4	SV+EW$_{20}$	1,241 a	52.03	13,583 d	111.49	10.95
LSD$_{0.05}$		242.497		341.847		

Note: S – soil; SV – soil + vermicompost, EW$_{10}$ – ten individuals of earthworms; LSD$_{0.05}$ – least significant difference at the level $\alpha = 0.05$; different letter behind a numerical value respond to the statistically significant difference at the level 95.0%.

Table 6 Impact of vermicompost and earthworms on content of total antioxidant activity of aboveground and underground carrot phytomass.

Treatment		Root		Leaf		Ratio
number	mark	(%)	(rel. %)	(%)	(rel. %)	L/R
1	S	27.40 d	100,00	89.46 a	100.00	3.26
2	SV	11.38 a	41,53	89.25 a	99.77	7.84
3	SV+EW$_{10}$	13.21 b	48,21	89.00 a	99.49	6.74
4	SV+EW$_{20}$	14.74 c	53,80	89.06 a	99.55	6.04
LSD$_{0.05}$		0.913		0.525		

Note: S – soil; SV – soil + vermicompost, EW$_{10}$ – ten individuals of earthworms; LSD$_{0.05}$ – least significant difference at the level $\alpha = 0.05$; different letter behind a numerical value respond to the statistically significant difference at the level 95.0%.

REFERENCES

Amador, J. A., Görres, J. H., Savin, M. C. 2006. Effects of *Lumbricus terrestris* L. on nitrogen dynamics beyond the burrow. *Applied Soil Ekology*, vol. 33, p. 61-66. https://doi.org/10.1016/j.apsoil.2005.09.008

Amossé, J., Bettarel, Y., Bouvier, C., Duc, T. T., Thu, T. D., Jouquet, P. 2013. The flows of nitrogen, bacteria and viruses from the soil to water compartments are influenced by earthworm activity and organic fertilization (compost vs. vermicompost). *Soil Biology & Biochemistry*, vol. 66, p. 197-203. http://dx.doi.org/10.1016/j.soilbio.2013.07.007

Arancon, N. Q., Edwards, C. A., Atyieh, R., Metzger, J. D. 2004. Effects of vermicomposts produced from food waste on the growth and yields of greenhouse peppers. *Bioresource Technology*, vol. 93, p. 139-144. https://doi.org/10.1016/j.biortech.2003.10.015

Arnold, R. E., Hodson, M. E. 2007. Effect of time and mode of depuration on tissue copper concentrations of the earthworms *Eisenia andrei*, *Lumbricus rubellus* and *Lumbricus terrestri. Environmental Pollution*, vol.148, no. 1, p. 21-30. http://dx.doi.org/10.1016/j.envpol. 2006.11.003

Bhat, S. A., Singh, S., Sing, J., Kumar, S. Bhawana, Vig, A. P. 2018. Bioremediation and detoxification of industrial wastes by earthworms: Vermicompost as powerful crop nutrient in sustainable agriculture. *Bioresource Technology*, vol. 252, p. 172-179. https://doi.org/10.1016/j.biortech.2018.01.003

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. https://doi.org/10.1016/S0023-6438(95)80008-5

Bremner, J. M. 1960. Determination of nitrogen in soil by the Kjeldahl method. *Journal of Agricultural Science*, vol. *55*, no. 1, p. 11-33. https://doi.org/10.1017/S0021859600021572

Brown, G. G., Edwards, C. A., Brussaard, L. 2004. How Earthworms Affect Plant Growth: Burrowing into the Mechanisms. In Edwards, C.A. et al. *Earthworm ecology*. 2nd Edition. Ch. 2, CRC Press : London, UK p. 13-45, ISBN: 9780849318191

Dziadowiec, H., Gonet, S. S. 1999. *A guide to the methods for determination of soil organic matter. Prace Komisie Naukowej*. PTG : Warszawa, Poland, 65. p.

Doan, T. T., Ngo, P. T., Rumpel, C., Nguyen, B. V. 2013. Interactions between compost, vermicompost and earthworms influence plant growth and yield: A one-year greenhouse experiment. *Scientia Horticulturae*, vol. 160, p. 148-154. http://dx.doi.org/10.1016/j.scienta.2013.05.042

Elmer, W. H. 2016. Effect of leaf mold mulch, biochar, and earthworms on mycorrhozal colonization and yield of asparagus affected by *Fusarium* crown and root rot. *Plant disease*. vol. 100, p. 2507-2512. http://dx.doi.org/10.1094/PDIS-10-15-1196-RE

Garg, P., Gupta, A., Satya, S. 2006. Vermicomposting of different types of waste using *Eisenia foetida* : A comparative study. *Bioresource Technology*. vol. 97, p. 391-395. http://dx.doi.org/10.1016/j.biortech.2005.03.009

Goswami, L., Nath, A., Sutradhar, S., Bhattacharya, S. S., Kalamdhad, A., Vellingiri, K., Kim, K-H. 2017. Application of drum compost and vermicompost to improve soil health, growth, and yield parameters for tomato and cabbage plants. *Journal of Environmental Management*, vol. 200, p. 243-252. http://dx.doi.org/10.1016/j.jenvman.2017.05.073

Friberg, H., Lagerlöf, J., Rämert, B. 2005. Influence of soil fauna on fungal plantpathogens in agricultural and horticultural systems. *Biocontrol Science and Technology*, vol. 15, no. 7, p. 641-658. http://dx.doi.org/10.1080/09583150500086979

Groenigen, J. W., Lubbers, I. M., Vos, H. M. J., Brown, G. G., De Deyn, G. B., Groenigen, K. J. 2014. Earthworms increase plant production: a meta-analysis. *Scientific Reports*, vol. 4, no. 6365, p. 1-7. https://doi.org/10.1038/srep06365

Gutiérrez-Miceli F. A., García-Gómez R. C., Rincón R. R., Abud-Archila M., Llaven O. M. A., Cruz M. J. G., Dendooven L. 2008**.** Formulation of a liquid fertilizer for sorghum (*Sorghum bicolor* L. Moench) using vermicompost leachate. *Bioresource Technology*, vol. 99, p. 6174-6180. http://dx.doi.org/10.1016/j.biortech.2007.12.043

Gutiérrez-Miceli, F. A., Santiago-Borraz, J., Molina, J. A. M., Nafatae, C. C., Abud-Archila, M., Llaven, M. A. O., Rosales, R. R., Dendooven, L. 2007. Vermicompost as a soil supplement to improve growth, yield and fruit quality of tomato (*Lycopersicum esculentum*). *Bioresource Technology*, vol. 98, no. 15, p. 2781-2786. http://dx.doi.org/10.1016/j.biortech.2006.02.032

Gunadi, B., Edwards, C. A. 2003. The effects of multiple applications of different organic wastes on the growth, fecundity and survival of *Eisenia fetida* (Savigny) (*Lumbricidae*). *Pedobiologia*, vol. 47, no. 4, 321-329. https://doi.org/10.1078/0031-4056-00196

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. https://doi.org/10.5219/412

Jouquet, P., Plumere, T., Thu, T. D., Rumpel, C., Duc, T. T., Orange, D. 2010. The rehabilitation of tropical soils using compost and vermicompost is affected by the presence of endogeic earth worms. *Applied Soil Ecology*, vol. 46, p. 125-133. https://doi.org/10.1016/j.apsoil.2010.07.002

Manh, V. H., Wang, C. H. 2014. Vermicompost as an important component in substrate: Effects on seedling quality and growth of muskmelon (*Cucumis melo* L.). *APCBEE Procedia*, vol. 8, p. 32-40. https://doi.org/10.1016/j.apcbee.2014.01.076

Khan, K., Pankaj, U., Verma, S. K., Gupta, A. K., Singh, R. P., Verma, R. K. 2015. Bio-inoculants and vermicompost influence on yield, quality of *Andrographis paniculata*, and soil properties. *Industrial Crops and Products*, vol. 70, p. 404-409. https://doi.org/10.1016/j.indcrop.2015.03.066

Kováčik. P. 2007. Brief history of agrochemistry and fertilization in Slovakia (*Stručná história agrochémie a úroveň hnojenia na Slovensku)*. Prešov : Vydavateľstvo Michala Vaška, Slovakia, 76 p. ISBN: 978-80-7165-608-1 (In Slovak)

Kováčik, P., Renčo, M., Šimanský, V., Hanáčková, E., Wiśniowska-Kielian, B. 2015 Impact of vermicompost extract application into soil and on plant leaves on maize phytomass formation. *Journal of Ecological Engineering*, vol. 16, no. 4, p. 143-153. https://doi.org/10.12911/22998993/59363

Kováčik, P., Šimanský, V., Wierzbowska, J., Renčo M. 2016. Impact of foliar application of biostimulator Mg-Titanit on formation of winter oilseed rape phytomass and its titanium content. *Journal of Elementology,* vol. 21, no. 4, p. 1235-1251. https://doi.org/10.5601/jelem.2016.21.2.1155

Kováčik, P., Šalamún, P., Wierzbowska, J. 2018. Vermikompost and *Eisenia foetida* as factors influencing the formation of radish phytomass. Agriculture (*Poľnohospodárstvo*), vol. 64, no. 2, p. 49-56. https://doi.org/10.2478/agri-2018-0005

Lalander, C. H. Komakech, A. J., VinnerÍs, B. 2015. Vermicomposting as manure management strategy for Urban small-holder animal farms – Kampala case study. *Waste Management*, vol. 39, p. 96-103. https://doi.org/10.1016/j.wasman.2015.02.009

Lachman, J., Proněk, D., Hejtmanková, A., Dudjak, J., Pivec, V., Faitová, K. 2003. Total polyphenol and main flavonoid antioxidant in different onion (*Allium cepa* L.) varieties. *Horticultural Science*, vol. 30, no. 4, p. 142-147. https://doi.org/10.17221/3876-HORTSCI

Lenková, M., Bystrická, J., Chlebo, P., Kovarovič, J. 2018. Garlic (*Allium Sativum* L.) – The content of bioactive compounds. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 405-412. https://doi.org/10.5219/830

Lichtenthaler, H. K. 1987. Chllorophylls and carotenoides: Pigments of photosynthetic biomembranes. *Methods Enzymology*, vol. 148, p. 350-382. https://doi.org/10.1016/0076-6879(87)48036-1

Musilová, J., Bystrická, J., Árvay, J., Harangózo, Ľ. 2017. Polyphenols and phenolic acids in sweet potato (*Ipomoea Batatas* L.) roots. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 82-87. https://doi.org/10.5219/705

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communication in Soil Science and Plant Analysis*, vol. 15, no. 12, p. 1409-1416. https://doi.org/10.1080/00103628409367568

Milcu, A., Schumacher, J., Scheu, S. 2006. Earthworms (*Lumbricus terrestris*) affect plant seedling recruitment and microhabitat heterogenity. *Functional Ekology,* vol. 20, p. 261-268. https://doi.org/10.1111/j.1365-2435.2006.01098.x

Najjari, F., Ghasemi, S. 2018. Changes in chemical properties of sawdust and blood powder mixture during vermicomposting and the effects on the growth and chemical composition of cucumber. *Scientia Horticulturae*, vol. 232, p. 250-255. https://doi.org/10.1016/j.scienta.2018.01.018

Nurhidayati, N., Ali, U., Murwani I. 2016. Yield and quality of cabbage *(Brassica oleracea* L. var. *c*apitata*)* under organic growing media using vermicompost and earthworm *Pontoscolex corethrurus* inoculation. *Agriculture and Agricultural Science Procedia*, vol. 11, p. 5-13. https://doi.org/10.1016/j.aaspro.2016.12.002

Nuutinen, V., Pöyhönen, S., Ketoja, E., Pitkänen, J. 2001. Abundance of the earthworm *Lumbricus terrestris* in relation to subsurface drainage pattern on a sandy clay field. *European Journal of Soil Biology*. vol. 37, no. 4, p. 301-304. https://doi.org/10.1016/S1164-5563(01)01105-0

Oszmianski, J., Kolniak-Ostek, J., Wojdyło, A. 2013. Characterization and content of flavonol derivarives of *Allium ursinum* L. plant. *Journal of Agricultural and Food Chemistry*, vol. 61, no. 1, p. 176-184. https://doi.org/10.1021/jf304268e PMid:23249145

Padmavathiamma, P. K., Loretta, Y. Li., Kumari, U. R. 2008. An experimental study of vermi-biowaste composting for agricultural soil improvement. *Bioresource Technology*. vol. 99, p. 1672-1681. https://doi.org/10.1016/j.biortech.2007.04.028

Rämert, B., Bugg, R. L., Clark, M. S., Werner, M. R., McGuinn, R. P., Poudel, D. D., Berry, A. M. 2002. Influence of *Lumbricus terrestris* inoculation on gree nmanure disappearance and the decomposer community in a walnut orchard. *Soil Biology and Biochemistry*, vol. 33, p. 1509-1516. https://doi.org/10.1016/S0038-0717(01)00066-9

Razaq, M., Zhang, P., Shen, H. L., Salahuddin. 2017 Influence of nitrogen and phosphorous on the growth and root morphology of *Acer mono*. *PLoS One*, vol. 12, no. 2, e0171321. https://doi.org/10.1371/journal.pone.0171321

Ražná, K., Khasanova, N., Ivanišová, E., Qahramon, D., Habán, M. 2018. Antioxidant properties of cumin (*Bunium Persicum* boiss.) extract and its protective role against ultrasound-induced oxidative stress tested by microrna based markers. *Potravinarstvo Slovak Journal of Food Sciences,* vol. 12, 2018, no. 1, p. 11-19. https://doi.org/10.5219/838

Santos, C., Fonseca, J., Aires, A., Coutinho, J., Trinidade, H. 2017. Effect of different rates of spent coffee grounds (SCG) on composting process, gaseous emissions and quality of end-product. *Waste Management*, vol. 59, p. 37-47. https://doi.org/10.1016/j.wasman.2016.10.020

Scheuerell S. J. 2004. Compost tea production practices, microbial properties, and plant disease suppression. In *Soil and compost eco-biology*, León–Spain, SoilAce : Spain, p. 41-51.

Spurgeon, D. J., Keith, A. M., Schmidt, O., Lammertsma, D. R., Faber, J. H. 2013. Landuse and land-management change: relationships with earthworm and fungi communities and soil structural properties. *BMC Ecoogy*. vol. 13, 46. https://doi.org/10.1186/1472-6785-13-46

Xiang, H., Zhang, J., Guo, L., Zhao, B. 2016. In situ earthworm breeding in orchards significantly improves the growth, quality and yield of papaya (*Carica papaya* L.). *PeerJ*, 4:e2752; https://doi.org/10.7717/peerj.2752

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VARIATION OF FRUITS MORPHOMETRIC PARAMETERS OF *ELAEAGNUS MULTIFLORA* THUNB. GERMPLASM COLLECTION

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ABSTRACT

The aim of this study was to determine morphometric parameters of fruits within some genotypes of *Elaeagnus multiflora* Thunb., which are growing in the Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of NAS of Ukraine (NBG). Their morphometric parameters were following: fruit weight from 0.32 to 1.89 g, fruit length from 7.60 to 19.54 mm, fruit diameter from 4.39 to 10.32 mm, seed weight from 0.10 to 0.41 g, seed length from 7.40 to 13.30 mm, seed diameter from 1.34 to 5.07 mm. The shape indexes of fruits and seeds were found ranging from 1.25 to 1.56 and from 2.90 to 4.04, respectively. The analysis of coefficient of variation showed the difference of variability in morphometric characteristics between *Elaeagnus multiflora* samples. Data showed that the most variability of important selection characteristics is the average cumulative seeds weight – from 18.72 to 36.61%, seeds diameter – from 10.46 to 24.29%, fruits weight – from 9.15 to 22.24%. The other characteristics are more or less stable. Collected quantitative data were subjected to principal hierarchical cluster analysis. The cluster analysis of morphometric parameters exhibited that the *Elaeagnus multiflora* genotypes fell into two clusters. These cluster analysis results suggest that broad morphologic diversity was found in *Elaeagnus multiflora* genotypes examined in this study. The introduction population of the *Elaeagnus multiflora*, was created in the M.M. Gryshko National Botanical Garden in Kyiv, has a sufficient potential for successful selection work. These preliminary results could open the interest of farmers in this *Elaeagnus multiflora* and will be precedent for future domestication and introduction of the species in the agroproductive system in Ukraine and the rest of the world.

Keywords: *Elaeagnus multiflora*; fruits; seeds; morphometric parameters

INTRODUCTION

Species of the genus *Elaeagnus* L. belong to the family *Elaeagnaceae* Juss. *Elaeagnus multiflora* Thunb. (cherry elaeagnus, cherry silverberry, goumi, gumi) has long been grown in China, Korea, and Japan a has for centuries been cultured as a decorative as well as for food and medicinal plant **(You et al., 1994)**. This species is known in Chinese traditional medicine **(Sakamura and Suga, 1987)**, where some diseases such as a cough, foul sours, diarrhoea, itch and cancer have been treated for a long time **(Lee et al., 2007)**. The fruits and leaves have characterized by high level of carbohydrate, crude protein, lipid, ash, reducing sugars, soluble proteins, and polyphenols **(Hong et al., 2006a; Yoon et al., 2007; Bieniek et al., 2017)**. The organic acids found in fruits were acetic, citric lactic, and malic, succinic acids. The content of citric acid was the highest among organic acids **(Hong et al., 2006a)**.

Fruits exhibit the antioxidant and anti-inflammatory activities **(Hong et al., 2006b; Chang et al., 2006; Lee et al., 2007, 2011)**, antiproliferative **(Kim et al., 2007; Lee et al., 2010)**, anticancer **(Lee et al., 2010)**, antimicrobial **(Patel, 2015)**. **Kim et al. (2014)**, these results suggest that *Elaeagnus multiflora* fruit extract is a potential possibility of application as a whitening functional cosmetic material through repression of melanin biosynthesis.

Biologically active compounds are not only in fruits but in different parts of the plant: bark, leaves, flowers, seeds **(Shin et al., 2008; Patel, 2015)**. *Elaeagnus multiflora* seeds are considered to be a candidate for preventative and dietetic treatment as an anticancer functional food **(Kim, Oh and Lee, 2008)**. The leaves, fruits and young branches of *Elaeagnus multiflora* could be exploited as phenolic, antioxidant additives and as nutritional supplements for prolonging existence **(Ismail et al., 2015)**. The fruits of *Elaeagnus multiflora* are used in fresh condition and from them are prepared pastes, jams, compote.

The unique biochemical characteristics of *Elaeagnus multiflora* are well documented. However, it is insufficient information about the morphological variability of *Elaeagnus multiflora* fruits. It is important to study the genetic variability of fruits for improving selected characteristics in the future.

The aim of this study was to separate, based on our research, the best genotypes from our collections *Elaeagnus*

multiflora, which can be successfully grown on plantations and can be utilized in future plant breeding programs.

Scientific hypothesis

In our experiment we have been support that fruit phenotyping variability of evaluated genotypes collection cherry elaeagnus not predomined only cultivation conditions but also genetical features.

MATERIAL AND METHODOLOGY

Locating trees and data collection

The objects of the research were 30-year-old plants of *Elaeagnus multiflora*, which are growing in the Forest-Steppe of Ukraine in M. M. Gryshko National Botanical Garden of NAS of Ukraine (NBG). They are well adapted to the climatic and soil conditions. Observations on the collections genotypes of *Elaeagnus multiflora* in the period 2016 – 2017 were performed during mass fruiting. We have described 10 genotypes (referred as EM-01 to EM-10) of *Elaeagnus multiflora*.

Morphometric characteristics

Pomological characteristics were conducted with four replications on a total 30 fruits per genotypes. In the study only one plant (bush) used for per genotype. The following measurements were taken: fruit weight, in g, fruit length, in mm, fruit diameter, in mm and seed weight, in g, seed length, in mm, seed diameter, in mm. Data, we are working with, were tested for normal distribution.

Statistical analyses

Basic statistical analyses were performed using PAST 2.17; hierarchical cluster analyses of similarity between phenotypes were computed on the basis of the Bray-Curtis similarity index; multi-dimensional scaling (MDS) analyses were performed in PRIMER **(Clarke and Gorley, 2006)**. Variability of all these parameters was evaluated using descriptive statistics. Level of variability determined by **Stehlíková (1998)**.

RESULTS AND DISCUSSION

Primarily, selection work of *Elaeagnus multiflora* started in Russia where are known the most widespread cultivars

Figure 1 Fruitage of *Elaeagnus multiflora* Thunb.

Figure 2 Variability in the shape of *Elaeagnus multiflora* Thunb. fruits.

Figure 3 Variability in the shape of *Elaeagnus multiflora* Thunb. seeds.

Table 1 The variability of some morphometric parameters of fruits for the whole collection of *Elaeagnus multiflora* Thunb. genotypes.

Characteristics	*Unit*	*n*	*min*	*max*	*mean*	*CV%*
Fruit weight	g	300	0.32	1.89	0.95	31.85
Fruit length	mm	300	7.60	19.54	10.39	11.88
Fruit diameter	mm	300	4.39	10.32	7.55	15.38
Seed weight	mm	300	0.10	0.41	0.25	24.90
Seed length	mm	300	7.40	13.30	10.77	8.09
Seed diameter	mm	300	1.34	5.07	2.95	19.46

Note: n – the number of measurements; min, max – minimal and maximal measured values; mean – arithmetic mean; CV – coefficient of variation (%).

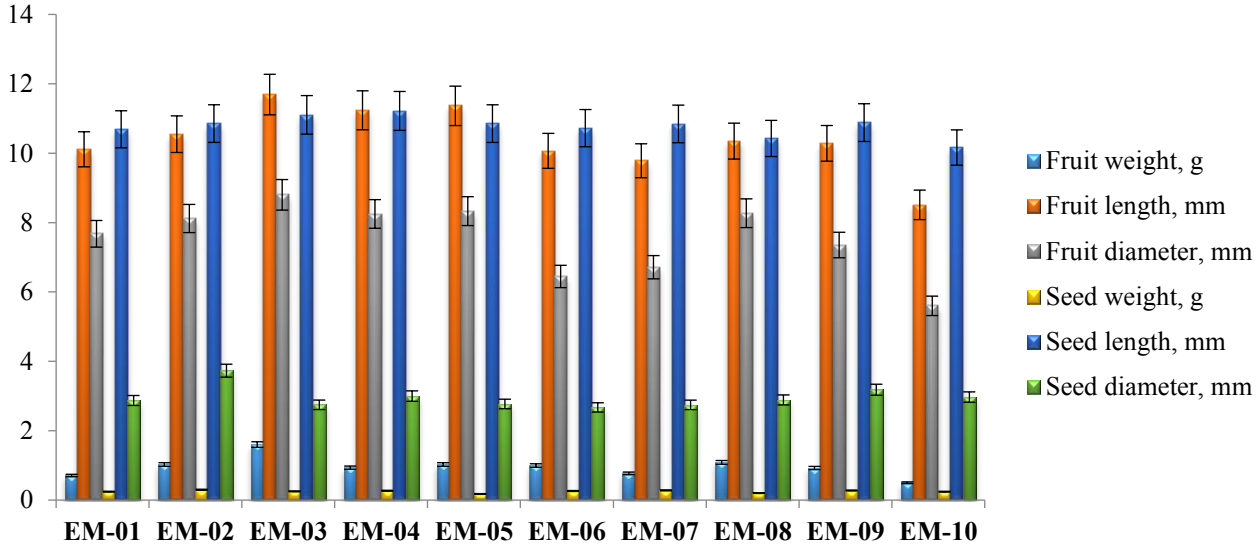

Figure 4 Mean values for various morphometric parameters of fruits and seeds of *Elaeagnus multiflora* Thunb. genotypes.

such as Sachaliński pierwyj, Moneron, Taisa, Kril'on, Szikotan, Juznyj, Kunaszir, Cunai, Paramushir. Cultivar Sweet Scarlet cultivates in the Europe and USA.

The collection of *Elaeagnus multiflora* has created at the M.M. Gryshko National Botanical Garden since 1980 – 1982. The primary material (seeds from free pollination) was imported from Sakhalin (Sakhalin Scientific Research Institute of Agriculture). Nowadays the collection of *Elaeagnus multiflora* includes 45 genotypes. We selected the most promising genotypes of this species.

The weight of the whole fruit is one of the significant production characteristics of plant species. These parameters of the *Elaeagnus multiflora* fruit varied significantly. The images of *Elaeagnus multiflora* fruits and seeds of various genotypes are shown in Figure 1, 2, 3. High variability of the size and shape of these fruits and seeds are evident.

The weight of *Elaeagnus multiflora* fruits of the present study was in the range from 0.32 (EM-10) to 1.89 (EM-03) g (Table 1, Figure 4).

The coefficient of variation was 31.85%, which shows a very high degree of variability of fruit weight. Investigations of **Bieniek et al. (2017)** established the range of fruits weight of variety from 1.03 to 1.29 g.

The fruit length in our analyses was determined in the range from 7.60 (EM-10) to 19.54 (EM-03) mm. The value of the coefficient of variation was 11.88%, which shows an average degree of variability of fruit weight. **Bieniek et al. (2017)** determined the length of the fruits in the range from 1.24 to 1.28 cm.

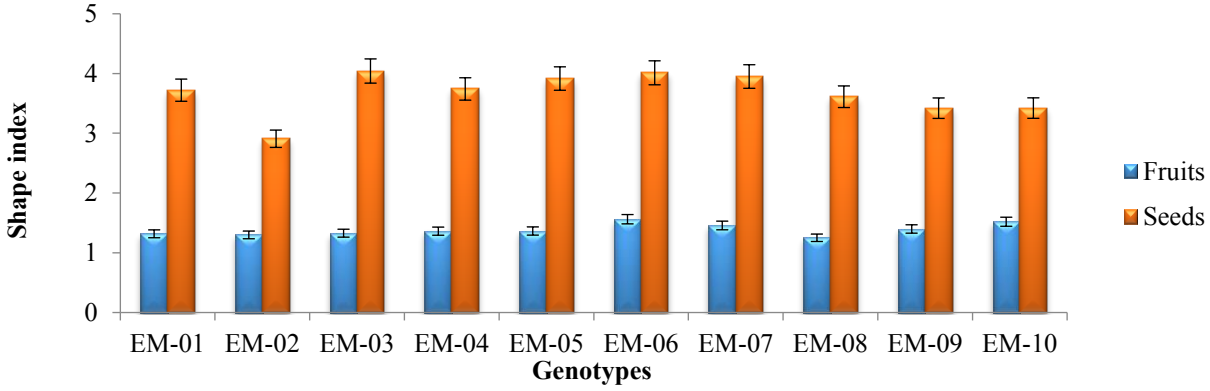

Figure 5 Comparison of the tested *Elaeagnus multiflora* Thunb. genotypes in the shape index of fruits and seeds.

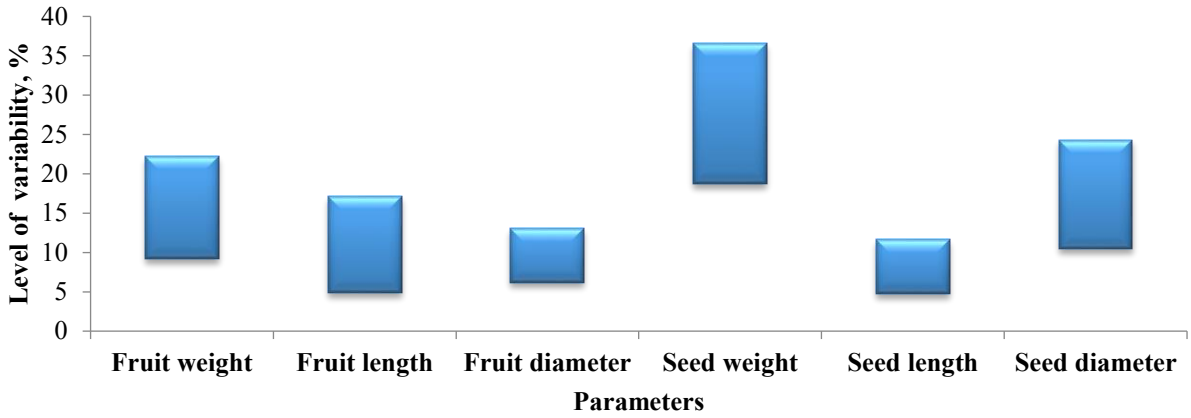

Figure 6 Level of the variability of morphometric parameters of fruits and seeds *Elaeagnus multiflora* Thunb. (%).

Table 2 The matrix of Pearson correlation coefficients for 15 pairs of variables.

Parameters	Fruit weight	Fruit length	Fruit diameter	Seed weight	Seed length
Fruit length	0.619*				
Fruit diameter	0.647*	0.689*			
Seed weight	0.054*	0.015	-0.056		
Seed length	0.160	0.202*	0.171*	0.157*	
Seed diameter	-0.040	0.020	0.080	0.170*	0.233*

Note: Significant according to the *t*-test (p <0.05).

In our experiments, the fruit diameter was determined in the range from 4.39 (EM-10) to 10.32 (EM-01) mm (Table 1). The variation coefficient (15.38%) confirmed an average degree of variability within the collection.

The seed weight in our analyses was determined in the range from 0.10 (EM-08) to 0.41 (EM-02) g. The value of the coefficient of variation was 24.90%, which shows a very high degree of variability of fruit weight. Investigations of **Bieniek et al. (2017)** established the range of seed weight of varieties from 0.10 to 0.12 g.

Seed length was identified in the range from 7.40 (EM-10) mm to 13.30 (EM-02) mm (Table 1). The variation coefficient characterizes the average degree of variability within the testable collection.

Seed diameter was identified in the range from 1.34 (EM-06) to 5.07 (EM-09) mm. The value of the coefficient of variation fixed the high degree of variability of this characteristic.

The shape of each object can be characterized by the shape index, i.e. the length to width ratio. Figure 5 represents the shape indexes of fruits and seeds. The shape index of the fruits was found in the range from 1.25 (EM-08) to 1.56 (EM-06). The shape index of the seed – ranged from 2.90 (EM-02) to 4.04 (EM-03), so the genotypes collection demonstrates significant variability in the shape of the seed, as seen in Figure 2. These parameters can be used for the identification of the genotypes.

The analysis of coefficient of variation showed the difference of variability of morphological signs between

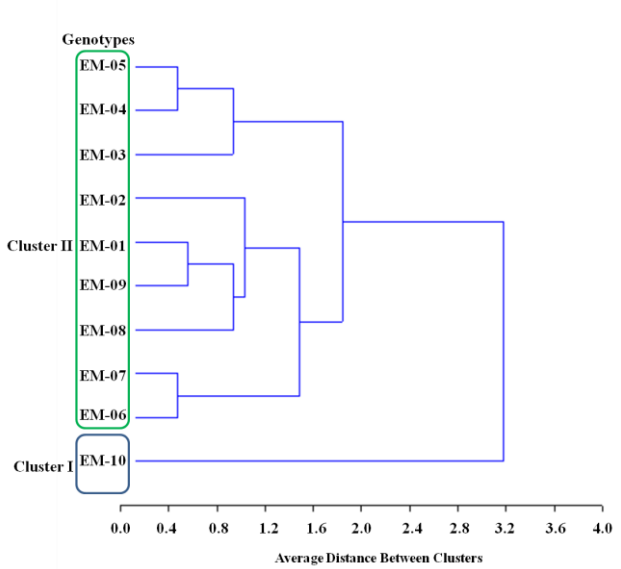

Figure 7 Cluster dendrogram based on morphometrics parameters of *Elaeagnus multiflora* Thunb. fruits genotypes.

Elaeagnus multiflora samples (Figure 6). Data showed that the most variable important selection signs are the seeds weight – from 18.72 to 36.61%, seeds diameter – from 10.46 to 24.29%, fruits weight – from 9.15 to 22.24%. These results indicate the promise of breeding in this way of investigations. The stable signs are seed length – from 4.77 to 11.66%.

The results indicated moderate positive correlations between the fruit diameter and the fruit length (r = 0.689), fruit weight (r = 0.647), fruit length and fruit weight (r = 0.619) (Table 2). The slight correlation was found between the seed diameter and seed length (r = 0.233).

The cluster analysis on the morphological characters have been carried out earlier for studying the genetic variability of some other plant species **(Milotic et al., 2005; Henderson, 2006; Abdali et al., 2014; Al-Ruqaie et al., 2016; Krishnapillai and Wijeratnam, 2016; Martinez-Nicolas et al., 2016; Grygorieva et al., 2017; Vinogradova et al., 2017)**.

The *Elaeagnus multiflora* genotypes were divided into two main clusters cluster I and cluster II (Figure 7). Cluster I contained the genotype (EM-10) only, which differs from other genotypes of collection by all parameters.

Cluster II was further sub-divided into two sub-clusters: A and B. Sub-cluster A was further sub-divided into sub-sub clusters A_1 and A_2. In sub-sub-cluster A_1 EM-06 and EM-07 were closely linked whereas in sub-sub-cluster A_2 EM-09 and EM-01 were connected in the same group, while EM-08 and EM-02 were linked as an outliner. Sub-cluster B contained only two *Elaeagnus multiflora* genotypes EM-04 and EM-05 were connected in the same group while EM-03 linked as an outliner.

CONCLUSION

Evaluating of 10 genotypes of *Elaeagnus multiflora* determined the weight of the fruits in the range from 0.32 to 1.89 g, fruit length from 7.60 to 19.54 mm, fruit diameter from 4.39 to 10.32 mm, seed weight from 0.10 to 0.41 g, seed length from 7.40 to 13.30 mm, seed diameter from 1.34 to 5.07 mm. Data showed that the most variability of important selection characteristics found for average cumulative seeds weight – from 18.72 to 36.61%, seeds diameter – from 10.46 to 24.29%, fruits weight – from 9.15 to 22.24%.

This study is significant as first selection work in Ukraine. Obtained results are important for breeding new varieties of *Elaeagnus multiflora* as well as their practical use. Study of adaptation characteristics will also be required for the selected *Elaeagnus multiflora* genotypes. The results of the study are helpful for understanding the variability and attempting the selection of superior desirable *Elaeagnus multiflora* accessions for bringing to commercial cultivation.

REFERENCES

Abdali, N., Ataei, S., Keihan, A., Motaghi, L., Naghavi, M., Kazemitabar, S. K., Rezazade, A., Hosseini-Mazinani, M. 2014. Comparative study of the morphological and molecular characteristics of 10 Iranian olive (*Olea europaea* L.) cultivars. *Annals of Biological Research*, vol. 5, no. 3, p. 76-83.

Al-Ruqaie, I., Al-Khalifah, N. S., Shanavaskhan, A. E. 2016. Morphological cladistic analysis of eight popular Olive (*Olea europaea* L.) cultivars grown in Saudi Arabia using Numerical Taxonomic System for personal computer to detect phyletic relationship and their proximate fruit composition. *Saudi Journal of Biological Sciences*, vol. 23, no. 1, p. 115-121. https://doi.org/10.1016/j.sjbs.2015.05.008 PMid:26858547

Bieniek, A., Piłat, B., Szałkiewicz, M., Markuszewski, B., Gojło, E. 2017. Evaluation of yield, morphology and quality of fruits of cherry silverberry (*Elaeagnus multiflora* Thunb.) biotypes under conditions of north-eastern Poland. *Polish Journal of Natural Science*, vol. 32, no. 1, p. 61-70.

Clarke, K., Gorley, R. 2006. PRIMER v6: User Manual/Tutorial. *Plymouth,* UK : *Primer-E,* Ltd. 192 p.

Grygorieva, O., Klymenko, S., Vergun, O., Hudz, N., Nikolaieva, N., Schubertová, Z., Palamarchuk, O., Brindza, J. 2017. Morphological characteristics and determination of volatile organic compounds of *Diospyros virginiana* L. genotypes fruits. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 612-622. https://doi.org/10.5219/808

Henderson, A. 2006. Traditional morphometrics in plant systematics and its role in palm systematics. *Botanical Journal of the Linnean Society*, vol. 151, no. 1, p. 103-111. https://doi.org/10.1111/j.1095-8339.2006.00526.x

Hong, J. Y., Nam, H. S., Kim, N. W., Shin, S. R. 2006a. Changes on the components of *Elaeagnus multiflora* fiuits during maturation. *Korean Journal of Food Preservation*, vol. 13, no. 2, p. 228-233.

Hong, J. Y., Nam, H. S., Lee, Y. S., Yoon, K. Y., Kim, N. W., Shin, S. R. 2006b. Study on the antioxidant activity of extracts from the fruit of *Elaeagnus multiflora* Thumb. *Korean Journal of Food Preservation*, vol. 13, 413-419.

Chang, Z. Q., Park, S. C., Oh, B. C., Lee, Y. S., Shin, S. R., Kim, N. W. 2006. Antiplatelet aggregation and anti-inflammatory activity for extracts of *Elaeagnus multiflora. Korean Journal of Medicinal Crop Science*, vol. 51, no. 1, p. 516-517.

Ismail, M., Hussain, M., Mahar, S., Iqbal, S. 2015. Investigation on Total Phenolic Contents of *Elaeagnus multiflora*. *Asian Journal of Chemistry*, vol. 27, no. 12, p. 4587-4590. https://doi.org/10.14233/ajchem.2015.19244

Kim, S. A., Oh, S. I., Lee, M. S. 2007. Antioxidative and cytotoxic effects of solvent fractions from *Elaeagnus multiflora*. *The Korean Journal of Food and Nutrition*, vol. 20, no. 2, p. 134-142.

Kim, S. T., Kim, S. W., Ha, J., Gal, S. W. 2014. *Elaeagnus multiflora* fruit extract inhibits melanin biosynthesis via regulation of tyrosinase gene on translational level. *Research Journal of Biotechnology*, vol. 9, no. 4, p. 1-6.

Krishnapillai, N., Wijeratnam, R. S. W. 2016. Morphometric analysis of mango varieties in Sri Lanka. *Australian Journal of Corp Science*, vol. 10, no. 6, p. 784-792. https://doi.org/10.21475/ajcs.2016.10.06.p7223

Lee, J. H., Seo, W. T., Cho, K. M. 2011. Determination of phytochemical contents and biological activities from the fruits of *Elaeagnus multiflora*. *Preventive Nutrition and Food Science*, vol. 16, no. 1, p. 29-36. https://doi.org/10.3746/jfn.2011.16.1.029

Lee, M. S., Lee, Y. K., Park, O. J. 2010. Cherry silver berry (*Elaeagnus multiflora*) extracts exert antiinflammatory effects by inhibiting COX-2 and Akt signals in HT-29 colon cancer cells. *Food Science and Biotechnology*, vol. 19, no. 6, p. 1673-1677. https://doi.org/10.1007/s10068-010-0237-1

Lee, Y. S., Chang, Z. Q., Oh, B. C., Park, S. C., Shin, S. R., Kim, N. W. 2007. Antioxidant activity, anti-inflammatory activity, and whitening effects of extracts of *Elaeagnus multiflora* Thunb. *Journal of Medicinal Food*, vol. 10, no. 1, p.

126-33. https://doi.org/10.1089/jmf.2006.145 PMid:17472476

Martinez-Nicolas, J. J., Melgarejo, P., Legua, P., Garcia-Sanchez, F., Hernández, F. 2016. Genetic diversity of pomegranate germplasm collection from Spain determined by fruit, seed, leaf and flower characteristics. *PeerJ.*, vol. 4, p. e2214. https://doi.org/10.7717/peerj.2214 PMid:27547535

Milotic, A., Setic, E., Persuric, D., Poljuha, D., Sladonja, B., Brscic, K. 2005. Identification and characterization of autochthonous olive varieties in Istria (Croatia). *Annales Ser. His. Nat.*, vol. 15, no. 2, p. 251-256.

Kim, S., Oh, S., Lee, M. 2008. Antioxidative and Cytotoxic Effects of Ethanol Extracts from *Elaeagnus multiflora*. The *Korean Journal of Food and Nutrition*, vol. 4, p. 403-409.

Patel, S. 2015. Plant genus *Elaeagnus* : underutilized lycopene and linoleic acid reserve with permaculture potential. *Fruits*, vol. 70, no. 4, p. 191-199. https://doi.org/10.1051/fruits/2015014

Sakamura, F., Suga, T. 1987. Changes in chemical components of ripening oleaster fruits. *Phytochemistry*, vol. 26, no. 9, p. 2481-2484. https://doi.org/10.1016/S0031-9422(00)83858-3

Shin, S. R., Hong, J. Y., Yoon, K. Y. 2008. Antioxidant properties and total phenolic contents of cherry elaeagnus (*Elaeagnus multiflora* Thunb.) leaf extracts. *Food Science and Biotechnology*, vol. 17, no. 3, p. 608-612.

Stehlíková, B. 1998. *Basics of biostatiscics (Biodiversity protection 51)*. Nitra, Sloavkia : SPU, 79 p. ISBN 80-7137-539-X.

Vinogradova, Yu., Grygorieva, O., Vergun, O., Brindza, J. 2017. Morphological characteristics for fruits of *Aronia mitschurinii* A. K. Skvortsov & Maitul. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 754-760. https://doi.org/10.5219/845

Yoon, K. Y., Hong, J. Y., Shin, S. R. 2007. Analysis on the Components of the *Elaeagnus multiflora* Thunb. Leaves. *Korean Journal of Food Preservation*, vol. 14, no. 6, p. 639-644.

You, Y. H., Kim, K. B., An, Ch. S., Kim, J. H., Song, S. D. 1994. Geographical Distribution and Soil Characteristics of Elaeagnus Plants in Korea. *The Korean Journal of Ecology*, vol. 17, no. 2, p. 159-170.

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BIOCHEMICAL COMPOSITION OF SWEET CHERRY (*PRYNUS AVIUM* L.) FRUIT DEPENDING ON THE SCION-STOCK COMBINATIONS

Galina Upadysheva, Svetlana Motyleva, Ivan Kulikov, Sergei Medvedev, Mariia Mertvishcheva

ABSTRACT

The results of the field and biochemical researches of sweet cherry *Prunus avium* L. scion Chermashnaya fruit grown on 8 clonal stocks (Kolt, Moskoviya, Izmaylovskiy, Stepnoy rodnik, AVCH-2, VSL-2, VTS-13, V-5-88) are presented. Trees productivity, the fruit average weight and the stone- fruit weight relation were studied, fruit degustation evaluation was fulfilled and the main biochemical parameters such as soluble solids, sugars sum, ascorbic acid concentration and antioxidant activity were determined. The influence of the stock on the fruit biochemical composition, the trees productivity and the fruit weight was determined. The sweet cherries productivity was 12,5 kg/tree at average, and depending on the stock it varied from 8,3 kg/tree (V-5-88) to 18,6 kg/tree (Izmaylovskiy). The biggest fruit were found on the trees with stocks VSL-2, Izmaylovskiy and AVCH-2. At average the proportion of the stone in the fruit weight was 5.3% with the variation depending on the stock from 5.2% (Kolt) to 5.5% (V-5-88, Stepnoy rodnik). According to the degustation results the fruit of scion Chermashnaya were characterized by the sweet harmonized taste and smell (4.5 points), there were not found either taste deterioration or bitterness on any stock. The best results according to the degustation evaluation by the parameters complex were found at scion Chermashnaya combinations on stocks Izmaylovskiy, VSL-2 and Kolt. The soluble solids content also depended on the stock and varied from 13.9 (Chermashnaya on AVCH-2) to 17.2% (Chermashnaya on Izmaylovskiy) at average value 16.0%. The sugars content in the sweet cherry fruit was within the range from 9.0 to 12.4%. The sugars higher accumulation belonged to the combinations of Chermashmaya on Stepnoy rodnik (11.1%) and Chermashmaya on Izmaylovskiy (12.4%). The ascorbic acid content in the sweet cherry fruit did not exceed 8 mg.100 g^{-1}. Depending on the stock this parameter varied from 5.9 to 9.3 mg.100 g^{-1}. The highest vitamin C content was found in the fruit at the grafting on the stock Stepnoy rodnik, the lowest one – on the stock Moskoviya. The sweet cherry fruit antioxidant activity according to DPPH method was not high and was in the range from 11.8 to 13.8%.

Keywords: sweet cherry; clonal stock; stock/scion combinations; biochemical structure of fruit; antioxidant activity

INTRODUCTION

Sweet cherry is one of the most perspective and popular stone fruit crops. The advantages of the sweet cherry fruit for the health nutrition are rather high mostly because of the concentration of such phenolic compounds as procyanidins, anthocyans and phenolic acids **(Liu et al., 2011; Usenik et al., 2010)**. Natural antioxidants such as soluble solids and vitamin C make a contribution into the struggle against oxidative stress **(Birt et al., 2001; Harborne and Williams, 2000; Halliwell et al., 2005)**. The researches have shown that the consumption of fruit with high antioxidant activity can low the risk of cancer (Kang et al., 2003) and other diseases **(Jacob et al., 2003)**. At the present time thanks to winter-hardy variety breeding it is cultivated not only in the southern areas, but northward as well – in the Central region of Russia **(Upadysheva, 2009)**. It is appreaciated for the high eating qualities of the fruit, their curative and dietic properties **(Morozova and Upadysheva, 2014)**. The fruit of the northern sweet cherry scions are smaller, but the concentration of the main nutritional and biologically active substances is equal to the southern scions **(Motyleva et al., 2016; Upadyshev, 2008; Zhbanova et al., 2015)**. Everywhere sweet cherry is cultivated in grafted culture, and in the last years generally on clonal stocks. The stock influences the grafted plants growth and development, but there is no consensus about the correlation between the fruitage quality and the stock **(Kamzolova et al., 1999; Upadysheva, Kolpakov, 2009)**. The majority of the stock forms have hybrid parentage and form bitter, not-edible fruit that may cause the deterioration of the grafted scions fruit taste and quality.

The purpose of our work is to study the productivity and sweet cherries fruit quality depending on the stock.

Scientific hypothesis

The biochemical composition, the sweet cherry fruit quality and yield depending on the stock-scion combination are not studied. We have checked the influence of the stock on the formation of yield, quality and nutritional value of scion Chermashnaya fruit grown in Moscow region conditions. We supposed that it is possible to achieve the productivization and the fruit quality characteristics improvement.

MATERIAL AND METHODOLOGY

The researches place and methods

The field researches were held in 2015 – 2017 on the experimental sweet cherry plantations of Federal State Budgetary Scientific Institution "All-Russian Horticultural Institute for Breeding, Agrotechnology and Nursery", Moscow in Figure 1.

The plantations overall area is 0,5 ha. The experimental researches object was the fruit of the sweet cherry scion Chermashnaya grafted on 8 clonal stocks (Kolt, Moskoviya, Izmaylovskiy, Stepnoy rodnik, AVCH-2, VSL-2, VTS-13, V-5-88). Not less than 5 trees on each stock were studied.

The biochemical researches were held in the Laboratory of Physiology and Biochemistry of Federal State Budgetary Scientific Institution "All-Russian Horticultural Institute for Breeding, Agrotechnology and Nursery".

The determination of the productivity and fruit weight and the sensor evaluation

In the period of fruit ripening the trees productivity was determined by the fruitage weighing from each tree in five time repetition. The average fruit weight and the stone-fruit weight relation were determined by the weighing of 100 fruit in three time repetition. The sensor evaluation was fulfilled by the group of high qualified specialists for the evaluating products. They estimated three main quality parameters: taste (sweet, sour, with bitterness), after taste and fruit external appearance – the form, colour, the surface condition.

Chemicals

All chemical substances chosen for the analysis were of analytical sort and were bought from Sigma Aldrich (USA) and Merck KgaA (Germany).

Sample preparation

From average 300 g probe 100 g fruit without stone were prepared and extracted by double-distilled water (to determine antioxidant activity) and metaphosphoric acid (for ascorbic acid determination) with the help of high-speed homogenizer (10 000 rpm, 1 min, UltraTurrax T25 Basic, IKA). After centrifugation at 4000 g (Sigma, Germany) within 10 min the supernatant was used for measuring. The extraction as well as the measurements were held in three time repetition.

Basic chemical analyses

The soluble solids content (SSC) was expressed by the index of refraction (°Bx). The sugars sum content – by Bertran method **(Ermakova, 1987)**.

Ascorbic acid (AsA) determination

Ascorbic acid determination was held using HELC method **(Stan et al., 2014)**, the chromatograph KNAUER (Germany) was used. Chromatoghraphic conditions: HELC column Silasorb C18 (5 mkm), 150 x 4.0 mm (Biohimmac, Russia), the column temperature is 25 ° C, flow speed 1,0 ml min-1, the detector UV, the wave length l = 251 nm, the mobile phase MeOH: water – 5:95 (r./r.), aliquote for injections 20 mkl, the retention time Rt = 4.4 mm.

Antioxidant activity (AA) determination

Antioxidant activity was measured by the **Brand-Williams et al. (1995)** method using a compound DPPH˙ (2,2-diphenyl-1-pikrylhydrazyl). The spectrometr Thermo

Figure 1 The sweet cherry plantations, blooming period.

Figure 2 The fruiting combinations of Chermashmaya on Izmaylovskiy.

Helios Ɣ (USA) was used. The homogenized by the distilled water samples were put on the shaker Lab-PU-01 (Russia) for 8 hours, and then they were filtered and the antioxidant activity was measured in 10 minutes after interaction between the extract and reagent at wavelength 515 nm.

The calculation of antioxidant activity values was fulfilled using the formula:

Inhibiting DPPH = (AC - AAt) = AC / 100 (%),

where:

AC – DPPH solution absorption;

AAt – absorption at the antioxidant presence.

Three time repetition.

Statisic analysis

As a minimum three repetitions of the analysis were held and the results were shown as arithmetic average with standard deviation (±SD). To determine the differences significance between the data one-way ANOVA test was used (p <0.05) via the program Statgraphics Centurion XV (USA).

RESULTS AND DISCUSSION

As a result it was determined that the sweet cherry trees productivity 12,5 kg/tr. at average and depending on the stock varied from 8,3 kg/tr. (V-5-88) to 18.6 kg/tr. (Izmaylovskiy) Figure 2.

Higher than the average value this parameter was at the trees grafted on the stocks Izmaylovskiy, VSL-2, Moscoviya and AVCH-2. It should be noted that the single tree fruitage reduction was observed not only for the weak-grown combination with stock V-5-88, but for the strong-grown VTS-13 stocks (Table 1). Scion Chermashnaya as the majority of early ripening northern scions has medium-sized fruit. A single fruit weight at average value of 4.1 g depending on the stock changed from 3.6 g (V-5-88) to 4.4 g (VSL-2). The fruit were significantly bigger under the influence of stocks VSL-2, Izmaylovskiy and AVCH-2. The portion of the stone in the fruit weight was 5.3% at average with variation depending on the stock from 5.2% (Kolt) to 5.5% (V-5-88, Stepnoy rodnik). According to the degustation results the fruit of scion Chermashnaya were characterized by the sweet harmonized taste and smell (4.5 points), no taste deterioration was observed on either of the stocks. The best degustation evaluation parameters in the complex were found at Chermashnaya combinations on stocks Izmailovskiy, VSL-2 and Kolt.

Sweet cherry fruit were characterized by the high content of soluble solids and sugars, which depended on the stock-scion combination. The soluble solids content variated from 13.9% (Chermashnaya on AVCH-2) to 17.2% (Chermashnaya on Izmaylovskiy) at average value of 16.0% (Figure 3).

The sugars content in the fruit were within 9.0 – 12.4%. The sugars higher accumuilation were found at Chermashnaya on Stepnoy rodnik (11.1%) and Chermashnaya on Izmaylovskiy (12.4%) (Figure 4).

Table 1 The productivity and organoleptic estimation of sweet cherry scion Chermashnaya fruit depending on the stock at average in 2015 – 2017.

Stock	Productivity, kg/tree	Parameters under study		
		Average fruit weight, g	Stone-fruit weight relation, %	Degustation evaluation, point
VSL-2	12.7	4.4	5.4	4.7
V-5-88	8.3	3.6	5.5	4.0
Izmaylovskiy	18.6	4.3	5.3	4.7
Moskoviya	13.4	4.0	5.2	4.3
AVCH-2	13.1	4.3	5.3	4.3
VTS-13	10.9	4.1	5.3	4.4
Stepnoy rodnik	11.6	4.1	5.5	4.5
Kolt	11.5	4.0	5.2	4.7
NSR_{05}	1.7	0.1	0.05	0.2

While evaluating the stock influence there should be noted the reduction of the soluble solids content on stocks AVCH-2, Moskoviya, VSL-2 and VTS-13 on 10% at average and the sugars content on AVCH-2, Moskoviya, VCL-2 and V-5-88 on 14.4% in comparison with Kolt, Stepnoy rodnik and Izmaylovskiy. The correlation coefficient between the soluble solids content and the sugars sum is high, r 0.88.

At average AsA content in sweet cherry scion Chermashnaya fruit is not relatively high – 8.1 mg.100 g^{-1}. However, depending on the stock this parameter was varied from from 5.9 to 9.3 mg.100 g^{-1}. The highest AsA content was fixed on the stock-scion combination Chermashnaya on Stepnoy rodnik. The lowest

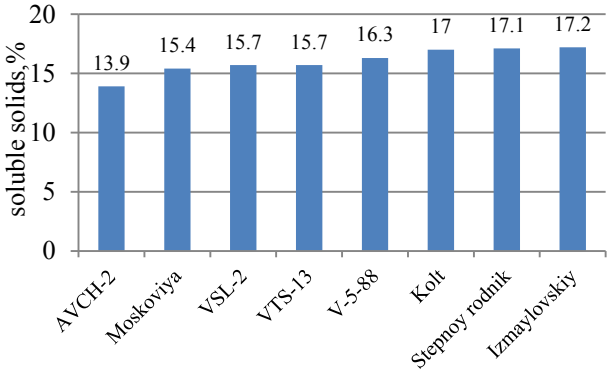

Figure 3 SS content in sweet cherry scion Chermashnaya fruit on different stocks.

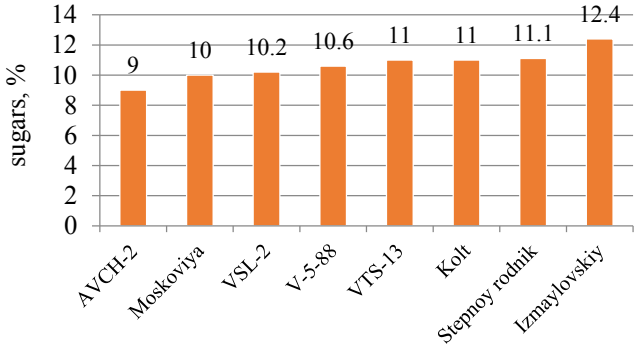

Figure 4 The sugars sum content in sweet cherry scion Chermashnay fruit on different stocks.

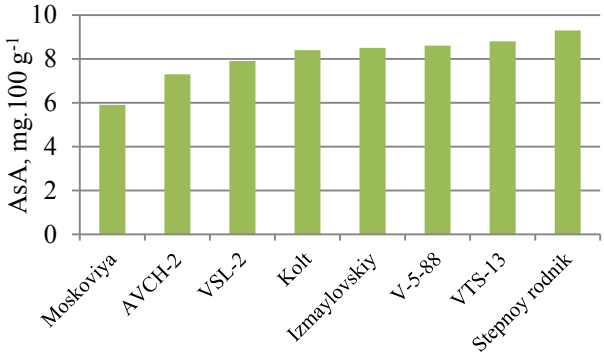

Figure 5 AsA content in sweet cherry scion Chermashnaya on different stocks.

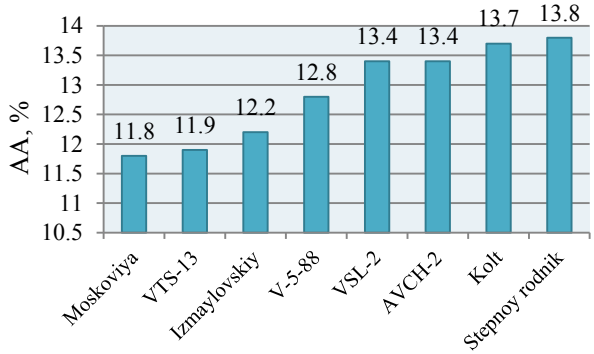

Figure 6 AA content in sweet cherry scion Chermashnaya fruit on different stocks.

accumulation was marked on Chermashnay on Moskoviya combination (Figure 5).

AA of sweet cherry fruit liquid extracts that determines their value for the functional nutrition was nearly 12,9% at average. The range of values depending on the used stock was - 9% (Moskoviya and VTS-13) and +7% (Kolt and Stepnoy rodnik) (Figure 6).

CONCLUSION

In the present paper the main focus was given to the study of the essential biochemical parameters that characterize the nutritional and dietic value of sweet cherry scion Chermashnaya fruit grown on different stocks. As the result of the researches the stock influence on the sweet cherry scion Chermashnaya fruit biochemical composition was identified, the limits of AsA, AA, sugars and soluble solids content variation depending on the used stock were determined. AsA and AA highest content was found in sweet cherry fruit on Stepnoy rodnik stock. According to the sugars sum values Chermashnaya on Izmaylovskiy stock-scion combination can be emphasized. While grafting on Moskoviya and AVCH-2 stocks the reduction of the parameters under study was observed. Using the data of the field and laboratory researches the conclusion can be made that the optimal stocks for scion Chermashnaya are Stepnoy rodnik, Izmaylovskiy and Kolt. The results received while working at this paper give new information about the stock influence on the biochemical characteristics of the scion fruit.

REFERENCES

Birt, D. F., Hendrich, S., Wang, W. 2001. Dietary agents in cancer prevention: Flavonoids and isoflavonoids. *Journal Pharmacology and Therapeutics*, vol. 90, no. 2-3, p. 157-177. https://doi.org/10.1016/S0163-7258(01)00137-1

Bushway, R. J., King, J. M., Perkins, B., Krishnan, M. 1988. High-Performance Liquid Chromatographic Determination of Ascorbic Acid in Fruits, Vegetables and Juices. *Journal of Liquid Chromatography*, vol. 11, no. 16, p. 3415-3423. https://doi.org/10.1080/01483918808082264

Horak, M., Golias, J., Nic, P., Nemcova, A., Kulichova, J. 2016. The effect of post-harvest treat ment on the qualily of seet cherries during storage. *Potravinarstvo Slovak Journal of Food Science*, vol.10, no 1, p. 570-577. https://doi.org/10.5219/667

Halliwell, B., Rafter, J., Jenner, A. 2005. Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects. Antioxidant or not. *The American Journal of Clinical Nutrition*, vol. 81, no. 1, p. 268-276. https://doi.org/10.1093/ajcn/81.1.268S

Harborne, J. B., Williams, C. A. 2000. Advances in flavonoid research since 2000. *Phytochemistry*, vol. 55, no. 6, p. 481-504. https://doi.org/10.1016/S0031-9422(00)00235-1

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. https://doi.org/10.1093/jn/133.6.1826 PMid:12771324

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/S0304-3835(02)00583-9

Kamzolova, O. I, Statskevich, I. M., Yaroshevich, I. V., Lipskaya, S. L. 1999. The chemical composition of plump fruit depending on the system of the soil content and stock-scion combination. *Fruit culture: Fruit culture BSRI scientific papers*, vol.12. p. 152-154.

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

Morozova, N. G., Upadysheva, G. Yu. 2014. The aspects of the sweet cherry growing in Moscow region. *Journal Gardening and wine growing*, vol. 3. p. 17-22.

Motyleva, S. M., Upadysheva, G. Yu., Mertvishcheva, M. E. 2016. The eduction of the signal function of ascorbic acid and substances-antioxidants in sweet cherry leaves under different stocks influence. In *Plants phytoimminity and cell signalization: Thes. rep. IV Russian symposium*. Kazan. p. 99-100.

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

Ermakova, A. I. L. 1987. *The methods of the plants biochemical researches*. Russia : Agropromizdat. 430 p.

Upadyshev, M. T. 2008. The role of phenolic compounds in the life processes of garden plants : monography. Moscow, Russia : VSTISP. 319 p. ISBN 978-5-902178-41-5.

Upadysheva, G. Yu. 2009. The sweet cherry productivity on clonal stocks. *Journal Gardening and wine growing*, vol. 4, p. 45-47.

Upadysheva, G. Yu., Kolpakov, N. S. 2009. The change of cherry fruit biochemical composition and quality under the stock influence. *Agricultural science*, vol. 4. p. 20-21.

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

Zhbanova, E. V, Savelev, N. I., Kovalenko, T. V., Kulikov, V. N. 2015. *The biochemical characteristicsof the modern sweet cherry and apricot corps in the conditions of the central part of Russia. Selection, seedage and technology of fruit-berry cultures and potato: scientific papers collection*. T.17. Chelyabinsk, Russia : FSBSI SUSRIGP. p. 20-27. ISBN 978-5-87184-633-9.

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PHYSICAL, THERMAL AND FUNCTIONAL PROPERTIES OF FLOUR DERIVED FROM UBI GEMBILI (*DIOSCOREA ESCULENTA* L.) TUBERS GROWN IN INDONESIA

Diah Susetyo Retnowati, Andri Cahyo Kumoro, Ratnawati Ratnawati

ABSTRACT

Gembili (*Dioscorea esculenta* L.) tuber is one of the important food sources in term of cultural, nutritional and economic perspectives for the people in the Africa, Caribbean islands, Asia, and Oceania regions. The tubers can be eaten after being boiled, roasted, fried or cooked. However, there is lack of information on the advanced utilizations of the tubers as raw material in the manufacture of modern foods. This study aims to characterize the physical, functional and thermal properties of Gembili flour, so that this information can be used as the basis in the development of novel foods. The microstructure, crystallinity, gelatinization temperature, swelling power and solubility of the flour were determined. The electron microscope observation revealed that Gembili flour consisted of smooth surface oval granules, which were light brown in color and having 23 μm average diameter. They are comprised of polygonals or some clusters of irregular fragments. Similar to most of tuber flours, Gembili flour also exhibited B-type crystallinity with approximately 31 ±3.7% crystallinity. The gelatinization temperature of Gembili flour was high and being comparable to that of cereal flour. The enthalpy of gelatinization of Gembili flour (9.52 ±0.80 $J.g^{-1}$) was comparable to that of *Dioscorea alata*. Unfortunately, Gembili flour exhibited low swelling power (3.90 ±0.01 $g.g^{-1}$). The Gembili flour granules were highly soluble in water (11.07 ±0.05%). Based on those reported properties, Gembili flour can be a suitable raw material for the manufacture of bakery, cookies, noodle and infant foods.

Keywords: *Dioscorea esculenta*; flour; physicochemical properties; functional properties; thermal properties

INTRODUCTION

Yams are the edible tubers of numerous species of the genus Dioscorea, which contain high content of starch as excellent sources of caloric energy **(Coursey and Ferber, 1979)**. The primary cultivation areas of yams are in the West, some parts of East, Central and Southern Africa **(FAO, 2001)**, which produce about 95% of the total world's yam production. The second largest yams producing areas are located in the Asian regions including China, Japan, Indonesia, Malaysia, Philippines and Oceania. The third growing areas include the Caribbean, Mexico, and parts of Central America **(FAO, 2001)**. Uwi (*Dioscorea alata*) and Gembili (*Dioscorea esculenta* L.) are the two main yam species commonly found in Indonesia, which can be harvested after being cultivated for 7 – 9 months. They are seasonal crops and generally planted in the end of September to October. The yellowing leaves and withered vines are strong indications of mature crop, which is usually ready to harvest around May to July **(Senanayake et al., 2012)**. Figure 1 (a) and (b) presents the Gembili plant and its tuber. Further, **Senanayake et al., (2012)** reported that the flour Gembili tubers planted in Sri Lanka contains 10.39 ±0.15% moisture, 1.50 ±0.20% lipid, 9.02 ±0.65% protein, 2.10 ±0.20% ash, 2.33 ±0.15% fiber and 74.66 ±0.66% carbohydrate. Similarly, **Ukpabi (2010)** also reported a comparable proximate composition of the flour of *D. esculenta* grown in Nigeria. Unfortunately, yam tubers are known to contain different toxic substances that affect both human and animals when they are consumed, despite their high nutritional values **(Polycarp et al., 2012)**. **Yang and Lin (2008)** reported that the age, the cultivar, the geographic locality of a plant or the storage condition after harvest could significantly affect its anti-nutritional content.

In most parts of Indonesia, yams tubers are only consumed as additional foods after being boiled, steamed, roasted, fried, baked or cooked. In contrast, yams are utilized as staple foods **(Coursey and Ferber, 1979)** and being important sources of ingredients for manufactured foods, which play a key role in the socio-economic and cultural lives of both growers and consumers in many

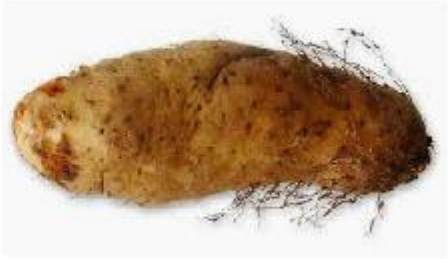

Figure 1 Ubi Gembili plant (a) and its tuber (b).

tropical countries in West Africa, the Caribbean islands, Asia, and Oceania **(Girardin et al., 1998)**. Primarily, the utilizations of yam have been limited to the preparation of traditional dishes, such as pounded yam and porridges. To prepare pounded yam, the yam tubers are usually sun-dried and powdered into flour for reconstituting into a stiff paste (amala), which is consumed with preferred vegetable soup **(Awoyale et al., 2010)**. In regard to their fiber contents, yam flours also provide health benefits especially in the prevention of obesity, constipation, cardiovascular disease, diabetes and colon cancer **(Chen et al., 2003)**. Interestingly, the absence of gluten has promoted yam flours as promising nutrition sources for those who suffer celiac disease (CD) **(Van Hung and Morita, 2005)**.

Considering that yam tubers are highly perishable and bulky, they are commonly processed into flour and starch as the most acceptable forms, in which they are usually consumed or stored. These types of product provide a higher possibility to prolong the supply of yam during the off-season, thereby decreasing the loss during storage and reducing the cost for marketing and transportation **(Coursey and Ferber, 1979)**. However, the potential utilizations of yam flours have not been fully understood, mainly due to lack of general knowledge on the suitable processing techniques and product development as well as the physicochemical and functional properties of these plant materials. Flour characteristics, such as granule size, crystallinity, swelling power and solubility pattern, pasting behavior and gelatinization properties are important in the design and manufacture of high quality food products **(Aprianita et al., 2014a)**. This valuable information could also enable the food processors to modify the flours if necessary to accommodate the product and processing demands. Interestingly, flour and starch from tubers and roots can be used to substitute wheat flour in certain food applications, especially in the manufacture of biscuits and bakery products such as cookies, bread and cakes to reduce the production cost **(Adeleke and Odedeji, 2010)**.

Due to its large population, Indonesia requires a relatively high quantity of rice and wheat as the main sources of carbohydrates, which is mostly fulfilled by import and triggered a large financial burden. This high import dependency is mainly caused by the unsuitability of the tropical climate for the cultivation of wheat in this country. Improving our knowledge of the properties of Gembili tubers grown in Indonesia and many other countries may result in the wider applications in the food or non-food industry. This will also contribute to the reduction of dependence on wheat flour as the main source of carbohydrate in Indonesia and other non-wheat growing countries.

Scientific hypothesis

The objective of this research was to determine the physical, thermal, and functional properties of Gembili (*D. esculenta*) tuber flour as an important part of efforts to widen the possible applications of Gembili flour within the food industries, particularly biscuits and noodles.

MATERIAL AND METHODOLOGY

Samples of plant material

The freshly harvested 9 months age Gembili tubers grown in Gunungpati, Semarang-Indonesia (geographical location: +7°5'13.39" S latitude, +110°21'27.69" E longitude and 285 m altitude) were purchased from the traditional market in Semarang-Indonesia, in the year 2017.

Chemicals and standard

Reagent grade of chemicals used for browning prevention (potassium metabisulfite) and Gembili flour characterization were the products of Merck-Indonesia and were purchased from an authorized chemical distributor in Semarang-Indonesia. Analytical grade (petroleum ether) and standard (amylose) with a purity >98 % manufactured by Merck (Germany) were used.

Gembili flour preparation

The Gembili tubers were thoroughly washed with clean tap water to remove adhering soil and other undesirable materials from the yam, and to reduce microbial growth on the final product. They were peeled and trimmed to remove defective parts, washed, and grated with semiautomatic machine to obtain thin slices (±5 mm). Then, the slices were soaked in potassium metabisulfite solution (0.075%) for 1 hour to prevent browning. After being washed with flowing water, the tuber slices were spread in a single layer on drying trays and dried in an air convection oven at 40 ºC for 3 days and were subsequently crushed in a locally fabricated crusher, milled into flour with hammer mill to obtain flour. The flour was then passed through -180 μm +250 μm sieves. Only the Gembili flour retained on the 250 μm was used in this

experiment. The produced flour was stored in zip-lock polyethylene bags and kept in covered plastic containers at 20 °C for further uses.

Flour properties analysis

Color measurement

The color analysis was determined by Hunter notation system, which characterized by three color parameters as L*, a*, and b*. L* is a measure of the degree of lightness from 0 (black) to 100 (white) color. The a* value states the red-green color (red is declared from 0 to +100 and green is stated from 0 to -80). Finally, the b* value defines components on the yellow-blue axis value (0 to +70 for yellow and 0 to -70 for blue). The color of flours was measured by Konica Minolta chromameter CR 400 as suggested by **Akissoe et.al. (2003)**.

Granule microstructure

The morphology and surface of Gembili flour granules were observed by scanning electron microscope (SEM JSM-6510 LA). Prior to SEM analysis, the flour samples were freed of granule clumps by sieving through a 250 μm mesh and spread evenly on Cambridge type circular aluminum stubs with carbon electro-conductive doubled-sided adhesive tape (Electron Microscopy Science, Hatfield, PA, USA) through a thorough inspection using a stereoscopic microscope (Carl Zeiss, Stemi 2000-C, Wek Gottingen, Germany), before being coated with gold powder (10 nm) for 60 s at 50 mA under vacuum using a EMS500 sputter coater (Electron Microscopy Science, Hatfield, PA, USA) to make the sample conductive. The freed clumps and gold coated flour granules within a horizontal field width of 54.08 μm were scanned and photographed using the method and conditions as suggested by **Jayakody et al. (2007)**, except the magnification of 2000x was used in this research.

Granule crystallinity

The Prior to X-ray diffraction analysis, the Gembili flours were kept in a desiccator (at 25 ^{o}C) over saturated K_2SO_4 solution (aw = 0.98) up to sorption equilibrium (3 weeks). X-ray diffractograms were obtained with a Rigaku RPT 300 PC X-ray diffractometer (Rigaku-Denki Co., Tokyo, Japan). The crystallinity patterns of Gembili flours were recorded with X-ray diffraction at Bragg angle 2θ between 4 – 40 ^{o} using a procedure previously described by **Jayakody et al. (2007)**. The crystallinity of the flour was then quantitatively estimated following the method of **Nara and Komiya (1983)** by using a software package (Orion-version 6.0 Microcal Inc., Northampton, MA, USA).

Thermal properties

The Thermal analysis of flour Gembili was conducted by differential scanning calorimetry (DSC) using a Seiko differential scanning calorimeter (DSC 210) (Seiko Instruments Inc., Chiba, Japan) equipped with a thermal analysis data station and data recording software. Before being subjected to DSC analysis, the Gembili flour of 20% water content was prepared by addition of 11 μL deionized water using a microsyringe to 3 mg flour sample (dry basis) in the DSC pans, which were then sealed, reweighed and allowed to stand overnight at room temperature at room temperature before analysis to ensure the equilibration of sample and water. The scanning temperatures range and heating rate applied in this analysis were respectively 25 – 300 ^{o}C and 10 ^{o}C.min^{-1}, as previously used by **Jayakody et al. (2007)**. The measurements were carried out under a dynamic nitrogen atmosphere (30 mL.min^{-1}) in pierced aluminum pans to avoid condensation. In all measurements, the thermogram was recorded with an empty aluminum pan as a reference.

Swelling power and solubility

The functional properties of Gembili flour, i.e. swelling power and solubility were measured by the method of **Afoakwa et al. (2012)**. Swelling power is defined as the weight (g) of the swollen sediment per g of dry flour, while solubility is expressed as the percentage (by weight) of the flour sample that is dissolved molecularly after being heated in water at 60 ^{o}C.

Statisic analysis

All measurements were conducted in tripilicates and the data obtained were expressed as mean ±Standard deviation. Significant differences between the mean values at significance level p <0.05 were compared using Student`s test using MS Excel version 2010.

RESULTS AND DISCUSSION

Color observation

Color may attribute to the quality of foodstuffs material, such as the degree of maturity and spoiledness. Finally, the color of food also affects the consumer's impressions. The color characteristics (L*, a*, and b*) of Gembili tuber flour are presented in Table 1. As a comparison, the same color characteristics of wheat flour are also tabulated in Table 1.

Visually, the Gembili flour is light brown in color and is even darker than the commercial wheat flour as indicated by L* value **(Ukpabi, 2010).** Lightness of flours can be affected by browning reactions, which occur during its processing, and this may have extensively affected Gembili flour and reduced its lightness. The

Table 1 Comparison of color parameters of Gembili flour and other flours.

Color parameter	This work	*D. esculenta*[a]	wheat flour[b]
L*	87.22 ±0.12	84.09 ±0.20	94.99 ±0.10
a*	1.84 ±0.00	-3.06 ±0.03	-0.78 ±0.00
b*	11.19 ±0.03	9.87 ±0.04	9.76 ±0.00

[a]Polycarp et al., (2016), [b]Ukpabi (2010).

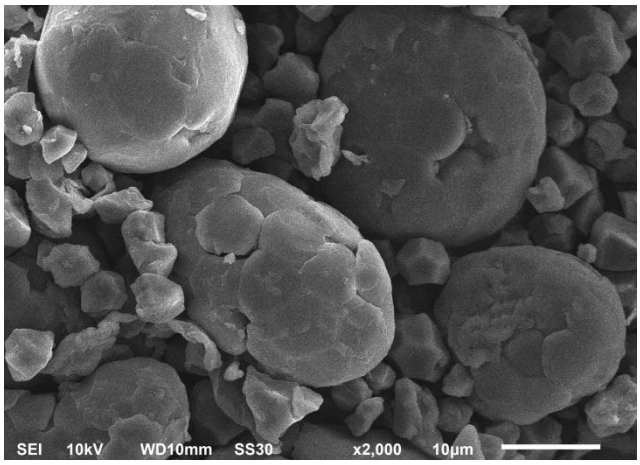

Figure 2 Scanning electron microscopy (magnification 2000×) of Gembili flour.

yellowness/blueness index (b*) value observed for Gembili flour was slightly higher than both commercial wheat flour and *D. esculenta* tuber flour grown in Ghana. This result confirms that Gembili flour appeared more yellowish than commercial wheat and *D. esculenta* tuber flours. Apart from the inherent color pigments present in yams, yellowness in yam flours has also been linked to total phenol content and the activity of polyphenoloxidase **(Akissoé et al., 2003)**. The redness/greenness index (a*) of Gembili flour was far higher than commercial wheat flour and *D. esculenta* tuber flour grown in Ghana. If the color of a food product is one of the important criteria, then the use of native Gembili flour for its manufacture should be less considered. This is because usually white flours are more preferred in various applications (as in white bread making). Theoretically, the substitution of wheat flours with Gembili flour will reduce the whiteness, but will increase the redness and yellowness of the flour composites **(Aprianita et al., 2014a)**.

Granule morphology

The scanning electron micrographs of Gembili flour is shown in Figure 2. It can be observed that the flour granules are oval in shape, which consists of polygonal or irregular form of fragments. Some fragments were scattered and were likely to be the result of the breakdown of the bigger oval structure. The surface of the granules appeared to be smooth and presented no evidence of fissures. According to observation by **Aprianita et al. (2014b)**, the smaller granule could be related to particle clusters. The average sizes of the oval structure and the polygonal fragments were 23 µm and 6 µm, respectively. With smaller starch granules size, the digestibility of Gembili flour is fairly high **(Szylit et al., 1978)**. Similarly, **Jayakody et al. (2007)** also observed that *D. esculenta* starch granules were polygonal in shape and the surfaces were smooth. The starch granules size of *D. esculenta* grown in Kukula, Java-ala, and Nattala was between 3 to 10 µm. In contrast, **Aprianita et al. (2014b)** observed a bigger average granule size of *D. alata* flour, which a high proportion of larger granules of 345 µm and a small portion of granules having 28 µm in size.

X-ray diffraction patterns

Figure 3 shows the crystallographic pattern of Gembili flour. The pattern has strongest and broad peaks centered at 17.1° and 24.2°, also has moderate peak at 14.9°, and weak peaks at 5.5° and 26° for 2θ angles. The crystallinity of Gembili flour was around 31 ±3.7%. These characteristics indicate that Gembili flour falls in the B type starch category **(Brunnschweiler et al., 2005)**. This observation is in good agreement with **Jayakody et al. (2007)** who explained that most of tuber flours, including *D. esculenta* flours exhibit B type starch. In general,

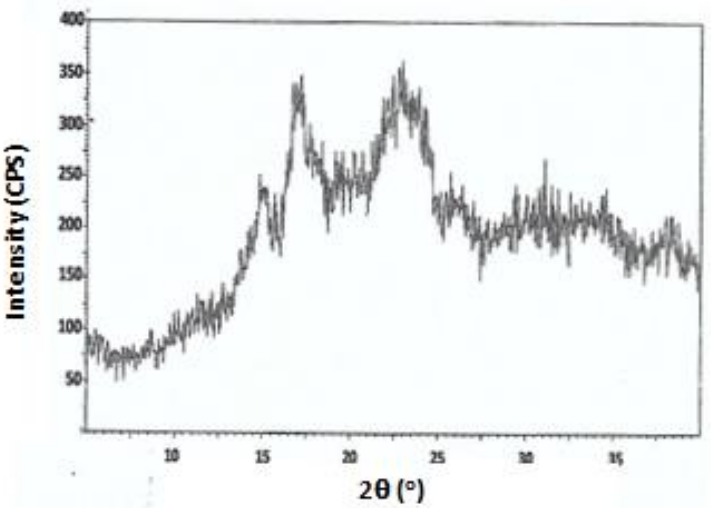

Figure 3 X-ray diffraction pattern of Gembili flour.

Table 2 The value of T_o, T_p, T_c and ΔH_{Gel} of some Dioscorea flours.

Botanical species (origins)	ΔT_o (^oC)	ΔT_p (^oC)	ΔT_c (^oC)	ΔH_{Gel} ($J.g^{-1}$)	Reference
D. alata (Indonesia)	62.00 ±1.72	73.90 ±0.94	81.80 ±1.12	7.40 ±0.82	**Aprianita et al. (2014b)**
D. alata (Brazil)	71.50 ±0.30	76.30 ±0.10	81.80 ±0.40	11.90 ±1.70	**Alves et al. (2002)**
D. esculenta (Indonesia)	74.34 ±0.20	79.65 ±0.50	85.83 ±0.10	9.52 ±0.80	This work
D. dumetorum (Nigeria)	NA	72.80 ±2.79	NA	NA	**Owuamanam et al. (2013)**
Low Protein Wheat	60.60 ±1.80	64.10 ±1.10	69.60 ±2.50	5.70 ±0.80	**Aprianita et al. (2014a)**
High Protein Wheat	57.80 ±1.50	63.90 ±1.10	70.30 ±1.50	5.00 ±0.90	**Aprianita et al. (2014a)**

Table 3 Swelling power and solubility of some Dioscorea flours.

Botanical species (origins)	Swelling power ($g.g^{-1}$)	Solubility (%)	Reference
D. dumetorum (Nigeria)	2.22 ±0.01	1.93 ±0.01	**(Abiodun et al., 2014)**
D. bulbifera (Côte d'Ivoire)	2.60 ±0.01	10.20 ±0.06	**(Achyet al., 2017)**
D. alata (Indonesia)	3.00 ±0.23	6.51 ±0.02	**(Harijono et al., 2013)**
D. esculenta (Indonesia)	3.90 ±0.01	11.07 ±0.05	This work
D. hispida Dennst (Indonesia)	4.67 ±0.18	6.53 ±0.15	**(Kumoro et al., 20102)**
D. rotundata (Ghana)	13.06 ±0.22	5.88 ±0.11	**(Tortoe et al., 2017)**
American Wheat	7.33 ±0.41	6.80 ±0.42	**(Chung et al., 2010)**

starches with B-type of crystallinity, such as sweet potato, taro, arrowroot and cassava have higher digestibility compared to canna and konjac flours that have A-type of crystallinity **(Liu et al., 2007)**. The high digestibility of Gembili flour is confirmed by high solubility value as discussed in the last section of this manuscript.

Thermal properties

The disruption of solid structure can be studied by heating with the presence of small amount water through differential scanning calorimetry (DSC). In this processes, the flour sample was heated at various temperature and heating rate. The onset (T_o), peak (T_p), conclusion (T_c) gelatinization temperatures and entahlpi of gelatinization (ΔH_{Gel}) of Gembili flour obtained from DSC analysis, and those of other dioscorea flours are tabulated in Table 2.

The gelatinization temperature of Gembili flour obtained in this research is slightly higher than that of *D. alata* planted in East Java – Indonesia **(Aprianita et al., 2014b)**, but significantly higher than *D. alata* farmed in Brazil **(Alves et al., 2002)** and *D. dumetorum* grown in Nigeria **Owuamanam et al. (2013)**. A number of factors may influence gelatinization temperature, including the molecular architecture of amylopectin, the formation of lipid complexes, degrees of crystallinity, and the proportion of crystalline regions **(Aprianita et al., 2014b)**. The high initial gelatinization temperature of Gembili flour indicates that the granules were slow in swelling due to high resistant swelling of the starch granules **(Alves et al., 2002)** and therefore requires longer cooking time. Gelatinization temperature of Gembili flour was higher than that of wheat flour **(Aprianita et al. (2014a)**, which indicates a higher stability of Gembili flour compared to that of wheat flour **(Srichuwong et al., 2005)**. The Gembili flour gelatinized at a high temperature range that could bring about its application as a thickening agent in retort foods or foods that require heat stable viscosity **(Tattiyakul et al., 2006)**.

Swelling power and solubility

The functional properties of the yam flours are important since they affect the end use of the flours. Swelling power is the ability of flour to absorb water and hold it in the swollen flour granule, whereas the solubility of flour is related to the extent of leaching of amylose out of starch granules during swelling and affected by intermolecular forces and the presence of surfactants and other related substances **(Moorthy, 2002)**. Swelling and solubility depend on the characteristic of the flour granules, such as granule size, the size distribution, amylose/amylopectin ratio and mineral content **(Singh et al., 2003)**. Both properties (swelling and solubilization) contribute to the some of characterictics of food product and play important role in the determination of flour's applications. Table 3 presents the swelling power and solubility of some dioscorea flours at 60 ^oC.

The swelling power of Gembili flour was lower than other yam flours, such as *D. rotundata* grown in Africa **(Tortoe et al., 2017)** and D. hispida Dennst **(Kumoro et al., 20102)**, but slightly higher than *D. dumetorum* **(Abiodun et al., 2014)**, *D. bulbifera* **(Achyet al., 2017)** and *D. alata* **(Harijono et al., 2013)**. According to **Schoch and Maywald (1968**) flour classification, Gembili flour falls in the highly-restricted swelling. **Aprianita et al. (2014b)** found that the swelling powers of *D. alata* var Krimbang flours were not significantly different at temperature range from 60 to 90 ^oC, at which the granules maintained their integrity. Lower value of swelling power of starch of the Gembili flour might be attributed to the protein-amylose complex formation in bean isolated starch and flour **(Pomeranz, 1991)**. This characteristic is desirable for the manufacture of value-added products

such as noodles and composite blends with cereals **(Garcia and Dale, 1999)**.

The solubility of Gembili flour is higher than any other dioscorea flours reported in the literature. The value is even higher than solubility of American wheat flour **(Chung et al., 2010)**. A higher value of solubility of flour signifies an improved digestibility. The high digestibility of Gembili flour might be beneficial for food preparations especially for infants and the elderly who require more readily digestible food **(Snow and O'Dea, 1981)**.

CONCLUSION

A comprehensive characterization of physicochemical, thermal and functional properties of Ubi Gembili (*Dioscorea esculenta* L.) flour has been successfully carried out. The Gembili flour was light brown incolor and was darker than commercial wheat flour. The Gembili flour granules were smooth surfaced oval structure having 23 µm average diameter, which comprised of polygonal or some clusters of irregular fragments. In accordance with typical tuber flours crystallinity, Gembili flour also exhibited B-type crystallinity with approximately 31 ±3.7% crystallinity. The gelatinization temperature of Gembili flour was high and being comparable to that of cereal flour. The enthalpy of gelatinization of Gembili flour (9.52 ±0.80 $J.g^{-1}$) was comparable to that of *D. alata*. Inherent with its crystallinity, Gembili flour exhibited low swelling power (3.90 ±0.01 $g.g^{-1}$). As expedted, the small size Gembili flour granules were highly soluble in water (11.07 ±0.05%). The low swelling power, but high solubility and gelatinization temperature suggests that Gembili flour is suitable for use as a raw material for the manufacture of bakery, cookies or noodle with hard bite and chewy texture and infant foods.

REFERENCES

Abiodun, O. A., Akinoso, R., Oluoti, O. J. 2014. Changes in functional and pasting properties of trifoliate yam flour during storage. *Journal of Applied Science & Environmental Management*, vol. 18, no. 2, p. 337-340. https://doi.org/10.4314/jasem.v18i2.26

Achy, J. Y., Ekissi, G. S. E., Koffi, P. K. B., Koné, F. M. T., Kouamé, L. P. 2017. Effect of boiling and baking times on the functional properties of aerial yam (*Dioscorea bulbifera*) flours cv Dugu-won har vested in Côte d'Ivoire. *International Journal of Agronomy and Agricultural Research*, vol.10, no. 2, p. 1-17.

Adeleke, R. O., Odedeji, J. O. 2010. Functional properties of wheat and sweet potato flour blend. *Pakistan Journal of Nutrition*, vol. 9, no. 6, p. 535-538. https://doi.org/10.3923/pjn.2010.535.538

Afoakwa, E. O., Budu, A., Asiedu, S., Chiwona-Karltun, C., Nyirenda, D. B. 2012. Viscoelastic properties and physicfunctional characterization of six high yielding cassava mosaic disease-resistant cassava (*Manihot esculenta* Crantz) genotypes. *Journal of Nutrition and Food Science*, vol. 2, no. 2, p. 129.

Akissoe, N., Hounhouigan J., Mestres C., Nago M. 2003. How blanching and drying affect the colour and functional characteristics of Yam (*Dioscorea cayenensis-rotunda*) flour. *Food Chemistry*, vol. 82, p. 257-264. https://doi.org/10.1016/S0308-8146(02)00546-0

Alves, R. M., Grossmann, M. V., Ferrero, C., Zaritky, N. E., Martini, M. N., Sierakoski, M. R. 2002. Chemical and functional characterization of products obtained from yam tubers. *Starch/Starke*, vol. 54, no. 10, p. 476-481. https://doi.org/10.1002/1521-379X(200210)54:10<476::AID-STAR476>3.0.CO;2-6

Aprianita, A., Vasiljevic, T., Bannikova, A., Kasapis, S. 2014a. Physicochemical properties of wheat-canna and wheat-konjac composite flours. *Journal of Food Science and Technology*, vol. 51, no. 9, p. 1784-1794. https://doi.org/10.1007/s13197-012-0696-x PMid:25190833

Aprianita, A., Vasiljevic, T., Bannikova, A., Kasapis, S. 2014b. Physicochemical properties of flours and starches derived from traditional Indonesia tubers and roots. *Journal of Food Science and Technology*, vol. 51, no. 12, p. 3669-3679. https://doi.org/10.1007/s13197-012-0915-5 PMid:25477633

Awoyale, W., Maziya-Dixon, B., Sanni, L. O., Shittu, T. A. 2010. Nutritional and sensory properties of amala supplemented with distiller's spent grain (DSG). *Journal of Food, Agriculture and Environment*, vol. 8, p. 66-70.

Brunnschweiler, J., Luethi, D., Handschin, S., Farah, Z., Escher, F., Conde-Petit, B. 2005. Isolation, physicochemical characterization and application of yam (*Dioscorea spp*.) starch a thickening and gelling agent. *Starch/Strarke*, vol. 57, p. 107-117.

Chen, H. I., Sheu, W. H. H., Tai, T. S., Liaw, Y. P. 2003. Konjac supplements alleviated hypercholesterolemia and hyperglycemia in type 2 diabetic subjects - a randomized double-blind trial. *Journal of the American College of Nutrition*, vol. 22, no. 1, p. 36-42. https://doi.org/10.1080/07315724.2003.10719273 PMid:12569112

Chung, S. Y., Han, S. H., Lee, S. W., Rhee, C. 2010. Physicochemical and bread-making properties of air flow pulverized wheat and corn flours. *Food Science and Biotechnology*, vol. 19, no. 6, p. 1529-1535. https://doi.org/10.1007/s10068-010-0217-5

Coursey, D. G., Ferber, C. E. M. 1979. The processing of Yams. In Pluckett, D. L. *Small-scale processing and storage of tropical root crops*. Boulder, Colorado, USA : Westview Press, p. 189-212. ISBN: 0891584174.

FAO. 2001. *FAO Production Yearbook 1999*, Rome, Italy : FAO. 321 p. ISBN-13: 9789250045207.

Garcia, M., Dale, N. 1999. Cassava root meal for poultry. *Journal of Applied Poultry Science*, vol. 8, no. 1, p. 132-137. https://doi.org/10.1093/japr/8.1.132

Girardin, O., Nindjin, C., Farah, Z., Eshcher, F., Stamp, P., Otokore, D. 1998. Effect of storage system and sprout removal on post-harvest yam (*Dioscorea spp*.) fresh weight losses. *Journal of Agriculture Science*, vol. 130, no. 3, p. 329-336. https://doi.org/10.1017/S0021859698005322

Harijono, E. T., Saputri, D. S., Kusnadi, J. 2013. Effect of blanching on properties of water yam (*Dioscorea alata*) flour. *Advance Journal of Food Science and Technology*, vol. 5, no. 10, p. 1342-1350. https://doi.org/10.19026/ajfst.5.3108

Van Hung, P., Morita, N. 2005. Physicochemical properties and enzymatic digestibility of starch from edible canna (*Canna edulis*) grown in Vietnam. *Carbohydrate Polymers*, vol. 61, no. 3, p. 314-321. https://doi.org/10.1016/j.carbpol.2005.04.021

Jayakody, L., Hoover, R., Liu, Q., Donner, E. 2007. Studies on tuber starches. II. Molecular structure, composition and physicochemical properties of yam (*Dioscorea* sp.) starches grown in Sri Lanka. *Carbohydrate Polymers,* vol. 69, no. 1, p.148-163. https://doi.org/10.1016/j.carbpol.2006.09.024

Kumoro, A. C., Retnowati, D. S., Budiyati, C. S., Manurung, T. and Siswanto. 2012. Water solubility, swelling and gelatinization properties of raw and ginger oil modified gadung (*Dioscorea hispida* Dennst) flour. *Research Journal of Applied Sciences, Engineering and Technology*, vol. 4, no. 17, p. 2854-2860.

Liu, Q., Donner, E., Yin, Y., Huang, R. L., Fan, M. Z. 2006. The physicochemical properties and in vitro digestibility of selected cereals, tubers, and legumes grown in China. *Food Chemistry*, vol. 99, no. 3, p. 470-477. https://doi.org/10.1016/j.foodchem.2005.08.008

Moorthy, N. S. 2002. Physicochemical and functional properties of tropical tuber starches: A review. *Starch – Stärke*, vol. 54, no. 12, p. 559-592. https://doi.org/10.1002/1521-379X(200212)54:12<559::AID-STAR2222559>3.0.CO;2-F

Nara, S., Komiya, T. 1983. Studies on the relationship between water saturated state and crystallinity by the diffraction method for moistened potato starch. *Starch*, vol. 35, no. 12, p. 111-114. https://doi.org/10.1002/star.19830351202

Owuamanam, C. I., Iwuoha, C. I., Onuegbu, N. G., Ogueke, C. C., Nwosu, J. N. 2013.Quality characteristics of processed flours from trifoliate yam (*Dioscorea dumetorum*) as influenced by steeping and boiling in varying concentration of Trona solution over time. *American Journal of Food Technology*, vol. 8, no. 3, p. 162-172. https://doi.org/10.3923/ajft.2013.162.172

Polycarp, D., Afoakwa, E. O., Budu, A. S., Otoo, E. 2012. Characterization of chemical composition and anti-nutritional factors in seven species within the Ghanaian yam (*Dioscorea*) germplasm. *International Food Research Journal*, vol. 19, no. 3, p. 985-992.

Pomeranz, Y. 1991. *Functional Properties of Food Components*. 2nd ed. NEW YORK, USA : Academic Press. 560 p. ISBN-13: 978-0125612814.

Schoch, T. J., Maywald, E. C. 1968. Preparation and properties of various legume starches. *Ceral Chemistry*, vol. 45, p. 564-573.

Senanayake, S. A., Ranaweera, K. K. D. S., Bamunuarchchi, A. Gunaratne, A. 2012. Proximate analysis and phytochemical and mineral constituents in four cultivars of yams and tuber crops in Sri Lanka. *Tropical Agricultural Research & Extension*, vol. 15, no. 1, p. 32-36. https://doi.org/10.4038/tare.v15i1.5240

Singh, N., Singh, J., Kaur, L., Sodhi, N. S., Gill, B. S., 2003. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry*, vol. 81, no. 2, p. 219-231. https://doi.org/10.1016/S0308-8146(02)00416-8

Snow, P., O'Dea, K. 1981. Factor affecting the rate of hydrolysis of starch in food. *American Journal of Clinical Nutrition*, vol. 34, no. 12, p. 2721-2727. https://doi.org/10.1093/ajcn/34.12.2721 PMid:6172034

Srichuwong, S., Sunarti, T. C., Mishima, T., Isono, N., Hisamatsu, M., 2005. Starches from different botanical sources II: Contribution of starch structure to swelling and pasting properties. *Carbohydrate Polymers*, vol. 62, no. 1, p. 25-34. https://doi.org/10.1016/j.carbpol.2005.07.003

Szylit, O., Durand, M., Borgida, L. P., Atinkpahoun, H., Prieto, F., Delort-Laval, J. 1978. Raw and steam- pelleted cassava, sweet potato and yam cayenensis as starch sources for ruminant and chicken diets. *Animal Feed Science Technology*, vol 3, no. 1, p.73-87. https://doi.org/10.1016/0377-8401(78)90025-1

Tattiyakul, J., Naksriarporn, T., Pradipasena, P., Miyawaki, O. 2006. Effect of moisture on hydrothermal modification of yam *Dioscorea hispida* Dennst starch. *Starch/Stärke*, vol. 58, no. 3-4, p. 170-176. https://doi.org/10.1002/star.200500462

Tortoe, C., Dowuona, S., Akonor, P. T., Dziedzoave, N. T. 2017. Examining the physicochemical, functional and rheological properties in flours of farmers' 7 key yam (*Dioscorea* spp.) varieties in Ghana to enhance yam production. *Cogent Food & Agriculture,* vol. 3, no. 1, p. 1371564. https://doi.org/10.1080/23311932.2017.1371564

Ukpabi, U. J. 2010. Farmstead bread making potential of lesser yam (*Dioscorea esculenta*) flour in Nigeria. *Australian Journal of Crop Science*, vol. 4, no. 2, p. 8-73.

Yang, D. J., Lin, J. T. 2008. Effects of different storage conditions on steroidal saponins in yam (*Dioscorea pseudojaponica* Yamamoto) tubers. *Food Chemistry*, vol. 110, p. 670-677. https://doi.org/10.1016/j.foodchem.2008.02.061

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MOLECULAR ANALYSIS OF BUCKWHEAT USING GENE SPECIFIC MARKERS

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ABSTRACT

Buckwheat (*Fagopyrium esculentum*) is a pseudo-cereal which has spread troughout the world and nowadays it represents cultural, economic and nutritionally important pseudocereal. It´s enviromentally friendly, characterized by high fiber, routine, protein and B vitamins, and is general-purpose. The goal of the present study was to analyze 17 genotypes of buckwheat by using 7 SCoT markers. In total, 52 fragments were detected, of which 38 were polymorphic. The average number of polymorphic fragments was 5.43. The most polymorphic fragments were detected in SCoT 26 and SCoT 29 markers, and the average percentage of polymorphism was 73.36 %. SCoT 29 reached the highest percentage of polymorphism (87.5 %) and SCoT 36 was lowest (60 %). The DI values ranged from 0.625 (SCoT 36) to 0.887 (SCoT 26) and the average DI value was 0.749. The average PIC value was 0.729 with PIC values ranging from 0.386 (SCoT 36) to 0.831 (SCoT 26). To determine the genetic diversity of 17 genotypes of the buckwheat, a dendrogram was created using the hierarchical cluster analysis. The genotypes were divided into two major clusters (I and II). Cluster I was divided into three other subgroups. Sixteen genotypes were included in cluster I and the genotype of Madawaska (USA) was genetically the farthest in cluster II. Genetically the closest were the varieties of Ballada (Russia) and Bamby (Austria). Used SCoT markers were sufficiently polymorphic, were able identify and differentiate chosen set of buckwheat genotypes.

Keywords: *Fagopyrium esculentum*; SCoT technique; genetic variability; DNA polymorphism; dendrogram

INTRODUCTION

Buckwheat (*Fagopyrium esculentum*), a diploid (2n = 16) annual, is a pseudo-cereal belonging to the family *Polygonaceae*. Buckwheat is an acient crop whose origins range from 5000 - 6000 years back in Asia. Common buckwheat is a traditional pseudo-cereal mainly grown in temperate regions of Asia, Europe, and North America. Due to short growth span, capability to grow at high altitudes, and the high quality protein of its grains it is an important crop in mountainous regions of India, China, Russia, Ukraine, Kazakhstan, parts of Eastern Europe, Canada, Japan, Korea, and Nepal **(Chrungoo et al., 2016)**. Recently common buckwheat is returning popular as a consequence of increasing gluten-free product market. The plant is a rich source of Zn, Cu, Mn, Se, vitamin B1, B2, E, and dietary proteins for gluten sensitive individuals and rich source of high biological-value proteins due to its balanced amino acids composition **(Wei et al. 2003; Stibilj et al. 2004)**. Common buckwheat seeds contain higher amounts of flavonoids, dietary fiber than cereals **(Przybylski and Gruczynska, 2009; Chen, 1999)**. As an important part of the human diet, common buckwheat seeds are a rich source of high biological-value proteins due to its balanced amino acids composition. The buckwheat grain is either consumed whole after boiling or steaming, or ground into a flour.

Despite the high nutritional and nutraceutical value of common buckwheat, the seed yield is low due to its self-incompatibility. Molecular breeding of common buckwheat has been impeded due to the lack of genomic resources and tightly linked markers for agronomically important genes. Molecular markers play an important role in genetic studies and marker-assisted selection (MAS) in crop breeding **(Shi et al., 2017)**.

Recently, the studies of genetic diversity are based mainly on the molecular analysis **(Žiarovská et. al., 2015; Vyhnánek et. al., 2015)**.

Worldwide collections of buckwheat were described by several types of dominant molecular markers, for example AFLP **(Yasui et al., 2004)**, **RAPD (Sharma and Jana, 2002)** and ISSR **(Kishore et al., 2013)**. Of the various DNA marker systems, start codon targeted (SCoT) polymorphism **(Collard and Mackill, 2009)** is gaining popularity for its superiority over other dominant DNA marker systems like RAPD and ISSR for higher polymorphism and better marker resolvability **(Gorji et al., 2011; Que et al. 2014; Satya et al., 2015; Zhang et al., 2015)**. A novel marker system called SCoT **(Collard**

and Mackill, 2009) was developed based on the short conserved region flanking the ATG start codon in plant genes. SCoT markers are generally reproducible, and it is suggested that primer length and annealing temperature are not the sole factors determining reproducibility. They are dominant markers like RAPDs and could be used for genetic analysis, quantitative trait loci (QTL) mapping and bulk segregation analysis **(Collard and Mackill, 2009)**. In principle, SCoT is similar to RAPD and ISSR because the same single primer is used as the forward and reverse primer **(Collard and Mackill, 2009; Gupta et al. 1994)**. Suitability of SCoT markers system has been successfully employed in genetic diversity analysis and fingerprinting of a number of agricultural and horticultural crop species, such as peanut **(Xiong at al., 2011)**, tomato **(Shahlaei et al., 2014)**, citrus **(Mahjbi et al., 2015)**, date palm **(Al-Qurainy et al., 2015)**, ramie **(Satya et al., 2015)**, castor **(Kallamadi et al., 2015; Vivodík et al., 2018)**, maize **(Vivodík et al., 2016)**, rye **(Petrovičová et al., 2017)**, mango **(Gajera et al., 2014)** and Indian jujube **(Singh et al., 2017)**, plantago **(Rahimi et al., 2018)**, taxus **(Hao et al., 2018)** and rose **(Agarwal et al., 2018)**.

Scientific hypothesis

The aim of our study was to detect genetic variability among the set of 17 buckwheat genotypes using 7 SCoT markers and to testify the usefulness of a used set of SCoT primers for the identification and differentiation of buckwheat genotypes. Molecular analyses are important source for crop breeders and can be useful for gene identification for crops improvement.

MATERIAL AND METHODOLOGY

Seventeen buckwheat (*Fagopyrium esculentum*) genotypes were used in the present study. Seeds of buckwheat were obtained from the Gene Bank of the Slovak Republic of the Plant Production Research Center in Piešťany.

Isolation of DNA

Genomic DNA of buckwheat cultivars was isolated from 100 mg freshly-collected leaf tissue according to GeneJET™ protocol (Thermo Scientific, 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.

PCR analysis

For analysis 7 SCoT primers were chosen (Table 2) according to the literature **(Collard a Mackill, 2009**). Amplification of SCoT fragments was performed according to **(Collard a Mackill, 2009)** (Table 2). Polymerase chain reaction (PCR) was performed in 15 µl mixture in a programmed thermocycler (Biometra, Germany). Amplified products were separated in 1 % agarose gels in 1× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system UVP PhotoDoc-t®. Size of amplified fragments was determined by comparing with standard lenght marker Quick-Load® Purple 2-Log DNA ladder (New England Biolabs, Inc).

Statistical analysis

For the assessment of the polymorphism between castor genotypes and usability of SSR markers in their differentiation diversity index (DI) **(Weir, 1990)**, the probability of identity (PI) **(Paetkau et al., 1995)** and polymorphic information content (PIC) **(Weber, 1990)** were used. The SCoT 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 SPSS professional statistics version 17 software package was constructed.

RESULTS AND DISCUSSION

In plant molecular genetic research, DNA markers have abundant usage for crop improvement in plant breeding **(Bhawna et al., 2017)**. DNA markers are commonly used for the assessment of genetic diversity in crop germplasm, population structure analysis **(Chen et al., 2012; Zhang et al., 2011)**, quantitative trait loci (QTL) or the linkage map construction for mapping genes **(Bhawna et al., 2017)**. For detecting polymorphisms a new molecular marker system called SCoT **(Collard a Mackill, 2009)** was developed which tag coding sequences of the genome. SCoT marker system had initially been validated in the model species rice (*Oryza sativa*) **(Collard and Mackill 2009)**.

For the molecular analysis of 17 buckwheat genotypes 7 SCoT primers were used. PCR amplifications using 7 SCoT primers produced total 52 DNA fragments that could be scored in all genotypes. The selected primers amplified DNA fragments across the 17 genotypes studied with the number of amplified fragments varying from 5 (SCoT36) to 11 (SCoT12) and the amplicon size varied from 200 to 3000 bp. Of the 52 amplified bands, 38 were polymorphic with an average of 5.43 fragments per primer (Table 3). The percentage of polymorphic bands ranged from 54 % (SCoT12) to 87.5 % (SCoT26 and SCoT29) with an average of 73.43 %. The polymorphic information content (PIC) values varied from 0.586 (SCoT36) to 0.831 (SCoT26) with an average of 0.729 and index diversity (DI) value ranged from 0.625 (SCoT36) to 0.837 (SCoT26) with an average of 0.749 (Tab.3). SCoT marker with the highest percentage of polymorphism (SCoT29) is showed on Figure 2.

Based the genetic distance matrix using profiles of the 7 SCoT primers and hierarchical cluster analysis using the unweighted pair-group method with the arithmetic average (UPGMA) method a dendrogram was constructed. According to analysis, the set of 17 diverse accessions of buckwheat was clustered into two main clusters (I, II) (Figure 1). Sixteen buckwheat genotypes were included in cluster I and the genotype of Madawaska (USA) was genetically the farthest and created cluster II. Cluster I was further subdivided into three other subgroups (Ia, Ib and Ic). Majority (87.5 %) of Polish genotypes grouped in the subgroup Ia. Genetically the closest were the varieties Ballada (Russia) and Bamby (Austria) and grouped along side in the subgroup Ia.

Table 1 List of analyzed genotypes of buckwheat.

No.	Genotype of buckwheat (*Fagopyrium esculentum*)	Country of origin
1.	Aiva	LVA
2.	Alex	DEU
3.	Ballada	RUS
4.	Bamby	AUT
5.	Bogatyr	RUS
6.	Branszczyk	POL
7.	Czerwone orzeszki	POL
8.	Darja	SVN
9.	Emka	POL
10.	Gema	POL
11.	Hruszowska	POL
12.	JANA C1	CZE
13.	Kora	POL
14.	Madawaska	USA
15.	Pulawska	POL
16.	Pyra	CZE
17.	St Jacut	FRA

Note: RUS – Russia, AUT – Austria, POL – Poland, CZE – Czech Republik, LVA – Latvia, FRA – France, SVN – Slovenia.

Table 2 List of used SCoT markers.

SCoT primer	Sequence of primers (5´-3´)	Anealing temperature [°C]
SCoT 12	ACGACATGGCGACCAACG	50 °C
SCoT 23	CACCATGGCTACCACCAG	50 °C
SCoT 26	ACCATGGCTACCACCGTC	50 °C
SCoT 28	CCATGGCTACCACCGCCA	50 °C
SCoT 29	CCATGGCTACCACCGGCC	50 °C
SCoT 30	CCATGGCTACCACCGGCG	50 °C
SCoT 36	GCAACAATGGCTACCACC	50 °C

Table 3 Statistical characteristics of the SCoT markers used in buckwheat.

SCoT marker	Number of all fragments	Number of polymorphic fragments	Percentage of polymorphic bands (%)	DI	PIC	PI
SCoT12	11	6	54.0	0.751	0.722	0.022
SCoT23	7	5	71.0	0.702	0.694	0.039
SCoT26	8	7	87.5	0.837	0.831	0.005
SCoT28	7	5	71.0	0.730	0.696	0.024
SCoT29	8	7	87.5	0.809	0.805	0.009
SCoT30	6	5	83.0	0.786	0.770	0.011
SCoT36	5	3	60.0	0.625	0.586	0.071
Average	**7.43**	**5.43**	**73.43**	**0.749**	**0.729**	**0.026**
Total	**52**	**38**				

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

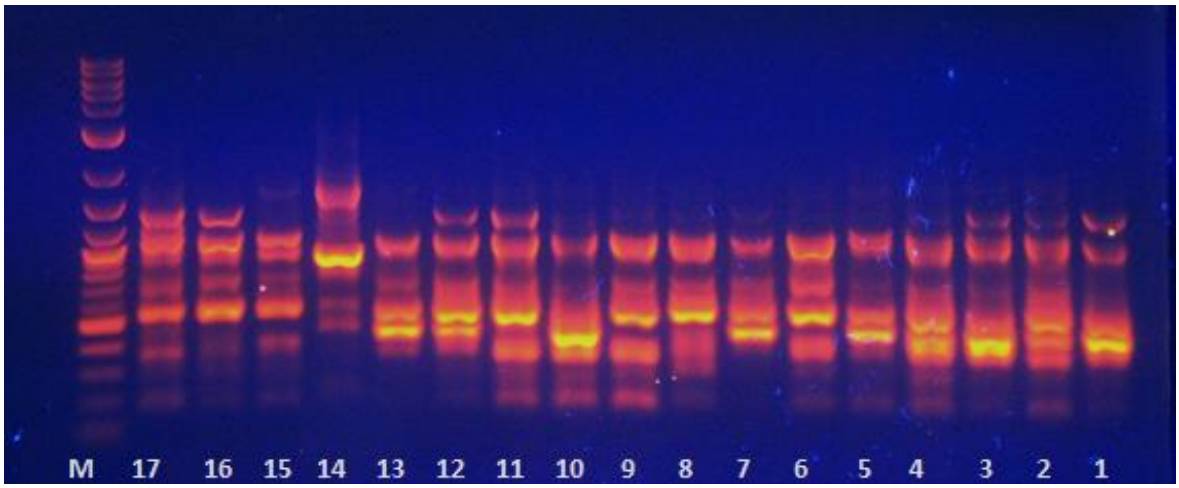

Figure 1 Electrophoreogram of SCoT29 marker.
Note: 1-17 are genotypes of buckwheat (tab.1), M is Quick-Load® Purple 2-Log DNA ladder.

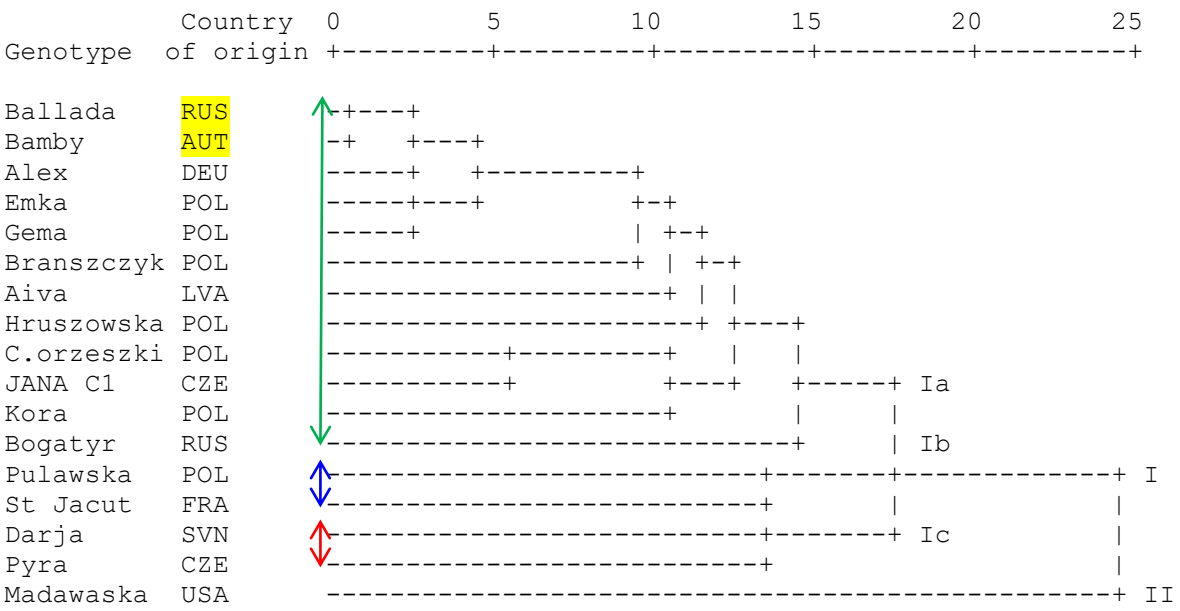

Figure 2 Dendrogram of 17 buckwheat genotypes prepared based on 7 SCoT markers.
Note: RUS – Russia, AUT – Austria, POL – Poland, CZE – Czech Republik, LVA – Latvia, FRA – France, SVN – Slovenia.

Lower average percentage of polymorphism (21 %) obtained **Kallamadi et al. (2015)** who analysed molecular diversity of castor (*Ricinus communis* L.) by SCoT technique. Out of 36 SCoT primers tested, all primers produced amplification products but only 10 primers resulted in polymorphic fingerprint patterns. Out of a total of 108 bands, 23 (21%) were polymorphic with an average of 2.1 polymorphic bands per primer. The total number of bands per primer varied from 5 and 20 in the molecular size range of 100–3000 bp. The PIC/DI varied from 0.06 for SCoT28 to 0.45 for SCoT12 with an average of 0.24.

On the other side, higher percentage of polymorphism with SCoT primers has been reported in crops like peanut **(Xiong et al., 2011)**, cicer **(Amirmoradi et al., 2012)**, mango **(Luo et al., 2010)**, ramie **(Satya et al., 2015)**, sugarcane **(Que et al., 2014)**, Chinese bayberry **(Fang-Yong and Ji-Hong, 2014)**, pepper **(Tsaballa et al., 2015)**, castor **(Kallamadi et al., 2015)**, maize **(Vivodík et al., 2016)** and taxus **(Hao et al., 2018)**.

Satya et al. (2015) used 20 SCoT markers to assess genetic diversity and population structure of indigenous, introduced and domesticated ramie (*Boehmeria nivea* L. Gaudich.). A total of 155 genotypes from five populations were investigated for SCoT polymorphism, which produced 136 amplicons with a range of 4 to 10 bands per primer, of which 119 (87.5%) were polymorphic. Polymorphism information content ranged from 0.25 to 0.93 with an average of 0.69. **Gajera et al. (2014)** used 19 SCoT primers for amplification among 20 mango cultivars which yielded a total of 117 clear and bright loci. Number of loci ranged from 4 to 10 with an average of 6.16 loci per primer. Of 117 loci, 96 loci (79.57 %) were polymorphic, the number of polymorphic loci varied from 2 to 10 with an average of 5.05 loci per primer. The detected

polymorphism per primer among the tested cultivars ranged from 50 % (SCoT26) to 100 % (SCoT-33, SCoT-40, and SCoT-51). In our study we detected by SCoT26 primer the percentage of polymorphic bands 87.5 %. **Que et al. (2014)** used 20 SCoT primers to assess the genetic diversity among 107 sugarcane accessions within a local sugarcane germplasm collection. Tventy SCoT primers amplified 176 DNA fragments, of which 163 were polymorphic (92.85%). **Fang-Yong and Ji-Hong (2014)** assessed the genetic diversity of 31 germplasm resources of *Myrica rubra* of China using 38 SCoT markers. Authors detected 298 reproducible bands of which 251 were polymorphic (84.23%). **Hao et al. (2018)** through a screening of 36 start codon targeted (SCoT) polymorphism primers, among 15 individuals of 4 *Taxus* species detected in 20 SCoT primers clear and repeatable polymorphism. The number of SCoT bands generated from *Taxus* samples was in the range of 5 – 10 for each SCoT primer. The ratio of polymorphic bands across the primers was 62.5 – 100%, with an average of 82.0%, indicating that SCoT markers provided a high level of information and could be employed for assessing genetic diversity and molecular identification of *Taxus* species.

Luo et al. (2010) found comparable percentage of polymorphism (76.2 %) using SCoT markers in analysis of diversity and relationships among mango cultivars and also **Agarwal et al. (2018)** who detected 72.49% percentage of polymorphism in the analysis of genetic diversity within 29 rose accessions using 32 SCoT markers.

To determine the level of polymorphism in analysed buckwheat genotypes polymorphic information content (PIC) was calculated (Table 3). Lower PIC values compare to our analysis (0.524) were detected by **Tsaballa et al. (2015), Kallamadi et al. (2015), Huang et al. (2014) and Hajibarat et al. (2015). Tsaballa et al. (2015)** analyzed genetic variability among the 30 landraces and one commercial Greek cultivar of pepper (*Capsicum annuum* L.) using 6 SCoT primers. They detected PIC values ranged from 0.123 (SCoT33) to 0.258 (SCoT15), with an average value of 0.232 per primer. **Kallamadi et al. (2015)** detected average PIC/DI vales from 0.06 (SCoT28) to 0.45 (SCoT12) with an average of 0.24 in analysis of genetic diversity in 31 accessions of castor representing seven geo-graphic areas by 36 SCoT markers. **Huang et al. (2014)** assessed the genetic diversity of six *Hemarthria* cultivars using seven SCoT primers. They calculated PIC values ranged from 0.471 to 0.758 with an average of 0.612. **Hajibarat et al. (2015)** used a set of 9 SCoT primers to fingerprint 48 chickpea genotypes. PIC values ranged from 0.43 to 0.47 with an average value of 0.45 per primer.

Higher values of PIC were detected by other authors **(Luo et al. 2010; Gajera et al. 2014; Que et al. 2014; Gao et al. 2014; Fang-Yong et al. 2014; Jiang et al. 2014; Satya et al., 2015)** and these values presented a high level of polymorphism of genotypes detected by SCoT markers. Higher PIC values were detecte by **Que et al. (2014)** who used assessed the genetic diversity among 107 sugarcane accessions using 20 SCoT markers and calculated PIC values from 0.783 to 0.907 with a mean of 0.861.

Agarwal et al. (2018) detected comparable polymorphic information content (PIC) ranged from 0.42 to 0.92 with an average of 0.78 in the identification and characterization of genetic variation within 29 rose accessions using 32 SCoT markers.

Kishore et al. (2013) used 13 ISSR markers to analyze genetic diversity and relatedness of 15 germplasms of *Fagopyrum tataricum*. They detected comparable average PIC value of the ISSR markers (0.812) which represents high level of polymorphism.

For the revealing of the genetic relationships among the cultivars a dendrogram is constructed. **Que et al. (2014)** to assess the genetic diversity among 107 sugarcane accessions within a local sugarcane germplasm collection used 20 SCoT primers. Using UPGMA cluster analysis of the SCoT marker data divided 107 sugarcane accessions into six clusters. **Jiang et al. (2014)** analyzed the diversity and genetic relationships among 95 orchardgrass accessions by using SCoT markers. In total, 273 polymorphic bands with an average of 11.4 bands per primer were detected. The UPGMA dendrogram separated 95 accessions into 7 main clusters according to the geographical origin. **Kallamadi et al. (2015)** analysed the genetic diversity of 31 accessions of castor using 36 SCoT markers with the aim to construct the UPGMA dendrogram in which the accessions of castor separated into two major clusters (11 and 17 accessions). Three accessions failed to cluster with others accessions. **Vivodík et al. (2018)** analyzed 56 genotypes of Tunisian castor using 37 SCoT primers. In the UGMA dendrogram, the collection of 56 Tunisian castor genotypes clustered into two main clusters (1 and 2). **Rajesh et al. (2015)** constructed dendrogram using genetic similarity coefficients obtained from UPGMA analysis among the coconut accessions. Coconut accessions grouped into two main clusters. Cluster analysis supported population genetic analysis and suggested close association between introduced and domesticated genotypes. **Gajera et al. (2014)** constructed dendrogram of the 20 mango cultivars using 19 SCoT primers which clustered into two major groups based on the SCoT data analysis with UPGMA.

CONCLUSION

The objective of this study was to determine the genetic variation among 17 rye varieties using 7 SCoT markers. Values of diversity index were higher than 0.7 in 85.7 % of SCoT markers that represents high level 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 into two main clusters where all buckwheat genotypes were distinguished. Clustering partially reflected geographic origin of studied buckwheat genotypes. Majority (87.5 %) of Polish genotypes grouped in the same subcluster Ia.

SCoT marker system is a simple and novel marker system belonging to gene-targeted and functional markers. Functional markers developed from the transcribed region of the genome have the ability to reveal polymorphism, which might be directly related to gene function. The technique is similar to RAPD or ISSR, is simple, is used simple primer which acts as the forward and the reverse. Visualisation of amplicons can be performed by standard

agarose gel electrophoresis. The higher primer lengths and subsequently higher annealing temperatures ensure higher reproducibility of SCoT markers, compared to RAPD markers. SCoTs markers are more informative and effective. Our result showed appreciably high genetic diversity among the buckwheat genotypes studied. This study showed high genetic diversity within the studied buckwheat genepool as an important source for crop breeders and indicated that there is value in sampling for useful genes for crops improvement.

REFERENCES

Agarwal, A., Gupta, V., Haq, S. U., Pradeep Kumar Jatav, P. K., Kothari, S.L. 2018. Assessment of genetic diversity in 29 rose germplasms using SCoT marker. *Journal of King Saud University.* https://doi.org/10.1016/j.jksus.2018.04.022

Al-Qurainy, F., Khan, S., Nadeem, M., Tarroum, M. 2015. SCoT marker for the assessment of genetic diversity in Saudi Arabian date palm cultivars. *Pak. J. Bot.,* vol. 47, no. 2, p. 637-640.

Amirmoradi, B., Talebi, R., Karami, E. 2012. Comparison of genetic variation anddifferentiation among annual Cicer species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. *Plant Syst. Evol.*, vol. 298, p. 1679-1688. https://doi.org/10.1007/s00606-012-0669-6

Bhawna, M., Abdin, Z., Arya, L., Verma, M. 2017. Use of SCoT markers to assess the gene flow and population structure among two different populations of bottle gourd. *Plant Gene*. vol. 9, p. 80-86. https://doi.org/10.1016/j.plgene.2016.09.001

Boczkowska, M., Tarczyk, E. 2013. Genetic diversity among Polish landraces of common oat (*Avena sativa* L.). *Genet Resour Crop Evol*., vol. 60, p. 2157. https://doi.org/10.1007/s10722-013-9984-1

Collard, B. C. Y., Mackill, D. J. 2009. Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Mol. Biol. Rep*., vol. 27, p. 86-93. https://doi.org/10.1007/s11105-008-0060-5

Fang-Yong, Ch., 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 Syst Evol*, vol. 300, p. 1011-1020. https://doi.org/10.1007/s00606-013-0939-y

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. *Am. J. Potato Res.*, vol. 88, p. 226-237. https://doi.org/10.1007/s12230-011-9187-2

Gupta, P. K., Y. S. Chyi, J. Romero-Severson, and J. L. Owen. 1994. Amplification of DNA markers from evolutionary diverse genomes using single primers of simple-sequence repeats. *Theoretical and Applied Genetics*, vol. 89, p. 998-1006. PMid:24178116

Hajibarat, Z., Saidi, A. Hajibarat, Z., Talebi, R. 2015. Characterization of genetic diversity in chickpea using SSR. *Physiol. Mol. Biol. Plants*, vol. 21, no. 3, p. 365-373. https://doi.org/10.1007/s12298-015-0306-2

Hao, J., Jiao, K., Yu, C., Guo, H., Zhu, Y., Yang, X., Zhang, S., Zhang, L., Feng, S., Song, Y., Dong, M., Wang, H., Shen, C. 2018. Development of SCoT-based SCAR marker for rapid authentication of Taxus media. *Biochem. Genet*., vol. 56, p. 255-266. https://doi.org/10.1007/s10528-018-9842-0

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. *Genet Resour Crop Evol.*, vol. 61, p. 1047-1055. https://doi.org/10.1007/s10722-014-0107-4

Chen, X., Min, D., Yasir, T. A., Hu, Y.-G., 2012. Genetic diversity, population structure and linkage disequilibrium in elite Chinese winter wheat investigated with SSR markers. *PLoS One.* vol. 7, No. 9, e44510. https://doi.org/10.1371/journal.pone.0044510

Chrungoo, N. K., Dohtdong, L., Chettry, U. 2016. Genome Plasticity in Buckwheat. In V. R. Rajpal et al. (eds.). *Gene Pool Diversity and Crop Improvement*, Springer International Publishing Switzerland. Sustainable Development and Biodiversity 10, p. 227-239. https://doi.org/10.1007/978-3-319-27096-8_7

Jiang, L. F., Qi, X., Zhang, X. Q., Huang, L. K., Ma, X., Xie, W. G. 2014. Analysis of diversity and relationships among orchardgrass (*Dactylis glomerata* L.) accessions using start codon-targeted markers. *Genet. Mol. Res*, vol. 13, no. 2, p. 4406-4418. https://doi.org/10.4238/2014.June.11.4

Kallamadi, P. R., Ganga, R., 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

Kishore, G., Pandey, A., Dobhal, R., Gupta, S. 2013. Population Genetic Study of *Fagopyrum tataricum* from Western Himalaya Using ISSR Markers. *Biochem Genet*. vol. 51, p. 750-765. https://doi.org/10.1007/s10528-013-9604-y

Luo, C., He, X. H., Chen, H., Ou, S. J., Gao, M. P. 2010. 2010. Analysis of diversity and relationships among mango cultivars using start codon targeted (SCoT) markers. *Biochem. Syst. Ecol.,* vol. 38, p. 1176-1184. https://doi.org/10.1016/j.bse.2010.11.004

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. https://doi.org/10.1016/j.bse.2015.07.017

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. PMiD:7663752

Petrovičová, L., Balážová, Ž., Vivodík, M., Gálová, Z. 2017. Detection genetic variability of *Secale cereale* L. by SCoT markers. *Potravinarstvo.* vol. 11, no. 1, p. 197-202. https://doi.org/10.5219/726

Poczai, P., Varga, I., Laos, M., Cseh, A., Bell, N., Valkonen, J. P., Hyvonen, J. 2013. Advances in plant gene-targeted and functional markers: a review. *Plant Methods*., vol. 9, no. 1, p. 6. https://doi.org/10.1186/1746-4811-9-6

Przybylski, R., Gruczynska, E. 2009. A review of nutritional and nutraceutical components of buckwheat. *Eur. J. Plant Sci. Biotechnol.*, vol. 3, p. 10-22.

Que, Y., Pan, Y., Lu, Y., Yang, C., Yang, Y., Huang, N., Xu, L. 2014. Genetic Analysis of Diversity within a Chinese Local Sugarcane Germplasm Based on Start Codon Targeted Polymorphism. *BioMed Research International*, vol. 2014,

Article ID 468375, 10 pages. https://doi.org/10.1155/2014/468375

Rahimi, M., Nazari, L., Kordrostami, M., Safari, P., 2018. SCoT marker diversity among Iranian Plantago ecotypes and their possible association with agronomic traits. *Sci. Hort.* vol. 233, p. 302-309. https://doi.org/10.1016/j.scienta.2018.01.009

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. *Biotech* vol. 5, p. 999-1006. https://doi.org/10.1007/s13205-015-0304-7

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. https://doi.org/10.1016/j.mgene.2015.01.003

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. https://doi.org/10.12692/ijb/5.2.14-22

Sharma, R., Jana, S. 2002. Species relationships in *Fagopyrum* revealed by PCR based DNA fingerprinting. *Theor Appl Genet*. vol. 105, p. 306-312. https://doi.org/10.1007/s00122-002-0938-9

Shi, T., Li, R., Chen, Q., Li, Y., Pan, F., Chen, Q. 2017. De novo sequencing of seed transcriptome and development of genic-SSR markers in common buckwheat (*Fagopyrum esculentum*). *Mol Breeding*. Vol. 37, p. 147. https://doi.org/10.1007/s11032-017-0743-4

Singh, S. K. Sidhika, CH., Rakesh, P., Bhatt, R. K., Kalia, R. K. 2017. Genetic diversity of Indian jujube cultivars using SCoT, ISSR, and rDNA markers. *Tree Genetics & Genomes.* 13 : 12. https://doi.org/10.1007/s11295-016-1092-x

Stibilj, V., Kreft, I., Smrkolj, P., Osvald, J. 2004. Enhanced selenium content in buckwheat (*Fagopyrum esculentum* Moench) and pumpkin (*Cucurbita pepo* L.) seeds by foliar fertilization. *Eur Food Res Technol.* vol. 219, no. 2, p. 142-144. https://doi.org/10.1007/s00217-004-0927-0

Tsaballa, A., Ganopoluos, I., Timplalexi, A., liki, X., Bosmali, I., Irini, N. O., Athanasios, T. Madesis, P. 2015. Molecular characterization of Greek pepper (*Capsicum annuum* L) landraces with neutral (ISSR) and gene-based (SCoT and EST-SSR) molecular markers. *Biochemical Systematics and Ecology*, vol. 59, p. 256-263. https://doi.org/10.1016/j.bse.2015.02.005

Vivodík, M., Gálová, Z., Balážová, Ž., Petrovičová, L. 2016. Start codon targeted (scot) polymorphism reveals genetic diversity in European old maize (*Zea mays* l.) genotypes. *Potravinarstvo*, vol. 10, no. 1, p. 563-569. https://doi.org/10.5219/660

Vivodík, M., Saadaoui, E., Balážová, Ž., Gálová, Z., Petrovičová, L. 2018. Genetic diversity and population structure in tunisian castor genotypes (*Ricinus communis* L.) detected using scot markers. *Potravinarstvo Slovak Journal of Food Sciences.* vol. 12, no. 1, p. 143-149. https://doi.org/10.5219/873

Vyhnánek, T., Trojan, V., Štiasna, K., Presinszká, M., Hřivna, L., Mrkvicová, E., Havel, L. 2015. Testing of DNA isolation for the identification of Hemp. *Potravinarstvo*, vol. 9, no. 1, p. 393-397. https://doi.org/10.5219/509

Weber, J. L. 1990. Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics,* vol. 7, p. 524-530.

Wei, Y., Hu, X., Zhang, G., Ouyang, S. 2003. Studies on the amino acid and mineral content of buckwheat protein fractions. *Nahrung/Food*. vol. 47, p. 114-116. https://doi.org/10.1002/food.200390020

Weir, B. S. *Genetic data analysis*. Sinauer Associated, Sunderland, UK : Massachusetts, 1990, 445 p. ISBN 0 87893 871 00 87893.

Xiong, F., Zhong, R., Han, Z., Jiang, J., He, L., Zhuang, W., Tang, R. 2011. Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) genotypes. *Mol. Biol. Rep*., vol. 38, p. 3487-3494. https://doi.org/10.1007/s11033-010-0459-6

Yasui, Y., Wang, Y., Ohnishi, O., Campbell, C. G. 2004. Amplified fragment length polymorphism linkage analysis of common buckwheat (*Fagopyrum esculentum*) and its wild selfpollinated relative *Fagopyrum homotropicum*. *Genome.* vol. 47, p. 345-351. https://doi.org/10.1139/g03-126

Zhang, J., Xie, W., Wang, Y., Zhao, X. 2015. Potential of Start Codon Targeted (SCoT) Markers to Estimate Genetic Diversity and Relationships among Chinese *Elymus sibiricus* Accessions. *Molecules*, vol. 20, p. 5987-6001. https://doi.org/10.3390/molecules20045987

Zhang, P., Li, J., Li, X., Liu, X., Zhao, X., 2011. Population structure and genetic diversity in a rice core collection (*Oryza sativa* L.) investigated with SSR markers. *PLoS One*, vol. 6, no. 12, e27565. https://doi.org/10.1371/journal.pone.0027565

Žiarovská, J., Grygorieva, O., Zeleňáková, L., Bežo, M., Brindza, J. 2015. Identification of sweet chesnut pollen in bee pollen pellet using molecular analysis. *Potravinarstvo*, vol. 9, no. 1, p. 352-358. https://doi.org/10.5219/497

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A STUDY OF THE QUALITY AND BIOLOGICAL VALUE OF MEAT OF DIFFERENT BREEDS OF RABBIT BRED IN GEORGIA

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ABSTRACT

The use of a rabbit meat is a perspective and accessible way of solving the problem of the shortage of domestic meat products in Georgia. Nowadays, there are sufficient on rabbit breeding in the country, but no data are available on the quality and technological properties of rabbit meat. Therefore, of scientific and practical interest is to study the quality indicators, safety, chemical composition and biological value of meat of different breeds of rabbits. The modern, standard, commonly accepted methods of the research have been used in performing this work, which are suitable for accomplishing the set tasks. Statistical processing of the results obtained and the evaluation of the reliability of the obtained data were carried out by the mathematical statistics methods using the Windows IBM SPSS Statistics software program. Based on the study of microbiological indicators of rabbit meat, it has been demonstrated that the number of mesophylic-aerobic and facultative anaerobic microorganisms does not exceed the values established by the sanitary standards and rules, no bacteria of intestinal bacillus and pathogenic microorganisms were detected, including salmonella, which is also is in compliance with the biological and hygienic safety requirements and standards. The safety of rabbit meat is also indicated by a study of its toxicity. It has been established that the breeds of rabbit bred in the country are distinguished by a high meat productivity, high protein and moderate fat contents and low caloric capacity. The biological value of rabbit meat has been determined: it is distinguished by the number of all essential amino acids and the protein-quality index, it does not have limited amino acids, and all essential amino acids scores, especially of lysine, tryptophan and leucine, exceed the "ideal protein" score. The level of satisfaction of human daily needs is also high with amino acids. The resulting data point to the high quality and biological value of the meat of rabbits bred in Georgia, and made it reasonable to use as raw material for domestic high-quality meat production.

Keywords: rabbit meat; safety; meat productivity; chemical composition; biological value

INTRODUCTION

Own natural resources and supplying the population with high quality foods is the most important strategic goal for all States, as shortages or inferiority of food cause serious economic and social consequences.

Recent years have seen deficiencies and imbalance of a number of nutrients – proteins, especially animal origin proteins, polyunsaturated fatty acids, vitamins and macro- and micronutrients.

An essential source of animal proteins and a number of biologically active substances is one of the mostly demanded food product – meat.

The population of Georgia traditionally consumes meat, pork, ovine and chicken meat.

At the same time, the real life of the country is the reduction in livestock numbers and an acute shortage of meat. The consumer market is saturated with meat and meat products imported from abroad.

Therefore, of high relevance is to search for the new, alternative sources for domestic production of meat products, which have a high biological and nutritional value.

Rabbit meat can be considered to be a prospective and affordable reserve of complete meat. In contrast to other agricultural animals, rabbit is distinguished by high reproduction capability, fast maturation and the feasibility of using its products in diet and preventive nutrition **(Aleksandrov and Kosova, 2006; Dalle Zotte, 2011; Hernández, 2008; Nikitin and Belchenko, 1994; Ulikhina, 2009; Zhitnikova, 2004)**.

The use of rabbit meat in food production is stemmed from the high content of easily digestible protein, the well-balanced aminoacid composition, the moderate fat content, and the substantial content of mineral substances and vitamins, low calorific capacity and high organoleptic indicators.

Compared to pork and poultry meat, the percentage of flesh in rabbit meat quite higher, but the percentage of connective tissue - significantly lower, and amount of sodium, purine compounds and the cholesterol content is smaller, the meat consistency itself is finefibered and delicate. Due to these properties, meat is considered to be a precious dietetic product that has no contraindications for arious diseases **(Tutelyan and Samsonov, 2002; Baranovsky, 2008; Hernández, 2008; Gerasimova and Golovaneva, 2013; Nistor, et al. 2013; Petracci and Cavani, 2013; Pla, Pascual and Ariño, 2004; Polak et al., 2006; Rafay and Prundeanu, 2013.; Mojto and Palanská, 1999; Vasilenko, 2004; Volkova, Inerbaeva and Motovilov, 2009)**.

It is noteworthy that rabbit production has been suspended for many years, but today, there has already been revealed the population's increased demand for rabbit meat, which was contributed considerably by information provided scientists, nutritionists and adherents of healthy eating about useful properties of rabbit meat. This is also demonstrated by our monitoring.

Rabbit meat is quite poorly represented on the Georgian consumer market, and besides products imported from abroad are dominating, while the country has all the necessary conditions for intensive development of rabbit breeding and rabbit meat production in large quantities. The implementation of this task corresponds to the important direction of of the country's economic policy – domestic safe food production.

In literature, there are sufficient data on rabbit breeding and nutritional value of rabbit meat, but there are no data on the quality of rabbit meat produced in Georgia.

Based on the above and taking into consideration that in consumption of food, the preference should be given to raw materials of local production, since people are genetically predisposed to their better digestion, and of scientific and practical importance is a comprehensive study of meat of different breeds of rabbits produced in Georgia.

The purpose of the work was to examine qualitative indicators of domestic rabbit meat production, as well as its safety, chemical composition and biological value.

Scientific hypothesis

(H1)There is a difference between the meat productivity of meat varieties and meat-skin varieties of rabbits.

(H2) Useful properties of rabbit meat is determined by the ratio of fat and protein, nutritional and biological value - by the content of amino acids and protein-quality index.

MATERIAL AND METHODOLOGY

Studies were conducted at the Akaki Tsereteli State University: in the laboratories of the Department of Food Technologies. The following breeds of rabbits bred in the cooperative "New Gurianta" were the subjects of these studies: meat varieties – Californian and New Zealand white rabbit, meat- skin varieties - White Giant and Gray Giant, their average age was 130 days.

We evaluated the quality of rabbit meat in a comprehensive manner – on the basis of organoleptic, microbiological, chemical indicators, aminoacid composition and biological value. To that end, we used the methods described in the appropriate standards, normative documents and special literature, which are widely accepted for analysis of meat products.

Organoleptic indicators were determined in accordance with the following characteristics: appearance and color, muscle condition on the section, consistency, smell, broth transparency and aroma.

During microbiological analysis, the quantities of mesophylic aerobic and facultative anaerobic microorganisms in rabbit meat were determined according to state standard "Food products. Methods for determining the quantities of mesophylic aerobic and facultative anaerobic microorganisms" – GOST 10444.15-94; number of bacteria of intestinal bacillus was determined according to State Standard "Food products. Methods for the detection and determination of the number of bacteria of the Escherichia coli group (coliform bacteria)" – GOST 30518-97; Salmonella was determined according to State standard "Food products. Methods for the detection of bacteria of the genus Salmonella " – GOST 30519-97.

To determine safety of rabbit meat, examination of samples on toxicity was carried out on the biological object *Tetraximena puriformis,* in compliance with "Methodological instructions on the accelerated determination of the toxicity of products and foods". The toxicity of test samples was determined by the presence of dead infusoria, altered forms, motion character and suppressed growth of *Tetraximena puriformi*. The absence of the death of infusoria or any other pathological change in *Tetraximena puriformi*, within 24 hours, indicates that meat is not toxic.

The meat productivity of rabbit was determined after slaughtering. We weighed and distributed the carcass, separated the meat from the bones and the cartilages from the flesh, then we determined the morphological composition of the rabbit carcass, based on the obtained data, and then we calculated the fleshing index.

The chemical composition was determined by determining the content of moisture, proteins, fats and ash in the mean sample of the flesh. Based on the obtained data, we calculated the energy value. The moisture content was determined at a temperature of 105 °C, by method of drying the weight up to the constant mass; the protein content was determined by Kjehldahl's method; the fat content was determined using Soxhlet method; the ash content was determined - by ashing method with preliminary drying **(Antipova, Glotova and Rogov, 2001)**.

The aminoacid composition of meat protein was determined by method of liquid chromatography on a chromatograph AGILENT 1200 according to the appliance instructions; the biological value of meat protein was determined according to the aminoacid composition wity further calculation of aminoacid scores, indexes E/N (the ratio of essential amino acids to the sum of nonessential amino acids) and E/T (the ratio of essential amino acids to the sum of all amino acids). In addition, we determined the protein-quality indicator – according to the ratio of triphophan/oxyproline.

Statisic analysis

To analyze the difference between the test parameters (meat productivity, chemical composition, biological and nutritional value) of meat of different varieties of rabbit, is conducted a statistical analysis of the obtained data, the

reliability of the obtained data was evaluated by the mathematical statistics methods using the Windows IBM SPSS Statistics software program (version 20.0). To describe the ordered sample, we used statistical functions of the average arithmetic value and average standard error. Graphical interpretation of the results was made by using Microsoft Excel.

In Tables and Figure, there are presented the data of typical tests, and each value is an average of at least three determinations.

RESULTS AND DISCUSSION

At the first stage of the work, we studied the organoleptic and microbiological indicators of the breeds bred in Georgia, such as Californian, New Zealand white, White Giant and Gray Giant.

By the organoleptic characteristics, the meat of individual breeds of rabbit did not differ from each other. On the surface of the carcasses of rabbits of all groups, there is formed a rose-pink dry crust of drying up. The serous membrane of the abdominal cavity is moist and shiny. Muscles are pink-rose, dense, elastic, the dimple formed when kneading with finger is quickly aligned. Meat has a specific peculiar smell characteristic of fresh rabbit meat, and when cooking, the broth is transparent and fragrant.

Microbiological analysis was carried out on the presence of mesophylic-aerobic and facultative anaerobic microorganisms, salmonellas and bacteria of intestinal bacillus. It has been established that in test samples, the quantities of mesophylic aerobic and facultative anaerobic microorganisms varied from 3.4·102 to 2.4·103 CFU.g-1 (colony forming unit/g) that does not exceed sanitary norms and rules. The established value of bacteria of intestinal bacillus (coliform) was not in 0.01 g sample, and met the hygienic requirements of microbiological safety, but pathogenic microorganisms including salmonella, have not been detected in 25 g samples, which also complied with microbiological safety norms and indicates safety of product.

The study of safety of test breeds of rabbit meat on the biological object *Tetraximena puriformis* showed that it is not toxic, because it does not have a negative impact on the survivorship rate infusoria, their motion and morphology of cells. The number of infusoria in the feeding area containing rabbit meat for each rabbit breed varied from 16.4 × 104 to 16.8 × 104 .

At the next stage, we studied the rabbit meat productivity and the morphological composition, as well as the content of individual tissues in the carcass. The results are presented in Table 1.

Analysis of data contained in the table reveals the high meat productivity of test breeds of rabbit.

Among individual tissues in the carcass, the highest percentage belongs to lean tissue, then followed by the contents of bone, fat and connective tissues, accordingly.

The New Zealand white and Californian breed of rabbit have the highest fleshing index (the ratio of the lean tissue content to the bone tissue content), and relatively lower – by 3.05% – White Giant and and by 2.5% – Gray Giant breeds (Figure 1). Thus, meat varieties of rabbit are characterized by higher meat productivity than meat – skin varieties.

The results of studying the chemical composition of different breeds of rabbit are presented in Table 2.

Analysis of data contained in the table reveals that all breeds of rabbit meat contain considerable amount of

Table 1 The yield of the rabbit meat carcass and individual tissues.

Indicator	Californian	New Zealand white	Gray Giant	White Giant
Live weight, g	3000 ±21.5	3260 ±21.5	3540 ±10.6	3420 ±8.6
Carcass body mass, g	1920 ±21.5	2119 ±14.83	2124 ±21.35	2018 ±17.85
Yield of the carcass, %	64	65	60	59
Lean tissue, g	1449 ±16.57	1626 ±16.57	1606 ±6.23	1542 ±19.29
Yield of lean tissue, %	75.45	76.75	75.60	76.40
Bone tissue, g	245 ±5.79	275 ±5.79	281 ±5.24	268 ±7.84
Yield of bone tissue, %	12.76	12.97	13.24	13.30
Connective tissue, g	76 ±4.32	83 ±4.32	86 ±2.62	86 ±5.6
Yield of connective tissue, %	3.98	3.95	4.05	
Fat tissue, g	143 ±10.2	133 ±10.2	150 ±4.78	121 ±5.73
Yield of fat tissue, %	7.45	6.30	7.05	6.01

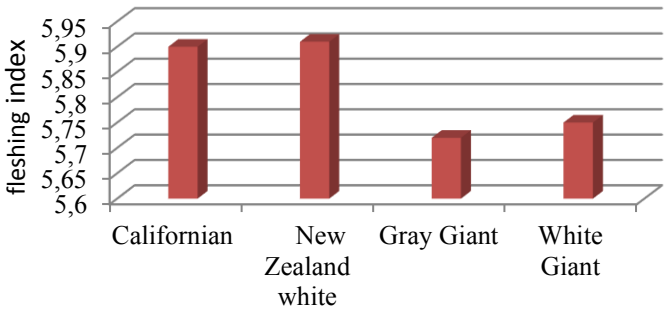

Figure 1 A fleshing index of different breeds of rabbit.

Table 2 Overall chemical composition and energy values of rabbit meat.

Substances	Rabbit breeds			
	Californian	New Zealand white	Gray Giant	White Giant
Moisture, %	71.20 ±0.42	69.2 ±0.36	70.3 ±0.61	70.2 ±0.58
Protein, %	21.1 ±0.08	20.4 ±0.10	19.1 ±0.12	19.6 ±0.19
Fat, %	7.1 ±0.14	7.4 ±0.23	8.3 ±0.18	8.7 ±0.15
Ash, %	1.22 ±0.18	1.2 ±0.22	1.0 ±0.16	1.0 ±0.14
Ratio of fat: protein	0.34	0.36	0.43	0.44
Energy value, kJ	538.4	673.8	629.3	656.6

proteins, and besides, the highest amount is observed in the meat of Californian breed of rabbit, but the same indicator for the New Zealand white rabbit breed is 3.3% lower, for the White Giant – 7.1% lower and for the Gray Giant breed – 8.5% lower.

The fat content in the meat of of the selected breeds is quite low. This indicates that it can be used as useful for health low-caloric raw material in diet and preventive nutrition.

The percentages of fat and protein in the meat of White and Gray Giant breeds are almost identical, but it is insignificantly lower in the meat of Californian and New Zealand white rabbit breeds.

The data obtained by us on the chemical composition of rabbit meat are confirmed by similar data existing in the literature. Thus, for example, in rabbit meat, the protein content is: 22.06% according to **Volkova (2009)**, 21.2%, according to **Nistor et al. (2013)**, 18.97% according to **Zhidik (2017)**, 22.3% – according to Sautkin's data **(Sautkin, 2010)**, According to these authors, the fat content is 1.29% 1.1%, 1.68% and 1.06%, respectively.

The above data indicate, in the same way that rabbit meat, compared to first-category beef and chicken meat and broiler chicken, is characterized by a higher protein and ash content (protein in beef 18.6, in chicken meat 18.2%, in chicken broiler – 19.7 %, ash – 0.9%, 0.8% and 1.1% respectively) and low fat content (fat – in beef 16.0%, in chicken meat – 18.4%, in chicken broiler – 13.8%) and low caloric content. A ratio of protein and fat in beef 0.9, in chicken meat – 1.0, in chicken broiler – 0.7 **(Vasilenko, 2004; Volkova, 2009)**.

To determine the biological value of rabbit meat, we studied its amino-acid composition (Table 3). There have been identified 19 amino acids in test breeds of rabbit meat, including all essential ones.

Analysis of the obtained data reveals that by the quantitative content of individual amino acids, the breeds did not exhibit many differences Of the concentrations of essential amino acids, the most are the amounts of lysine, in a sufficient amount contains tryptophan, which are almost fully absorbed and of nonessential amino acids – the amounts of glutamine, asparagine and alanine The amounts

Table 3 Amino-acid composition of rabbit meat, g.100 g^{-1} of protein.

Amino acid	Rabbit breeds			
	Californian	New Zealand white	Gray Giant	White Giant
Essential amino acids:				
Valine	5.02 ±0.20	5.04 ±0.22	5.05 ±0.23	5.03 ±0.24
Izoleitsin	4.01 ±0.19	4.02 ±0.18	4.03 ±0.17	4.10 ±0.20
Leitsin	8.05 ±0.22	8.02 ±0.20	8.04 ±0.25	8.08 ±0.26
Lysine	10.28 ±0.32	10.23 ±0.33	10.35 ±0.31	10.58 ±0.34
Methionine	2.61 ±0.15	2.58 ±0.12	2.65 ±0.16	2.69 ±0.14
Threonine	4.25 ±0.13	4.22 ±0.14	4.28 ±0.17	4.32 ±0.15
Tryptophan	1.51 ±0.04	1.48 ±0.06	1.54 ±0.07	1.59 ±0.08
Phenylalanine	3.90 ±0.13	3.89 ±0.12	3.93 ±0.14	3.97 ±0.13
Nonessential amino acids:				
Arginine	6.51 ±0.35	6.48 ±0.33	6.53 ±0.34	6.59 ±0.23
Asparagine	8.5 ±0.27	8.47 ±0.28	8.4 ±0.25	8.6 ±0.29
Histidine	2.3 ±0.05	2.29 ±0.07	2.6 ±0.09	2.9 ±0.30
Serine	3.62 ±0.19	3.60 ±0.21	3.68 ±0.23	3.73 ±0.08
Glutamine	14.44 ±0.32	14.42 ±0.38	14.49 ±0.39	14.55 ±0.28
Proline	3.72 ±0.04	3.70 ±0.03	3.78 ±0.04	3.82 ±0.22
Oxyproline	0.25 ±0.05	0.22 ±0.06	0.23 ±0.04	0.27 ±0.01
Alanine	7.72 ±0.31	7.71 ±0.33	7.78 ±0.34	7.89 ±0.34
Glycine	4.03 ±0.22	4.01 ±0.23	4.08 ±0.25	4.15 ±0.23
Cystine	0.92 ±0.18	0.90 ±0.15	0.96 ±0.16	0.99 ±0.05
Tyrosine	2.65 ±0.22	2.62 ±0.21	2.69 ±0.23	2.73 ±0.11
Total amino acid sum	94.29	93.9	95.05	96.58 ±2.16
Total amount of essential amino acids	39.63 ±1.27	39.48 ±1.25	39.83 ±1.32	40.35 ±1.34
Ratio of tryptophan / Oxyproline	6.04	6.73	6.70	5.90

Table 4 Aminoacid score of the rabbit meat proteins.

Amino acid	"Ideal protein" score	Rabbit breeds							
		Californian		New Zealand white		Gray Giant		White Giant	
Izoleitsin	4.0	4.01	100.3	4.02	100.5	4.03	100.8	4.10	102.5
Leitsin	7.0	8.05	115.0	8.02	114.6	8.04	119.1	8.08	115.4
Lysine	5.5	10.28	186.9	10.23	186.0	10.35	188.2	10.58	192.4
Methionine + cystine	3.5	3.53	100.9	3.48	116.0	3.61	103.1	3.68	105.1
Phenylalanine+ tyrosine	6.0	6.55	109.2	6.51	108.5	6.62	110.3	6.70	111.7
Threonine	4.0	4.25	106.3	4.22	105.5	4.28	107.0	4.32	108.0
Tryptophan	1.0	1.51	151.0	1.48	148.0	1.54	154.0	1.59	159.0
Valine	5.0	5.02	100.4	5.04	100.8	5.05	101.0	5.03	100.6

Table 5 Data on the biological value of the rabbit meat protein.

Rabbit breeds	Limited Amino acid	E/N,%	E/T,%	protein-quality index,%
Californian	does not have	0.72	0.42	5.90
New Zealand white	does not have	0.73	0.42	6.73
Gray Giant	does not have	0.72	0.42	6.70
White Giant	does not have	0.73	0.42	6.04

Table 6 Formula of balanced nutrition satisfaction level of rabbit meat by the content of amino acids.

Amino acids	Daily demand, g	Rabbit breeds			
		Californian	New Zealand white	Gray Giant	White Giant
Esencial aminoacids:					
Valine	3.5	30.3	29.4	27.4	28.0
Izoleitsin	3.5	24.9	23.4	21.7	22.6
Leitsin	5.0	34.0	32.6	30.6	31.6
Lysine	4.0	55.7	52.0	49.3	50.3
Methionine	3.0	19.0	17.3	16.6	17.0
Threonine	2.5	36.4	34.4	32.4	33.2
Tryptophan	1.0	34.0	30.0	29.0	30.0
Phenylalanine	3.0	28.0	26.3	25.0	25.3
Nonesencial aminoacids:					
Arginine	5.5	25.3	24.1	22.7	23.2
Asparagine	6.0	30.2	28.8	26.8	27.8
Histidine	1.5	40.8	31.1	33.1	30.1
Serine	3.0	26.2	24.5	23.4	23.7
Glutamine	16.0	19.2	18.4	17.3	17.6
Proline	5.0	16.1	15.1	14.4	14.6
Alanine	3.0	55.5	52.4	49.5	50.4
Glycine	3.0	29.2	27.3	26.0	26.3
Cystine	2.5	8.4	7.3	7.3	7.2
Tyrosine	3.5	16.5	3	14,7	14,8

of essential amino acids is 42%, the ratio of essential and nonessential amino acids for the Grey Giant and Californian breeds is 0.72, and for White Giant and New Zealand White breeds – 0.73. The obtained data do not contradict the literary data. For example, according to Volkova **(2009)**, the E / N value is 0.72, according to **Vasilenko (2004.)** – within 0.71 – 0.76, according to **Zhidik (2017)** – 0.72.

The results of calculation of amino acids (Table 4) and the data on the biological value of rabbit protein (Table 5) indicate that the rabbit meat does not have limited amino acids, the scores of all essential amino acids are higher than the "ideal protein" score.

One of the significant characteristics of meat protein quality is the protein-quality indicator – the ratio of trifluorane and oxyproline. In this ratio, triflufan characterizes the content of high-grade proteins, and oxyproline – deficient. It should be noted in the sample samples that the protein content oxyproline of the connective tissue is 0.22 – 0.27%, which determines the stiffness of the meat when its content in the beef is 1.56% and in the chicken meat is 0.83% **(Antipova and Zherebtsov-Voronezh, 1991; Tutelyan and Samsonov, 2002; Volkova, 2009)**.

There have been calculated the formula of balanced nutrition satisfaction level of rabbit meat by the content of amino acids (Table 6).

Table data indicate that amino acids have the highest satisfaction level of human daily demand for lysine, which,

along with other useful properties, contributes to the complete absorption of calcium by the organism and the growth of bone tissue, and for the selected breeds of rabbit it varies from 49.3% to 55.7%.

Of high importance are also the balanced nutrition formula's satisfaction levels for threonine and tryptophan, which cover the human daily demand, accordingly, from 33.2% to 36.4% and 29% to 34%.

The level of satisfaction of the daily demand is also quite high for both all essential and almost all nonessential amino acids.

Thus, the resulting data point to the high quality and biological value of the meat of rabbits bred in Georgia.

CONCLUSION

1. There have been established the quality indicators, safety, chemical composition and biological value of the meat of the relatively under-investigated and non-traditional for the population rabbit breeds bred in Georgia.
2. Study of the microbiological characteristics and toxicity of rabbit meat points to its safety.
3. The yield of the rabbit carcass and the content of individual tissues in the carcass point to a high meat productivity of rabbit. At the same time, was revealed the difference between meat and meat- skins breeds – the meat productivity of California and New Zealand white breeds was 2.7 – 3% higher than for gray giant and white giant breeds. These results confirmed our first hypothesis (H1) and showed higher meat productivity in meat varieties of rabbits.
4. Study of the chemical composition showed that rabbit meat is distinguished by a high protein content (19.1 – 21.1%), a moderate fat content (7.1 – 8.7%) and a relatively low energy value (538.4 – 673.8 kJ); contains a complete set of essential amino acids, does not have limited amino acids.
5. An advantageous ratio of fat and protein in rabbit meat, a significant content of protein and essential amino acids, including lysine, indicates the beneficial properties of rabbit meat, its high nutritional and biological value. This confirms our second research hypothesis (H2).
6. The chemical composition of rabbit meat, low caloric capacity and high amounts of essential amino acids, especially of lysine, have made it reasonable to use as raw material for domestic high-quality meat production, and as a protein enricher in the production of dietary and medicinal-prophylactic culinary foodstuffs.

REFERENCES

Aleksandrov, S. N., Kosova, T. I. 2004. *Rabbits: Breeding, rearing, feeding*. Moscow, Russia : AST, Stalker. 120 p. ISBN 966-696-643-3.

Antipova, L. V., Glotova, I. A., Rogov, I. A. 2001. *Methods for studying meat and meat products*. Moscow, Russia : Kolos. 376 p. ISBN: 5-9532-0187-7.

Antipova, L. V., Zherebtsov-Voronezh, N. A. 1991. *Biochemistry of meat and meat products*. Estonia : Voronezh Publishing House, 184 p.

Baranovsky, A. Yu. 2008. *Dietology*. St. Petersburg, Russia : Peter. 893 p. ISBN: 9785469016816.

Dalle Zotte, A., Szendrő, Z., 2011. The role of rabbit meat as functional food. *Meat Science*, vol. 88, no. 3, p. 319-331. https://doi.org/10.1016/j.meatsci.2011.02.017 PMid:21392894

Gerasimova, N. Y., Golovaneva T. V. 2013. Optimization of rabbit meat functional and technological properties by means of whey and apple acid treatment. *Scientific Journal of KubSAU*, vol. 85, no. 1, p. 337-346.

Hernández, P. 2008. Enhancement of nutritional quality and safety in rabbit meat. In *9th World Rabbit Congress*. Verona, Italy, p. 1287-1299.

Methodical instructions for the accelerated determination of the toxicity of livestock products and feed. Approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation, 16.10.2000. no. 13-7-2156.

Nikitin, B. I., Belchenko, N. B. 1994. *Processing of poultry, rabbits and production of poultry products*. Moscow, Russia: Kolos. 320 p.

Nistor, E., Bampidis, V. A., Păcală, N., Pentea, M., Tozer, J., Prundeanu H. 2013. Nutrient Content of Rabbit Meat as Compared to Chicken, Beef and Pork Meat. *Journal of Animal Production Advances*, vol. 3, no.4, p. 172-176. https://doi.org/10.5455/japa.20130411110313

Petracci, M., Cavani, C. 2013. Rabbit meat processing: historical perspective to future directions1. *World Rabbit Science*. vol. 21, no. 4, p. 217-226. https://doi.org/10.4995/wrs.2013.1329

Pla, M., Pascual, M., Ariño, B. 2004. Protein, fat and moisture content of retail cuts of rabbit meat evaluated with the NIRS methodology. *World Rabbit Science*, vol. 12, no. 3, p.149-158.

Polak, T., Gašperlin, L., Rajar, A., Žlender, B. 2006. Influence of genotype lines, age at slaughter and sexes on the composition of rabbit meat. *Food Technology and Biotechnolology*, vol. 44, no. 1, p. 65-73.

Sautkin, A. V. 2010. *Veterinary and sanitary assessment of rabbit meat when using "Emil" in their diet* : theses. Moscow, Russia. 137 p.

State Standard GOST 10444.15-94: 2010. *Food products. Methods for determining the amount of mesophilic aerobic and facultative-anaerobic microorganisms*.

State Standard GOST 30518-97: 2009. *Food products. Methods for the detection and determination of the number of bacteria of the Escherichia coli group (coliform bacteria)*.

State standard GOST 30519-97: 2005. *Food products. Methods for the detection of bacteria of the genus Salmonella*.

Tutelyan, V. A., Samsonov, M. A. 2002. *Guide to Dietology*. 3rd ed. Russia : Clinical Medicine, 544p. .ISBN 5-225-04723-8.

Ulikhina, L. I. 2009. *Handbook of rabbit breeder from A to Z*. Moscow, Russia : Aquarium-Print, 256 p. ISBN: 978-5-9934-0221-5.

Vasilenko, O. A. 2004. Development of meat products production technologies on the basis of the rational cuttingof rabbit towels : abstract of theses. Russia, 22 p.

Volkova, O. V., Inerbaeva, A. T., Motovilov, K. Y. 2009. Morphological composition and biological value of rabbit meat. *Siberian Herald of Agricultural Science*, vol. 8, p. 97.

Zhidik, I. Yu. 2017. *Veterinary-sanitary evaluation of rabbit meat by the use of mineral additive "Zeolite Natural" of Kholinsky deposit* : abstract of theses. Russia : UFA. 21p.

Zhitnikova, I. 2004. *Rabbits: breeds, breeding, maintenance, care. Rostov-on-Don*. Russia : Phoenix. 256 p. ISBN 5-222-05603-1.

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EFFECT OF DIFFERENT STORAGE TIMES ON JAPANESE QUAIL EGG QUALITY CHARACTERISTICS

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ABSTRACT

The aim of this research was to monitor selected quality parameters of Japanese quails (*Coturnix coturnix japonica*) – the loss of egg weight, changing yolk and white index, Haugh units, egg yolks color. Quail eggs were stored for 0, 1, 2, 4, 6 and 8 weeks at 4 °C. The weight of the quail eggs ranged from 11.67 to 12.27 g. The ratio of the shell range 7.60 to 8.16 % (resp. 0.89 – 0.96 g), ratio of egg white from 59.33 to 62.10 % (resp. 6.31 – 6.66 g) and a ratio yolk of 30.13 – 32.88 % (resp. 3.68 – 3.91 g). The lost of egg weight ranged from 0.47 to 2.93 % during the quail eggs storage, corresponding to a weight loss of 0.26 – 0.58 g of the total weight of the eggs. The average values of the yolk index ranged from 42.67 – 48.53 % and the average values of the quail egg white index ranged from 6.77 to 11.35 %. The average Haugh units were set between 56.93 and 73.72. The color of quail egg yolk was determined using the La Roche scale with the most frequent value 3. During the quail eggs storage, a statistically significant difference was found with most of the quality traits observed.

Keywords: quail eggs; egg quality parameters; storage time; storage temperature

INTRODUCTION

Egg as a nutritionally balanced and easily digestible nutritional ingredient is one of the frequent foods appearing on the menu. Hen eggs are the most commonly consumed, although Japanese quail eggs (*Coturnix coturnix japonica*) are also readily available today, which are considered to be nutritionally important to consumers, mainly due to their rich vitamin and mineral content. Despite the small size (approximately 10 – 12 g), quail eggs are also rich in proteins, amino acids, macro and microelements (calcium, selenium and zinc) and have low triglyceride and saturation fatty acids. Some countries have a long tradition of consuming quail eggs such as Japan, where quail eggs are considered to be almost natural medicine, especially to reduce cholesterol, blood pressure, increased immunity and allergy treatment **(Baumgartner and Hetényi, 2001; Angelovičová et al., 2013)**.

In the Czech Republic, the production of quail eggs is not as wide as it is in other countries. The most significant producer quail eggs are China, Japan, Brazil and France. The production and consumption of eggs bear certain requirements that are necessary to maintain egg quality. These include good hygienic practice, in particular length and temperature of storage. The storage period for fresh eggs is set at 28 days and must be sold to the consumer within 21 days at the latest, so that they have enough time to process them **(Regulation (EC) No. 853/2004)**. Temperature requirements vary slightly from country to country and are determined by temperatures ranging from 5 °C to 18 °C for the Czech Republic **(Decree No. 69/2016 Coll. of Czech Republic)**.

The quality of the egg is usually given in relation to the requirements of consumers and determines the general characteristics which are easily identifiable without breaking the egg, especially the freshness, weight, size, shape and appearance of the eggshell. More precisely, the quality characteristics are determined by the individual egg fluids and the shells can be distinguished. The quality of quail eggs is mainly dependent on the breed, the age laying hens, the composition of the feed, and also the storage time and temperature. For the consumer, one of the most important features is the weight of the egg, as well as the visual aspect of the egg purity and the integrity of the eggshell.

Japanese quails are due to their easy care, early sexual maturity and above all a high degree of egg production used in research. And in some countries (Poland, Hungary) are increasingly used in the food industry.

Egg quality parameters include general characteristics – in particular weight, size, egg shape and shell appearance. Another very important feature is the shell integrity, which is important not only from the economic point of view, but also with regard to the safety of human health **(Yanakopolous, 1986)**.

Quail eggs quality is divided into external and internal quality parameters **(Arpášová et al., 2012)**. The outer

quality features include the weight, the shape of the egg, the quality of the egg shell, the structure of the shell, the strength, the porosity and the color. **Baumgartner and Hetényi (2001)** presented in his study weight whole egg 10.16 g, shaped index 85.70 % and shell strength 24.40 N.

The weight of quail eggs is a significant indicator of total egg production and is about 5 times lower than that of hen eggs. The average weight of quail eggs ranges from 10 to 12 g, which is about 8 % of the total weight of the hen **(Panda and Singh, 1990)**. The size and hence the weight of the quail eggs is influenced by several factors such as the age of the laying hens, the breed, genetics, climatic conditions, the season, the composition of the feed or the length of the laying **(Nowaczewski et al., 2010b)**.

However, quail eggs are not a constant indicator and are dependent on a number of working indicators such as genotype, length and storage temperature and age of laying **(Alkan et al., 2008).**

The internal characters are divided into individual egg components, ie the egg albumen is determined by the albumen index, Haugh unit and its weight is calculated. **Baumgartner and Hetényi (2001)** reported values of index albumen 11.30 %, Haugh units 114.50, and weight of egg albumen 5.12 g for quail eggs. For the yolk part was monitored the yolk index, weight and their color, subjectively determined by the La Roche scale, are followed. These values are reported in **Baumgartner and Hetényi (2001)**, where the yolk index was 50.40 % and the yolk weight 3.22 g. Neither quail eggshell are neglected in terms of quality and their weight is followed after thorough cleaning and drying, but also the thickness of the shell at the blunt and sharp end of the egg.

The quality of quail eggs, however, also depends on a number of internal as well as external influences, whether in pre-laying or laying on eggs. This quality can therefore be affected by the age of laying hens, feed composition, controlled farming conditions, storage temperature and time, relative humidity and other influences **(Baumgartner and Hetényi, 2001).**

Scientific hypothesis

The main hypothesis of this work is to determine qualitative parameters quail eggs during 8 week storage with constant temperature 4 °C.

MATERIAL AND METHODOLOGY

To determine the selected qualities of quail eggs and their changes during storage was used of Japanese quail egg (*Coturnix coturnix japonica*) the breed Pharaon in 20th week of age from cages in South Moravia, which supplies quail eggs to the market network. Quails were fed a complex feed mixture throughout the laying season. Complex feed mixture was composit from wheat (30 %), maize (28 %), soybean extruded toast (18 %), fish meal (6 %), wheat bran (4%), alfalfa meal (3 %), calcium carbonate (4 %), L-lysine, DL-methinine, vitamin A, D3, E, copper sulfate pentahydrate (CuSO4.5H2O), butylhydroxyanisole, butylhydroxytoulene, etoxyquin.

Quail eggs were removed and imported on the day of laying. All fresh eggs were first weighed, labeled and stored at 4 °C at 75 % relative humidity. The length of quail eggs storage was 0, 1, 2, 4, 6 and 8 weeks, and a total of 120 quail eggs were used to monitor the quality characteristics. In each storage week, the following quality parameters were analyzed 20 eggs.

At sampling, eggs were weighed and broken on to a flat surface where the height of the albumen was measured by using albumen height gauge. The thick of albumen were measured using micrometer. The yolk was separated from the albumen and was weighed. The shells were dried at 130 °C temperature during 60 minutes and weighed. The shell thickness were measured from the three different parts of shell in each (sharp, blunt and equator end of egg) using a micrometer and was averaged and recorded as shell thickness. The weight of the albumen was calculated as the difference between the weight of the egg and the weight of the yolk and shell. The color of yolk was determined by the La Roche color scale.

Egg quality parameters were calculated as:

Haugh unit = $100 \times \log (\text{Albumen height} + 7.57 - 1.7 \times \text{Egg weight}^{0.37})$ **(Haugh, 1937).**

Albumen index = Albumen height (mm) / [Albumen length (mm) + Albumen width (mm)] × 100.

Yolk index = Yolk height (mm) / Yolk diameter (mm) × 100.

Egg weight loss = [(Egg weight - Egg broken weight) / Egg weight] × 100.

Shape index = maximum width (mm) / maximum length (mm) × 100.

Statisic analysis

Statistical analysis of the differences was based on Statistica12 (StatSoft, Czech Republic), namely single-factor ANOVA – Duncan's test. Microsoft Excel version 2010 (Microsoft) was used to evaluate the results. The statistically inconclusive difference was considered to be a result whose probability value reached $p > 0.05$.

RESULTS AND DISCUSSION

The egg weight and egg weight loss of quail eggs

The average weights of the fresh quail eggs (12.00 g) are considerably higher, then values **Panda and Singh (1990)** and **Nowaczewski et al. (2010b)**. On the contrary, **Alkan et al. (2011)** and **Genchev (2012)** indicate the quail eggs weighing values lower, due to the age and breed of Japanese quail. **Baylan et al. (2011)** is the average weight of quail eggs at the same level as our measurements. Our results correlate with the results of **Nowaczewski et al. (2010b)** where the average weight of quail eggs during storage gradually decreased from 11.37 to 11.00 g. The weight loss values of quail eggs increased from 0.47 % (1st week of storage) to 2.93 % (8th week of storage) which is caused when the water contained in the eggs had evaporated. The weight loss in quail eggs during storage was almost linearly increased, which corresponds to the claim by **Roriz et al. (2016)**, which also reported the highest weight loss in the 6th week of storage with a loss of 2.40 % of the original weight. The statistical significant of weight and weight loss during storage is given in the Table 1.

The egg shell thickness, shell weight and shell ratio of quail eggs

The ratio of the shell from the total weight of quail eggs ranged from 7.60 to 8.16 %. The lowest individual egg shell ratio for quail eggs was 5.34 %, whereas the highest individual shell ratio was 9.58 %. Creation and shell thickness favorably affects feeds rich on minerals, especially calcium, while high chlorine content in feed, uneasiness of laying hens, and high ambient temperatures can have a negative impact on the thickness and strength of the shell. The average shell thickness for quail eggs ranges from 0.10 to 0.14 mm. The lowest individual shellfish shell thickness of the quail eggs was 0.05 mm, while the highest individual value was 0.22 mm.

The ratio of the shell from quail eggs coincides with that of **Akpinar et al. (2015)**, where the ratio of quail eggs was 8.14 – 8.40 %. Our results ranged from 7.60 % (8th week of storage) to 8.16 %, which was found in eggs at the 4th week of storage. In the **Olgun (2015)** study, the shell ratio values are closest to our closest values, with a difference of only 0.01 %, which correlates with the shell thickness, which in this case was 0.17 mm. The statistically significant of weight and weight loss during storage is given in the Table 1.

However, the shell ratio is lower than **Genchev (2012)**, **Wilkanowska and Kokoszyński (2012)**, which is attributed to the quail eggs of another breed. **Baumgartner and Hetényi (2001)** show the shell ratio values also higher than those we achieved in the measurement, up to 9.51 % and similarly determined shell share in **El-Tarabany et al. (2015)**, when the difference to our results was 2.47 %. Deviations between results may be due to different breeding factors, which is related to egg weight and feed composition, which correlates with egg shell thickness and therefore its ratio. However, the results show that the shelf life of egg shells in quail eggs has minimal demonstrable effect.

Albumen weight and albumen ratio of quail eggs

The average ratio of egg albumen in quail eggs ranged from 60.13 % (Table 2) in freshly eggs, followed by an increase in the albumen ratio by an average of 1.8 % after the first week of storage. The increase in the proportion of egg whites in quail eggs was recorded only after the end of the 2nd week of storage, and thereafter the amount of albumen began to decrease, which correlates with the increasing proportion of yolk when osmotic pressure is due to the diffusion of water from the part of the albumen to the yolk. The average values of the albumen ratio of the quail eggs range from 59.33 to 62.10 %, corresponding to a weight of 6.31 – 7.31 g. The proportion of albumen from the total weight of quail eggs (60.50 %) corresponds to the weight categories of eggs according to **Nowaczewski et al. (2010b)** between egg sizes S and M. These values are slightly higher than reported by **El-Tarabany et al. (2015)** and **Zeweil et al. (2016)**. The **Baylan et al. (2011)** reached slightly higher scores versus 60.50 % with albumen ratio of 61.68 %. The decrease in the proportion of the albumen also dependent on the storage temperature, when **Inci et al. (2015)** for stored eggs at 28 °C shows a ratio of albumen after storage of 58.58 % after storage for ten days and 55.78 % under the same conditions after 20 days of storage.

Different values of the ratio of albumen in quail eggs may be different temperature and relative humidity of storage as well as breed or age of laying hens. **Nowaczewski et al. (2010b)** shows the weight of the albumen before storage of 7.05 g and after 8 days of storage 6.70 g, of which the effect of shelf life on the weight of egg albumen which is due to the migration of water contained in the white part into the yolk.

Albumen index and Haugh units of quail eggs

One of the most important qualities of quail eggs is the albumen egg index, which closely correlates with the height and width of the albumen, when it is easy to determine the albumen index and, therefore, its quality quail eggs during storage. However, the age of the laying hen and especially the storage lenght and temperature affect by the value of the albumen index. The average values of the dense albumen index ranged from 6.77 % (8th week of storage) to 11.35 % achieved at week 2nd storage (Table 2). The lowest individual index of albumen index for quail eggs was recorded at the 8th week of storage, namely 0.18 %, while the highest individual index values of white were reached in the 4th week of storage with a value of 20.07 %. The values of the albumen index of our quail eggs almost coincide with the results of **Baumgartner and Hetényi (2001)** for Japanese quail eggs stored at 4 °C. In contrast, **Baylan et al. (2011)** also monitored the albumen index of samples stored at 20 °C and proved the negative effect the temperature, where this value decreased compared to samples stored at refrigerated temperature by 4.75 %. **Bagh et al. (2016)** also

Table 1 Effect of storage time on exterior characteristics of quail eggs.

Length of storage [week]	0	1	2	4	6	8
Egg weight, [g]	12.27	12.17	12.18	11.80	11.94	11.67
Egg weight loss, [%]	-	0.47^{b}	1.11^{a}	1.20^{a}	1.93^{c}	2.93^{d}
Shape index, [%]	74.85	76.43	77.33	78.85	76.86	76.61
Egg shell thickness, [mm]	0.12^{b,c}	0.14^{c}	0.11^{a,b}	0.14^{c}	0.10^{a,b}	0.10^{a}
Shell weight, [g]	0.99^{b}	0.94^{a,b}	0.94^{a,b}	0.96^{a,b}	0.95^{a,b}	0.89^{a}
Shell ratio, [%]	8.08^{a,b}	7.71^{a,b}	7.76^{a,b}	8.16^{b}	7.94^{a,b}	7.60^{a}

Note: $^{a, b, c, d}$ – different superscripts in a line indicate a statistically significant difference at p <0.05.

Table 2 Effect of storage time on interior characteristics of quail eggs.

Length of storage [week]	0	1	2	4	6	8
Albumen weight, [g]	6.67^{a}	7.01^{b}	6.66^{a}	$6.85^{a,b}$	7.31^{b}	6.31^{a}
Albumen ratio, [%]	$60.13^{a,b}$	$61.21^{b,c}$	62.10^{c}	$60.69^{a,b,c}$	59.33^{a}	$59.52^{a,b}$
Yolk weight, [g]	3.91	3.78	3.68	3.68	3.91	3.84
Yolk ratio, [%]	$31.79^{a,b}$	$31.07^{a,b}$	30.13^{b}	$31.15^{a,b}$	32.73^{a}	32.88^{a}
Yolk index, [%]	$47.04^{c,d}$	$46.34^{b,c,d}$	48.53^{d}	$44.27^{a,b,c}$	42.67^{a}	$43.60^{a,b}$
Albumen index, [%]	$9.37^{a,b}$	11.35^{b}	$10.19^{a,b}$	$10.47^{a,b}$	$8.56^{a,c}$	6.77^{c}
Haugh unit	$66.25^{a,b}$	73.72^{b}	$67.57^{a,b}$	$66.34^{a,b}$	$61.52^{a,c}$	56.93^{c}
Yolk color	3	3	3	3	3	4

Note: $^{a, b, c, d}$ – different superscripts in a line indicate a statistically significant difference at $p < 0.05$.

reached slightly higher values which are caused to the different breeds of lay hens between experiments. Somewhat higher values of the quail egg albumen index were achieved by **Nowaczewski et al. (2010a)**, by only 1.33 %. Haugh units are based on the weight of fresh quail eggs and the height of the dense albumen. The average Haugh unit values quail eggs was the range from 56.93 (8th week of storage) to 73.72 (1st week of storage). In freshly laying quail eggs, the average Haugh units reached 66.25. The lowest individual value of Haugh units during storage was set at the 8th week of storage, namely 23.81. The highest individual value of the Haugh units showed samples at the 4th week of storage, when this quality indicator reached the 94.53. Initial values of Haugh units are significantly lower than stated in their **Baumgartner and Hetényi (2001)** study, which states the average value of Haugh units of 114.40. The closest to the results of the study is **Kumari et al. (2008)**, which shows the average value of the Haugh units of 59.50. **Nowaczewski et al. (2010b)** have shown in their study that Haugh units are being reduced during storage, with which our results are identical, which is related to changes in dense egg albumen during storage when it is thinned.

The yolk weight, yolk ratio, and yolk index of quail eggs

The average yolk index values for quail eggs during storage were 42.67 % (6th week) and 48.53 % (2nd week of storage), with average yolk weight 3.68 - 3.91 g (Table 2). For fresh eggs was yolk index 47.04 %. However, from the 4th week of storage, the yolk index was reduced by up to 3.44 % (8th week of storage) compared to fresh eggs. The yolk index for quail eggs almost coincides with those reported in **El-Tarabany et al. (2015)**, **Wilkanowska and Kokoszyński (2012)** and **Inci et al. (2015)**. Higher values of the yolk index at the beginning of the experiment were showed by **Nowaczewski et al. (2010a)**, where the freshly layed eggs had an index value of 3.26 % higher than that of our samples at the same time. In all of these studies, the yolk index during storage decreases, which is due to the migration of water between the egg albumen and egg yolks.

Yolk color

The yolk color of Japanese quail eggs is one of the important characteristics of egg quality. However, this fact is very easily influenced, especially by feeding a feed, either in the form of natural, ie green feed, or in the form of carotenoid-enriched compound feeds. The most common yolk color in quail eggs is 3 (Table 2). During stocking, honey yolk quark color values were recorded using a La Roche scale ranging from 1 to 5. The highest shade of 4 was determined in egg yolks of quail eggs at the 8th week of storage. In the other weeks of storage, the highest color values of yolk quail eggs were identical, with a value of 3. The lowest shade of value 1 was, in addition to the 4th week of storage, set in all the remaining weeks. The most common color of egg yolk in Japanese quail eggs coincides with the results of **Kara et al. (2016)**. Similar results were obtained in **Pereira et al. (2016)** with a color shade of 4. The significantly higher values compared to our results were obtained in the **Cayan and Erener (2015)** studies, which determined the average frequency of egg yolk color 12. **El-Tarabany study (2016)** indicates the range of egg yolk color in Japanese quails between 7 and 9.

CONCLUSION

This contribution was focused on the change of qualitative parameters of quail eggs during storage.

The results of this study show the increase in egg weight loss during storage, the increase the yolk ratio during storage, the decrease the albumen ratio, the reduction of white and yolk index, the reduction of Haugh units during storage.

The loss of egg weight during storage occurs due to the evaporation of water from the egg, but also due to the loss of carbon dioxide, ammonia, nitrogen and hydrogen sulphide. During storage, the yolk index is also reduced, which is caused by the diffusion of water into the white part, which also leads to a decrease in the albumen index and leads thinned and the structure changes. The decrease in the albumen index is correlated with the decrease in Haugh units.

These changes during a storage lead to a deterioration in the quality of quail eggs and it is recommended low temperature storing to preserve quality parameters during 8 week storing.

REFERENCES

Angelovičová, M., Alfaig, E., Král, M., Tkáčová, J. 2013. The effect of the probiotics *Bacillus subtilis* (PB6) on the selected indicators of the table eggs quality, fat and cholesterol. *Potravinarstvo,* vol. 7, no. 1, p. 80-84. https://doi.org/10.5219/271

Akpinar, G. C., Canogullari, S., Baylan, M., Alasahan, S., Aygun, A. 2015. The use of propolis extract for the storage of quail eggs. *The Journal of Applied Poultry Research*, vol. 24, no. 4, p. 427-435. https://doi.org/10.3382/japr/pfv043

Alkan, S., Karabağ, K., Galiç, A., Karsli, T., Balcioğlu, M. S. 2011. Effects of selection for body weight and egg production on egg quality traits in Japanese quails (*Coturnix coturnix japonica*) of different lines and relationships between these traits. *Kafkas Universitesi Veteriner Fakultesi Dergisi*. vol. 17, no. 5, p. 239-244.

Alkan, S., Karabağ, K., Galiç, A., Karsli, T., Balcioğlu, M. S. 2008. Predicting yolk height, yolk width, albumen length, eggshell weight, egg shape index, eggshell thickness, egg surface area of Japanese quails using various eggs traits as regressors. *International Journal of Poultry Science*, vol. 7, no. 1, p. 85-88. https://doi.org/10.3923/ijps.2008.85.88

Arpášová, H., Kačániová, M., Haščík, P., Šidlová, V. 2012. Effect of selected feed additives on internal quality parameters of table eggs. *Potravinarstvo*, vol. 6, no. 4, p. 52-61. https://doi.org/10.5219/235

Bagh, J., Panigrahi, B., Panda, N., Pradhan, C. R., Mallik, B. K., Majhi, B., Rout, S. S. 2016. Body weight, egg production, and egg quality traits of gray, brown, and white varieties of Japanese quail (*Coturnix coturnix japonica*) in coastal climatic condition of Odisha. *Veterinary World*, vol. 9, no. 8, p. 832. https://doi.org/10.14202/vetworld.2016.832-836

Baumgartner, J., Hetényi, L. 2001. *Japanese Quail* (*Prepelica japonská)*. 1st ed., Nitra, Slovakia : SPU, 73 p. ISBN 80-88872-16-2. (In Slovak)

Baylan, M., Canogullari, S., Ayasan, T., Copus, G. 2011. Effects of dietary selenium source, storage time, and temperature on the quality of quail eggs. *Biological Ttrace Element Research*, vol. 143, no. 2, p. 957-964. https://doi.org/10.1007/s12011-010-8912-x PMid:21136198

Cayan, H., Erener, G. 2015. Effect of olive leaf (*Olea europaea*) powder on laying hens performance, egg quality and egg yolk cholesterol levels. *Asian-Australasian Journal of Animal Sciences*, vol. 28, no. 4, p. 538. https://doi.org/10.5713/ajas.14.0369 PMid:25656181

Decree No. 69/2016 Coll. Of Czech Republic of 17 February 2016 on requirements for meat, meat products, fishery and aquaculture products and products thereof, eggs and products thereof.

El-Tarabany, M. S., Abdel-Hamid, T. M., Mohammed, H. H. 2015. Effects of cage stocking density on egg quality traits in Japanese quails. *Kafkas niversitesi Veteriner Fak ltesi Dergisi*, vol. 21, no. 1, p. 13-18.

Genchev, A. 2012. Quality and composition of Japanese quail eggs (*Coturnix japonica)*. *Trakia Journal of Sciences*, vol. 10, no. 2, p. 91-101.

Haugh, R. R. 1937. The haugh unit for measuring egg quality. *US Egg Poultry Magazine*, vol. 43, p. 522-555.

Inci, H., Sogut, B., Sengul, T., Sengul, A. Y., Taysi, M. R. 2015. Comparison of fattening performance, carcass characteristics, and egg quality characteristics of Japanese quails with different feather colors. *Revista Brasileira de Zootecnia*, vol. 44, no. 11, p. 390-396. http://doi.org/10.1590/S1806-92902015001100003

Kara, K., Kocaoglu Güclü, B., Şentürk, M., Konca, Y. 2016. Influence of catechin (flavan-3-ol) addition to breeder quail (*Coturnix coturnix japonica*) diets on productivity, reproductive performance, egg quality and yolk oxidative stability. *Journal of Applied Animal Research*, vol. 44, no. 1, p. 436-441. https://doi.org/10.1080/09712119.2015.1091337

Kumari, B. P., Gupta, B. R., Prakash, M. G., Reddy, A. R. 2008. A study on egg quality traits in Japanese quails (*Coturnix coturnix japonica*). *Tamil Nadu Journal of Veterinary and Animal Sciences,* 2008, vol. 4, no. 6, p. 227-231.

Nowaczewski, S., Kontecka, H., Rosiński, A., Koberling, S., Koronowski, P. 2010a. Egg quality of Japanese quail depends on layer age and storage time. *Folia biologica*, vol. 58, no. 3, p. 201-207. https://doi.org/10.3409/fb58_3-4.201-207

Nowaczewski, S., Witkiewicz, K., Kontecka, H. E., Krystianiak, S., Rosiński, A. 2010b. Eggs weight of Japanese quail vs. eggs quality after storage time and hatchability results. *Archiv Tierzucht*, vol. 53, no. 6, p. 720-730. https://doi.org/10.5194/aab-53-720-2010

Olgun, O. 2015. The effect of dietary cadmium supplementation on performance, egg quality, tibia biomechanical properties and eggshell and bone mineralisation in laying quails. *Animal*, vol. 9, no. 8, p. 1298-1303. https://doi.org/10.1017/S1751731115000579

Panda, B., Singh, R. P. 1990. Developments in processing quail meat and eggs. *World's Poultry Science Journal*, vol. 46, no. 3, p. 219-234. https://doi.org/10.1079/WPS19900022

Pereira, A. A., Da Silva, W. A., De Lima júnior, D. M., Lima, C. B., Júnior, D. N. G., Lana, G. R. Q., Oliveira, L. P. 2016. Broken rice in feeds for laying Japanese quails. *Semina: Ciências Agrárias*, vol. 37, no. 4, p. 2831-2838. https://doi.org/10.5433/1679-0359.2016v37n4Supl1p2831

Regulation (EC) No 853/2004 of the European arliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.

Roriz, B. C., Sgavioli, S., Garcia, R. G., Nääs, I. A., Domingues, C. H. F., Caldara, F. R., Bernnecke, K. 2016. Storage period affects weight loss of Japanese quail eggs. *Revista Brasileira de Ciência Avícola*, vol. 18, no. 4, p. 589-592. https://doi.org/10.1590/1806-9061-2015-0178

Wilkanowska, A., Kokoszyński, D. 2012. Layer age and quality of Pharaon quail eggs, *Journal of Central European Agriculture*, vol. 13, no. 1, p. 10-21. https://doi.org/10.5513/jcea.v13i1.1066

Yanakopolous, A. L., Tserveni-Gousi, A. S. 1986. Quality characteristics of quail eggs. *British Poultry Science*, vol. 27, no. 2, p. 171-176. https://doi.org/10.1080/00071668608416870

Zeweil, H. S., Eid, Y. Z., Zahran, S., Dosoky, W., Abu hafsa, S. H., Girges, A. 2016. Effect of different levels of Aspergillus awamori as probiotic on the production and egg quality of laying Japanese quail under summer conditions. *Egyptian Poultry Science Journal*, vol. 36, no. 1, p. 53-65. https://doi.org/10.21608/epsj.2016.5415

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HYPOLIPIDEMIC ACTION OF THE MEAT PRODUCT: *IN VIVO* STUDY

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ABSTRACT

Results of meat product influence on the serum lipid profile of hyperlipidemic rats are presented. Meat product for specialized nutrition content porcine aortas and hearts in ratio 1:3. Thirty male Wistar rats (380 ±20 g) aged approximately 1 year were kept in conventional standard conditions; water and feed were available ad libitum. Animals were randomly divided in 3 groups: group 1 – negative control (n=10); group 2 – positive control (n=10) and group 3 – experimental animals (n=10). Animals in group 2 and 3 were modeled an alimentary hyperlipidemia by adding cholesterol, fat and vitamin D2 into diet. After modeling, rats in group 2 were fed with standard chow, in group 3 – meat product (8g/kg b.w.) with standard chow. On the 42nd day serum lipid profile was investigated and immunoassay was carried out. It was found that the developed meat product given to the hyperlipidemic rats led to a decrease in the concentration of cholesterol, triglycerides and atherogenic fractions of lipoproteins by 31.8% (p <0.05), 28.2% and 2.4 times (p <0.05), respectively. Estimation of the concentration changes in apolipoproteins, forming lipoprotein particles, allowed to indirectly determining the main lipoprotein reduction that contributed to the total decrease in the atherogenic index of serum, which reached 41.3% (p <0.05). Previous proteomic study revealed the presence of a number of specific proteins and peptides in tissues of porcine aortas and heart. The hypothesis that tissue-specific proteins could decomposed into active peptides with antiatherogenic action is considered.

Keywords: meat product; lipids; hyperlipidemia; apolipoproteins; cholesterol

INTRODUCTION

Modern technologies are actively implemented in the food industry, among which a special place is occupied by the functional and specialized products. Major number of publications highlights the results of studies aimed at the modification of the recipe by adding essential nutrients as well as ingredients of vegetable and animal origin in order to achieve a certain biological effect **(Hui, 2012; Weiss et al., 2010)**.

Meat is a functional system including protein, conjugated linoleic acid (CLA), minerals (iron, zinc and selenium), vitamins (B, E), glutathione, ubiquinone, lipoic acid etc. **(Arihara, 2006; Arihara and Ohata, 2011)**. Nowadays, the functional properties of meat are associated with biologically active peptides, such as L-carnitine, carnosine, anserine, creatine, taurine etc. **(Arihara, 2006; Udenigwe and Howard, 2013; Lafarga and Hayes, 2014)**. Moreover, meat proteome is a source of bioactive sequences possessed hypotensive, antioxidant, opioid, immunomodulatory, prebiotic, mineral-binding, cholesterol-lowering and antimicrobial activity **(Bauchart et al., 2006; Ahhmed and Muguruma, 2010; Toldrá et al., 2012; Udenigwe and Howard, 2013; Lafarga and Hayes, 2014)**.

During the last decade a special emphasis is paid on peptides, native contained in raw materials or formed during the enzymatic hydrolysis or food processing **(Mine and Shahidi, 2006)**. Moreover, advanced methodological approaches, particularly proteomics, confirmed that proteome and peptidome of any tissue are formed by not only constitutive structural and functional proteins and peptides, but also are characterised by a number of specific molecules involved in maitance of normal physiological condition **(Fagerberg et al., 2014)**.

In this regard, the study of by-products as sources of bioactive sequences involved in normalization of the metabolic disorders is very relevant to the food industry as well as development specialized and functional products on their basis.

Scientific hypothesis

Previously, authors revealed that introduction of native tissues of cattle and porcine hearts and the aortas into the diet of hyperlipidemic rats led to a significant decrease of total cholesterol, triglycerides and atherogenic fractions of lipoproteins in serum **(Chernukha et al., 2014)**. Porcine tissues possessed the greatest efficiency.

Proteomic study revealed the presence a number of specific proteins in tissues of porcine aortas: apolipoprotein A-1 involved in the formation of high density lipoproteins, peroxiredoxin-1 (in mixture with transgelin) involved in the

suppression of oxidative stress, galectin-1 induced apoptosis of T-lymphocytes, a number of heat shock proteins as well as about 22 tissue-specific peptides with unknown function.

Fatty acid-binding protein and about 6 tissue-specific peptides with unknown function were detected in tissues of porcine heart. However, it was found that these bioactive substances are decomposed after sterilization process of the product, except fatty acid-binding protein and several peptides **(Chernukha et al., 2016)**.

Therefore we hypothesized that tissue-specific proteins could decomposed into active peptides with similar biological action. This fact could make it possible to create a functional meat product based on porcine heart and aortas.

MATERIAL AND METHODOLOGY

Meat product production

Meat product for specialized nutrition was produced on ZAO "Yoshkar-Olinskiy Myasokombinat". Porcine hearts were chopped with a particle size of 2-3 mm and salted for 12 h. Porcine aortas were chopped with a particle size of 2-3 mm and homogenized in cutter at 3000rpm for 2-3 min. Minced hearts with the juice were quantitatively transferred in the cutter and homogenized at 3000rpm for 6-8 min (ratio of aorta to hearts 1:3). Obtained mince was packed in cans of lamister and sterilized at 115 °C, a pressure of 0.23 MPa for 40 min. Meat product contained 17.53 ±0.95% protein, 3.82 ±0.13% fat, 0.305 ±0.015% sodium chloride, and 2.35 ±0.25% starch.

Animal experiments

Thirty male Wistar rats (380 ±20 g) aged approximately 1 year were kept in conventional standard conditions; water and feed were available ad libitum. Animals were randomly divided in 3 groups: group 1 – negative control (n=10); group 2 – positive control (n=10) and group 3 – experimental animals (n=10). Animals in group 1 (negative control) got a standard chow (Labkorm, Russia) ad libitum during the experiment. Rat model of alimentary hyperlipidemia was developed by adding cholesterol (2.0-10.0%) and fat (10.0 – 25.5%) to the standard diet and vitamin D2 injection per os (35,000 IU/kg b.w.). After modeling, rats in group 2 (positive control) were fed with standard chow, in group 3 – meat product (8g/kg b.w.) with standard chow.

On the 42nd day rats were euthanized in VETtech camera according to the rules of the animal welfare, blood samples biochemical investigations were taken.

Biochemical analysis

Biochemical investigations were carried out on automatic analyzer BioChem FC-360 (HTI, USA) according to instructions applied to measurement kits (HTI, USA). Total cholesterol (TCL), triglyceride (TG), cholesterol low-density lipoproteins (CL LDL) and cholesterol high-density lipoproteins (CL HDL) levels were measured in rat serum. Cholesterol non-LDL and non-HDL was calculated as the difference between TCL and CL LDL and HDL. Atherogenic index (AI) = (TCL - CL HDL)/ CL HDL.

Immunoassay

Evaluation of apolipoprotein A-1 (Apo A-1), apolipoprotein B-100 (Apo B-100), and apolipoprotein E (Apo E) levels in the serum were measured on ImmunoChem 2100 (HTI, USA) using the respective commercial ELISA kits, according to the respective instructions (Cloud-Clone Corp., China).

Statisic analysis

STATISTICA 10.0 software was used in this study for the statistical analyses. Significant differences were tested by using two-way analysis of variance (ANOVA), followed by Duncan's test. Differences with p-values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Long-term consumption of a diet enriched with cholesterol and animal fats led to the increase of TCL and of atherogenic fractions of lipoproteins and TG in rat serum. On the 42nd day after the cancellation of proatherogenic diet the concentration of TCL and TG in rat serum of group 2 exceeded the group 1 level by 35.8% (p <0.05) and 17.0% (p <0.05). Redistribution of lipoprotein fractions was also noticed: CL LDL cholesterol increased by 15.5% (p <0.05) as well as CL non-LDL and non-LDL by 2.3 fold (p <0.05) (Table 1).

Introduction of meat product into rat diet (group 3) led to a decrease in the serum concentration of TCL (31.8%, p <0.05) and TG (by 28.2%, p <0.05) compared with group 2. Redistribution of lipoprotein fractions was also noticed: CL LDL cholesterol decreased by 21.6% (p <0.05) as well as CL non-LDL and non-LDL by 2.4 fold (p <0.05) (table 1).

Elevation of athergenic lipoproteins rate in group 2 resulted in a significant increase of AI by 59.5% compared to group 1, while in group 3 it reduced by 41.3% compared with group 2 (table.1).

Apolipoprotein levels directly correlate with the concentration of lipoproteins circulating in the blood. Apo A-I is the major protein of HDL and provide HDL binding with receptor, but also detected in chylomicrons (minor). Apo B-100 is the main ligand of very low and middle density lipoproteins (VLDL, MDL) and LDL to corresponding receptor. Apo E is the protein part of chylomicrons, VLDL, MDL and HDL (minor) and also provides ligand-specific regulation of lipoprotein-receptor binding process responsible for cholesterol and cholesterol ether transfer from blood to internal organs and tissues, mostly in liver **(Olofsson et al., 2007; Camejo et al., 2014)**.

It is interesting to note that a significant increase of Apo A-I by 36.5% (p <0.05) and 38.0% (p <0.05) was revealed in group 2 compared with group 1 and 3, while CL HDL between all groups was not significantly changed. On the contrary, changes in the content of Apo B-100 were not observed. However, the concentration of CL LDL in group 3 was lower by 21.6% (p <0.05) than in group 2.

On the other hand, the content of APO E in group 2 was significantly increased by 53.2% (p <0.05), while in group 3 decreased to group 1 level (Table 1). These changes correlated with a decrease in the concentration of CL non-LDL and non-LDL, characterizing a content of atherogenic lipoproteins such as chylomicrons, VLDL and MDL.

Table 1 Serum lipid profile and apolopoprotein concentration in rat serum.

Group	AI (relative units ±SD)	TG (mmol.L^{-1} ±SD)	CL (mmol.L^{-1} ±SD)			
			Total	LDL	HDL	non-LDL and non-HDL
Group 1	1.58 ±0.09	1.76 ±0.27	2.18 ±0.12	0.84 ±0.05	0.85 ±0.03	0.49 ±0.06
Group 2	2.52 ±0.12*	2.06 ±0.33	2.96 ±0.08*	0.97 ±0.02	0.88 ±0.06	1.12 ±0.03*
Group 3	1.48 ±0.22$^{\#}$	1.48 ±0.18	2.02 ±0.19$^{\#}$	0.76 ±0.11$^{\#}$	0.81 ±0.03	0.47 ±0.14$^{\#}$

Apolipoproteins

Group	Apo A-I (mg.mL^{-1} ±SD)	Apo B-100 (mg.mL^{-1} ±SD)	Apo E (µg.mL^{-1} ±SD)
Group 1	0.444 ±0.016	2.506 ±0.207	21.449 ±0.795
Group 2	0.586 ±0.020*	2.504 ±0.200	32.851 ±1.701*
Group 3	0.424 ±0.027$^{\#}$	2.632 ±0.177	25.326 ±3.644$^{\#}$

Note: *Significant different while compared with group 1, $^{\#}$Significant different while compared with group 2.

Thus, despite of CL LDL reduction in group 3, there were no significant differences in the change of Apo B-100 concentration between the experimental and control groups. Therefore this observation may be linked to an increase of MDL and VLDL, whereas the increased content of Apo A-I in the serum of positive control animals may be correlated not only with increase of HDL, but also with elevated chylomicron content. The hypothesis is confirmed by the increase of Apo E in the serum of positive control rats. Apo E content is correlated with the level of chylomicrons, MDL and VLDL summarized in CL non-LDL and non-LDL, and TG, which were in positive control group by 2.3 times (p <0.05) and 28.2% higher than in group 1.

According to scientific hypothesis mentioned above, identified tissue-specific proteins could be decomposed into active peptides with similar biological action or observed hypolipidemic effect could be linked with saved after sterilization tissue-specific peptides (**Chernukha et al., 2016**). More of them were identified in porcine aorta.

In two reference studies, authors investigated the influence of fractions containing target proteins and peptides in animal experiments. It was revealed that *per os* injection of low-molecular ultrafiltrate (Mw<30kDa) during only 14 days to hyperlipidemic Guinea pigs lead to total cholesterol content and atherogenic index reduction by 44.1% and 43.7%, respectively, due to CL LDL and CL non-LDL and non-LDL levels decrease by 43.7% and 79.5%, respectively. Moreover, such inflammatory markers as serum C-reactive protein (CRP) and vascular endothelial growth factor (VEGF) was also reduced by 34.0% and 58.3% on average, respectively **(Chernukha et al., 2015)**. CRP is knewn as an acute phase protein involved in general atherosclerosis inflammation by attracting monocytes into atherosclerotic plaques zone by binding to a specific receptor **(Seidova 2005; Huang et al., 2014)**. VEGF is a key regulator of the formation of microvessels and also known as a factor of endothelium disfunction **(Mangilova, 2012; Swirski and Robbins, 2013)**.

In second reference study authors revealed that *per os* injection of peptide ultrafiltrate (Mw<5kDa) during only 14 days to hyperlipidemic rats mainly contributed to normalization of inflammation and demonstrated regulatory activity, while that *per os* injection of ultrafiltrate containing middle weight proteins (Mw=5-30kDa) lead to total cholesterol content and atherogenic index reduction by 30.1% and 40.7%, respectively, due to CL non-LDL and non-LDL levels decrease by 66.9% **(Kotenkova, 2017)**.

Thus, in both two reference studies hypolipidemic effect was observed after 14 days of per os injection of fractions containing target substanses, while meat product introduction into diet of rats with hyperlipidemia lead to noticeable serum lipids reduction only on 42nd day. This fact could be explained by target protein breakdown into peptides with less residual biological activity. On the other hand, numerous publications also confirmed structural proteins as a good sourse of bioactive peptides, including peptides with lipid-lowering action **(Bauchart et al., 2006; Ahhmed and Muguruma, 2010; Toldrá et al., 2012; Udenigwe and Howard, 2013; Lafarga and Hayes, 2014)**. In this regard, heart tissue is enriced with muscle tissue proteins, while aorta – collagen and elastine, which are a source of GLY-PRO peptides with hypolipidemic action **(Lyapina et al., 2015)**. Presumably, active peptides could be generated both during meat product processing and digestion processes.

CONCLUSION

Developed meat product contributed to serum lipid level reduction in hyperlipidemic rats, mainly due to the decrease of chylomicrons and VLDL content, which is resulted in AI reduction by 41.3% (p <0.05). Despite on the decomposition of functional protein and peptides compounds after heat treatment process, a pronounced lipid-lowering effect of the developed product was noted. This effect can be linked with protein breakdown during meat product processing and digestion processes which occur on peptides with less residual biological activity.

REFERENCES

Ahhmed, A. M., Muguruma, M. 2010. A review of meat protein hydrolysates and hypertension. *Meat Science*, vol. 86, no. 1, p. 110-118. https://doi.org/10.1016/j.meatsci.2010.04.032

Arihara, K. 2006. Strategies for designing novel functional meat products. *Meat Science*, vol. 74, no. 1, p. 219-229. doi: https://doi.org/10.1016/j.meatsci.2006.04.028

Arihara, K., Ohata, M. 2011. Functional foods: concept to products. In Functional Foods. 2nd ed. Cambridge: Woodhead Publishing, UK, p. 512-533. ISBN: 978-1-84569-690-0. https://doi.org/10.1533/9780857092557.3.512

Bauchart, C., Rémond, D., Chambon, C., Patureau Mirand, P., Savary-Auzeloux, I., Reynès, C., Morzel, M. 2006. Small peptides (<5 kDa) found in ready-to-eat beef meat. *Meat Science*, vol. 74, no. 4, p. 658-666. doi: https://doi.org/10.1016/j.meatsci.2006.05.016

Camejo, G., Hurt-Camejo, E. 2014. Macrophages, extracellular matrix, and lipoproteins in arterial cholesterol balance. *Journal of Lipid Research*, vol. 55, no. 1, p. 1-3. doi: https://doi.org/10.1194/jlr.E045732

Chernukha, I. M., Fedulova, L. V., Kotenkova, E. A. 2014. Study on the molecular-biological principles of hypolipidemic activity of cardiac and aortic tissues of pigs and cattle. *Archivos Latinoamericanos de Produccion Animal,* vol. 22, no. 5, p. 22, 594-597.

Chernukha, I. M., Fedulova, L. V., Kotenkova, E. A. 2015. Meat by-product is a source of tissue-specific bioactive proteins and peptides against cardio-vascular diseases. *Procedia Food Science*, vol. 5, p. 50-53.

Chernukha, I. M., Fedulova, L. V., Kotenkova, E. A., Shishkin, S. S., Kovalyov, L. I., Mashentseva, N. G., Klabukova, D. L. 2016 Influence of heat treatment on tissue specific proteins in the Sus Scrofa cardiac muscle and aorta. *Russian Journal of Biopharmaceuticals*, vol. 8, no. 6, p. 38-44.

Fagerberg, L., Hallström, B. M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J., *et al*. 2014. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Molecular and Cellular Proteomics,* vol. 13, no. 2, p. 397-406. doi: https://doi.org/10.1074/mcp.M113.035600

Huang, C., Cen, C., Wang, C. X., Zhan, H., Ding, X. 2014. Synergistic effects of colchicine combined with atorvastatin in rats with hyperlipidemia. *Lipids in Health and Disease,* vol. 13, no. 67, p. 1-6. https://doi.org/10.1186/1476-511X-13-67

Hui, Y. H. 2012. *Handbook of Meat and Meat Processing*. 2nd ed. USA : Taylor & Francis Group. 1000 p. ISBN 9781439836835. https://doi.org/10.1201/b11479

Kotenkova, E. A. 2017. The study of substances contained in porcine aorta tissues for creation of functional food additive. *Issues of nutrition, biotechnology and food safety*. Russia : Federal Reseach Centre of Nutrition and Biotechnology. 302 p. ISBN 978-5-9909049-1-0.

Lafarga, T., Hayes, M. 2014. Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. *Meat Science,* vol. 98, no. 2, p. 227-239. https://doi.org/10.1016/j.meatsci.2014.05.036

Lyapina, L. A., Grigor'eva, M. E., Obergan, T. Y., Shubina, T. A., Andreeva, L. A., Myasoedov, N. F. 2015. Peptide regulation of metabolic processes under hypercholesterinemia conditions of an organism. *Biology Bulletin,* vol. 42, no. 6, p. 546-555. https://doi.org/10.1134/S1062359015060060

Mangilova, T. O. 2012. The system of vascular endothelial growth factor and hypertension. *Heart and Vessels,* vol. 41, p. 107-115.

Mine, Y., Shahidi, F. 2006. *Nutraceutical Proteins and Peptides in Health and Disease*. USA : Taylor & Francis Group, CRC Press. 688 p. ISBN 9781420028836.

Olofsson, S. O., Wiklund, O., Borén, J. 2007. Apolipoproteins A-I and B: biosynthesis, role in the development of atherosclerosis and targets for intervention against cardiovascular disease. *Vascular Health and Risk Management,* vol. 3, no. 4, p. 491-502. PMid:17969379

Seidova, G. B. 2005. C-reactive protein and its correlation with metabolic syndrome associated with coronary heart disease in women in pre- and postmenopausal women. *Bulletin of Arrhythmology*, no. 41, p. 47-50.

Swirski, F. K., Robbins, C. S. 2013. Neutrophils Usher Monocytes Into Sites of Inflammation. *Circulation Research*, vol. 112, no. 5, p. 744-745. https://doi.org/10.1161/CIRCRESAHA.113.300867

Toldrá, F., Aristoy, M. C., Mora, L., Reig, M. 2012. Innovations in value-addition of edible meat by-products. *Meat Science,* vol. 92, 290-296. https://doi.org/10.1016/j.meatsci.2012.04.004

Udenigwe, C. C., Howard, A. 2013. Meat proteome as source of functional biopeptides. *Food Research International*, vol. 54, no. 1, p. 1021-1032. https://doi.org/10.1016/j.foodres.2012.10.002

Weiss, J., Gibis, M., Schuh, V., Salminen, H. 2010. Advances in ingredient and processing systems for meat and meat products. *Meat Science*, vol. 86, no. 1, p. 196-213. https://doi.org/10.1016/j.meatsci.2010.05.008

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EGGS AND THEIR CONSUMPTION AFFECTED BY THE DIFFERENT FACTORS OF PURCHASE

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ABSTRACT

Consumers' buying behavior and preferences are affected by many factors such as the product quality, price, labeling and country of origin. The objective of this paper was to examine the opinions of the Slovak consumers about the purchase and consumption of eggs and identify their preferences at egg purchase. The input data were obtained using a questionnaire survey. The method of descriptive statistics and selected methods of measuring dependence or associations were used. The existence of statistically significant relationships was verified by the Pearson chi-square test or by the Fisher's exact test. The statistical proof of relationships was evaluated based on the significance of the test characteristic (*p*-value). The existence of dependence between the most important factor affecting purchasing of eggs - a price of eggs, and the age of the respondent - was confirmed. Further, the relationship between the preferred place of buying eggs and the classification criterion was also proved, i.e. whether a respondent is a breeder of the egg type of hens or not. The relationship between the preferred place of buying eggs and the gender of a respondent was confirmed as well. There was determined the dependence on the gender of a respondent in the question related to eggs consumption.

Keywords: egg consumption; consumer behavior; preferences; purchasing place; Fisher's exact test

INTRODUCTION

Eggs are one of nature's the most nutritious foods, with low content of saturated fat and high content of protein. It remains a popular ingredient in cooking worldwide **(Fang et al., 2012)**. Egg white proteins (EWPs) are well established as a valuable source of dietary nitrogen, but recently, these proteins have reclaimed scientist's interests due to a number of new discovered biological functions **(Ruan et al., 2010)**. Additionally, EWPs possess very important functional properties, which make them very important ingredients for food products **(Jing et al., 2011).** Eggs are also good dietary sources of choline, vitamin A, vitamin D, iron, conjugated linoleic acid (CLA) and lutein **(Fang et al., 2012)**.

The Food and Agriculture Organization of the United Nations estimates that about 795 million people of the 7.3 billion people in the world, or one in nine, were suffering from chronic undernourishment in 2014 – 2016. Millions of children were malnourished in the same time **(World Hunger, 2015)**.

The idea of providing cheap, accessible and valuable source of protein and energy is also dominant in "bottom of the pyramid market approach" **(Prahalad 2014; Horská et al., 2014; Paluchová and Prokeinová, 2014)**. Even more, many research studies were accomplished regarding enrichment of eggs with different micronutrients as omega-3 fatty acids, selenium and vitamin E through nutritional manipulations without deteriorating egg internal and organoleptic qualities **(Hayat et al., 2014)**. **Angelovičová et al. (2013)** observed the effect of dietary probiotics *Bacillus subtilis (PB6)* on egg weigh, egg mass weigh, egg fat content and cholesterol content in egg yolk in laying hens. **Arpášová et al. (2012)** evaluated the influence of probiotic preparation based on lactobacillus, oregano essential oil, sumac (*Rhus coriaria*), propolis and pollen on egg quality parameters of laying hens.

In accordance with the law, from January 1st, 2012 Slovak producers of eggs do not keep laying hens in barren battery cages and sell only eggs from enriched breeding. Laying hens had to be moved from barren cages into bio breeding, or in barn systems with free range and barn systems cages with perch – enriched cages. Implementation of these EU directives ensured the welfare in breeding of laying hens. Welfare provides physical and mental health of the animal, comfort in accordance with the environment in which it lives, as well as reducing the incidence of stressful situations to a minimum. Due to changes in technology of keeping hens there was a significant increase in the cost of egg production, which is

reflected in the price of table eggs and the purchasing patterns of Slovak consumers.

Scientific hypothesis

The purpose of this study was to monitor consumer's preferences at buying eggs. We verified the validity of the following hypotheses:

Hypothesis H1: We assume the existence of dependence between the place of buying eggs and a respondent's age (or gender, or layer breeding).

Hypothesis H2: We assume the existence of dependence between the impact factor egg purchase and respondent's age (or gender, or layer breeding).

Hypothesis H3: We assume the existence of dependence between the frequency of egg consumption and respondent's age (or gender, or layer breeding).

MATERIAL AND METHODOLOGY

The primary data originated from the questionnaire survey, performed during the year 2016 in the Slovak districts of Turčianske Teplice, Martin and Nitra. The sample of 200 respondents answered to seven questions, out of them three were classification ones (age and gender). The questions were aimed at finding consumers` opinions and their preferences at purchase and consumption of eggs.

1. Which factor is the most important at egg purchase?
2. Which is the most preferred place for your egg purchase?
3. How often do you eat eggs?
4. Do you keep laying hens?

In the selected sample of 200 respondents there was approximately equal representation of both genders (54.5% women and 45.5% men). By the age structure the most represented group was from 20 to 30 years (24%), the second one was from 31 to 40 years (22.5%), followed by the category from 41 to 50 years (21%) and from 51 to 60 years (19.5%). The smallest one was the age group from 61 and more (13 %). In our survey we were also interested in the percentage of consumers who keep hens. We determined that 24.5% of respondents breed hens at home.

Statisic analysis

The first method, used for testing the hypotheses, was the statistical method – Chi-Square Goodness of Fit Test test of independence. The chi-squared statistic of this test is given by the following formula: $\chi^2 = \Sigma [(O_i - E_i)^2 / E_i]$, where O_i is the observed frequency count for the *i*th level of the categorical variable, and E_i is the expected frequency count for the *i*th level of the categorical variable.

The basic prerequisite for the use of this test is that expected value of the number of sample observations in each level of the variable is at least 5. If this condition is not met, then the incorrect conclusions can be obtained. In this case it is possible to use the Fisher's exact test, which is the part of output SAS Enterprise Guide after identification. Fisher's exact test is also known as Freeman-Halton test, which is described in detail in the source: http://support.sas.com/documentation/cdl/en/procstat/63963/HTML/default/. In the paper the conclusions of hypotheses were achieved based on p-value, which is the part of outputs in the tables. It is stated: if p-value < a, then H0 hypothesis is refused.

Within the measurement of associations we also dealt with the degree of association between the studied characteristics. It is possible to measure the intensity (or power) of dependences by several means of statistics. In our paper we consider the measures which are the part of the output of SAS Enterprise Guide. The measures are following: Phi Coefficient, Contingency Coefficient, and Cramer's V Coefficient. As we utilized the table r x s, the given coefficients acquire the values from the interval from 0 to 1, where the high value indicates the high degree of association. The particular measures are also described in detail in the source: http://support.sas.com/documentation/cdl/en/procstat/63963/HTML/default/viewer.htm#procstat_freq_a0000000561.htm.

Analyzes, calculations and graphical outputs were carried out in statistical software SAS Enterprise Guide 5.1 and in MS Excel 2013.

RESULTS AND DISCUSSION

The annual egg consumption per inhabitant should be 11.2 kg. The egg consumption in Slovakia was 200 pieces (12.2 kg) in 2015, that means higher recommended dose by 1.0 kg. Until now the highest egg consumption (13.3 kg) was recorded in 2012. As **Jamborová (2016)** states the egg consumption can be influenced by the supply on the domestic market, purchasing power of inhabitants and price.

We asked about the place where the respondents do shopping of eggs. 43 respondents (21.5%) claimed that they buy eggs directly from the hens` farms, 62 respondents (31%) buy them from poultry plants, 76 respondents (38%) in the food stores and 19 (9.5%) buy eggs elsewhere (Figure1). We can conclude that the consumers buy eggs most frequently from food stores (38%) or poultry plants (31%).

We can compare our results with **Fiľa and Tóthová (2013)**; they state about the direct sale advantage in promoting the local economy, increasing employment in the region, reduction of transport costs as well as consolidation relations in the region. In relation farmer - consumer, there is a direct relationship between the seller and the buyer. Other benefits for producer are demand, development regarding consumers' needs, supporting social network. Food quality, transparent prices, contact with soil and animals belong to benefits for consumer **(Chreneková et al., 2015)**.

Research objective was focused also on the fact if the age of consumers (or gender, or breeding resp. non-breeding) has the impact on the shopping place, i. e. we verified the validity of our first hypothesis (H1). The obtained results of the statistical testing are given in the Table 1.

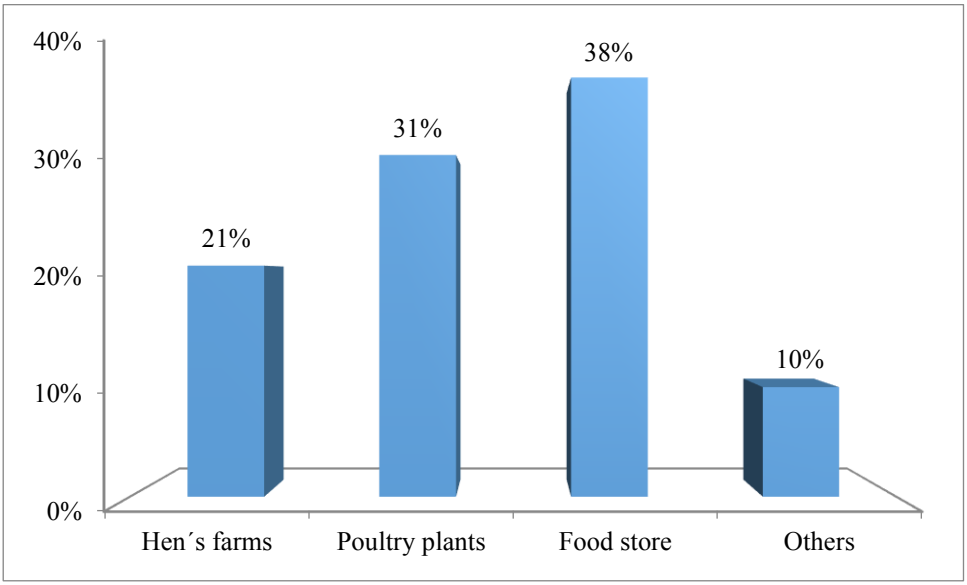

Figure 1 The most preferred place for the egg purchase. Source: own processing in MS Excel.

Table 1 The results of the statistical testing to the question: The most preferred place for the egg purchase.

	Ownership of laying hens		***Gender***		***Age***	
Statistic	Value	*p*-value	Value	*p*-value	Value	*p*-value
Chi-Square	43.2421	**0.0000**	17.1911	**0.0006**	10.8538	0.5415
Fisher's Exact Test		1.08E-08		6.62E-04		
Phi Coefficient	0.4650		0.2932		0.2330	
Contingency Coefficient	0.4216		0.2813		0.2269	
Cramer's V	0.4650		0.2932		0.1345	

Note: Source: Own calculation in the system SAS Enterprise 5.1.

The results in the Table 1 indicate that statistically significant dependence was proved between preferred place and the customer gender (p-value 0.0006); moreover, significant dependence exists between preferred place and layer breeding (*p*-value 0.0000). When evaluating the degrees of the tightness of dependence (Phi Coefficient, Contingency Coefficient, Cramer´s V) more important factor on the place of buying eggs is the fact if the respondents keep or do not keep layers (we can say it is medium strong dependence). A moderate dependence (around value 0.3) occurred in the gender of respondents.

Figure 2 shows that women prefer buying eggs from the food stores and poultry plants, while men prefer buying eggs directly from the layer breeders.

Regarding the preferred purchase place with respect to the own keeping of hens (Figure 3), if consumer does not have the possibility to have own production, they prefer to buy eggs from the poultry plants and food stores. Part of respondents buys sometimes eggs from farmers; the majority of respondents still prefer food stores. As **Fil'a et al. (2013)** state this may be caused by the lack of advertising marketing activities from the side of producers. As it is generally known, main reason why people prefer large shopping centers is the fact they can find everything they need under one roof. They do not have time to travel to several different shops neither to visit some local farms.

The next question related to the different factors influencing the respondents at egg purchase. Figure 4 demonstrates that the respondents considered to be the most important factor the price (34% respondents). The following significant factor was own experience (24%), or the origin (22%).

At food purchase, price seems to be the most important factor. However, we can see that also some sustainable attributes show a certain growing dominance, especially the methods of rearing of laying hens. Sustainable consumption patterns should be the part of the solution to the sustainability problem, including animal welfare as the food value for consumers **(De Bakker and Dagevos, 2012)**. Also in research of **Annunziata and Scarpato (2014)** was found that among the factors affecting consumers' attitude towards food products with sustainable attributes belong animal welfare, together with environmental impact.

The existence of dependence in the question about the importance of different factors for respondents was also verified by the statistical procedures (Table 2).

The results in Table 2 indicate that there is the dependence between the considered factors which have impact on the egg purchase and the respondents` age. Based on the degrees of tightness we can claim the moderate even medium strong dependence.

Figure 2 Preferred purchase place with respect to the gender. Source: own processing in MS Excel.

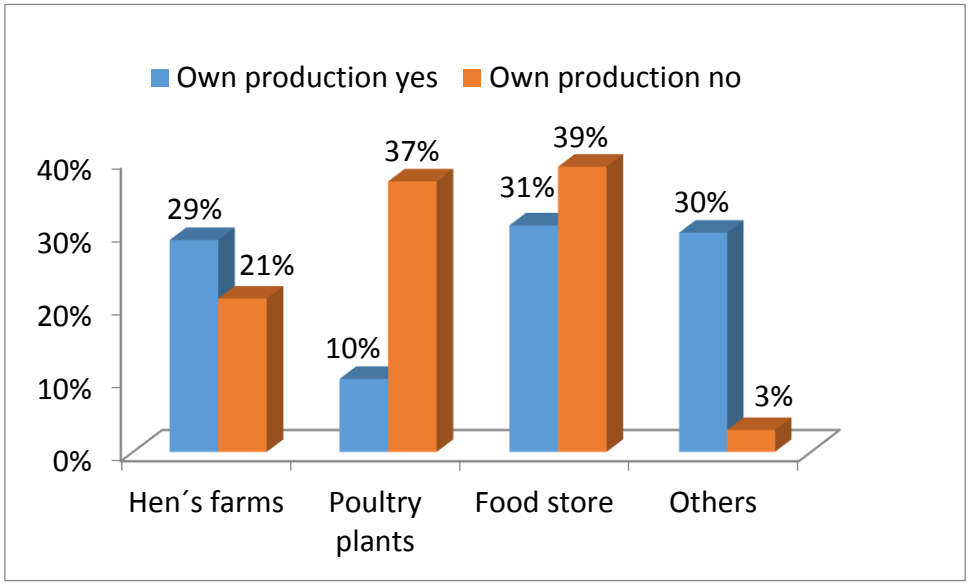

Figure 3 Preferred purchase place with respect to the own keeping hens. Source: own processing in MS Excel.

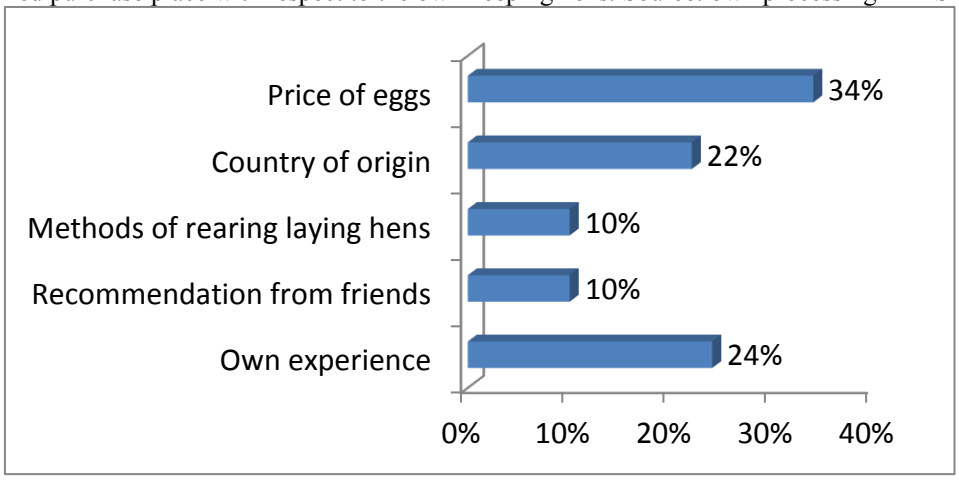

Figure 4 Preferred factor at egg purchase. Source: own processing in MS Excel.

Table 2 The results of the statistical testing to the question: *Preferred factor at egg purchase.*

	Ownership of laying hens		***Gender***		***Age***	
Statistic	Value	*p*-value	Value	*p*-value	Value	*p*-value
Chi-Square	9.0708	0.0594	7.5728	0.1085	32.7537	**0.0080**
Fisher's Exact Test		5.62E-02		1.11E-01		
Phi Coefficient	0.2157		0.1971		0.4098	
Contingency Coefficient	0.2108		0.1933		0.3792	
Cramer's V	0.2157		0.1971		0.2049	

Note: Source: Own calculation in system SAS Enterprise 5.1.

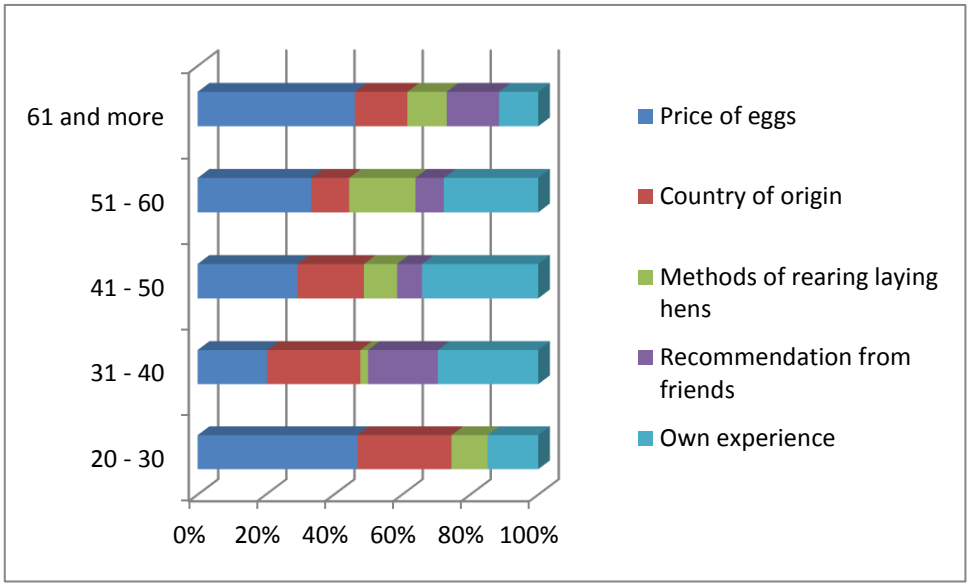

Figure 5 Preferred factors at egg purchase with respect to the age. Source: own processing in MS Excel.

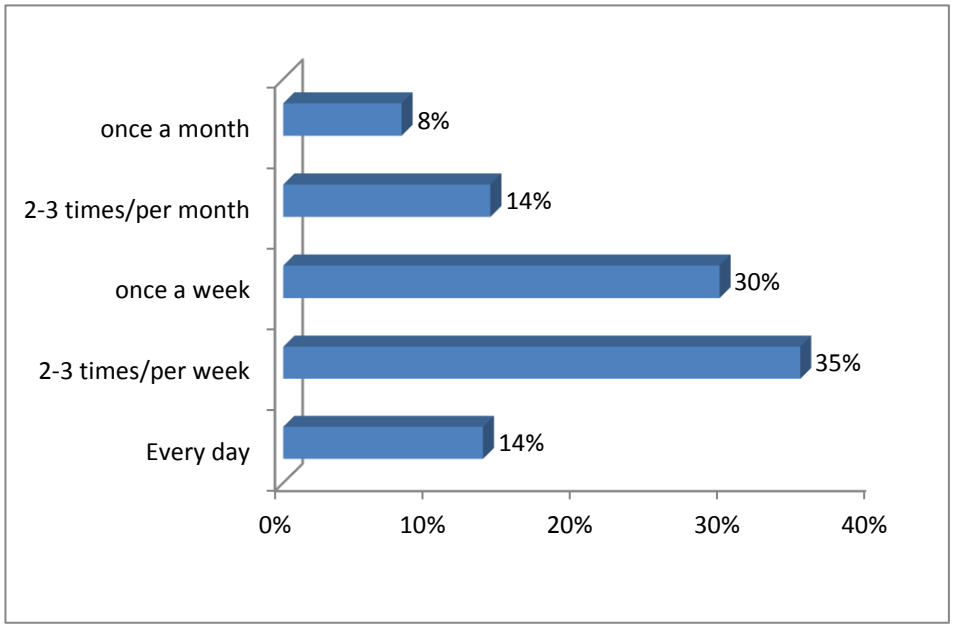

Figure 6 Frequency of egg consumption. Source: own processing in MS Excel.

Table 3 The results of the statistical testing to the question: *The frequency of egg consumption.*

	Ownership of laying hens		***Gender***		***Age***	
Statistic	Value	*p*-value	Value	*p*-value	Value	*p*-value
Chi-Square	3.1603	0.5314	11.5898	**0.0207**	16.7043	0.4050
Fisher's Exact Test		5.50E-01		1.89E-02		
Phi Coefficient	0.1257		0.2407		0.2890	
Contingency Coefficient	0.1247		0.2340		0.2776	
Cramer's V	0.1257		0.2407		0.1445	

Note: Source: Own calculation in SAS Enterprise 5.1 system.

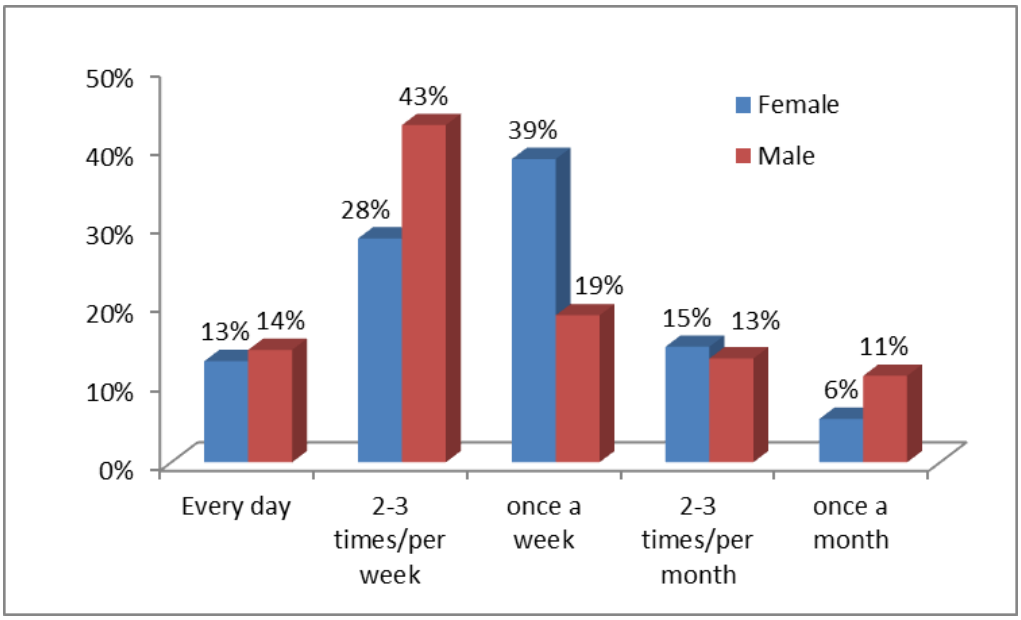

Figure 7 The consumption frequency with respect to the gender. Source: own processing in MS Excel

Figure 5 shows that the egg price is the most important for the young people (20 – 30 years old), older people and the retired (61 and more). The essential factor for the people in the productive age (31 – 50) is their own experience. Therefore the hypothesis H2 was affirmed only in the relation to age. From the viewpoint of gender, or breeding/non-breeding of layers we did not refuse the null hypothesis, i.e. men and women are influenced by the considered factors of egg purchase equally. The fact, whether they breed or not breed the layers, similarly it does not have impact on the consumers from the aspect of the determined factors, which can influence at egg purchase.

The last presented question is the issue related to the frequency of egg consumption. The respondents were offered to select among five options: from the daily egg consumption to the consumption once a month. The Figure 6 indicates that the consumers eat eggs mostly 2 – 3 times a week (35%), or once a week (30%). Further, 8% of respondents consume eggs once a month minimally; on the contrary, 14 % respondents consume eggs every day.

Apart from the positive impact of the nutritional value of eggs it is possible to remind that hens´eggs contain allergens which can cause the immediate reaction (type I, IgE - mediate) at the sensitive patients. The symptoms of egg allergy can vary from mild to serious ones (e.g. anaphylaxis) **(Sakai et al., 2015)**. Egg white (albumin) is more allergenic than yolk **(Halaj and Golian, 2011)**.

In many expert studies the impact of egg consumption on human health was analyzed. **Missmer et al. (2002)** report that eggs contain particularly high level of cholesterol (425 mg per 100 g) and the recommended daily dose is 300 mg, for children and persons at risk of 100 mg. The increased level of LDL (low density lipoproteins) cholesterol tends to develop vascular deposition and atherosclerosis. Cholesterol is a precursor of steroid hormones and can affect the risk of breast cancer through the formation of estrogen **(Rong et al., 2013)**. According **Shin et al. (2013)** consumption of up to one egg per day can have a significant effect on coronary heart disease or brain stroke. However, some researchers believe that eggs are helpful for reducing breast cancer risk, as eggs are sources of certain amino acids, lutein zeaxanthin, omega-3

and omega-6 fatty acids which may be used to prevent cancer **(Bao et al., 2012)**. Egg yolks are also a significant source of choline, the consumption of which has been found to be associated with a lower risk of breast cancer **(Zhang et al., 2013)**.

The results of verification of H3 hypothesis validity are given in the Table 3. The obtained values of statistical testing indicate that there exists dependence on 5% level of significance between the frequency of egg consumption and a respondent`s gender. Based on the degrees of tightness of dependence we can state that there is the weak dependence (0.2340, or 0.2407 by the applied coefficient). The statistically significant dependences were not confirmed between the frequency of eggs consumption and the other classification criteria (age, breeding/non-breeding of layers).

Figure 7 indicates the relationship between the frequency of egg consumption and gender. Based on the Figure 7 we can claim that women consume eggs once a week most frequently, while men eat eggs 2 – 3 times per week.

The survey showed that 30% of respondents do not take into account the way how the layers are kept.

CONCLUSION

Eggs belong to a food that Slovak consumers often buy and like to consume. Results indicate that preferences of consumers are affected by many factors while some of them are considered to be significant.

The frequency of eating eggs demonstrates that 90% of respondents consume eggs. The price of eggs is the most important factor at egg purchase (34% respondents) followed by the own experience of consumers. The research was focused on the most preferred place for the egg purchase as well. Results confirmed the statistically significant dependence between preferred place of purchase and a customer's gender. We can conclude that the consumers buy eggs as the most frequently from large shops and only 21% directly from farms.

Regarding the consumption, women consume eggs once a week the most frequently, while men eat eggs 2 - 3 times per week. 8% of respondents consume eggs once a month minimally; on the contrary, 14% respondents consume eggs every day.

The survey showed that 30% of respondents do not take into account the way how the layers are kept. Country of origin as preferred factor at purchasing of eggs stated only 22% of our respondents.

REFERENCES

Angelovičová, M., Alfaig, E., Král, M., Tkáčová, J. 2013. The effect of the probiotics Bacillus subtilis (PB6) on the selected indicators of the table eggs quality, fat and cholesterol. *Potravinarstvo*, vol. 7, no. 1, p. 80-84. https://doi.org/10.5219/271

Annunziata, A., Scarpato, D. 2014. Factors affecting consumer attitudes towards food products with sustainable attributes. *Agricultural Economics – Czech*, vol. 60, no. 8, p. 353-363.

Arpášová, H., Kačániová,M., Haščík, P., Šidlová, V. 2012. Effect of selected feed additives on internal quality parameters of table eggs. *Potravinarstvo*, vol. 6, no. 4, p. 52-61. https://doi.org/10.5219/235

Bao, P. P., Shu, X. O., Zheng, Y., Cai, H., Ruan, Z. X., Gu, K., Su, Y., Gao, Y.-T., Zheng, W., Lu, W. 2012. Fruit, vegetable, and animal food intake and breast cancer risk by hormone receptor status. *Nutr. Cancer Int. J.*, vol. 64, no. 6, p. 806-819. https://doi.org/10.1080/01635581.2012.707277 PMid:22860889

De Bakker, E., Dagevos, H. 2012. Reducing meat consumption in today's consumer society: questioning the citizen-consumer gap. *Journal of Agricultural and Environmental Ethics,* vol. 25, p. 877-894. https://doi.org/10.1007/s10806-011-9345-z

Fang, D., Tan, F., Wang, Ch., Zhu, X., Xie, L. 2012. Egg intake and bladder cancer risk: A meta-analysis. *ExpTherMed*., vol. 4, p. 906-912. https://doi.org/10.3892/etm.2012.671 PMid:23226747

Fiľa, M., Tóthová, V. 2013. Possibility of using the CSA as an alternative farmers' business model in Slovak Republic. *Journal on Law, Economy & Management*., vol. 3, no. 1, p. 19-26.

Fiľa, M., Tóthová, V., Slíšková, S. 2013. Slovak consumers' preferences in relation to CSA in comparison with some international results. *Journal of Management and Marketing*, vol. 1, no. 1, p. 35-41.

Halaj, M., Golian, J. 2011. Vajce - biologické, technické a potravinárske využitie (Egg - biological, technical and food use). Nitra, Slovakia : Garmond, 222 p. ISBN 978-80-89148-70-7.

Hayat, Z., Nasir, M., Rasul, H. 2014. Egg quality and organoleptic evaluation of nutrient enriched designer eggs. *Pakistan Journal of Agricultural Sciences*, vol. 51, no. 4, p. 1085-1089.

Horská, E., Ondieki-Mwaura, F., Krasnodębski, A. 2014. The Role of Cross-Border Heterogeneity, Location Specificity and Distance Related Variables: Lessons Learnt from V4 Marketers: In: International business from the Central European Perspective, edit. by Blaženka Kneževic and Krzysztof Wach, Zagreb 2014, p. 129-144. ISBN 978-953-6025-89-03.

Chreneková, M., Dirgasová, K., Fáziková, M. 2015 Selected development factors of peripheral districts of the Žilina region. In *XVIII. mezinárodní kolokvium o regionálních vědách*. 1st ed, CD-ROM, p. 521-531. ISBN 978-80-210-7861-1.

Jamborová, M. 2016. Poultry and eggs. Situation and outlook report to the date 31/12/2015. Bratislava, Slovakia : NPP VÚEPP. Available at: http://www.vuepp.sk/dokumenty/komodity/2016/Hyd06_16.pdf

Jing, H., Yap, M., Wong, P. Y., Kitts, D. 2011. Comparison of physicochemical and antioxidant properties of egg-white proteins and fructose and inulin Maillard reaction products. *Food and Bioprocess Technology*, vol. 4, no. 8, p. 1489-1496. https://doi.org/10.1007/s11947-009-0279-7

Missmer, S. A., Smith-Warner, S. A., Spiegelman, D., Yaun, S. S., Adami, H. O., Beeson, W. L., van den Brandt, P. A., Fraser, G. E., Freudenheim, J. L., Goldbohm, R. A., Graham, S., Kushi, L. H., Miller, A. B., Potter, J. D., Rohan, T. E., Speizer, F. E., Toniolo, P., Willett W. C., Wolk, A., Zeleniuch-Jacquotte, A., Hunter, D. J. 2002. Meat and dairy food consumption and breast cancer: a pooled analysis of cohort studies. *International journal of epidemiology*, vol. 31, no. 1, p. 78-85. https://doi.org/10.1093/ije/31.1.78 PMid:11914299

Paluchová, J., Benda Prokeinová, R. 2014. Creation of responsible behavior and impact on sustainable customer

buying behavior in retail sector. *Visegrad journal on bioeconomy and sustainable development,* vol. 3, no. 1, p. 39-45.

Prahalad, C. K. 2014. The fortune at the bottom of the Pyramid, USA : Prentice Hall, 407 p. ISBN 013-3829136.

Rong, Y., Chen, L., Zhu, T., Song, Y., Yu, M., Shan, Z., Sands, A., Hu, F. B., Liu, L. 2013. Egg consumption and risk of coronary heart disease and stroke: dose-responsemeta-analysis of prospective cohort studies. *BMJ, p.* 346:e8539. https://doi.org/10.1136/bmj.e8539

Ruan, Ch. Q., Chi, Y. J., Zhang, R. D. 2010. Kinetics of hydrolysis of egg white protein by pepsin. *Czech Journal of Food Sciences,* vol. 28, p. 355-363. https://doi.org/10.17221/228/2009-CJFS

Sakai, S., Adachi, R., Teshima, R. 2015. Detection and control of eggs as a food allergen. In A volume of *Woodhead Publishing Series in Food Science, Technology and Nutrition* [online]. p. 313-340. https://doi.org/10.1533/9781782420217.3.313

SAS Product Documentation, Freeman-Halton test. Available at: http://support.sas.com/documentation/cdl/en/procstat/63963/HTML/default/

SAS Product Documentation. Available at: http://support.sas.com/documentation/cdl/en/procstat/63963/HTML /default/viewer.htm#procstat_freq_a0000000561.htm

Shin, J. Y., Xun, P., Nakamura, Y., He, K. 2013. Egg consumption in relation to risk of cardiovascular disease and diabetes: a systematic review and meta-analysi. *Am. J. Clin. Nutr*., vol. 98, p. 146-159. https://doi.org/10.3945/ajcn.112.051318

World Hunger and Poverty Facts and Statistics. 2015. Available at: http://www.worldhunger.org/articles/Learn/world%20hunger%20facts%202002.htm.

Zhang, C. X., Pan, M. X., Li, B., Wang, L., Mo, X. F., Chen, Y. M., Lin, F. Y., Ho, S. C. 2013. Choline and betaine intake is inversely associated with breast cancer risk: a two-stage case–control study in China. *Cancer Sci*. vol. 104, p. 250-258. https://doi.org/10.1111/cas.12064

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THE EFFECT OF EXPLANT, BAP AND 2,4-D ON CALLUS INDUCTION OF *TRACHYSPERMUM AMMI*

Bahman Fazeli-Nasab

ABSTRACT

Ajowan (*Trachyspermum ammi*) has been considered as an important medicinal plant because it contains many alkaloids such as Thymol. In vitro culture of Ajowan provides new tissue sources such as callus, cell suspension and seedlings to produce secondary metabolites. The present study describes callus production optimization procedures experiment that was a factorial experiment based on completely randomized design at three levels with four explants (root, shoot, leaf and cotyledon) on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP (0.25, 0.5 and 1 $mg.L^{-1}$) and 2,4-D (2, 4 and 8 $mg.L^{-1}$). Comparison of means showed that the maximum callus production was obtained from shoot explants, the cotyledon and leaf explants were in the second orders. In overall, 0.25 $mg.L^{-1}$ BAP with 2 $mg.L^{-1}$ 2,4-D concentrations proved to be optimal for the production of maximum callus and also were more effect on callus weight, callus volume and callus color. The best explant based on callus weight was cotyledon explant and for callus volume was shoot explant. The result were shown that the effective hormone combination and explant was 2 $mg.L^{-1}$ 2,4-D with 0.25 $mg.L^{-1}$ BAP concentrations were more effect on callus induction and shoot explant, respectively.

Keywords: *Carum copticum* (L.); cotyledon; Plant Hormone; Thymol

INTRODUCTION

The consumption of herb and herbal medicines is increasing in different countries day by day and this is due to their proven effectiveness in scientific communities and admissibility in majority of human societies. Because of increasing concerns about the side effect of chemical medicines an ineffectiveness of number of them in long-term consumption, use of natural ingredients has been paid more and more attention alternatively or as a supplement treatment method. Use of herbs as medicament has continuing from the beginning of the human civilization **(Dattner, 2003)**. Herbal medicines are being used as an alternative treatment with less side effects and variety features and in some cases as the only effective treatment **(Huseini et al., 2006)**.

Like many plants which are important economically, Ajowan laboratory proliferation with using of tissue culture techniques can be considered as an alternative for traditional reproduction methods.

Ajowan, one of the herbal medications, is highly considered which due to secondary metabolic production and its high importance in medical consumption, cosmetic and hygienic industries. The utilization of Ajowan has been globally outspreaded **(Fazeli-nasab and Fooladvand, 2016)**. Therefore, applying correctional techniques is essential matter to breed Ajowan as well as improve of the quantitative and qualitative of important properties of the secondary metabolite. Given the economic and environmental benefits and also the intensive use of pasture and forest resource and limited crop cultivation and due to the requisite of use of the correctional optimization methods of preparatory courses of breeding a herb to be able take advantages or breeding methods and molecular agronomy to optimize achieving the secondary metabolite and related products of herbs **(Afolabi et al., 2018a; Afolabi et al., 2018b)**. According to not being presented any report of Ajowan tissue culture and even there is a little number of tissue culture of Apiaceae. in this research has been proceeded in finding cost-effective and economical methods to callus implantation in Ajowan in tissue culture condition to using of breeding methods such as somatic-clonal diversity, radiation and mutation, chimer and polyploidy production, production and extraction of essence and also molecular breeding be possible.

Karegar et al., (2011) obtained the highest weight of the callus of fennel plant from treatment 1 $mg.L^{-1}$ of 2,4-D and 0.2 $mg.L^{-1}$ BAP using root explant, **Anzidei et al., 2000; Anzidei et al., (1996)** obtained the best callus induction from hypocotyl explant and **Sarkheil et al., (2009)** obtained from the hypocotyl explant of apical meristem.

To form callus in different species of bean, the best explant fragment according to Malik and **(Malik and Saxena,**

1991) is leaf, **(Amiri and Fahimi, 2003)** hypocotyl, **(Ahmed et al., 2002)** cotyledon nodule, **(El-Shemy et al., 2002)** epicotyl and also **(Veltecheva et al., 2005)** is leaf.

In bee balm (*Monarda didyma*) the best percentage of callus induction obtained from the treatment of 1 mg.$L^{-1}$of 2,4-D hormone and 1 mg.L^{-1} of BAP for the explant of internode and petiole **(Soltani pol et al., 2011)** while in spinach the best obtained only from the 0.5-1 mg.L^{-1} of 2,4-D hormone for leaf explants **(Khayatzadeh et al., 2011)**.

In Silybum marianum the best percentage of callus induction obtained from 1 mg.$L^{-1}$of 2,4-D and 1.5 mg.L^{-1} KIN for root explant **(Arekhi et al., 2012)** and the best in Salsola arbuscula from the treatment of (0.5 and 1 mg.L^{-1}) of 2,4-D only for stem explant **(Amini et al., 2013)**. In fennel the best percentage of callus induction obtained from 2 mg.L^{-1} of 2,4-D hormone and 0.25 mg.L^{-1} of BAP for hypocotyl and apical meristem **(Sarkheil et al., 2009)**. In eucalyptus, the best percentage of callus induction obtained from 1 mg.L^{-1} of TDZ for stem explant **(Ghadiri Sardrood et al., 2012)**.

MATERIAL AND METHODS

Plant cultivation

To conduct the experiment, in year 2016, the Ajowan seeds including the Sistan local mass (origin of Ajowan **(Fazeli-nasab and Fooladvand, 2016))** was supplied from the Gene Bank of the Agricultural Biotechnology Research Institute of Zabol.

To the study germination used MS medium as well as instillation of the callus. In all condition, the density of sucrose was 30 g.$L^{-1}$and the density of agar was 7.5 g.L^{-1}.

The PH of the medium for germination and callus instillation was adjusted by in order 5.64 and 5.8

To sterilize seeds, submergence was done in sodium-hypo chloride 1% in laminar-flow-hood, after 10 minutes hypo chloride was evaporated and washing the seeds was done with sterilized distilled water in four step including a one minute pre-washing and three 5, 10 and 15 minutes sterilization washing with distilled water. After this stage, absorb the extra water and preparing for cultivation the seeds were located on filter paper. After cultivation, the medium was covered with parafilm and was kept in growth chamber under light condition, 16 hours light, 8 hours darkness and temperature of 15 °C. Finding appropriate germination environment in order that seeds be prepared for the wide cultivation In Vitro.

To carry out callus induction experiment, factorial in a completely randomized design was used with explant factors including root, stem, leaf and cotyledon, s, 4-D hormone in three levels of 2, 4 and 8 mg.L^{-1} and BAP hormone in three levels of 0.25, 0.5 and 1 mg.L^{-1} in 3 replication. The prepared explant from the 48 days grown plants growing In Vitro was inoculated on MS culture under different treatment. Containers containing the explants, were kept in growth chamber under temperature 25 ºC and a light period of 16 hours light and 8 hours darkness. Sub culturing every two weeks. After two months from growth explant some traits such as percentage of callus induction (generate or non-generate of callus), callus volume, callus weight, callus quality (fragile of watery) and callus color were recorded.

Data analysis

Data was analyzed with R and student statistic software and used Excel to draw the charts and Duncan's multiple range test (LSD) to compare the average means with probability of 1 and 5%

RESULTS

Germination

Based on qualitative observation, germination of Ajowan seeds on MS medium was more appropriate either the number of germination seeds or the required time for germination and development of the plant (Figure 1 (section A and B). However, 1/2MS medium has been found more appropriate rather than MS because it seems high levels of salt in medium can limit germination of different plants **(Reis et al., 2015)** but reported that Ajowan is resistant to salinity because it tolerates the saline environments up to 140 mg.L^{-1} and even in presence of Kinetin could increase the level of tolerance by 210 mg.L^{-1} of saltiness (highest level of the used treatment in the **experiment (Fazeli-Nasab et al., 2016)** consequently can say that Ajowan can growth well in MS medium because of higher level of Vitamin and macro and micro nutrition minerals as the result of the experiment approve that

Callus induction

To study the callus induction process, the explants segments including root, stem, leaf and cotyledon located on the base MS medium containing sucrose 3%, Vitamin B and 16 different density of a combination of 2,4-D and BAP hormones. The first reaction to forming callus was observed after 24 days and this completed after 60 days (Figure 1(section C and D)) and feature such as percentage of callus induction, callus color, callus volume and callus quality of the callus was recorded. After data analysis, results indicated that both hormone 2,4-D and BAP in most of the densities caused the callus induction in majority of explant segments except the root (Table 1 and Figure 2). So that the highest percentage of callus induction (100 %) was observed in stem explant and the least (zero percent) in root. Also regarding to callus induction a significant different of probability of 1 percent was between different level of BAP and different level of 2,4-D

BAP, singly, in density of 0.5 mg.L^{-1} had the highest effect (55%) on stem callus induction and the least role on root explant in density of 1 mg.L^{-1} (Figure 2). As well as, 2,4-D in a density of 2 mg.L^{-1} the most production of callus in stem explant segment (66%) and the least role in the density of 8 mg.L^{-1} in root explant. Meanwhile with increasing the density of 2,4-D its effect on the amount of callus was getting down as the highest percentage of callus in density of 2 mg.L^{-1} and the least in density of 8 mg.L^{-1} (Figure 3).

Effect of BAP, singly, on callus induction was less than 2,4-D but BAP could stimulate the callus induction in a lower density. In interaction of BAP (0.25 mg.L^{-1}) with 2,4-D (2 mg.L^{-1}) the most callus was obtained from stem explant and the least in density of BAP (1 mg.L^{-1}) with 2,4-D (8 mg.L^{-1}) in root explant (Figure 4).

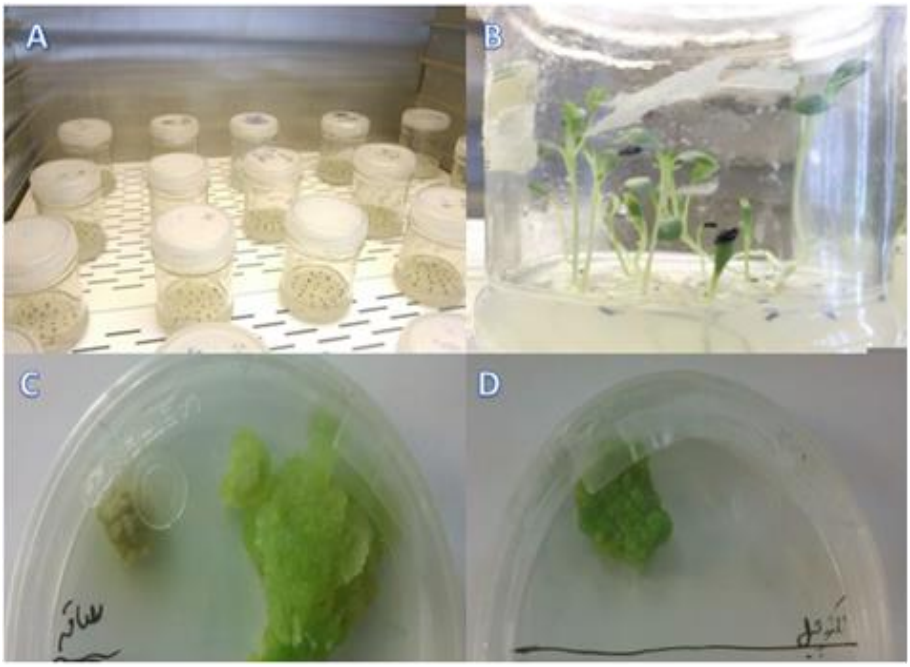

Figure 1 germination in ½ MS medium (A & B) and callus induction from Stem (C) and Cotyledon (D) explant.

Table 3 analysis variance of callus induction based on explants and hormones.

SOV	df	SS	MS	F
Explant	3	14074	4691.34	3.688**
BAP	2	2222.24	1111.12	8.7349**
2,4-D	2	2222.21	1111.11	8.7348**
BAP * Explant	6	10370.4	1728.4	1.3587**
2,4-D * Explant	6	1481.46	246.91	1.941**
BAP * 2,4-D	4	555.561	138.89	1.0918**
BAP * 2,4-D * Explant	12	9073.96	756.164	5.9445**
Error	72	9.159	1.272	
Total	107	39999.9		

Note: ** significant in p <0.01.

In means because of interaction, BAP reduced the effect of 2,4-D on callus induction. Therefor recommended first, to obtain the best and most callus in herb Ajowan, 2,4-D (2 mg.L^{-1}) should be used with BAP (0.25 mg.L^{-1}) on stem explant. Secondary, other admixture of 2,4-D with other different hormone such as NAA, IAA should be used to obtain a large and absolute hormone regarding to the most effective hormone or a combination of.

According to reported **(Schultz et al., 1990)** that callus induction begins from the cutting edges and spread out to other parts of the explant, so over the current study the explant were cut and the result indicated that the callus induction was appropriate so can be resulted the cutting in explant causes increasing in callus. So is recommended cut the surface of the explant before inoculation.

Weight, color and quality of the callus

There was a significant different of probability of 1% between explant, BAP and 2,4-D hormone, singly and interaction between hormone in the weight of the obtained callus of explant (Table 2). Meanwhile, LSD analysis indicated that there was a significant different between explant including root, stem, leaf and cotyledon based on callus weight and cotyledon was the best explant and then stem (Figure 5) The most effective level of BAP and 2,4-D on callus weight was in order 0.25 and 2 mg.L^{-1} (Figure 6).

Ki square test showed that explant regarding to color and quality had a significant different in probability of 1 percent 2,4-D also was effective on color and quality of callus at the probability of 5 percent. But BAP wasn't effective on color and quality of the callus as the obtained callus from green stem explant was lighter rather than other explant then cotyledon and after that leaf. On the other hand, treatment 2 mg.L^{-1} was more effective on color. As long as density of 2,4-D increased the color callus was getting darker obtained callus from stem, was more fragile and 2,4-D at level of 2 mg.L^{-1} was more effective on callus.

Callus volume

It was a significant different at probability of 1% between explant, BAP and 2,4-D hormone, individually and hormone interaction in volume of obtained callus of explant (Table 3). LSD results showed that there was a significant different between explant (root, stem. Leaf and cotyledon) regarding volume of callus and stem was the best explant then cotyledon (Figure 7). The most effective level of BAP and 2,4-D on volume was in order 0.25 and 2 mg.L^{-1} (Figure 8).

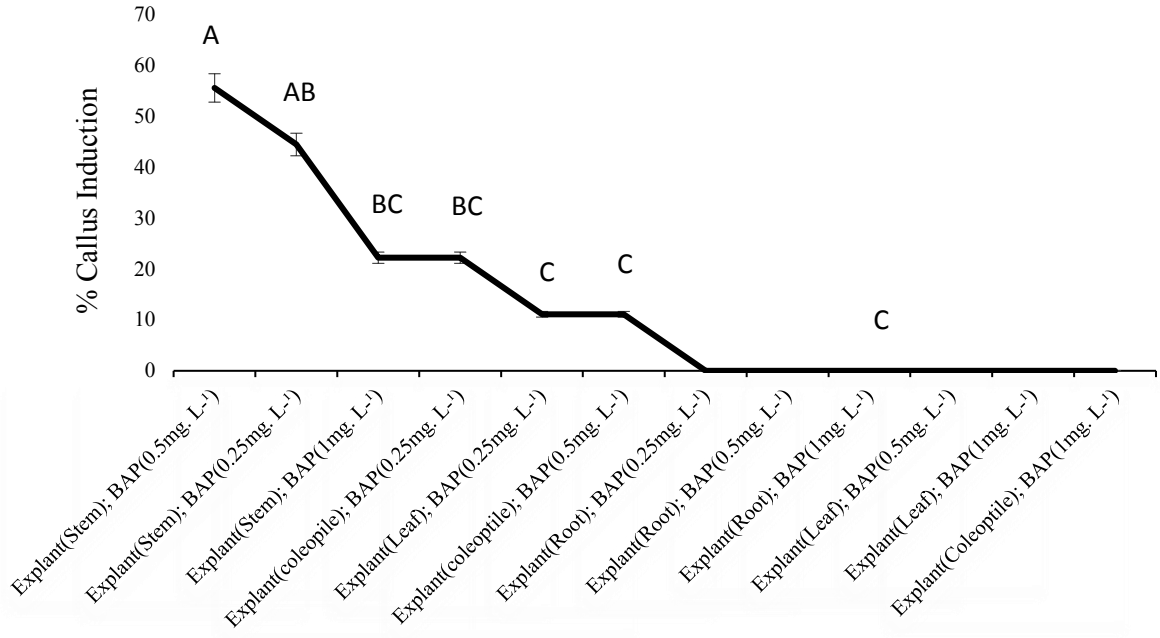

Figure 2 studying of different levels of BAP hormone and explant on percent of callus induction.

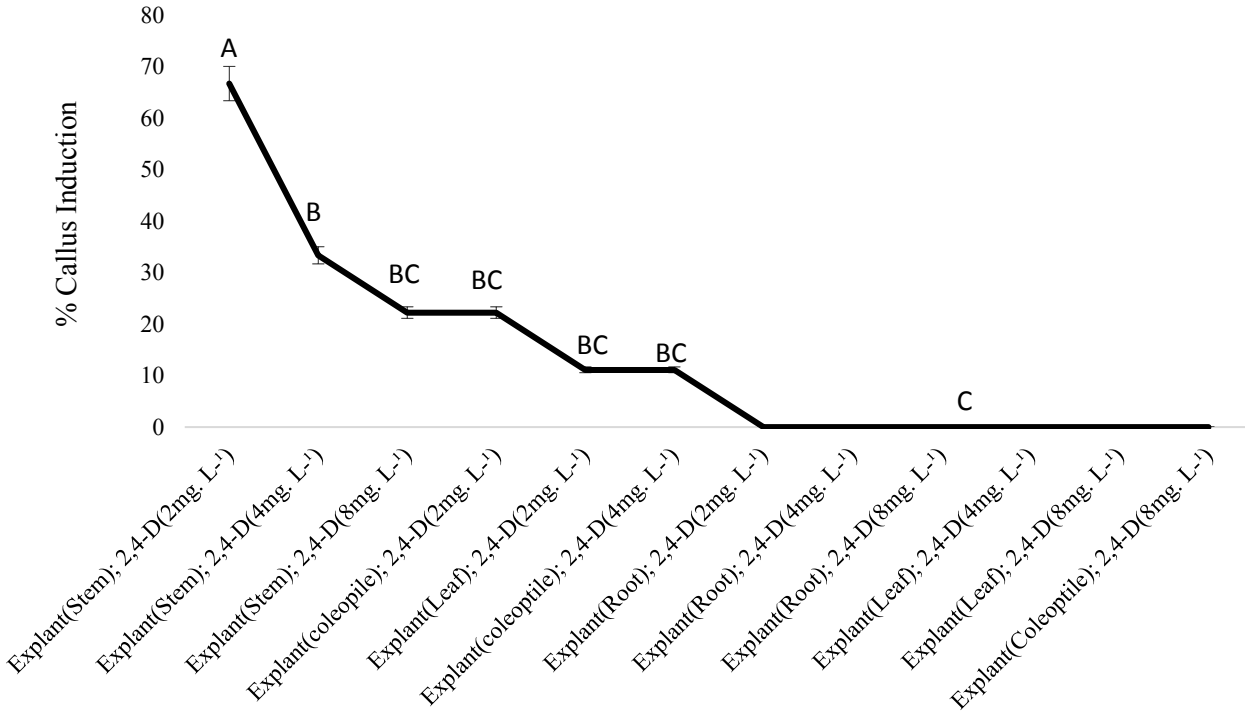

Figure 3 studying of different levels of 2,4-D hormone and explant on percent of callus induction.

DISCUSSION

Study of explants segments showed different results as root explant couldn't generate callus after a while necrotic and disappeared but the highest callus was in stem and then in order cotyledon and leaf. According to (**Malik and Saxena, 1991)** study the best explant was leaf, as well as hypocotyl by **(Amiri and Fahimi, 2003)**, Cotyledon node by **(Ahmed et al., 2002)**, hypocotyl by **(El-Shemy et al., 2002)** but has not been report regarding Ajowan so far the current report is the first report ever with regarding to Ajowan tissue culture. The root explant was the most effective explant.

In some results **(Karami et al., 2013)** that no callus in bean leaf explant was formed as well as the highest effect of BAP (in 4 $mg.L^{-1}$) for callus induction was 11 % but in combined form with NAA could increase the callus induction by 42 % while in this study effect of BAP on Ajowan callus was 55 % in a low density of 0.5 $mg.L^{-1}$.
The common point was that combination of BAP and 2,4-D in Ajowan and BAP with NAA in bean productized a callus of 42 %.

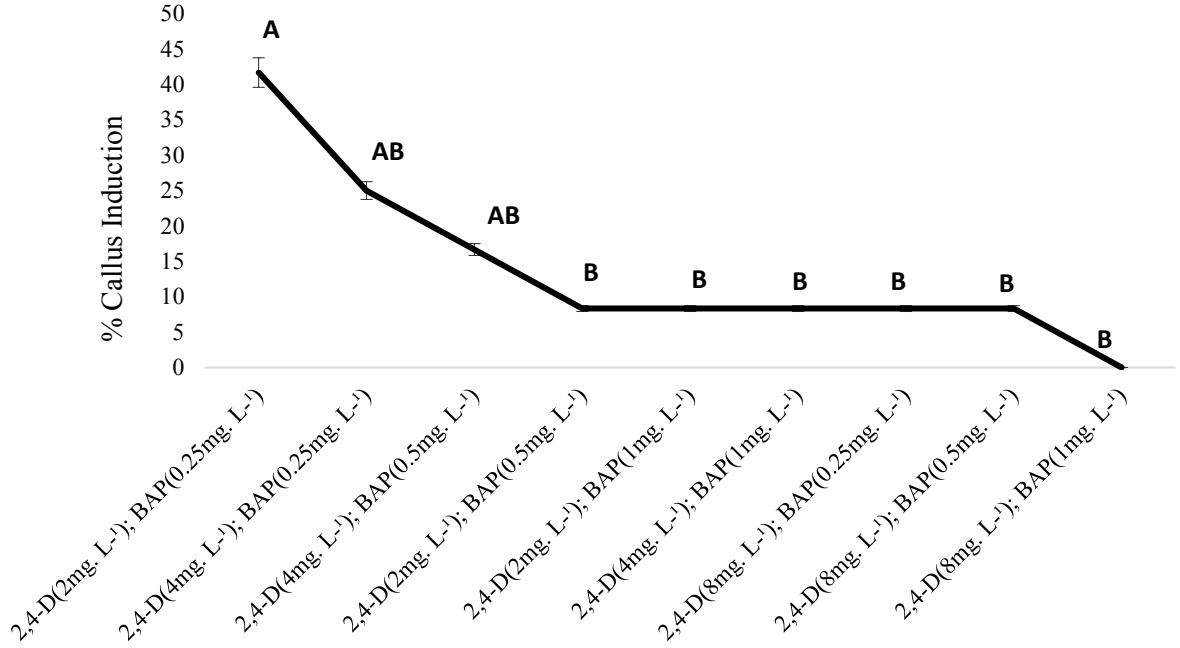

Figure 4 studying of the interaction effects of BAP and 2,4-D hormones on percent of callus induction.

Table 2 analysis variance of callus weight based on explants and hormones.

SOV	df	SS	MS	F
Explant	3	3.36	1.121	21.96**
BAP	2	0.888	0.444	8.69**
2,4-D	2	1.862	0.931	18.23**
BAP * Explant	6	1.994	0.332	6.51**
2,4-D * Explant	6	2.744	0.457	8.96**
BAP * 2,4-D	4	1.118	0.2797	5.48**
BAP * 2,4-D * Explant	12	3.713	0.3094	6.06**
Error	54	2.758	0.051	
Total	89	18.439		

Note: ** significant in $p < 0.01$

As reported that salts in medium has the negative effect on growth and callus induction to some extent **(Reis et al., 2015)** therefore the reason for callus induction in Ajowan can be due to salinity resistance **(Fazeli-Nasab et al., 2016)** and could product more appropriate callus in present of hormones.

In another results **(Ahmed et al., 2002)** obtained the best results in the MS medium contained BAP with the density of 1 $mg.L^{-1}$ and NAA with the density of 0.1 $mg.L^{-1}$. the highest product of callus either in **(Ahmed et al., 2002)** or **(Karami et al., 2013)** research was the result of interaction of Auxin and Cytokinin and also in current study the best result obtained from the combination of Auxinic and Cytokinin hormone but the result of this study was much higher that the mentioned researches, apparently seems that because the used explant in both experiments was partly different as well as the optimized densities that was applied. Naturally, can't be ignored the possibility of the main reasons of observed different in optimized densities of hormones caused by the different used species and laboratory conditions. In dissimilar results **(Amiri and Fahimi, 2003)** obtained the best callus in hypocotyl, NAZ variety in the density of 5 $mg.L^{-1}$ of 2,4-D and 2.5 $mg.L^{-1}$ of Kin. Noteworthy that NAA and Kin are weaker hormone and 2,4-D and BAP more appropriate in most studies.

Although 2,4-D normally considered as a strongest Auxin and the high level of Auxin causes the enlargement of cell length and increase the cell division **(Sobhanizadeh et al., 2017)** but in this study observed that increasing in 2,4-D didn't increase the callus induction so this disagreed with the result of **(Kurniati, 2013)** but agree with result of **(Soltani pol et al., 2011)**.

Regarding to the importance of hormones in callus induction **(Amini et al., 2013)** showed to product callus the presence is a necessity and in a medium without hormone and Auxin no callus obtained similar results related to this has been recorded by other scientist in a few species of plants **(Elaleem et al., 2009)**. Some studies indicated that in a medium with present of Auxin and not Cytokinin, callus can be produced but not without Auxin **(Hohtola, 1988)** in walnut cotyledon, also, reported that without Auxin callus couldn't be produced and also in this study showed that callus could inducted by collaboration of Auxin and Cytokinin that this achievement revealed similar to the former studies. Meanwhile, in a dissimilar study. **(Khayatzadeh et al., 2011)** reported that highest callus induction obtained from leaf explant, Orai variety with a

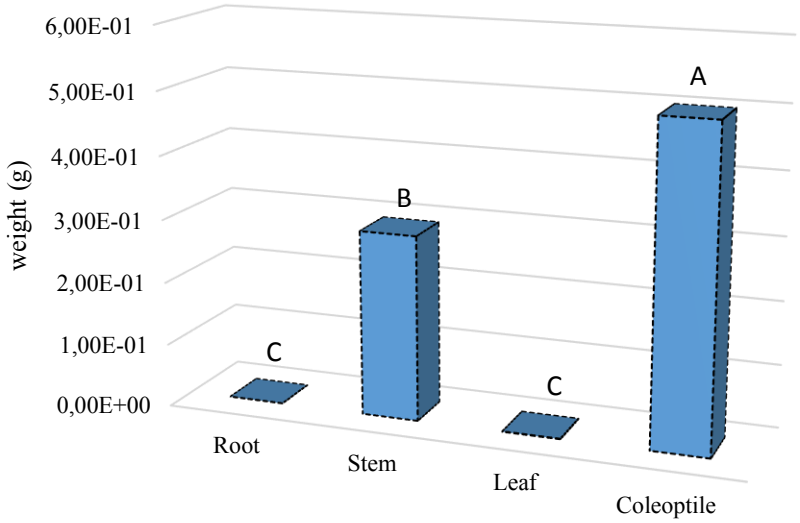

Figure 5 mean compare of effect of explant on callus weight in all explant of Ajowan.

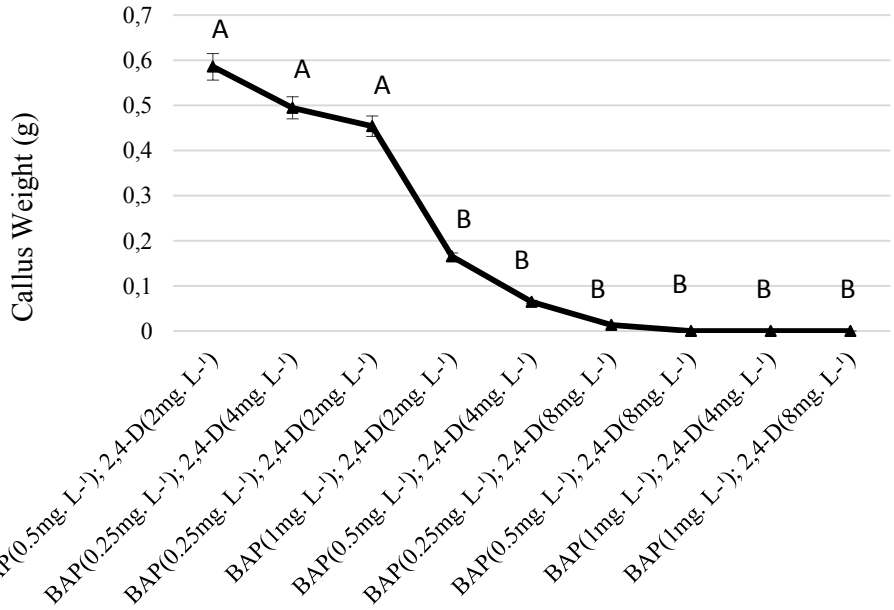

Figure 6 mean compare of interaction effect of BAP and 2,4-D hormones on callus weight in all explant of Ajowan.

density of 0.1 – 0.5 mg.L^{-1} of 2,4-D and Viroflay variety with a density of 0.5 – 1 mg.L^{-1} of 2,4-D.

Regarding to hormone combination, to induce an effective callus in Lemon balm, the best treatment obtained from applying 1 mg.L^{-1} of 2,4-D with 1 mg.L^{-1} of BAP for internodes and petioles explants under light condition as well as the treatment of 0.5 mg.L^{-1} it of 2,4-D with 1 mg.L^{-1} of BAP in the dark and then light for leaf explant **(Soltani pol et al., 2011)**. **Khayatzadeh et al., (2011)** reported that the highest callus induction obtained from leaf explant of Orai variety with a density of 0.1 – 0.5 mg.L^{-1} of 2,4-D and Viroflay variety with a density of 0.5 – 1 mg.L^{-1} of 2,4-D. **(Arekhi et al., 2012)** reported that the highest percentage of callus induction (98%) observed in root explant in a medium containing 1 and 1.5 mg.L^{-1} of 2,4-D and Kin. They also reported that the highest percentage of callus induction (97%) observed in root explant in a medium containing 1.5 and 1.5 mg.L^{-1} of NAA and Kin hormone. **Amini et al., (2013)** reported that the best explant was root and the most appropriate medium was the 1mg.L^{-1} of 2,4-D and 1 mg.L^{-1}of Kin as well as direct regeneration in stem in medium with density of 0.5 and 1 mg.L^{-1} of BAP. In separate researches, **Ebrahimie et al., (2003)** and **Mafavi Fard et al., (2010)** studied the effect of different explants, combination of different hormone and medium for callus generation in cumin. They were able to callus induction and regeneration in cumin with a combination of BAP, NAA and IAA in order 0.1, 0.2 and 0.4 mg.L^{-1} of each.

Sarkheil et al., (2009) studied the effect of 2,4-D and BAP on callus induction and regeneration on leaf, Hypocotyl, apical meristem, root and crown in fennel reporting 2 mg.L^{-1} of 2,4-D and 0.25 mg.L^{-1} BAP as the best combination as well as hypocotyl and apical meristem as the best explant **(Fatahi moghadam et al., 2011)**.

Table 3 analysis variance of callus volume based on explants and hormones

SOV	df	SS	MS	F
Explant	3	75.977	25.325	785.15**
BAP	2	10.322	5.161	160.01**
2, 4-D	2	21.096	10.548	327.02**
BAP * Explant	6	18.1482	3.024	93.77**
2, 4-D * Explant	6	15.278	2.546	78.94**
BAP * 2, 4-D	4	5.05	1.262	39.14**
BAP * 2, 4-D * Explant	12	42.365	3.53	109.45**
Error	54	1.741	0.032	
Total	89	189.9772		

Note: ** significant in p <0.01.

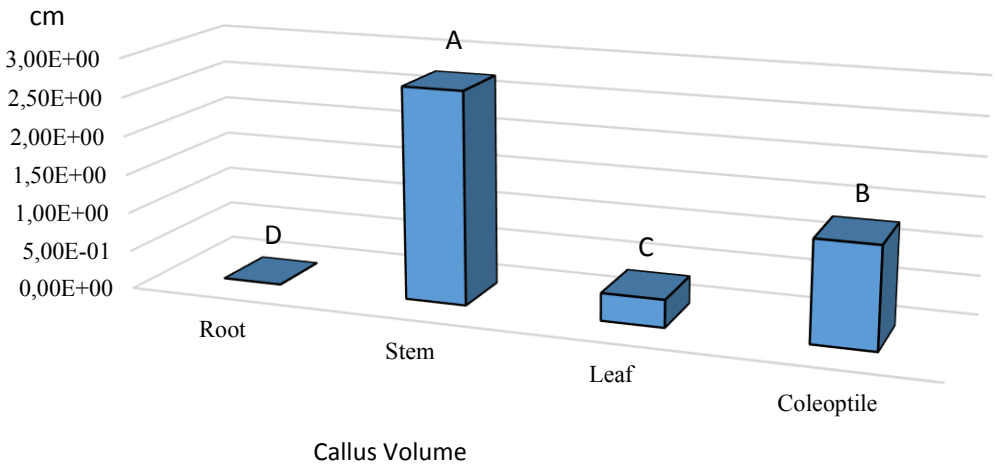

Figure 7 mean compare of callus volume based on Stem, Root, Leaf and cotyledon explants of Ajowan.

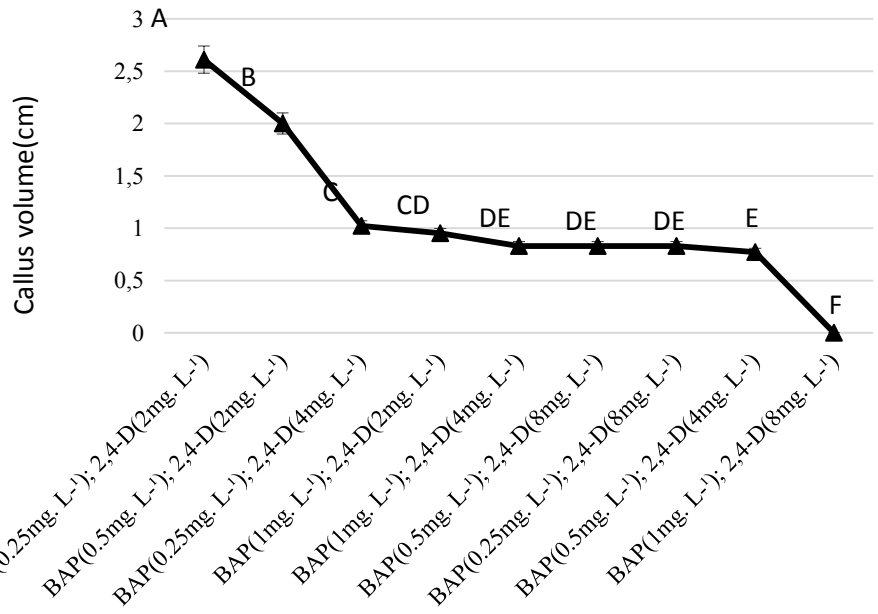

Figure 8 mean compare of interaction effect of BAP and 2,4-D hormones on callus volume.

Considered the effect of TDZ and 2,4-D on callus induction and regeneration in eucalyptus concluding 1 $mg.L^{-1}$ of 2,4-D and 0.5 $mg.L^{-1}$ TDZ the best combination of hormone and stem the best explant. In the present study, both 2,4-D and BAP in most often of density caused the callus induction process in majority of the explant except the root. Therefor the highest (100 %) callus induction was observed in stem explant as well as BAP in the density of 0.25 $mg.L^{-1}$ and 2,4-D in density of 2 $mg.L^{-1}$ had the highest callus generation.

CONCLUSION

Nowadays, indiscriminate and unprincipled harvesting on the one hand and the other hand the difficulties and complexities of germination of the herb seeds, put this plants in the danger of inexistence. On the other side, variability of drug compounds from clone to clone, enforced scientists to propagate after finding appropriate and prevalent clone and this micro-reproduction can be achieved only by applying tissue culture methods. Ajowan is one of the most important and local plant of Iran **(Fazeli-nasab and Fooladvand, 2016)** that in recent years a particular attention has been paid to so in the present study, focused on micro propagation. Obtained results indicated that the most effective treatment in Ajowan callus induction was 2 $mg.L^{-1}$ of 2,4-D with 0.25 $mg.L^{-1}$ of BAP and the best explant was stem.

Recommendations based on this study:

Advised study the biochemical pathway responsible for the production of valuable secondary metabolites of the herb such as Thymol and finally, application of engineering techniques to optimize cell suspension culture in terms of production of most of valuable secondary metabolites and also studying of secondary metabolites of the herb through the different stages of the tissue culture and preparing of the suspension medium.

It should be noted that given that Ajowan is one of the best herbs of Iran and rich of thymol and other secondary metabolites compounds.

The author of this article is on a comprehensive plan of action describing the optimization of production of secondary metabolites in Ajowan including studying the amount of generation of secondary metabolites in different vegetative stages and surface of the tissue culture, evaluation of level of secondary metabolites in Ajowan based on duplicated chromosomes then diploid state, evaluation of different genotypes of Ajowan in terms of secondary metabolites generation, the expression of genes involved in production of secondary metabolites and so on.

RERFERENCES:

Afolabi, O. B., Ibidun, O. O., Ibitayo, O. A., Bolaji, A. O., Idowu, O. I., Damilola, B. B., Abimbola, F., Olabisi, O. T., Joseph, A. O., Funmilayo, A. 2018a. Evaluation of antioxidant potentials of different solvent-fractions of *Dialium indium* (African Black velvet tamarind) fruit pulp–in vitro. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 70-78. https://doi.org/10.5219/825

Afolabi, O. B., Oloyede, O. I., Ojo, A. A., Onansanya, A. A., Agunbiade, S. O., Ajiboye, B. O., Johnson, J., Peters, O. A. 2018b. In vitro antioxidant potential and inhibitory effect of hydro-ethanolic extract from African black velvet tamarind (*Dialium indium*) pulp on type 2 diabetes linked enzymes. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 413-421. https://doi.org/10.5219/485

Ahmed, E. E., Bisztray, G., Velich, I. 2002. Plant regeneration from seedling explants of common bean (*Phaseolus vulgaris* L.). *Acta Biologica Szegediensis*, vol. 46, no. 3-4, p. 27-28.

Amini, F., Ganbarzade, Z., Askary, M. M. 2013. Optimization of Callus production and Plant regeneration in *Salsola Aebuscula* Pall. *Jornal of Cell and Tissue*, vol. 4, no. 2, p. 129-137.

Amiri, H., Fahimi, H. 2003. The effect of different concentrations of potassium salt tolerance bean in tissue culture. *Science Journal of Tehran University*, vol. 29, p. 320-326.

Anzidei, M., Bennici, A., Schiff, S., Tani, C., Mori, B. 2000. Organogenesis and somatic embryogenesis in *Foeniculum vulgare*. *Plant Cell, Tissue & Organ Culture*, vol. 61, no. 1, p. 69-79. https://doi.org/10.1023/A:1006454702620

Anzidei, M., Vivona, L., Schiff, S., Bennici, A. 1996. In vitro culture of Foeniculum vulgare: callus characteristics in relation to morphogenesis. *Plant cell, tissue and organ culture*, vol. 45, no. 3, p. 263-268. https://doi.org/10.1007/BF00043640

Arekhi, S., Aghdasi, M., Khalafi, M. 2012. Optimization of Silybum Marianum Tissue Culture for Production of Medicinal. *Journal of Plant production (Journal of Agricultural Sciences and natural resources)*, vol. 19, no. 2, p. 69-87.

Dattner, A. M. 2003. From medical herbalism to phytotherapy in dermatology: back to the future. *Dermatologic therapy*, vol. 16, no. 2, p. 106-113. https://doi.org/10.1046/j.1529-8019.2003.01618.x PMid:12919112

Ebrahimie, E., Habashi, A., Ghareyazie, B., Ghannadha, M., Mohammadie, M. 2003. A rapid and efficient method for regeneration of plantlets from embryo explants of cumin (*Cuminum cyminum*). *Plant cell, tissue and organ culture*, vol. 75, no. 1, p. 19-25. https://doi.org/10.1023/A:1024676507010

El-Shemy, H. A., Khalafalla, M., Wakasa, K., Ishimoto, M. 2002. Reproducible transformation in two grain legumes-soybean and azuki bean-using different systems. *Cellular and Molecular Biology Letters*, vol. 7, no. 2B, p. 709-720. PMid:12378231

Elaleem, K. G. A., Modawi, R. S., Khalafalla, M. M. 2009. Effect of plant growth regulators on callus induction and plant regeneration in tuber segment culture of potato (*Solanum tuberosum* L.) cultivar Diamant. *African journal of biotechnology*, vol. 8, no. 11, p. 2529-2534.

Fatahi Moghadam, J., Hamid Oghli, Y., Fotohi Ghazvini, R., Ghasem Nezhad, M., Bakhshi, D. 2011. Assessment of physicochemical and anti-oxidant of crest in some joinary cultivars of citrus. *Journal of Horticultural Science*, vol. 25, no. 2, p. 211-217. (In Persian).

Fazeli-Nasab, B., Davari, A., Nikoei, M. 2016. The effect of Kinetin on seed germination and seedling growth under salt stress in Sistan *C. copticum*. In Proceeding of the Second International & Fourteenth National Iranian Crop Science Congress, University of Guilan, Rasht, Iran. p. 1-4. Available at: https://www.researchgate.net/publication/312497783_The_effect_of_Kinetin_on_seed_germination_and_seedling_growth_under_salt_stress_in_Sistan_C_copticum/related

Fazeli-Nasab, B., Fooladvand, Z. 2016. A Review on Iranian *Carum copticum* (L.): Composition and Biological Activities. *European Journal of Medicinal Plants*, vol. 12, no. 1, p. 1-8. https://doi.org/10.9734/EJMP/2016/17584

Ghadiri Sardrood, S., Ghamari Zare, A., Assareh, M. H., Shahrzad, S., Bakhshi Khaniki, G. 2012. Effects of TDZ and 2,4-D growth regulators on callus production and organogenesis in *Eucalyptus rubida*. *Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research*, vol. 20, no. 2, p. 304-313.

Hohtola, A. 1988. Seasonal changes in explant viability and contamination of tissue cultures from mature Scots pine. *Plant cell, tissue and organ culture*, vol. 15, no. 3, p. 211-222. https://doi.org/10.1007/BF00033645

Huseini, H. F., Larijani, B., Heshmat, R. A., Fakhrzadeh, H., Radjabipour, B., Toliat, T., Raza, M. 2006. The efficacy of *Silybum marianum* (L.) Gaertn. (silymarin) in the treatment of type II diabetes: a randomized, double-blind, placebo-controlled, clinical trial. *Phytotherapy research*, vol. 20, no. 12, p. 1036-1039. https://doi.org/10.1002/ptr.1988 PMid:17072885

Karami, M., Bagherieh-Najjar, M. B., Aghdasi, M. 2013. Optimization of conditions suitable for bean (*Phaseolus vulgaris* L.) regeneration. *Journal of Plant Biology*, vol. 5, no. 15, p. 1-14.

Karegar, M., Sajjadi, S. R., Mobasheri, S., Sadeghi, H., Behrouznam, B. 2011. Study of tissue culture and organogenesis in fennel explants. In proceeding of the National conference of agricultural management, Islam Azad University, Jahrom branch. Iran, p. 1390. (In Persian) Available at: https://www.civilica.com/Paper-NCAMJAHROM01-NCAMJAHROM01_166.html

Khayatzadeh, M., Nabati-Ahmadi, D., Rajabi-Memari, H., Abdollahi, M. R. 2011. Optimization of Callogenesis and Regeneration of Two Spinach (*Spinacia oleracea* L.) Cultivars Utilizing Three Different Explants. *Agricultural biotechnology*, vol. 10, no. 2, p. 1-8.

Kurniati, E. 2013. Case induction and capsaicin incoming in nutrien MS virtual various and combinations sower growth supply (Induksi Kalus dan Penghasilan Capsaicin pada Variasi Kadar Nutrien MS dan Kombinasi Zat Pengatur Tumbuh), UAJY. Available at: http://e-journal.uajy.ac.id/id/eprint/4833

Mafavi Fard, M. R., J, A., Beiki, A. H. 2010. callus Production and plant regeneration in Three Iranian Cumin Landraces (*Cuminum Cyminum*). *Plant Production Technology (Agricultural Research)*, vol. 9, no. 2, p. 11-19.

Malik, K. A., Saxena, P. K. 1991. Regeneration in *Phaseolus vulgaris* L. Promotive role of N6-benzylaminopurine in cultures from juvenile leaves. Planta, vol. 184, no. 1, p. 148-150. https://doi.org/10.1007/BF00208248 PMid:24193941

Reis, É. S., Pinto, J. E. B., Rosado, L. D. S., Corrêa, R. M. 2015. Influence of culture medium on In vitro seed germination and multiplication rate of *Melissa officinalis* L. Revista Ceres, vol. 55, no. 3, p. 160-167.

Sarkheil, P., Omidi, M., Peyghambari, S., Davazdahemami, S. 2009. The effects of plant growth regulators and explants on callogenesis, regeneration and suspension culture in *Foeniculum vulgare* Mill. *Iranian Journal of Medicinal and Aromatic Plants*, vol. 25, no. 3, p. 364-375.

Schultze, W., Hose, S., Abou-Mandour, A. and Czygan, F.-C. 1993. *Melissa officinalis* L. (Lemon Balm): In: Bajaj Y. P. S. (ed) *Medicinal and Aromatic Plants V. Biotechnology in Agriculture and Forestry*, vol. 24. Berlin, Heidelberg, germany : Springer, p. 242-268. eISBN: 978-3-642-58062-8 https://doi.org/10.1007/978-3-642-58062-8_18

Sobhanizadeh, A., Solouki, M. and Fazeli-Nasab, B. 2017. Optimization of Callus Induction and Effects of Biological and Non- biological Elicitors on Content of Phenol/ Flavonoid Compounds in *Nigella sativa* under In-Vitro Conditions. *Journal of Cell & Tissue*, vol. 8, no. 2, p. 165-184.

Soltanipol, M. M., Mohammadi, A., Rahnama, H., Abbaszadeh, B. 2011. Callusogenesis investigation of lemon balm (*Melissa officinalis* L.). *Journal of Agronomy and Plant Breeding*, vol. 7, no. 1, p. 45-54.

Veltecheva, M., Svetleva, D., Petkova, S. P., Perl, A. 2005. In vitro regeneration and genetic transformation of common bean. *Scientia Horticulturae*, vol. 107, p. 2-10.

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ASSESSMENT OF THE FUNCTIONAL QUALITY AND SAFETY OF YOGHURTS PRODUCED WITH STARTER CULTURES OBTAINED FROM SELECTED COMMERCIALLY SOLD YOGHURTS

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ABSTRACT

The study focused on the examination of laboratory prepared yoghurts which were fermented with selected starter cultures from commercially sold yoghurt. The starter cultures were molecularly identified (16s rRNA) as *Enterococcus lactis, Lactobacillus plantarum, Lactobacillus pentosus, Pediococcuspentosaceus* and *Enterococcus durans*. The isolates were examined for bile tolerance as an indicator of their ability to survive in the gut. The starter cultures were used to produce different yoghurts in the following order: *Enterococcus lactis* produced yoghurt, *L. plantarum* and *L. pentosus* produced yoghurt, *Pediococcuspentosaceus* produced yoghurt, *E. durans* produced yoghurt and yoghurt produced with all starter cultures. All yoghurts were examined for nutritional quality (vitamin A, B12 and C content, soluble and casein bound magnesium and calcium and proximate nutrient composition). At $p \leq 0.05$, there was statistical significant difference in the nutritional content with *P. pentosaceus* contained yoghurt, E. durans contained yoghurt and yoghurt produced with a combination of all isolates recording the highest nutritional values and the lowest was observed with the control. Safety tests such as haematology and histology were carried out on wistar rats. After 7 days of feeding the rats in groups with the different yoghurts and a control without yoghurt, there were marked improvements in the red blood cell counts, white blood cell counts but no significant difference in the differentials at $p \leq 0.05$. The isolates were also observed to have no disruptive effect on the morphology and structure of the small intestine. Overall, the use of these lactic acid bacteria strains showed immense benefits in their use as starter cultures and the study demonstrated safety of the final products for consumption.

Keywords: bile tolerance; nutritional quality; haematology; histology

INTRODUCTION

Yoghurt is a fermented milk product obtained from the milk or the milk products by the lactic acid fermentation through the action of *Streptococcus salivarius* subsp. *Thermophilus, Lactobacillus delbrueckii subsp. Bulgaricus* **(FAO/WHO, 1977)**. When a sufficient quantity of lactic acid is produced then the milk coagulates and this coagulated milk is called yoghurt. The probiotic yoghurt, having probiotic effect is a fermented milk product with adjuvant microorganisms. There are numerous advantages of consuming fermented dairy products containing probiotic bacteria. A high population of probiotic organisms in the colon contributes to good intestinal health. Consequently, consumption of products such as yoghurt containing viable probiotic organisms adds benefit to human gut health. Moreover, yoghurt supplies good quality proteins, also an excellent source of calcium, phosphorus, potassium and contains significant quantities of general vitamins. Yoghurt could be used for feeding, owing to its higher Ca/Na ratio **(Demott, 1985)**.

Yoghurts vary in appearance, flavor and ingredients. The quality and composition of yoghurt of applied bacterial cultures affects the quality of the yoghurt obtained as the result of the milk fermentation processes. There is a symbiotic relationship between the two species of bacteria i.e *Lactobacillus bulgaricus* and *Streptococcus thermophilus* that is why there is more rapid acid development than in the single strain culture **(Rasic et al., 1978; Tamime et al., 1980)**.

Various combinations of starter cultures are selected during manufacturing of yoghurt to achieve desirable characteristics of product and also to provide the consumers with a wide choice of therapeutic benefits. Depending on its activity, manufacturer usually adds 2 – 4% yoghurt starter culture. These days, there has been increasing trends to fortify the dairy product with fruits (natural fruit juice, pulp, dry fruits) **(Desai et al., 1994;**

Ghadge et al., 2008). Aesthetic value of new product can be increased by using fruit juice as a functional pigment in fermented milks with array of colors and flavor properties. **Coissonet al., (2005)** used *Euterpeoleracea* juice as functional pigment for yoghurt, which is dark purple in color having high anthocynin and phenolic content.

Yoghurt is a functional food. The functional food includes probiotics, prebiotics and synbiotics. Probiotics can be defined as "live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance" **(Champagne et al., 2005)**.

Prebiotics is defined as "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". Symbiotic is a combination of probiotics and prebiotics that "beneficially affects the host by improving the survival and the implantation of live microbial dietary supplements in the gastro-intestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria" **(Di Rienzo et al., 2000)**.

Yoghurt is one of the oldest fermented milk products known. Fermentation of milk involves the action of microorganisms, principally the lactic acid bacteria. These microorganisms sour the milk by converting the milk sugar lactose to lactic acid **(Kagan, 1985)**. Yogurt gels are built of clusters of aggregated casein particles formed as a result of gradual fermentation of lactose by lactic acid bacteria **(Horne, 1993)**. The Food and Drug Administration **(FDA, 2008)** standard of identity for yogurt drinks specifies >8.25% milk solids-not-fat and fat levels to satisfy nonfat yogurt (<0.5%), low-fat yogurt (2%), or yogurt (>3.25%) before the addition of other ingredients **(Chandan et al.,2006)**.Yogurt is among the most common dairy products consumed around the world **(Saint-Eve et al., 2006)**.

As the popularity of yogurt products continues to grow, manufacturers are continuously investigating value-added ingredients to entice health-conscious consumers **(Allgeyer et al., 2010)**.

Scientific hypothesis

The present study investigates the functional quality and safety of yoghurts produced from starter cultures obtained from randomly sampled commercial yoghurts and conducted with the following objectives:

1. Molecular identification of starter cultures Yoghurt production with identified starter cultures
2. Evaluation of physicochemical and nutritional properties that contribute to final product quality such as pH, titratable acidity, reducing sugar concentration, vitamin content, mineral composition and proximate nutrient composition
3. Assessment of probiotic potential of the yoghurts with antibacterial assay and safety to the intestine

MATERIAL AND METHODS

The media that were used in this work include nutrient agar, Macconkey agar, Eosinemethyleneblue agar (EMB), peptone water. The composition of the media, their method of production and the list of other equipment and reagents used in this work are presented in the appendix.

Sample Collection

Six different brands of bottle packaged yoghurt were brought from hawkers and beverage stores in Ikeji Ara-keji, Osun State. Isolates from each brand was used and the brands were designated A, B, C, D and E. The samples were brought to the laboratory, stored in the refrigerator and analyzed within 6 hours of collection.

Analysis of Sample

Each sample was serially diluted using sterile distilled water as diluents according to **(Prescott *et al.,* 2002)** and 1 milliliter of 10^{-3} sample was plated in duplicate using the pour plate method on nutrient agar media. The plates were incubated at 37 °C for 24hrs. After incubation the colonies developedon the nutrient agar plates were counted and used to determine the total bacterial count of the yoghurt samples ($CFU.ml^{-1}$). The representative colonies on the plates were sub-cultured on fresh nutrient agar to obtain pure cultures of the isolates. The pure cultures were then transferred into nutrient agar slants for molecular characterization.

Identification of Isolates

Molecular identification of the lactic acid bacteria isolate involved Denaturing Gradient Gel Electerophoresis, Polymerase Chain reaction and pure sequencing of 16s rRNA (bacteria) genes as described by **Akabanda et al. (2013)** and **Cocolin et al. (2000).**

Inoculum Preparation

Pure cultures of the bacterial isolates were inoculated in sterilized lactose broth and incubated at 37 ^{o}C for 18 hours

Preparation of Yoghurt

One hundred milliliteroutof whole milk collected from lactating cow was poured into a 500 mL beaker; was brought to boiling point without being allowed to boil and immediately cooled to 60 °C. 3.5 grams of non fat dry milk was added to the milk and stirred vigourously with a glass rod to dissolve the powder. The mixture was allowed to cool to 45 °C. 10 milliliter of 18 hour culture of the lactic acid bacteria was added in single, double and allied fermentation trials. This was done separately for the purpose of comparison. The beakers were covered with aluminium foil and incubated for 6 hours at 45 °C until it becomes firm **(Fassara, 2010)**.

Determination of Physicochemical parameter

The determination of physic-chemical parameters such as pH, total acidity, reducing sugar and optical density were determined according to the **Association of Analytical Chemists, (2000)**.

Proximate Analysis and Analysis of Nutrient Composition

The proximate analysis and determination of nutrient composition of the prepared yoghurt were determined according to **A.O.A.C (2000)** and **ASEAN (2011)**.

Determination of Calcium and Magnesium

The analytical method used for the analysis of heavy metal concentration was the Atomic Absorption Spectroscopy (AAS) using the calibration plot method. For each element, the instrument was auto-zeroed using the blank (distilled water) after which the standard was aspirated into the flame from the lowest to the highest concentration. The corresponding absorbance was obtained by the instrument and the graph of absorbance against concentration was plotted. The samples were analyzed in duplicates with the concentration of the metals present being displayed in parts per million (ppm) after extrapolation from the standard curve **(Greenberg et al., 1985)**.

In-vitro antibacterial activity

The in-vitro antibacterial activity of the yoghurts was carried out by agar well diffusing assaywith Mueller-Hinton agar (Lab M) **(Clinical and Laboratory Standards 2013)**. The yoghurts were centrifuged and the supernatant was tested against *Salmonella typhimurium* ATCC 14028*, Escherichia coli* ATCC 29929 and *Staphylococcus aureus* ATCC 29293.

Biological material

This was done by modifying the methodology of **Hounkpatin et al. (2013)**. The animal material was composed of 14 male albino Wistar rats weighing about 250 grams. These rats were purchased from a local rat farmer in Ado-Ekiti, Nigeria and were acclimated for 3 days before the experiments. They wereplaced in designed sterile polypropylene cagesat room temperature (25 to 30 °C). Thecages were illuminated with a sequence of 12 h light and 12h darkness. The rats 63 had free access to water and food.

Treatment of Wistar rats with the yoghurts

The wistar rats in duplicates were fed differently with the different yoghurts twice daily and supplemented with grains while the control was fed only with grains and water. The animals were fed for a period of 7 days after which they were sacrificed for haematological and histological assays.

Blood collection and haematological analysis

This was done by modifying the methodology of **Hounkpatin et al. (2013)**.

After 7 days of treatment, rats were fastedovernight. They were weighed before the collection of blood and sacrifice. All samples were taken between 7 and 9 am to avoid variations due to circadian rhythm. Whole blood was obtained from a puncture of the retro-orbital sinus by the conventional method **(Van Hercket al., 1992)**. Blood samples collected in ethylene diamine tetra-acetic acid (EDTA)anticoagulant tubes (8.5%) wasquickly returned bymixing with anticoagulant in the tube. All blood samples were labeled and immediately conveyed to the laboratory for analysis. Hematological parameters were analyzed: Packed Cell Volume (PCV), white blood cell count (WBC) and its differentials such as: Leukocyte, Eosinophil, Neutrophil, Basophils and Monocyte counts. All hematological parameters were analyzed in the "Haematology Unit, Federal Polytechnic Ado-Ekiti, Ekiti State, Nigeria Medical Laboratory using the automated method withthe automatic analyzer "Haematology auto analyzer Sysmex KX-21N".

Histology

The tissue were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. The deparaffinised sections were stained routinely with haematoxylin and eosin. Photomicrographs of the desired results were obtained using digital research photographic microscope in the laboratory **(Eweka and Om'Iniabohs, 2011)**.

Analysis of data

The results were presented as the mean standard values of three replicates. A one-way analysis of variance (ANOVA) was carried out using SPSS 16.0. Significance was accepted at $p \leq 0.05$.

RESULTS AND DISCUSSION

The commercial yoghurts used in the study are presented as a randomsampling of five different brands. All of five samples were used as starter cultures for the production of yoghurts in the laboratory. An assessment of the physical properties of the commercial yoghurt showed that sample A and B were thick and sour, sample C was very thick and very sour while sample D and E were slightly thick and sour. Samples A, B and E showed smooth consistency while samples C and D were coarsely smooth. The isolates were identified through the characterization of the 16S rRNA sequence and were analysed with the Basic Local Alignment Search Tool for sequence alignment (Table 1) while figure 1.0 shows the phylogenetic relationship between the organisms. The lactic acid bacterial isolates used in the study were isolated from the commercial yoghurts and their cell morphology and Gram stain reaction were observed. All isolates were observed to be Gram positive short rods and coccobacilli. The bacterial isolates were tested for their ability to survive and grow on bile salts and tested positive. The isolates used for the laboratory production of yoghurt in this study are lactic acid bacteria which have been reported as probiotic in functional dairy. *Enterococci* are lactic acid bacteria in large numbers and are naturally present in vegetables, plant material and food stuff; especially those of animal origin such as dairy products **(Girraffa, 2003); Katie and Carol, 2009)**. Because they produce bacteriocins, *Enterococcus* species have been used widely over the last decade in the food industry as probiotics or as starter cultures **(Foulquie, et al., 2006)**. Studies on the microbiota of traditional cheeses of Mediterranean countries produced mainly from raw milk of sheep, goats and cows indicate that *Enterococci* are a relevant component of the natural cultures involved in fermentation and play important role in the final quality of the product. They are also used to extend product shelf life and improve the hygienic safety of foodstuffs because they produce antimicrobial substances such as lactic acid, hydrogen peroxide, and bacteriocins (enteroxins) **(Franz et al., 2007)**.

Table 1 MolecularlyidentifiedLacticAcidbacteriaisolates.

ISOLATE/CODE	Name of Isolate	ACCESSION NUMBER
A1	*Enterococcuslactis*	LC318433.1
B1	*Lactobacillus planetarium*	MF942369.1
B2	*Lactobacillus Pentosus*	CP022130.1
C1	*Pediococcuspentosaceus*	MF942373.1
D1	*Pediococcuspentosaceus*	MF942373.1
D2	*Enterococcusdurans*	MF628998.1
E1	*Enterococcusdurans*	MF628998.1
E2	*Enterococcusdurans*	MF583025.1

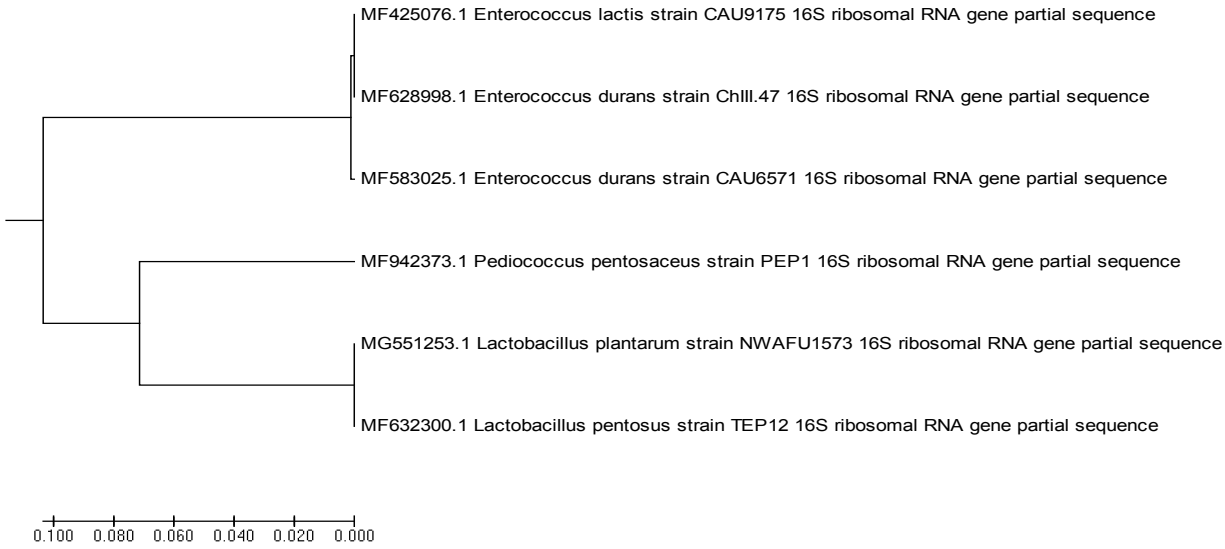

Figure 1 Phylogenetictree of thelacticacidbacteriaisolates.

According to **Franz et al. (2007)** and **Naoualet al. (2010)**, *Enterococcus durans* and *Enterococcus lactis* are reported as non pathogenic **Fabianova, (2010)** reported the presence of members of the enterobacteriaceae in raw cow milk and their presence may be as a result of their presence in the raw milk used for the production of the yoghurts. **Kumar et al. (2016)** reported the use of *lactobacillus plantarium* in yoghurt fermentation properties and was said to give excellent antimicrobial and final product quality. **Swain et al. (2014)** and **Lilis (2015)** described *Pediococcuspentosus* and *Pediococcuspentosaceus* to be of high probiotic quality and were isolated from Indonesian fermented foods in a bid to review health promoting lactic acid bacteria.

After 24 hours of incubation at 37 ^{o}C on MacConkey agar with bile salt, all isolates were observed to survive the growth condition. After 18 hours of incubation of the laboratory prepared yoghurts at 37 ^{o}C, physical parameters such as texture, taste, consistency and colour were determined while taste was determined as the only organoleptic property. Yoghurt produced with *Enterococcus lactis* (L.A) and yoghurt produced with *Lactobacillus plantarum* and *L. pentosus* (L.B) as starter cultures were thick and sour; yoghurt produced with *Pediococcuspentosaceus* (L.C) as starter culture was slightly thick and very sour while yoghurt produced with *Pediococcuspentosaceus* and *E. durans* as starter cultures (L.D) and yoghurt produced with *E. durans* as starter culture (L.E) were slightly thick and sour. They all showed a smooth consistency with a creamy colouration. The pH of the yoghurts (Table 2) after 8 hours of fermentation was found to be within the range of 5.51 in *Pediococcuspentosaceus* produced yoghurt and 5.76 in *Enterococcus durans* produced yoghurt. This finding corresponds with the report of **Obi et al. (2016)** who reported pH 4 – 5.5 and 5.5 as optimum. The study is also in agreement with the report of **Charles et al. (2016)**, who reported titratable acidity between 0.02 – 0.06%. The control revealed the progress of fermentation and decrease in pH of the samples inoculated with the lactic acid bacteria as starter cultures. The study also revealed increasing optical density in all samples and decreasing trend in the reducing sugars as a result of the fermentative activities of the starter cultures and this is supported by the report of **Shao-Chi et al. (2007)**.

The vitamin content (A, B_{12} and C) as determined in all the yoghurts had varying differences ($p \leq 0.05$) which was as a result of different starter cultures and combinations of the starter cultures (Table 3). The vitamin A and B_{12} content of yoghurt produced with *Pediococcuspentosaceus, Enterococcusdurans*and a combination of all the isolates resulted in higher vitamin A content when compared with others. The vitamin A and B_{12} content recorded in this study is higher than the record found in the report of **Ihemeje et al. (2015**).

Table 2 Physicochemical assessment of the laboratory prepared yoghurts.

Yoghurt code	pH ±SD	Total titratable acidity (moles) ±SD	Optical density ±SD	Reducing sugar (mg/g) ±SD
L.A	5.52 ±0.005[b]	0.050 ±0.000[b]	1.32 ±0.005[b]	15.1 ±0.294[a]
L.B	5.67 ±0.005[d]	0.053 ±0.000[c]	1.69 ±0.005[f]	16.5 ±0.005[b]
L.C	5.41 ±0.005[a]	0.056 ±0.000[e]	1.42 ±0.005[d]	18.7 ±0.005[d]
L.D	5.61 ±0.005[c]	0.055 ±0.000[d]	1.40 ±0.005[c]	15.1 ±0.005[a]
L.E	5.76 ±0.005[e]	0.060 ±0.000[f]	1.60 ±0.005[e]	16.5 ±0.010[b]
L.ABCDE	5.42 ±0.005[a]	0.059 ±0.000[e]	1.80 ±0.005[g]	17.52 ±0.010[c]
Control	7.05 ±0.005[f]	0.000 ±0.000[a]	0.60 ±0.005[a]	25.2 ±0.050[e]

Note: SD: Standard deviation, L.A: Yoghurt produced with *Enterococcus lactis* as starter culture, L.B: Yoghurt produced with *Lactobacillus plantarum* and *Lactobacillus pentosus* as starter cultures, L.C: Yoghurt produced with *Pediococcus pentosaceus* as starter culture,L.D: Yoghurt produced with *Pediococcuspentosaceus* and *Enterococcus durans* as yoghurt starter cultures,L.E: Yoghurt produced with two isolates of *Enterococcus durans* as starter culturesand ABCDE: Yoghurt produced with all isolates as starter cultures.

Table 3 Vitamin content of the laboratory prepared yoghurts.

Yoghurtcode	Vitamin A (IU/100G) ±SD	Vitamin B12 (µg/100) ±SD	Vitamin C (mg/100g) ±SD
L.A	297.20 ±0.007[b]	1.95 ±0.007[b]	2.90 ±0.007[d]
L.B	258.50 ±0.353[a]	1.84 ±0.007[a]	2.71 ±0.007[c]
L.C	401.80 ±0.007[e]	3.40 ±0.007[f]	3.50 ±0.00[7]
L.D	370.00 ±0.007[c]	3.20 ±0.007[e]	2.30 ±0.007[a]
L.E	396.50 ±0.007[d]	2.55 ±0.007[d]	2.33 ±0.007[b]
L.ABCDE	612.00 ±0.014[g]	3.82 ±0.007[g]	3.88 ±0.007[f]
RAW	479.00 ±0.007[f]	2.46 ±0.007[c]	3.64 ±0.007[e]

Note: ND: Not Determined, L.A: Yoghurt produced with *Enterococcuslactis* as starterculture, L.B: Yoghurtproduced with *Lactobacillus plantarum* and *Lactobacillus pentosus* as startercultures, L.C: Yoghurt produced with *Pediococcus pentosaceus* as starter culture, L.D: Yoghurt produced with *Pediococcus pentosaceus* and *Enterococcus durans* as yoghurt starter cultures, L.E: Yoghurt produced with two isolates of *Enterococcus durans* as starter cultures and ABCDE: Yoghurt produced with all isolates as starter cultures.

Table 4 Determination of soluble and casein bound magnesium and calcium.

Yoghurtcode	Soluble Magnesium(ppm)	Soluble calcium	Caseinbound Magnesium	Caseinbound calcium
L.A	4.147 ±0.001[a]	48.600 ±0.007[c]	4.250 ±0.001[a]	48.550 ±0.007[b]
L.B	5.756 ±0.000[f]	39.800 ±0.007[b]	4.260 ±0.001[b]	66.200 ±0.007[d]
L.C	5.555 ±0.007[b]	62.500 ±0.007[f]	5.080 ±0.003[e]	75.600 ±0.007[e]
L.D	5.567 ±0.001[d]	59.800 ±0.007[d]	4.510 ±0.003[d]	85.400 ±0.007[f]
L.E	5.713 ±0.002[e]	60.100 ±0.007[e]	4.960 ±0.001[f]	58.100 ±0.007[c]
L.ABCDE	4.140 ±0.007[a]	66.500 ±0.007[g]	4.300 ±0.007[c]	48.450 ±0.007[a]
RAW	5.650 ±0.007[c]	32.400 ±0.007[a]	ND	ND

Note: ND: Not Determined, Superscript a-g denotes statistical difference in mean at P≤0.01.L.A: Yoghurt produced with *Enterococcus lactis* as starter culture, L.B: Yoghurt produced with *Lactobacillus plantarum* and *Lactobacillus pentosus* as starter cultures, L.C: Yoghurt produced with *Pediococcus pentosaceus* as starter culture,L.D: Yoghurt produced with *Pediococcuspentosaceus* and Enterococcus duransas yoghurt starter cultures,L.E: Yoghurt produced with two isolates of *Enterococcus durans* as starter cultures and ABCDE: Yoghurt produced with all isolates as starter cultures.

Table 5 Proximate analysis of the laboratory prepared yoghurts.

SAMPLE	MOISTURE (%) ±SD	ASH (%) ±SD	FAT (%) ±SD	CRUDE FIBRE (%)	PROTEIN (%) ±SD	CARBOHYDRATE (%) ±SD
RAW MILK	81.94 ±0.05^{g}	0.68 ±0.01^{c}	0.00 ±0.00^{a}	ND	16.91 ±0.01^{g}	0.48 ±0.02^{a}
ABCDE	79.59 ±0.02^{f}	0.29 ±0.01^{a}	0.02 ±0.01^{b}	ND	5.19 ±0.03^{a}	14.93 ±0.04^{c}
A	78.01 ±0.01^{e}	1.14 ±0.01^{f}	0.02 ±0.01^{b}	ND	9.4 ±0.01^{f}	11.43 ±0.05^{b}
B	71.71 ±0.01^{d}	0.95 ±0.05^{d}	0.06 ±0.04^{e}	ND	8.39 ±0.00^{e}	18.9 ±0.04^{d}
C	61.73 ±0.01^{a}	0.96 ±0.05^{e}	0.55 ±0.01^{d}	ND	7.81 ±0.01^{d}	28.96 ±0.60^{g}
D	67.31 ±0.00^{c}	0.54 ±0.05^{b}	0.03 ±0.01^{c}	ND	7.51 ±0.01^{c}	24.62 ±0.02^{e}
E	64.36 ±0.00^{b}	1.03 ±0.05	0.03 ±0.01^{c}	ND	6.87 ±0.01^{b}	27.71 ±0.02^{f}

Note: SD: Standard Deviation, ND: Not Determined, Superscript a-g denotes statistical difference in mean at $p \leq 0.01$. A: *Enterococcus lactis* as yoghurt starter culture. B: *Lactobacillus plantarum* and *Lactobacillus pentosus* as yoghurt starter culture. C: *Pediococcuspentosaceus*as yoghurt starter culture. D: *Pediococcuspentosaceus* and Enterococcus duransas yoghurt starter culture. E: Two isolates of *Enterococcus durans* as yoghurt starter culture. ABCDE: All isolates

Table 6 Antibacterial assay of the laboratory prepared yoghurts on selected clinical isolates using well agar diffusion.

Clinicalisolates/	Zones of inhibition (mm)					
Test organisms	**A**	**B**	**C**	**D**	**E**	**ABCDE**
Staphylococcus aureus	9	7	7	6	7	12
Escherichia coli	4	7	5	4	5	6
Salmonella typhi	4	3	3	3	3	3

Note:A: *Enterococcuslactis*as yoghurt starter culture, B: *Lactobacillus plantarum* and *Lactobacillus pentosus* as yoghurt starter culture. C: *Pediococcus pentosaceus* as yoghurt starter culture. D: *Pediococcus pentosaceus* and *Enterococcus durans* as yoghurt starter culture. E: Twoisolates of *Enterococcus durans* as yoghurt starter culture, ABCDE: All isolates.

Table 7 Hematologicalevaluation of Wistarratstreatedwiththepreparedyoghurts.

			DIFFERENTIAL COUNT (dl)				
SAMPLE	**PCV (%)**	**TOTAL WBC**	**NEUTROPHIL**	**LEUCOCYTES**	**EOSINOPHILS**	**MONOCYTES**	**BASOPHILS**
A	38	11.8 X 10^{9}	52	42	1	5	0
B	30	11.5 X 10^{9}	53	42	1	4	0
C	32	10.9 X 10^{9}	51	43	1	5	0
D	36	11.3 X 10^{9}	51	41	1	7	0
E	32	10.7 X 10^{9}	52	41	1	6	0
ABCDE	40	11.2 X 10^{9}	53	42	1	4	0
CONTROL	24	12.3 X 10^{9}	52	42	1	5	0

Note: A: *Enterococcus lactis* as yoghurt starter culture. B: *Lactobacillus plantarum* and *Lactobacillus pentosus* as yoghurt starter culture. C: *Pediococcuspentosaceus*as yoghurt starter culture. D: *Pediococcuspentosaceus* and Enterococcus duransas yoghurt starter culture. E: Two isolates of *Enterococcus durans* as yoghurt starter culture. ABCDE: All isolates.

The combination of all the starter cultures resulted in higher vitamin C contentWhich is lower when compared to the report of **Ihemeje *et al.* (2015)**. This increase may be as a result of more efficient oxidation of sugar by the consortium of lactic acid bacteria to ascorbic acid in a fermentative path way of L-galactose to L-ascorbic acid (vitamin C) as reported by **Berry (2002)**. *Pediococcus pentosaceus* used as starter culture also produced yoghurt at a close range with the combined isolates, vitamins are essential organic compounds required in very small amounts to maintain the fundamental functions of the body **(Hassen et al., 2010)**.

The soluble and casein bound calcium and magnesium of the yoghurts showed varying concentrations of magnesium and calcium which showed statistically significant differences ($p \leq 0.05$) (Table 4.0). The soluble magnesium recorded lied between 4.140 ±0.007 – 5.713 ±0.002 while the raw milk before fermentation was 5.650 ±0.007. Therefore, there was depletion in the soluble magnesium content as it is a mineral requirement of lactic acid bacteria **(Boyaval, 1989)** but yoghurt produced with *Enterococcus durans* as starter culture recorded improved magnesium content and was found to have the highest magnesium content. The highest soluble calcium in the study was observed with yoghurt produced with all the isolates followed by *Enterococcus durans* and *Pediococcus pentosaceus* with relatively high values.

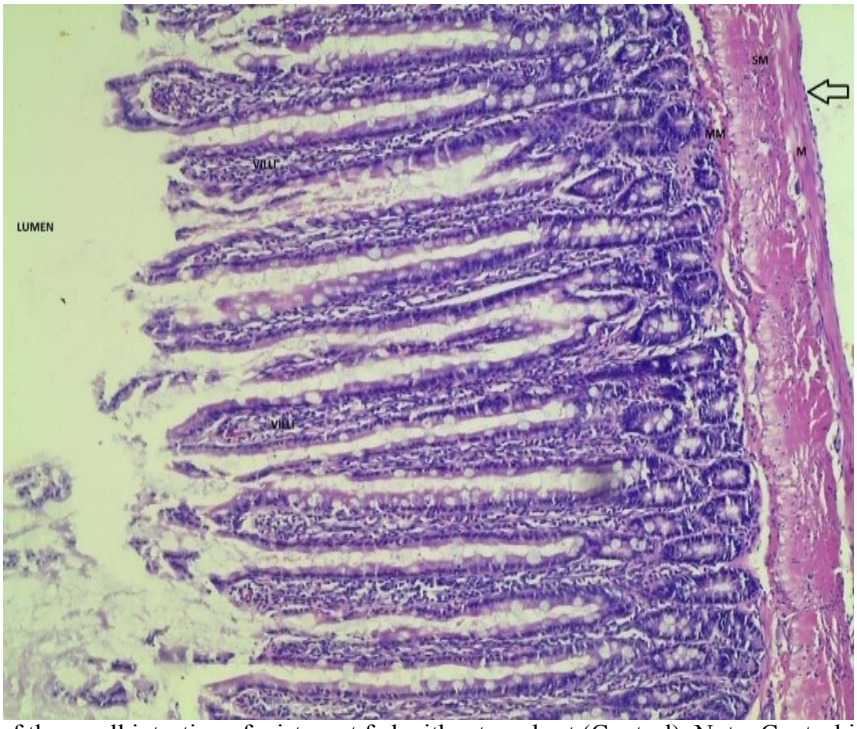

Figure 2 Histology of the small intestine of wistar rat fed without yoghurt (Control) .Note: Control intestine, X100, HE STAIN. HE (Haematoxylin and Eosin stain).

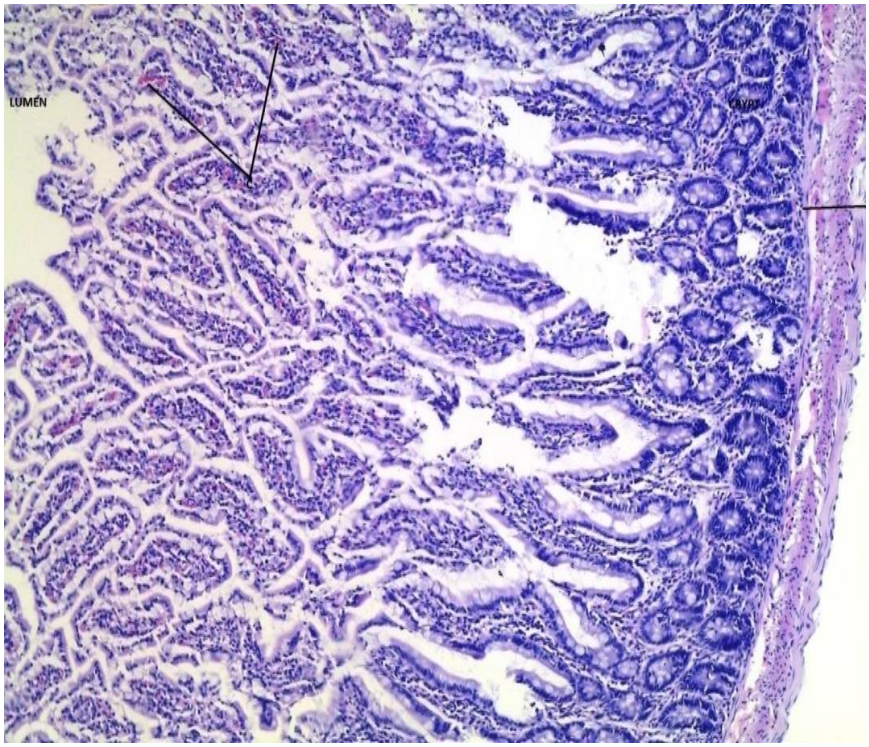

Figure 3 Histology of the small intestine of wistar rat fed with *Enterococcus lactis* containing yoghurt. Intestine, X100, HE STAIN.

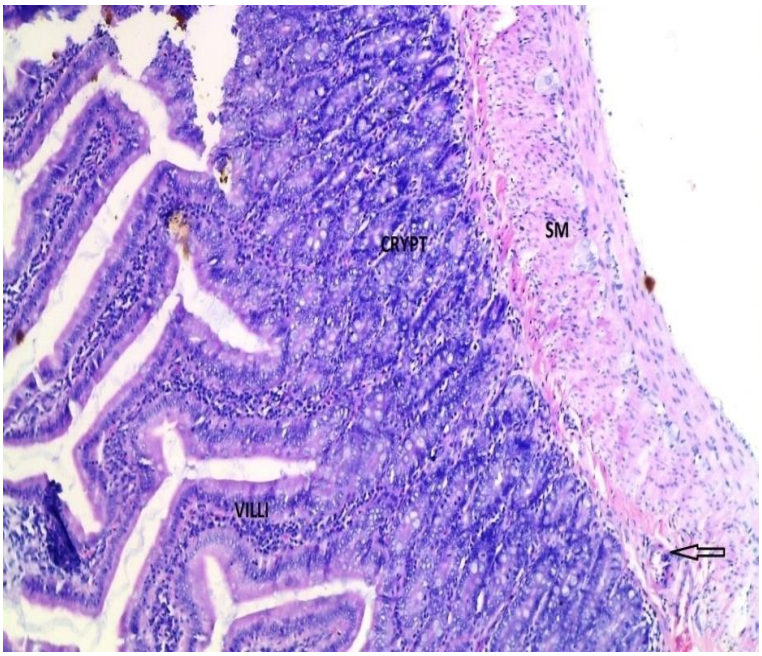

Figure 4 Histology of the small intestine of wistar rat fed with *Lactobacillus plantarum* and *Lactobacillus pentosus* containing yoghurt. Intestine, X100, HE STAIN.

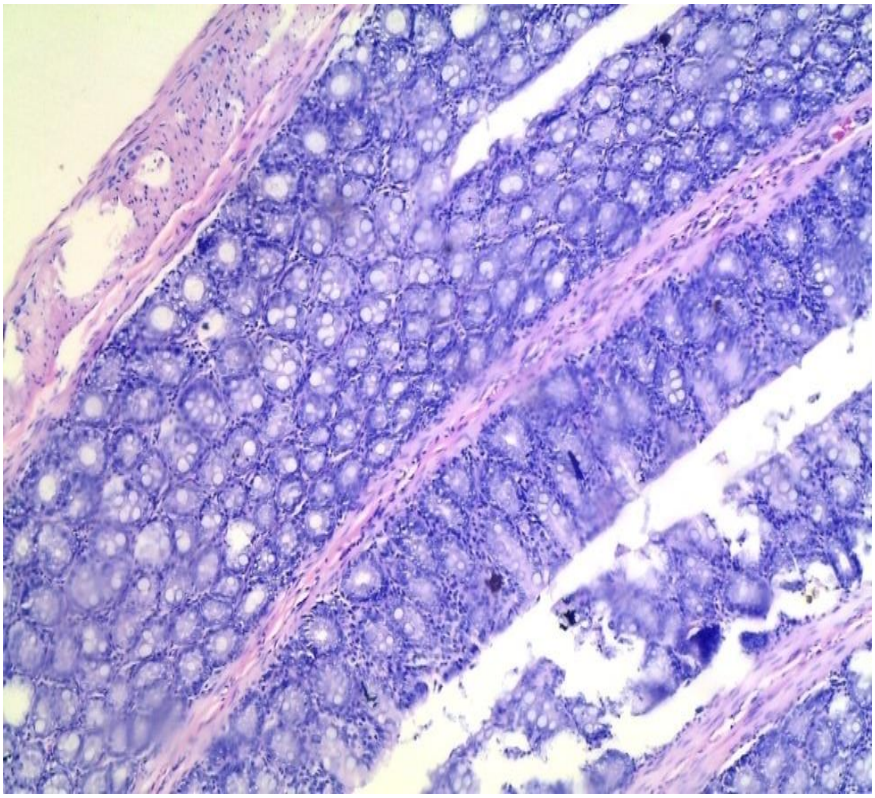

Figure 5 Histology of the small intestine of wistar rat fed with *Pediococcus pentosaceus* containing yoghurt. Intestine, X100, HE STAIN.

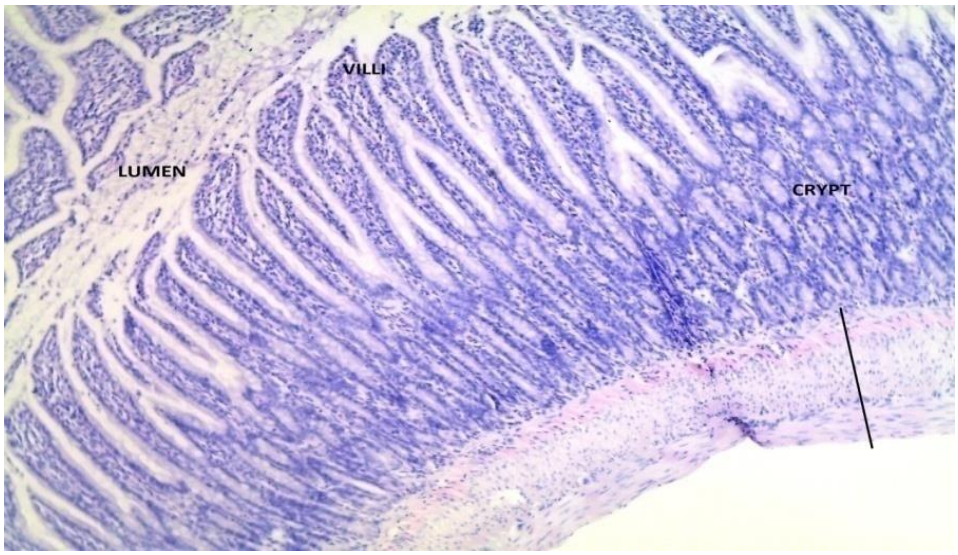

Figure 6 Histology of the small intestine of wistar rat fed with *Pediococcus pentosaceus* and *Enterococcus durans* containing yoghurt. Intestine, X100, HE STAIN.

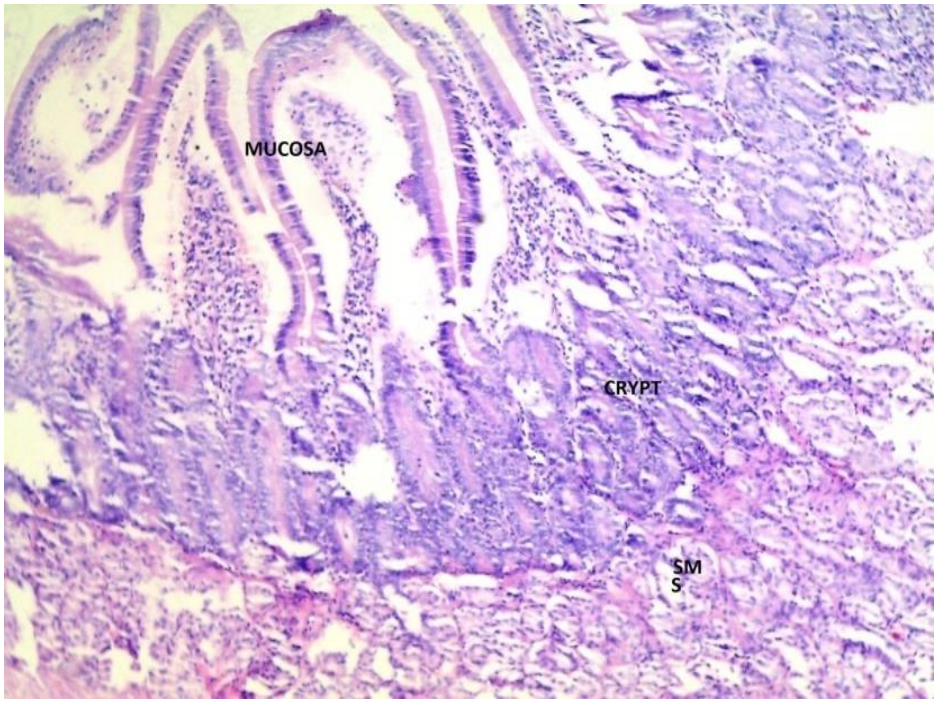

Figure 7 Histology of the small intestine of wistar rat fed with *Enterococcus durans* containing yoghurt. Intestine, X100, HE STAIN.

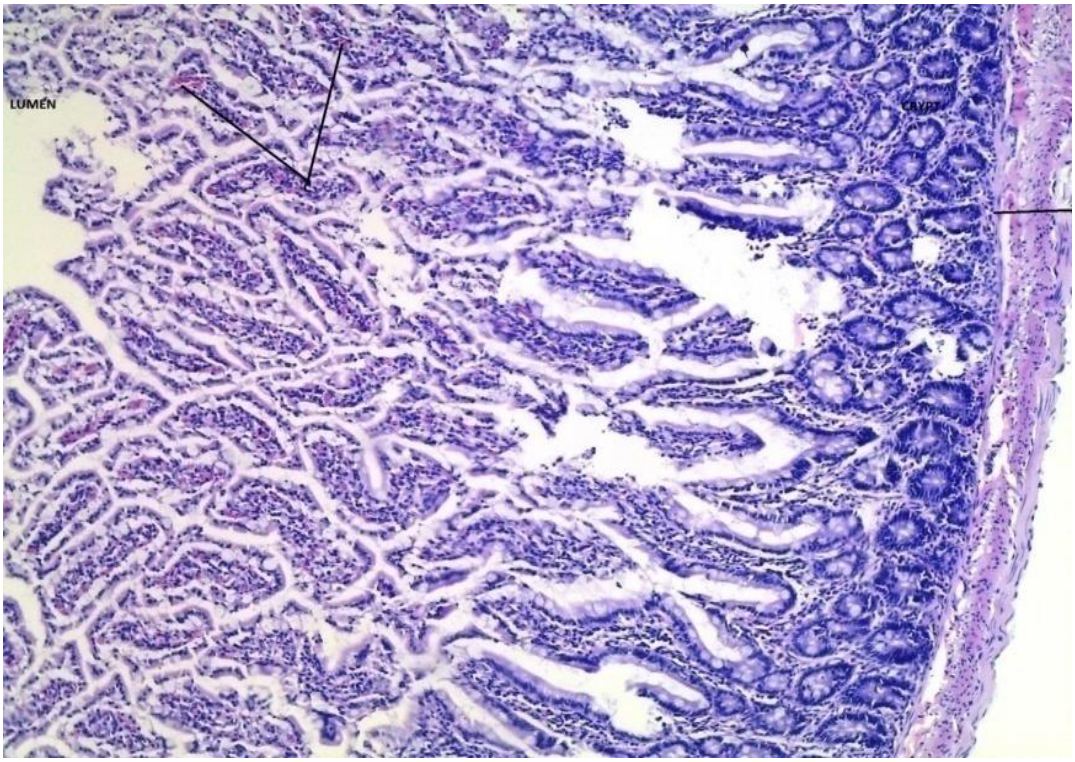

Figure 8 Histology of the small intestine of wistar rat fed with *Enterococcus lactis* containing yoghurt. Intestine, X100, HE STAIN.

The casein bound calcium in this study were relatively higher than the concentrations found in the soluble calcium content in yoghurt which had starter cultures of *Lactobacillus plantarium* and *L. pentosus* as dual starter cultures, *Pediococcus pentosaceus* and *Enterococcus durans* as dual starter culture and *Enterococcus durans* as starter culture. However, *Enterococcus lactis* and the combination of the isolates gave improved magnesium content in the casein bound magnesium. The result of the study in terms of calcium and magnesium content is in contrast with the work of **Gad et al. (2010)** who reported higher calcium and magnesium values.

The report of this study agrees with the work of **Miguel et al. (2003)** who reported such variations between the soluble and casein bound magnesium and calcium.

The proximate composition of the yoghurts as determined in comparison with the raw, recorded a decrease. However, the carbohydrate content increased in all the yoghurts produced. The highest protein content was found in the yoghurt produced with *Enterococcus lactis* while the highest carbohydrate content was observed with the yoghurt produced with Pediococcuspentosaceus as starter culture. There was significant difference in all tested parameters of the different yoghurts ($p \leq 0.05$) (Table 5). The fat content of the yoghurts were relatively low. Fat plays an important role in improving the consistency of yoghurt and also provides twice the energy of carbohydrate and protein **(Ehirim and Onyeneke, 2013)**. The findings of this study in terms of proximate composition agree with the report of **Igbabul et al. (2014)** and **Dairy Council (2013)**.

The yoghurts were observed to exhibit antibacterial activity against selected bacterial isolates representative of the Gram positives and Gram negatives as in *Staphylococcus aureus* and *Escherichia coli* and *Salmonella typhi* respectively (Table 6). The yoghurts were observed to express antibacterial activity although at minimal levels while the highest inhibitory activity was found in an assay performed using the synergistic potential of all the laboratory prepared yoghurts. This is in agreement with the report of **Hami (2011)** and **Okiki et al. (2018)** who established such antibacterial properties in nono fermented spontaneously by naturally occurring lactic acid bacteria**.** A number of studies have found probiotic consumption to be useful in the treatment of many types of diarrhea and intestinal disorders **(Isolauri et al., 1991; Oksanen et al., 1990 and Siitonen et al., 1990)**. Antimicrobial effects of lactic acid bacteria are formed by producing some substances such as organic acids, carbondioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances and bacteriocins **(Ouwehand and Vesterlund, 2004; Romanova and Urminska 2017)**.

The examination of the yoghurts influence on haematological parameters using Wistar ratsShowed improvements in the tested parameterswhich was demonstrated in the difference between the control and the treated samples (Table 7). The study revealed that combined use of the isolates always yielded better outcomes in terms of parameters examined. In a previous study, **Shu et al. (1999)** reported that 4 weeks of consumption of *Lactobacillus rhamnosus*, *Lactobacillus*

acidophilus, and *Bfidobacterium lactis* had no adverse effect on mice general health status including haematology. In this study there was general increase in total white blood cell, packed cell volume but there was no significant effect on differentials such as the neutrophils, leukocytes, eosinophils, monocytes and basophil count. The report of this study differs from **Hamid et al. (2008)** as the study recorded higher blood cell counts as a result of longer exposure to treatment. A high level of packed cell volume is an indication that the rats are not anemic while a lower level is an indicationOf anemia **(Aboderin and Oyetayo, 2006)**. The white blood cell is important in defending our body against infection **(Aboderin and Oyetayo, 2006)**. However, the leukocyte counts cannot give specific information and this necessitated the differential counts. The lymphocytes did not change significantly and this is in agreement with the report **Hamid et al. (2008)**.

Figure 2 shows a section of small intestinal tissue, the submucosa (SM), muscularis mucosa (MM) and serosa arrow. The projection of the intestinal villi into the lumen together with the intestinal glands are lined by columnar epithelium, numerous goblets cells are seen. No sign of intestinal inflammation. Figure 3 shows a section of intestinal tissue with marked disruption of the mucosa layer (line), other layers of the intestine appear normal. Figure 4 shows a section of small intestinal tissue, the submucosa (SM), muscularis mucosa (MM) and serosa. The projection of the intestinal villi into the lumen together with the intestinal glands are lined by columnar epithelium, numerous goblets cells are seen. Arrow shows vascular leucocytes Margination. Figure 5 shows a section of the small intestinal tissue, the submucosa (SM), muscularis mucosa (MM) and serosa arrow. The projection of the intestinal villi into the lumen together with the intestinal glands which are lined by columnar epithelium, numerous goblets cells are seen. No sign of intestinal inflammation. Figure 6 shows a section of intestinal tissue with intestinal villi projection into the lumen, the intestinal crypt and adjoining layers (line) appear essentially normal. Figure 7 shows a section of unremarkable intestinal layers, mucosa, sub-mucosa (SM), muscularis and mucosa appear essentially normal. Figure 8 shows a section of intestinal tissue with some disruption of the mucosa layer (line), other layers of the intestine appeared normal. The projection of the intestinal villi into the lumen together with the intestinal glands is lined by columnar epithelium. The lactic acid bacteria strains were reported to cause no morphological abnormalities to the small intestine. The report of this study is in the affirmative to the reports of **Ramiahet al., (2009)** who reported the safety examination of *Enterococcus mundtii* and *Lactobacillus plantarum* and the organisms were found to have caused no histological abnormalities or inflammation. **Cheng-Chihet al., (2014)** also examined *Lactobacillus acidophilus*, *L. pentosus, L. plantarum, L. reuteri* and *Enterococcus faecium* and their toxicity. The small intestines of the Wistar rats fed with the different yoghurts which contained starter cultures; *Enterococcus lactis, L. pentosaceus, L. pentosus, L. plantarum* and *E. durans*showed no abnormality, inflammation or damage.

CONCLUSION

As determined in this study, the final product quality of yoghurt or functional dairy is dependent on the selected lactic acid bacteria (LAB) cultures; therefore the choice of organism(s) to drive the fermentation is as important as the product itself. The study also demonstrated the importance of using animal models to determine the safety of the LAB cultures to be employed during yoghurt production. The use of more than one starter culture results in enhanced product quality as use of combined isolates in this study gave better outcomes in terms of nutritional quality. Finally, yoghurt is an important source of nutrients required for the body and also a remedy for gastrointestinal disorders caused by pathogens.

RERFERENCES:

Aboderin, F. I., Oyetayo, V. O. 2006. Haematological studies of rats fed different doses of probiotic, *Lactobacillus plantarum*, isolated from fermenting corn slurry, *Journal of Nutrition*, vol. 5, no. 2, p. 102-105.

Anupama, G., Nevedita, S. 2017. Characterization of Potential Probiotic Lactic Acid Bacteria-Pediococcus acidilactici Ch-2 Isolated from Chuli- A Traditional Apricot Product of Himalayan Region for the Production of Novel Bioactive Compounds with Special Therapeutic Properties. *Journal of Food: Microbiology, safety and hygiene*, vol. 2. no. 1, p. 9-11.

ASEAN, 2011. Manual of Food Analysis. Regional centre of ASEAN network of food data system. Institute of nutrition, Mahidol University, Thailand.

Berry A., Running, J. A., David, K. S., Richard, P. B. 2002. Vitamin C production in microorganisms. United States patent publication. 0012979 A1, p. 1-12.

Boyaval, P. 1989. Lactic acid bacteria and metal ions. INRA Editions, vol. 69, no. 2, p. 87-113 https://doi.org/10.1051/lait:198927

Champagne, C. P., Gardener, N. J. 2005. Challenges in the Addition of Probiotic Cultures to Foods. *Critical Reviews in Food Science and Nutrition*, vol. 45, p. 61-84. https://doi.org/10.1080/10408690590900144 PMid:15730189

Charles, B., Ernest, C. I., Mamudu, H. B., Ohene, B., Vincent, G. Z. 2016. Microbial ecology and effectiveness of different starter cultures in yoghurts produced from whole cow milk and soybeans. *African Journal Food Science and Technology*, vol. 7, no. 5, p. 107-117.

Cheng-Chih, T., Sew-Feni, L., Quen-Rong, H., Lan-Chun, C., Chun-Chih, H. 2014. Safety evaluation of multiple strains of *Lactobacillus plantarum* and *Pediococcus pentosaceus* in wistar rats based on the Ames test and 28-day feeding study. *The Scientific World Journal*, vol. 2, no. 1, p. 9-11.

Clinical Laboratory Standards Institute, CLSI 2013. Performance standard for antimicrobial susceptibility testing: seventeenth informational supplement M100-S17. Clinical Laboratory Standards Institute, Wayne, PA, USA, vol. 27, no. 1.

DiRienzo, D. B. 2000. Symposium: Probiotic Bacteria: Implications for Human Health. *Journal of Nutrition*, vol. 130, p. 382S-383S.https://doi.org/10.1093/jn/130.2.382S

Ehirim, F. N., Onyeneke, E. N. 2013. Physicochemical and organoleptic properties of yoghurt manufactured with Cow milk and Goat milk. Natural and Applied Science, vol. 4, no. 4, p. 10-15.

Eweka, A. O., Om'Iniabohs F. A. E. 2011. Histological studies of the effects of monosodium glutamate on the ovaries

of adult wistar rats. A*nnals of Medical and Health Sciences Research*, vol. 1, no. 1. p. 37-43. PMid:23209953

Fabianová, J., Ducková, V., Čanigová, M., Kročko, M. 2011. Presence of *Enterococci* in cow milk and their antibiotic resistance. *Potravinarstvo*, vol. 4, no. 2, p. 17-21. https://doi.org/10.5219/45

FAO/WHO, 1977. Report joint FAO/WHO expert committee on the code of principlesconcerning milk and milk products. FAO/WHO, Rome.

Foulquie, M. R., Sarantinopoulos, P., Tsakalidou, E., De Vuyst, L. 2006. The role and application of enterococci in food and health. *International Journal of Food Microbiology*, vol. 106, p. 1-24. https://doi.org/10.1016/j.ijfoodmicro.2005.06.026 PMid:16216368

Franz, Z. C. M., van Belkum, M. J., Holzapfel, W. H., Abriouel, H., Galvez, A. 2007. Diversity of Enterococcal Bacteriocins and their Grouping in a New Classification Scheme. *FEMS Microbiology Review*, vol. 31, p. 293-310. https://doi.org/10.1111/j.1574-6976.2007.00064.x PMid:17298586

Gad, S., Kholif, A. M., Sayed, A. F. 2010. Evaluation of the Nutritional Value of Functional Yoghurt Resulting Combination of Date Palm Syrup and Skimmed milk. American *Journal of Food Technology*, vol. 5, p. 250-259. https://doi.org/10.3923/ajft.2010.250.259

Ghadge, P. N., Prasad, K., Kadam, P. S. 2008. Effect of fortification on the physico chemical and sensory properties of buffalo milk yoghurt. *Electronic Journal of Environmental Agriculture and Food Chemistry,* vol. 7, p. 2890-2899.

Giraffa, G. 2003. Functionality of enterococci in dairy products. *International Journal of Food Microbiology*, vol. 88, p. 215-222. https://doi.org/10.1016/S0168-1605(03)00183-1

Greenberg, A. E., Trussell, R. R., Clesceri L. S. 1985. Standard methods for the examination of water and waste water, 16th edition. *American Public Health Association, Washington, DC*, p.146-150.

Hami, K. 2011. Antibacterial effects of yoghurts isolated from yoghurts against some common bacterial pathogens. *African Journal of Microbiology Research*, vol. 5, no. 25, p. 4363-4367.

Hamid, M., Amouoghli, T., Ali, H., Alireza, B., Guitty, K. 2008. Study on the effect of consuming different amount of fermented milk with *Lactobacillus casei* 01 on haematological parameters in rats. *Research Journal of Biological Sciences*, vol. 3, no. 12, p. 1376-1380.

Hassen, B., Shanmugam, S., Tarek T. 2010. Vitamin A from Physiology to Disease Prevention. *International Journal of Pharmaceutical Sciences Review and Research*, vol. 1 no. 1, p. 68-73.

Ida Muryany, M. Y., Ina Salwany, M. Y., Ghazali, A. R., Hing, H. L., Nor Fadilah, R. 2017. Identification and characterization of the Lactic Acid Bacteria isolated from Malaysian fermented fish (Pekasam). *International Food Research Journal*, vol. 24, no. 2, p. 868-875.

Igbabul, B., Shember, J., Amove, J. 2014. Physicochemical, microbiological an sensory evaluation of yoghurt sold in Makurdi Metropolis. *African Journal of Food Science and Technology*, vol. 5, no. 6, p. 129-135.

Ihemeje, A., Nwachukwu, C. N., Ekwe, C. C. 2014. Production and quality evaluation of flavoured yoghurts using carrot, pineapple and spiced yoghurts using ginger and pepper fruit. *African Journal of Food Science*, vol. 9, no. 3, p. 163-169. https://doi.org/10.5897/AJFS2014.1244

Isolauri, E., Juntunen, M., Rautanen, T., Sillanaukee, P., Koivula, T. 1991. A human *Lactobacillus* strain (*Lactobacillus casei* sp. Strain GG) promotes recovery from acute diarrhea in Children. *Pediatrics*, vol. 88, p. 90-97. PMid:1905394

Kai, S. H., Bongard, V., Simon C. 2014. Low-fat and high-fat dairy products are related to blood lipids and cardiovascular risk score. *Eur. J. Prev. Cardiol*., vol. 21, p. 1557-1567.

Katie, F., Carol, P. 2009. The ecology, epidemiology and virulence of *Enterococcus*. *Microbiology*, vol. 155, p. 1749-1757. https://doi.org/10.1099/mic.0.026385-0 PMid:19383684

Kumar, M., Dhaka, P., Vijay, D., Vergis, J., Mohan, V. 2016. Antimicrobial effects of *Lactobacillus plantarum* and *Lactobacillus acidophilus* against multidrug-resistant entero-aggregative *Escherichia coli*. *International Journal of Antimicrobial Agents*, vol. 48, no. 3, p. 265-270. https://doi.org/10.1016/j.ijantimicag.2016.05.014 PMid:27451088

Lilis, N. 2015. A review: Health promoting lactic acid bacteria in traditional Indonesian fermented foods. *Food Science and Human Wellness*, vol. 4, p. 47-55. https://doi.org/10.1016/j.fshw.2015.06.001

Lim, S., Dong-Soon, I. 2008. Screening and characterization of probiotic lactic acid bacteria isolated from Korean fermented foods. *Journal of Microbiology and Biotechnology*, vol. 19, no. 2, p. 178-186. https://doi.org/10.4014/jmb.0804.269

McBean, L. D. 1999. Emerging Dietary Benefits of Dairy Foods. Nutrition, vol. 34, p. 47-53. https://doi.org/10.1097/00017285-199901000-00008

Miguel, A., Fernando, M., Gonzalo, G., Manuela, J. 2003. Total and Soluble contents of calcium, magnesium, phosphorus and zinc in yoghurts. *Food Chemistry*, vol. 80, no. 4, p. 573-578. https://doi.org/10.1016/S0308-8146(02)00505-8

Naoual, J., Abdelaziz, B., Roberta, C., Elisabetta, D., Mohammed, B. 2010. Characterization of *Enterococci* isolated from Moroccan Dairy Products. *African Journal of Microbiology Research*, vol. 4, no. 16, p. 1708-1774.

Obi, C. N., Olugbue, V. U, Mpamugo, C. P. 2016. Yoghurt Production from Powdered Milk using Mixed Lactic Acid Bacteria Starter Cultures. *Saudi Journal of Pathology and Microbiology*, vol. 1, no. 2, p. 42-49.

Okiki, P. A., Adeniji, C. A., Oyetunji, O. A., Yusuf, O. A., Peters, O. A. 2018. Assessment of the physicochemical and bacteriological qualities of nono- a fermented cow milk. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 26-32.https://doi.org/10.5219/839

Oksanen, P. J., Salminen, S., Saxelin, M., Hamalainen, P., Ihantola-Vormisto, A., Muurasniemi- Isoviita, L. N. S., Oksanen, T. Porsti, I., Salminen, E. 1990. Prevention of travelers diarrhea by *Lactobacillus* GG. *Annals of Medicine*, vol. 22, p. 53-56. https://doi.org/10.3109/07853899009147242 PMid:2184847

Ouwehand, A. C., Vesterlund, S. 2004. Antimicrobial components from lactic acid bacteria. In Salminen, S., Wright, A. *Lactic Acid Bacteria Microbiological and Fuctional Aspects*, 3rd ed., Boca Raton, USA, CRC Press, 656 p. http://dx.doi.org/10.1201/9780824752033.ch11

Rasic, J. L., Kurmann, J. A. 1978. *Yogurt*. Copenhagen, Denmark : Technical Dairy Publishing House. p. 55-68.

Romanova, K., Urminska, D. 2017. Potential of *Lactobacillus plantarum* CCM 3027 qnd *Lactobacillus brevis* CCM 1815 for fermentation of cereal substrates.

Potravinarstvo Slovak Journal of Food Sciences, vol. 11, no. 1, p. 544-549. https://doi.org/10.5219/696

Shao-Chi, W., Fu-Jin, W., Chorng-Liang, P. 2007. Growth and survival of lactic acid bacteria during the fermentation and storage of sea weed oligosaccharides solution. *Journal of Marine Science and Technology*, vol. 15, no. 2, p. 104-114.

Shu, Q., Zhou, J. S., Rutherfurd, K. J., Britles, M. J., Prasad, J., Gopal, P. K., Gill, H. S. 1999. Probiotic lactic acid bacteria (*Lactobacillus acidophilus* HNO17, *Lactobacillus rhamnosus* HN001 and *Bifidobacterium lactis* HNO19) have no adverse effects on the health of mice, *International Dairy Journal*. vol. 9, no. 11, p. 831-836 https://doi.org/10.1016/S0958-6946(99)00154-5

Siitonen, S., Vapaatalo, H., Salmknen, S., Gordin, A., Saxelin, M., Wikberg, R., Kirkkola, A. L. 1990. Effects of *Lactobacillus* GG yoghurt in prevention of antibiotic associated diarrhea. *Annals of Medicine*, vol. 22, p. 57-59. https://doi.org/10.3109/07853899009147243 PMid:2184848

Swain, M. R., Anandharaj, M., Ray, R. C., Rani, R. P. 2014. Fermented fruits and vegetables of Asia: a potential source of probiotics. *Biotechnology Research International*, vol. 25, no. 4, p. 24. https://doi.org/10.1155/2014/250424 PMid:25343046

Tamime, A. Y., Deeth, H. C. 1980. Yoghurt: Technology and Biochemistry. *Journal of Food Protection*, vol. 43, no. 12, p. 939-977. https://doi.org/10.4315/0362-028X-43.12.939

The Dairy Council, 2013. The Nutritional Composition of Dairy Products. London, UK : The Dairy Council.

Van Herck, H., Baumans, V., Van Der, C. N. R. 1992. Histological changes in the orbital region after orbital puncture. *Laboratory Animal*, vol. 26, p. 53-58.

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RELATIONSHIP BETWEEN VISCOSITY AND SUGAR CONTENT OF MUST DURING RIPENING PERIOD OF GRAPES

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ABSTRACT
The relationship between dynamic viscosity and sugar content of the must is important indicator during the ripening of the vine grapes. For the experiment were selected and used only grape vine varieties. The grape vine varieties are divided into blue and white. The varieties of Blaufränkisch, Blauer Portugieser, and Cabernet Moravia were used in the blue varieties. Representatives of the white varieties were used Pinot Blanc, Pinot Gris, and Sauvignon. Country of origin was the Czech Republic, wine region Moravia (sub-region Slovácko). The grapes were collected and analyzed four times week after week during their ripening period. After grapes harvesting the individual berries were cut out of grape using the scalpel. These berries were then weighed and then the must was squeezed using a mechanical presser. Weight of berries, dynamic viscosity (in shear strain rate 100 s^{-1}), sugar content, and density of must were measured and evaluated. From the values of berries weight it can be observed the variations in weight depending especially on the weather change – the water content in the berries. The observed sugar content did not change a lot during maturity, which can be explained by a more mature phase of the grapes. The results of viscosity and sugar content (for all varieties) demonstrate the viscosity dependence on the sugar content of must – with increasing viscosity of the must the sugar content of the must increase and conversely. The knowledge of the physico-mechanical properties os wine must is very important for for technocologists, producers, but also wine consumers.

Keywords: viscosity; sugar content; density; ripening; must; vine variety.

INTRODUCTION
Wine production in the Czech Republic has long been around 60 million litres, where 63% is production of white wine, 28% is red wine, and 9% is pink wine. The average annual consumption of wine has reached 20 litres per person **(Šrédl et al., 2017)**. For food quality is necessary knowledge of the properties of the raw materials and foodstuffs **(Nedomová, 2009; Severa et al, 2010; Božiková and Hlaváč, 2013)**. The same case is with wine, each grape and table grape varieties has specific properties and dispositions that make it unique. It is therefore necessary to know the characteristics of the individual grape varieties **(Kumbár and Votava, 2015; Hlaváč et al., 2016)**.

Grapes have a huge impact on the end product. The varietal diversity, together with the processing method and the yeast used, ensures some variability among products **(Mlček et al., 2018)**. Grape must is a juice containing a large amount of natural substances – contains water, sugars, acids, tannins, aromatics, nitrogen and minerals, dyes, enzymes, fatty substances, and waxes, see Table 1 **(Poracova et al., 2016)**.

Many ingredients of grape must are very valuable for human nutrition, especially for the natural content of easily extractable phenolic substances, the grape must has antioxidant properties. Therefore, this juice in the beverage industry is used to produce refreshing beverages and syrups **(Yadav et al., 2009; Iriti and Varoni, 2016)**.

Table 1 Substances of grape berries.

Substance	Content (mg/berry)
Water	750
Sugars	240
Acids	6
Mineral substances	5
Phenol substances	2
Fragrant aromatic substances	0.1
Nitrogenous substances	2

Sugar is produced in the grapes by CO_2 assimilation – photosynthesis. From the carbohydrates are then form organic acids in the grapes. These are, for example, tartaric acid, malic acid and succinic acid **(Flores et al., 2012)**. The sweet taste in grape must is caused by the two most common

monosaccharides, D-glucose and D-fructose, with more than 90% soluble berries, see more in **Bangaraiah and Ashok Kumar (2017)**. The presence of carbohydrates directly affects the fullness, texture and extract of the future wine. Conversely, reducing carbohydrates results in bitterness, acidity, and tarseness. In mature berries, the sugar content across the varieties is above 250 $g.L^{-1}$ **(Delgado Cuzmar et al., 2018)**.

Scientific hypothesis

The main hypothesis of this work is to determine if the viscosity of the must is depend on the sugar content of the must from the grape berries. Experiment deals with the properties of must from six varieties of grapevine. The selected properties were carried out (in three weeks replicates) for the berries: sugar content, viscosity, and density of must. Observed was also berry weight. The results were subsequently evaluated, focusing on the viscosity dependence on the sugar content in must from grape berries.

MATERIAL AND METHODOLOGY

For the experiment were selected and used only grape vine varieties. The grape vine varieties are divided into blue and white. The varieties of Blaufränkisch, Blauer Portugieser, and Cabernet Moravia were used in the blue varieties. Representatives of the white varieties were used Pinot Blanc, Pinot Gris, and Sauvignon. Country of origin is the Czech Republic, wine region Moravia – sub-region Slovácko.

Grapes were collected in the four terms – September 4th, September 11th, September 18th, and September 25th in 2017. These terms correspond with mature period of these grape vine varieties **(Bautista-Ortín et al., 2006; Maoz eta l., 2018)**. After grapes harvesting the individual berries were cut out of grape using the scalpel. These berries were then weighed and then the must was squeezed using a mechanical presser. Immediately after then the must was analysed using several equipment and method.

Precision values of berries weight was carried out using digital scale GX-2000-EC (A&D, Japan) with accuracy 0.001 g. Sugar content in the must was measured using digital refractometer RDBS1-ATC (JLab, China) with automatic temperature compensation. In this meauserement the unit °Bx (degree of Brix) was used. The unit °Bx means same as g/100g – for example 25 °Bx expresses 25% sugar and 75% of water in 100g solution. The density of the must was measured using digital densitometer Densito 30 PX (Mettler Toledo, USA) with accuracy 0.001 $g.cm^{-3}$.

Viscosity measurements were carried out using the DV-2T rotary viscometer (Brookfield, USA) equipped with a coaxial cylinder sensor system with precision small samples adapter and standard spindle number 18 (according to Brookfield). The shear strain rate was set to 100 s^{-1} and the geometry of the measuring device it can be seen in **Kumbár and Dostál (2014)**.

All experiment were conducted at the room temperature 22 °C.

Statisic analysis

Statistical analysis were carried out using the software MATLAB® R2012a with Statistics toolbox (MathWorks, USA) – paired t-test and analysis of variance (ANOVA) with interaction, testing on the significance level of $p = 0.05$.

RESULTS AND DISCUSSION

The first step of processing results was to find correlation between density, sugar content, and dynamic viscosity of grape must.

Table 2 indicates whether the calculated paired correlation coefficient is statistically significant at the chosen significance level ($p < 0.05$).

Table 2 Matrix with correlation coefficients of measured properties

Properties	Density	Sugar content	Viscosity
Density	1.00	**0.98**	**0.57**
Sugar content	**0.98**	1.00	**0.56**
Viscosity	**0.57**	**0.56**	1.00

The bold values in the Table 2 represents a statistically significant correlations on the level of significance $p = 0.05$.

The result values of all analysis and measurements are shown in the Table 3.

From the values of berries weight could be observed the variations in weight depending especially on the weather changes which caused the water content in the grape berries (**McCarthy and Coombe, 199**9; **Auzmendi and Holzapfel, 2016**).

For each of six grape vine varieties was created the graph illustrated the dependence of the dynamic viscosity and the sugar content of grape must, see Figure 1 (blue varietes) and Figure 2 (white varietes).

Obtained trends were modelled using the basic mathematical model – linear function – which can be describe:

$$SC = a \cdot \eta + b \quad (1)$$

Where SC [°Bx] is sugar content, η is dynamic viscosity [mPa·s], a [°Bx·(mPa·s)$^{-1}$] and b [°Bx] are regression coefficients. In the Table 4 there are values of regression coefficients a, b and coefficients of determination R^2 of the used mathematical model.

The most varieties shows the same trend – with the gradual maturation the dynamic viscosity decreased and the sugar content was not changed significantly ($p < 0.05$). Due to non-grading sugar content, the dynamic viscosity dependence on sugar content cannot be directly assessed, but the data obtained for this experiment suggest that the dynamic viscosity should increase with increasing sugar content. These trends agree with the studies **Lopéz et al. (1989)**, **Nurgel and Pickering (2005)**, **Trávníček et al. (2016)** and **Nedomová et al. (2017)**. The other paper witch deals with the ice wines **(Cliff et al., 2002)** supplement the claim that increasing the sugar content affects the viscosity increase over density. At the other hand, there were published several studies dealing with a sucrose of fruit juice where different sugar contents have no influence on viscosity, see **Neto et al. (2005)**, **Tarzia et al. (2010)**, and **Steiner et al. (2011)**.

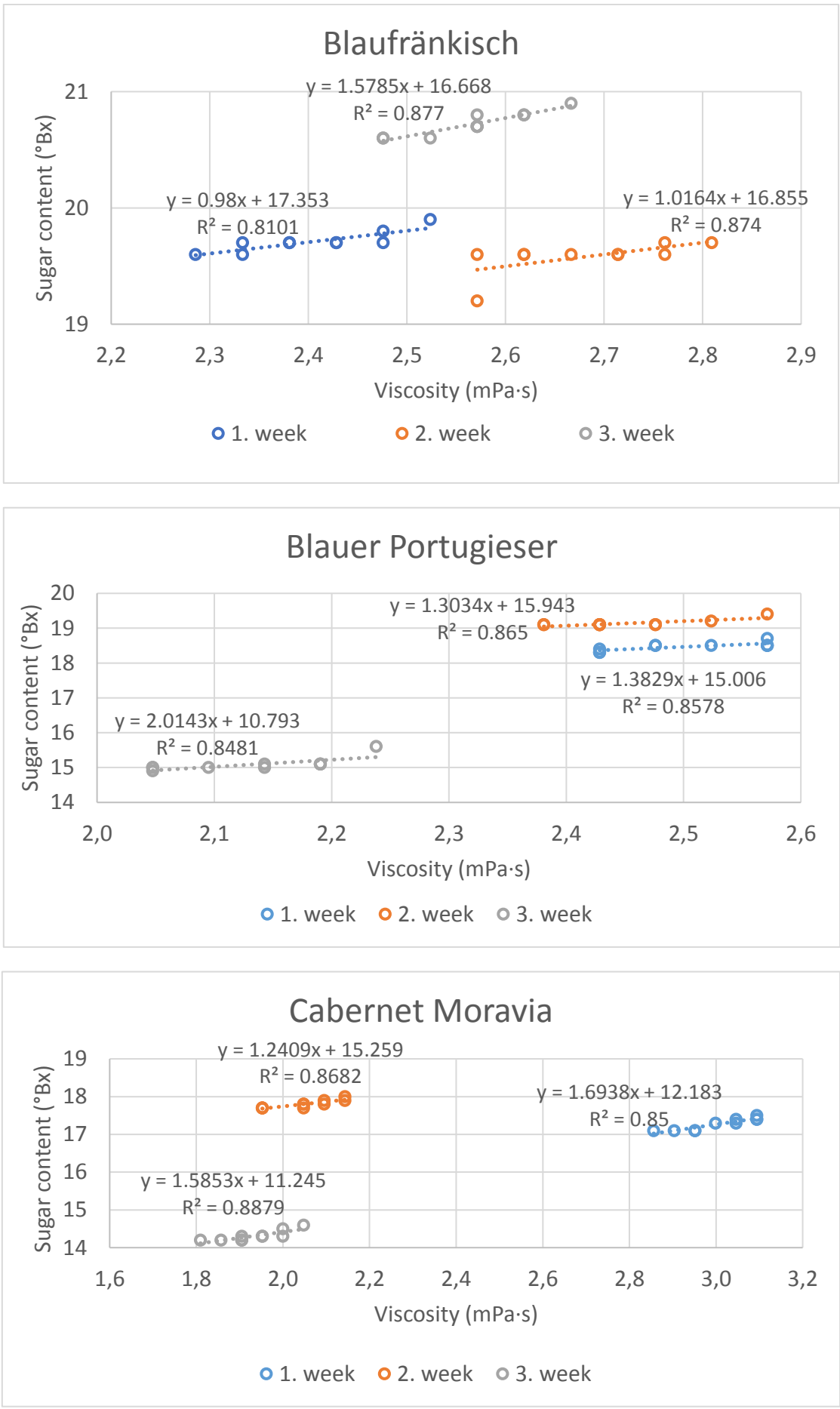

Figure 1 Dependence viscosity and sugar content of must – Blaufränkisch, Blauer Portugieser, Cabernet Moravia.

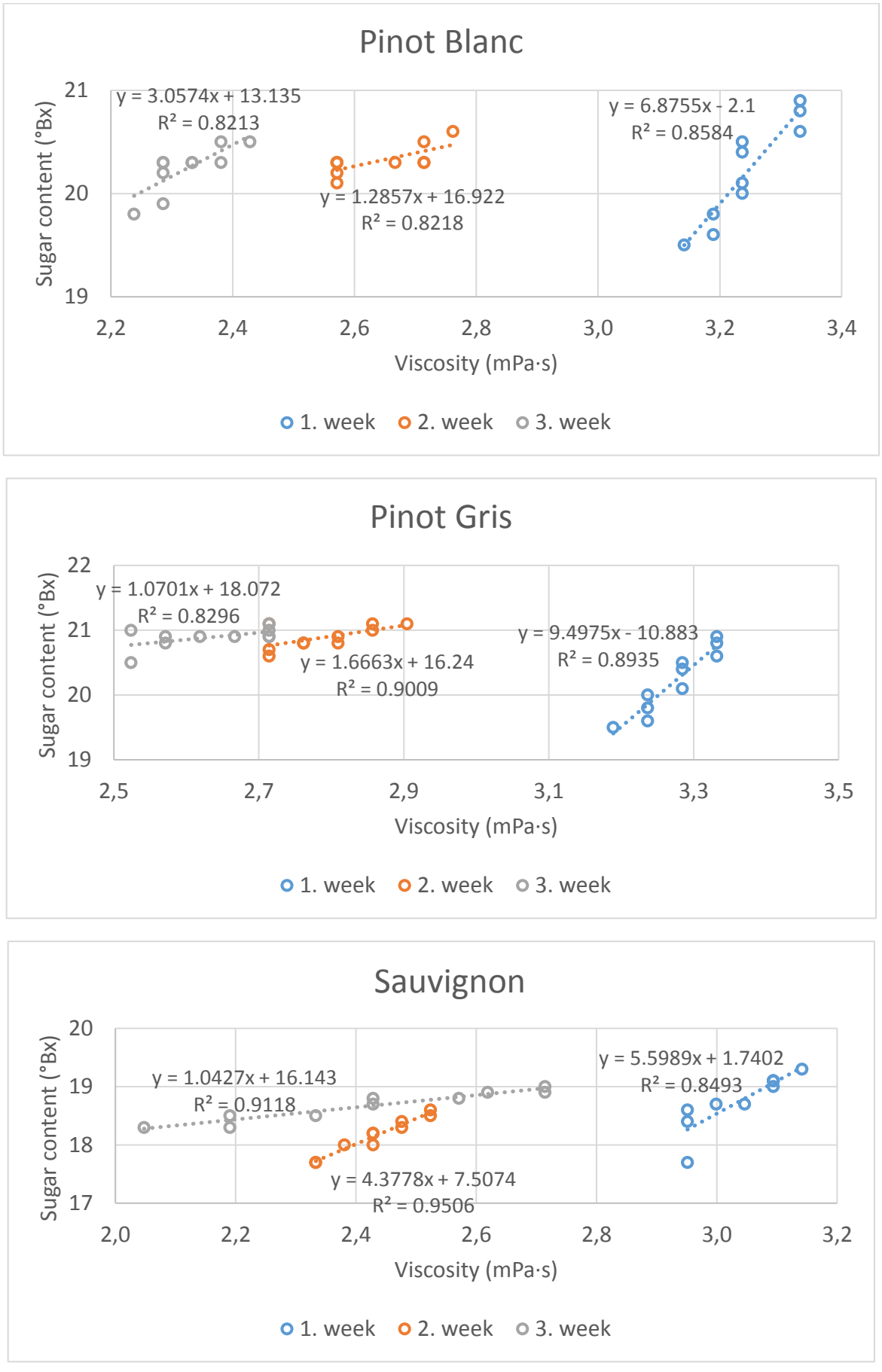

Figure 2 Dependence viscosity and sugar content of must – Pinot Blanc, Pinot Gris, Sauvignon.

Table 3 Experimental values (n = 10; results are shown as average ±standard deviation).

Date	Properties	Units	Blaufränkisch	Blauer Portugieser	Cabernet Moravia	Pinot Blanc	Pinot Gris	Sauvignon
04.09.2017	Berry weight	g	-		2.374 ±0.1584	1.567 ±01432	1.972 ±0.2298	1.877 ±0.2148
	Sugar content	°Bx	-		17.27 ±0.16	20.22 ±0.49	20.62 ±0.13	18.77 ± 0,47
	Density	kg.m^{-3}	-		1074.13 ±0.19	1089.67 ±0.41	1089.67 ±0.41	1075.99 ±0.07
	Viscosity	mPa·s	-		3.004 ±0.085	3.246 ±0.067	3.275 ±0.049	3.042 ±0.073
11.09.2017	Berry weight	g	2.82 ±0.3788	2.886 ±0.2954	1.995 ±0.3619	1.947 ±0.2708	1.775 ±0.3064	1.302 ±0.3086
	Sugar content	°Bx	19.71 ±0.09	18.47 ±0.12	17.80 ±0.11	20.32 ±0.14	20.89 ±0.18	18.16 ±0.31
	Density	kg.m^{-3}	1082.50 ±0.05	1077.10 ±0.07	1075.08 ±0.08	1086.83 ±0.05	1088.77 ±0.05	1076.74 ±0.07
	Viscosity	mPa·s	2.405 ±0.075	2.505 ±0.064	2.048 ±0.074	2.643 ±0.079	2.790 ±0.068	2.433 ±0.069
18.09.2017	Berry weight	g	2.766 ±0.3252	2.764 ±0.5826	2.310 ±0.3086	2.216 ±0.2321	2.046 ±0.2287	1.828 ±0.2372
	Sugar content	°Bx	19.58 ±0.14	19.14 ±0.10	14.31 ±0.14	20.24 ±0.23	20.89 ±0.16	18.67 ±0.25
	Density	kg.m^{-3}	1084.76 ±0.17	1081.17 ±0.53	1059.63 ±0.21	1087.67 ±0.66	1089.39 ±0.14	1080.51 ±0.43
	Viscosity	mPa·s	2.681 ±0.084	2.452 ±0.060	1.933 ±0.072	2.324 ±0.059	2.633 ±0.081	2.424 ±0.233
25.09.2017	Berry weight	g	2.426 ±0.3211	2.300 ±0.3498		-		
	Sugar content	°Bx	20.72 ±0.10	15.09 ±0.19		-		
	Density	kg.m^{-3}	1090.76 ±0.26	1065.54 ±0.61		-		
	Viscosity	mPa·s	2.567 ±0.061	2.133 ±0.070		-		

Table 4 Regression coefficients and coefficient of determination.

Variety	Week	a (°Bx·(mPa·s)$^{-1}$)	b (°Bx)	R^2
Blaufränkisch	1.	0.9800	17.353	0.8101
	2.	1.0164	16.855	0.8740
	3.	1.5785	16.668	0.8770
Blauer Portugieser	1.	1.3829	15.006	0.8578
	2.	1.3034	15.943	0.8650
	3.	2.0143	10.793	0.8481
Cabernet Moravia	1.	1.6938	12.183	0.8500
	2.	1.2409	15.259	0.8682
	3.	1.5853	11.245	0.8879
Pinot Blanc	1.	6.8755	-2.100	0.8584
	2.	1.2857	16.922	0.8218
	3.	3.0574	13.155	0.8213
Pinot Gris	1.	9.4975	-10.883	0.8935
	2.	1.6663	16.240	0.9009
	3.	1.0701	18.072	0.8296
Sauvignon	1.	5.5989	1.7402	0.8493
	2.	4.3778	7.5074	0.9506
	3.	1.0427	16.143	0.9118

CONCLUSION

At the present time it is necessary to know up-to-date information from scientific research in the food industry, because the characteristics and understanding of the properties of the foodstuffs is the key to product innovation and optimization of industrial foodstuff processing. Of course, this information is also helpful in the field of winemaking for the development of new equipment and equipment, in particular the chemical and thermos-physical properties of the wine.

From the values of berries weight could be observed the variations in weight depending especially on the weather changes – the water content in the berries.

The observed varieties were shown the same trend – with the gradual maturation the viscosity decreased and the sugar content was not changed significantly (p <0.05).

The sugar content was not changed a lot during ripening period, which can be explained by the higher degree of ripeness of the grapes.

At the finally, the relationship between the viscosity and sugar content demonstrate the viscosity dependence on the sugar content of must – with increasing viscosity of the must the sugar content of the must increase and conversely (for all varieties).

REFERENCES

Auzmendi, I., Holzapfel, B. P. 2016. Leaf area to fruit weight ratios for maximising grape berry weight, sugar concentration and anthocyanin content during ripening. *Acta Horticulturae*, vol. 1115, p. 127-132. https://doi.org/10.17660/ActaHortic.2016.1115.19

Bangaraiah, P., Ashok Kumar, P. 2017. Production of white wine from grapes using sacharomyces cervisiae. *Research Journal of Pharmacy and Technology*, vol. 10, no. 2, p. 391-395. https://doi.org/10.5958/0974-360X.2017.00079.8

Bautista-Ortín, A. B., Fernández-Fernández, J. I., López-Roca, J. M., Gómez-Plaza, E. 2006. The effect of grape ripening stage on red wine color. *Journal International Des Sciences De La Vigne Et Du Vin*, vol. 40, no. 1, p. 15-24. https://doi.org/10.20870/oeno-one.2006.40.1.879

Božiková, M., Hlaváč, P. 2013. Temperature and storing time influence on selected physical properties of milk and acidophilus milk. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 61, no. 6, p. 1589-1595. https://doi.org/10.11118/actaun201361061589

Cliff, M., Yuksel, D., Girard, B., King, M. 2002. Characterization of Canadian ice wines by sensory and compositional analyses. *American Journal of Enology and Viticulture*, vol. 53, no. 1, p. 46-53.

Delgado Cuzmar, P., Salgado, E., Ribalta-Pizarro, C., Olaeta, J. A., López, E., Pastenes, C., Cáceres-Mella, A. 2018. Phenolic composition and sensory characteristics of cabernet sauvignon wines: Effect of water stress and harvest date. *International Journal of Food Science and Technology*, vol. 53, no. 7, p. 1726-1735. https://doi.org/10.1111/ijfs.13757

Flores, P., Hellín, P., Fenoll, J. 2012. Determination of organic acids in fruits and vegetables by liquid chromatography with tandem-mass spectrometry. *Food Chemistry*, vol. 132, no. 2, p. 1049-1054. https://doi.org/10.1016/j.foodchem.2011.10.064

Hlaváč, P., Božiková, M., Hlaváčová, Z., Kardjilova, K. 2016. Changes in selected wine physical properties during the short-time storage. *Research in Agricultural Engineering*, vol. 62, no. 3, p. 147-153. https://doi.org/10.17221/7/2015-RAE

Iriti, M., Varoni, E. M. 2016. Grape bioactives for human health. In Watson, R. R., Preedy, V. R. *Fruits, vegetables, and herbs: Bioactive foods in health promotion*. 1st ed. Cambridge, USA : Academic press, pp. 221-238. ISBN 978-0-12-802972-5. https://doi.org/10.1016/B978-0-12-802972-5.00011-1

Kumbár, V., Dostál, P. 2014. Temperature dependence density and kinematic viscosity of petrol, bioethanol and their blends. *Pakistan Journal of Agricultural Sciences*, vol. 51, no. 1, p. 175-179.

Kumbár, V., Votava, J. 2015. Influence of storage temperature on the viscous behaviour of partially fermented wine must (*Pinot Gris*). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 63, no. 3, p. 781-785. https://doi.org/10.11118/actaun201563030781

López, A., Ibarz, A., Pagán, J., Vilavella, M. 1989. Rheology of wine musts during fermentation. *Journal of Food Engineering*, vol. 10, no. 2, p. 155-161. https://doi.org/10.1016/0260-8774(89)90033-2

Maoz, I., Kaplunov, T., Beno-Mualem, D., Lewinsohn, E., Lichter, A. 2018. Variability in volatile composition of crimson seedless (*Vitis vinifera*) in association with maturity at harvest. *American Journal of Enology and Viticulture*, vol. 69, no. 2, p. 125-132. https://doi.org/10.5344/ajev.2017.17069

McCarthy, M. G., Coombe, B. G. 1999. Is weight loss in ripening grape berries cv. Shiraz caused by impeded phloem transport? *Australian Journal of Grape and Wine Research*, vol. 5, no. 1, p. 17-21. https://doi.org/10.1111/j.1755-0238.1999.tb00146.x

Mlček, J., Trágeová, S., Adámková, A., Adámek, M., Bednářová, M., Škrovánková, S., Sedláčková, E. 2018. Comparison of the content of selected mineral substances in czech liturgical and common wines. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 150-156. https://doi.org/10.5219/901

Nedomová, Š. 2009. Response of the potato tubers to impact loading. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 57, no. 4, p. 63-72. https://doi.org/10.11118/actaun200957040063

Nedomová, Š., Kumbár, V., Pavloušek, P., Pytel, R., Lampíř, L., Buchar, J. 2017. Effect of harvest date on composition and geometry of grape berries. *European Journal of Horticultural Science*, vol. 82, no. 1, p. 21-30. https://doi.org/10.17660/eJHS.2017/82.1.3

Neto, F. S. P. P., de Castilhos, M. B. M., Telis, V. R. N., Telis-Romero, J. 2005. Effect of ethanol, dry extract and reducing sugars on density and viscosity of Brazilian red wines. *Journal of the Science of Food and Agriculture*, vol. 95, no. 7, p. 1421-1427. https://doi.org/10.1002/jsfa.6835

Nurgel, C., Pickering, G. 2005. Contribution of glycerol, ethanol and sugar to the perception of viscosity and density elicited by model white wines. *Journal of Texture Studies*, vol. 36, no. 3, p. 303-323. https://doi.org/10.1111/j.1745-4603.2005.00018.x

Poracova, J., Sedlak, V., Gogalova, Z., Ondekova, J., Blascakova, M. M., Fejer, J., Grulova, D. 2016. The antioxidant activity of vitis vinifera L. and vaccinium myrtillus L. extracts. *Acta Horticulturae*, vol., 1125, p. 283-285. https://doi.org/10.17660/ActaHortic.2016.1125.35

Severa, L., Buchar, J., Nedomová, Š., Šustová, K. 2010. Rheological profile of rawwhey. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 58, no. 1, p. 167-174. https://doi.org/10.11118/actaun201058010167

Steiner, E., Arendt, E. K., Gastl, M., Becker, T. 2011. Influence of the malting parameters on the haze formation of beer after filtration. *European Food Research and Technology*, vol. 233, no. 4, p. 587-597. https://doi.org/10.1007/s00217-011-1547-0

Šrédl, K., Kučírková, L., Svoboda, R. 2017. The competition on wine markets in the Czech Republic. *Journal of Wine Research*, vol. 28, no. 4, p. 278-293. https://doi.org/10.1080/09571264.2017.1383887

Tarzia, A., dos Santos Scholz, M. B., de Oliveira Petkowicz, C. L. 2010. Influence of the postharvest processing method on polysaccharides and coffee beverages. *International Journal of Food Science and Technology*, vol. 45, no. 10, p. 2167-2175. https://doi.org/10.1111/j.1365-2621.2010.02388.x

Trávníček, P., Burg, P., Krakowiak-Bal, A., Junga, P., Vítěz, T., Ziemiańczyk, U. 2016. Study of rheological behaviour of wines. *International Agrophysics*, vol. 30, no. 4, p. 509-518. https://doi.org/10.1515/intag-2016-0018

Yadav, M., Jain, S., Bhardwaj, A., Nagpal, R., Puniya, M., Tomar, R., Yadav, H. 2009. Biological and medicinal properties of grapes and their bioactive constituents: An

update. *Journal of Medicinal Food*, vol. 12, no. 3, p. 473-484. https://doi.org/10.1089/jmf.2008.0096

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FUNCTIONAL AND THERMAL PROPERTIES OF FLOUR OBTAINED FROM SUBMERGED FERMENTATION OF DURIAN (*DURIO ZIBETHINUS* MURR.) SEED CHIPS USING *LACTOBACILLUS PLANTARUM*

Andri Cahyo Kumoro, Jefri Pandu Hidayat

ABSTRACT

Durian (*Durio zibethinus* Murr.) is one of the most popular seasonal fruits in South East Asia, mainly Thailand, Indonesia, Malaysia and the Philippines. After consuming of the fruit pulp, the abundant durian seeds are usually disposed. However, they can be eaten after being boiled, roasted, baked, fried or cooked. Unfortunately, there is no sufficient information on the innovative utilizations of the durian seeds as raw materials in the preparation of modern foods. This study aims to characterize the functional and thermal properties of native and fermented durian seed flour obtained from submerged fermentation of durian seed chips using *Lactobacillus plantarum*, so that this information can be used as the basis in the development of functional foods. The effect of solid consistency (5 – 25% w/v), inoculum size (2.5 – 15% w/v) and time (0 – 40 h) on the swelling power, water solubility, water absorption capacity, oil absorption capacity, carbonyl and carboxyl content, degree of substitution and gelatinization temperature of the flour were investigated. Fermentation was found to alter all of the functional and thermal properties of durian seed flour. Although fermentation increased the gelatinization temperature and oil absorption capacity of durian seed flour, their value were still lower than that of wheat flour. Based on the carboxyl group content and degree of substitution, the fermented durian seed flour obtained from fermentation of durian seed chips at 15% w/v solid consistency and 5% v/v inoculums size for 24 h is safe for consumption. Fermented durian seed flour exhibits similar functional properties to that of wheat flour and offers its superiority and high potential applications in the food industry over wheat flour due to its high fiber content (8.50 ±0.26%), but low fat content (0.63 ±0.03%). However, rigorous research should be conducted to ensure the acceptability and practical implementation of fermented durian seed flour as raw material for the manufacture of bread, cookies, cake and noodle.

Keywords: *Lactobacillus plantarum*; durian seed flour; functional property; thermal property; fermentation

INTRODUCTION

Wheat flour is the main raw material for the manufacture of noodle and baked products, such as breads, cakes, biscuits, and cookies. This is because wheat flour contains gluten, a specific protein that gives a unique nature and functional properties of wheat flour **(Tharise et al., 2014)**. Unfortunately, the climate differences with its origin hardly hinder the mass cultivation of wheat in the tropical countries, such as Indonesia and Malaysia **(Amin et al., 2007)**. In fact, Indonesia had to import 11.48 million ton of wheat flour or equivalent to US $ 2.65 billion in 2017 to supply the domestic demand, including the need in diversification of food products **(Bizteka, 2018)**. Because some individuals with celiac disease may often experience undesirable reactions to gluten, many attempts have been devoted to develop gluten free food products. **Larrosa et al. (2013)** proposed the reduction of gluten from food products through substitution of wheat flour with other food materials having similar physicochemical properties to gluten.

Durian (*Durio zibethinus* Murr.) is one of the most favorite seasonal fruits in South East Asia, especially Thailand, Indonesia, Malaysia and the Philippines **(Azima et al., 2017)**. **Statistics Indonesia (2014)** reported that Indonesian durian production in 2013 was approximately 1,818,949 tons and was estimated to increase every year. Durian fruit pulp has a unique flavor and strong aroma, which can be consumed fresh or processed into ice cream, pancake and different kind of traditional food products. The edible part of durian fruit is only about 30 – 35% of its total weight, whereas the seeds (20 – 25%) and the shell are usually discarded. It is estimated that the durian seed as by-product of durian fruit production can reach 454,737 tons per year, but its potential has not been extensively utilized. Ripe

durian seeds may comprise up to 51.1% moisture, 43.6% carbohydrates, dietary fiber, polysaccharide gum (20 – 25% w/w), and protein (3 – 5% w/w) **(Brown, 1997)**. Durian seeds can be consumed after being boiled, roasted, fried, baked or cooked **(Berg, 1979)**. Durian seed also contains heteropolysaccharide-protein polymer composed of galactose and glucose as major monosaccharide, which is believed to have similar roles to gluten in the food preparation **(Amid and Mirhosseini, 2011)**. **Amin and Arshad (2009)** reported that the whole and dehulled durian seed flour contains approximately 73.90% and 76.8% of carbohydrate. They also found that based on its pasting properties, durian seed flour may withstand tough cooking condition. In addition, it has also been used as a source of dietary fiber, as dough and as a thickening agent in the food manufacturing **(Amid and Mirhosseini, 2011)**. Hence, durian seed flour exhibits a promising potential to substitute the use of wheat flour in various food applications.

Lactobacillus is the most common strain in a class of lactic acid bacteria (LAB), which perform an essential role in the preservation and production of healthy foods. Lactic acid fermentation using a variety of strains of LAB is the oldest conventional method for preparation of fermented vegetables, meat products, dairy products and cereal foods **(Romanová and Urminská, 2017)**. During the fermentation processes, LAB generates particular metabolites such as enzymes, acids, alcohols, antibiotics, carbohydrates, and inhibitory compounds that are responsible to the safety, sensory and nutritional quality of fermented foods. Since chemical modifications of cereal, tuber and seed flours are being avoided due to the presence of undesirable residues in the products, lactic acid fermentation is likely to be the better option to modify the functional properties of durian seed flour.

The aim of the current study was to investigate the effect of solid consistency (5– 25% w/v), inoculum size (2.5 – 15% v/v), and fermentation time (0 – 40 h) on the swelling power, water solubility, carbonyl and carboxyl group content, water absorption capacity, oil absorption capacity, degree of substitution and gelatinization temperature during fermentation of durian seed chips using *Lactobacillus plantarum*.

Scientific hypothesis

During fermentation *Lactobacillus plantarum* may produce specific metabolites that contribute to the safety, sensory, nutritional and functional properties of fermented foods. Fermentation of durian seed chips using *Lactobacillus plantarum* is expected to alter the proximate composition, functional and thermal properties of the flour obtained. This information will be beneficial in broadening the potential applications of durian seed flour within the food industries, especially cake, biscuits, cookies and noodles.

MATERIAL AND METHODOLOGY

Plant material, microorganism and chemicals

The durian (*Durio zibethinus* Murr.) seeds used in this study were obtained from durian sellers in Gunungpati, Semarang, Indonesia. They were cleaned from the remaining fruit pulp and washed carefully with flowing water. To prevent sprouting, the seeds were dried in a convective drying oven at ambient temperature (30 °C). The air dried seeds were collected and packed in plastic bags and kept in a dry and cool place at 5 °C for 24 h as recommended earlier **(Larrosa et al., 2013)**. *Lactobacillus plantarum* sp CCRC 12251 was obtained Food and Nutrition Inter-University Center, Universitas Gadjah Mada, Yogyakarta-Indonesia and maintained in Mann Rogassa Sharpe (MRS) agar slant at 4 °C. All of the chemicals and reagents used for analyses were the products of Sigma-Aldrich with analytical grade (purity ≥ 98% w/w) and were purchased from an authorized chemicals distributor in Semarang.

Inoculum preparation

The inoculum was made in a 250 mL Erlenmeyer flask containing 100 mL of modified MRS liquid medium (peptone, 10; beef extract 10; yeast extract 5; glucose 20; Na_2HPO_4; sodium acetate 5; triammonium citrate 2; $MgSO_4$ 0.2; $MnSO_4$ 0.2; and $CaCO_3$ 4 $g.L^{-1}$, Tween 80 0.1 mL, and pH 6.8) by transferring a loop full of microorganisms (*Lactobacillus plantarum*) from a stock culture and incubated at 35 °C and 120 rpm for 48 h in an orbital incubator-cum-shaker. The number of viable bacteria was quantified by the total plate count (TPC) method as suggested by previous researchers **(Rizzello et al., 2010)**. The inoculums were found to contain 3×10^7 $CFU.mL^{-1}$.

Durian seed chips fermentation

Durian seeds were sliced to obtain chips with ±5 mm thickness. They were then introduced into 200 mL distilled water in 500 mL Erlenmeyer flasks to obtain various solid consistencies (5 – 25% w/v). The durian seed chips were inoculated with various sizes (2.5 – 15% v/v) of freshly prepared inoculums and covered with aluminum foil. To avoid starch gelatinization, no thermal sterilization was performed. The fermentation flaks were mounted on a horizontal shaker waterbath to control the temperature (35 °C) and to provide sufficient mixing. Fermented durian chip samples were withdrawn from the fermentation system at 8, 16, 24, 32 and 40 h. After being thoroughly washed with flowing water, the fermented durian seed chips were overlaid as a single layer on drying pans and were dehydrated in an electric oven at 40 ºC for 3 days. Then, the dried chips were subjected to size reduction using a locally fabricated crusher before milling them in a ball mill to obtain fine flour. The flour was then sieved through -180 μm +250 μm screens from which only flour parctiles retained on the 250 μm were used in this study. The flour was stockpiled in zip-lock polyethylene plastic bags and deposited in covered plastic containers at 20 °C for further uses and analyses as recommended earlier by **Retnowati et al. (2018)**.

Raw material and product analysis

Chemical properties

The proximate composition of all flour samples was analyzed following the official method of analysis **(Latimer, 2016)**. The carboxyl content of the flour was

Table 1 Effect of fermentation time on functional and thermal properties of fermented durian seed flours.

Functional properties	Fermentation time (h)						Korean wheat flour (KWF)
	0 (native)	8	16	24	32	40	
WS (%)	7.42 ±0.18	8.60 ±1.08	8.35 ±1.56	7.84 ±0.33	6.79 ±1.05	5.97 ±1.61	7.86 ±0.42
SP ($g.g^{-1}$)	8.03 ±0.13	11.33 ±0.20	10.80 ±0.08	8.50 ±0.26	7.11 ±0.40	6.26 ±0.20	8.50 ±0.50
WAC ($g.g^{-1}$)	1.51 ±0.20	2.13 ±0.02	2.04 ±0.04	1.62 ±0.08	1.52 ±0.21	1.42 ±0.26	1.50 ±0.05
OAC ($g.g^{-1}$)	0.24 ±0.03	0.31 ±0.08	0.29 ±0.05	0.29 ±0.06	0.27 ±0.01	0.25 ±0.07	0.81 ±0.04
Carbonyl (%)	0.27 ±0.06	0.35 ±0.08	0.40 ±0.04	0.48 ±0.05	0.33 ±0.05	0.30 ±0.03	n.a.
Carboxyl (%)	0.12 ±0.08	0.28 ±0.01	0.38±0.04	1.03 ±0.11	0.62 ±0.18	0.56 ±0.10	n.a.
DS	n.a.	0.03 ±0.02	0.04 ±0.00	0.08 ±0.02	0.05 ±0.01	0.02 ±0.01	n.a.
T_o (°C)	51.69 ±0.85	n.a.	n.a.	56.59 ±1.21	n.a.	n.a.	57.80 ±1.50
T_p (°C)	56.91 ±1.20	n.a.	n.a.	59.35 ±1.13	n.a.	n.a.	63.90 ±1.10
T_c (°C)	63.37 ±1.30	n.a.	n.a.	66.24 ±1.05	n.a.	n.a.	70.30 ±1.50

Note: n.a.: data not available. KWF: korean wheat flour **(Chung et al., 2010)**.

determined as previously described **(Chattopadhyay et al., 1997)**, while the carbonyl content was measured according to the titrimetric hydroxylamine method **(Smith, 1967)**. The degree of substitution (DS) of the fermented durian seed flour was determined titrimetrically, following the method of **Sodhi and Singh (2005).**

Swelling power and water solubility

The swelling power (SP) and water solubility (WS) of durian seed flour were determined by the method of **Afoakwa et al. (2012)**. Swelling power is measured as the weight (g) of the swollen sediment per g of dry flour, whereas water solubility is reported as the percentage (by weight) of the flour sample that is dissolved molecularly upon heating in water at 60 °C.

Water/oil absorption capacity

Water and oil absorption capacities (WAC/OAC) for each durian seed flour sample were measured by the method of **Abbey and Ibeh (1988)**. The sample (10% w/v) was carefully weighed into a clean conical flask and was mixed rigorously with distilled water/oil using a warring mixer for 30 s. The sample was then let to stand for 30 min at ambient temperature, after which it was centrifuged at 5000 rpm for 30 min. The free water or oil (supernatant) was read directly from the graduated centrifuge cuvette. The absorbed water/oilwas converted to weight (in grams) by multiplying by the respective density (water, 1 g. mL^{-1} and soybean oil, 0.924 $g.mL^{-1}$).The water and oil absorption capacities were reported in grams of water/oil absorbed per gram of durian seed flour sample.

Thermal properties

The thermal analysis of durian seed flour was carried out by differential scanning calorimetry (DSC) using a Seiko differential scanning calorimeter (DSC 210) (Seiko Instruments Inc., Chiba, Japan) supported with a thermal analysis data station and data recording software. Before being subjected to DSC analysis, the durian seed flour of 20% water content was prepared by addition of 11 μL deionized water using a microsyringe to 3 mg flour sample (dry basis) in the DSC pans, which were then sealed, reweighed and let to stand overnight at ambient temperature before analysis to achieve sample and water equilibrium. The analysis used scanning temperatures range of 25 – 300 °C and heating rate of 10 $°C.min^{-1}$ as previously described by **Retnowati et al. (2018)**. The measurements were performed under a dynamic nitrogen atmosphere (30 $mL.min^{-1}$) in punctured aluminum pans to avoid condensation. In all measurements, the thermogram was recorded with an empty aluminum pan as a reference.

Statistical analyses

All measurements were performed in tripilicates and the data obtained were reported as mean ±Standard deviation. Significant differences between the mean values at significance level p <0.05 were compared using Student`s test facility available in MS Excel version 2010.

RESULTS AND DISCUSSION

Effect of fermentation time

This investigation was performed by fermentation of durian seed chips using solid consistency of 15% w/v and inoculums size of 5% v/v from 0 – 40 h at ambient temperature. The results are presented in Table 1. The swelling power, water solubility and oil absorption capacity of native durian seed flour were lower than those of Korean wheat flour. Surprisingly, the water absorption capacity of native durian seed flour was comparable to that of Korean wheat flour **(Chung et al., 2010)**. During fermentation, the swelling power, water solubility, water absorption capacity, oil absorption capacity and carbonyl content of durian seed flour increased with time to a maximum value and leveled off. However, the time to reach the maximum value of those parameters was different one to the others. In addition, the oil absorption capacity of fermented durian seed flour remained far below the oil absorption capacity of Korean wheat flour.

Table 1 shows that the swelling power, water solubility and water absorption capacity of the fermented durian seed flour were close to those of Korean wheat flour when fermentation was carried out for 24 h. According to the European Union Scientific Committee for Food (EU SCF), the safe level of carboxyl group in food material is a maximum of 1.1% **(Commission of the European**

Table 2 Effect of solid consistency on functional and thermal properties of fermented durian seed flours.

Functional properties	Solid consistency (% w/v)						Korean wheat flour (KWF)
	0 (native)	5	10	15	20	25	
WS (%)	7.42 ±0.18	6.95 ±0.04	8.35 ±1.56	7.84 ±0.33	7.68 ±0.36	8.00 ±0.13	7.86 ±0.42
SP ($g.g^{-1}$)	8.03 ±0.13	8.05 ±0.13	10.80 ±0.08	8.50 ±0.26	7.74 ±0.08	7.50 ±0.37	8.50 ±0.50
WAC ($g.g^{-1}$)	1.51 ±0.20	1.52 ±0.02	2.04 ±0.04	1.62 ±0.08	1.64 ±0.03	1.65 ±0.03	1.50 ±0.05
OAC ($g.g^{-1}$)	0.24 ±0.03	0.25 ±0.06	0.28 ±0.05	0.29 ±0.06	0.29 ±0.02	0.29 ±0.02	0.81 ±0.04
Carbonyl (%)	0.27 ±0.06	0.20 ±0.02	0.25 ±0.04	0.48 ±0.05	0.31 ±0.05	0.23 ±0.02	n.a.
Carboxyl (%)	0.12 ±0.08	0.63 ±0.04	0.78±0.04	1.03 ±0.11	0.83 ±0.08	0.81 ±0.11	n.a.
DS	n.a.	0.09 ±0.02	0.04 ±0.00	0.08 ±0.02	0.04 ±0.00	0.04 ±0.01	n.a.
T_o (°C)	51.69 ±0.85	n.a.	n.a.	56.59 ±1.21	n.a.	n.a.	57.80 ±1.50
T_p (°C)	56.91 ±1.20	n.a.	n.a.	59.35 ±1.13	n.a.	n.a.	63.90 ±1.10
T_c (°C)	63.37 ±1.30	n.a.	n.a.	66.24 ±1.05	n.a.	n.a.	70.30 ±1.50

Note: n.a.: data not available. KWF: korean wheat flour **(Chung et al., 2010)**.

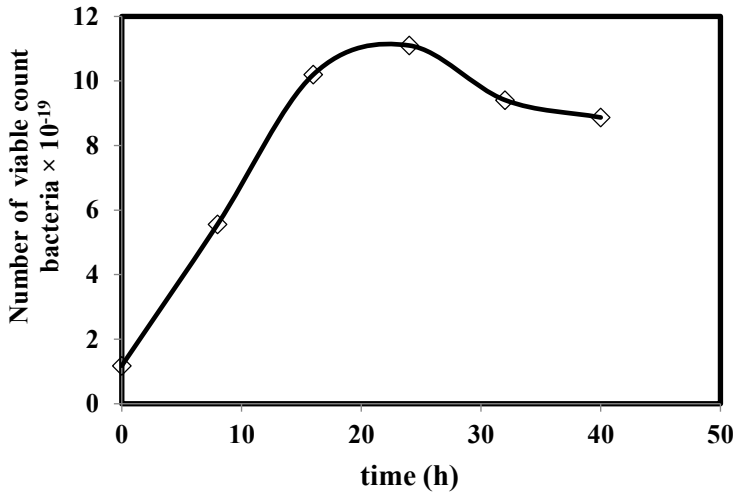

Figure 1 Profile of *Lactobacillus plantarum* growth in durian seed flour media.

Communities, 1976). In addition, the allowable degree of substitution of a specific functional group in the modified flour for food application is between 0.01 – 0.2 **(de Graaf et al., 1998)**. Therefore, 24 h is selected as the best fermen tation time for fermentation using *Lactobacillus plantarum*. This phenomenon is confirmed by the growth profile of *Lactobacillus plantarum* in durian seed chips media shown in Figure 1. As seen in Figure 1, the viable count of *Lactobacillus plantarum* reached a maximum value at 24 h fermentation. Similar observation was reported by **Passos et al. (1994)** on the fermentation of cucumber juice using *Lactobacillus plantarum*. **Gupta et al. (2010)** also observed a maximum value of viable count of *Lactobacillus plantarum* at the end of 16 – 24 h of fermentation of edible two Irish brown seaweeds. The production level of degrading enzymes used for flour granules modification is the highest during the exponential phase of LAB growth **(Chookietwattana, 2014**), which exists in the first 10 h of fermentation (**Passos et al., 1994)**.

The thermal property of durian seed flour as reflected by the onset (T_o), peak (T_p) and conclusion gelatinization temperatures (T_c) was lower than that of korean wheat flour **(Chung et al., 2010)**. As expected, fermentation of durian seed chips for 24 h slightly increased the onset, peak and conclusion gelatiniziation temperatures of durian seed flour to a closer value to gelatinization temperature of Korean wheat flour. **Alonso-Gomez et al. (2016)** also found an increase in the gelatinization temperature of cassava starch during fermentation for production of sour starch. The increase in the onset and peak gelatinization temperatures is due to the acidic condition caused by lactic acid as a main product of fermentation, which alter the starch composition and morphology. Fermentation process may also cause dramatic changes of the macromolular structure and/or conformation of amylose and amylopectin of the starch in the flour granules, which lead to change the gelatinization temperature **(Metres et al., 1997)**.

Effect of solid consistency

This study was conducted by fermentation of durian seed chips at various solid consistencies (5 – 25% w/v) using inoculums size of 5% v/v for 24 h. The results are presented in Table 2.

Table 2 shows that the water solubility and carbonyl content of the fermented flour obtained from fermentation using 5% w/v solid consistency were lower than the native durian seed floud. Similar to the other functional properties, the water solubility and carbonyl content of the fermented

Table 3 Effect of inoculum size on functional and thermal properties of fermented durian seed flours.

Functional properties	Inoculum size (% v/v)					Korean wheat flour (KWF)
	0 (native)	2.5	5	10	15	
WS (%)	7.42 ±0.18	6.58 ±0.20	7.84 ±0.33	7.63 ±0.23	7.55 ±0.04	7.86 ±0.42
SP ($g.g^{-1}$)	8.03 ±0.13	8.20 ±0.23	8.50 ±0.26	8.40 ±0.20	8.54 ±0.07	8.50 ±0.50
WAC ($g.g^{-1}$)	1.51 ±0.20	1.56 ±0.10	1.62 ±0.08	1.66 ±0.02	1.69 ±0.17	1.50 ±0.05
OAC ($g.g^{-1}$)	0.24 ±0.03	0.26 ±0.02	0.29 ±0.06	0.29 ±0.03	0.29 ±0.02	0.81 ±0.04
Carbonyl (%)	0.27 ±0.06	0.12 ±0.07	0.48 ±0.05	0.16 ±0.13	0.11 ±0.03	n.a.
Carboxyl (%)	0.12 ±0.08	0.31 ±0.15	1.03 ±0.11	0.95 ±0.11	0.86 ±0.12	n.a.
DS	n.a.	0.05 ±0.02	0.08 ±0.02	0.10 ±0.01	0.01 ±0.01	n.a.
T_o (°C)	51.69 ±0.85	n.a.	56.59 ±1.21	n.a.	n.a.	57.80 ±1.50
T_p (°C)	56.91 ±1.20	n.a.	59.35 ±1.13	n.a.	n.a.	63.90 ±1.10
T_c (°C)	63.37 ±1.30	n.a.	66.24 ±1.05	n.a.	n.a.	70.30 ±1.50

Note: n.a.: data not available. KWF: korean wheat flour **(Chung et al., 2010)**.

flour increased gradually with solid consistency and achieved a maximum value and then leveled off. When solid consistencies were higher than 10% w/v, no clear effect of solid consistency on oil absorption capacity was observed. Unfortunately, all values of the oil absorption capacity of the fermented durian seed flour were still far lower than that of wheat flour. As expected, the water solubility, water absorption capacity and carboxyl group content of the fermented durian seed flours were higher than those of the native one. These phenomena were likely due to the effect of fermentation and acidification. A similar result was reported by **Putri et al. (2011)** during their study on the fermentation of cassava starch. However, the carboxyl group content decreased when the solid consistency was increased further, which was due to inhibitions caused by the high substrate concentration. The other reason of decreasing the utilization of starch beyond 15% w/v consistency might be due to the increase in osmotic effects or due to hydrolysis of starch to reducing sugars or the microorganisms were incapable to hydrolyze the starch present in durian seed chips at high consistency because they generally grow and being productive at higher water activity **(Ray et al., 2009)**.

Based on the functional properties value compared to those of wheat flour, food regulations, economical and technological applications, the solid consistency of 15% w/v was chosen as the best fermentation condition. The thermal property of durian seed flour obtained from this condition has been discussed in the previous section.

Effect of inoculums size

This investigation was carried out by fermentation of durian seed chips at various inoculum sizes (2.5 – 15% v/v) using solid consistency of 15% w/v for 24 h. In industrial practices, the microbe loading range for lactic acid fermentation is usually between 3 – 10% of the fermentation broth volume **(Clark and Blanch, 1991)**. A proper microbe loading would reduce the probable existence of lag phase or shorten the lag phase period. The results are reported in Table 3.

In their study on the lactic acid production from paneer whey by *Lactobacillus delbrueckii* in a submerged fermentation process, **Tripathi et al. (2015)** reported that the lactic acid production increased substantially from 2.8 to 5.6 $g.L^{-1}$, when the inoculum size was increased from 3 to 8% v/v. However, no significant effect on lactic acid production was noticed when the inoculum size was further increased beyond 8% v/v. A long lag phase is not preferred in the fermentation process because it is time-wasting and the medium is consumed to maintain a viable culture prior to the growth. Therefore, 5% v/v inoculum size performed better than 10% v/v because the lag phase of 5% v/v inoculum size was a little shorter than that of 10% v/v during fermentation of whey using *Lactobacillus bulgaricus* for lactic acid production **(Taleghani et al., 2016)**. In submerged liquid fermentation, an appropriate inoculum size is an absolutely important factor for obtaining high product yield and productivities. At low value of inoculum size, the substrate is slowly utilized by microorganisms and prolongs the incubation time. On the other hand, a large value of inoculum size will lead to competition of growth of microorganisms over the limited substrate supply.

Based on the functional properties value compared to those of wheat flour, food regulations, economical and technological applications, the inoculums size of 5% v/v was selected as the best fermentation condition. The thermal property of durian seed flour obtained from this condition has been discussed in the earlier section.

Proximate composition of durian seed flour

The proximate composition was determined for native and durian seed flour obtained from fermentation of durian seed chips at 15% w/v solid consistency, 5% v/v inoculums size for 24 h. The results are compared with proximate composition of whole durian seed flour (WDSF) and wheat flour reported in the literature by **Amin and Arshad (2009)** and **Chung et al. (2010)** presented in Table 4. In general, the proximate composition of native durian seed flour used in this study is comparable to the whole durian seed flour (WDSF) reported by **Amin and Arshad (2009).**

The moisture contents of durian seed flour obtained from drying in an electric oven at 40 ºC for 3 days were lower than 10%. Foods with moisture content higher than 14% are vulnerable to bacterial attacks and mould growth, which produce undesirable changes **(Ihekoronye and Ngoddy, 1985)**. Therefore, the moisture contents of durian seed flours obtained in this study were within the acceptable

Table 4 Proximate composition of native and fermented durian seed flours in comparison to wheat flour

Flour samples	Content (%)					
	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
NDSF	9.18 ±0.14	6.30 ±0.06	0.64 ±0.03	9.64 ±0.33	3.25 ±0.11	70.99 ±1.61
WDSF	6.50 ±0.05	6.00 ±0.13	0.40 ±0.03	10.10 ±0.10	3.10 ±0.03	73.90 ±0.02
FDSF	9.56 ±0.37	6.47 ±0.10	0.63 ±0.03	8.50 ±0.26	1.94 ±0.07	72.90 ±0.20
KWF	8.80 ±0.21	13.26 ±0.10	1.47 ±0.15	0.99 ±0.10	1.27 ±0.11	74.21 ±0.16

Note: NDS : native durian seed flour, WDSF: whole durian seed flour **(Amin and Arshad, 2009)**: FDSF: fermented durian seed flour, KWF: korean wheat flour **(Chung et al., 2010)**.

values for dried foods. The protein content of content of durian seed flour is about half of that of Korean wheat flour. This shows that the durian seed flour may not be appropriate for application in bread making; however, it could be suitable for the preparation of cookies, cakes or noodle. The lack of gluten in durian seed flour will be advantageous for people with celiac disease. The ash content of durian seed flour is more than two times of that of wheat flour, which indicates rich in mineral content. The reduction in ash content may be caused by either leaching of soluble minerals into water during fermentation or consumed by microorganisms that need nutrients and minerals for growth and development. As expected, the fat content of durian seed flour was low and comparable with the fat content in other tropical fruit seeds, such as jackfruit seed (0.94%) and cempedak (0.96%) **(Meethal et al., 2017; Aziz and Zabidi, 2011)**. The fat content of wheat flour is about 2 – 2.5 times higher compared to that of native and fermented durian seed flour. From this point of view, durian seed flour is considered as a healthier raw material for food preparation compared to wheat flour. Compared to wheat flour, the fiber content in durian seed flour is about ten times higher in durian seed flour, which indicates that durian seed hull contained a lot of fiber. This indicated that durian seed flour has a promising potential to be used as a source of dietary fiber in the food industries. In addition to its high fiber content, the texture of native and fermented durian seed flour is less gritty compared to Korean wheat flour. Carbohydrate content of native durian seed flour obtained in this study was slightly lower than that reported by **Amin and Arshad (2009)**. As expected, fermentation increased the carbohydrate content of fermented durian seed flour to a closer value to that of wheat flour **(Chung et al., 2010)** and jackfruit seed flour with brown spermoderm (75.8%) **(Tulyathan et al., 2002)**.

CONCLUSION

A study on the effect of solid consistency (5 – 25% w/v), inoculum size (2.5 – 15% w/v) and time (0 – 40 h) on the submerged fermentation of durian seed chips using *Lactobacillus plantarum* has been successfully conducted. Obviously, fermentation changed all of the functional and thermal properties of durian seed flour. Fermentation increased the gelatinization temperature and oil absorption capacity of durian seed flour, but their value was still lower than that of wheat flour. The fermented durian seed flour obtained from fermentation of durian seed chips at 15% w/v solid consistency and 5% v/v inoculums size for 24 h is safe for consumption and exhibits similar functional properties to that of wheat flour. Therefore, durian seed flour offers its advantages and high potential applications in the food industry to substitute wheat flour as raw material. However, further research needs to be conducted to ensure the acceptability and practical application of fermented durian seed flour as raw material for the manufacture of bread, cookies, cake and noodle.

REFERENCES

Abbey, B. W., Ibeh, G. O. 1988. Functional properties of raw and heat processed cowpea flour. *Journal of Food Science*, vol. 53, no. 6, p. 1774-1777. https://doi.org/10.1111/j.1365-2621.1998.tb07840.x

Afoakwa, E. O., Budu, A., Asiedu, S., Chiwona-Karltun, C., Nyirenda, D. B. 2012. Viscoelastic properties and physicfunctional characterization of six high yielding cassava mosaic disease-resistant cassava (*Manihot esculenta* Crantz) genotypes. *Journal of Nutrition and Food Science*, vol. 2, no. 2, p. 129. http://dx.doi.org/10.4172/2155-9600.1000129

Alonso-Gomez, L., Nino-Lopez, A. N., Romero-Garzon, A. M., Pineda-Gomez, P. 2016. Physicochemical transformation of cassava starch during fermentation for production of sour starch in Colombia. Starch/Stärke, vol. 68, no. 11-12, p. 1-9. https://doi.org/10.1002/star.201600059

Amid, B. T., Mirhosseini, H. 2011. Optimization of aqueous extraction of gum from Durian (*Durio zibethinus*) seed: a potential, low cost source of hydrocolloid. *Food Chemistry*, vol. 132, no. 3, p. 1258-1268. https://doi.org/10.1016/j.foodchem.2011.11.099

Amin, A. M., Ahmad, A. S., Yin, Y. Y., Yahaya, N., Ibrahim, N. 2007. Extraction, purification and characterization of durian seed gum. *Food Hydrocolloids*, vol. 21, no. 2, p. 273-279. https://doi.org/10.1016/j.foodhyd.2006.04.004

Amin, A. M., Arshad, R. 2009. Proximate composition and pasting properties of durian (*Durio zibethinus*) seed flour. *International Journal of Postharvest Technology and Innovation*, vol. 1, no. 4, p. 367-375. https://doi.org/10.1504/IJPTI.2009.030685

Latimer Jr., G. W. 2016. *Official methods of analysis of AOAC international*. 20th ed. Gaithersburg: AOAC International, p. 3172. ISBN: 0935584870.

Azima, A., Anggraini, T., Syukri, D., Septia, R. A. 2017. Effects of sodium bisulfite soaking on the quality of durian seed flour and its application to dakak-dakak production (West Sumatra's Traditional Snack). *Pakistan Journal of Nutrition*, vol. 16, no. 3, 175-178. https://doi.org/10.3923/pjn.2017.175.178

Aziz, N. A. A., Zabidi, M. A. 2011. Partial substitution of wheat flour with chempedak (*Artocarpus integer*) seed flour in bread. In Preedy, V. R. et al. *Flour and breads and their fortification in health and disease prevention*. London, UK : Academic Press, p. 365-374. ISBN: 9780123808868 https://doi.org/10.1016/B978-0-12-380886-8.10033-9

Berg, R. Y. 1979. Legume seed and myrmecochorous dispersal in *Kennedia* and *Hardenburgia* (Fabaceae), with a remark on the durian theory. *Norwegian Journal of Botany*, vol. 26, no. 4, p. 229-254.

Bizteka. 2018. *Perkembangan impor tepung terigu*. Jakarta, Indonesia : PT. Citra Cendekia Indonesia.

Brown, M. J. 1997. *Durio - A bibliographic review*. New Delhi, India : International Plant Genetic Resources Institute, 188 p. ISBN-13: 978-9290433187.

Chattopadhyay, S., Singhal, R. S., Kulkarni, P. R. 1997. Optimization of conditions of synthesis of oxidized starch from corn and amaranth for use in film-forming applications. *Carbohydrate Polymers*, vol. 34, no. 4, p. 203-212. https://doi.org/10.1016/S0144-8617(97)87306-7

Chookietwattana, K. 2014. Lactic acid production from simultaneous saccharification and fermentation of cassava starch by *Lactobacillus plantarum* MSUL 903. APCBEE Procedia, vol. 8, p. 156-160. https://doi.org/10.1016/j.apcbee.2014.03.019

Chung, S. Y., Han, S. H., Lee, S. W., Rhee, C. 2010. Physicochemical and bread-making properties of air flow pulverized wheat and corn flours. Food Science and Biotechnology, vol. 19, no. 6, p. 1529-1535. https://doi.org/10.1007/s10068-010-0217-5

Clark, D. S., Blanch, H. W. 1997. *Biochemical engineering*. 2nd ed. New York, USA : CRC Press, 716 p. ISBN 13: 9780824700997.

Commission of the European Communities. 1976. *Reports of the scientific committee for food on some chemically modified starches*. 2nd ed. Brussels: ECSC, 23 p.

de Graaf, R. A., Broekroelofs, A., Janssen, L. B. M. 1998. The acetylation of starch by reactive extrusion. Starch, vol. 50, no. 5, p.198-205. https://doi.org/10.1002/(SICI)1521-379X(199805)50:5<198::AID-STAR198>3.0.CO;2-O

Gupta, S., Abu-Ghannam, N., Scannell, A. G. M., 2011. Growth and kinetics of *Lactobacillus plantarum* in the fermentation of edible Irish brown seaweeds. Food and Bioproducts Processing, vol. 89, no. 4, p. 346-355. https://doi.org/10.1016/j.fbp.2010.10.001

Ihekoronye, A. I., Ngoddy, P. O. 1985. *Integrated food science and technology for the tropics*. New York: Macmilian Publishers, 386 p. ISBN 13: 9780333388839.

Larrosa, V., Lorenzo, G., Zaritzky, N., Califano, A. 2013. Optimization of rheological properties of gluten-free pasta dough using mixture design. *Journal of Cereal Science*, vol. 57, no. 3, p. 520-526. https://doi.org/10.1016/j.jcs.2013.03.003

Mestres, C., Zakhia, N., Dufour, D. 1997. Functional and physic-chemical properties of sour cassava starch. In Frazier, P. J. et al. *Starch: Structure and Functionality*. London, UK : Royal Society of Chemistry, p. 163-171. ISBN-13: 978-0854047420.

Meethal, S. M., Kaur, N., Singh, J., Gat, Y. 2017. Effect of addition of jackfruit seed flour on nutrimental, phytochemical and sensory properties of snack bar. Current Research Nutrition and Food Science, 2017; vol. 5, no. 2, p. 154-158. http://doi.org/10.12944/CRNFSJ.5.2.12

Passos, F. V., Fleming, H. P., Ollis, D. F., Felder, R. M., McFeeters, R. F. 1994. Kinetic and modeling of latic acid production by *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, vol. 60, no. 7, p. 2627-2636. PMid:16349339

Putri, W. D. R., Haryadi, Marseno, D. W., Cahyanto, M. N. 2011. Effect of biodegradation by lactic acid bacteria on physical properties of cassava starch. *International Food Research Journal*, vol. 18, no. 3, p. 1149-1154.

Ray, R. C., Sharma, P., Panda, S. H. 2009. Lactic acid production from cassava fibrous residue using *Lactobacillus plantarum* MTCC 1407. *Journal of Environmental Biology*, vol. 30, no. 5, p. 847-852. PMid:20143717

Retnowati, D. S., Kumoro, A. C., Ratnawati, R. 2018. Physical, thermal and functional properties of flour derived from ubi gembili (*Dioscorea esculenta* L.) tubers grown in Indonesia. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 539-545. https://doi.org/10.5219/937

Rizzello, C. G., Nionelli, L., Coda, R., De Angelis, M., Gobbetti, M. 2010. Effect of sourdough fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ. *Food Chemistry*, vol. 119, no. 3, p. 1079-1089. https://doi.org/10.1016/j.foodchem.2009.08.016

Romanová, K., Urminská, D. 2017. Potential of *Lactobacillus plantarum* ccm 3627 and *Lactobacillus brevis* ccm 1815 for fermentation of cereal substrates. Potravinarstvo Slovak Journal of Food Sciences, vol. 11, no. 1, p. 544-549. https://doi.org/10.5219/696

Statistics Indonesia. 2014. *Fruits Production by Province* (ton). Jakarta, Indonesia : Statistics Indonesia.

Smith, R. J. 1967. Production and used of hypochlorite oxidized starches. In Whistler, R. L. et al. *Starch Chemistry and Technology*. New York: Academic Press, p. 620-625. ISBN 13: 9780127462622.

Sodhi, N. S., Singh, N. 2005. Characteristics of acetylated starches prepared using starches separated from different rice cultivars. *Journal of Food Engineering*, vol. 70, no. 1, p. 117-127. https://doi.org/10.1016/j.jfoodeng.2004.09.018

Taleghani, H. G., Najafpour, G. D., Ghoreyshi, A. A. 2016. A study on the effect of parameters on lactic acid production from whey. *Polish Journal of Chemical Technology*, vol. 18, no. 1, p. 58-63. https://doi.org/10.1515/pjct-2016-0010

Tharise, N., Julianti, E., Nurminah, M. 2014. Evaluation of physico-chemical and functional properties of composite flour from cassava, rice, potato, soybean and xanthan gum as alternative of wheat flour. *International Food Research Journal*, vol. 21, no. 4, p. 1641-1649.

Tripathi, A. D., Srivastava, S. K., Singh, P., Singh, R. P., Singh, S. P., Jha, A., Yadav, P. 2015. Optimization of process variables for enhanced lactic acid production utilizing paneer whey as substrate in SMF. Applied Food Biotechnology, vol. 2, no. 2, p. 46-55. http://doi.org/10.22037/afb.v2i2.7612

Tulyathan, V., Tananuwong, K., Songjinda, P., Jaiboon, N. 2002. Some physicochemical properties of jackfruit (*Artocarpus heterophyllus* Lam.) seed flour and starch. Science Asia, vol. 28, no. 1, pp. 37-41. https://doi.org/10.2306/scienceasia1513-1874.2002.28.037

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THE IMPORTANCE OF HIGHER ALCOHOLS AND ESTERS FOR SENSORY EVALUATION OF RHEINRIESLING AND CHARDONNAY WINE VARIETIES

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ABSTRACT

For a consumer, one of the first characters for evaluation of wine is its scent. A pleasant aroma of wine associated with the subsequent taste experience can be remembered by the consumer for a long time and appreciated appropriately. For this reason, the aromatic properties of wine are very important to both consumers and producers. The question, however, is to assess the evaluation of wine sensory evaluation based on a rapidly developing chemical analysis without the use of a panel of evaluators. This study has dealt with the problem of the correlation of sensory evaluation of wine with the total content of higher alcohols and esters in wine prepared from the same wine varieties (Rheinriesling and Chardonnay) on the same vineyard under the same climatic conditions and processed using the same production technology in the years 2008 - 2012. The total content of higher alcohols and esters was determined by gas chromatography with a mass spectrometer (GC/MS). The correlation between the sensory evaluation and the total content of higher alcohols has not been established. However, the direct effect of the total content of the esters on the level of the sensory analysis of the two varieties studied was demonstrated. This can be an important economic indicator for a manufacturer who can estimate the sensory quality of the wine only on the basis of chemical analysis and thus estimate the success of the wine on the consumer market.

Keywords: wine; Chardonnay; Rheinriesling; total content of higher alcohols; total ester content

INTRODUCTION

Products of grapevine are a significant source of biologically active substances with a positive effect on a human health **(Snopek et al., 2018a)**. A wine is considered as one of those products with a long tradition and popularity amongst consumers **(Soyollkham et al., 2011; Mlček et al., 2018)**. A food made from grapes of grapevine or a food where sensorial or technological attributes can be enhanced by their addition is counted between these products **(Boudová Pečivová et al., 2014)**.

The quality of grape wines and fruit spirits is assessed not only by the results of physico-chemical analyzes but also by sensory assessment of their properties (sensory analysis). From the sensory point of view, the content of flavorings in the food is important for the consumer. Aromatic substances here are all fragrances and flavors that create a complex sensory sensation called flavor (aroma) of food. Aromatic substances are either a natural ingredient of food (as primary flavorings) or are formed during food and beverage processing and storage, enzymatic and chemical reactions (as secondary or tertiary aromatics) **(Hampl, Rádl and Paleček, 2007; Patra, Kim and Baek, 2015; Zhang, 2017)**.

Perception of smell is one of the important psychological aspects in eating foods that you remember for a long time unlike other senses. This can be used in sensory evaluation, especially when evaluating foods with a wide range of aromatic substances, such as wine **(Lawless and Heymann, 2010)**.

There are many different flavors for the wine, especially the esters that are formed by the reaction of alcohols and acids. The finished wine has more than 300 different esters. Esters in wine are produced in two different ways: enzymatic esterification during fermentation and chemical esterification during long-term ripening **(Jacobson, 2006; Ribéreau-Gayon et al., 2006)**. There are several factors contributing to the formation of esters during fermentation, such as grape maturity and sugar content, yeast strains used, fermentation temperature, vinification methods, variety, pH of must and sulfur dioxide. Biochemical reactions occurring during maturation and storage may affect the aroma and quality of the wine. The fruit character of the wine can be lost very quickly depending on the storage temperature **(Pavelková, 2005)**. Yeasts, due to their esterase activity, form different esters (in a quantity of several $mg.L^{-1}$). Higher ester content is found in white wines compared to red wines, especially when a

lower temperature is used during vinification **(Clarke and Bakker, 2004)**.

Chardonnay is an old grape variety for a white wine production, originating from Burgundy. This grape variety is adaptable. It is favored among winemakers because it is easier to cultivate than other varieties. This grape variety is resilient to extreme climate conditions and can adapt to various types of a soil. It ripens quite reliably and has a good yield. In comparison to other grape varieties, Chardonnay has a neutral taste. It can also retain fruitlike flavor **(Callec, Ptáček and Svobodová, 2007)**.

Riesling is an old grape variety with a production of high-quality white wines **(Lapčíková, 2017)**. This grape variety originates from Rhine basin area in Germany. Its flavor may vary from steel-like, in unripe vintages, through peppery, crisply spicy to maturely fresh. Peach, both green and yellow apple, orange, lemon peel, quince, and in softer wines apricot, pineapple, honey, marzipan, almonds and even raisins can be found in its flavor **(Kraus et al., 2005)**.

From the consumer's point of view, the sensory quality of the wine is very important. Sensory evaluation of wine can be subjective and influenced by many aspects. On the contrary, the rapidly developing analytical methods are objective methods under different circumstances. Based on the correlation between analytical values and sensory evaluation, the sensory quality of the product and the consumer's interest could be estimated. This could become one of the key economic indicators in introducing a given vintage wine into the consumer market.

Scientific hypothesis

Scientific hypothesis is: The overall sensory evaluation of the selected type of wine correlates with the content of aromatic substances (higher alcohols and esters) in wine. (Correlation coefficient is greater than 0.95).

The aim of the study was to find out the correlation between the total point assessment of sensory analysis and the total content of higher alcohols and esters in selected wine varieties. Wine samples were selected with respect to their comparability - they are made from the same varieties, grapes for their production come from the same vineyard and are made using the same technology.

MATERIAL AND METHODOLOGY

Wine samples

In total 10 samples of wine – 5 samples of Chardonnay (further marked as CH + respective vintage) and 5 samples of Riesling (further marked as RR + respective vintage) – was used to determine the content of individual aromatic substances. **Table 1** shows a description of individual wine samples.

Presented wine samples were of the same grape variety from the same vineyard track and made using the same technology. Grapes come from vineyard track "Horní hory – Pohany", city Bzenec, region Slovácko, area Morava. This vineyard track is one of the most suitable for cultivation of grape varieties, typical for this winemaking region.

Methodology of sensory evaluation of wine

The evaluation of the wine was performed according to the 100 points scale of the International Union of Oenologists UIOE **(Kuttelvašer, 2003)**. The evaluation was carried out by a twelve-member panel of trained assessors with practical experience of seven men and five women. The age of the evaluators ranged from 28 to 71 years. Prior to the evaluation, a short briefing was conducted by the Chair of the Panel to specify the evaluation tasks.

Table 1 Description of individual wine samples.

Sample	Date of harvest	Sugar content [°NM]	Total alcohol content [% vol.]	Reducing sugars content [g.L^{-1}]	Classes according to remaining sugar	Quality
			Riesling			
RR 2008	10.11.2008	21.6	12.78	2.2	dry	PS
RR 2009	29.10.2009	23	13.74	9.9	medium dry	PS
RR 2010	23.10.2010	22	12.31	1.5	dry	PS
RR 2011	24.10.2011	22.2	12.97	7.9	medium dry	PS
RR 2012	13.10.2012	22.2	12.94	4.1	medium dry	PS
			Chardonnay			
CH 2008	21.10.2008	22.6	13.32	4.50	dry	PS
CH 2009	23.10.2009	24.6	14.57	10.6	medium dry	VH
CH 2010	17.10.2010	22.8	12.62	2.3	dry	PS
CH 2011	05.10.2011	24	13.61	11.7	medium dry	VH
CH 2012	02.10.2012	24.2	13.7	3.6	dry	PS

Note: VH – special selection of grapes, PS – Late harvest, RR – Riesling + respective vintage, CH – Chardonnay + respective vintage.

Method of aromatic substances content determination using GC/MS

Individual aromatic substances content in wine samples was determined using gas chromatography with a mass spectrometer (GC/MS). Used apparatus was GCMS – QP2010 Ultra (Shimadzu, Japan) with Supelco SP MTM – PUFA column sized 30 m × 0.25 mm × 0.25 μm. Each vintage was analyzed three times. The sum of all the monitored higher alcohols found in the sample was used to calculate the correlation. Higher alcohols – isobutyl alcohol, propyl alcohol, pentyl alcohol, isobornyl alcohol, phenylethyl alcohol and n-butanol were found in the samples. The sum of all observed esters that were found in the sample (19 in total) was used to calculate the correlation.

Chromatographic conditions were following:

Injector

Injection temperature	200 °C
Flow regulation	Linear flow velocity
Column flow	1.22 mL.min^{-1}
Flow velocity	39.9 cm

Column

Temperature program	40 °C – 6 min
	57 °C – 4 min
	180 °C – 0 min

Detector

Interface temperature	220 °C
Ion source temperature	200 °C
Detector voltage	0.80 kV

Statistical analysis

The data were analyzed using Excel 2013 (Microsoft Corporation, USA) and STATISTICA Cz version 12 (StatSoft, Inc., USA). Results were expressed by average ± standard deviation. The correlation between the overall score of the sensory analysis and the total content of higher alcohols and esters in selected wine varieties was determined using the Pearson correlation coefficient.

RESULTS AND DISCUSSION

Basic results

The basic results of the sensory analysis of the monitored wines samples and their total content of higher alcohols and esters are given in **Table 2**. The table shows the average number of points obtained by sensory evaluation of samples of individual wine samples and the total content of higher alcohols and the total content of esters obtained from chemical analysis of wine using gas chromatography with a mass spectrometer (GC/MS).

As reported by **Francis and Newton (2005)** and **Melherbe (2011)**, higher alcohols significantly affect the variability of wine aromas (honey, spicy, whiskey, pink and similar scents) at concentrations below 300 mg.L^{-1}. In the case of higher concentrations (above 600 mg.L^{-1}), they can cause an unpleasant aroma of wine that resembles a dissolving agent. Our measured values in our study did not exceed 600 mg.L^{-1}and are consistent with the total amount of higher alcohols in the 150 – 700 mg.L^{-1}wine reported by **Steidel (2010)**.

According to **Farkaš (1983)**, young wine ranges from 2 to 6 mg.L^{-1} and older wines around 10 mg.L^{-1}. On the other hand, **Tomanová (2015)** reports the measured content of the individual esters in wine at 10 and 100 mg.L^{-1}, so the total content of the measured esters is higher than that reported by **Farkaš (1983)**. The results measured in this study are in accordance with **Tomanová (2015)**. The reason for this wide spread may be several factors, such as grape maturity and sugar content, yeast strains used, fermentation temperature, vinification methods, variety, pH of must and sulfur dioxide. Biochemical reactions occurring during maturation and storage may affect the aroma and quality of the wine. The fruit character of the wine can be lost very quickly depending on the storage temperature **(Pavelková, 2005)**.

In spite of the effort to adhere to the strict conditions of the experiment (the same cultivation site, the same agrotechnical technology, the same technological process of production), unconscious increases in the quality of these production conditions (e.g. subconscious

Table 2 Basic results of the sensory analysis of the monitored vines samples and their total content of higher alcohols and esters.

Sample	Sensory evaluation [points]	Total content of higher alcohols [mg.L^{-1}]	Total content of esters [mg.L^{-1}]
RR 08	92	182	438
RR 09	91	162	420
RR 10	89	298	406
RR 11	73	438	310
RR 12	78	251	339
CH 08	71	183	162
CH 09	85	335	331
CH 10	93	210	438
CH 11	82	438	290
CH 12	80	162	271

Note: RR – Riesling + respective vintage, CH – Chardonnay + respective vintage.

improvements in the quality of workers who can gain more experience each year) The results of the sensory evaluation of individual samples of grape wines do not show any significant dependence according to years, which shows the independence of the wine production process in case of possible improvement of production.

Results of sensory evaluation of grape wines in context to the total content of higher alcohols

In the fermentation process, ethanol, carboxylic acids and higher alcohols (the so-called "blooms") are formed in the third phase. Higher alcohols significantly contribute to the bouquet of mature wine. The total content is from 150 to 700 mg.L^{-1} **(Steidl, 2010)**.

The correlation between the sensory evaluation of samples and the total content of higher alcohols was analyzed for the Rheinriesling variety and the Pearson correlation coefficient was determined. The Pearson correlation coefficient was r = -0.8052 and the correlation equation:

Sensory evaluation [points] = 101.23 – (0.0625 × Total content of higher alcohols [mg.L^{-1}])

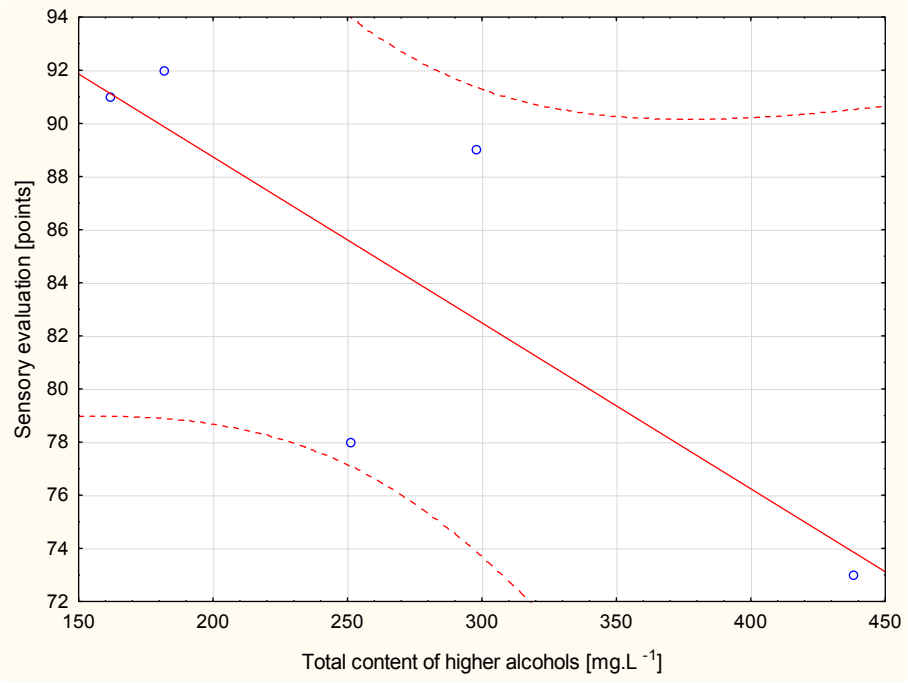

Figure 1 Graph of the correlation between the total content of higher alcohols [mg.L^{-1}] and the sensory evaluation [point] of the Rheinriesling.

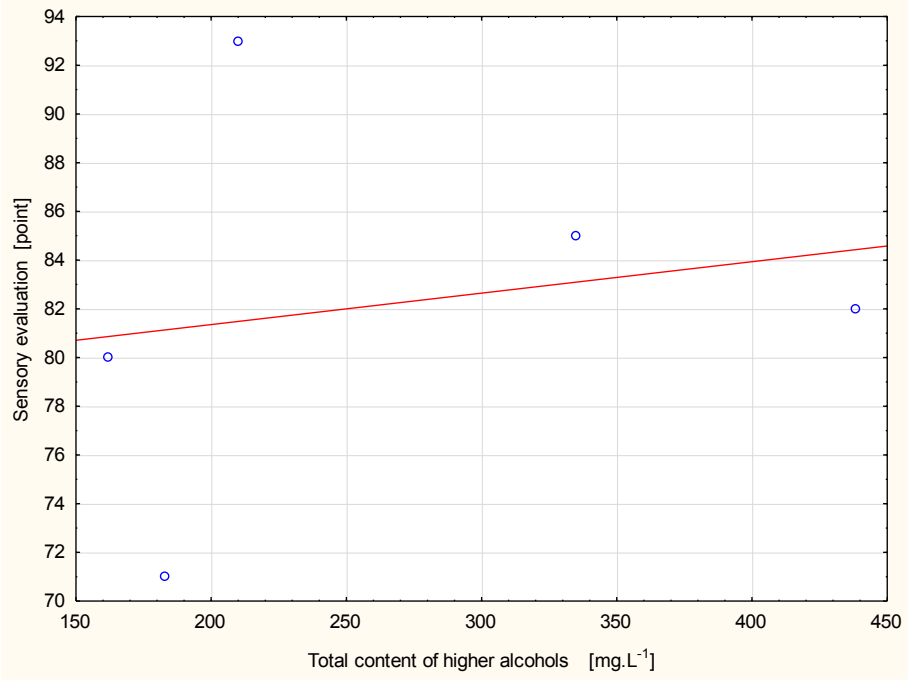

Figure 2 Graph of the correlation between the total content of higher alcohols [mg.L^{-1}] and the sensory evaluation [point] of the Chardonnay variety.

The results show a intermediate correlation of the total score of sensory analysis with the total content of higher alcohols in the sample of wine.

This fact is confirmed by the graph in **Figure 1**.

For the Chardonnay variety, the Pearson correlation coefficient was determined by r = 0.18998 and the correlation equation:

Sensory evaluation [points] = 78.771 + (0.01291 × Total content of higher alcohols [mg.L^{-1}])

The results for the Chardonnay variety show that the total score of sensory analysis does not correlate with the total content of higher alcohols in the sample of wine.

This fact is again confirmed by the graph in **Figure 2**.

Results of sensory evaluation of grape wines in context to the total content of esters

Esters are one of the most important categories of volatile substances in wine and they represent the primary source of wine fruity aroma, namely attributed to derivatives of esters **(Pavelková, 2005)**. Esters are therefore of great

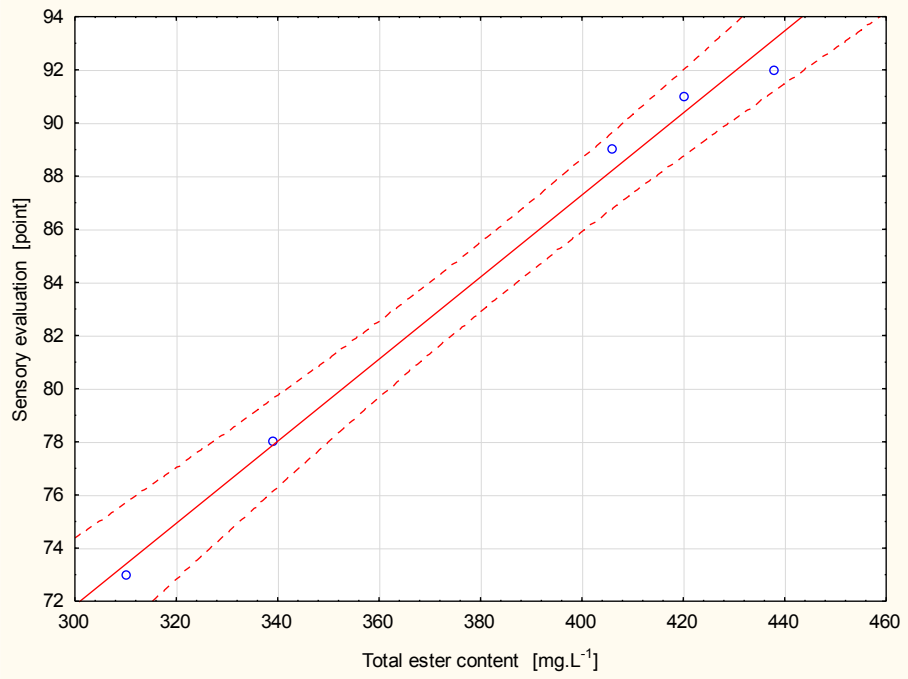

Figure 3 Graph of the correlation between the total ester content [mg.L^{-1}] and the sensory evaluation [point] of the Rheinriesling.

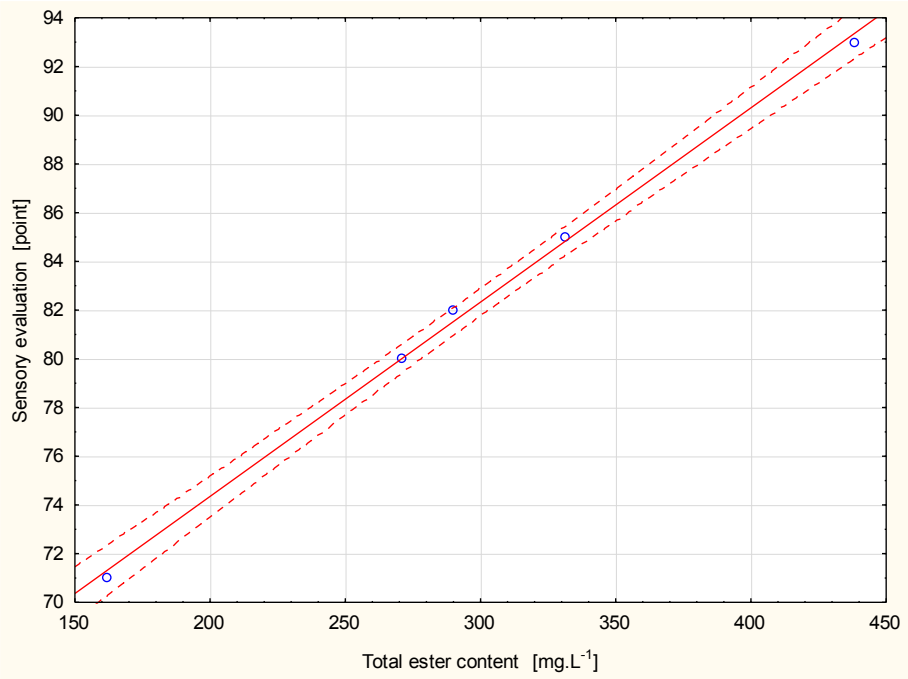

Figure 4 Graph of the correlation between the total ester content [mg.L^{-1}] and the sensory score [point] of the Chardonnay variety.

importance for sensory evaluation of wine properties. Sensory evaluation of wine has been dealt with by **Fikselova et al. (2018)** and **Snopek (2018b)**, who emphasizes the importance of flavorings at the first contact of wine with the consumer. Esters are produced in wine in two ways. They can be formed by enzymatic esterification during alcohol fermentation or during malolactic fermentation by yeast and bacteria or by chemical esterification by aging **(Ribéreau-Gayon et al., 2006)**. The most important esters of higher alcohols with acetic acid are isoamylaceate with banana flavor and phenylethyl acetate having rose aroma **(Ribéreau-Gayon et al., 2006)**. **Francis and Newton (2005)** report the concentration of 118 – 4300 $\mu g.L^{-1}$ in the case of isoamylacetate in young wine and248 - 3300 $\mu g.L^{-1}$ in the old wine with a perception threshold of 30 $\mu g.L^{-1}$. Similarly, apple flavor ethyl butyrate has a perception threshold of 20 $\mu g.L^{-1}$ and a young wine concentration of69 – 371 $\mu g.L^{-1}$ and of old wine of20 – 1118 $\mu g.L^{-1}$. In addition, ethyl acetate, which, when exceeding the limit of 150 – 200 $mg.L^{-1}$ causes a wine defect, which is characterized by an acetic odor **(Pavelková, 2005)**. This ester is undesirable in the wine **(Jacobson, 2006)**.

The Pearson correlation coefficient between the sensory evaluation and the total content of the esters r = 0.99571 was found in the Rheinriesling species. From the observed value it is clear that the sensory evaluation of the wine is dependent on the total content of the esters in the samples. This is documented by the graph in **Figure 3**. Corresponding equation was determined for this variety:

Sensory evaluation [points] = 25.511 + (0.15444 × Total ester content [$mg.L^{-1}$])

Similarly to the Rheinriesling variety, the Chardonnay variety was found to depend on sensory evaluation of wine on the total content of esters in the samples. Given the Pearson correlation coefficient r = 0.99907, this dependence is even more pronounced than for the Rheinriesling variety. This fact is confirmed by the graph in **Figure 4**. The correlation equation was determined as:

Sensory evaluation [points] = 58.399 + (0.07976 × Total ester content [$mg.L^{-1}$])

CONCLUSION

This study has dealt with the problem of the correlation of wine sensory evaluation with the total content of higher alcohols and esters in wine prepared from the same wine varieties (Rheinriesling and Chardonnay) on the same vineyard under the same climatic conditions and processed using the same production technology in the years 2008 – 2012. The correlation of the sensory evaluation with the total content of higher alcohols has not been demonstrated, as opposed to the direct dependence of the height of the sensory analysis point score on the total ester content. This can be an important economic indicator for a manufacturer who can estimate the sensory quality of the wine only on the basis of chemical analysis and thus estimate the success of the wine on the consumer market.

REFERENCES

Boudová Pečivová, P., Kráčmar, S., Kubáň, V., Mlček, J., Juríková, T., Sochor, J. 2014. Effect of Addition of Grape Seed Flour on Chemical, Textural and Sensory Properties of Bread Dough. *Mitteilungen Klosterneuburg*, vol. 64, no. 3, p. 114-119.

Callec, Ch., Ptáček, L., Svobodová, L. 2007. *Ví o: velký obrazový lexikon (Wine: a large image lexicon.)*. Čestlice, Czech Republic: Rebo. 528 p. ISBN-13: 978-80-7234-889-3 (In Czech)

Clarke, R. J., Bakker, J. 2004. *Wine flavour chemistry*. Oxford, UK: Blackwell Publishing Ltd., 324 p. ISBN 1-4051-0530-5.

Farkaš, J. 1983. *Biotechnológia vína* (*Biotechnology of Wine)*. 2nd ed. Bratislava, Slovak Republic: Alfa. 984 p. ISBN 63-076-83. (In Slovak)

Fikselova, M., Czako, P., Gažo, J., Mendelová, A., Mellen, M. 2018. Sauvignon wine quality as affected by its processing and storage. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 299-303. https://doi.org/10.5219/885

Francis, I., Newton, J. 2005. Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research*, vol. 11, no. 2, p. 114-126. https://doi.org/10.1111/j.1755-0238.2005.tb00283.x

Hampl, F., Rádl, S., Paleček, J. 2007. *Farmakochemie (Farmakochemistry)*. 2nd ed. Prahague, Czech Republic: VŠCHT Praha, 450 p. ISBN-13: 978-80-7080-639-5 (In Czech)

Jacobson, J. L. 2006. *Introduction to wine laboratory practices and procedures*. New York, USA: Springer Science+Business media, LLC., 375 p. ISBN-13: 978-0387-24377-1.

Kraus, V., Foffová, Z., Vurm, B., Krausová, D. 2005. *Nová encyklopedie českého a moravské o ví a* (*New Encyclopedia of Czech and Moravian Wines)*. 1st ed. Praha, Czech Republic: Praga Mystica. 304 p. ISBN-13: 978-8086-76700-0 (In Czech)

Kuttelvašer, Z.. 2003. *Abeceda vína (Alphabet of wine)*. 2nd ed. Praha, Czech Republic: Radix, 279 p. ISBN-13: 978-80-86031-43-9 (In Czech)

Lapčíková, B., Lapčík, L., Hupková, J. 2017. Physico-chemical characterisation of Slovak wines. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 216-222. https://dx.doi.org/10.5219/727

Lawless, H. T., Heymann, H. 2010. *Sensory Evaluation of Food. Principles and Practices*. New york, USA: Springer Science+Business media, LLC. 596 p. ISBN-13: 978-1-4419-6488-5.

Malherbe, S. 2011. *Investigation of the impact of commercial malolactic fermentation starter cultures on red wine aroma compounds, sensory properties and consumer preference* : dissertation theses. Stellenbosch, South Africa: University of Stellenbosch. 121 p.

Mlček, J., Trágeová, S., Adámková, A., Adámek, M., Bednářová, M., Škrovánková, S., Sedláčková, E. 2018. Comparison of the content of selected mineral substances in Czech liturgical and common wines. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 150-156. https://doi.org/10.5219/901

Patra, J. K., Kim, S. H., Baek, K.-H. 2015. Antioxidant and free radical-scavenging potential of essential oil from Enteromorpha linza L. Prepared by Microwave-Assisted Hydrodistillation. *Journal of Food Biochemistry*, vol. 39, no. 1, p. 80-90. https://doi.org/10.1111/jfbc.12110

Pavelková, I. 2005. Kvasinky a aroma vína (Yeasts and wine aroma). *Vinařský obzor*, vol. 98, no. 11, p. 557-559. (In Czech)

Ribéreau-Gayon, P., Dubourdieu, D., Donèc e, B., Lonvaud, A. 2006. *Handbook of enology*. 2nd ed. Hoboken, NJ, USA : John Wiley and Sons. 512 p. ISBN-13: 978-0470-01034-1.

Snopek, L., Mlcek, J., Sochorova, L., Baron, M., Hlavacova, I., Jurikova, T., Kizek, R., Sedlackova, E., Sochor, J. 2018a. Contribution of red wine consumption to human health protection. *Molecules*, vol. 23, no. 7, p. 1-16. https://doi.org/10.3390/molecules23071684

Snopek, L., Mlček, J., Fic, V., Hlaváčová, I., Škrovánková, S., Fišera, , M., Velichová, H., Ondrášová, M. 2018b. Interaction of polyphenols and wine antioxidants with its sulfur dioxide preservative. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 180-185. https://doi.org/10.5219/899

Soyollkham, B., Valášek, P., Fišera, M., Fic, V., Kubáň, V., Hoza, I. 2011. Total polyphenolic compounds contents (TPC), total antioxidant activities (TAA) and HPLC determination of individual polyphenolic compounds in selected Moravian and Austrian wines. *Central European Journal of Chemistry*, vol. 9, no. 4, p. 677-687. https://doi.org/10.2478/s11532-011-0045-3

Steidl, R. 2010. *Sklepní hospodářství*. (*Cellar Management*). 2nd ed. Valtice, Czech Republic : Národní vinařské centrum. 309 p. ISBN: 978-80-903201-9-2 (In Czech)

Tomanová, B. 2015. *Aromatický profil mladých vín (Aromatic profile of young wines*) : diploma theses. Lednice, Czech Republic : Mendel University in Brno. 74 p.

Zhang, W., Si, G., Ye, M., Feng, S., Cheng, F., Li, J., Mei, J., Zong, S., Wang, J., Zhou, P. 2017. An Efficient Assay for Simultaneous Quantification of Ethyl Carbamate and Phthalate Esters in Chinese Liquor by Gas Chromatography-Mass Spectrometry. *Food Analytical Methods*, vol. 10, no. 11, p. 3487–3495. https://doi.org/10.1007/s12161-017-0906-2

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EFFECT OF TiO_2 NANOPARTICLES IN THYME UNDER REDUCED IRRIGATION CONDITIONS

Bahman Fazeli-Nasab, Josep Antoni Rossello, Amir Mokhtarpour

ABSTRACT

The nanotechnology is a relatively new technology that has recently entered the field of agriculture. Nanotechnology covers the integration or manipulation of individual atoms, molecules or molecular masses to a diverse array of structures allowing the production of new characteristics and traits of interest. The aim of this study was to evaluate the effects of foliar application of TiO_2 nanoparticles on quantitative traits (plant height, number of branches, dry weight of shoots and roots) and the essential oil content of thyme under different levels of field capacity. Our results showed that the application of TiO_2 nanoparticles had significant effects on thyme growth, while the essential oil concentrations not affected. These results imply that the application of TiO_2 nanoparticles in plants increase agronomic value under reduced irrigation conditions but has not different significant on essential oil.

Keywords: *Thymus vulgaris*; essence; nano dioxide titanium; nanotechnology

INTRODUCTION

Health concerns have been one of the main drivers increasing the knowledge and use of medicinal plants worldwide in folk medicine since prehistorically times **(Trebichalský et al., 2015; Fazeli-Nasab and Mirzaei. 2018)**. Currently, economic, social, and ethical demands have shaped the use of herbal medicines. Some drugs can hardly be synthesized in the lab, or their artificial production is costly. Furthermore, the social perceptions that herbal medicines have fewer negative collateral effects on human health and reduce more concerns towards their use than artificial drugs have been installed in the human minds of modern societies **(Alizadeh-Salteh et al., 2010; Omidbeigi, 2002)**.

The increasing consumption of medicinal plants in the last decades is a challenge as, inevitably, agronomic practices should be applied to optimize the production rates of several species under culture to fulfill the current demands on a world-wide scale **(Fazeli-Nasab et al., 2017)**.

Medicinal plants usually need full vegetative and reproductive growth to produce enough yields of active compounds **(Eftimova et al., 2018)**. These requirements may be critical in arid and semi-arid regions, where limited water resources for agriculture cause drought stress which can lead to reduction in the quantity and quality of the desired plant components **(Khodadadi Dehkordi, 2016a; Khodadadi Dehkordi, 2016b)**.

Plants respond to drought stress at the physiological, cellular and molecular level. This response depends on the genotype of the plant **(Distelfeld et al., 2014)**, the duration and severity of water shortages **(Kunrath et al., 2018)** and the age and developmental stage at which drought stress was imposed **(Sheoran et al., 2014)**.

Nanotechnology (the application of single atoms, molecules, molecular aggregates to produce new structures with different characteristics) is considered as a promising field in agricultural practices. Specifically, considerable attention has recently been devoted to the use of Nano-titanium dioxide particles in plants because of their reported growth stimulation effects **(Wang et al., 2016)**.

In this study we evaluated the effects of titanium dioxide nanoparticles (TiO_2 nanoparticles) on growth features and the amount of essential oil in the medicinal plant thyme (*Thymus vulgaris* L.) under drought stress conditions.

Thyme (*Thymus vulgaris* L.,) belong to Lamiaceae and also is a small shrub native to the Western Mediterranean basin. Its use is reputed to alleviate different diseases such as cough, sore throat, bronchitis and asthma, and it shows anti-inflammatory, antiseptic, and antispasmodic properties **(Adwan et al., 2007; Lixandru et al., 2010)**. Essential oil is produced in all parts of the plant; however, the highest yields occurred in the flowering branches at anthesis. The essential oil shows anti-rheumatic and anti-aseptic properties, as well as antioxidant and antifungal effects **(Shokri and Sharifzadeh, 2017)**. The essential oil components in this plant varies depending on genetic and agronomic conditions **(Kouchaki, 2009; Kouchaki et al., 2008)**.

Scientific hypothesis

The purposes of this study were to assess whether the foliar application of TiO_2 nanoparticles stimulates plant growth, and whether it increases essential oil yield in thyme plants subjected to different levels of field capacity.

MATERIAL AND METHODS

Material

Seeds of *Thymus vulgaris* were obtained from Pakan Bazr Ishafan (Ishafan, Iran). Twenty healthy seeds were sown in 20 cm (diameter) x 30 cm (height) filled with commercial substrate. The soil samples were analyzed (Laboratory of Soil Science, Faculty of Agriculture, and University of Zabol) to assess several physical and chemical characters (Supplementary Table 1). The contents of minerals were determined by a Varian SpectrAA-400 plus atomic absorption spectrometer. After radicle emergence and seedling establishment (plants growth at fresh air) six plants were kept per pot. Before starting the experimental work all plants were watered regularly at field capacity according to **Haghighi and Daneshmand. (2012)**. The experimental work was conducted during the 2015 summer in Sareyn (Iran; 38° 09' 05'' N, 48° 04' 15" E).

Table 1 Variance analysis of quantitative traits and essential oil yield under different treatments.

Significance of effects	Items						
	Essential oil percentage	Root dry weight	Root fresh weight	Shoot dry weight	Shoot fresh weight	Number of lateral branches	Plant height
Reduced Irrigation (RI)	0.069**	9.17**	56.75*	122.88**	3185.67**	350.96**	48.29*
Titanium dioxide (T)	0.026ns	1.9**	6.43*	16.36**	84.95**	18.22ns	8.42**
RI * T	0.024ns	0.78*	4.95ns	14.42*	48.59*	0.36ns	2.03ns

Note: ns – non-significant, * – significant at p <0.5, and ** – significant at p <0.01.

Experimental design

TiO_2 nanoparticles (30nm; were obtained from US Research Nanomaterial's (US-Nano) of course with collaboration of Iranian nano-Pishgaman Company) at three concentrations (0, 0.25 and 0.5 mg per liter) were applied to plants subjected to three reduced irrigation levels (50, 70 and 90% of field capacity) using a completely randomized design with a three-factorial arrangement. Titanium nanoparticles were sprayed weekly for three weeks (started from Four leaves stage) **(Nezami et al., 2012)**. For each treatment four replicates were performed.

Quantitative character assessment

After completion of the growth stage (about 60 days), four plants from each pot were randomly selected and the plant height, number of branches, fresh and dry weight of the aerial part and roots, and the essential oil concentration were assessed. Essential oil concentration was assessed from dried leaves and stems according to hydro distillation method in a Clevenger-type apparatus **(Arazmjo et al., 2010)** In this way, 30 gr of shoots and dried leaves of thyme harvested and then poured into a 500 mm balloon, then added to 300 ml of sterilized water and placed on a stove for 2 hours and after extraction of essential oil, Its value was measured.

Statistical analysis

Results were analyzed using the general linear model (GLM) procedure implemented in the SAS/STAT package **(SAS Institute, 2003)**. Least square means (LSMEANS) was used to detect difference between treatments at p <0.05 level.

RESULTS

Experimental results are summarized in Table 1, Table 2, Figure 1 and Figure 2. Overall, reduced irrigation significantly decreased plant height, number of branches, fresh and dry weight of the aerial part and roots in thyme plants and the lowest of them were observed in plants under a 50% field capacity (water restriction). However, a positive effect on plant height and number of branches, fresh and dry weight of roots was noted as the level of titanium applications increased. Increases in fresh and dry weight of shoots were detected at 90% FC when concentrations of 0.5 mg. L^{-1} titanium dioxide were added to plants. In contrast, reduction in water supply significantly decreases root biomass of plants. However, the addition of titanium dioxide at high concentration (0.5 mg. L^{-1}) increased root fresh and dry weight.

The concentracion of essential oils was significantly changed under reduced irrigation, and the highest amounts were recorded at 70% water restriction levels. No reversal of this trend was observed when titanium nanoparticles were added to thyme plants.

In Figure 1, similar letters within each column are non-significant values according to Duncan's Multiple Range Test at p <5%.

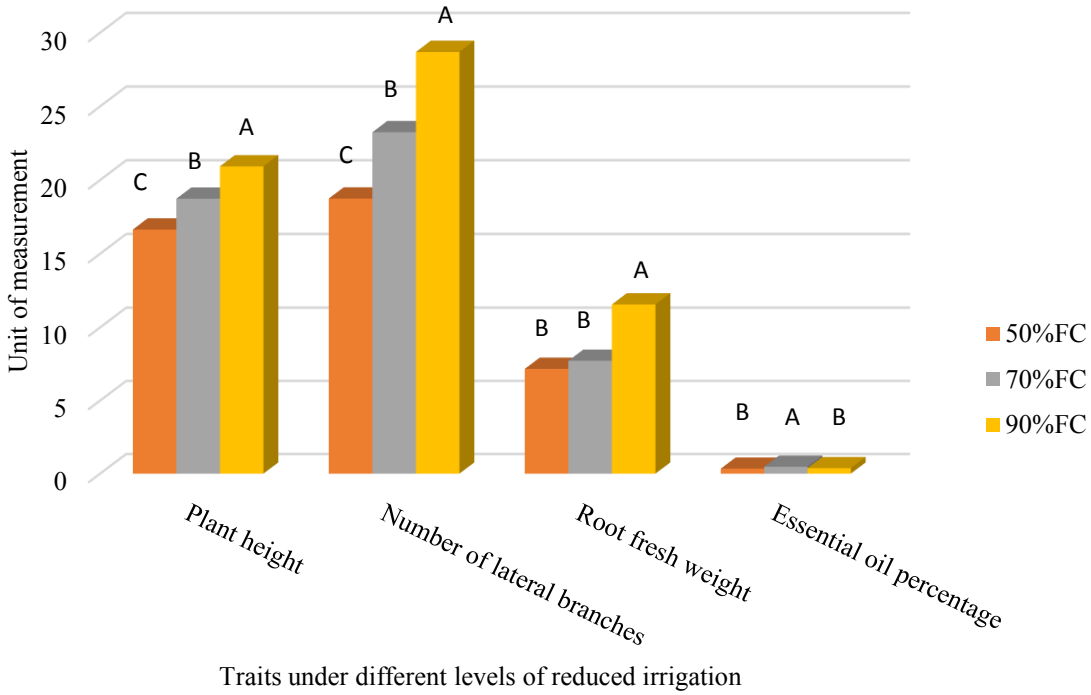

Figure 1 Mean comparison of four measured traits under different levels of reduced irrigation.

Note: similar letters within each column are non-significant values according to Duncan's Multiple Range Test at p <5%.

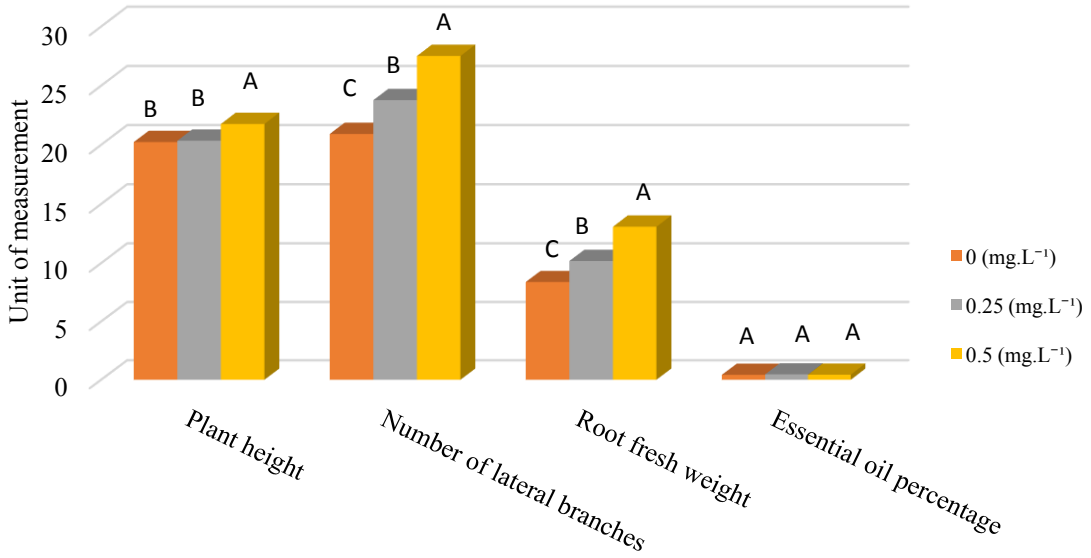

Figure 2 Mean comparison of four measured traits under different levels of foliar application of titanium dioxide.

Table 2 Mean comparison of double interaction effects, reduced irrigation and titanium, on four measured traits.

Irrigation Level (% FC)	Titanium dioxide (mg. L^{-1})	Root dry weight	Shoot dry weight	Shoot fresh weight
50	0	3.84^{d}	20.06^{c}	44.19^{e}
	0.25	4.09^{d}	21.15^{c}	49.29^{d}
	0.50	4.13^{d}	21.38^{c}	52.12^{d}
70	0	3.91^{d}	25.24^{b}	58.47^{cd}
	0.25	4.18^{d}	25.21^{b}	64.1^{c}
	0.50	4.42^{cd}	25.32^{b}	67.78^{b}
90	0	4.86^{c}	26.08^{ab}	75.82^{ab}
	0.25	5.42^{b}	26.29^{ab}	84.85^{a}
	0.50	6.42^{a}	31.31^{a}	84.52^{a}

Note: Similar letters within each column are non-significant values according to Duncan's Multiple Range Test at p <5%.

DISCUSSION

The application of titanium nanoparticles in *Thymus vulgaris* individuals under reduced irrigation conditions revealed a diversity of effects which were not fully concordant neither between the scored morphological characters nor with the level of reduced irrigation.

TiO_2 nanoparticles increased plant height, but not lateral branch growth under different drought regimes.

Reduced irrigation can reduce the active plant growth period retarding development processes involving stem elongation and leaf growth, the number of nodes and internode length **(Ribas-Carbo et al., 2005)**. **Pourmousavi et al., (2007)** reported that both titanium dioxide and reduced irrigation decrease plant height in soybean. These observations are in contrast with those obtained in wheat by **Moaveni et al., (2011)** who reported that plant height increased in response to different treatments of TiO_2 nanoparticles.

Our results in thyme agree with the later report and suggest that the application of titanium nanoparticles may partly counteract the decrease in stem height as a result of water drought. However, the behavior of other aerial growth parameters, like stem diameter and lateral branch number, remains unchanged when titanium nanoparticles were added. Our observations agree with the results **of Haghighi and Daneshmand, (2012)** who reported that Nano-titanium dioxide had no significant effects on the stem diameter on tomato plants.

Since fresh and dry weight of the aerial parts are partially related to plant height it is reasonable to assume that congruent effects on the effects of titanium nanoparticles would be found. Our results showed that this is the case, in agreement with other works reporting that spraying TiO_2 nanoparticles on leaves positively affects the overall growth rate as measured by the increase in fresh and dry weights of plants from several species **(Martínez-Sánchez et al., 1991; Murbach Teles Andrade et al., 2014)**. However, negative effects on shoot dry weight and length of stem in canola were reported when high concentrations of TiO_2 nanoparticles were supplied **(Shafiei and Tadayon. 2014)**.

Moreover, it should be stated that the method of application of the TiO_2 nanoparticles to plants could have influence on the results obtained. Thus, it has been reported that the application of titanium as a nutrient solution showed no effects on growth rate in tomato **(Haghighi and Daneshmand, 2012)**. It has been suggested that better performances on growth rates are obtained when spraying TiO_2 nanoparticles rather than adding them to conventional nutrient solutions.

The growth rate of fresh roots weight was not affected by the addition of TiO_2 nanoparticles.

Previous results on soybean plants showed that the transportation of titanium across cells was very slow and was more effective on the organs of the plant which were subjected to titanium treatment **(Lu et al., 2001)**. Since in our experimental design we sprayed titanium dioxide on the aerial parts of thyme it might be expected a higher growth rate in the aerial parts than in the root system. Our results agree with this prediction and they were consistent with other experimental work conducted on canola **(Mahmoodzadeh et al., 2013)**, cowpea **(Owolade et al., 2008)** and apple **(Grzyb et al., 2014; Wojcik and Wojcik, 2001)**.

However, opposite data were obtained by several authors and it is still controversial whether a consistent pattern in the growth rate of roots may be predicted. Even, it has been reported that the inclusion of titanium dioxide only increased the growth rate in apple when the plants showed an optimal nutritional state **(Wojcik and Klamkowski, 2005)**. Also, the threshold concentrations of titanium dioxide used may show contrasting results in different species **(Bagheri et al., 2010; Shafiei and Tadvin, 2014)**.

TiO_2 nanoparticles show no effects on the essential oil content of thyme under reduced irrigation.

Several studies have reported a positive association between the essential oil yield and the levels of irrigation in medicinal species from Lamiaceae **(Charles et al., 1990; Taarit et al., 2010)**. However, these observations appears to be genotype specific, since the highest content of essential oils and the most diversity of the individual components of the essential oil have been obtained in some

species at moderate reduced irrigation conditions (e.g., 55% field capacity irrigation; **(Omidbeigi, 2007)**). Our results obtained in thyme agree with this view and suggest that reduced irrigation may increase the overall concentration of essential oils of course only up to 70% FC.

Apparently, the application of titanium nanoparticles resulted in no significant quantifiable changes in oil content. However, our study was not addressed to assess the metabolic turnover of the individual components of the essential oil. Therefore, it should be further assessed whether several chemical compounds of the essential oil may change their relative concentration when TiO_2 nanoparticles are supplied to plants.

CONCLUSION

Our experimental results in thyme has shown that the addition of titanium nanoparticles in cultivated thyme plants may be an interesting choice for the agricultural practices of this species in arid areas. Nevertheless, the effects of spraying of titanium nanoparticles in this species are varied. On the one hand, growth and height of the aerial parts were significantly affected by TiO_2 nanoparticles. On the other, changes in the growth rate of roots were not observed. In addition, titanium dioxide did not increase the yield of the essential oils. Our data suggest that while the use of titanium dioxide may improve the agronomic features linked to the vegetative aerial parts of thyme, other substances should be screened to increase the essential oil concentration.

REFERENCES:

Adwan, G. M., Abu-Shanab, B., Adwan, K., Abu-Shanab, F. 2007. Antibacterial effects of nutraceutical plants growing in Palestine on *Pseudomonas aeruginosa*. *Turkish Journal of Biology*, vol. 30, no. 4, p. 239-242.

Alizadeh-Salteh, S., Arzani, K., Omidbeigi, R., Safaie, N. 2010. Essential oils inhibit mycelial growth of *Rhizopus stolonifer*. *European Journal of Horticultural Science*, vol. 76, no. 5, p. 278-282.

Arazmjo, A., Heidari, M., Ghanbari, A. 2010. The effect of water stress and three sources of fertilizers on flower yield, physiological parameters and nutrient uptake in chamomile (*Matricaria chamomilla* L.). *Iranian Journal of Medicinal and Aromatic Plants*, vol. 25, no. 4, p. 482-494.

Bagheri, M., Yeganeh, H., Ghasemi Aryan, Y., Yari, R. 2010. The physiological effects of stress caused by polyethylene glycol Atylm on germination characteristics of medicinal species *Thymus koteschanus* and *Thymus daenensis*. The National Conference of medicinal plants, Sari, Mazandaran University Jihad Uni, Available at: https://www.civilica.com/Paper-HERBAL01-HERBAL01_738.html

Charles, D. J., Joly, R. J., Simon, J. E. 1990. Effects of osmotic stress on the essential oil content and composition of peppermint. *Phytochemistry*, vol. 29, no. 9, p. 2837-2840. https://doi.org/10.1016/0031-9422(90)87087-B

Distelfeld, A., Avni, R., Fischer, A. M. 2014. Senescence, nutrient remobilization, and yield in wheat and barley. *Journal of Experimental Botany*, vol. 65, no. 14, p. 3783-3798. https://doi.org/10.1093/jxb/ert477 PMid:24470467

Fazeli-Nasab, B., Mirzaei, N. 2018. Evaluation of Total Phenol and Flavonoid Content in a Wide Range of Local and imported Plants. *Scientific Journal of Ilam University of Medical Sciences*, vol. 26, no. 2, p. 141-154. http://doi.org/10.29252/sjimu.26.2.141

Fazeli-Nasab, B., Sirousmehr, A., Mirzaei, N., Solimani, M. 2017. Evaluation of total phenolic, flavenoeid content and antioxidant activity of Leaf and Fruit in 14 different genotypes of *Ziziphus mauritiana* L. in south of Iran. *Eco-Phytochemical Journal of Medicinal Plants*, vol. 4, no. 4, p. 1-14.

Grzyb, Z. S., Piotrowski, W., Sas-Paszt, L. 2014. Treatments Comparison of Mineral and Bio Fertilizers in the Apple and Sour Cherry Organic Nursery. *J. Life Sci. USA*, vol. 8, p. 889-898.

Haghighi, M., Daneshmand, B. 2012. Comparison of titanium and nano-titanium on growth and photosynthesis of tomato changes in hydroponics. *Journal of Science and Technology of Greenhouse Culture*, vol. 4, no. 13, p. 73-79.

Khodadadi Dehkordi, D. 2016a. The Effects of Superabsorbent Polymers on Soils and Plants. *Pertanika Journal of Tropical Agricultural Science*, vol. 39, no. 3, p. 267-298.

Khodadadi Dehkordi, D. 2016b. Tropical Agricultural Science. *Pertanika Journal of Tropical Agricultural Science*, vol. 39, no. 3, p. 267-298.

Kouchaki, A. R. 2009. *Agriculture in arid areas: cereals, industrial crops and forage plants*. Masshad, Iran : Sientific Information Database Press of University of Mashhad, 202 p., ISBN; 964-6023-08-8.

Kouchaki, A. R., Tabrizi, L., Ghorbani, R. 2008. Effect of biofertilizers on agronomic and quality criteria of Hyssop (*Hyssopus officinalis*). *Iranian Journal of Filed Crops Research*, vol. 6, no. 1, p. 127-137.

Kunrath, T. R., Lemaire, G., Sadras, V. O., Gastal, F. 2018. Water use efficiency in perennial forage species: Interactions between nitrogen nutrition and water deficit. *Field Crops Research*, vol. 222, p. 1-11. https://doi.org/10.1016/j.fcr.2018.02.031

Lixandru, B. E., Dracea, N. O., Dragomirescu, C. C., Dragulescu, E. C., Coldea, I. L., Anton, L., Dobre, E., Rovinaru, C., Codita, I. 2010. Antimicrobial activity of plant essential oils against bacterial and fungal species involved in food poisoning and/or food decay. *Roum. Arch. Microbiol. Immunol.*, vol. 69, no. 4, p. 224-230. PMid:21462837

Lu, C., Zhang, C., Wen, J., Wu, G., Tao, M. 2001. Research of the effect of nanometer materials on germination and growth enhancement of Glycine max and its mechanism. *Soybean Science*, vol. 21, no. 3, p. 168-171.

Mahmoodzadeh, H., Nabavi, M., Kashefi, H. 2013. Effect of nanoscale titanium dioxide particles on the germination and growth of canola (*Brassica napus*). *Journal of Ornamental and Horticultural Plants*, vol. 3, no. 1, p. 25-32.

Martínez-Sánchez, F., Carvajal, M., Frutos, M., Giménez, J., Alcaraz, C. 1991. Titanium in the nutrition of *Capsicum annuum* L. plants. *Ciencia Agronómica*, vol. 11, p. 73-78.

Moaveni, P., Aliabadi-Farahani, H., Maroufi, K. 2011. Effect of TiO_2 Nanoparticles Spraying on Wheat (*Triticum aestivum* L.) Under Field Condition. *Advances in Environmental Biology*, vol. 5, no. 8, p. 2208-2210.

Murbach Teles Andrade, B. F., Nunes Barbosa, L., da Silva Probst, I., Fernandes Júnior, A. 2014. Antimicrobial activity of essential oils. *Journal of Essential Oil Research*, vol. 26, no. 1, p. 34-40. https://doi.org/10.1080/10412905.2013.860409

Nezami, S., Neamati, H., Aroei, H., Bagheri, A. 2012. Three genera of mint response to water stress under controlled conditions. *Agricultural Science and Technology*, vol. 26, no. 14, p. 1053-1061.

Omidbeigi, R. 2002. Pharmaceutical plants cultivation. *Raazi Magazine*, vol. 5, p. 24-39.

Omidbeigi, R. 2007. *Production and processing of medicinal plants.* Tehran, Iran : Tarrahan Nashe press, 348 p., ISBN; 9789640208274.

Owolade, O., Ogunleti, D., Adenekan, M. 2008. Titanium dioxide affects disease development and yield of edible cowpea. *EJEAF Chem*., vol. 7, no. 50, p. 2942-2947.

Pourmousavi, S., Galavi, M., Daneshian, J., Ghanbari, A., Basyrani, N. 2007. Effect of drought and manure on moisture content, the stability of the cell membrane and chlorophyll content of soybean leaves. *Journal of Agricultural Sciences and Natural Resources*, vol. 14, no. 4, p. 327-337.

Ribas-Carbo, M., Taylor, N. L., Giles, L., Busquets, S., Finnegan, P. M., Day, D. A., Lambers, H., Medrano, H., Berry, J. A., Flexas, J. 2005. Effects of water stress on respiration in soybean leaves. *Plant Physiology*, vol. 139, no. 1, p. 466-473. https://doi.org/10.1104/pp.105.065565 PMid:16126857

SAS Institute, I. 2003. SAS version 9.1, SAS institute Cary, NC.

Shafiei, M., Tadayon, M. R. 2014. Nano-titanium dioxide plant germination effect on some indices Okapi rapeseed oil. In: Proceeding of the Thirteenth meeting of the Conference of Crop Seeds of Science and Technology, Iran. Available at: https://www.civilica.com/Paper-NABATAT13-NABATAT13_0059.html

Sheoran, I. S., Koonjul, P., Attieh, J., Saini, H. S. 2014. Water-stress-induced inhibition of α-tubulin gene expression during growth, and its implications for reproductive success in rice. *Plant Physiology and Biochemistry*, vol. 80, p. 291-299. https://doi.org/10.1016/j.plaphy.2014.04.011 PMid:24814750

Shokri, H., Sharifzadeh, A. 2017. *Zataria multiflora Boiss*.: A review study on chemical composition, anti-fungal and anti-mycotoxin activities, and ultrastructural changes. *Journal of Herbmed Pharmacology*, vol. 6, no. 1, p. 1-9.

Taarit, M. B., Msaada, K., Hosni, K., Marzouk, B. 2010. Changes in fatty acid and essential oil composition of sage (*Salvia officinalis* L.) leaves under NaCl stress. Food chemistry, vol. 119, no. 3, p. 951-956. https://doi.org/10.1016/j.foodchem.2009.07.055

Trebichalský, P., Molnárová, J., Bajčan, D., Timoracká, M., Musilová, J., Harangozo, Ľ. 2015. Total polyphenols content in fruits of selected cultivars of strawberries in relation to concentrations of cadmium and lead in soil. *Potravinarstvo*, vol. 9, no. 1, p. 480-486. https://doi.org/10.5219/507

Wang, P., Lombi, E., Zhao, F.-J., Kopittke, P. M. 2016. Nanotechnology: a new opportunity in plant sciences. *Trends in Plant Science*, vol. 21, no. 8, p. 699-712. https://doi.org/10.1016/j.tplants.2016.04.005 PMid:27130471

Wojcik, P., Klamkowski, K. 2005. "Szampion" Apple Tree Response to Foliar Titanium Application. *Journal of Plant Nutrition*, vol. 27, no. 11, p. 2033-2046. https://doi.org/10.1081/PLN-200030108

Wojcik, P., Wojcik, M. 2001. Growth and nutrition of M. 26 EMLA apple rootstock as influenced by titanium fertilization. *Journal of Plant Nutrition*, vol. 24, no. 10, p. 1575-1588. https://doi.org/10.1081/PLN-100106022

Conflict of interest

The authors declare that they have no conflict of interests.

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CHANGES IN CHOSEN PROPERTIES OF SOFT CHEESES WITH CHILLI PEPPER DURING STORAGE

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ABSTRACT

The aim of this study was to evaluate chosen physicochemical and sensory properties of soft cheeses with addition of chilli peppers *Fatalii*. These samples were packed into plastic vacuum packages and analysed during 14 days of storage at cooling temperature (6 ±1 °C). Within the physicochemical properties, dry matter content, fat content, moisture in fat-free-substance, fat in dry matter and pH values were determined. Physicochemical analyzes, except pH value measure, were carried out only on the 1th day following the cheeses production. Textural properties hardness and stickiness were measured by the texture analyser. Within the sensory properties, consistency (hard, spreadable and friable) and taste (salty and spicy) were evaluated. Measurements of pH value, textural and sensory analysis were carried out on the 1st, 7th and 14th day of storage. All of cheese samples were classified as full-fat (FDM 48.21%) soft (MFFS 71.92%) cheeses. Their pH values decreased during 14 days of storage. The hardness of cheese samples decreased (p <0.05), while their stickiness statistic significantly increased during storage. From the view of sensory evaluation, the hard consistency of cheeses statistic significantly decreased and the spreadable consistency increased (p <0.05) during storage. The changes of friable consistency were no statistic significant. During storage of cheese samples were observed statistic significantly increase in the spicy taste and statistic significantly decrease in the salty taste.

Keywords: cheese; chilli pepper; analysis

INTRODUCTION

Cheese is a dairy product that has played a key role in human nutrition. The broad range of different cheeses available is based mainly on regional conditions and production technology, which has been repeatedly adapted and optimized. The ambition to provide nutritive rich foodstuffs with appetizing flavour increased with the development of technologies and the growing competition. The different ingredients are added to modify the taste and smell of cheese. Addition of sodium chloride is necessary for sensory properties and texture development of cheeses. The texture and appearance of cheese are as important as the taste and they are ones of the first properties that consumers use to judge the kind and quality of cheeses **(Elsamani et al., 2014; Remeňová et al., 2017; Hailu et al., 2018)**.

Chilli peppers were originated and domesticated in the American tropics, and cultivated in New Zealand, South Africa, Malaysia and other Asian counties. All spices of them are still not known, there are to be 25–27 spices of *Capsicum*, of which five are namely and domesticated *Capsicum annuum* L., *Capsicum frutescens* L., *Capsicum baccatum* L., *Capsicum chinense* and *Capsicum pubescens* Ruiz and Pavon. Within these species there are several cultivars, these differ in shapes, sizes, colours and flavours **(Ali et al., 2016)**. Chilli peppers *Fatalii* are the kind of chilli peppers, which belong to species *Capsicum chinense* and come from South Africa. After maturation, colour of fatalii is yellow, red or orange **(Peter, 2001)**.

Chilli pepper is used as a food ingredient to add pungency due to the accumulation of capsaicin and spicy flavour, as well as a food colourant or natural preservative. The colours of chilli pepper are due to a mixture of esters of capsanthin, capsorubin, zeaxanthine, cryptoxanthine and other carotenoids. These extractable colours are used in the food processing industry to wide range of products such as meat products, cheeses, butters and condiment mixtures. Chilli pepper may be used as a spice and flavouring when it is dried and ground up, but also can be employed whole and alone or in combination with other flavouring agents. Capsaicin is the main active component of chilli peppers, followed by dihydrocapsaicin, homocapsaicin and others. These are called capsaicinoids together and are responsible for the pungency of chilli peppers. Chilli peppers contain

vitamins A, B-complex, C and E, and are rich in beta carotene and minerals like molybdenum, manganese and potassium. Chilli peppers have a number of biological properties and potential health benefits, such as antioxidant, anti-inflammatory, anti-arthritic, anticancer and antifungal properties **(Kothari et al., 2010; Tunde-Akintunde, 2010; Sricharoen et at., 2017; Ghanimah et al., 2018)**.

The physical and textural properties of cheese are influenced by the milk composition and manufacturing procedures. During storage, the textural properties of various cheeses can change as a result for biochemical processes **(Nedomová et al., 2017; Alinovi et al., 2018)**. Cheese ripening is a complex process consisting of microbiological, biochemical and chemical reactions that result in physicochemical changes, such as changes of pH value and breakdown of protein. These changes lead to loses firmness and toughness of cheese **(Aminifar et al., 2010)**. Hardness and stickiness belong to the monitored properties of cheeses. Hardness (firmness) is defined as high resistance to deformation by applied stress. Stickiness (adhesiveness) is defined as the tendency of cheese to resist separation from another material with which it makes contact **(Fox et al., 2004)**. The consistency of cheeses is affected by the final composition of cheese (dry matter content, fat content, fat in dry matter and pH value), the composition of raw materials, by the production technology and the storage conditions of cheese **(Černíková et al., 2017)**. In the development of texture, two distinct phases were identified. Within the first 7-14 days, residual coagulant enzymes are responsible for hydrolysis of α_{s1}-casein to the soluble fraction. This process reduces the rubbery texture of the cheese. The second phase includes proteolysis of the protein **(Hort and Le Grys, 2001)**. To produce a cheese with a suitable flavour and texture properties, the dairy industry has to monitor all the outcomes of the whole process, from the herd to distribution network. In monitoring and assessing sensory properties of cheese, the interaction between milk quality and the type of cheese to be produced is necessary to consider **(Cipolat-Gotet et al., 2018)**. Sensory methods study the sensory attributes of products, for example cheese, giving a profile consisting of taste, smell and texture for the product. In soft cheeses, flavour compounds such as ketones, acids, alcohols, esters are especially important **(Westling et al., 2016)**.

The aim of this study was to evaluate chosen physicochemical, textural and sensory properties of soft cheese with addition of chilli peppers during 14 days of storage.

Scientific hypothesis

We tested the changes of physicochemical, textural and sensory properties of soft cheeses with chilli addition during refrigerated storage in plastic vacuum packages. We expect that flavouring additive (chilli peppers) will have a positive effect on sensory and textural properties of cheeses.

MATERIAL AND METHODOLOGY

Preparation of chilli peppers

Chilli peppers *Fatalii* were obtained from a self-employed grower. Before application into cheese, chilli peppers were dried by hot air dryer at 50 °C. Chilli peppers were grinded up also with seeds and kept in dark until the use.

Preparation of soft cheese

Soft cheeses were made and assessed in Department of Evaluation and Processing of Animal Products, Slovak University of Agriculture in Nitra. Before production, experimental samples with different additions of chilli peppers *Fatalii* (0.1 g, 0.2 g and 0.351 g per 100 g of cheese) were made for determination of optimal addition. As optimal addition of chilli peppers was determined 0.351 g. For this experiment was used raw cow milk from dairy vending machine. Milk was heated up to 72 °C during 30 sec and then milk temperature was adjusted to 35 °C for the addition of calcium chloride (Reachem Slovakia, s. r. o., Slovak Republic, 40% w/v) and starter culture (Laktoflora®, Milcom a. s., Czech Republic). After a rest period of 30 min, microbial coagulant (Milase®, CSK Food enrichment, Netherlands) was added. After coagulation, the curd was cut into cubes, reheated at 39 °C and then drained. The curd was mixed with chilli peppers, formed and then dripped out. The cheese was salted in saline solution (NaCl 6% w/v), dried, packed into plastic vacuum packages and stored at 6 ±1 °C for up to 14 days.

Physicochemical analysis

Dry matter content, fat content and pH values of cheese were determined. Analyzes were performed at least in duplicate. Dry matter content and fat content were determined on the 1st day following cheese production. Dry matter content was determined by a gravimetric reference method **(ISO 5534:2004)** by drying to constant weight at 102 ±2 °C and content of fat by Gerber´s acidobutyrometric method **(Cvak et al., 1992)**.

FDM – fat in dry matter (Eq. 1) and

MFFS – moisture in fat-free-substance (Eq. 2) were calculated according to the following equations **(Regulation MARD SR No 343/2016)**:

$$(1) \qquad \text{Fat in dry matter } (\%) = \frac{\text{fat (g) x 100}}{100 - H_2O\ (g)}$$

$$(2) \qquad \text{Moisture in fat} - \text{free} - \text{substance } (\%) = \frac{\text{moisture in 100 g of cheese (g)}}{100 - \text{fat in 100 g of cheese (g)}} \text{ x } 100$$

The pH values of the cheese were measured by pH meter Orion Star A211 (Thermo Fisher Scientific, USA). The pH measurement was carried out on the 1st, 7th and 14th day following the cheese production.

Textural analysis

Textural properties were measured on a texture analyser TA.XT Plus (Stable Micro Systems LTD., UK). The texture analyser was used to measure the chosen textural properties such as hardness and stickiness of cheeses. The texture analysis was carried out on the 1st, 7th and 14th day

following the cheeses production. The test was conducted on pieces of cheese (2 cm x 2 cm), using spherical probe (P/1S) and 5 kg load cell. Test speed of probe was 2 mm per sec and a distance reached in the pieces of cheese was 5 mm. Temperature of cheese was 15 °C. The course of measurement was recorded through the curves by Texture Exponent software 6.1.4.0 (Stable Micro Systems LTD., UK).

Sensory analysis

Sensory properties, consistency (hard, spreadable and friable) and taste (salty and spicy) were evaluated. Sensory analysis was performed by ten-member committee of assessors who evaluated selected parameters by five point scale. Evaluation was carried out on the 1st, 7th and 14th day following the cheeses production.

Statisic analysis

The entire experiment was replicated three times and the resulting value was calculated as the mean value, standard deviation and variation coefficient of these measurements. Obtained results were processed by variation-statistical method in ANOVA of Statistica CZ9.1 software (Stat Soft Ltd., CZ). The differences were considered significant at the p <0.05 level.

RESULTS AND DISCUSSION

Physicochemical analysis

On the first day of storage, experimental samples of cheese had dry matter content 42.98% ±2.10% and fat content 20.73% ±1.79% (Table 1). Fat in dry matter of all experimental cheeses was higher than 45% and lower than 60% and therefore all cheeses were classified as full-fat cheese. All experimental samples of cheese were classified as soft cheeses from the point of view of moisture in fat-free-substance **(Regulation MARD SR No 343/2016)**.

The pH measurement is important in the cheese production and storage. The pH values were in range from 5.15 to 4.90 during 14 days of storage (Figure 1). During first 7 days of storage, the pH value decrease was major compared to decrease after next 7 days. Twenty-four hours after cheese production, the pH values of cheeses are an indicator of right preliminary ripening of cheeses.

Textural analysis

During 14 days of refrigerated storage, a change in consistency of cheeses is to be expected **(Černíková et al., 2017)**. The texture of cheese is determined by its composition, microstructure and processing, including its microflora **(Aryana and Haque, 2005)**.

Table 1 Chemical analysis of cheese samples on the first day of storage.

	Dry matter content g.100 g^{-1}	Fat content g.100 g^{-1}	Fat in dry mater %	Moisture in fat-free-substance %
1. experimental sample	40.01	19.00	47.49	74.04
2. experimental sample	44.38	20.00	45.06	69.52
3. experimental sample	44.55	23.20	52.08	72.21
Average	42.98	20.73	48.21	71.92
Standard deviation	2.10	1.79	2.91	1.86
Coefficient of variation (%)	4.89	8.64	6.04	2.58

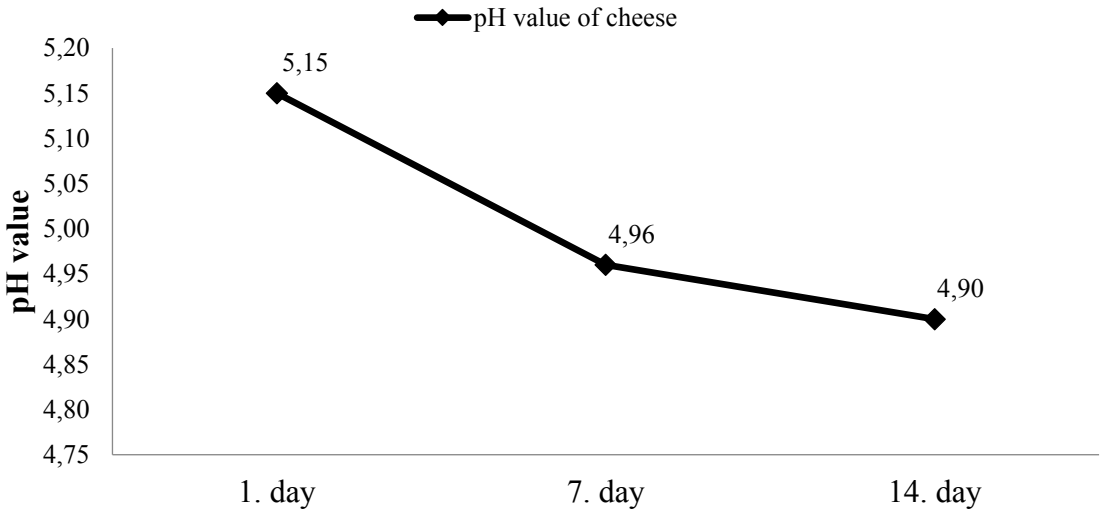

Figure 1 The pH values of cheese samples during 14 days of storage.

Table 2 Textural properties of cheese samples during 14 days of storage.

Experimental sample		1. day		7. day		14. day	
		Hardness g	Stickiness g	Hardness g	Stickiness g	Hardness g	Stickiness g
1	**Average**	420.41	-3.05	267.42	-8.01	243.62	-12.28
	Standard deviation	43.82	0.81	4.34	3.94	12.94	5.59
	Coefficient of variation (%)	10.42	-26.56	1.62	-49.19	5.31	-45.52
2	**Average**	412.39	-4.18	261.94	-9.68	194.82	-20.22
	Standard deviation	22.15	2.20	13.68	7.48	9.93	4.45
	Coefficient of variation (%)	5.37	-52.63	5.22	-77.27	5.10	-22.01
3	**Average**	415.39	-3.81	262.94	-9.45	214.82	-18.28
	Standard deviation	25.22	1.75	10.28	5.36	11.97	4.85
	Coefficient of variation (%)	6.07	-45.93	3.91	-56.72	5.57	-26.53

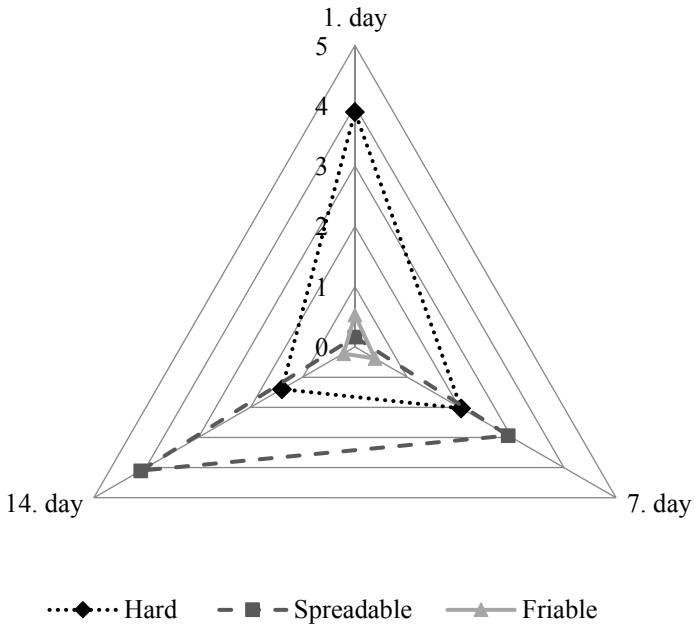

Figure 2 Sensory evaluation of consistency of cheese samples during 14 days of storage.

In all experimental samples of cheese decreased their hardness during 14 days of storage (Table 2). On the last day of storage, the hardness of cheeses showed statistically significant decrease (p <0.05) compared to the first day of storage. We suppose that the changes are due to the cheese ripening and the change of protein structure. **Eroglu et al. (2015)** reported that the texture of cheeses depends on the chemical composition of cheese, biochemical changes occurring throughout ripening and their textural properties are influenced by pH value during ripening period.

The highest stickiness of cheese was determined on the last day of storage (Table 2). During storage it was found statistically significant increase (p <0.05) in the stickiness of all experimental samples of cheese.

Sensory analysis

The assessors provided a lower score for hard and friable consistency of cheeses on the 7th and 14th day of storage compared on the 1st day (Figure 2). By contrast, the spreadable consistency statistically significant increased (p <0.05) during storage (Figure 2).

On the last day of storage, the hard consistency showed statistic significantly decrease (p <0.05) compared to the first day. This evaluation of hard consistency is in accordance with textural analysis of cheeses.

Brown et al. (2003) reported that the correlation of sensory and textural analysis associated with hardness is not surprising because the evaluations are very similar between instrumental and human techniques.

The sensory evaluation showed no statistic significantly decrease (p >0.05) of friable consistency throughout the storage. **Delgado et al. (2010)** reported that proteolysis contributes to cheese matrix textural changes due to the protein network breakdown.

Our results of pH values, textural analysis of hardness and sensory analysis of hard consistency are comparable with findings of **Chen et al. (2015)**, whoreported that

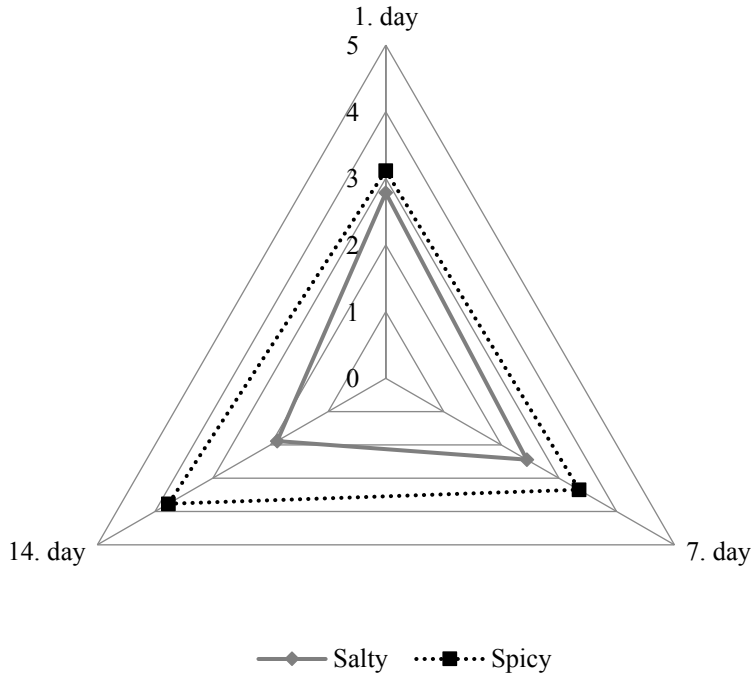

Figure 3 Taste of cheese samples during 14 days of storage.

cheeses with lower pH normally became less firm and their hardness decreased. There is a significant correlation between the pH and hardness of soft cheeses.

Changes in salty and spicy taste of cheese were observed during 14 days of storage (Figure 3). During storage period, the salty taste of cheese gradually decreased while the spicy taste gradually increased. After 14 days of storage, the score of spicy taste was statistic significantly higher (p <0.05) compared to the score on the 1st day of storage. The salty taste of cheese samples was statistic significantly lower (p <0.05) at the end of storage. We suppose that these changes are related to the diffusion of salt and capsaicinoids into the whole cheese during storage.

CONCLUSION

Addition of dried, crushed chilli peppers *Fatalii* had no negative effect on ongoing biochemical processes in full-fat soft cheeses stored during 14 days. The proofs are the changes of pH values and mainly the changes of textural properties. From the view of sensory evaluation, the textural properties of cheeses were evaluated better on the last day of storage compared to the first day. The spicy taste of cheeses became more intensive during storage however this change was unacceptable for some assessors. Our results show that the spicy taste becomes more intensive during storage and therefore the addition of chilli peppers *Fatalii* need to be reduced in cheese production. According to the Scoville heat scale, the pungency of chilli peppers Fatalii is from 125,000 to 350,000 units. The next possibility of using chilli pepper by the cheese production is to use another kind of chilli peppers with less pungency.

REFERENCES

Ali, A., Bordoh, P. K., Singh, A., Siddiqui, Y., Droby, S. 2016. Post-harvest development of anthracnose in pepper (*Capsicum* spp): Etiology and management strategies. *Crop Protection*, vol. 90, p. 132-141. https://doi.org/10.1016/j.cropro.2016.07.026

Alinovi, M., Rinaldi, M., Mucchetti, G. 2018. Spatiotemporal Characterization of Texture of Crescenza Cheese, a Soft Fresh Italian Cheese. *Journal of Food Quality*, vol. 2018, p. 1-8. https://doi.org/10.1155/2018/5062124

Aminifar, M., Hamedi, M., Emam-Djomeh, Z., Mehdinia, A. 2010. Microstructural, compositional and textural properties during ripening of Lighvan cheese, a traditional raw sheep cheese. *Journal of Texture Studies*, vol. 41, no. 4, p. 579-593. https://doi.org/10.1111/j.1745-4603.2010.00244.x

Aryana, K. J., Haque, Z. Z. 2005. Texture and microflora of vallagret cheese during maturation. *International Journal of Dairy Technology*, vol. 58, no. 1, p. 47-50.https://doi.org/10.1111/j.1471-0307.2005.00181.x

Brown, J. A., Foegeding, E. A., Daubert, C. R., Drake, M. A., Gumpertz, M. 2003. Relationships Among Rheological and Sensorial Properties of Young Cheeses. *Journal of Dairy Science*, vol. 86, no. 10, p. 3054-3067. https://doi.org/10.3168/jds.s0022-0302(03)73905-8

Cipolat-Gotet, C., Cecchinato, A., Drake, M. A., Marangon, A., Martin, B., Bittante, G. 2018. From cow to cheese: Novel phenotypes related to the sensory profile of model cheeses from individual cows. *Journal of Dairy Science,* vol. 101, no. 7, p. 5865-5877. https://doi.org/10.3168/jds.2017-14342

Cvak, Z., Peterková, Ľ., Černá, E. 1992. *Chemické a fyzikalně chemické metódy v kontrole jakosti mléka a mlékárenských výrobků*. (Chemical and physicochemical methods in the quality control of milk and dairy products) Praha, Czech Republic: VÚPP. 221 p. (In Czech)

Černíková, M., Nebesářová, J., Salek, R. N., Řiháčková, L., Buňka, F. 2017. *Journal of Dairy Science*, vol. 100, no. 6, p. 4300-4307. https://doi.org/10.3168/jds.2016-12120

Delgado, F. J., Rodríguez-Pinilla, J., González-Crespo, J., Ramírez, R., Roa, I. 2010. Proteolysis and texture changes of a Spanish soft cheese ('Torta del Casar') manufactured with raw ewe milk and vegetable rennet during ripening. *International Journal of Food Science & Technology*, vol. 45, no. 3, p. 512-519. https://doi.org/10.1111/j.1365-2621.2009.02157.x

Elsamani, M. O., Habbani, S. S., Babiker, E. E., Ahmed, I. A. M. 2014. Biochemical, microbial and sensory evaluation of white soft cheese made from cow and lupin milk. *LWT - Food Science and Technology*, vol. 59, no. 1, p. 553-559. https://doi.org/10.1016/j.lwt.2014.04.027

Eroglu, A., Toker, O. S., Dogan, M. 2015. Changes in the texture, physicochemical properties and volatile compound profiles of fresh Kashar cheese (<90 days) during ripening. *International Journal of Dairy Technology*, vol. 69, no. 2, p. 243-253. https://doi.org/10.1111/1471-0307.12250

Fox, P. F., McSweeney, P. L. H., Cogan, T. M., Guinee, T. P. 2004. *Cheese Chemistry, Physics and Microbiology*. 3rd ed.London, England: Elsevier Ltd. 617 p. ISBN 0-1226-3652-X

Ghanimah, M., Hanafy, N., Hassanein, A., Hashim, M. 2018. Utilisation of water extract of green chilli pepper in the manufacture of low-fat fresh cheese. *International Journal of Dairy Technology*, vol. 71, p. 1-6. https://doi.org/10.1111/1471-0307.12505

Hailu, Y., Hansen, E. B., Seifu, E., Eshetu, M., Petersen, M. A., Lametsch, R., Rattray, F., Ipsen, R. 2018. Rheological and sensory properties and aroma compounds formed during ripening of soft brined cheese made from camel milk. *International Dairy Journal*, vol. 81, p. 122-130. https://doi.org/10.1016/j.idairyj.2018.01.007

Hort, J., Le Grys, G. 2001. Developments in the textural and rheological properties of UK Cheddar cheese during ripening. *International Dairy Journal*, vol. 11, no. 4-7, p. 475-481. https://doi.org/10.1016/s0958-6946(01)00074-7

Chen, B., Lewis, M. J., Grandison, A. S. 2015. Effect of seasonal variation on the properties of whipping cream, soft cheese and skim milk powder in the UK. *International Journal of Dairy Technology*, vol. 68, no. 1, p. 30-37. https://doi.org/10.1111/1471-0307.12181

ISO 5534:2004. *Cheese and processed cheese - Determination of the total solids content*.

Kothari, S. L., Joshi, A., Kachhwaha, S., Ochoa-Alejo, N. 2010. Chilli peppers — A review on tissue culture and transgenesis. *Biotechnology Advances*, vol. 28, no. 1, p. 35-48. https://doi.org/10.1016/j.biotechadv.2009.08.005

Nedomová, Š., Kilián, L., Pytel, R., Kumbár, V. 2017. Effect of ripening time on colour and texture properties in cheese. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 296-301. https://doi.org/10.5219/744

Peter, K. V. 2001. *Handbook of herbs and spices*. Cambridge, England, UK : Woodhead Publishing limited. 365 p.

RegulationNo 343/2016 of the Ministry of Agriculture and Rural Development of the Slovak Republic of 8thDecember 2016 on certain dairy products. 1.4.2017, p. 1-16.

Remeňová, Z., Čanigová, M., Kročko, M., Ducková, V., Šimková, A. 2017. Effects of floral honey and pressed flax seeds on intensity of yogurt aroma, sweetness and sour taste of yogurts during storage. *Potravinarstvo*, vol. 11, no. 1, p. 436-440. https://doi.org/10.5219/760

Sricharoen, P., Lamaiphan, N., Patthawaro, P., Limchoowong, N., Techawongstien, S., Chanthai, S. 2017. Phytochemicals in *Capsicum* oleoresin from different varieties of hot chilli peppers with their antidiabetic and antioxidant activities due to some phenolic compounds. *Ultrasonics Sonochemistry*, vol. 38, p. 629-639. https://doi.org/10.1016/j.ultsonch.2016.08.018

Tunde-Akintunde, T. Y. 2010. Effect of pretreatment on drying time and quality of chilli pepper. *Journal of Food Processing and Preservation*, vol. 34, p. 595-608. https://doi.org/10.1111/j.1745-4549.2008.00360.x

Westling, M., Danielsson-Tham, M. L., Jass, J., Nilsen, A., Öström, A., Tham, W. 2016. Contribution of Enterobacteriaceae to Sensory Characteristics in Soft Cheeses Made from Raw Milk. *Procedia Food Science*, vol. 7, p. 17-20. https://doi.org/10.1016/j.profoo.2016.02.075

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THE EFFECT OF FOLIAR FEEDING ON PHYSIOLOGICAL CONDITION OF APPLE TREES AND CHEMICAL CONTENT OF FRUITS

Valentina Popova, Olesya Yaroshenko, Natalya Sergeeva

ABSTRACT

Chemical content of leaves and fruits of the low growing apple trees on the rootstocks SK4 and M9 in the result of application of foliar feeding with water solutions of various mineral fertilizers was studied. Special ballastless multi-nutrient fertilizers in solid and liquid forms containing a wide range of chelated microelements were used. The effect of foliar feeding on the content in plants and fruits of macro- and microelements at the fruit maturity stage was estimated with the help of the method of leaf analysis. Significant stable increase of the content of nitrogen and potassium was observed in the leaves of apple trees on the rootstocks SK4 and M9. The content of potassium and calcium in apples became higher. With the help of statistical analysis it is shown that there exists close correlation between the content of elements in leaves and fruits: (N) $r = 0.79$; (K) $r = 0.77$; (Ca) $r = 0.94$; (Cu) $r = 0.75$; (Mn) $r = 0.89$; (Zn) $r = 0.75$; (B) $r = 0.70$. In the result of our physiological and biochemical tests positive effect of mineral feeding on the functional condition of apple trees during summer season when being subjected to intensive hydrothermal stress factors was established. The foliar feeding effect appeared as changing of the ratio of water fractions in the apple tree leaves at the background of increasing atmospheric drought, which in some years in July and August reached the criteria of "hazardous weather". Sufficient level of water content in the cells of apple trees on the rootstocks SK4 and M9 in case of foliar application of fertilizers was ensured as a result of bound water increasing. Statistic analysis of the experimental data showed significant changes in the ratio of bound and free water fractions. In August, in the environment of strong impact of negative abiotic factors, at higher level of water content in the tissues it was found that the content of pigments in leaves was much higher than in the reference version. The functional changes confirm that application of mineral nutrients contributes to activation of the adaptation mechanism. When foliar feeding is used the content of biologically active substances in apples is the highest: content of vitamin C increases by 13.6 – 15.2%, vitamin P – by 8.7 – 24.6%.

Keywords: apple tree; abiotic stress; foliar fertilizer; adaptability; quality of apples

INTRODUCTION

In the southern regions of Russia the period of summer vegetation of plants in the recent years is characterized by durable hyperthermia alongside with high insolation, drought conditions and dry hot winds. For perennial fruit plants negative impact of abiotic factors causes destruction of physiological homeostasis and results in shifts in the process of form-building, failure of reproductive function, decrease of fruits quality **(Gudkovsky, 2005; Nenko et al., 2014, 2015; Doroshenko, Zakharchuk and Ryazanov, 2000, 2010; Goncharova, 2011, Srinivasa Rao, Laxman and Shivashankara, 2016)**. Current importance of finding proper solutions for enhancement of the fruit plants adaptability under conditions of physical stresses is regularly covered in scientific literature **(Zhu, 1997; Vardanov, 2003; Wood, 2005; Šircelj, 2007; Shanker, 2011; Doroshenko, Chumakov and Maksimtsov, 2012; Yushkov, 2016, Sofo and Palese, 2012)**. One of the most efficient methods for normalization of functional status of perennial fruit plants consists in optimization of the content in plants of biogenic elements, participating in biochemical and physiological metabolism **(Trunov et al. 2009, 2011, Abilfazova and Belous, 2015)**. Based on the known mechanism of retention and transportation by leaves of ions from solutions of nutritive salts there was developed the method of foliar feeding, ensuring prompt introduction of mineral elements into metabolic processes at different stages of plant growing **(Rogachev, 2008; Nenko, 2015, Ryndin et al., 2017)**. Systematic implementation in the technological procedure of fruit plans growing of foliar feeding with the help of water solutions of macro- and microelements represents a perspective method for enhancement of the plants adaptability, stabilization of agrocoenosis functioning in general.

In the process of investigation of the possibility of actual controlling the physiological parameters of fruit plants under unstable environmental conditions with the help of foliar feeding method, during 2014 – 2017 we performed field tests with fruit-bearing low growing apple trees of a group of varieties.

Scientific hypothesis

The principal assumption taken as basis for the objective of the performed researches was the working hypothesis of regulatory function of nutritive salts on the adaptability of apple trees, which is of crucial importance for production of fruits of high quality having valuable economic traits.

MATERIAL AND METHODOLOGY

Experimental works were carried out with the help of field and laboratory research methods. For performance of field and analytical works there were used biological, agrochemical and physiological procedures. The field tests were located in the experimental production enterprise "Central" of the North-Caucasian Scientific Research Institute of Horticulture and Viticulture (Krasnodar) in the garden set out in 2009. Geographically, the experimental site is located in the central plains of the Krasnodar Territory. Height above sea level varies from 19 to 32 m. The climate is temperate-continental. In the southern part, there is a subtropical climate at times, especially in the summer and deep autumn. Winter is short and warm. Summer here is long and hot. In the off-season, rain falls often and the winds blow. The annual amount of precipitation is in the flat part from 400 to 600 mm. The average air temperature is +12.1 °C (in recent years, the average annual temperature is kept at 13.3 °C).

The object of researches in 2014 – 2015 were low growing apple trees on the rootstock SK4 (Idared, Prikubanskoe varieties), in 2016 – 2017 - apple trees on the rootstock M9 of Szampion variety (Figure 1).

The apple trees varieties and rootstocks were released for the regional conditions. The plantation of fruit-bearing apple trees occupies an even plot of land. The garden soils are represented by extra-thick low-humic leached chernozem. The key parameters of the garden soil are as follows: pH value of aqueous extract is neutral, 7.2 – 7.3 at the depth of 0 – 20 cm, 7.2 at the depth of 20 – 40 cm. Humus content in the surface soil is 2.9 – 3.3%. Content of nitrate nitrogen for (0 – 20 cm) is 5.4 – 5.5 mg.kg^{-1}; (20 – 40 cm) 0.9 – 2.4 mg.kg^{-1}. Quantity of labile phosphorus for (0 – 20 cm) is 385 – 397 mg.kg^{-1}; (20 – 40 cm) 304 – 308 mg.kg^{-1}. Content of exchangeable potassium for (0 – 20 cm) is 266 – 345 mg.kg^{-1}; (20 – 40 cm) 133 – 239 mg.kg^{-1}.

The tests were performed in four replications. For each replication there were 6 estimated plants. Trees were sprayed tree times with the help of a back-pack sprayer: 15 days after blossom, at the stage of fruit inception and growing, after the June fruit reduction.

In 2014 – 2015 the apple trees were sprayed with 0.5% water solution of polynutrient salts of "Aquarin" series (manufactured by JSC "Buisk chemical plant", Russia), having the following chemical compounds: N12P12K35Mg2S0,7. The fertilizers include chelated microelements of Fe, Cu, Zn, Mn, Mo.

In 2016 – 2017 there were applied foliar feedings with liquid polynutrient nitrogen-calcium fertilizer "SeliKa" (manufactured by LLC "Kuban-agro-humates") including: N18Ca19 + chelated microelements (Fe, Al, Ni, Mn, Zn, Mo, Co, Cu). The fertilizer dosage for trees spraying was as follows: 1 version – 10 L.ha^{-1}; 2 version – 15 L.ha^{-1} at the consumption of spray solution 800 L.ha^{-1}.

As a reference version there was taken the version of spraying trees with pure water free of any fertilizers.

The chemical content of apple leaves and fruits was studied after accelerated wet ashing **(Voskresenskaya, 2006)**. In the ashed material the total content of nitrogen was measured with the help of chloramine-T method, of phosphorus – by "blue" phosphatomolybdic complex with colorimetric endpoint determination on photocolorimeter KFK-3-01 ("Zagorsk optical and mechanical plant", Russia), of potassium – using the method of flame photometry at the spectrophotometer PFA-354 (OOO "UNICO-SIS", Russia) of calcium and magnesium – with the help of complexometry **(Voskresenskaya, 2006)**.

Physical characteristics of the apple tree condition were determined with classical methods: the factor of thermal drought – by Kushnirenko **(Kushnirenko, 1986)** (Moisture Analyzer ML-50, A&D Company Limited, Japan), the content of photosynthetic pigments – with spectral method (UNICO 2800 SpectroQuest, UNITED PRODUCTS & INSTRUMENTS (USA)).

Statisic analysis

Content in apples of total sugars, organic acids, vitamin C, bioflavonoids was measured with the help of appropriate methodological guidelines **(Volobueva, 2008)**. Processing of experimental data was carried out by methods of correlation, regression and dispersion analysis out in compliance with the recommended procedures **(Volkov, 2005)**. All calculations were made with the help of Microsoft Office 2010 software package ("Microsoft, Inc.", USA).

Figure 1 The picture of Apple fruit researched in the experience.

RESULTS AND DISCUSSION

The intensity and duration of stress factors were reported on the basis of regular registration of variations of the daytime air temperature, quantity and periodicity of atmospheric precipitations falling during summer season. Observations showed that within the period of 2014 – 2015 the hydrothermal stress factors were increasing starting from June. Every year in the first decade of June the maximum daytime air temperature was about 28 – 31 °C. Minor precipitations were falling from time to time (0.3 – 10.3 mm), therefore during a month the air humidity sometimes increased.

At the beginning of the first decade of July the air temperature reached 31 – 32 °C. During the second and third decades it was sometimes above 34 – 36 °C. Precipitations fell with intervals of 3 – 5 days in quantity of 0.1 – 1.3 and 3.4 – 22.0 mm.

In 2014 and 2015 there were no rainfalls in August, for a long time the maximum air temperature was registered at the level of 34 – 40 °C, maximum air humidity sometimes was 12 – 14%.

The summer seasons of 2016 – 2017 were also characterized by hydrothermal stresses. In 2016, starting from the second decade of July till the second decade of September, the maximum daytime air temperature reached 34 – 38 °C. Duration of rainless period exceeded one and a half months. In 2017 from July till the end of August the air temperature regularly increased up to 33 – 40 °C. Rainless period lasted over 40 days. The atmospheric drought meeting the criteria of "hazardous weather" was reported.

Under such conditions at the initial stage of experiment the chemical content of indicative plant organs and content of mineral elements in apple fruits were analyzed in dynamics at the background of feeding with foliar-applied fertilizers **(Popova, 2014; Sergeeva, 2015; Yaroshenko, 2016)**. It was ascertained that the leaf-dressing with special mineral fertilizers had effect on the chemical content of apple leaves and fruits, analyzed at the maturity stage (Table 1). Such tendency was maintained within the whole period of researches. Stable significant increase of the content of nitrogen and potassium was found in the leaves of apple trees on the rootstocks SK4 and M9. For apple trees on the rootstock M9 the most increase of potassium in leaves was determined in case of feeding with aqueous solution of fertilizer at the dosage of 15 $L.ha^{-1}$. The highest as compared with the reference version increase of the content of potassium in apples was determined in all versions of the experiment with application of fertilizers. The content of

Table 1 Effect of fertilizers on the content of macronutrients in the leaves and fruits of apple, %.

Variation	N		P		K		Ca		Mg	
	Leaf*	**Fruit**	**Leaf***	**Fruit**	**Leaf***	**Fruit**	**Leaf***	**Fruit**	**Leaf***	**Fruit**
					Rootstock SK 4					
Control	2.0 ±0.05	0.45 ±0.019	0.21 ±0.002	0.021 ±0.001	1.0 ±0.026	0.68 ±0.015	2.2 ±0.05	0.18 ±0.001	0.30 ±0.003	0.083 ±0.001
N12P12K35Mg2S0,7+Fe, Cu, Zn, Mn, Mo, B	2.4 ±0.02	0.48 ±0.017	0.22 ±0.003	0.019 ±0.001	1.1 ±0.029	0.78 ±0.012	2,3 ±0.33	0.19 ±0.002	0.34 ±0.026	0.083 ±0.026
LSD ($p \leq 0.05$)	0.11	0.05	0.01	0.002	0.08	0.04	0.23	0.01	0.05	0.006
					Rootstock M 9					
Control	2.2 ±0.016	0.34 ±0.006	0.23 ±0.009	0.059 ±0.009	0.64 ±0.004	0.57 ±0.008	1.99 ±0.025	0.19 ±0.005	0.53 ±0.01	0.03 ±0.005
10 $L.ha^{-1}$	2.3 ±0.007	0.35 ±0.006	0.23 ±0.01	0.061 ±0.001	0.75 ±0.023	0.57 ±0.006	1.96 ±0.037	0.16 ±0.005	0.54 ±0.018	0.022 ±0.005
15 $L.ha^{-1}$	2.3 ±0.025	0.38 ±0.006	0.24 ±0.009	0.062 ±0.001	0.88 ±0.03	0.69 ±0.04	2.04 ±0.023	0.21 ±0.003	0.57 ±0.015	0.02 ±0.003
LSD ($p \leq 0.05$)	0.10	0.03	0.04	0.003	0.13	0.03	0.14	0.018	0.05	0.018

Note: * The content of mineral elements in leaves in the second decade of August, pouring fruit.

Table 2 Effect of fertilizers on the content of micronutrients in the leaves and fruits of apple, $mg.kg^{-1}$.

Variation	Cu		Zn		Mn		B		Fe
	Leaf*	Fruit	**Leaf***	Fruit	**Leaf***	Fruit	**Leaf***	Fruit	**Leaf***
				Rootstock SK 4					
Control	2.9 ±0.15	3.0 ±0.15	15.9 ±0.18	2.2 ±0.19	19.3 ±0.82	0.3 ±0.03	9.4 ±0.57	4.1 ±0.12	33.4 ±0.95
N12P12K35Mg2S0,7+Fe, Cu, Zn, Mn, Mo, B	3.1 ±0.1	2,9 ±0.2	15.5 ±1.07	2.5 ±0.39	19.7 ±0.35	0.5 ±0.33	10.7 ±0.15	4.5 ±0.17	34.4 ±0.80
LSD ($p \leq 0.05$)	0.4	0.5	2.1	0.8	1.8	0.1	1.2	0.4	2.4

Note: * The content of mineral elements in leaves in the second decade of August, pouring fruit.

calcium also increased (by 5.6%), but slightly. Foliar feeding had no effect on the content of phosphorus and magnesium in fruits. In apples of Szampion variety apparent increase of the content of nitrogen at the maturity stage was established only at the background of feeding with nitrogen-calcium fertilizer in the maximum dosage. Accumulation of nitrogen did not exceed the maximum permissive limits.

Correlation analysis of quantitative parameters of the mineral content in apple leaves (X) and fruits (Y) revealed rather close interrelation between such values. The correlation factors are as follows: (nitrogen) r = 0.79; (potassium) r = 0.77; (calcium) r = 0.94.

Our researches have shown that for the apple trees on the rootstock CK4 certain accumulation of microelements was observed in the leaves and fruits in the result of application of the solution of special multi-nutrient fertilizer "Aquarin" (Table 2). Availability in the fertilizer formula of chelated microelements contributed to increasing the content in the leaves of ferrum (3%), copper (7%), manganese (2%), boron (14%). Apparent increase of the content of ferrum, copper and manganese in the leaves was not confirmed statistically, however in the apples at maturity stage there was measured significant increase of quantity of manganese and boron as compared to the reference version. The resulting experimental data were subjected to correlation analysis and interrelation between the parameters in the "leaf-fruit" system was established: (copper) r = 0.75; (manganese) r = 0.89; (zinc) r = 0.75; (boron) r = 0.70.

Further stage of researches was dealing with study of the effect of mineral foliar feeding and changes of the apple feeding schedule on physiological condition of apple trees and their functional stability during the period of summer abiotic stresses **(Popova, 2013, 2014, 2017; Yaroshenko, 2014, 2017; Sergeeva, 2014, 2015)**. The interrelation between increase of the content of macro- and micronutrients in the apple trees and dynamics of fractional water composition in the leaves under conditions of intensification of hydrothermal stress factors was analyzed (Table 3). Such parameter is helpful for identification of the water balance, it characterizes the level of water supply for an apple tree in the drought environment and can be regarded as a factor of plant stability. In May, in the absence of negative impact of abiotic factors, prior to application of water solutions of fertilizers to the apple trees on the rootstock CK4, the content of water in leaves of the reference version was much higher. The ratio of water fractions was 4.1 and 6.1 respectively. Already in June, after

Table 3 Seasonal dynamics of fractional water composition in apple tree leaves, %.

Variation	May		June		July		August	
	free form of water	bound form of water	free form of water	bound form of water	free form of water	bound form of water	free form of water	bound form of water
			Rootstock SK 4					
Control	17.8	82.2	38.0	62.0	38.8	61.2	21.1	77.9
N12P12K35Mg2S0,7+ Fe, Cu, Zn, Mn, Mo, B	14.0	86.0	34.7	65.3	31.2	68.8	20.1	79.9
LSD ($p \leq 0.05$)	2.9	4.4	2.7	3.4	4.3	6.5	3.3	1.7
			Rootstock M 9					
Control	-	-	30.3	69.7	49.0	51.0	25.6	74.4
10 L.ha^{-1}	-	-	32.7	67.3	41.1	58.9	22.2	77.8
15 L.ha^{-1}	-	-	35.0	65.0	34.1	65.9	20.8	79.2
LSD ($p \leq 0.05$)	-	-	3.9	4.0	6.3	7.4	3.4	4.7

Table 4 Characteristics of the pigmentary complex of apple leaves in connection with application of sheet dressings, mg.g^{-1} of dry matter.

Variation	May		June		July		August	
	chloro-phyll (a +b)	carotenoids	chloro-phyll (a +b)	carotenoids	chloro-phyll (a +b)	carotenoids	chloro-phyll (a +b)	carotenoids
			Rootstock SK 4					
Control	2.49	1.62	3.59	1.83	4.05	1.91	2.66	1.49
N12P12K35Mg2S0,7+Fe, Cu, Zn, Mn, Mo, B	3.60	2.02	3.98	2.07	4.04	1.95	4.32	2.29
LSD ($p \leq 0.05$)	0.52	0.26	0.65	0.30	0.14	0.32	0.27	0.23
			Rootstock M 9					
Control	-	-	5.16	1.77	4.32	2.11	3.69	1.77
10 L.ha^{-1}	-	-	5.38	1.81	4.54	2.11	3.90	2.01
15 L.ha^{-1}	-	-	5.68	2.07	5.17	2.30	4.10	2.13
LSD ($p \leq 0.05$)	-	-	1.20	0.36	0.67	0.40	0.42	0.35

application of foliar feeding, the content of free water increased by 2.5 times, which can be regarded as evidence of activation of physiological activity of plants. The ratio of free and bound water in the experimental versions was 1.6 and 1.9. During such period in the apple trees on the rootstock M9 at the background of mineral feeding the content of free water was much higher than in the reference version. Such value was substantially higher when maximum fertilizer dosage of 15 L.ha^{-1} was applied. Correlation analysis of the results at this stage of researches established close direct interrelation between quantity of potassium in the leaves and content of free water. The correlation factor is: $r = 0.76 – 0.83$.

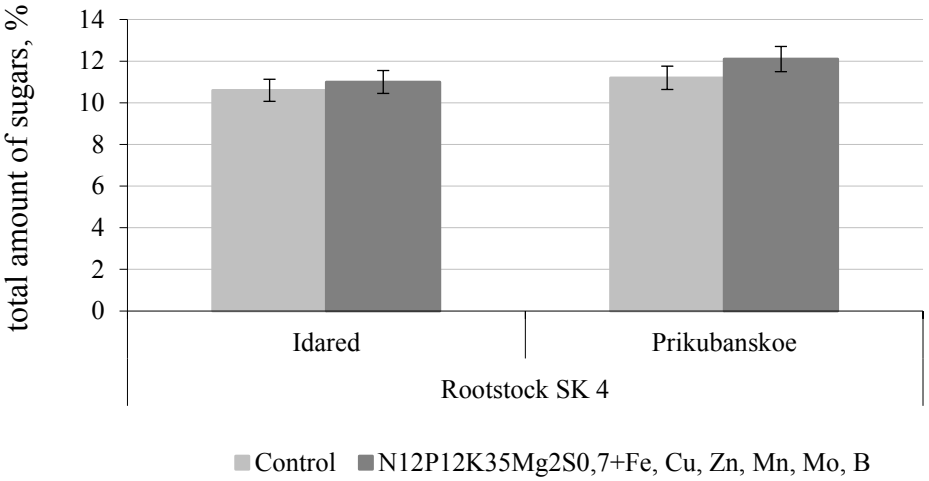

Figure 2 The total amount of sugars in apples per 100 g of raw material, LSD ($p \leq 0.05$) = 0.76 (Idared); 0.40 (Prikubanskoe).

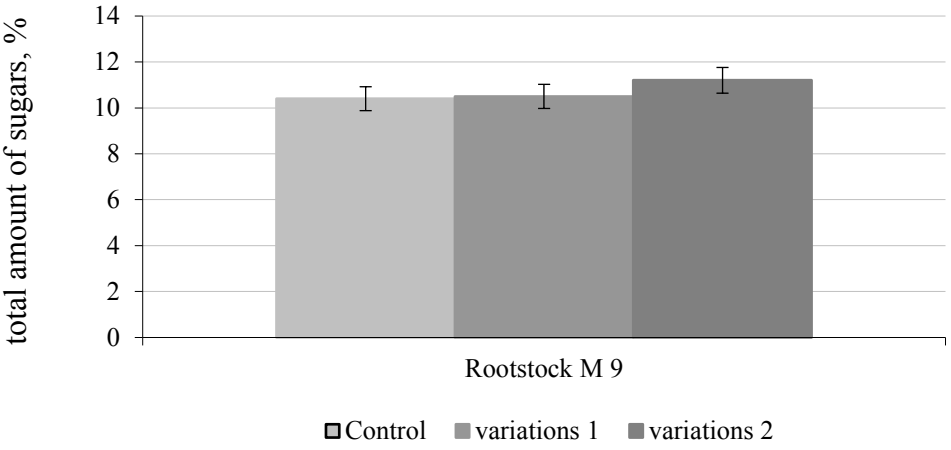

Figure 3 The total amount of sugars in apples Szampion per 100 g of raw material, LSD ($p \leq 0.05$) = 1.23.

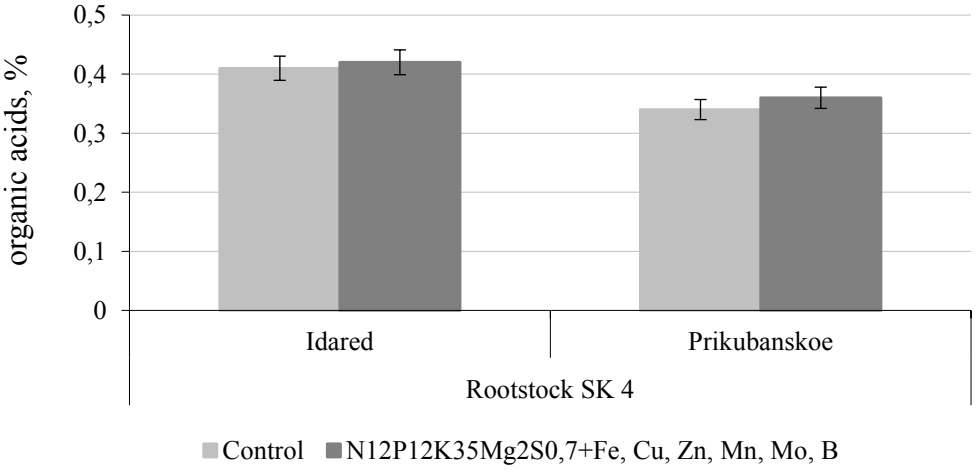

Figure 4 The content of organic (titrated) acids in apples per 100 g of raw material, LSD ($p \leq 0.05$) = 0.03 (Idared); 0.06 (Prikubanskoe).

During the second half of summer, alongside with increasing stress of hydrothermal factors, the content of free water in the leaves after being subjected to mineral feeding was decreasing at the highest rate. Such tendency was maintained during the whole period of researches for the apple trees on the rootstocks SK4 and M9. Under conditions of maximum intensity of hydrothermal stress factors (in August) sufficient water content in the cells of apple trees on the rootstocks SK4 and M9 upon foliar application of fertilizers was ensured due to increase of bound water. Statistic analysis of the experimental data showed significant changes in the ratio of bound and free water fractions. The correlation factor during such period in the reference version and in the version with application of fertilizers was already respectively 3.7 and 4.0 (for apple trees on the rootstock CK4); 2.9 and 2.5 – 3.8 (for apple trees on the rootstock M9). The functional changes in the apple trees ascertained under impact of abiotic stress characterize application of foliar

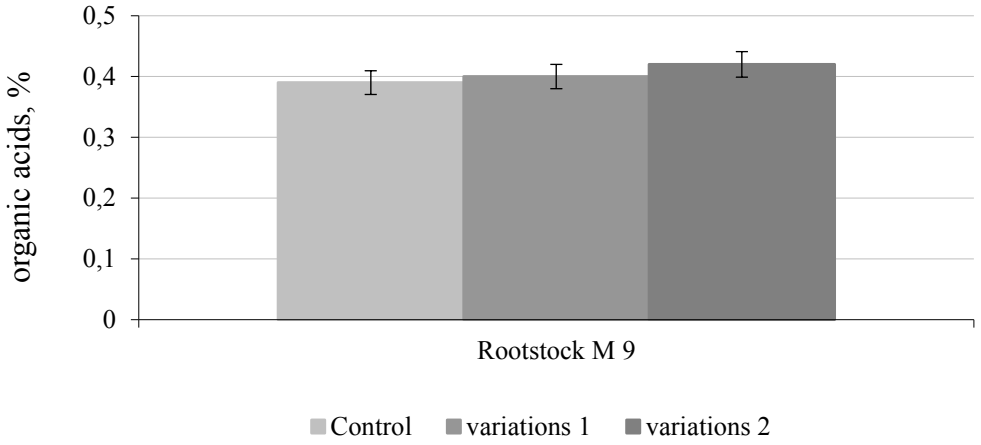

Figure 5 The content of organic (titrated) acids in apples Szampion per 100 g of raw material, LSD ($p \leq 0.05$) = 0.05.

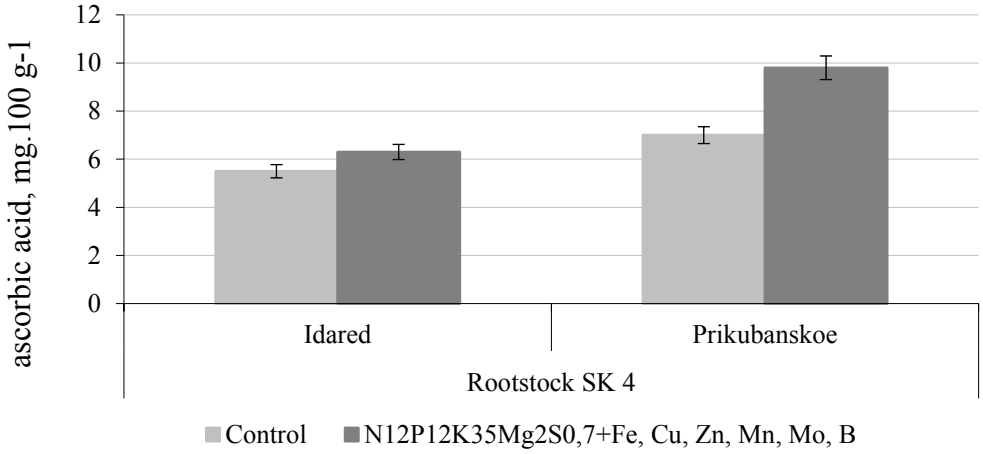

Figure 6 The content of ascorbic acid (vitamin C) in apples per 100 g of raw material, LSD ($p \leq 0.05$) = 0.42 (Idared); 0.29 (Prikubanskoe).

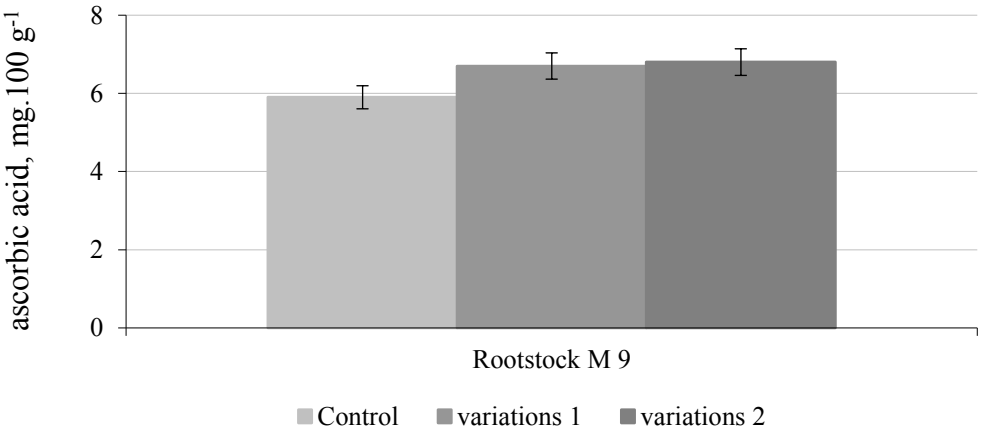

Figure 7 The content of ascorbic acid (vitamin C) in apples Szampion per 100 g of raw material, LSD ($p \leq 0.05$) = 0.34.

feeding with special mineral fertilizers as a factor, increasing resistance of plants.

Seasonal dynamics of fractional water composition in apple leaves had influence on the intensity of synthetic processes. Photosynthetic activity of plants was studied (Table 4).

After foliar application of mineral fertilizer solutions in May the apple trees on the rootstock SK4 formed better pigmentary complex. In July, during the period of differentiation of fruit buds, the content of chlorophyll and carotinoids in apple leaves became approximately equal in the reference and experimental versions. In August, at the background of intensive negative impact of abiotic factors, at higher level of water content in tissues the content of pigments in leaves was much higher than in the reference version. The established functional changes give evidence of more intensive activation of adaptation mechanism under the influence of mineral feeding.

For the apple trees on the rootstock M9 application of mineral feeding already in July caused significant increase of the content of chlorophyll in leaves as compared to the reference version. In August the content of chlorophyll increased less, however the content of carotinoids increased substantially.

Activation of the adaptive system of apple trees for overcoming abiotic stress conditions upon application of mineral feeding facilitated enhancement of the reproductive function. Average growth of productivity for the apple trees on the rootstock SK4 was 10 – 12 %, on the rootstock M9 – up to 22%.

At the maturity stage the properties of apples, characteristic of specific taste and nutritive value of the fruits, were analyzed. It was found that variation of some parameters of qualitative characteristics of apples to a greater extent was dependent on the variety. Apples of Idared and Prikubanskoe varieties from the apple trees on the rootstock SK4 in the reference version had various content of total sugars (Figure 2).

Upon application of mineral feeding to the trees the content of sugar in the fruits was higher, however slightly. And the sugar-acid index (SAI) in the experimental versions was for the Idared variety: 25.8 in the reference samples, and upon application of feedings – 26.2; for the Prikubanskoe variety: 32.9 in the reference samples, and upon application of feedings – 33.9. For the apple trees of Szampion variety the total sugar content slightly changed in various versions of tests (Figure 3). SAI varied within the range of 26.2 – 26.7.

The content of organic acids in apples had less variations (Figure 4). In the fruits of Prikubanskoe apple trees on the rootstock SK4 the annual total quantity of acids in apples at the maturity stage was 14 – 17% less than as compared with

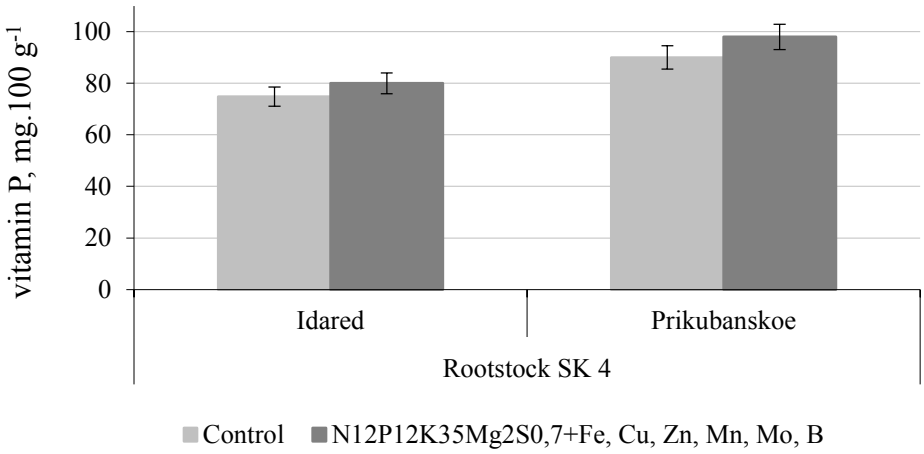

Figure 8 The content of bioflavonoids (vitamin P) in apples per 100 g of raw material, LSD ($p \leq 0.05$) = 5.02 (Idared); 8.42 (Prikubanskoe).

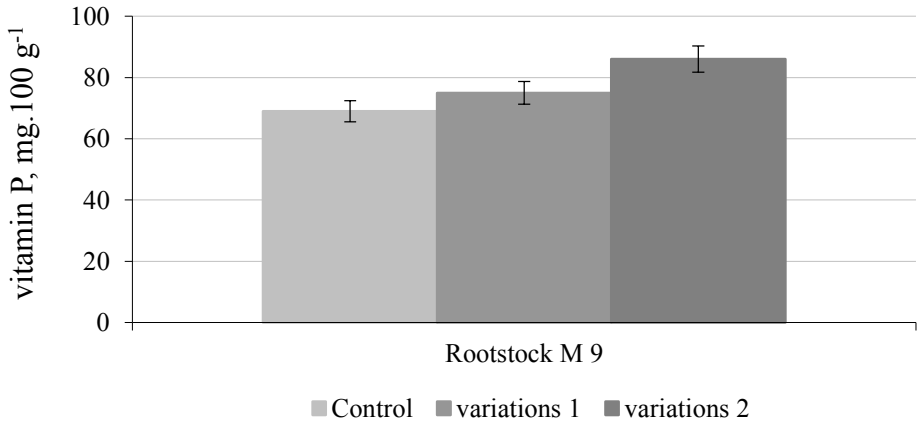

Figure 9 The content of bioflavonoids (vitamin P) in apples of the grade Szampion per 100 g of raw material, LSD ($p \leq 0.05$) = 6.22.

the Idared variety. Meanwhile according to the tasting assessment results the apples at the eating-ripe stage had quite balanced taste. For Szampion apple trees stable content of organic acids in fruits was typical annually. No significant variations of such parameter were measured for different versions (Figure 5).

Analysis of the content of ascorbic acid in the apples of Idared and Prikubanskoe varieties revealed significant advantage of the apples in the version with application of multi-nutrient mineral fertilizer "Aquarin" as foliar feeding (Figure 6). Growth of the content of vitamin C in the apples of Szampion variety was not confirmed statistically (Figure 7).

The effect of foliar feeding resulted in increase of the content of such important for a human food antioxidants as bioflavonoids in the apples of Idared variety (Figure 8). The quantity of biologically active substances in the apples of Prikubanskoe variety increased to a lesser extent.

In the fruits of Szampion variety considerable growth of the content of vitamin P as compared to the reference samples was found in the version with application of the maximum fertilizer dosage of 15 $L.ha^{-1}$ (Figure 9).

CONCLUSION

Thus, experimental testing of the efficient method for optimization of physiological condition of apple trees subjected to negative physical factors during summer season allowed to ascertain that it is possible to enhance adaptability of such plants with the help of regular application of foliar feeding with water solutions of mineral fertilizers. It was established that in the result of application in 2014 – 2015 of the multi-nutrient fertilizer "Aquarin" with the formula N12P12K35Mg2S0,7 + micronutrients on the low growing apple trees engrafted on the rootstock SK4 facilitated higher accumulation of nitrogen and potassium in the leaves, increase of the content of bound water in the tissues of leaves during the period of maximum stress of hydrothermal factors. In the same version a stronger pigmentary complex was formed. Foliar feeding ensured substantial as compared to the reference version accumulation of potassium in the fruits, the content of ferrum, copper, manganese increased slightly. Nutritive value of the fruits was improved due to significant increase of the content of vitamin C and bioflavonoids.

Usage during 2016 – 2017 for foliar feeding of a multi-nutrient liquid potassium-and-calcium fertilizer with a wide range of microelements also facilitated enhancement of the adaptability of apple trees on the rootstock M9. The best efficiency was ensured in case of application of fertilizers in the dosage of 15 $L.ha^{-1}$, which resulted in growth of the content of nitrogen and potassium in the leaves. At the maturity stage the content of nitrogen in the fruits increased within the tolerable limits in comparison with the reference samples, the content of calcium increased by 5.6%.

Changing of the schedule of feeding for the apple trees on the rootstock M9 was connected with activation of physiological processes during summer season. In the first half of summer, in the absence of negative impact of any physical factors, the free water content in the apple leaves of Szampion variety at the background of application of mineral feeding was much higher than that in the reference version. Upon enhancement in August of the stress impact of drought the ratio of bound and free water fractions changed significantly, thus providing evidence of activation of the adaptation mechanism under the influence of mineral nutrients. More intensive photosynthetic activity of the plants was observed during the whole period of researches.

Application of fertilizers in the experiments had no apparent effect on the content of sugars and organic acids in the apples. The highest content of biologically active substances was measured in the apples of the version subjected to foliar feeding: the content of vitamin C increased by 13.6 – 15.2%, vitamin P – by 8.7 – 24.6%.

Based on the results of the researches it can be concluded that regular foliar application of multi-nutrient fertilizers, including macro- and microelements, has effect on the functional condition of low growing apple trees and stabilization of the production processes in general.

REFERENCES

Abilphazova, Y., Belous, O. 2015. Adaptability of grades and hybrids of tangerine in a subtropical zone of Russia. *Potravinarstvo,* vol. 9, no. 1, p. 299-303. https://doi.org/10.5219/485

Doroshenko, T. N. 2000. *Physiological and ecological aspects of southern fruit growing*. Krasnodar, Russia : KubGAU. 235 p.

Doroshenko, T. N., Zakharchuk, N. V., Ryazanova, L. G. 2010. *Adaptive potential of fruit plants in the south of Russia*. Krasnodar, Russia : Enlightenment-Yug. 123 p.

Doroshenko, T. N., Chumakov, S. S., Maksimtsov, D. V. 2012. Features of foliar nutrition of fruit plants under the influence of temperature stress factors in the spring-summer period. *Fruit growing and berry-culture in Russia*, vol. 30. p. 22-30.

Goncharova, E. A. 2011. Strategy of diagnosis and prediction of resistance of agricultural plants to weather and climate anomalies. *Agricultural Biology*, vol. 1. p. 24-31.

Gudkovsky, V. A, Kashirskaya, E. M., Tsukanova, N.Y. 2005. *Stress of fruit plants*. Michurinsk, Russia : Quart. 127 p.

Kushnirenko, M. D., Kurchatova, G. P., Stepinca, A. A. 1986. *Rapid methods of diagnosis of heat-, drought- and the timing of watering the plants*. Kishinev : Stiinta. 39 p.

Nenko, N. I, Kiseleva, G. K., Ulyanovskaya, E. V. 2014. Physiological and biochemical research of apple cultivars of various ecological and geographical origin in the conditions of the south of Russia. In *Materials of the X International Conference "News of advanced science"*. Sofia : Bial GRAD-BG OOD, p. 32-37.

Nenko, N. I., Kiseleva, G. K. 2014. Physiological and biochemical characteristics of the conjugate stability of apple trees to abiotic stresses in the south of Russia. In *The International Scientific and Practical Congress "Scientific resources management of countries and region*". ISA Science & Genesis : Copenhagen, p. 123-130. ISBN: 978-966-53268-09.

Nenko, N. I., Kiseleva, G. K., Karavaeva, A. V., Ulyanovskaya, E. V. 2015. Features of the water regime of apple varieties of various ploidy in connection with adaptation to drought. *Fruit growing and viticulture in the South of Russia*, vol. 31, no. 1, p. 98-109.

Nenko. N. I., Kiseleva, G. K., Ulyanovskaya, E. V. 2014. Adaptation of apple cultivars of different ecological and geographical origin to the stress factors of the North Caucasus region of Russia. In *Fundamental and applied aspects of modern ecology-biological and medical-technological research*. Rishon le Zion, Israel : MEDIAL, p. 83-111.

Nenko, N. I, Sergeeva, N. N., Kiseleva, G. K., Sergeev, Y. I. 2015. Effect of fertilizers and growth regulator "Furolan" on the dynamics of metabolites in apple tree leaves. *Herald of the agrarian and industrial complex of Stavropol,* vol. 19, no. 3, p. 166-170.

Nenko, N. I. Sergeeva, N. N., Karavaeva. A. V. 2015. Study of adaptive reactions of apple varieties on the background of sheet treatments with special fertilizers and growth regulators. *Fruit growing and viticulture of the South of Russia*, vol. 35 no. 5, p. 83-94.

Nenko, N., Sergeeva, N., Kiseleva, G., Karavaeva. A. 2015. Effect of growth regulators and mineral foliar application apple on stability fruit cenosis. *News of Science and Education*, vol. 29, no 5, p. 53-61.

Popova, V. P., Yaroshenko, O. V., Pestova, N. G. 2014. The content of macro- and microelements in the "soil-leaf-fetus" system under intensive apple tree cultivating technologies. *Fruit Growing and Berry-Culture in Russia*, vol. 40, no. 1. p. 255-260.

Popova, V. P., Fomenko, T. G., Petrov, I. A. 2013. Efficiency of the use of fertilizers and growth regulators of a new generation for increasing the productivity of apple trees. *Fruit Growing and Viticulture in The South of Russia*, vol. 19, no. 1, p. 75-87.

Popova, V. P., Yaroshenko, O. V., Pestova, N. G. 2014. Seasonal dynamics of the content of macro- and microelements in the "soil-leaf-fruit" system of apple plantations in conditions of intensive cultivation technologies. *Scientific Works of SSO NCRRIH&V,* vol. 5. p. 98-104.

Popova, V. P., Yaroshenko, O. V. 2017. The quality of apple nutrition in conditions of intensive cultivation technology. *Fruit Growing and Berry-culture in Russia*, vol. 51, p. 292-298.

Rogachev, M. A. 2008. The influence of mineral fertilizers on the balance of nutrients in leaves and its relationship with the productivity and growth of apple trees. *Agro XXI,* no. 4-6, p. 38-39.

Ryndin, A., Belous, O., Abilfazova, Y., Prytula, Z. 2017. The regulation of the functional state of subtropical crops with micronutrients. *Potravinarstvo Slovak Journal of Food Sciences*. vol. 11, no. 1, p. 175-182. https://doi.org/10.5219/669

Sergeeva, N. N., Nenko, N. I., Pestova, N. G., Karavaeva A. V. 2014. Criteria of stability of fruit agrocenosis to stressors of the summer period depending on the plant nutrition regime *Scientific Works of SSO NCRRIH&V,* vol. 5. p. 105-112.

Šircelj, H., Tausz, M., Grill, D., Batič, F. 2007. Detecting different levels of drought stress in apple trees (Malus domestica Borkh.) with selected biochemical and physiological parameter. *Scientia Horticulturae*, vol. 113. no. 4, p. 362-369. https://doi.org/10.1016/j.scienta.2007.04.012

Shanker, A., Venkateswarlu B. 2011. *Abiotic Stress in Plants-Mechanisms and Adaptations*. Rijeka, Croatia : InTech. 428 p. ISBN 978-953-307-394-1.

Sergeeva, N. N., Nenko, N. I., Sergeev, Y. I. 2014. Stability of fruit agrocenosis to stressors of the summer period depending on the plant nutrition regime in the south of Russia. In *III International Scientific and Practical Conference "Scientific perspectives of the XXI century: achievements and prospects of the new century"*. Novosibarsk, p. 119-123.

Sergeeva, N. N., Nenko, N. I., Sergeev, Y. I. 2015. The content of free proline in the apple tree leaves with application of foliar fertilization. In *Materials of the XI international research and practice conference "Science and civilization-2015"*. Sheffield : Science and Education LTD, p. 50-53.

Sergeeva, N. N., Buntsevich, L. L., Nenko, N. I., Sergeev, Y. I. 2015. Nutrition and morphophysiological development of apple trees in the application of sheet dressings. *Scientific Works of RRIFSC*, vol. 53. p. 175-186.

Sergeeva, N. N., Pestova, N. G. 2015. Parameters of the content of the basic mineral elements in the apple tree leaves, depending on the stage of individual plant development and plantation design. *Fruit growing and viticulture of the South of Russia,* vol. 33, no. 3. p. 86-96.

Sofo, A., Palese, A. M., Casacchia, T., Dichio, B., Xiloyannis, C. 2012. *Sustainable Fruit Production in Mediterranean Orchards Subjected to Drought Stress. Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability*. New York, USA : Springer-Verlag. p. 105-129. ISBN 978-1-4614-0633-4.

Srinivasa Rao, N. K., Laxman, R. H., Shivashankara, K. S. 2016. Physiological and Morphological Responses of Horticultural Crops to Abiotic Stresses. *Abiotic Stress Physiology of Horticultural Crops*, p. 3-17.

Trunov, Y. V., Greznev, O. A., Solomakhin, A. L., Sergeeva N. N., Ulianich L. P. 2009. Study of the effectiveness of using elements of mineral nutrition to diagnose the functional state of perennial fruit plants. *Achievements of Science and Technology of Agroindustrial Complex*, vol. 10, p. 65-67.

Trunov, Y. V., Tsukanova, E. M., Tkachev, O. A., Greznev, O. A., Sergeeva, N. N. 2011. Activation of the adaptive mechanisms of apple plants under the influence of special fertilizers. *Fruit Growing and Viticulture of The South of Russia,* vol. 61 no. 12, p. 78-89.

Vardanov, I. Velikova, V., Tsonev T. 2003. Plants responses to drought and stress tolerance. *European Workspop on environmental stress and sustainable agriculture*, vol. 38, no. 2, p. 187-206.

Volkov, F. A. 2005. *The methodology of research in gardening.* Moscow, Russia : VSTISP. 94 p.

Volobueva, V. F., Shatilova, T. I. 2008. *Workshop on the biochemistry of vegetable, fruit, berry, ethereal and medicinal crops* Moscow, Russia : FGOU HPE RGAU-MAAA them. K. A. Timiryazev. 135 p.

Voskresenskaya, O. L., Alyabysheva, E. A., Polovnikova M. G. 2006. *A large workshop on bioecology.* 1rd ed. Yoshar-Ola, Russia : Mari State University. 107 p.

Wood, A. J. 2007. Eco-physiological adaptations to limited water environments. In Jenks M. A. et al. *Plant abiotic stress.* USA : Blackwell Publishing. p. 21-33. ISBN-13: 978-14051-2238-2

Yaroshenko, O. V., Popova, V. P. 2016. Formation of chemical composition and commodity qualities of fruits of apple tree under the conditions of the intensive technologies of the cultivation. *Technology for the food and processing industry of AIC – healthy food.* vol. 13, no 5, p. 15-23.

Yushkov, A. N., Borzykh, N. V., Butenko, A. I. 2016. Evaluation of Resistance of Horticultural Plants to Destabilizing Effects Based on Analysis of Leaf Reflection Spectra. *Journal of Applied Spectroscopy,* vol. 83, no. 2, p 302-306. https://doi.org/10.1007/s10812-016-0286-1

Zhu, L., Borsboom, O., Tromp, J. 1997. The effect of temperature on flower-budformation in apple including some morphological aspects. *Scientia Horticulturae*, vol. 70, no. 1, p. 1-8. https://doi.org/10.1016/S0304-4238(97)00018-6

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THE EFFECT OF THE REGULAR CONSUMPTION OF LARD FROM FATS OF CROSSBREED MANGALITSA AND BREED OF MEAT TYPE PIG ON THE LIPID PROFILE OF CONSUMERS

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ABSTRACT

The aim of this study was to evaluate the influence of consumption of lard processed from crossbreed of the original mangalitsa genotype and the breed of meat type pigs on selected biochemical parameters: total cholesterol (T-C), LDL cholesterol, HDL cholesterol and triacylglycerols (TAG). Twenty-nine probands participated in the study, of which 14 women and 15 men (aged 21 – 59) who consumed regularly lard four times a week during 5 weeks. By evaluating the lipid profile of probands, we observed elevated hypercholesterolemia at the beginning of the study in 43% women and 27% men. Statistically significant differences were recorded in this group of probands, where we recorded a decrease in TC of 0.55 mmol.L^{-1} in the whole sample of probands with p <0.05 probability in the 2nd sample of study after 5 weeks of consumption of lard. Borderline high (up to 3.35 mmol.L^{-1}) was found in 21% of women and 33% of men, and above LDL cholesterol was found in 79% of women and 60% of men. This high level of LDL cholesterol is considered a risk factor for the development of atherosclerosis. We can conclude from the results that regular consumption of lard has significantly reduced the total cholesterol levels, especially in women, this effect has been associated with lowering LDL cholesterol (p <0.01) and lowering HDL cholesterol.

Keywords: lard; cholesterol; lipid profile; mangalitsa

INTRODUCTION

Dietary fatty acids play significant roles in the cause and prevention of cardiovascular disease (CVD). Trans fatty acids from partially hydrogenated vegetable oils have well-established adverse effects and should be eliminated from the human diet. CVD risk can be modestly reduced by decreasing saturated fatty acids (SFA) and replacing it by a combination of polyunsaturated fatty acids (PUFA) and mono-unsaturated fatty acids (MUFA) **(Michas, 2014)**. The clinical consequences of atherosclerosis in the form of cardiovascular diseases still remain despite significant advances in treatment of the major causes of mortality in developed countries **(Vohnout and Rašlová, 2009)**. Significant is the effect of fatty acids in the diet on blood lipoprotein levels **(Béder et al., 2005)**.

Lard is considered as high-nutritional food that is part of human nutrition. It contains 40% of saturated and 59% unsaturated MK, of which 44% monounsaturated and 11% polyunsaturated fats have better ratio of omega 3 to omega 6 MK **(Gunstone., 1996)**. It contains vitamin D, which helps in absorbing nutrients in the intestine, prevents rachitis and osteomalacia, help to absorb calcium which prevents osteoporosis and arthritis. Another important vitamin in lard is tocopherol **(Kasper, 2015)**. Lard is one of the most widely used raw materials of animal origin derived from fatty tissues of pigs. The fatty tissue includes the bacon and the internal saddle, which are grouped into qualitative classes used in the food industry or in the technical industry. Pig lipids are an important source of conjugated linoleic acid, which in the light of recent studies can provide protection against some civilization diseases **(Nistor et al., 2012)**. From animal fats, lard has the highest thermal stability with a relatively high content of unsaturated fatty acids, especially oleic acid **(Jurkovičová, 2005, Wollmannová et al., 2018)**.

According to the Statistical Office of the Slovak Republic (2016), the consumption of edible fats and oils per capita rose by 2.8%, compared to 2015 and amounted to 21.7 kg. With regard to recommended food doses (ODP), the total fat and oil consumption falls within the rational consumption range of 19.8-23.1 kg. The structure of consumption of individual fats is as follows: vegetable fats and oils represent 64.9%, butter and lard 34.6%, and other fats 0.5%. Consumption of lard from 2011 has an ascending character, reaching a consumption of 3.7 kg per person/year in 2016 **(SO SR, 2018)**.

At present, primitive breeds can keep interest in market demand from an economic point of view. The specific characteristics of meat products and the fact that they are derived from traditional breeding systems increase their value. Consumers today seek and value products obtained from the most natural conditions. Sensory indicator values are important when choosing a product for consumers as well as for economic value. Nevertheless, scientific evidence is required to prove the biochemical properties of the mangalitsa meat. Meat and mangalitsa products begin to gain public and media attention. As it has been mentioned, in the past the production of this breed has been reduced. Since 2011, the number of mangalitsa breed has started to increase because of the quality of the products obtained from traditional fattening **(Cordis et al., 2015)**. In recent years, a number of publications has been published in relation to fatty acid and cholesterol content in meat and fatty deposits. Hungarian scientists studied fatty acids and cholesterol containing fatty tissue in mangalitsa and mangalitsa crosses. They determined that the content of unsaturated fatty acids exceeded 60% in mangalitsa fat and reached almost the same percentage in crosses **(Parunovič et al., 2015)**. **Nistor et al. (2012)** report that mangalitsa lard has 12-16% less saturated fatty acids and 8-10% more unsaturated fatty acids (omega 3 and omega 6) than modern breeds of pigs. Research results of **Debreceni et al. (2016)** suggest that the use of the mangalitsa for crossbreeding with pork meat breeds can improve the quality of the meat and fat of hybrids that are desirable for the production of special meat products. **Parunovič (2015)** in research has shown that nutrition and condition affect the ratio of omega 6 and omega 3 polyunsaturated fatty acids in mangalitsa meat.

Scientific hypothesis

This study was designed to investigate the effects of consumption of lard processed from crossbreed of the original mangalitsa genotype and the breed of meat type pigs on the lipid profile of consumers.

MATERIAL AND METHODOLOGY

The research was carried out on the basis of a clinical study conducted at the Department of Animal Nutrition and the Department of Animal Husbandry at the Slovak Agricultural University in Nitra aimed at monitoring the effect of regular consumption of lard processed from crossbreed of the original genotype of mangalitsa and breed of meat type pigs with regard on selected biochemical parameters of the probands. The study was aimed at assessing the lipid profile of the probands with the determination of the essential lipid parameters in the blood. The probands were volunteers from the ordinary population. The condition for participation in the research was the consent of probands with the conditions of the study and the examinations that they had to undergo during the research. Twenty nine probands, of which 14 women and 15 men participated in the survey. The monitored group was made up of employees and students of the Slovak University of Agricultural in Nitra, aged 21 – 59. The mean age of females was 45.8 ±9.6 years and the mean age of males was 38 ±11.4 years. The monitored group of probands was comprised of persons without pathological changes in the basic haematological and biochemical parameters of blood.

Prior to commencement of regular consumption of lard (1st sample), the venous blood was collected by probands and the anthropometric measurements were performed a scheduled. The second sampling and measurement took place immediately after the end of 5 weeks of consumption and the third collection and the measurements were performed with one month delay. Probands consumed a dose of lard with a bakery product 4 times a week (3 times on weekdays and 1 time during the weekend) during 5 weeks. The daily dose for women was determined at 20 g of lard and 40 g for men. Lard was processed by frying at a constant temperature of 130 °C.

Fatty acid content determination was performed by Capillary Gas Chromatography (GC) with Agilent Technologies 6890N (Agilent, Waldbronn, Germany) with a flame ionization detector and a 5973 Network mass-selective detector. Capillary column 100m x 0.25 mm i.d. x 0.2 μm stationary phase HP-88 thick film (J & W Scientific, Agilent Technologies, CA, USA) was used to separate FAME.

Blood collection, anthropometric measurements as well as analyzes were performed in the laboratories of the Department of Human Nutrition of FAPZ SPU in Nitra. Blood serum lipid profiles (T-C, HDL-C and TG) were determined using the DiaSys commercial kits (Diagnostic Systems GmbH, Holzheim, Germany) of Randox with Biolis 24i Premium Biochemical Analyzer (Tokyo Boeki Machinery Ltd., Japan). The LDL-C level was calculated by the Friedewald equation.

Statisic analysis

The results were evaluated with appropriate standard mathematical - statistical methods and are listed in the following tables. The STATISTICA Cz program 10 was used for the statistical programs in MS Excel 2007. All data were expressed as the mean standard deviation (SD), differences between the values before and after the consumption were tested by a paired Student t-test. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Content of fatty acids in lard is presented in Table 1.

To assessment of the effect of consumption of lard on the level of total cholesterol

From the biochemical blood indicators, we established an average total cholesterol of 6.07 ±1.02 mmol.L^{-1} prior to the beginning of regular consumption of lard (1st sample), with women having a higher TC level of 6.4 ±0.85 mmol.L^{-1}, as for men 5.81 ±1.07 mmol.L $^{-1}$. Only 7% of women and 26% of men (up to 5.2 mmol.L^{-1}) were in the blood T-C reference range. 36% of women and 47% of men had elevated T-C (5.2 – 6.2 mmol.L^{-1}) and a risk level (above 6.2 mmol.L^{-1}) was found in 57% of females and 27% of males. Hypercholesterolemia (values above 6.2 mmol.L^{-1}) were observed in 41% of the probands, averaging 7.03 ±0.60 mmol.L^{-1}, which signalizes that probands were at increased risk of developing cardiovascular disease (CVD). Similar data on the risk of developing cardiovascular disease is reported by **Aiglova (2017)**. After 5 weeks of consumption of lard (2nd sample), we observed a decrease in TC level by an average of 0.22 mmol.L^{-1} (in

Table 1 Content of fatty acids in lard.

Fatty acids	g.100g^{-1} FAME	Fatty acids	g.100g^{-1} FAME	Fatty acids	g.100g^{-1} FAME
myristic acid	1.57	oleic acid	41.19	polyunsaturated	9.58
palmitic acid	26.43	linoleic acid	8.34	monounsaturated	44.80
palmitoleic acid	2.70	α-linolenic acid	0.52	saturated	41.59
heptadecanoic acid	0.30	arachidic acid	0.17	ratio Σn3/Σn6	0.07
stearic acid	13.12	arachidonic acid	0.17	ratio Σn6/Σn3	14.24

Note: FAME - fatty acid methyl ester

Table2 The comparison of the lipid profile of men during the experiment.

Lipid profile	1st sample	2nd sample	3rd sample
Total cholesterol (mmol.L^{-1})	5.77 ±1.11	5.72 ±1.10	5.98 ±1.10
LDL cholesterol (mmol.L^{-1})	3.49 ±0.71	3.51 ±0.84	3.55 ±0.76
HDL cholesterol (mmol.L^{-1})	1.37 ±0.33	1.39 ±0.31	1.45 ±0.33
Triacylglycerol (mmol.L^{-1})	1.99 ±1.35	1.77 ±0.79	2.13 ±1.23

Table 3 The comparison of the lipid profile of women during the experiment.

Lipid profile	1st sample	2nd sample	3rd sample
Total cholesterol (mmol.L^{-1})	6.40 ±0.88	6.00 ±1.06*^{a}	5.88 ±0.80*^{b}
LDL cholesterol (mmol.L^{-1})	3.94 ±0.83	3.67 ±0.97*^{a}	3.57 ±0.81**^{b}
HDL cholesterol (mmol.L^{-1})	1.85 ±0.43	1.72 ±0.42	1.69 ±0.36*^{b}
Triacylglycerol (mmol.L^{-1})	1.35 ±0.69	1.32 ±0.68	1.36 ±0.50

Note: 1st sample – baseline, 2nd sample - after 5 weeks of consumption, 3rd sample - 1 months after end of consumption, the levels of statistical significance chosen for the comparisons were p <0.05 (*), p <0.01 (**); a – intra- group differences;

women by 0.40 mmol.L^{-1} and 0.05 mmol.L^{-1} in men) (Table 2 and Table 3). Statistically significant differences were observed in the group of probands with hypercholesterolaemia where we noticed lower values in second sample in average by 0.55 mmol.L^{-1} in the whole batch of probands with p <0.05 (Figure 1). The p <0.05 (Figure 1) also corresponds with results in women where it represents a reduction in total cholesterol averaging 5.88 ±0.80 mmol.L^{-1} (Table 3) 4 weeks after ending their consumption.

The evaluation of the effect of consumption of lard on LDL cholesterol levels

Prior to consumption of the lard (1st sample), we recorded an average LDL cholesterol level of 3.70 ±0.79 mmol.L^{-1}. In females an average value at 3.94 ±0.83 mmol. L^{-1} whereas in males 3.49 ±0.71 mmol.L^{-1}. Optimal LDL cholesterol levels which should be up to 2.5 mmol.L^{-1}, were not present in any of the women and only in 7% of men. Upper borderline (up to 3.35 mmol.L^{-1}) was found in 21% of women and 33% of men. Above this limit, LDL cholesterol levels were detected in 79% of women and 60% in men. This high level of LDL cholesterol is considered to be a risk factor for the development of atherosclerosis **(Kaško et al., 2017)**. Total reduction in LDL cholesterol was 0.11 mmol.L^{-1} (in females by 0.27 mmol.L^{-1}) after 5 weeks of consumption of lard (2nd sample) and a slight increase of 0.02 mmol.L^{-1} in males. Four weeks after ending of consumption (3rd sample), we recorded a slight decrease in LDL cholesterol by 0.03 mmol.L^{-1}, in women by 0.10 mmol.L^{-1}. In men, on the other hand, an increase of 0.04 mmol.L^{-1} compared to the second sample (Table 2). From the results we can see that consumption of lard 20 g in women and 40 g in men 4 times per week for 5 weeks has significantly manifested in lowering of total cholesterol levels, especially in women, which was associated with LDL cholesterol lowering (p <0.01) (Figure 2), and lowering of HDL cholesterol. Our results compared with **Stewart et al. (2001)** confirmed the similarity between consumption of lard and LDL cholesterol in women.

Nelson (2013) puts emphasis on reducing LDL cholesterol, leading to a significant decrease in cardiovascular morbidity and mortality. However, despite the emphasis on controlling LDL cholesterol, a number of cardiovascular diseases occur in people without clinically abnormal LDL cholesterol levels. One way to prevent disease and improve treatment is to concentrate on HDL cholesterol rather than on LDL cholesterol.

The evaluation of the effect of consumption of lard on HDL cholesterol levels

The HDL cholesterol level was 1.60 ±0.45 mmol.L^{-1} on average before lard consumption, namely 1.85 ±0.43 mmol.L^{-1} in females and 1.37 ±0.33 mmol.L^{-1} in males. Reference values up to 1.55 mmol.L^{-1}, were detected only in 29% of women and 66% in men, while other probands had higher values. Low values below 1.0 mmol.L^{-1} were not determined in any women and 7% in men (Table 2 and Table 3). Optimal level of HDL cholesterol showed 52% of the probands, which could protect them from the onset of cardiovascular disease. These results are confirmed by the findings of **Aigl's research (2017)**. After 5 weeks of consumption with probands, HDL cholesterol was reduced to 1.55 mmol.L^{-1} ±0.76 mmol.L^{-1} (in women to 1.72 ±0.42 mmol.L^{-1}, in men to the contrary, a positive increase to 1.39 mmol.L^{-1} ±0.31 mmol.L^{-1}) (Figure 3).

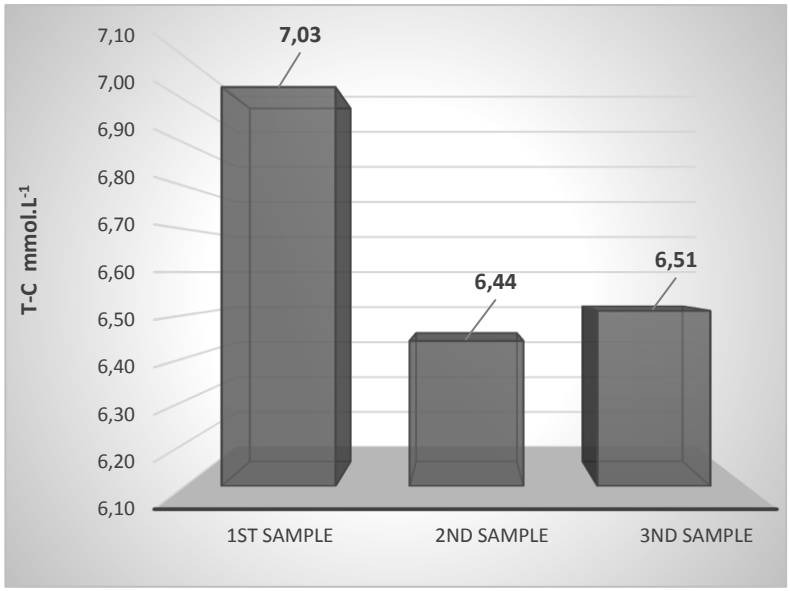

Figure 1 The comparison average value of cholesterol of probands with hypercholesterolemic.

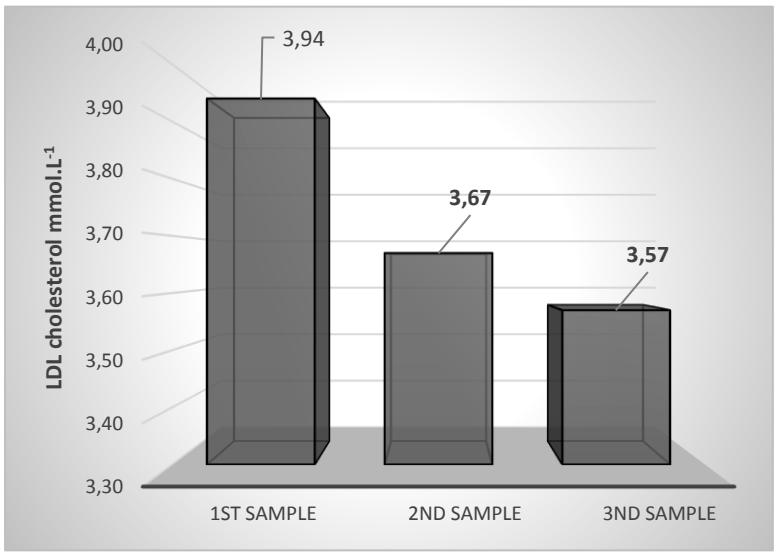

Figure 2 The comparison of average values of LDL cholesterol of women.

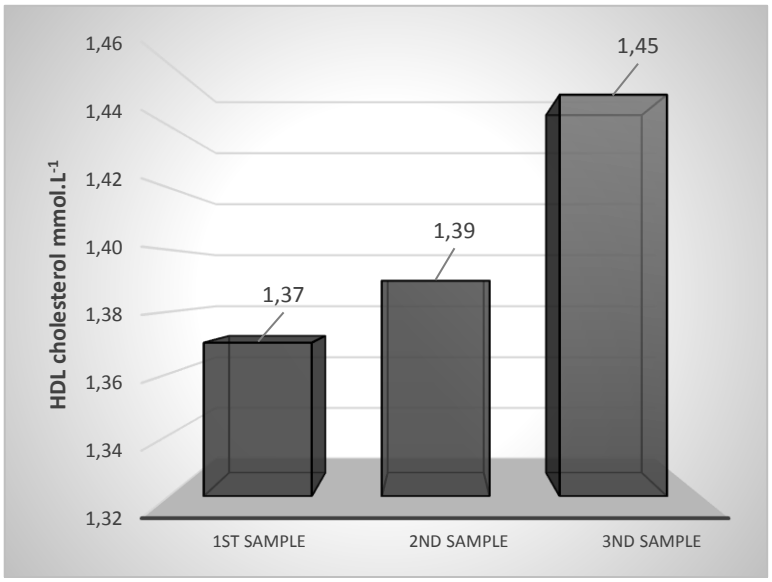

Figure 3 The comparison of average values of HDL cholesterol of men.

From the results it is conclusive that the reduction of total cholesterol by the regular consumption of lard in women is related to lipid metabolism probably within a certain age range from 20 to 60 years. At the same time, the results also indicate that the rise in LDL cholesterol in men is proportional to the rise in HDL cholesterol.

Similar results of the lipid spectrum in men, together with consumption of lard are reported by **Imaki et al. (1989)**. It is important to highlight the different HDL cholesterol values measured in men and women. In general, men have lower HDL cholesterol than women. From the results measured 9 weeks after onset of consumption (3rd sample), we found a decrease in HDL cholesterol by an average of 0.04 mmol.L^{-1}, in famels by 0.16 mmol.L^{-1} and in males an increase by 0.08 mmol.L^{-1} (Table 2 and Table 3). **Biju et al. (2015)** report that metabolic syndrome increases with the age and is more frequent in women. Low HDL cholesterol and elevated levels of triacylglycerols are associated with the prevalence of metabolic syndrome. Low HDL cholesterol occurs more frequently in women, whereas hypertriacylglycerolimia is more common in men.

The evaluation of the effect of consumption of lard on the level of triacylglycerols

In the 1st blood serum sample, the mean triacylglycerol levels were 1.68 ±1.11 mmol.L^{-1}, in women 1.35 ±0.69 mmol.L^{-1} and in men 1.99 ±1.35 mmol.L^{-1}. A borderline of up to 2.25 mmol.L^{-1} was recorded in 93% of women and 60% of men. Above this value, the level of triacylglycerols was measured in 7% of women and 40% of men. In the 2nd sample, we observed a decrease in the average level of triacylglycerols by 0.13 mmol.L^{-1}, in women by 0.03 mmol.L^{-1} and in men by 0.22 mmol.L^{-1}. In 3 rd blood serum sampling, the mean level of triacylglycerols was 1.76 ±1.01 mmol.L^{-1}, with women almost unchanged and 0.14 mmol.L^{-1} (Table 2) in men.

The effect of eating lard on lipid and hormonal parameters of women was studied by **Jansen et al. (1999)**. They found that obese women had significantly higher fasting concentration and postprandial responses of plasma total triacylglycerol, compared to the normal-weight women. The obese women had a fasting leptin concentration of four times the normal-weight women. Postprandial changes in leptin concentrations did not occur. The results of HDLcholesterol are significantly lower in obese women than in normal-weight women. These results provide the evidence that obese women have exaggerated lipid and hormone responses compared to normal-weight women.

CONCLUSION

In recent years, cholesterol views have been reevaluated as well as opinions on food with higher cholesterol levels and are no longer very problematic. No scientific research has so far substantiated or rebutted the view that consumption of lard is detrimental. The results of our research, as well as from other sources did not confirm the current views that exclude lard from a rational diet with a justification for a negative effect on the healthy human organism. As we observed 29 probands, we did not detect negative health changes after a regular 5 week lard consumption. From the results of our study, we can conclude that consumption of lard processed from the transgenic genotype of the original mangalitsa genotype and the breed meat-type pigs in the recommended amount is beneficial in human nutrition.

REFERENCES

Aiglová, R. 2017. HDL cholesterol a kardiovaskulární onemocnění. HDL cholesterol and cardiovascular disease. *Medicine after graduation*, vol. 18, no. 4, p. 376-379.

Béder, I., Babinská, K., Béderová, A., Bukovský, I., Gabašová, E., Hájek, J., Slezáková, O., Važan, R. 2005. *Výživa a dietetika. Nutrition and dietetics*, 1st ed. Bratislava, Slovakia : UK. 188 p. ISBN 80-223-2007-2.

Biju, J. Alvin T., George, A. T. P., Regi, J., Shaliet, R. S. 2015. Prevalence of Metabolic Syndrome in Newly Detected Type 2 Diabetes Mellitus. *Academic Medical Journal of India*, vol. 3, no. 1, p. 8-12.

Cordis, I., Mihaiu, M., Tăbăran, A., Mihaiu, R., Dan, S. D., Reget, O., Cordea, D., Mureşan, C. 2015. Compositional Studies on Mangalitsa Meat Products for Public Consumption. *Bulletin UASVM Veterinary Medicine*, vol. 72, no. 1. p. 57-61. https://doi.org/10.15835/buasvmcn-vm:10568

Debrecéni, O., Komová, P., Bučko, O. 2016. Comparison the physicochemical quality indicators of Musculus Longissimus Dorsi from Mangalitsa Breed and their crossbreeds. *Journal of Central European Agriculture*, vol. 17, no. 4, p. 1253-1263. https://doi.org/10.5513/jcea01/17.4.1840

Gunstone, F. D. 1996. Fatty acid and lipid chemistry. London, UK : Blackie Academic & Professional, 1996, 252 p. ISBN 978-1-4613-6852-6. https://doi.org/10.1007/978-1-4615-4131-8

Imaki, M. Miyoshi T, Tanada S, Nakamura T, Yamada Y, Yamasaki R, Terada H.1989. Effect of lard and corn oil intake on serum lipids in young men. *Acta Biologica Hungarica Kaposvariensis*, vol. 40, no. 3, p. 271-282.

Jansen, J. Anette Bysted, A., Dawids, S., Kjeld Hermansen, K.1999. The effect of palm oil, lard, and puff-pastry margarine on postprandial lipid and hormone responses in normal-weight and obese young women. *British Journal of Nutrition*, vol 82, p. 469-479. https://doi.org/10.1017/S0007114599001725

Jurkovičová, J. 2005. Vieme zdravo žiť? Výskyt rizikových faktorov kardiovaskulárnych chorôb v slovenskej populácii a možnosť prevenci. Do we live healthy? The incidence of risk factors for cardiovascular diseases in the Slovak population and the possibility of prevention, 1st ed. Bratislava, Slovakia : Univerzita Komenského. 166 p. ISBN 80-223-2132-X (In Slovak)

Kasper, H. 2015. Výživa v medicíne a dietetike. Nutrition in medicine and dietetics. Praha, Czech Republic : Grada, p. 555. ISBN 978-80-247-4533-6. (In Czech)

Kaško, M., Kaško, V. 2017. HDL mystery. *Cardiology Letters,* vol. 26. no 5. p. 282-287.

Mandovec, A. 2008. *Kardiovaskulární ochoření u žen. Cardiovascular diseases in women*. Praha, Czech Republic : Grada, 128 p. ISBN 80-2427-807-0.

Metger, O. J., Meier, A. R. M. 2016. Fats and oils as renewable feedstock for the chemical industry. *European Journal of Lipid Science and Technology,* vol. 118, no.1, p. 1-2. https://doi.org/10.1002/ejlt.201500535

Michas, G., Micha, R., Zampelas, A. 2014. Dietary fats and cardiovascular disease: Putting together the pieces of a complicated puzzle. *Atherosclerosis*, vol. 234, no. 2, p. 320-328. https://doi.org/10.1016/j.atherosclerosis.2014.03.013

Nelson, H. R. 2013. Hyperlipidemia as a Risk Factor for Cardiovascular Disease. *Primary Care: Clinics in Office Practice*, vol. 40, no. 1, p. 195-211. https://doi.org/10.1016/j.pop.2012.11.003

Nistor, E. Bampidis, V., Pentea, M., Prundeanu, H., Ciolac, V. 2012. Nutritional Quality of Pork Produced by Mangalitsa Breed. *Scientific Papers Animal Science and Biotechnologies*, vol. 45, no. 2, p. 386-389.

Parunovič, N. Petrovic, M., Djordjevic, V.,Petronijevic, R., Lakicevic, B., Petrovic, Z., Savic, R. 2015. Cholesterol Content and Fatty Acids Composition of Mangalitsa Pork Meat. *Procedia Food Science*, vol. 5, no. 1, p. 215-218. https://doi.org/10.1016/j.profoo.2015.09.021

Stewart, W. J., Kaplan, L. M., Beitz, C. D. 2001. Pork with a high content of polyunsaturated fatty acids lowers LDL cholesterol in woman. *The American Journal of Clinical Nutrition*, vol. 74, no. 2, p. 179-187. https://doi.org/10.1093/ajcn/74.2.179

ŠÚ SR. 2018. Spotreba potravín v SR. Consumption of SR food. Bratislava, Slovakia : Statistical Office of The Slovak Republic. Available at: http://www.statistics.sk/pls/elisw/web.extSearch

Vohnout, B., Rašlová, K. 2009. Risk factors for the development of atherosclerosis: lipids and their relationship to atherosclerosis. *Via practica*, vol. 6, no. 3, p. 116-119.

Wollmannová, A. Musilová, J., Urminská, D. Bajčan, D., Bobková, A., Bojňanská, T., Bystrická, J., Čanigová, M., Kročko, M., Mašková, Z., Mendelová, A., Stanovič, R., Szabová, E., Tančinová, D., Tóth, T. 2018. *Chémia potravín. Food chemistry*. Nitra, Slovakia: SPU, 543 p. ISBN 978-80-552-1814-4.

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PHTHALIC ACID ESTERS CONTENT IN YOGHURT WITH CHIA FLOUR AND BAMBOO FIBER DURING STORAGE TIME

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ABSTRACT

Phthalic acid esters are plasticizers, they can migrate freely from plastic to their surroundings. They have negative health effects. European legislation sets specific migration limits for phthalic acid esters. In our study, we deal with two esters of phthalic acid, dibutylphtalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP). It was studied the effect of storage of four types of yoghurt on the concentration of phthalic acid esters. Yoghurts contained 1% chia flour, 5% chia flour, 1% bamboo fiber and 5% bamboo fiber. Yoghurts were stored in plastic cups, which contained both examined phthalic acid esters. Esters of phthalic acid were determined after 1 week of storage then after 2 weeks of storage and in the original raw material. Furthermore, the pH of the yoghurt was determined. The pH values were correlated with phthalate concentrations: the correlation coefficient for DBP with a pH of -0.0265 and for DEHP with a pH of 0.3075. Mean concentrations of DEHP decreased over time, while DBP decreased for yoghurt with 1% chia flour, while in other cases they increased. The mean DBP concentrations in yoghurt were higher than the average concentrations of DEHP. Comparing the mean sample values with t-test for dependent samples for yoghurt of the same type, when comparing the DBP or DEHP concentration in week 1 with the DBP or DEHP concentration at week 2, the mean values were consistent. It can be noted that there was no increase in DEHP concentrations from cups to yoghurts, which was probably due to a lower concentration in cups than the DBP concentration. DBP concentrations increased in 3 of the 4 types of yoghurt. The determined pH in yoghurts did not differ significantly. Apparently a greater effect on the migration of phthalic acid esters will have in our case a different yoghurt consistency than pH. It would be appropriate to examine the effect of food density on the migration of phthalic acid esters. Likewise, it would be appropriate to examine the effect of pH but in the same food with different pH on the migration of phthalic acid esters.

Keywords: yogurt; dietary fiber; contaminant; dibutylphthalate; di-(2-ethylhexyl) phthalate

INTRODUCTION

Phthalic acid esters represents important plasticizers. They are also present in food packaging materials. Yet they are not covalently bonded which means that they can migrate into their surroundings **(Benson, 2014)**. In case of plastic food packaging, which is in contact with food, plasticizers can migrate both in the plastic, and to the surrounding area, respectively into food **(Piotrowska, 2005)**. When components of plastic packaging material – mostly monomers and additional components – migrate, the material is not completely destroyed, it still preserves technologically significant properties. At the same time, migrate the ingredients of the food in the package, which can affect the migration speed of the ingredients from the package to the food **(Velíšek, 2002)**.

Phthalic acid ester migration from packaging materials into fatty food is very important and closely monitored **(Petersen, 2003)**, as phthalic acid esters are strongly lipophilic **(Velíšek and Hajšlová, 2009)**. Being plasticizers, phthalic acid esters are present in a very large amount in softened PVC where they can make up to 50 % of the weight. Food packaging foil is made of softened PVC as well. Phthalates are used abundantly also for non-food purposes, which causes environmental expansion and the possibility of secondary contamination from the environment **(Brimer, 2011)**. Phthalates are also used in nail polish, perfumes, print inks, toys for children, and flooring **(Mikula et al., 2011)**. Phthalates are also found in the room facilities, but there is found a difference in the time. At the time, when the phthalates had not regulated, and today's when is existing phthalate control and regulation. Exposure values dropped compared with measurements fifteen years ago, it was the age without regulation **(Larsson et al., 2017)**. European legislation lays down limits for phthalic acid ester migration **(Brimer, 2011)**. The Commission Regulation (EU) no. 10/2011 defines specific migration limits (SML) which represents the largest permissible amount of released substance that is

set free from objects into food. The SML value for DBP is 0.3 mg·kg^{-1} and for DEHP it is 1.5 mg·kg^{-1} food. The overall migration limit represents the highest permitted amount of released non-volatile matter from materials into food. The overall migration limit is defined as 10 mg per 1 dm^2 **(Commission Regulation (EU) No 10/2011)**.

Phthalic acid ester occurrence in food is caused especially by the food getting in touch with the material, be it during processing, handling or transport, but also contamination or fallout on the agricultural crops may occur **(Benson, 2014)**. Yet it has been found out, that even manually milked milk contains phthalic acid esters although it is being presupposed that the cattle digestive tract is to hydrolyse phthalic acid esters to mono-esters and alcohols and excrete them in urine as glucuronide conjugates **(Petersen, 2003)**.

Of course, phthalic acid esters are not sole plasticizers, as plasticizers are used: too adipates, trimellitates, citrates, polymerics, etc. They number about 8 types of commonly used plasticizers **(Andrady and Neal, 2009)**.

Humans are exposed to phthalic acid esters via their skin, they can inhale or swallow it **(Clark et al., 2011)**. Phthalic acid esters have a negative impact especially on the reproductive system; administering phthalates to male rats during the perinatal stadium developed cryptorchism, a low number of sperms and hypospadias **(Martino-Andrade and Chahoud, 2010)**. Hepatotoxicity of phthalates was particularly solved, finally it was found that phthalates are endocrine disruptors **(Latini, 2005)**. In animal studies, in which was the animals exposed of DBP had occurred the development and reproductive changes, decrease fertility, increase the number of fetal malformation, exposure of DBP cause disease of liver, kidney and haematology **(ATSDR, 2001)**. It was found low toxicity in inhalation exposure of DEHP, nevertheless was found mortality in rats, after 2–4 hours of inhalation exposure, lung lesions were observed in neonates with mechanical ventilation where respiratory tubes were made of PVC. Pulmonary mass was increased by rats after inhalation of aerosol with DEHP. By oral exposure, the product of metabolism mono-(2-ethylhexy) phthalate (MEHP) is cardiotoxic. Acute exposure in humans causes diarrhoea and abdominal pain. In mice and rats it causes hyperplasia and hypertrophy of liver cells. Negative effects on the liver were found more in mice and rats than in dogs and monkeys, males have been always more prone than females. It has also been established that administration of DEHP to the rat feed dose resulted in a reduction of body weight. Oral administration of DEHP has a negative effect on the reproductive system - it reduces the weight of the testicles, causes deformities and abnormalities. The effect on the female reproductive system was also found when female mice were unable to have litters. In addition, developmental toxicity was found in mice and rats - malformation or resorption of foetuses, lower fetal and juvenile weight, increased abortion rates. Long-term administration of DEHP led to the formation of liver tumours in mice and rats **(ATSDR, 2002)**.

Animal studies indicate that the developmental and reproductive toxicity of phthalates is most affected by the individual as a fetus. As well as the phthalic acid esters are investigated for suspicion that they cause premature births in humans and impair sperm quality, etc. **(Hauser and Calafat, 2005)**. However, studies of the effects of phthalates on humans are limited by the low number of data and inconsistencies of the data **(Meeker et al., 2009)**. Due to the abundant use of phthalates, they have become ubiquitous **(Velíšek, 2002)**. Phthalic acid esters are easily released, and are found in the environment, e.g. in plants, soil, air, water, sewage, etc. **(Przybylińska and Wyszkowski, 2016)**. In the German study, most of the DEHP was found in surface wastewater and sewage and sludge water, and DBP was found only in low concentrations **(Fromme et al., 2002)**.

Degradation of phthalic acid esters from the environment can be through biodegradation – many organisms can catalyse the hydrolysis of phthalates **(Velíšek, 2002)**. Easy biodegradable are all commonly used phthalic acid esters **(Cadogan, 2002)**. In mammals, up to 90% of phthalates are excreted during metabolism from the organism, but also they are partially deposited in the organism **(Velíšek, 2002)**. Daily estimated phthalate intake of DEHP is around 4.9–18 μg·kg^{-1} and day for humans and daily estimated phthalate intake of DBP is at 0.48 μg·kg^{-1} and day **(Schettler, 2006)**. The tolerated daily intake of DEHP is at 37 μg·kg^{-1} body weight and day **(Koch et al., 2003**). The tolerated daily intake of DBP is at 10 μg·kg^{-1} body weight and day **(Hines et al., 2011)**. Nevertheless, some studies suggest that the negative effect of phthalic acid esters on the human body is low, yet they are set up legislative limits for articles for human use, especially for items intended for children **(Kamrin, 2009**).

Yogurt, a fermented milk product, is considered to be a healthy food, both because contained probiotic bacteria and nutrients, vitamins and minerals, especially calcium, which is a good utilisable by the human organism. Nutritional values of yogurt are similar to the milk from which yoghurt is produced, however, it may vary when ingredients are added to yoghurt, such as fruits, cereals, etc. **(McKinley, 2005)**, e.g. fiber. Dietary fiber is important for the prevention of civilization diseases, it is also the prevention of coronary-heart diseases, supports the maintenance of proper body weight, right levels of blood lipids and sugars, promotes the growth of beneficial intestinal microflora, while reducing the number of pathogenic microorganisms. The recommended daily intake of dietary fiber is set at 38 g·day^{-1} for men and 25 g·day^{-1} for women **(Betteridge et al., 2012**). Yoghurts with fiber, which were found as acceptable for consumers, were with apple, wheat and bamboo fiber, inulin, as well as with date fiber **(Dhingra et al., 2012; Jandlová et al., 2017; Pytel et al., 2018)**. Yogurts are usually wrapped in HDPE (high-density polyethylene) or in PS (polystyrene) plastic cups **(Holm, 2010)**.

Scientific hypothesis

It was studied the effect of storage in four types of yoghurt with different addition of fiber on the concentration of phthalic acid esters. The assumption is that with the storage time, the concentration of phthalic acid esters in yoghurts will increase due to migration from the packaging. Further, the question arises which is the correlation of the concentrations of phthalic acid esters with pH.

MATERIAL AND METHODOLOGY

The yoghurts were produced at the Department of Food Technology at Mendel University. Content of the used milk (Holstein cattle, South-Moravian region, CZ) was determined fat to 3.50%, protein to 3.42% and lactose to 4.50%. The milk was pasteurized for 5 minutes at 85 °C, after that the milk was cooled down to 36 °C, and inoculated with a starter culture (0.5wt%) of original Bulgarian yogurt (GENESIS LABORATORIES, Bulgary). The mixture was fermented at 36 °C for 18 hours. The yoghurt was homogenized for 5 minutes, and divided into groups with additions of chia flour (ADVENI MEDICAL, spol. s.r.o., CZ) or bamboo fiber (J. Rettenmaier & Söhne GmbH & Co. KG, DE). One group was natural yoghurt with chia flour 1%, second yoghurt with chia flour 5%, next yoghurt with bamboo fiber 1% and last group yoghurt with 5% of bamboo fiber. The yoghurts were stored at 4 °C and were analysed in the 1st and 2nd week of storage. They were analysed also mixtures of yoghurts with chia flour/or bamboo fiber before filling into cups. All samples of yoghurts in cups were analysed in six repeats. The samples of yoghurts were analysed according to **Jarošová et al., (1999).** The samples were homogenized, weighed out 25 g into aluminium bowls, in which they were frozen. Afterwards the samples were lyophilized and extracted with hexane (PENTA s.r.o., CZ): acetone (PENTA s.r.o., CZ) (V:V= 1:1). The obtained solvent-free fat was weighed 0.5 grams out, from which the phthalate fraction was separated by gel permeation chromatography (ECOM spol. s.r.o., CZ). The fraction was further purified by sulphuric acid (Lach-Ner s.r.o., CZ): 1 ml of 96% sulphuric acid was added to 1 ml of sample dissolved in hexane, shaken for 10 minutes, 300 rpm, on a shaker (GFL 3005, GFL Gesellschaft für Labortechnik mbH, DE), centrifuged on centrifuge (Hettich-Zentrifugen D-78532 Tuttlingen-universal 32R, DE) (at -4 ° C, 10 min, 3000 rpm). The upper layer was given away as a waste. Then 2 ml of 68% sulfuric acid and 1 ml of hexane were added, all was shaken and centrifuged under the same conditions. The top layer was taken as the sample into 1.5 ml vials. Next, 1 ml of hexane was added, shaken and centrifuged under the same conditions, and taken as the sample into a vial. Subsequently, 1 ml of hexane was added again, shaken and centrifuged under the same conditions, and taken as a sample into a vial. The vials were dried with nitrogen (SIAD Czech spol. s r.o., CZ), and put 1 ml of acetonitrile (SIGMA-ALDRICH spol. s r.o., CZ). Measurement was performed on high-performance liquid chromatography (HPLC) (Agilent Technologies 1100 Series, DE), with UV detection at 224 nm, Zorbax Eclipse C8 column (Agilent Technologies, USA), mobile phase acetonitrile.

The cups from PS (polystyrene) (JEPA Plastics a.s., CZ), which were used, content plasticizer DBP (average concentration 59.45 $\mu g \cdot g^{-1}$) and DEHP (average concentration 8.99 $\mu g \cdot g^{-1}$) measured by **Gajdůšková et al., (1996).** The analyses were carried out in chemical laboratory at the Department of Food Technology at Mendel University. The evaluation was realized by software Agilent Chemstation for LC and LC / MS systems (Agilent Technologies, US).

In every type of yoghurts was determined pH by pH meter Portavo 907 Multi pH (KNICK, DE).

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Statistic analysis

The results were processed by Microsoft Excel 2010 (Microsoft Corporation, USA) and by Statistica 12 (StatSoft, USA).

Normality was tested by Shapiro–Wilk test. It was used Grubbs' test for to detect outliers data.

The t-test for comparison of mean values for dependent samples ($\alpha = 0.05$) was used to compare dibutyl phthalate concentrations and di-(2-ethylhexyl)phthalate concentrations in week 1 and week 2 by the same group of yoghurts.

Furthermore, a correlation coefficient was established between the average DBP concentrations in yoghurts with the measured pH of yoghurts, as well as the correlation coefficient between the average concentrations of DEHP in yoghurts with the measured pH of yoghurts. The correlation coefficient was determined even though the correlation does not imply causality.

RESULTS AND DISCUSSION

The concentrations of phthalic acid esters (DBP and DEHP) in mixtures of yoghurts with chia flour/or bamboo fiber before filling (Table 1) were higher (except for mixture of yogurt with 5% chia flour) than the yoghurts, which were stored.

The concentrations of phthalic acid esters is given in Table 2 and Table 3. Comparing the mean values of samples by t-test for dependent samples in yoghurts of the same type, when the DBP or DEHP concentrations in 1st week were compared with the DBP or DEHP concentrations in 2nd week. It was found no statistic difference ($p > 0.05$).

Table 1 Concentration of DBP and DEHP in mixtures of yoghurts before filling

Mixtures of yoghurts with	DBP [$\mu g \cdot g^{-1}$ of dry matter]	DEHP [$\mu g \cdot g^{-1}$ of dry matter]	DBP [$\mu g \cdot g^{-1}$ of original matter]	DEHP [$\mu g \cdot g^{-1}$ of original matter]
Chia flour 1%	36.43	16.84	6.09	2.82
Chia flour 5%	22.02	9.88	3.71	1.67
Bamboo fiber 1%	6.56	10.66	1.91	3.11
Bamboo fiber 5%	14.26	6.03	2.78	1.18

Table 2 Concentration of DBP and DEHP in yoghurts with chia flour

Yogurts with	1st week DBP [μg·g^{-1} of original matter]	2nd week DBP [μg·g^{-1} of original matter]	1st week DEHP [μg·g^{-1} of original matter]	2nd week DEHP [μg·g^{-1} of original matter]
Chia flour 1%	1.53	1.03	0.90	0.79
	1.21	1.30	0.88	1.16
	1.91	0.78	0.72	0.48
	1.21	1.34	0.60	0.62
	1.90	1.43	1.01	0.81
	1.55	0.84	0.93	0.69
Chia flour 5%	3.35	4.36	1.63	1.21
	4.07	4.50	5.60	0.85
	5.84	4.18	1.46	1.63
	5.21	5.48	1.94	1.99
	2.65	4.15	0.86	2.56
	3.31	2.81	0.90	1.49

Table 3 Concentration of DBP and DEHP in yoghurts with bamboo fibre

Yogurts with	1st week DBP [μg·g^{-1} of original matter]	2nd week DBP [μg·g^{-1} of original matter]	1st week DEHP [μg·g^{-1} of original matter]	2nd week DEHP [μg·g^{-1} of original matter]
Bamboo fiber 1%	4.22	5.81	1.80	2.93
	3.51	4.49	1.45	1.14
	3.16	4.29	1.26	1.21
	2.17	3.54	0.86	1.21
	3.72	2.94	1.45	0.76
	3.12	0.16	1.28	0.04
Bamboo fiber 5%	4.11	2.38	1.60	0.53
	2.33	1.93	0.57	0.40
	1.61	2.62	0.72	0.59
	1.43	3.92	0.88	0.89
	3.91	3.57	1.26	0.81
	2.24	3.65	0.78	0.90

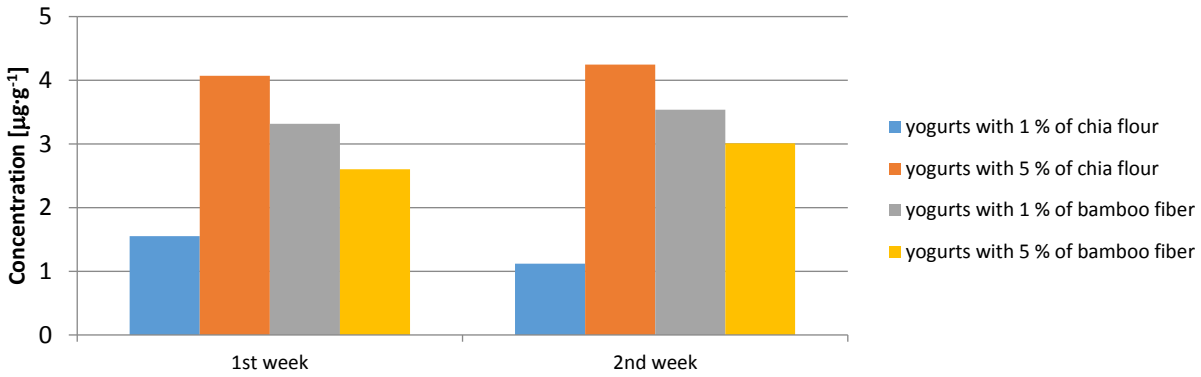

Figure 1 Average concentrations of DBP in yogurts.

Average concentrations of DBP with time of storage increased in yogurts with chia flour 5%, bamboo fiber 1%, and with bamboo fiber 5%. While the concentrations of DBP in yogurt with chia flour 1% decreased (Figure 1). Average concentrations of DEHP in all types of yogurt decreased over time (Figure 2).

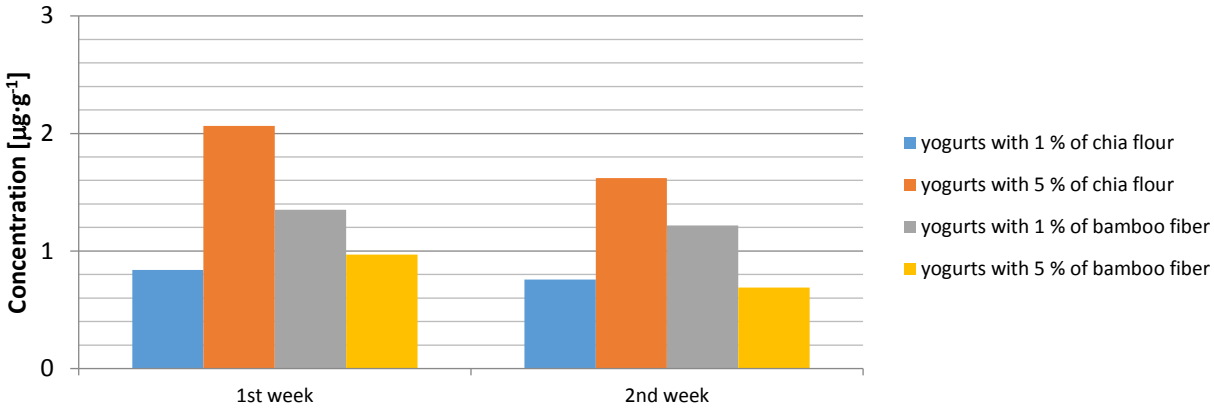

Figure 2 Average concentrations of DEHP in yogurts.

Table 4 pH of yoghurts in first week and in second week of storage.

Yogurts with	pH in 1st week	pH in 2nd week
Chia flour 1%	3.74	3.74
Chia flour 5%	3.78	3.76
Bamboo fiber 1%	3.51	3.62
Bamboo fiber 5%	3.51	3.62

In yoghurts, the average concentrations of DBP have always been found to be higher than the DEHP concentration, which corresponds to the fact that the DBP concentration in the cups was higher than the DEHP concentration.

The pH of each type of yoghurt was not very different (Table 4), but the correlation coefficient was determined. The correlation coefficient for the relationship between average DBP concentrations with pH was -0.0265 and for average DEHP concentrations with pH 0.3075.

In further research, it would be appropriate to test the effect of different pH in the same food on the migration of phthalic acid esters. Or make the study influence of different added concentrations on the migration of phthalic acid esters.

We were looking for some similar research to our research for better comparison, but we did not find any similar research with concentrations of phthalates in yoghurt enriched with flour or fiber. In study by **Rastkari et al., (2017)**, where they examined the transfer of phthalates from PET and HDPE into the acidic fluid during storage (0, 2, 4 and 6 months of measurement), the DEHP concentration increased with time in the liquids, the DBP concentration increased to 2 months, then dropped in the packaged fluid. In **Schecter et al., (2013)** study, the average concentration of DEHP was determined at 144 $ng \cdot g^{-1}$ and the average concentration of DBP was determined at 104.4 $ng \cdot g^{-1}$ in the category "other dairy products" purchased in New York, this category included yoghurt.

In study by **Sørensen, (2006)** was detected in raw milk concentration of DBP less than 9 $\mu g \cdot kg^{-1}$ and DEHP from 7 to 30 $\mu g \cdot kg^{-1}$, and in fruit yoghurt concentration of DBP below 9 $\mu g \cdot kg^{-1}$ and DEHP from 15 to 37 $\mu g \cdot kg^{-1}$.

In other study by **Sharman et al., (1994)** was measured in Norwegian raw milk the total amount of phthalate esters from 0.12 to 0.28 $mg \cdot kg^{-1}$. Concentration of DEHP in Norwegian cream was determined up to 1.93 $mg \cdot kg^{-1}$ in United Kingdom cream from 0.2 to 2.7 $mg \cdot kg^{-1}$. And in Spanish dairy products was detected the concentration of DEHP from below 0.01 to 0.55 $mg \cdot kg^{-1}$.

A study examining the transfer of phthalic acid esters from plastic articles to liquid simulants represented by distilled water, 3% acetic acid and 10% ethanol. The highest phthalate migration values were found in distilled water, followed in the solution of acetic acid and finally in the ethanol solution. However, the established concentrations did not exceed the limits set by legislation **(Bošnir et al., 2003)**.

CONCLUSION

Mean concentrations of di-(2-ethylhexyl) phthalate in yoghurts with time of storage decreased. Mean concentrations of di-(2-ethylhexyl) phthalate was detected in youghurt with 1% of chia flour in the 1st week of 0.84 $\mu g \cdot g^{-1}$ in the 2nd week of 0.76 $\mu g \cdot g^{-1}$, youghurt with 5% of chia flour in the 1st week of 2.06 $\mu g \cdot g^{-1}$ and in the 2nd week of 1.62 $\mu g \cdot g^{-1}$, youghurt with 1% of bamboo fiber in the 1st week of 1.35 $\mu g \cdot g^{-1}$ and in the 2nd week of 1.22 $\mu g \cdot g^{-1}$, youghurt with 5% of bamboo fiber in the 1st week of 0.97 $\mu g \cdot g^{-1}$ and in the 2nd week of 0.69 $\mu g \cdot g^{-1}$. It was compared with t-test mean values of concentrations in the 1st week and 2nd week. It was found no statistic difference between mean values of concentrations ($p > 0.05$).

Average dibutylphthalate concentrations dropped during storage for yoghurt with chia flour at 1% (in the 1st week of 1.55 $\mu g \cdot g^{-1}$ in the 2nd week of 1.12 $\mu g \cdot g^{-1}$) and rose in

yoghurts with chia flour at 5% (in the 1st week of 4.07 μg·g^{-1} in the 2nd week of 4.25 μg·g^{-1}) bamboo fiber at 1% (in the 1st week of 3.32 μg·g^{-1} in the 2nd week of 3.54 μg·g^{-1}) and 5% of bamboo fiber (in the 1st week of 2.60 μg·g^{-1} and in the 2nd week of 3.01 μg·g^{-1}). The concentrations were compared with t-test mean values in 1st week and 2nd week. It was found no statistic difference between mean values of concentrations (p >0.05).

The increase of dibutylphthalate concentration in yogurts caused migration from the plastic cups, where the dibutylphthalate concentration was more than 6 times higher than the di-(2-ethylhexyl) phthalate concentration. While the decline of di-(2-ethylhexyl) phthalate has most likely caused either dissociation or, reaction di-(2-ethylhexyl) phthalate with another food ingredient.

It was tested the correlation between the pH and concentrations of phthalic acid, however the pH of the yoghurt was too similar and the yoghurt too different. The correlation coefficient for the relationship between average dibutylphthalate concentrations with pH was -0.03 and for average di-(2-ethylhexyl) phthalate concentrations with pH 0.31. It would be advisable to monitor the migration of phthalic acid esters from the packaging to the same food with different of pH. And it would also be appropriate to determine the effect of migration of phthalates at different fiber concentrations.

REFERENCES

Andrady, A. L., Neal, M. A. 2009. Applications and societal benefits of plastics. *Philosophical Transactions of the Royal Society B: Biological Sciences,* vol. 364, no. 1526, p. 1977-1984. https://doi.org/10.1098/rstb.2008.0304

ATSDR, 2001. Toxicological profile for di-*n*-butyl phthalate [online] 2001-10-04. [cit. 2017-12-04] Available at: https://www.atsdr.cdc.gov/toxprofiles/tp135.pdf.

ATSDR, 2002. Toxicological profile for di(2-ethylhexyl)phthalate [online] 2001-06-06. [cit. 2017-12-05] Available at: https://www.atsdr.cdc.gov/toxprofiles/tp9.pdf.

Benson, R. W. 2014. Hazards of Food Contact Material: Phthalates. In Motarjemi, Y., Moy, G., Todd, E. *Encyclopedia of Food Safety*, London, UK : Elsevier, p. 438-443. ISBN 9780123786135. https://doi.org/10.1016/B978-0-12-378612-8.00219-5

Betteridge, V. A., Caers, W., Lupton, J. R., Slavin, J. L., Devries, J. 2012. Implementation Issues of the Codex Definition of Dietary Fiber: Degree of Polymerization, Physiological, and Methodological Considerations. In Cho, S. S., Almeida, N. *Dietary fiber and health*. Boca Raton, FL : CRC Press, p. 19-32. ISBN 9781439899373. https://doi.org/10.1201/b12156-4

Bošnir, J., Puntarić, D., Škes, I., Klarić, M., Šimić, S., Zorić, I. 2003. Migration of phthalates from plastic products to model solutions. *Coll. Antropol.*, vol. 27, p. 23-30. PMid:12955888

Brimer, L. 2011. Contaminants from processing machinery and food contact materials. In Brimer, L. *Chemical food safety*. Cambridge, MA : CABI, p. 191-195. ISBN 978-1-84593-676-1. https://doi.org/10.1079/9781845936761.0191

Cadogan, D. 2002. Health and environmental impact of phthalates. *Plastics, Additives and Compounding*, vol. 4, no. 6, p. 28-29. https://doi.org/10.1016/S1464-391X(02)80091-5

Clark, K. E., David, R. M., Guinn, R., Kramarz, K. W., Lampi, M. A., Staples, C. A. 2011. Modeling Human Exposure to Phthalate Esters: A Comparison of Indirect and Biomonitoring Estimation Methods. *Human and Ecological Risk Assessment: An International Journal*, vol. 17, no. 4, p. 923-965. https://doi.org/10.1080/10807039.2011.588157

Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1-89.

Dhingra, D., Michael, M., Rajput, H., Patil, R. T. 2012. Dietary fibre in foods: a review. *Journal of Food Science and Technology*, vol. 49, no. 3, p. 255-266. https://doi.org/10.1007/s13197-011-0365-5

Fromme, H., Küchler, T., Otto, T., Pilz, K., Müller, J., Wenzel, A. 2002. Occurrence of phthalates and bisphenol A and F in the environment. *Water Research*, vol. 36, no. 6, p. 1429-1438. https://doi.org/10.1016/S0043-1354(01)00367-0

Gajdůšková, V., Jarošová, A., Ulrich, R. 1996. Occurrence of phthalic acid esters in food packaging materials. *Potrav. Vědy*, vol. 14, no. 2, p. 99-108.

Hauser, R., Calafat, A. M. 2005. Phthalates and human health. *Occupational and Environmental Medicine*, vol. 62, no. 11, p. 806-818. https://doi.org/10.1136/oem.2004.017590

Hines, C. J., Hopf, N. B. N., Deddens, J. A., Silva, M. J., Calafat, A. M. 2011. Estimated daily intake of phthalates in occupationally exposed groups. *Journal of Exposure Science & Environmental Epidemiology*, vol. 21, no. 2, p. 133-141. https://doi.org/10.1038/jes.2009.62

Holm, V. K. 2010. Shelf Life of Foods in Biobased Packaging. In Robertson, G. L. *Food packaging and shelf life: a practical guide*. Boca Raton, FL : CRC Press/Taylor & Francis Group, p. 353-365. ISBN 1420078445.

Jandlová, M., Kumbár, V., Jarošová, A., Pytel, R., Nedomová, Š., Ondrušíková, S. 2017. The sensory evaluation of yoghurts with chia flour, quinoa flour, nopal powder, apple fiber and bamboo fiber. *MendelNet 2017: Proceedings of International PhD Students Conference.* Brno : Mendel University in Brno, p. 547-552. ISBN 978-80-7509-529-9.

Jarošová, A., Gajdůšková, V., Razsyk, J., Sevela, K. 1999. Di-2-ethylhexyl phthalate and di-n-butyl phthalate in the tissues of pigs and broiler chicks after their oral administration. *Veterinary Medicine*, vol. 44, no. 3, p. 61-70.

Kamrin, M. A. 2009. Phthalate Risks, Phthalate Regulation, and Public Health: A Review. In *Journal of Toxicology and Environmental Health, Part B*, vol. 12, no. 2, p. 157-174. https://doi.org/10.1080/10937400902729226

Koch, H. M., Drexler, H., Angerer, J. 2003. An estimation of the daily intake of di(2-ethylhexyl)phthalate (DEHP) and other phthalates in the general population. *International Journal of Hygiene and Environmental Health*, vol. 206, no. 2, p. 77-83. https://doi.org/10.1078/1438-4639-00205

Larsson, K., Lindh, C. H., Jönsson, B. A. G., Giovanoulis, G., Bibi, M., Bottai, M., Bergström, A., Berglund, M. 2017. Phthalates, non-phthalate plasticizers and bisphenols in Swedish preschool dust in relation to children's exposure. *Environment International*, vol. 102, p. 114-124. https://doi.org/10.1016/j.envint.2017.02.006

Latini, G. 2005. Monitoring phthalate exposure in humans. *Clinica Chimica Acta*, vol. 361, no. 1-2, p. 20-29. https://doi.org/10.1016/j.cccn.2005.05.003

Martino-Andrade, A. J., Chahoud, I. 2010. Reproductive toxicity of phthalate esters. *Molecular Nutrition & Food Research*, vol. 54, no. 1, p. 148-157. https://doi.org/10.1002/mnfr.200800312

McKinley, M. C. 2005. The nutrition and health benefits of yoghurt. *International Journal of Dairy Technology*, vol. 58, no. 1, p. 1-12. https://doi.org/10.1111/j.1471-0307.2005.00180.x

Meeker, J. D., Sathyanarayana, S., Swan, S. H. 2009. Phthalates and other additives in plastics: human exposure and associated health outcomes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 364, no. 1526, p. 2097-2113. https://doi.org/10.1098/rstb.2008.0268

Mikula, P., Svobodová, Z., Smutná, M. 2011. Phthalates: toxicology and food safety – a review. *Czech Journal of Food Sciences*, vol. 23, no. 6, p. 217-223. https://doi.org/10.17221/3394-CJFS

Petersen, J. H. 2003. Migration of Compounds from Food Contact Materials and Articles. In D'Mello, J. P. F. *Food safety: contaminants and toxins*. Cambridge, MA : CABI Publishing, p. 271-291. ISBN 0851996078. https://doi.org/10.1079/9780851996073.0271 PMid:14568993

Piotrowska, B. 2005. Toxic Components of Food Packaging Materials. In Dąbrowski, W. M., Sikorski, Z. E. *Toxins in Food.* Boca Raton, FL : CRC Press, p. 313-333. ISBN 978-0-8493-1904-4.

Przybylińska, P. A., Wyszkowski, M. 2016. Environmental contamination with phthalates and its impact on living organisms. *Ecological Chemistry and Engineering S*, vol. 23, no. 2, p. 347-356. https://doi.org/10.1515/eces-2016-0024

Pytel, R., Cwiková, O., Ondrušíková, S., Nedomová, Š., Kumbár, V. 2018. Effect of additives to microbiological quality of yogurts. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 186-194. https://doi.org/10.5219/886

Rastkari, N., Jeddi, M. Z., Yunesian, M., Ahmadkhaniha, R. 2017. The Effect of Storage Time, Temperature and Type of Packaging on Release of Phthalate Ester into Packed Acidic Juice. *Food Technology and Biotechnology*, vol. 55, no. 4, p. 562-569. https://doi.org/10.17113/ftb.55.04.17.5128

Schecter, A., Lorber, M., Guo, Y., Wu, Q., Yun, S. H., Kannan, K., Hommel, M., Imran, N., Hynan, L. S., Cheng, D., Colacino, J. A., Birnbaum, L. S. 2013. Phthalate Concentrations and Dietary Exposure from Food Purchased in New York State. *Environmental Health Perspectives*, vol. 121, no. 4, p. 473-479. https://doi.org/10.1289/ehp.1206367

Schettler, T. 2006. Human exposure to phthalates via consumer products. *International Journal of Andrology*, vol. 29, no. 1, p. 134-139. https://doi.org/10.1111/j.1365-2605.2005.00567.x

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, no. 3, p. 375-385. https://doi.org/10.1080/02652039409374236

Sørensen, L. K. 2006. Determination of phthalates in milk and milk products by liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry,* vol. 20, no. 7, p. 1135-1143. https://doi.org/10.1002/rcm.2425

Velíšek, J. 2002. *Food chemistry III.* 2nd ed. Tábor, Czech Republic : OSSIS. 343 p. ISBN 80-86659-03-8.

Velíšek, J., Hajšlová, J. 2009. *Food chemistry II.* 3rd ed. Tábor, Czech Republic : OSSIS. 623 p. ISBN 978-80-86659-16-9.

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ASSESMENT OF MOLECULAR DIVERSITY OF INTERNAL TRANSCRIBED SPACER REGION IN SOME LINES AND LANDRACE OF PERSIAN CLOVER (*TRIFOLIUM RESUPINATUM* L.)

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ABSTRACT

Clover which is an herbaceous, annual, and self-pollinated plant belongs to fabaceae family (legumes) and has become naturalized in Iran, Asia Minor and the Mediterranean eastern suburban countries. The aim of the present study is ITS molecular evaluation of the nuclear ribosomal genes of lines and landraces of Persian Clover. The sequences were aligned using ClustalW method and by MegAlign software and the dendrogram of different phylogenetic and matrix relationships between the sequences were drawn. The results showed little genetic diversity between the lines and the landrace. The conserved sequence of the analyzed gene in the Persian clover is 561 base. Totally, 740 loci (69 and 671 loci, respectively, with and without removal and addition), 9 Singletons, and 5 haplotypes were identified. The highest rate of transfer was observed in pyrimidine (%16.3). The numerical value of the ratio (dN/dS) was 0.86, and since it was less than 1, the pure selection on the studied gene happened. The lines and landraces were not separated based on their geographic locations. In general, the results indicated that the highest rate of the regional diversity belonged to the clover plants in Lorestan region. Moreover, ITS markers did not seem suitable enough for evaluating the intra- species genetic variation, but it was quite well- suited for inter-species or intergeneric evaluation.

Keywords: Persian clover; haplotype; genetic diversity; Ribosomal DNA; dN/dS

INTRODUCTION

After alfalfa, Clover, with about 100,000 hectares of cultivation area, is the most important forage plant and has a special place in the country **(Lala et al., 2018)**. They are located in three main centers of diversity: Eurasia, South Africa, and the Americas.

Iran is one of the most important centers of the clover genetic diversity at the main origin of Eurasian diversity **(Yousefi et al., 2018)**. The 60% of clover species are Eurasian- originated and 7% are endemic species of Iran, Tūrān, and Euro-Siberia **(Abbasi et al., 2012)**.

Based on the latest statistics, the total mass of forage plants kept in the National Plant Gene Bank of Iran is 5989 landrace among which clover with a mass of 1859 has got the second place, after alfalfa with a mass of 2285. In the clover collection, 416 landrace belong to the Persian clover (one- cut or multi- cut) **(Yousefi et al., 2018)**. Clover or trefoil are is a species of plants in the *leguminous pea* family in Fabaceae of the genus *Trifolium* (Latin, *tres* "three" + *Folium* "leaf"); thus, all species belong to this trifoliate genome. It consists of 238 species of plants of which 49 species are distributed medicinally in Iran **(Roma-Marzio et al., 2018)**. About a third of the clover species is annual and one of these annual species is Persian clover (*Trifolium resupinatum*) **(Hussain et al., 2017; Yousefi et al., 2018)**.

According to many classification systems, including APG III system, Fabacea species is located in Fabales with the following three sub-species **(Shahverdi, 2014; Yousefi et al., 2018)**:

1- Cercis siliquastrum subspecies (*Caesalpinioideae*), with about 166 genera and 400 species, spread all over the world: e.g. *Cesalpinia*, *Senna,Bauhinia*, and *Amherstia*

2- Caesalpinia subspecies (*Mimosoideae*), with about 60 genera and 3,200 species, mostly grow in the tropics and warm temperate Asia and America: such as *Mimosa* and *Acasia*.

3- Acacia Senegal subspecies (*Papilionoideae*), with about 430 genera and 9000 species, are scattered all over the world. Plants such as *Astragalus, Lupines*, and Persian clover (*Trifolium Resupinatum*) are also known with the same subspecies.

According to **Chapman and Oldham, (2018)** and **Yousefi et al., (2018)** clovers have different species among which the most important ones are Berseem clover (Egyptian clover) (*Trifolium alexandrium*); red clover

(*Trifolium pratense* L); white clover (*Trifolium repense* L.); Crimson clover (clove) (*Trifolium incarnatum*); Alsike clover (Lsyk) (*Trifolium hybridum*); strawberry clover (*Trifolium fragiforum*); Alpine clover (*Trifolium alpestre*); mountain white clover (*Trifolium mentanum*); Largehop clover (*Trifolium campestre*); Lesser yellow clover (*Trifolium dubium*); Harest foot or Bottlegrass clover (*Trifolium arvense*); subterranean clover (*Trifolium subterraneum*); Arrow leaf colver (*Trifolium squarosum*), Persian clover (*Trifolium Resupinatum*) (Figure 4), and wild clover (Trifolium Clusii).

Given that the registered genotypes are so-called cultivars or varieties **(Bazdar and Sadeghi, 2018)**, the registered genotypes of Persian clover (*Trifolium Resupinatum*) which is the same crop clover is one-cut and multi- cut **(Chapman and Oldham, 2018; Yousefi et al., 2018)** with the following cultivars **(Shahverdi, 2014)**: (1) Kermanshahi 1; (2) Kermanshahi 2; (3) Haft tan; (4) P513; (5) Isfahanian triple- cut; (6) Isfahanian 7- cut; (7) Nahavandi (8) Hanedanian 7- cut; (9) Chegni Lorestan, (10) Doroud Lorestan, (11) Harati Boroujerd 1; (12) 7- cut Boroujerd; (13) Deh pir Lorestan, (14) Silakhor Boroujerd; (15) Elashtar Lorestan; (16) Kazeroon (17) Shazand; (18) Alavijan Markazi; (19) Reihan Markazi; (20) Tajra Markazi; (21) 7- cut Anaj; (22) 7- cut Qurchi Bashi Tabriz; (23) one- cut Kurdistan; (24) Surian Abade Fars; (25) Lordgan Bakhtiari, and (26) Kyambro (Persian clover cultivar in Australia). Of course, there are other cultivars which are less important, so their names will be discarded.

Genetic diversity, as the basis for the development and evolution of all creatures, is important for all those who somehow, theoretically or practically, deal with the genetic modification in organisms **(Vivodík et al., 2018; Fazeli-Nasab et al., 2013)**. Plant breeding which is both a science and an art is the most applicable science in this field and its efficient activity and continued viability depends greatly on genetic variation in the genetic species under investigation **(Sani et al., 2018; Yousef et al., 2018)**. Modifyaction of the plants was conducted with the aim of improving their quantity and quality, but it requires passing many steps among which the first and foremost ones are collecting the genetic resources of the desired plant, conserving the collection and determining the traits and the genetic diversity of the gathered samples **(McKain et al., 2018; Yousef et al., 2018)**.

ITS marker has never been used for genetic assessment of clover, whereas similar plants of the same family such as beans **(De Luca et al., 2018)**, Acacia **(Úrbez-Torres et al., 2016)**, Glycine and Flemingia **(Wu et al., 2013)**, *Brassica napus* **(Abdelmigid and El-Sayed, 2016)**, Subtribe Diocleinae **(Varela and Albornoz, 2013)**, chickpea **(Yadav et al., 2017)**, and other fungi including *Lallemantia* Kamrani, 2018 #5282}, *Myrothecium roridum* **(Jordan et al., 2018)**, *Rhizoctonia cerealis* **(Ji et al., 2017)** and *Alternaria burnsii* **(Singh et al., 2018)** were used frequently.

Presence of high levels of diversity in the first (T. *resupinatum*), the second (T. *clusii*), and the third (T. *fragiferum*) gene pools of Persian clover available in the National Plant Gene Bank of Iran, grounds for the users of this valuable germplasm to develop the superior varieties of Persian clover **(Abbasi, 2008)**. Moreover, the present study has used the clover samples gathered from different habitats of the country, especially the ones available in Borujerd Agricultural Research Center which were under modification and in the final stage of providing superior lines **(Shahverdi, 2014)**.

MATERIAL AND METHODS

Plant materials

The Persian clover lines and landraces used in this study (Table 1) are the results of a ten-year research project conducted to create superior lines in Boroujerd Agricultural Research Center **(Shahverdi, 2014)**.

DNA extraction and polymerase chain reaction (PCR)

Genomic DNA extraction method was performed based on SDS **(Dellaporta et al., 1983)**. DNA quality and quantity were determined respectively by using agarose gel (1%) and a UV–VIS spectrophotometer (LUV-300, Labnika, USA) and for amplification of the ITS1, ITS2 and 5.8 S fragments, the AB101primer with a forward sequence of 5'-ACGAATTCATGGTCCGGTGAAGTGTTCG-3' and the AB102 primer with a backward sequence of 5'-TAGAATTCCCCGGTTCGCTCGCCGTTAC-3' (as reported by **Robinson et al., (2001)**) were used. The PCR mixture provided by **Li et al., (2010)** methods and contained 20 ngDNA, 5 µL mastermix (Amplicon, Denmark), 0.7 µL of each primer (10 pmol) and water to a final volume of 15 µL. PCR amplification was carried out on Eppendorf Thermal Cycler (22331 Humburg) with the thermal profile: dena-turation step at 95°C for 5 min, then followed by 35 cycles of 55 s at94°C, the optimized annealing temperature (60°C) for 50 s and72°C for 55 s, finishing extension at 72°C for 10 min. To ensure successful amplification of 2% agarose gel, staining was performed using the Gel Red Staining.

Sequencing and data analysis

The final PCR products were sent to Macrogen Co. in South Korea for sequencing. To make corrections in the sequences, Sequencing Analysis Software ver 5.1 software and Chromas 2.3 software were used. Moreover, to assess individual sequences with the sequences in the NCBI database, BLAST online software was carried out. The sequences were aligned and clustered and their similarity and genetic distance were defined using ClustalW method and by DNASTAR software package and MEGA6 software.

Table 1 The name of Lines and Landrace of Persian Clover were used.

Sample Code	Line/ Landrace	Sample Name	Province	Accession Number	Geography Area
1	Line	Kazeroon	Fars	KY021172	N, 51° 39′ 29.99″ E, 29° 37′ 5.99″
2	Line	Kordestan	Kordestan	KY021173	N, 46° 59′ 45.6″ E, 35° 18′ 40.68″
3	Line	Eghlid	Fars	KY021168	N, 52° 41′ 9.27″ E, 30° 54′ 21.79″
4	Line	Abadeh	Fars	KY021161	N, 52° 40′ 12″ E, 31° 10′ 48″
5	Line	Aligodarz	Lorestan	KY021163	N, 49° 40′ 12″ E, 33° 22′ 12″
6	Line	Ghoorchi Bashi	Markazi	KY021170	N, 49° 52′ 22.08″ E, 33° 39′ 10.08″
7	Line	Aleshtar	Lorestan	KY021162	N, 48° 15′ 0″ E, 33° 52′ 12″
8	Line	Dorood	Lorestan	KY021167	N, 48° 42′ 0″ E, 33° 24′ 0″
9	Line	Dochin Borujerd	Lorestan	KY021166	N, 48° 30′ 0″ E, 33° 48′ 0″
10	Line	Shazand	Markazi	KY021175	N, 49° 25′ 12″ E, 33° 55′ 48″
11	Line	Harati Borujerd	Lorestan	KY021171	N, 48° 50′ 34″ E, 33° 55′ 46″
12	Line	Dehkalan	Kordestan	KY021165	N, 47° 24′ 57.6″ E, 35° 16′ 30″
13	Line	Lordegan	Charmahal-Bakhteiari	KY021164	N, 50° 49′ 41.88″ E, 31° 30′ 34.92″
14	Line	Hafti Chin Borujerd	Lorestan	KY021169	N, 48° 35′ 23″ E, 33° 54′ 59″
15	Landrace	Native Borujerd	Lorestan	KY021174	N, 48° 30′ 0″ E, 33° 48′ 0″

DnaSP5 software was also used to perform and calculate additional analyses including dN and ds. Nucleotide substitution was also calculated based on the Tamura-Nei model pattern **(Tamura and Nei, 1993; Tamura et al., 2011)** as transition and Transversion.

RESULTS

Sequencing of ITS1-5.8s rRNA-ITS2 regions

After sequencing, the homology rate of the examined sequences with the sequences of the reference species available in the NCBI was measured and their overall similarity was in the range of 98 – 99%. Following the sequence alignment, a 695 bp fragment was obtained in which 561 was preserved. It should be noted that all the sequences were registered in the NCBI and their access numbers are mentioned in Table 1.

Using the sequences obtained with the help of DnaSP software, a total of 740 positions (671 positions without removal and addition (10 polymorphic loci, 661 monomorphic loci) and 69 loci with removal and addition), 9 Singletons (29 33 37 59 622 623 624 628 698) were identified. The proportion of 5 haplotype (haplotype diversity index 77/0) were identified.

Nucleotide substitution was calculated as transmission and cross in which the maximum and the minimum transfer rates belonged respectively to pyrimidine (16.03%) and purine (9.76%) (Table 2). Moreover, the ratio of various nucleotides to the entire nucleotides was calculated (Table 3), and the average ratios of 26.8% (thymine), 22.5 % (cytosine), 23.7% (adenine), and 26.9% (guanine) was obtained in the population.

In comparison with the Nucleotide variations that have no impact on the resulting amino acid (dS), the results of the Nucleotide variations that alter the amino acids (dN) can be a more useful and highly efficient method for detection process of natural selection during genetic evolution. If the ratio is greater than one, the selection is positive, if less than one, it is a pure selection, and if equal to one, it will show a neutral selection during the evolution of these genes (Dasmeh et al., 2014). In this study, the numerical value of this ratio (dN/dS) was 0.86 which was less than one indicating that the pure selection has occurred on the desired gene without any key changes.

Comparing Genetic Data between Paired Regions

Genetic distance matrix between the studied samples indicated that the genetic distance among the samples was from 0 to 0.15 (Table 4). It was found the lines and the landraces in clover were not clearly separated, but Aligoodarz line was in completely separated group (Figure 1). Moreover, the highest rate of the regional diversity was observed in the clovers of Lorestan region (Table 5), so it can be concluded that Lorestan could be regarded as the origin of clover.

Of the samples collected from different areas in the same clusters of genetic similarity or physical interchange seed can due to between different regions is the interpretation based on physical currency is more justified than genetic similarity **(Ninou et al., 2017)**.

Since ITS was unable to separate the lines of a species from each other, the sequences of some plants in other sub-families of *Fabaceae* available at NCBI were used for further assessments of the ITS ability in assessing the genetic diversity of different crops. They were then compared with the sequences of the present study. It was also found that each sub-species of the family was in a separate group (Figure 2); therefore, it can be concluded that ITS is a useful tool for genetic assessments, both inter-species and intergeneric.

Due to the fact that Iran has two types of clover dominant Persian clover (*Trifolium Resupinatum / persian clover*) and Wild clover (*Trifolium Clusii*) and it is likely that Persian clover has originated from the wild species, categorizing the Iranian clovers beside the wild clover (*Trifolium Clusii*) (Figure 2) proves the accuracy of this testing, so it can be concluded that there is a lot of genetic similarity between the Persian clover and the wild clover.

In the subsequent studies, the accuracy of the evolutionary process of the emergence of Persian clover from the wild clover will be discussed.

Table 2 The Percentage of displacement nucleotide sequences of ribosomal genes Persian clover on the pattern of Tamura-Nei model (**Tamura and Nei, 1993**).

	A	T	C	G
A	-	*6.83*	*5.65*	**11.43**
T	*5.65*	-	**13.26**	*6.62*
C	*5.65*	**16.03**	-	*6.62*
G	**9.76**	*6.83*	*5.65*	-

Note: Black and italicized numbers represent transition and transversion substitutions respectively.

Table 3 The base pair ratio of ITS sequences in all lines and landrace of Persian Clover.

Genotypes	T(U)	C	A	G
1	27.1	22.6	23.3	27.1
2	27.0	22.6	23.4	27.1
3	26.7	22.4	23.8	27.1
4	26.8	22.3	23.8	27.1
5	27.5	23.0	22.3	27.2
6	26.8	22.3	23.7	27.2
7	26.8	22.4	23.7	27.1
8	26.7	22.4	24.0	27.0
9	26.8	22.3	24.1	26.8
10	26.7	22.6	24.1	26.7
11	26.8	22.4	24.1	26.7
12	26.7	22.6	24.1	26.7
13	26.7	22.6	24.1	26.7
14	27.0	22.6	23.4	27.1
15	26.7	22.6	24.1	26.7

Table 4 Distance Matrix of ITS region sequence variation among genotypes of Persian Clover.

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	0.000													
3	0.001	0.001												
4	0.001	0.001	0.000											
5	0.015	0.015	0.014	0.014										
6	0.001	0.001	0.001	0.001	0.015									
7	0.001	0.001	0.001	0.001	0.015	0.000								
8	0.001	0.001	0.001	0.001	0.015	0.001	0.001							
9	0.001	0.001	0.001	0.001	0.015	0.000	0.000	0.001						
10	0.001	0.001	0.000	0.000	0.014	0.001	0.001	0.001	0.001					
11	0.001	0.001	0.001	0.001	0.015	0.000	0.000	0.001	0.000	0.001				
12	0.001	0.001	0.000	0.000	0.014	0.001	0.001	0.001	0.001	0.000	0.001			
13	0.001	0.001	0.000	0.000	0.014	0.001	0.001	0.001	0.001	0.000	0.001	0.000		
14	0.000	0.000	0.001	0.001	0.015	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	
15	0.001	0.001	0.000	0.000	0.014	0.001	0.001	0.001	0.001	0.000	0.001	0.000	0.000	0.001

Table 5 Distance Matrix of ITS region sequence variation among genotypes of Persian Clover based on provinces that were collected.

Province	Genetic distance
Fars	0.000994657
Kordestan	0.001491986
Lorestan	0.004567513
Markazi	0.001494834

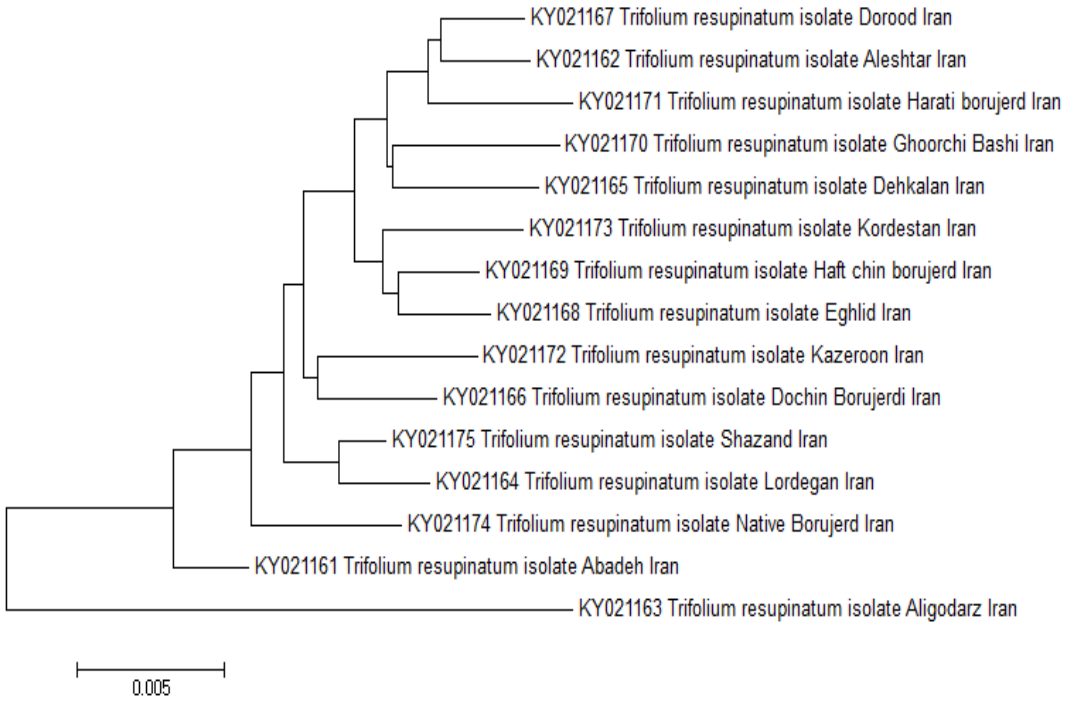

Figure 1 Cluster analysis of the genotypes of Persian Clover based on ITS sequences using ClustalW and NJ methods using MEGA6 software.

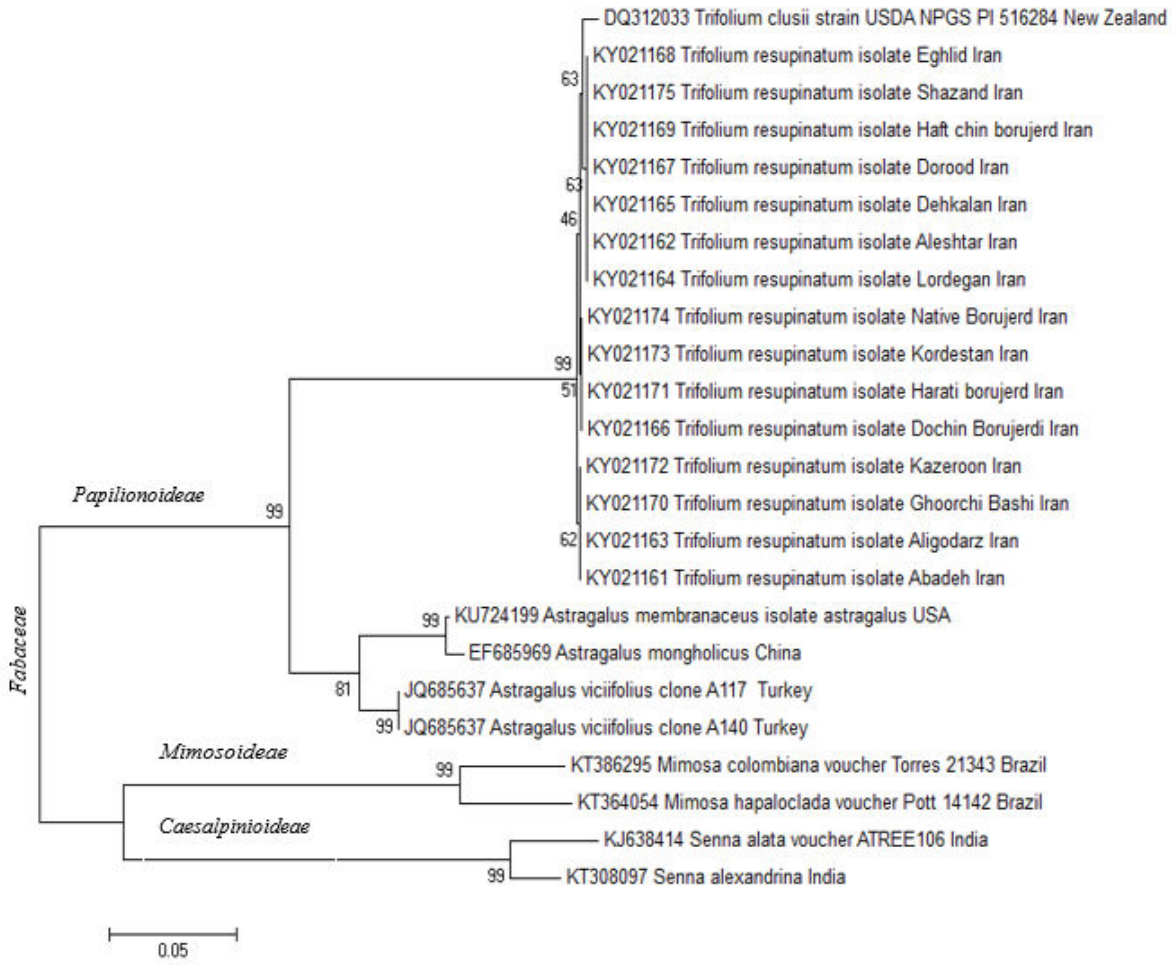

Figure 2 Cluster analysis of the genotypes of Persian Clover in this research with some other sub-family's clover ITS sequences were related from NCBI using ClustalW and NJ methods using MEGA6 software.

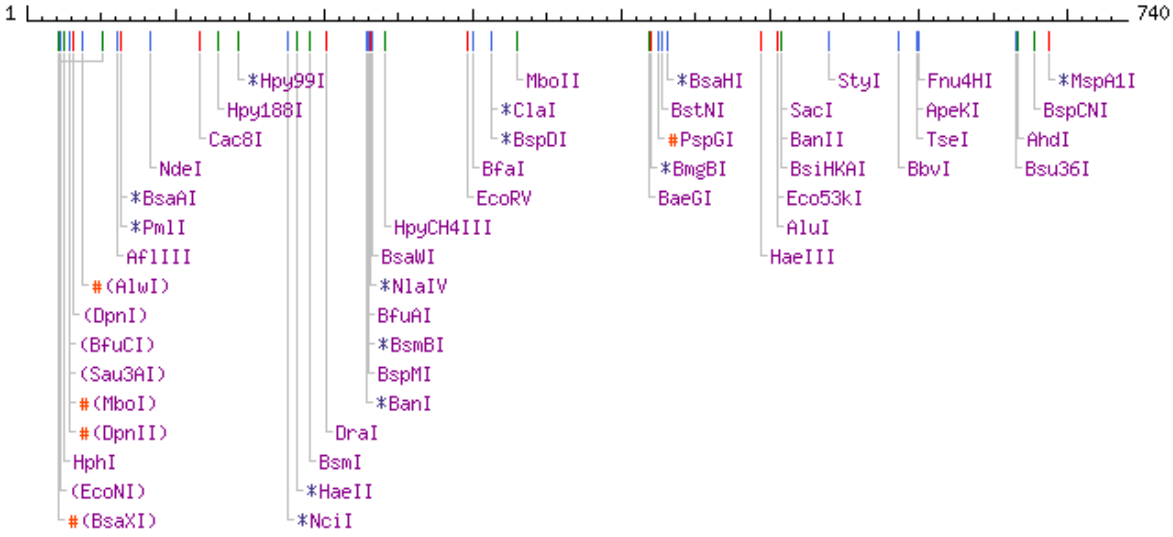

Figure 3 The restriction enzymes cut position on ITS gene sequence.

Table 6 Introduce the affect restriction enzymes on ITS gene in this research.

Enzyme Name	Cut Number	Cut Site	Fragment Length
EcoNI	1	5'..CCTNN▼NNNAGG…3' 3'..GGANNN▲NNTCC…5'	24 Nucleotide and 716 Nucleotide
MspA1I	1	5'..CMG▼CKG…3' 3'..GKC▲GMC…5'	51 Nucleotide and 689 Nucleotide
HphI	1	5'..GGTGA(N)^{8}▼…3' 3'..CCACT(N)7▲…5'	26 Nucleotide and 714 Nucleotide

Figure 4 Persian clover *Trifolium resupinatum* L., Photography by: Dr. Mohammad shahverdi.

In Silico Analysis of Clover Samples

Consensus Sequence of all samples, analyzed by using BioEdit Sequence Alignment Editor 7 software **(Hall, 1999)**, was entered in Nebcutter (http://tools.neb.com/NEBcutter2/) to identify the specific restriction enzymes that are able to recognize haplotypes (Figure 3). The results showed that different human enzymes are capable of cutting in the amplified area, so three types of restrictions enzymes (EcoNI, MspA1I and HphI) can cut and distinguish the amplified area in different samples (Table 6).

DISCUSSION

The results of the present study showed that the clove lines and landrace were not separated based on their geographic loci, yet the highest intra-region diversity belonged to the clovers in Lorestan region. The results showed that ITS marker was not suitable for intraspecific evaluation but it was useful for inter-species and intergeneric assessments. Moreover, the Persian clover (*Trifolium Resupinatum / Persian clover)* and wild clover (*Trifolium Clusii*) were put together in a separate group and given that Iran's dominant species of clover are *Trifolium Resupinatum / persian clover* and *Trifolium Clusii*, it is likely that the crop clover has emerged from wild clover.

Given that most genes responsible for resistance to diseases, pests, environmental stresses and genes responsible for product quality are usually found in the centers of diversity, so plant breeders having accurate information about the genetic diversity of each plant can engage more effectively using the genetic resources, so they can directly collect the required genetic resources. In addition, such information has led the plant breeders and those involved in conservation of these plant resources to the issue of biodiversity issues to use them. Moreover, according to Vavilov's theory, the origin of these plants belongs to the centers with the greatest diversity (Lalramnghaki). Therefore, in collecting clover germplasm, it is highly recommended to pay more attention to these geographic regions of Iran (especially Lorestan).

Several ecological factors have led to the accumulation of genetic differences between both populations. Different geographical locations vary in terms of some ecological characteristics, including latitude and longitude, temperature, and humidity. These factors, so- called eco-geographic, cause genetic variation between the two populations. According to the results, using more populations in the geographical areas is necessary to confirm the existing pattern.

Due to new combinations of genes, in the cross-pollinated species, the high genetic flow and the environmental pressure governing the region have established a series of specific genes, but the genetic distance within the populations is so sparse. Instead, the diversity of the population is relatively low **(Rauf et al., 2010)**. However, in the self-pollinated species, because of the different alleles within a population, there are more changes within populations **(Mirzaei and Mirzaghaderi, 2017; Petrova et al., 2017)**. In general, in both self-pollinated and cross-pollinated plants, the variation within the population was mostly greater than the variation between the populations, but this variation in self-pollinated plants was greater than in the cross-pollinated species.

Some key factors used to explain the greater intra-population diversity in the plants are self-pollination, annual, increased by seed, number of studied allelic sites studied, allelic and genotypic loci of the population, type of crosses, and population size **(Thormann et al., 2017)**. Analysis of the genetic diversity in populations faces difficulties due to the interference of several factors, including integration, cross kinship, migration and differences between the individuals of the populations **(Ndjiondjop et al., 2018)**.

In some studies, it has been indicated that ITS marker was suitable for both inter-species and intergeneric assessments so that in a research a different species of 6 genera belonging to the family *Subtribe Diocleinae* were evaluated using the 5.8S ribosomal genome, distance matrix, and Furthest Neighbor method and finally, it was concluded that the genomes so- called *Calopogonium* and *Pachyrhizus* which belonged to this family till the beginning of this study were clearly separated and diagnosed Meanwhile, the phytochemical and morphological results were obtained after this separation and the accuracy of the ITS result was also confirmed and it was concluded that ITS in section of 5.8S could be a useful tool for evaluating various plants in the genus level **(Varela et al., 2004)**.

In another study, four species of Glycine and two species of *Flemingia* were evaluated using ITS1 and ITS2. Fragments with a length of 595 to 622 were replicated and the cluster analyses of two species were placed in separate clusters. It was also concluded that ITS can be useful for intraspecific evaluation (Wu et al., 2013). Using ITS sequencing data in the nuclear ribosomal DNA as well as plastid DNA sequencing (*psbA-trnH intergenic spacer* regions) and morphological markers (the number of edges and seam width of fruit), the phylogenetic relationships between 39 species of *Bunium* were studied and the results showed that the genome *Bunium* has more than one subtype. It is composed of two main sub-branches. The results of molecular, morphological and carbologic studies in this study have shown similar patterns of phylogenetic relations of the species and have also confirmed each other **(Degtjareva et al., 2009)**.

In another study, 5 lanraces of *Dracocephalum* with 2 landraces of *D. moldavica* and *Basil* plants (as excluded plants) were studied using ITS regions (Internal Transcribed Spacers) and then the results showed that ITS marker was appropriated to intergeneric evaluation **(Haidari et al., 2014)**.

In other studies, it was also indicated that ITS marker is not suitable for intra species evaluation so that in a research, 44 population of Egyptian bean along with 5 other species were assessed using ITS. ITS marker showed an unexpectedly kinship between *Angustifolia* and *Lutei*. Likewise, based on the geographic areas, the results of ITS made a distinction between the clovers in the East and West regions of the US, but the other groups were various and even in the group of the South East of America, there were perennial beans. In general, the results indicated that except for some cases, intergeneric phylogenetic is not resolved by ITS **(Ainouche and Bayer, 1999)**.

Meanwhile, in another study, 51 species of Acacia of the family Phylodina (*leguminous* family) and a different kind of *Lysiloma divaricate* were assessed by using ITS. Cluster analysis showed that the two groups were in two main species: the first species were quite unified in terms of the morphologic features, whereas the second group were different **(Brown et al., 2010; Murphy et al., 2003)**.

In another study, six different breeds of species *Glycine tomentella* were evaluated using ITS, yet no significant difference was observed between ITS regions of these diverse species (Rauscher et al., 2004). Potential variability in the ITS region, ribosomal DNA of the isolates of *Fusarium solani* of potato and its relation to pathogenesis and its geographical origin in the provinces of Khorasan and Northern Razavi were investigated and the results showed that PCR-ribotyping technique showed no relationship in separate groups between the geographical areas and pathogenesis of the isolates **(Baghaee Ravari et al., 2007)**.

CONCLUSION

According to the results of the ITS marker in lack of separation of the lines and the landraces in Persian clover based on the geographical location and other key traits, it can be concluded that the ITS marker is not so suitable for analyzing the intraspecific genetic diversity, but on the basis of a comparison between the lines and the landraces used in this study as well as other plants of different genera of the *Fabaceae* family (provided on NCBI site), it was determined that ITS can be regarded as a useful tool for genetic assessments, both inter-species and intergeneric. In addition, in this study, the numerical value ratio (dN/dS) was 0.86, indicating that the pure selection has occurred on the studied gene, yet has made no key changes. On the other hand, of 740 loci, 671 loci were without removal and addition and only 69 loci had removal and addition. As a result, the fact that the ratio of dN/dS is less than 1 and there are few loci for removal and addition indicates that there are small variations between different lines; thus, it can be the possible reason for the inability of ITS in separating the lines and the landraces.

Since the source or origin of the plants belong to the centers with the highest diversity and regarding the point that Lorestan lines are the most diverse lines, it is necessary to reconsider this region while collecting clover germplasm used for exploitation of eugenics.

REFERENCES

Abbasi, M. R. 2008. Genetic diversity of Persian clover (*Trifolium resupinatum*) gene pools in National Plant Gene Bank of Iran. *Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research*, vol. 16, no. 1, p. 37-49.

Abbasi, M. R., Mirakhorli, A., Mahdipur, A., Hasanzadeh, A., Kanani, R., Alitabar, R. A., Mokhatarpur, H., Safaei, H., Fathi, A., Khakizad, G. R., Nadali, F., Nakhaei, A., Kamaledin Abbasi, M., Dadfar, S., Hajgholizadeh, G., Taheriun, G. R., Safari, S., Hamzenejad, A., Abaduz, G. R., Zamanian, M., Jahanbani, F., Samani, M., Darkhal, H. 2012. Seed collection and centers of diversity of *Trifolium* genetic resources in Iran. *Electronic Journal of crop Production*, vol. 5, no. 4, p. 191-204.

Abdelmigid, H. M., El-Sayed, A. 2016. Molecular phylogenetic implications in *Brassica napus* based on internal transcribed spacer sequences. *J. Genet.,* vol. 95, no. 2, p. 447-451. https://doi.org/10.1007/s12041-016-0631-4 PMid:27350690

Ainouche, A.-K., Bayer, R. J. 1999. Phylogenetic relationships in Lupinus (*Fabaceae*: *Papilionoideae*) based on internal transcribed spacer sequences (ITS) of nuclear ribosomal DNA. *American Journal of Botany*, vol. 86, no. 4, p. 590-607. https://doi.org/10.2307/2656820 PMid:10205079

Baghaee Ravari, S., Falahati Rastegar, M., Jafarpour, B., Shokoohifar, F. 2007. The study of probable variation in ITS-rDNA region of *Fusarium solani* in potato and its correlation with pathogenicity and geographical origin in Razavi and Northern Khorasan Provinces. *Journsl of Agrictural Science and Natural Resource*, vol. 14, no. 3, p. 1-9.

Bazdar, M., Sadeghi, H. 2018. Antioxidant activity of Trifolium resupinatum L. exposed to different extracts from leaves, flowers and shoots of Prangos ferulacea. *Spanish Journal of Agricultural Research*, vol. 15, no. 4, p. 0303.

Brown, G. K., Clowes, C., Murphy, D. J., Ladiges, P. Y. 2010. Phylogenetic analysis based on nuclear DNA and morphology defines a clade of eastern Australian species of *Acacia s. s.* (section *Juliflorae*): the 'Acacia longifolia group'. *Australian Systematic Botany*, vol. 23, no. 3, p. 162-172. https://doi.org/10.1071/SB09037

Dasmeh, P., Serohijos, A. W., Kepp, K. P., Shakhnovich, E. I. 2014. The influence of selection for protein stability on dN/dS estimations. *Genome biology and evolution*, vol. 6, no. 10, p. 2956-2967. https://doi.org/10.1093/gbe/evu223 PMid:25355808

De Luca, D., Cennamo, P., Del Guacchio, E., Di Novella, R., Caputo, P. 2018. Conservation and genetic characterisation of common bean landraces from Cilento region (southern Italy): high differentiation in spite of low genetic diversity. *Genetica*, p. 1-16. https://doi.org/10.1007/s10709-017-9994-6

Degtjareva, J. V., Kljuykov, E. V., Samigulli, T. H., Valiejo-Roman, C. M., Pimenov, M. G. 2009. Molecular Appraisal of Bunium and Some related arid and sub-arid geophilic *Apiaceae-Apioideae* taxa of ancient Mediterranean. *Botanical Journal of the Linnean Society*, vol. 160, p. 149-170. https://doi.org/10.1111/j.1095-8339.2009.00970.x

Dellaporta, S. L., Wood, J., Hicks, J. B. 1983. A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter*, vol. 1, no. 4, p. 19-21. https://doi.org/10.1007/BF02712670

Fazeli-Nasab, B., Naghavi, M. R., Mehrabi, A. A. 2013. Allelic Variation of Microsatellite Markers from Linkage Group A Genome in Wild Populations of Einkorn and Hexaploid Wheat. *Agricultural Biotechnology*, vol. 4, no. 1, p. 53-62. http://doi.org/10.13140/RG.2.1.1434.4164

Haidari, P., Mehrabi, A. A., Nasrollah Nejad Ghomi, A. A. 2014. Genetic Diversity of Balm (*Melissa Officinalis* L.) Landraces and Genetic Relationship Within and Between Them using ITS Markers. *Journal of Crop Breeding*, vol. 6, no. 13, p. 29-39.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, p. 95-98, [London]: Information Retrieval Ltd., c1979-c2000.

Hussain, S., Verry, I., Jahufer, M., Williams, W. 2017. Cytological and Morphological Evaluation of Interspecific Hybrids between *Trifolium repens* and *T. uniflorum*. *Crop Science*, vol. 57, no. 5, p. 2617-2625. https://doi.org/10.2135/cropsci2017.05.0314

Chapman, C. J., Oldham, M. J. 2018. Reversed Clover, *Trifolium resupinatum* L. (*Fabaceae*), Confirmed in Canada. *The Canadian Field-Naturalist*, vol. 131, no. 4, p. 328-330. https://doi.org/10.22621/cfn.v131i4.1866

Ji, L., Liu, C., Zhang, L., Liu, A., Yu, J. 2017. Variation of rDNA internal transcribed spacer sequences in *Rhizoctonia cerealis*. *Curr. Microbiol.*, vol. 74, no. 7, p. 877-884. https://doi.org/10.1007/s00284-017-1258-2 PMid:28474105

Jordan, B., Culbreath, A., Brock, J., Dutta, B. 2018. First Report of Myrothecium Leaf Spot Caused by *Myrothecium roridum* on Pepper in the United States. *Plant Disease*, vol. 102, no. 1, p. 246. https://doi.org/10.1094/PDIS-06-17-0918-PDN

Lala, S., Amri, A., Maxted, N. 2018. Towards the conservation of crop wild relative diversity in North Africa: checklist, prioritisation and inventory. *Genetic Resources and Crop Evolution*, vol. 65, no. 1, p. 113-124. https://doi.org/10.1007/s10722-017-0513-5

Lalramnghaki, H. C. Morphological and molecular characterization of *entomopathogenic nematode*, *Heterorhabditis baujardi* (*Rhabditida, Heterorhabditidae*) from Mizoram, northeastern India. *Journal of Parasitic Diseases*, p. 1-9.

Li, X., Xie, R., Lu, Z., Zhou, Z. 2010. The origin of cultivated citrus as inferred from internal transcribed spacer and chloroplast DNA sequence and amplified fragment length polymorphism fingerprints. *Journal of the American Society for Horticultural Science*, vol. 135, no. 4, p. 341-350.

McKain, M. R., Johnson, M. G., Uribe-Convers, S., Eaton, D., Yang, Y. 2018. Practical considerations for plant phylogenomics. *Appl. Plant Sci.,* vol. 6, no. 3, p. e1038. https://doi.org/10.1002/aps3.1038 PMid:29732268

Mirzaei, K., Mirzaghaderi, G. 2017. Genetic diversity analysis of Iranian *Nigella sativa* L. landraces using SCoT markers and evaluation of adjusted polymorphism information content. *Plant Genetic Resources*, vol. 15, no. 1, p. 64-71. https://doi.org/10.1017/S1479262115000386

Murphy, D. J., Miller, J. T., Bayer, R. J., Ladiges, P. Y. 2003. Molecular phylogeny of *Acacia subgenus Phyllodineae* (*Mimosoideae: Leguminosae*) based on DNA sequences of the internal transcribed spacer region. *Australian Systematic Botany*, vol. 16, no. 1, p. 19-26. https://doi.org/10.1071/SB01042

Ndjiondjop, M. N., Semagn, K., Sow, M., Manneh, B., Gouda, A. C., Kpeki, S. B., Pegalepo, E., Wambugu, P., Sié, M., Warburton, M. L. 2018. Assessment of Genetic Variation and Population Structure of Diverse Rice Genotypes Adapted to Lowland and Upland Ecologies in Africa Using SNPs. *Front. Plant Sci.*, vol. 9, no., p. 1-13.

Ninou, E. G., Mylonas, I. G., Tsivelikas, A. L., Ralli, P. E. 2017. Phenotypic diversity of Greek dill (*Anethum graveolens* L.) landraces. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, vol. 67, no. 4, p. 318-325.

Petrova, G., Petrov, S., Delcheva, M., Bancheva, S. 2017. Genetic diversity and conservation of Bulgarian endemic *Verbascum tzar-borisii* (*Scrophulariaceae*), *BioOne*, p. 307-316,

Rauf, M. A., Meetani, M. A., Khaleel, A., Ahmed, A. 2010. Photocatalytic degradation of methylene blue using a mixed catalyst and product analysis by LC/MS. *Chemical Engineering Journal*, vol. 157, no. 2-3, p. 373-378. https://doi.org/10.1016/j.cej.2009.11.017

Robinson, J., Harris, S., Juniper, B. 2001. Taxonomy of the genus *Malus Mill.*(*Rosaceae*) with emphasis on the cultivated apple, Malus domestica Borkh. *Plant Systematics and Evolution*, vol. 226, no. 1-2, p. 35-58. https://doi.org/10.1007/s006060170072

Roma-Marzio, F., D'Antraccoli, M., Astuti, G., Maccioni, S., Amadei, L., Peruzzi, L. 2018. Typification of the names in Trifolium described by Gaetano Savi. *Taxon*, vol. 67, no. 2, p. 411-421. https://doi.org/10.12705/672.10

Sani, S. G. A. S., Chang, P. L., Zubair, A., Carrasquilla-Garcia, N., Cordeiro, M., Penmetsa, R. V., Munis, M. F. H., Nuzhdin, S. V., Cook, D. R., von Wettberg, E. J. 2018. Genetic Diversity, Population Structure, and Genetic Correlation with Climatic Variation in Chickpea (*Cicer arietinum*) Landraces from Pakistan. *The Plant Genome*, vol. 11, no. 1, p. 1-11. https://doi.org/10.3835/plantgenome2017.08.0067 PMid:29505627

Shahverdi, M. 2014. Evaluation of final forage yield lines of Persian clover. Lorestan Natural resources and Agriculture Research Center, (Final report of the research project; 059-03-87089 Code), p. 1-36.

Singh, N. K., Patel, D. B., Chaudhari, S. R., Morad, B. G., Rabari, S. M., Chattopadhyay, A., Tetarwal, M. L. 2018. Morphometric and internally transcribed spacer gene sequence-based characterization of *Alternaria burnsii*. *Curr. Sci.,* vol. 114, no. 9, p. 1927-1933.

Tamura, K., Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, vol. 10, no. 3, p. 512-526. PMid:8336541

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, vol. 28, no. 10, p. 2731-2739. https://doi.org/10.1093/molbev/msr121 PMid:21546353

Thormann, I., Reeves, P., Thumm, S., Reilley, A., Engels, J. M., Biradar, C. M., Lohwasser, U., Börner, A., Pillen, K., Richards, C. 2017. Genotypic and phenotypic changes in wild barley (*Hordeum vulgare* subsp. *spontaneum*) during a period of climate change in Jordan. *Genetic Resources and Crop Evolution*, vol. 64, no. 6, p. 1295-1312. https://doi.org/10.1007/s10722-016-0437-5

Úrbez-Torres, J., Castro-Medina, F., Mohali, S., Gubler, W. 2016. Botryosphaeriaceae species associated with cankers and dieback symptoms of Acacia mangium and Pinus caribaea var. hondurensis in Venezuela. Plant Disease, vol. 100, no. 12, p. 2455-2464. https://doi.org/10.1094/PDIS-05-16-0612-RE

Varela, E., Lima, J., Galdino, A., Pinto, L. d. S., Bezerra, W., Nunes, E., Alves, M., Grangeiro, T. 2004. Relationships in subtribe Diocleinae (*Leguminosae*; *Papilionoideae*) inferred from transcribed spacer sequences from nuclear ribosomal DNA. *Phytochemistry*, vol. 65, no. 1, p. 59-69. https://doi.org/10.1016/j.phytochem.2003.08.005 PMid:14697271

Varela, R. O., Albornoz, P. L. 2013. Morpho-anatomy, imbibition, viability and germination of the seed of *Anadenanthera colubrina* var. cebil (*Fabaceae*). *Rev. Biol. Trop.*, vol. 61, no. 3, p. 1109-1118. https://doi.org/10.15517/rbt.v61i3.11907 PMid:24027911

Vivodík, M., Saadaoui, E., Balážová, Ž., Gálová, Z., Petrovičová, L. 2018. Genetic diversity and population structure in tunisian castor genotypes (*Ricinus communis* L.)

Detected using scot markers. Potravinarstvo Slovak Journal of Food Sciences, vol. 12, no. 1, p. 143-149. https://doi.org/10.5219/873

Wu, C.-T., Hsieh, C.-C., Lin, W.-C., Tang, C.-Y., Yang, C.-H., Huang, Y.-C., Ko, Y.-J. 2013. Internal transcribed spacer sequence-based identification and phylogenic relationship of I-Tiao-Gung originating from Flemingia and Glycine (*Leguminosae*) in Taiwan. *Journal of Food and Drug Analysis*, vol. 21, no. 4, p. 356-362. https://doi.org/10.1016/j.jfda.2013.08.002

Yadav, P., Koul, K., Srivastava, N., Mendki, M., Bhagyawant, S. 2017. ITS-PCR sequencing approach deciphers molecular phylogeny in chickpea. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, vol. 151, no. 3, p. 429-435. https://doi.org/10.1080/11263504.2016.1179694

Yousef, E. A., Mueller, T., Börner, A., Schmid, K. J. 2018. Comparative analysis of genetic diversity and differentiation of cauliflower (*Brassica oleracea* var. botrytis) accessions from two ex situ genebanks. *PLoS One*, vol. 13, no. 2, p. e0192062. https://doi.org/10.1371/journal.pone.0192062 PMid:29420661

Yousefi, S., Saeidi, H., Assadi, M. 2018. Genetic Diversity Analysis of Red Clover (*Trifolium pratense* L.) in Iran Using Sequence Related Amplified Polymorphism (SRAP) Markers. *Journal of Agricultural Science and Technology*, vol. 20, no. 2, p. 373-386.

Availability of data and material

The Persian clover lines and landraces used in this study are the results of a ten-year research project conducted to create superior lines in Agricultural and Natural Resources Research Center of Lorestan Province by Dr. Mohammad Shahverdi.

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BOVINE MUCOUS MEMBRANES AS A SOURCE OF ANTIMICROBIAL COMPOUNDS

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ABSTRACT

Loss of food quality, deterioration of organoleptic properties and accumulation of anti-alimentary compounds are in focus of modern food science. Nowadays, such traditional methods as processing, physical and chemical treatment are used for improving of shelf life. An alternative ways of shelf life increasing are quite a sharp problem. Antimicrobial peptides (AMPs) could be an actual alternative. According to Antimicrobial Peptide Database (http://aps.unmc.edu/AP/main.php), 2884 antimicrobial peptides from six kingdoms were found and identified. Mucous membranes of farm animals due to their border position and intensive contact with different pathogens could be a capacious source of such substances. Objects of the study were bovine oral cavity mucosa, nasal cavity mucosa, tracheal cavity mucosa, rectal mucosa, tongue mucosa, saliva gland and submandibular lymph nodes. Two-dimensional electrophoresis (2DE) was performed according to the method of O'Farrell, 35 protein fractions were identified by MALDI-TOF MS and MS/MS mass spectrometry. A number of qualitative and quantitative differences were revealed. A large number of histones (H2bd-like, H2BC, HIST1H2BD, HIST2H2AC, HIST1H2AH, histone H3.3 and H2bl-like, HIST2H2AC and histone H3.3, mixture HIST1H2AJ, HIST2H2BE and histone H2A type 2-C) were found in all mucous membranes as well as several tissue-specific proteins (proteins S100-A12 and AGR2, isoforms of ribosomal proteins, myelin P2, odorant-binding protein, secretoglobin), which could be a precusors of bioactive peptides.

Keywords: AMPs; storage; shelf-life; mucous membranes; proteins

INTRODUCTION

Progressive technologies are actively implemented in the food industry. Significant part of researches is aimed on reduction of losses, stabilization of quality and increase of shelf life. Loss of food quality, deterioration of organoleptic properties and accumulation of anti-alimentary compounds directly correlate with initial quality of raw materials, storage conditions and final shelf life. One of the main reasons is microbiological contamination **(Kameník, 2013; Dikeman and Devine, 2014; Popelka et al., 2016)**.

Nowadays, the following traditional methods are used for improving of shelf life: processing (sterilization, smoking, freezing, refrigeration, salting - wet and dry), physical (low frequency and ionizing treatment, gas-modified package, cryo-treatment, etc.), chemical treatment (sulphur dioxide, benzoic acid, sorbic acid, etc.) **(Zolotokopova and Palagina, 2007; Syasin, 2011; Nesterenko and Kayatskaya, 2012; Tuniyeva, 2013, 2015; Zaitseva et al., 2014; Bilek et al., 2016)**. These methods are very effective, but, nevertheless, its implementation may lead to quality lowering, accumulation of anti-alimentary factors, activity reduction of introduced or native bioactive substances, etc.

Modern concept of foods development is primarily focused on creation of products with high quality and nutritional value. A special attention is paid in specialized and functional products with limited shelf life. In this regard, an alternative ways of shelf life increasing are quite a sharp problem. Antimicrobial peptides (AMPs), whose existence has been known for more than 60 years could be an actual alternative. According to Antimicrobial Peptide Database (http://aps.unmc.edu/AP/main.php), 2884 antimicrobial peptides from six kingdoms (333 bacteriocins/peptide antibiotics from bacteria, 4 from archaea, 8 from protists, 13 from fungi, 342 from plants, and 2184 from animals) were found and identified **(Wang, 2010)**.

A wide range of AMPs were found in tissues of mammals and are classified into histatins, cathelicidins and defensins. Mainly, antimicrobial compounds were identified in blood cells (leukocytes, neutrophils, platelets) **(Tecle et al., 2010; Wang, 2010; Wang, 2014; Bahar and Ren, 2013; Jarczak et al., 2013; Zhao and Lu, 2014; Shamova et al., 2014)**. But mucous membranes of

farm animals due to their border position and intensive contact with different pathogens could be also a capacious source of such substances.

Scientific hypothesis

Despite of high availability and low cost of farm animal's by-products, the question of its use as a source of substances with antimicrobial action is not enough in focus. In this regard, the study of antimicrobial proteins and peptides contained in the mucous membranes is highly relevant due to their border position and, as a result, intensive contact with a wide range of biological agents (pathogenic and opportunistic microorganisms, viruses, fungi). It is known that the reaction of nonspecific protection is formed including signaling, regulatory and primary active substances. In this case, border tissues of animals are rich source both antimicrobial constitutive sequences and variable compounds accumulated and induced by pathogens. It is important to note that significant genomic resources of epithelium as a "first line of defense" is able to form a unique combination of proteomic profiles and capacious peptide pools both as the result of acute inflammation and a component of a booster effect.

MATERIAL AND METHODOLOGY

Objects of the study were bovine oral cavity mucosa, nasal cavity mucosa, tracheal cavity mucosa, rectal mucosa, tongue mucosa, saliva gland and submandibular lymph nodes.

Proteomic study

Two-dimensional electrophoresis (2DE) was performed according to the method of O'Farrell with isoelectric focusing in ampholine pH gradient (IEF-PAGE). The subsequent detection of the proteins was carried out by staining with Coomassie R-250 (Applichem, USA) and silver nitrate (Panreac, Spain) as described previously **(Kovalyov et al., 2006)**. The resulting digital images were edited in a graphic editor and the quantitative protein content was calculated using ImageMaster 2D Platinum version 7 ("GE Healthcare", Switzerland).

Protein fractions were excised from the gel, grinded and undergone trypsinolysis (Sigma, Germany) **(Zvereva et al., 2015)**. Obtained peptides were investigated by MALDI-TOF MS and MS/MS mass spectrometry on Ultraflex MALDI-TOF mass spectrometer (Bruker, Germany) with UV laser (336 nm) in the positive ion mode in molecular weight range of 500 – 8000 Da with calibration according to known peaks of trypsin autolysis.

Bioinformatics analysis

Analysis of obtained tryptic peptides mass spectra was performed using Peptide Fingerprint option in Mascot software (Matrix Science, USA) with MH+ mass determination accuracy of 0.01%; search was performed in databases of the National Center for Biotechnology Information, USA (NCBI).

Comparative analysis of obtained proteomic profiles was carried out with use of information module "Proteins of skeletal muscle of cows (Bos Taurus)" of the Database "Proteomics of muscle organs" (http://mp.inbi.ras.ru).

RESULTS AND DISCUSSION

A number of identified protein fractions were qualitatively or quantitatively different between the studied mucous membranes (Figure 1 and Table 1).

It was found that at least 10% muscle tissue was also presented in samples. Thus, troponin I, fast skeletal muscle (5 and 24), myosin regulatory light chain 2, skeletal muscle isoform (10), myoglobin (1) were identified. A number of major proteins, which was not detected in muscle tissues, were detected in mucous membranes. Presumably, these proteins could be a sourse a bioactive peptides.

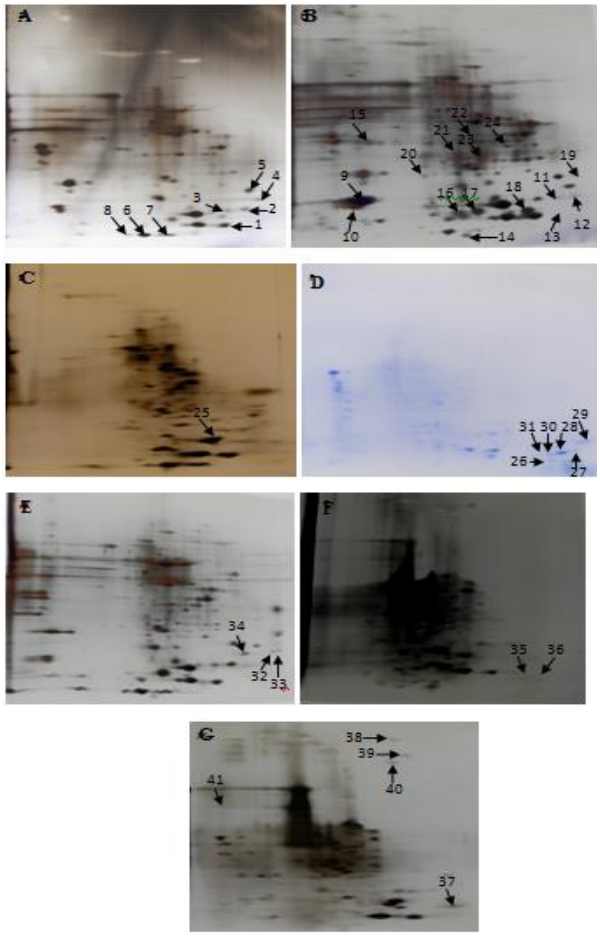

Figure 1 2DE proteins of bovine mucous membranes. Note: A – oral cavity mucosa, B – nasal cavity mucosa, C – tracheal cavity mucosa, D – rectal mucosa, E – tongue mucosa, F – submandibular lymph nodes, G – saliva gland.

Histones formed one of these groups. It's known that these proteins possessed an antimicrobial activity and may decompose into peptides with the same action **(Tagai et al., 2011)**. Some fractions were coincided in different objects. H2bd-like were identified in submandibular lymph nodes (27) and nasal cavity mucosa (11), but MW was different. MW of H2bd-like (11) was higher, presumably, due to its glycosylation in nasal cavity. The same phenomenon was revealed in relation to ubiquitin (14), which MW was arisen due to accession of hexoses.

Table 1 The results of mass spectrometric identification (MALDI-TOF MS и MS / MS) of protein fractions.

№	Protein name; (Gene symbol)	Number in Protein NCBI	S / M/ C *	Мм/pI (exp.)**	Мм/pI (calc.)**
	Oral cavity mucosa				
1	Mixture of 50s ribosomal protein l13 (RPLM)****(2) [Pseudomonas] and fragment 1-140 myoglobin [Bos taurus](MB)***(1)	WP 047273761.1 133/9/	187/4/52 133/9/51	15,0/9,60	15,8/9,79 17,1/6,90
2	Mixture of fragment 40 -166 anterior gradient protein 2 homolog isoform X1 (AGR2 and 50S ribosomal protein L13 (RPLM)****(2) [Pseudomonas]	XP 005205231.1 WP 047273761.1	159/13/62 131/13/33	18,5/9,90	20,0/8,82 15,8/9,79
3	peptidyl-prolyl cis-trans isomerase A (PPIA)***(2)	XP 006051354.1	173/21/63	18,2/9,75	17,9/8,34
4	Mixture of fragment 75 – 191 protein HP-20 homolog (MGC137014) and fragment 38 – 207 peptidyl-prolyl cis-trans isomerase B (PPIB)	NP 001040049.1 DAA25310.1	107/9/48 102/11/37	20,0/10,10	20,6/8,85 22,7/9,23
5	troponin I, fast skeletal muscle (TNNI2)	NP 001179023.1	162/21/55	22,0/10,00	21,4/8,88
6	protein S100-A12 (S100A12)***(2)	NP 777076.1	144/20/77	10,5/6,00	10,7/5,92
7	protein S100-A12 (S100A12)***(2)	NP 777076.1	210/15/63	10,0/6,50	10,7/5,92
8	protein S100-A12 (S100A12)***(2)	NP 777076.1	217/17/76	10,0/5,90	10,7/5,92
	Nasal cavity mucosa				
9	odorant-binding protein (OBP)	XP 001253219.3	118/9/53	20,0/5,20	20,0/5,20
10	myosin regulatory light chain 2, skeletal muscle isoform (MYLPF)	NP 001069115.1	173/20/78	19,0/4,90	19,0/4,91
11	Mixture histone cluster 1, H2bd-like (LOC100138359)***(2) and anterior gradient protein 2 homolog isoform X1 (AGR2)	DAA21814.1 XP 005205231.1	150/5/34 116/10/48	18,5/9,90	12,4/9,74 20,0/8,82
12	Mixture of fragment 36-93 calponin-1 (CNN1)***(2) and histone cluster 1, H2bc-like (LOC520044)***(3)	NP 001039844.1 DAA21889.1	137/3/13 146/5/24	18,5/10,20	33,3/9,05 13,8/10,11
13	myelin P2 protein (PMP2)	NP 001068707.1	214/19/77	17,0/9,90	15,0/9,67
14	Ubiquitin (LOC101902760)***(1) with signs of glycosylation (hexose)	XP 005195085.1	76/12/90	10,5/6,60	8,70/6,56
15	immunoglobulin gamma 1 heavy chain constant region	ABE68619.1	112/12/42	34,0/5,40	35,5/6,49
16	Mixture of histidine triad nucleotide-binding protein 1 (HINT1)***(1), secretoglobin family 1D member (SCGB1D)***(5)и cytochrome c oxidase subunit 5B, mitochondrial ()***(2)	NP 787006.1 NP 001071275.1 NP 001029218.1	99/5/53 199/15/63 154/12/70	16,5/6,55	13,9/6,03 11,3/8,98 13,8/8,80
17	Mixture fatty acid-binding protein, epidermal (FABP5)***(1)и secretoglobin family 1D member (SCGB1D)***(3)	DAA22652.1 NP 001071275.1	34/22/71 140/5/40	17,0/6,80	11,9/6,58 11,3/8,98
18	secretoglobin family 1D member (SCGB1D) ***(4)+ the hexose-to-peptide 2912	NP 001071275.1	201/14/65	16,5/9,50	11,3/8,98
19	troponin I, fast skeletal muscle (TNNI2)	XP 005903574.1	109/13/40	22,0/10,40	22,1/9,30
20	Mixture of immunoglobulin light chain variable region***(2) with modification Gln->pyro-Glu (Pyro-glu from Q) and immunoglobulin lambda light chain variable region (VlAMBDA1B)***(2)	AAB66578.1 AAC48559.1	247/6/60 178/15/92	29,0/6,80	11,3/6,23 13,3/4,93

№	Protein name; (Gene symbol)	Number in Protein NCBI	S / M/ C *	Мм/pI (exp.)**	Мм/pI (calc.)**
21	Mixture of alcohol dehydrogenase [NADP(+)](AKR1A1), fragment keratin, type II cytoskeletal 68 kDa, component IA (KRT76) and fragment aflatoxin B1 aldehyde reductase member 2 (AKR7A2)***(1).	NP 001069981.1 XP 002687308.1 NP 001095419.1	221/34/86 113/28/54 101/7/22	34,0/7,00	36,6/6,80 64,7/7,59 40,5/8,57
22	Mixture of immunoglobulin light chain variable region with midification + Gln->pyro-Glu (N-term Q)***(2), immunoglobulin lambda light chain constant region 3 allotypic variant IGLC3a ***(1), C-end fragment creatine kinase M-type (CKM)***(1) and calcyclin-binding protein (CACYBP)***(1)	AAB66568.1 AEMO5841.1 NP 777198.2 NP 00102998.1	194/2/42 105/4/66 84/8/30 50/1/10	28,0/7,10	11,3/6,23 11,3/8,49 43,0/6,63 26,3/8,29
23	ATP synthase subunit d, mitochondrial (ATP5PD)	NP 777149.1	150/13/79	24,0/6,20	18,7/5,99
24	Mixture of malate dehydrogenase, mitochondrial isoform X1 (MDH2)***(2), voltage-dependent anion-selective channel protein 1 (VDAC1)и keratin, type II cytoskeletal 59 kDa, component IV (KRT6B).	XP 005225065.1 NP 776910.2 NP 001244333.1	128/2/10 218/23/77 112/18/33	31,0/9,00	35,6/8,82 30,8/8,82 60,8/8,58
	Tracheal cavity mucosa				
25	anterior gradient protein 2 homolog isoform X1 (AGR2)	XP 005205231.1	144/9/46	19,5/8,90	20,0/8,82
	Rectal mucosa				
26	Fragment 48-117 histone H2B type 1-D (HIST1H2BD)***(3)	NP 001039711.1	191/15/55	8,0/10,30	14,0/10,31
27	histone H2A type 2-C (HIST2H2AC)***(4)	DAA31670.1	545/12/49	12,0/10,50	12,90/11,02
28	Fragment 22 -119 histone H2A type 1-H (HIST1H2AH)***(3)	XP 010823702.1	381/13/61	12,5/10,70	13,90/10,88
29	Mixture of fragment 17 – 163 desmin (DES)***(1), histone H3.3 (LOC100297725)***(1) and histone cluster 1, H2bl-like (LOC100299996)***(2)	NP 001075044.1 XP 002684244.1 DAA19208.1	120/6/15 147/8/34 145/6/29	14,0/10,90	53,50/5,21 15,20/11,14 14,00/10,22
30	Mixture of histone H2A type 2-C (HIST2H2AC)****(1), histone H3.3 (LOC100297725)****(1) and cytochrome c oxidase subunit NDUFA4 (NDUFA4)***(1)	DAA31670.1 XP 002684244.1 NP 787014.1	251/10/43 147/7/30 161/778/	11,5/9,90	12,90/11,02 15,20/11,14 9,30/9,57
31	Mixture of histone cluster 1, H2aj (HIST1H2AJ)****(1), histone H2B type 2-E (HIST2H2BE)****(3) and histone H2A type 2-C ()***(4)	XP 010823687.1 NP 001092854.1 DAA31670.1	233/9/41 256/12/62 545/16/68	11,5/10,40	14,0/10,88 13,9/10,31 12,9/11,02
	Tongue mucosa				
32	60S ribosomal protein L11 isoform X1 (RPL11)***(2)	XP 005203175.1	167/13/52	20,0/10,30	21,3/9,72
33	40S ribosomal protein S10 (RPS10)***(2)	NP 001029888.1	185/5/29	20,0/10,40	18,9/10,15
34	troponin I, fast skeletal muscle (TNNI2)	NP 001179023.1	113/12/25	21,0/10,20	21,4/8,88
	Saliva gland				
35	Mixture of myelin P2 protein (PMP2)и histone H2A type 2-C (HIST2H2AC)***(2)	NP 001068707.1 DAA31670.1	162/11/50 204/3/29	15,0/10,00	15,0/9,67 12,8/11,02

№	Protein name; (Gene symbol)	Number in Protein NCBI	S / M/ C *	Мм/pI (exp.)**	Мм/pI (calc.)**
36	Aggregate hemoglobin subunit beta (HBB)	NP 776342.1	171/11/68	120,0/9,50	16,0/7,01
37	Aggregate anterior gradient protein 2 homolog isoform X1 (AGR2)	XP 005205231.1	103/6/46	100,0/7,70	19,9/8,82
38	Aggregate hemoglobin subunit beta (HBB)	NP 776342.1	195/13/82	85,0/9,30	16,0/7,01
39	protein disulfide-isomerase (P4HB)***(1)	NP 776560.1	56/39/74	55,0/4,80	57,0/4,80
	Submandibular lymph nodes				
40	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2 (NDUFA2)***(1)	NP 787009.1	119/2/25	14,0/10,20	11,1/9,93
41	histone cluster 1, H2bd-like (LOC100138359)***(2)	DAA21814.1	149/4/21	12,0/10,30	12,4/9,74

Note: * S/M/C: Score – indicator of conformity or «scorecard»; Match peptides – the number of matched peptides; Coverage – % coverage of the entire amino acid sequence of the protein by identified peptides.
**mM/pI (experiment) – scores obtained as a result of electrophoretic mobility on the DE and mM/pI (calculation) – estimates made based on amino acid sequence data with consideration of signal peptide removal, but with no consideration of other post-synthetic modifications using the ExPASy Compute pI/Mw tool software.
***msms – indication of identification by tandem mass spectrometry, the number of sequenced tryptic peptides in parentheses.

Histones of other types, identified in mucous membranes in different combinations, can also make a great contribution to the formation of bioactive peptides. The following histones were identified: №12 (H2BC), №16 (HIST1H2BD), №17 (HIST2H2AC), 18 (HIST1H2AH), №19 histone H3.3 и H2bl-like, №20 (HIST2H2AC) and histone H3.3, №21 mixture (HIST1H2AJ), (HIST2H2BE) and histone H2A type 2-C (Table 1).

Protein S100-A12 (№6-8) is localized in cell membranes and cytoplasm, and is represented in the mucous membranes in large quantities in the form of three electrophoretic fractions. Its activity is associated with the production of chemo- and cytokines. He is involved in antimicrobial humoral immune response mediated by antimicrobial peptides **(Cole et al., 2001)**.

Protein fractions AGR2 were identified in oral, nasal and tracheal mucous membranes (2, 11 and 15, respectively). This protein is most fully studied in humans (O95994 in UniProt Database), and performs various biological functions. It is required for MUC2 posttranscription synthesis and secretion, plays a role in mucus production, in migration, differentiation and cell growth, and promotes their adhesion. It is usually presented in small quantities. But in mucous tissues it is presented in significant quantities, and can be an important source of active biopeptides.

There were found two another groups of proteins. Isoforms of ribosomal proteins (1, 2 and 22, 23) were identified in the oral and tongue mucosa. Moreover, the fractions № 1 and 2 belonged to the *Pseudomonas* bacteria. Myelin P2 protein (PMP2) was detected in nasal mucosa (13) and salivary gland (25). Odorant-binding protein (9) was identified in nasal mucosa, as well as 3 fraction of secretoglobin (16,17 and 18) and immunoglobulin (15,20 and 22). Presumably, all identified tissue-specific proteins can be a source of bioactive peptides

CONCLUSION

More than 35 protein fractions were identified in investigated samples. A number of qualitative and quantitative differences were revealed. A large number of histones were found in all mucous membranes as well as several tissue-specific proteins, which would be a precusors of bioactive peptides.

REFERENCES

Bahar, A. A., Ren, D. 2013. Antimicrobial peptides. *Pharmaceuticals (Basel),* vol. 6, no. 12, p. 1543-1575. https://doi.org/10.3390/ph6121543

Bilek, M., Vietoris, V., Ilko, V. (2016) Shelf life extension and sensory evaluation of birch tree sap using chemical preservatives. *Potravinarstvo*, vol. 10, no. 1, p. 499-505. https://doi.org/10.5219/649

Cole, A. M., Kim, Y. H., Tahk, S., Hong, T., Weis, P., Ganz, T. 2001. Calcitermin, a novel antimicrobial peptide isolated from human airway secretions. *FEBS Letters*, vol. 504, no. 1-2, p. 5-10. https://doi.org/10.1016/S0014-5793(01)02731-4

Dikeman, M., Devine, C. 2014. *Encyclopedia of Meat Sciences*. 2nd ed. London, UK : Academic Press, 1712 p. ISBN 978-0-12-384734-8.

Jarczak, J., Kościuczuk, E. M., Lisowski, P., Strzałkowska, N., Jóźwik, A., Horbańczuk, J., Krzyżewski, J., Zwierzchowski, L., Bagnicka, E. 2013. Defensins: Natural component of human innate immunity. *Human Immunology*, vol. 74, no. 9, p. 1069-1079. https://doi.org/10.1016/j.humimm.2013.05.008

Kameník, J. 2013. The microbiology of meat spoilage: a review. *Maso International – Journal of Food Science and Technology*, vol. 2013, no. 1, p. 1-9.

Kovalyov, L. I., Kovalyova, M. A., Kovalyov, P. L., Serebryakova, M. V., Moshkovskii, S. A., Shishkin, S. S. 2006. Polymorphism of delta3,5-delta2,4-dienoyl-coenzyme A isomerase (the ECH1 gene product protein) in human striated muscle tissue. *Biochemistry (Moscow)*, vol. 71. no. 4, p. 448-453. https://doi.org/10.1134/S0006297906040146

Nesterenko, A. A., Kayatskaya A. S. 2012. Pickles of meat and meat products. *Vestnik NGIEI,* no. 8, p. 46-54.

Popelka, P., Jevinová, P., Marcinčák, S. 2016. Microbiological and chemical quality of fresh and frozen whole trout and trout fillets. *Potravinarstvo*, vol. 10, no. 1, p. 431-436. https://doi.org/10.5219/599

Shamova, O. V., Orlov, D. S., Balandin, S. V., Shramova, E. I., Tsvetkova, E. V., Panteleev, P. V., Leonova, Yu, F., Tagaev, A. A., Kokryakov, V. N., Ovchinnikova, T. V. 2014. Acipensins – novel antimicrobial peptides from leukocytes of the Russian sturgeon Acipenser gueldenstaedtii. *Acta Naturae*, vol. 6, no. 4, p. 99-109.

Syasin, I. E. 2011. Features of cryopreservation and cryoseparation of food raw materials. *Polythematic online scientific journal of Kuban State Agrarian University,* vol. 66, no 2, p. 1-12.

Tagai, C., Morita, S., Shiraishi, T., Miyaji, K., Iwamuro, S. 2011. Antimicrobial properties of arginine- and lysine-rich histones and involvement of bacterial outer membrane protease T in their differential mode of actions. *Peptides,* vol. 32, no. 10, p. 2003-2009. https://doi.org/10.1016/j.peptides.2011.09.005

Tecle, T., Tripathi, S., Hartshorn, K. L. 2010. Review: Defensins and cathelicidins in lung immunity. *Innate immunity*, vol. 16, no. 3, p. 151-159. https://doi.org/10.1177/1753425910365734

The Antimicrobial Peptide Database. Available at: http://aps.unmc.edu/AP/main.php.

Tuniyeva, E. K. 2013. Ingredients and packaging at IFFA 2013: the easy consumption of natural products. *Vse o myase,* vol. 2013, no. 3, p. 5-7.

Tuniyeva, E. K. 2015. To a safety issue of food additives. *Vse o myase*, vol. 2015, no. 4, p. 10-13.

Wang, G. 2010. *Antimicrobial Peptides: Discovery, Design and Novel Therapeutic Strategies.* Oxfordshire, UK: CABI. 240 p. ISBN-13: 978-1845936570. https://doi.org/10.1079/9781845936570.0000

Wang, G. 2015. Improved methods for classification, prediction, and design of antimicrobial peptides. *Methods in molecular biology*, vol. 1268, p. 43-66. https://doi.org/10.1007/978-1-4939-2285-7_3

Zaitseva, U. A., Girina, E. G., Ponomarenko, A. V. 2014. Types of pickles and its application in the meat industry. *Molodoi ychenyi,* vol. 63, no. 4, p. 164-167.

Zhao, L., Lu, W. 2014. Defensins in innate immunity. *Current Opinion in Hematology,* vol. 21, no. 1, p. 37-42. https://doi.org/10.1097/MOH.0000000000000005

Zolotokopova, S. V., Palagina, I. A. 2007. Theoretical study of preservative action mechanism of smoke components in the extracts. *Izvestia vuzov. Pishevaya tekhnologia*, vol. 298, no. 3, p. 36-42.

Zvereva, E. A., Kovalev, L. I., Ivanov, A. V., Kovaleva, M. A., Zherdev, A.V., Shishkin, S. S., Lisitsyn, A. B., Chernukha, I. M., Dzantiev, B. B. 2015. Enzyme immunoassay and proteomic character-ization of troponin I as a marker of mammalian muscle compounds in raw meat and some meat products. *Meat Science*, vol. 105, p. 46-52. https://doi.org/10.1016/j.meatsci.2015.03.001

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CONSUMER SENSORY EVALUATION OF HONEY ACROSS AGE COHORTS IN SLOVAKIA

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ABSTRACT

The growing popularity of honey among consumers has caused many frauds and cases when honey of high quality is adulterated by cheap imported honey of very low quality. The aim of this research paper was to study consumer perception of honey quality based on sensory attributes such as taste, aroma, colour and consistency. The primary research comprised the sensory blind test conducted as a part of questionnaire survey at selected shopping mall and at university. Research sample reached 400 respondents living in the Nitra region between 18 and 70 years. Respondents tested sensory attributes of two samples. Sample A represented Slovak honey from a local beekeeper and sample B was honey purchased from selected supermarket with country of origin „blend of EU and non – EU honeys“ and represented imported honey. Besides descriptive statistics, the following statistical tests were applied: Fisher's Exact Test, Chi-Square Test of Independence, Cramer´V coefficient and Mann-Whitney U test. Results showed significant differences in perception of honey quality across age cohorts. Respondents older than 40 years (Generation X and Babyboomers) evaluated better the local honey from a beekeeper (sample A) than younger generations (Generation Y and Generation Z). Imported honey from selected supermarket (sample B) obtained the best evaluation in case of colour in both age cohorts while sample A obtained it in terms of aroma. The majority of respondents in both age cohorts mostly decided their preference according to taste, however there exist some differences. While Generation X and Baby boomers took into consideration also aroma, the generations Y and Z considered consistency. More interesting observation appealed in case of aroma where more than 90% of respondents, who decided according to aroma, preferred sample A – local Slovak honey. According to physico-chemical analysis, both samples fulfilled standard of EU legislation, however better parameters were reached in sample A. All in all, better perception of honey quality through sensory attributes in older age cohorts could be caused by the deeper experience in honey consumption as well as due to the fact that younger cohorts consume more semiproducts and industrial food products characterised by intensive, sweet taste which could confuse their assessments.

Keywords: consumer research; sensory evaluation; honey; generations; Slovakia

INTRODUCTION

In recent years, the popularity of honey has been increasing continuously at global level, mainly due to trends in food market connected with healthy eating habits, lifestyle or sustainable consumption **(Kubeláková and Košičiarová, 2016, Paluchová and Prokeinová, 2013; Gálová et al., 2012)**. The increasing consumption of honey can be seen also in Slovakia where according to **Statistical Office of the Slovak Republic (2018)** in the past 10 years the annual consumption of honey per capita has nearly doubled. Nevertheless, younger generations tend to consume less quantities than consumers older than 30 years **(Guziy et al., 2017)** and the similar situation occurs in many other European countries **(Pocol, 2011;Šanová et al., 2015; Vanyi et al., 2010; Pidek, 2001; Pocol and Teselios, 2012)**.

Furthermore, honey is perceived as a sweetener with added value as it contains many vitamins, minerals and enzymes. In many countries, honey is used not only as food, but also in medicine due to its healing properties **(Aparna and Rajalakshmi, 1999; Crittenden, 2011;Martinovski and Gulevska, 2017)**.

Increased popularity of honey among consumers is connected with frequent cases of honey adulteration and quality issues **(Šedík et al., 2018)**. According to **Phipps (2017)**, honey is the third most adulterated food in the world. Despite the fact that the European Union declares, protects, supports, registers and controls certain food and agricultural products according to quality policy and legislation **(Nagyová et al., 2014)**, the legislation allows producers to mix the imported honey from third countries with Eurpean honey and sell it as product with country of origin, formulated as follows: blend of EC honeys, blend of non – EC honeys or blend of EU and non – EU honeys **(Council Directive 2001/110/EC of 20 December 2001 relating to honey)**. The current situation provides opportunities for honey with lower quality or even fake honey, as aproximately 40% of overall consumption in the

European Union is covered by the imported honey **(European Commission, 2016)**. Honey adulteration is mostly proved in sophisticated laboratories therefore the only method for honey consumers how to determine the quality of honey is their sensory evaluation, which involves attributes such as taste, aroma, colour and consistency **(Kaakeh and Gadelhak, 2005)**.

Scientific hypothesis

The aim of this research paper is to identify consumers' perception of honey quality based on intrinsic attributes such as taste, aroma, colour and consistency of imported and local honey between age cohorts.

MATERIAL AND METHODOLOGY

Consumer research was based on questionnaire survey and blind sensory test both conducted at selected shopping mall and the University during the period of November 2017 – February 2018. The research was aimed at honey consumers living in the Nitra region. The research sample comprised 400 respondents between 18 – 70 years.

The socio-demographic profile of respondents is described as follows: 64.75% women and 35.25% men, 54.25% obtained secondary education and 45.75% higher education, 53.50% living in the city and 53.50% in the countryside. Economic activity: 53.00% students, 25.25% employed, 18.25% pensioners and 3.50% other. According to age, respondents were divided into four age cohorts – generations: 26.75% Baby boomers (51 – 70 years), 9.50% Generation X (41 – 50 years), 37.50% Generation Y (25 – 40 years) and 26.25% Generation Z (18 – 24 years).

In the sensory test, respondents tested two samples of honey. Sample A represented Slovak honey from the local beekeeper while sample B was honey purchased from selected supermarket with country of origin marked as a blend of EU and non-EU honeys which represents imported honey. Respondents evaluated sensory attributes such as taste, aroma, colour and consistency of both samples on the 5-point scale (see Tab. 1) from 1 – very good to 5 – very bad. Furthermore, respondents had to decide which sample they prefer more and indicate which sample is from a beekeeper and which from a supermarket.

We formulated several assumptions, same for both investigated countries:

Hypothesis 1: – There exist differences in sensory evaluation of honey between samples.

Hypothesis 2: – There exist differences in sensory evaluation of honey among age cohorts.

Hypothesis 3: – There exists dependence between sample preference and age cohorts.

Hypothesis 4: – There exists dependence between sensory quality criteria and sample preference.

Hypothesis 5: – There exists dependence between sensory quality criteria and age cohorts.

Statistical testing was performed in the statistical program – SAS Enterprise Guide 7.1 and the following non-parametric tests were applied:

- Fisher's Exact Test,
- Chi-Square Test of Independence,
- Cramer'V coefficient,
- Mann-Whitney U test.

The physico-chemical analysis of honey consists of water content, free acidity (FA), electrical conductivity (EC) and hydroxymethylfurfural (HMF) determinations.

Water content was measured according to **IHC (2009)** by laboratory refractometer. Crystallized samples were dissolved in a water bath at 50 °C, then cooled to room temperature. The sample was covered on the surface of the prism of the refractometer and the refractive index was read. Each sample was measured twice and the average value was taken. If the temperature of honey was not 20 °C we did correction: for temperatures above 20 °C we added 0.00023 per °C; for temperatures below 20 °C we subtracted 0.00023 per °C. The corresponding water content was read from the table according to the refractive index.

Free acidity was detected according to **IHC (2009)** by titration with a measure of pH to 8.3. We dissolved 10 g of sample in 75 mL of distilled water in a 250 mL beaker. We mixed it and titrated with 0.1 M NaOH to pH 8.3. Each sample was measured twice and the average value was taken. Free acidity was expressed as milliequivalents acid.kg^{-1} honey (mL of 0.1 M NaOH multiplied by 10).

Electrical conductivity was measured according to **IHC (2009)** by conductometer. Honey, equivalent to 20.0 g anhydrous honey (the amount was calculated according water content), was dissolved in distilled water and transferred to 100 mL volumetric flask and made up to volume with distilled water. The conductivity cell was immersed in the sample solution and the conductance in mS was read. Electrical conductivity (mS/cm) was calculated – conductance was multiplied by cell constant.

Hydroxymethylfurfural (HMF) is a heterocyclic aldehyde which occurrence depends on the heat and period of storage (Kňazovická, 2011). HMF was measured by reflectometer (Merck) and HMF test (Merck). Both reaction zones of test strip were immersed in the honey for 1 s and then we followed the instructions of the producer. The HMF in mg.L-1was found by this way. The HMF in $mg.kg^{-1}$ was calculated - HMF in $mg.L^{-1}$ was divided by the specific gravity of honey (depending on water content).

Results were compared to the EU honey limits – **Council Directive 2001/110/EC** (water content, free acidity, HMF, electrical conductivity) and with standard of SZV (the Slovak Association of Beekeepers) no. 1 (water content – max 18%, HMF content – max 20 $mg.kg^{-1}$).

RESULTS AND DISCUSSION

Table 1 Sensory blind test.

	Sample A	Sample B
Taste		
Aroma		
Colour		
Consistency		

Results of the sensory blind test showed significant differences in evaluation between age cohorts. Respondents older than 40 years representing Generation X and Baby boomers evaluated sample A (local honey) better than sample B (imported honey) in average (see Figure 1). The best evaluation was obtained by aroma A (1.5), followed by taste A (1.6), consistency A (1.7) and colour A (1.8). The worst evaluation was obtained by aroma B (3.5), followed by taste B (3.1), consistency B (2.8) and colour B (2.7). Respondents younger than 40 years representing Generation Y and Generation Z (Figure 2) evaluated the samples similarly, in average, than previous age cohorts, however differences between the evaluation of the samples are smaller. Even in case of colour, sample B obtained better evaluation. The best evaluation was obtained by consistency A (2.0) followed by taste A (2.1), colour B (2.3) and colour A (2.4). The worst evaluation was obtained by aroma B (3.3), followed by aroma A (3.0), taste B (2.8) and consistency B (2.5).

Furthermore, the differences in evaluation between the samples A and B of each age cohort were statistically tested by applying Mann-Whitney U test. According to the Table 2, statistically significant differences (<0.0001) in case of Generation Y and Babyboomers were proven in all sensory attributes, while in case of younger generations – Generation Y and Generation Z it was proven in taste (<0.0001), aroma (<0.0001) and consistency (<0.0001). The first hypothesis was confirmed and there exist differences in sensory evaluation of honey between samples. The second formulated hypothesis examined whether the evaluation of the samples differs between age cohorts. Based on the same test, the statistically significant differences exist in the evaluation of all sensory attributes besides consistency B (0.0949) therefore hypothesis 2 was confirmed. Different sensory perception between age cohorts is shown by sample preference, where 80% of respondents older than 40 years preferred local honey (sample A) while only 53% of respondents younger than 40 years preferred sample A. These differences (hypothesis 3) were statistically tested by applying Fisher's Exact Test which proved (Table 3) that there exists dependence between sample preference and age cohorts (<0.0001). The similar results were obtained in a study dealing with sensory marketing and its impact on consumer's purchasing behavior. Sensory perception as well as perception of sensory marketing varies across the age cohorts **(Géci et al., 2017)**.

Besides sensory evaluation, respondents indicated according to which sensory attributes they decided their preference (quality of honey). The most decisive attributes regarding the quality of honey (Figure 3) is taste (58.8%) followed by aroma (16.5%), consistency (14.8%). The least important attribute is the colour (7.9%). Moreover, the study examined if there exists the depedence between sensory attributes as quality criteria and sample preference (hypothesis 4). By applying Chi-Square Test of Independence, the statistically significant dependence was proven (<0.0001) with weak strength of dependence where Cramer´s V coefficient = 0.2083 (Table 3). Interesting observation occurred with aroma attribute where the majority of respondents (≥90%) preferred local honey (sample A).

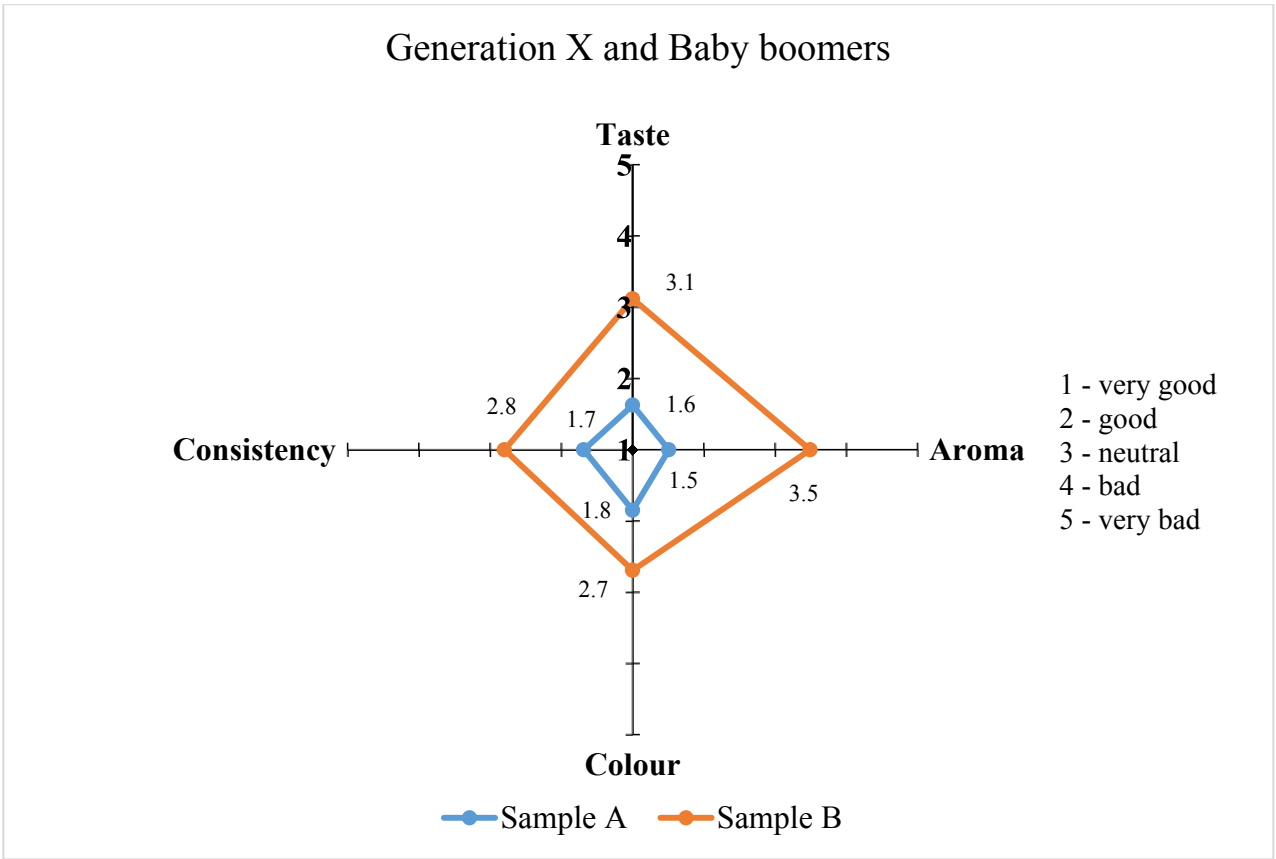

Figure 1 Sensory evaluation of honey samples by Generation X and Baby boomers.

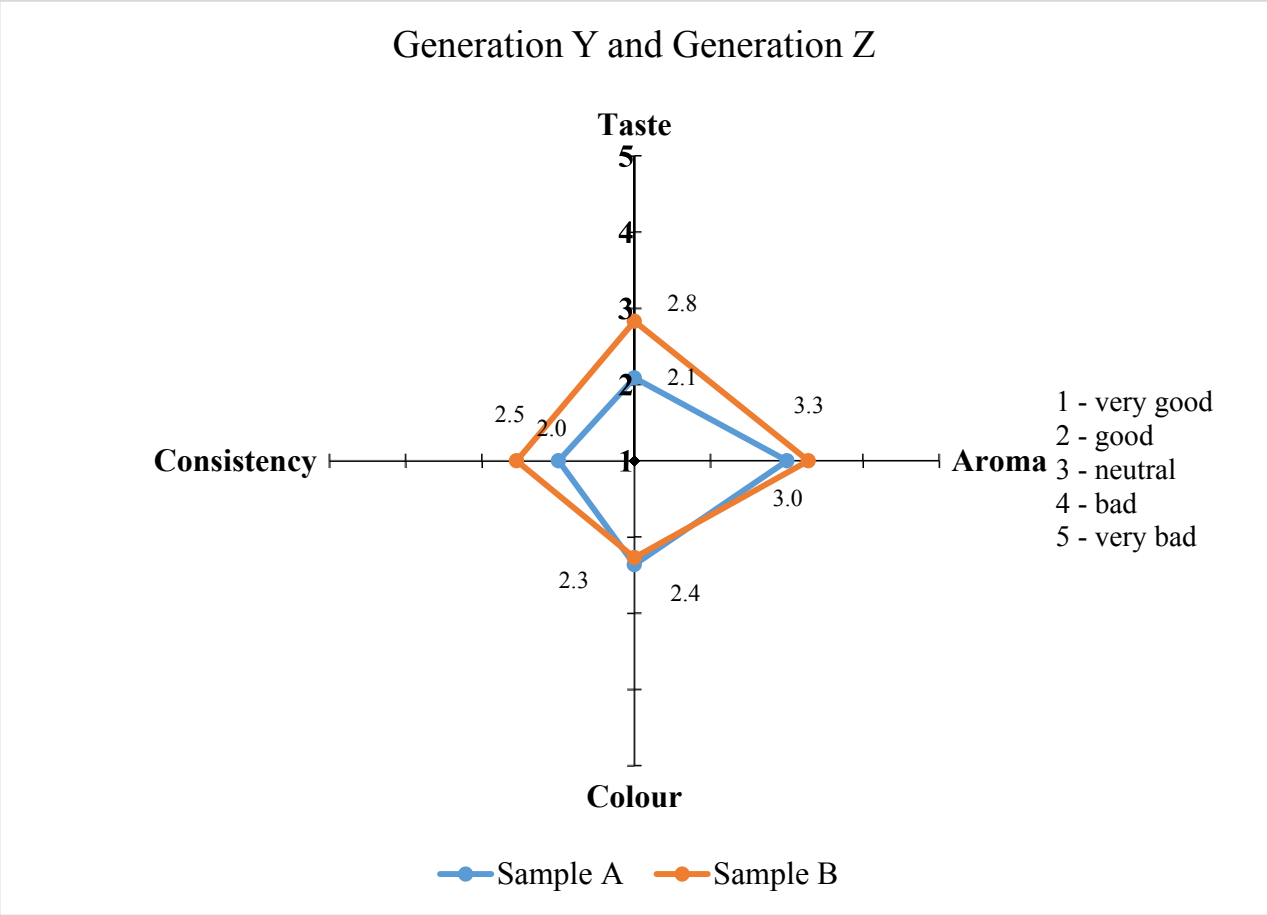

Figure 2 Sensory evaluation of honey samples by Generation Y and Generation Z.

Table 2 Results of Mann-Whitney U test.

Sensory evaluation between samples	Generation X and Baby boomers (*p*-value)	Generation Y and Generation Z (*p*-value)	Sensory evaluation between cohorts	Sample A (*p*-value)	Sample B (*p*-value)
Taste	<0.0001	<0.0001	**Taste**	<0.0001	0.0428
Aroma	<0.0001	<0.0001	**Aroma**	0.0377	0.0321
Colour	<0.0001	0.0921	**Colour**	<0.0001	0.0051
Consistency	<0.0001	<0.0001	**Consistency**	0.0006	0.0949

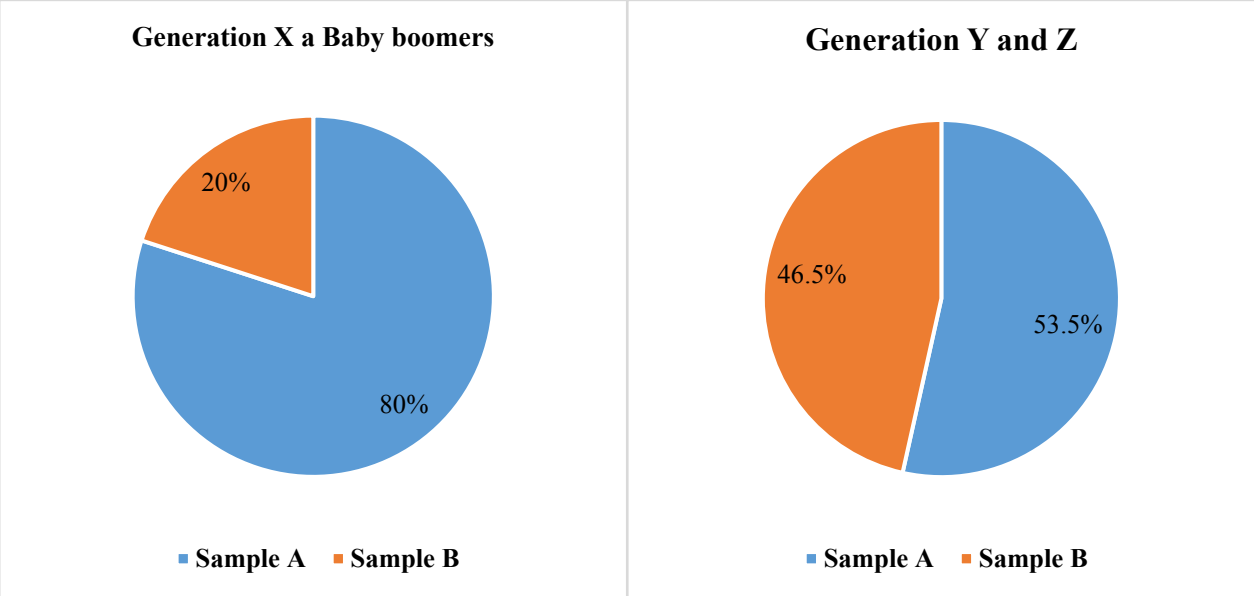

Figure 3 Annual consumption of honey in Slovakia and Russia.

Moreover, decisive criteria – sensory attributes were compared between age cohorts. Chi-Square Test of Independence has proven statistically significant dependence (<0.0001) between sensory attributes and age cohorts (hypothesis 5) with weak strength of dependence where Cramer´s V coefficient = 0.1845 (Table 3). The results illustrated in Figure 4 show differences between age cohorts. Taste is the most frequent decisive factor regarding the honey quality, and the colour is the least frequent for both age cohorts. Nevertheless, for Generation X and Baby boomers the second frequent decisive factor is aroma while for Generation Y and Z it is consistency.

The sensory blind test which represents the consumer point of view on the honey quality was supported by laboratory analyses of physico-chemical parameters of both samples to determine honey quality according to the EU honey legislation – **Council Directive 2001/110/EC**. Physico-chemical parameters included water content, Free acidity, electrical conductivity and HMF. Results showed that both samples fulfilled the selected criteria (Table 4), nevertheless local honey – sample A possesses better

Table 3 Results of Chi-Square Test of Independence and Fisher's Exact Test.

	p-value	correlation	Cramer´s V coefficient
sample preferences and age cohorts	<0.0001	yes	-
sensory attributes and sample preferences	<0.0001	yes	0.2083
sensory attributes and age cohorts	0.0001	yes	0.1845

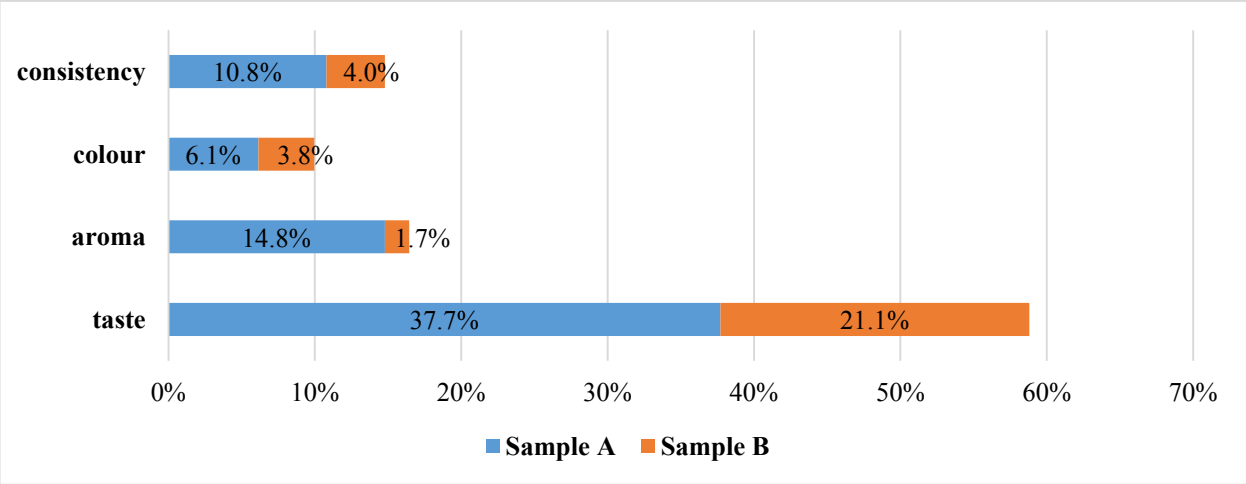

Figure 3 Respondents' sensory quality criteria according to sample preference.

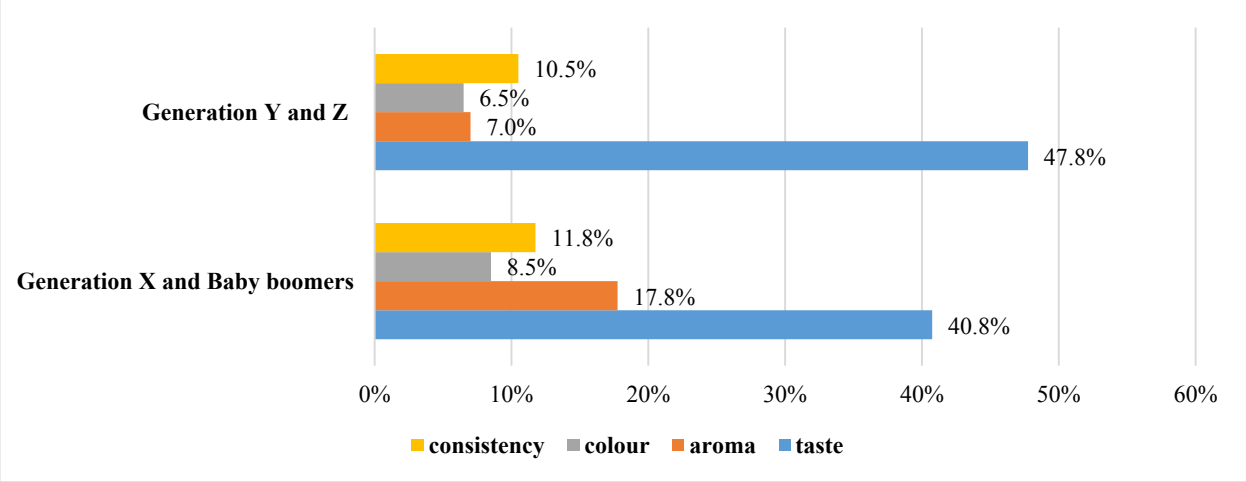

Figure 4 Respondents' sensory quality criteria according to age cohorts.

Table 4 Results of physico-chemical parameters.

	Water content (%)	**Free acidity** (meq kg^{-1})	**Electrical conductivity** (mS cm^{-1})	**HMF** (mg.kg^{-1})
EU Standard (SZV standard)	max 20 (18)	max 50	blossom - max 0.8	40 (20)
Sample A	15.4	15.8	0.37	11.9
Sample B	18.8	6.3	0.22	22.7

parameters and therefore has higher quality than imported honey – sample B. Moreover, the results of physico-chemical parameters were compared with standard of Slovak Association of Beekeepers (SZV standard) which was created as trademark „Slovak honey“ and as a variant which is more strict than EU legislation mainly due to frequent scandals in food industry and as consumer dissatisfaction with food safety **(Golian et al., 2018)**. According to this standard in terms of water content and HMF the only sample which has fulfilled the requirements was the sample A – local Slovak honey. Customers should be more aware of the uniqueness and the quality of domestic products and prefer them rather than products from abroad **(Nagyová et al., 2018)**.

Sensory test is an esential tools in determining the quality of honey, however, it has been mostly used for determining botanical origin, regions or sensory profile **(Kaakeh and Gadelhak, 2005)**. The most commonly used method to identify the botanical and geographical origin of honey is to apply the physicochemical parameters **(Popek, 2002)** as well as sensory analyses to differentiate and declare honey quality and profile **(Piana et al., 2004; Castro-Vazquez et al., 2009; Lorente et al., 2008; Oddo and Piro 2004)**. Sensory perspective of honey was studied in several countries such as Spain **(Galán-Soldevilla et al., 2005; González-Vinas et al., 2003)**, Italy **(Esti, 1997)** or India **(Anupama et al., 2003)**, Denmark **(Stolzenbach et al., 2012)**, Hungary **(Szabó et al., 2016)**, and Serbia **(Popov-Raljic et al., 2015)**, and all studies implemented ratings of colour, texture, taste, flavour or aftertaste. Nevertheless, sensory test provides information about perception of product quality **(Stolzenbach et al. 2011)**.

Understanding the consumer's expectations and how they perceive product attributes can be applied in the development of product strategy **(Stolzenbach et al. 2012)**.

CONCLUSION

Consumer research has proven different sensory perception among consumer based on sensory blind test involved in the questionnaire survey realised in Slovakia.

Quality perception of honey based on sensory attributes showed significant differences between age cohorts.It could be concluded that different perception among age cohorts could be caused by several reasons. Respondents older than 40 years – Generation X and Baby boomers preferred more local honey (sample A) due to their deeper experience in honey consumption and the ability to recognise honey of Slovak origin. Respondents younger than 40 years – Generation Y and Generation Z were more confused in sensory blind test and only 53.5% preferred local honey. It could be caused by the fact that younger consumers consume less quantity and therefore do not have many experience with Slovak honey. Furthermore, taste perception among age cohorts could be influenced by different eating habits and structure of the diet. Younger generations are more used to intensive, sweet taste by consuming semiproducts and industrial food products than older consumers. In conclusion, consumers should consider the fact that in many cases natural products do not have an intensive taste or colour as commercialised products. Moreover, in sensory evaluation they should decide not only according to taste, but also aroma as honey from beekeeper should have at least some aroma.

REFERENCES

Anupama, D., Bhat, K. K., Sapna, V. K. 2003. Sensory and physico-chemical properties of commercial samples of honey. *Food Research International*, vol. 36, no. 2, p. 183-191. https://doi.org/10.1016/S0963-9969(02)00135-7

Aparna A. R., Rajalakshmi, D. 1999. Honey – its characteristics, sensory aspects, and applications. *Food Reviews International*, vol. 15, no. 4, p. 455-471. https://doi.org/10.1080/87559129909541199

Castro-Vázquez, L., Díaz-Moroto, M. C., González-Viñas, M. A., Pérez-Coello, M. S. 2009. Differentiation of monofloral citrus, rosemary, eucalyptus, lavender, thyme and heather honeys based on volatile composition and sensory descriptive analysis. *Food Chemistry*, vol. 112, no. 4, p. 1022-1030. https://doi.org/10.1016/j.foodchem.2008.06.036

Council Directive 2001/110/EC of 20 December 2001 relating to honey.

Crittenden, N. A. 2011. The importance of honey consumption in Human Evolution. *Food and Foodways*, vol. 19, no. 4, p. 257-273. https://doi.org/10.1080/07409710.2011.630618

Esti, M., Panfili, G., Marconi, E., Trivisonno, M. C. 1997. Valorization of the honeys from the Molise region through physico-chemical, organoleptic and nutritional assessment. *Food Chemistry*, vol. 58, no. 1-2, p. 125-128. https://doi.org/10.1016/S0308-8146(96)00228-2

European Commission. 2016. *Agriculture and Rural Development*, ISAMM CM.

Galán-Soldevilla, H., Ruiz-Pérez-Cacho, M. P., Serrano Jiménez, S., Jodral Villarejo, M., Bentabol Manzanares, A. 2005. Development of a preliminary sensory lexicon for floral honeys. *Food Quality and Preference*, vol. 16, no. 1, p. 71-77. https://doi.org/10.1016/j.foodqual.2004.02.001

Gálová, J., Berčík, J., Vilhanová, L. 2012. Sustainable marketing in the practice of selected brands. *Marketing in Practise of Agro-Food Sector*, p. 16-30.

Géci, A., Nagyová, Ľ., Rybanská, J. 2017. Impact of sensory marketing on consumer´s buying behaviour. *Potravinarstvo Slovak Journal of Food Science*, vol. 11, no. 1, p. 709-717. https://doi.org/10.5219/835

Golian, J., Nagyová, Ľ., Andocsová, A., Zajác, P., Palkovič, J. 2018. Food safety from consumer perspective: health safety. *Potravinarstvo Slovak Journal of Food Science*, vol. 12, no. 1, p. 313-322. https://doi.org/10.5219/917

González-Viñas, M. A., Moya, A., Cabezudo, M. D. 2003. Description of the sensory characteristics of Spanish unifloral honeys by free choice profiling. *Journal of Sensory Studies*, vol. 18, no. 2, p. 103-113. https://doi.org/10.1111/j.1745-459X.2003.tb00378.x

IHC. 2009. Harmonised methods of the International Honey Comission, 63 p.

Kaakeh, W., Gadelhak, G. 2005. Sensory evaluation and chemical analysis of Apis mellifera honey from the Arab Gulf region. *Journal of Food and Drug Analysis*, vol. 13, no. 4, p. 331-337.

Kňazovická, V., Kačániová, M., Miluchová, M., Gábor, M., Dovičičová, M., Melich, M., Trakovická, A. 2011. *Honey and Microorganisms: Monitoring of Microorganisms in Slovak Honey by Molecular-Biological Methods in Relation to Physico-Chemical Properties*. Saarbrücken : LAP Lampert Academic Press. p. 192. ISBN 978-3-8473-2310-5.

Kubelaková, A., Košičiarová, I. 2016. Organic food and its position in retail stores in Slovak Republic. In *The agri-food*

value chain: challenges for natural resources management and society, Nitra: Slovak University of Agriculture, p. 1036-1042. ISBN 978-80-552-1505-1.

Lorente, G. M., De Lorenzo Carretero, C., Pérez Martin, R. A. 2008. Sensory attributes and antioxidant capacity of Spanish honeys. *Journal of Sensory Studies*, vol. 23, no. 3, p. 293-302. https://doi.org/10.1111/j.1745-459X.2008.00156.x

Martinovski, S., Gulevska, F. 2017. Nutritive Marketing with a Special Review on Honey. *International Journal of Business and Management Invention*, vol. 6, no. 7, p 05-11.

Nagyová, Ľ., Golian, J., Géci, A., Palkovič, J., Čapla, J., Kádeková, Z. 2018. Food safety from a consumers´point of view: food quality. *Potravinarstvo Slovak Journal of Food Science*, vol. 12, no. 1, p. 355-363. https://doi:10.5219/918

Nagyová, Ľ., Košičiarová, I., Kádeková, Z. 2014. *Quality Label: Food Quality Guarantee at National Level of EU Countries. Značka kvality: Garancia kvality potravín na národnej úrovni krajín EÚ*. Nitra : Slovenská poľnohospodárska univerzita v Nitre. p. 154. ISBN 978-80-552-1283-8. (In Slovak)

Paluchová, J., Prokeinová, R. B. 2013. *Sustainable trends in consumer behavior: association rules, sustainable marketing and responsible consumption. Udržateľné tendencie v spotrebiteľskom správaní: asociačné pravidlá, udržateľný marketing a zodpovedná spotreba*. Nitra : Slovenská poľnohospodárska univerzita v Nitre, p. 112. ISBN 978-80-552-1125-1. (In Slovak)

Persano Oddo, L., Piro, R. 2004. Main European uniforal honeys: Descriptive sheets. *Apidologie*, vol. 35, suppl. no. 1, p. 38-81. https://doi.org/10.1051/apido:2004049

Phipps, R. 2017. International honey market: A Second Point of Inflection has been attained. A Point of Equilibrium is Approaching. The NMR test is gaining international attention and acceptance. *American Bee Journal*, p. 597-604.

Piana, M. L., Persano Oddo, L., Bentabol, A., Bruneau, E., Bogdanov, S., Guyot Declerck, C. 2004. Sensory analysis applied to honey: State of the art. *Apidologie*, vol. 35, suppl. no. 1, p. 26-37. https://doi.org/10.1051/apido:2004048

Pidek, A. 2001. Youth preferences in honey consumption. *Journal of apicultural science,* vol. 45, p. 115-119.

Pocol, C. B. 2011. Modelling the honey consumption behaviour in Romania by using socio-demographic determinants. *African Journal of Agricuture Research*. vol. 6, no. 17, p. 4069-4080.

Pocol, C. B., Teselios C. M. 2012. Socio-economic determinants of honey consumption in Romania. *Journal of Food, Agriculture & Environmen,* vol.10, no. 2, p. 18-21.

Popek, S. 2002. A procedure to identify a honey type. *Food Chemistry*, vol. 79, no. 3, p. 401-406. https://doi.org/10.1016/S0308-8146(02)00391-6

Popov-Raljic, J., Arsic, N., Zlatkovic, B., Basarin, B., Mladenovic, M. Lalicic-Petronijevic, J., Ivkov, M., Popov, V. 2015. Evaluation of color, mineral substances and sensory uniqueness of meadow and acacia honey from Serbia. *Romanian Biotechnological Letters*, vol. 20, no. 5, p. 10784-10799.

Statistical Office of the SR. 2018. [online] s.a. [cit. 2018-04-18] Available at: https://slovak.statistics.sk, Accessed 18.05.11.

Stolzenbach, S., Bredie, W. L. P., Byrne, D. V. 2013. Consumer concepts in new product development of local foods: Traditional versus novel honeys. *Food Research International*, vol. 52, no. 1, p. 144-152. https://doi.org/10.1016/j.foodres.2013.02.030

Stolzenbach, S., V. Byrne, D., Bredie, W. L. P. 2011, Sensory local uniqueness of Danish honeys, *Food Research International*, vol. 44, no. 9, p. 2766-2774. https://doi.org/10.1016/j.foodres.2011.06.006

Szabó, R. T., Mezes, M., Szalai, T., Zajácz, E., Weber, M. 2016. Colour identification of honey and methodical development of its instrumental measuring. *Journal of Agricultural and Environmental* Sciences, vol. 3. no. 1. p. 29-36. https://doi.org/10.18380/SZIE.COLUM.2016.3.1.29

Šánová. P., Nový, J., Svobodová, J., And Šeráková, P. 2015. Pilot analysis of key factors in honey consumption. In *Agrarian perspectives XXIV: Global agribusiness and the rural economy*. Prague, Czech Republic : Czech University Life Science, p. 416-424. ISBN 78-80-213-2581-4.

Vanyi G. V., Csapo, Z., Karpati, L. 2010. Honey consumption in Europe with especial regard to Hungary. In *45th Croatian & 5th International Symposium on Agriculture: Agricultural Economics and Rural Sociology*. Opatija, Croatia : Josip Juraj Strossmayer University, p. 200-204. ISBN 978-953-6331-80-2.

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PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF VARIETY GRAPES FROM KURDISTAN IRAQ

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ABSTRACT

This study was carried out to determine the amount of total phenols, total flavonoid and antioxidant activity of 7 grape cultivars grown in Kurdistan rejoin of Iraq. The results showed that total phenols, flavonoid and antioxidant activity in the berries varied among the investigated cultivars. Total phenolic content, total flavonoid content ranged from 112.77 to 249.19 mg GAE/100g FW, 584.23 to 288.55 mg of rutin equivalents/100 g of (fresh sample) respectly and antioxidant capacity value ranged from 41.79 to 92.30%. Tahlik cultivar had the highest value of antioxidant capacity, flavonoid and total phenolic content. The lowest total phenolic, flavonoid content and the lowest value of antioxidant capacity were found in Abhar cultivar. Present results showed statistically significant correlations with the free radical scavenging activity. There was a very strong positive correlation (R^2 =1, p <0.05) between the antioxidant activity and total phenolic content and a strong positive correlation (R^2 =0.9735, p <0.05) between the antioxidant activity and total flavonoid content.

Keywords: grape; phenolic; antioxidant activity; flavonoid

INTRODUCTION

Grape is a fruit grown in different parts of the world and widely cultivated because of its economic and importance in making juice, wine and raisins. South America has significant role in production and export of grapes **(Ruiz, 2011)**. Table grape used for eaten and other kind grapes are used mainly to produce wine. Italy is the main wine producer and France is the second wine producer in the World **(FAO, 2010)**. Grapes are rich sourse of flavonoids, anthocyanins and phenolic compounds, which have significant role for health benefits **(Yang et al., 2009)**. The anthocyanins participate in several reactions that responsible for changes the color of grape products, due to formation of polymeric pigments and through co pigmentation **(Wrolstad, et al., 2005)**. Grape has antioxidant properties it can prevent the oxidative damage of cells **(Park et al., 2003)**. The consumption of fresh grape has the health benefits which have associated with broadly known and connected to the extravagance of phenolic compounds, for example, anthocyanins, Gallic corrosive, catechin and a wide assortment of procyanidins. These mixes have been set up to have an extensive variety of biochemical and pharmacological impacts, for example, antiatherogenic, anticarcinogenic, cell reinforcement exercises and calming **(Dell Agli et al., 2004; Darra et al., 2012)**. Right now phenolic compounds are accepting much consideration because of their helpful wellbeing impacts identified with their capacity to ensure against oxidative cell harm if happened master oxidant and cancer prevention agent awkward nature. Truth is told responsive species play both a poisonous and gainful part and the harmony between them must be looked after **(Forman et al., 2010)**. Phenolic compounds have a wide spectrum of health benefits such as anti-mutagenic, anti-bacterial, anti-inflammatory, and antioxidant activity and minimize oxidative stress **(Celep and Rastmanesh, 2013)**. Polyphenolic compounds product the free radicals in the body and reduce damage to DNA **(Bub et al., 2003)**. Antioxidant properties of grapes are attributed at least partly to their phenolic content. Consumption of fruits rich in antioxidant substances such as phenolic nalyses proved that frequent adequate intake of fruits could help to prevent cardiovascular diseases **(Rautiainen et al. 2012)**. diabetes **(Hegde et al., 2013)** and cancer **(Wang et al., 2014)**. The majority of agro industrial residues of grape are mostly solid by products including stalks and the liquid filtrate; residues are composed of carbohydrates, vitamins, minerals, lipids, water, and compounds with important biological properties such as phenolic compounds and fiber, depending on the types of wastcompounds and vitamin C is inversely associated with risks of non communicable diseases. Epidemiological studies and meta-ae, climatic and cultivar **(Ahmad and Ali Siahsar, 2011)**.

Scientific hypothesis

The main object of this study was to evaluate the total phenolictotal content (TPC), total flavonoid content (TFC) and antioxidant activity using the spectrophotometrical determination of different varieties grape from different location in Kurdistan Rejoin of Iraq Republic. We assume there are different concentrations of flavonoids in different varieties of grapes.

MATERIAL AND METHODOLOGY

Chemicals and reagents

Folin Ciocalteu reagent (FCR), Gallic acid (GA), [DPPH=2, 2-diphenyl-1-picrylhydrazyl], rutin, [Na_2CO_3 = Sodium carbonate], Methanol (CH_3OH), Sodium nitrite, [$AlCl_3$ = Aluminum chloride], Sodium hydroxide were acquired from Sigma Chemical Co. in Bratislava, Slovakia Republic). The reagents and chemicals utilizes as a part of this investigation were of explanatory review (99%).

Material

Grape samples harvested at fully ripened and matured, Seven grape varieties including four of varieties black colors (Mula Hassan, Awilka. Mamarik, Tahlik) and three red colors (Sadani, Abhar, Kamali) were obtained from different location in (three Cities) in Kurdistan rejoin of Iraq Republic. The seven grape varieties are described in Table 1.

Extraction

For extraction of bioactive mixes from new grapes utilizing separated by the altered strategy depicted by **Yang et al. (2009)**. 200 g of grapes were mixed for 3 min in 200 g of 80% CH_3COCH_3) by utilizing Warring blender with low speed for expelling seeds. Included another sum (200 g of 80% CH_3COCH_3) after evacuation of the seeds, at that point the grapes were mixed for 2 min by a similar blender with fast, at that point the blend was homogenized by homogenizer for 5 min and sifted with vacuum under an ice shower. To expel (CH_3COCH_3) in the filtrate utilized a rotating evaporator at 40 °C until the point that the heaviness of the vanished filtrate break even with 20% of original filtrate weight. The concentrates were put away at -40°C until utilize.

Estimation of total phenolic content

Add up to phenolics decided utilizing Folin-Ciocalteu reagent (FCR) by the strategy portrayed **Lachman et al. (2003)**. Test removed (0.05 g to 1 mL of 80% methanol then 2.5 mL of (FCR) and 4 mL of distilled water added to a 50 mL carafe. Following 5 minutes 7.5 mL of [Sodium Carbonate=Na_2CO_3 (25%)] added to the carafe then the volume moved toward becoming 50 mL with distilled water. The blend was (permitted) brooded for (2.5 hours) at research temperature. At that point the absorbance was measured at 765 nm against a blank (80% methanol) by a spectrophotometer (Shimadzu710 Japan). The result was calculated as (mg GAE/100fw) the average content polyphenol compounds in samples were obtained from ±SD six replicates.

Estimation of total flavonoid content

For determination flavonoid content of grapes extract used the modification method according to **(Jia et al., 1999)**. 0.20 mL of 1:20 weakened grape separates was blended with 1.10 mL of refined water and with 0.070 mL of 4 % sodium nitrite arrangement at that point permitted to respond for 7 min. From that point forward, a 0.12 mL of 10% aluminum chloride was included and permitted extra respond for 7 min after that 0.60 mL of 1 M NaOH was included. The last volume make to 3 mL by including refined water. Absorbance of the blend was measured using spectrophotometer at a wavelength 510 nm against arranged clear from rutin standered. For assurance flavonoid content utilizing rutin standard bend and communicated as the mean ±SD for six replications of the sample (mg rutenE/100g Fw).

Determination of antioxidant activity

For the analysis of free radical scavenging activity 2, 2-diphenyl-1picryl hydrazyl (DPPH) was utilized by a changed technique for **Brand-Williams et al. (2005)**. To get a stock arrangement: 0.020 g DPPH was diuted to 100 mL methanol at that point kept in a cool and dim place. Prior to the examination, 1:10 dilution of the stock was made with methanol, at that point 3.9 cm^3 of the arranged DPPH was added to a cuvette and the absorbance at 515.6 nm was measured by spectrophotometer (UV/ VIS 710) at time At0 was written. After that 0.1 cm^3 of the solution (sample) was added and mixed then the absorbance was measured at 10 minutes (At10). The absorbance of time 0 and 10 minutes at 515.6 nm was measured. The dependence A = f (t) was measured.

The percentage of inhibition in given time (%) = (At0 – At10 / At0) x 100

Statisic analysis

The results were evaluated statistically using the Analysis of Variance. Procedure compares the data in seven varieties. The results assays were expressed as mean ±SD of six repeated samples. The data was utilized the F-test in the restricted investigation of change (ANOVA) by the F-test, if the *p*-value is less than 0.05, there is a statistically significant difference between the means at the 95% level; the Multiple Range Tests will indicate which implies are essentially unique in relation to others. This technique was utilized to separate between the methods for Fisher's minimum huge contrast (LSD) methodology. Examination was utilizing SAS programming 9.4. Connections among different parameters were likewise utilized and decided at $p < 0.05$, of six rehashed tests.

RESULTS AND DISCUSSION

Total polyphenolic content

The total phenol content (TPC) for seven grape varieties was determined using the Folin-Ciocalteau method. The outcomes found that the levels of phenolic compound in the grape tests essentially vary ($p < 0.05$) among all examples utilized from the distinctive grape assortments. In the present investigation as appeared in (Table.1), it was distinguished, that aggregate polyphenols content in tests ran from (112.77 ±0.34 to 249.19 ±0. 29 mg GAE/100FW) mg of Gallic corrosive reciprocals per100 g fresh weight.

Table 1 The total phenolic, total flavonoid, contents and antioxidant capacity of 7 grape varieties.

Varieties	Locality	Color	Total Phenolics (TPC) ($mg.100g^{-1}$)	Total Flavonoid (TFC) ($mg.100g^{-1}$)	Antioxid antcapacity (TAC) (%)
MULA HASSAN	Erbil	Black	212.91 ±0.59a	498.96 ±0.48a	78.89 ±0.27a
AWILKA	Erbil	Black	124.36 ±0.37b	291.83 ±0.22b	46.41±0.40b
MAMARIK	Dohuk	Black	173.83 ±0.53c	380.91 ±0.30c	64.30 ±0.44c
TAHLIK	Dohuk	Black	249.19 ±0.29d	584.23 ±0.07d	92.30 ±0.56d
SADANI	Erbil	Red	128.31 ±0.4e	294.49 ±0.31e	47.67 ±0.29e
ABHAR	Sulaimanya	Red	112.77 ±0.34f	288.55 ±0.29f	41.79 ±0.25f
KAMALI	Dohuk	Red	236.14 ±0.41g	503.37 ±0.25g	87.22 ±0.64g

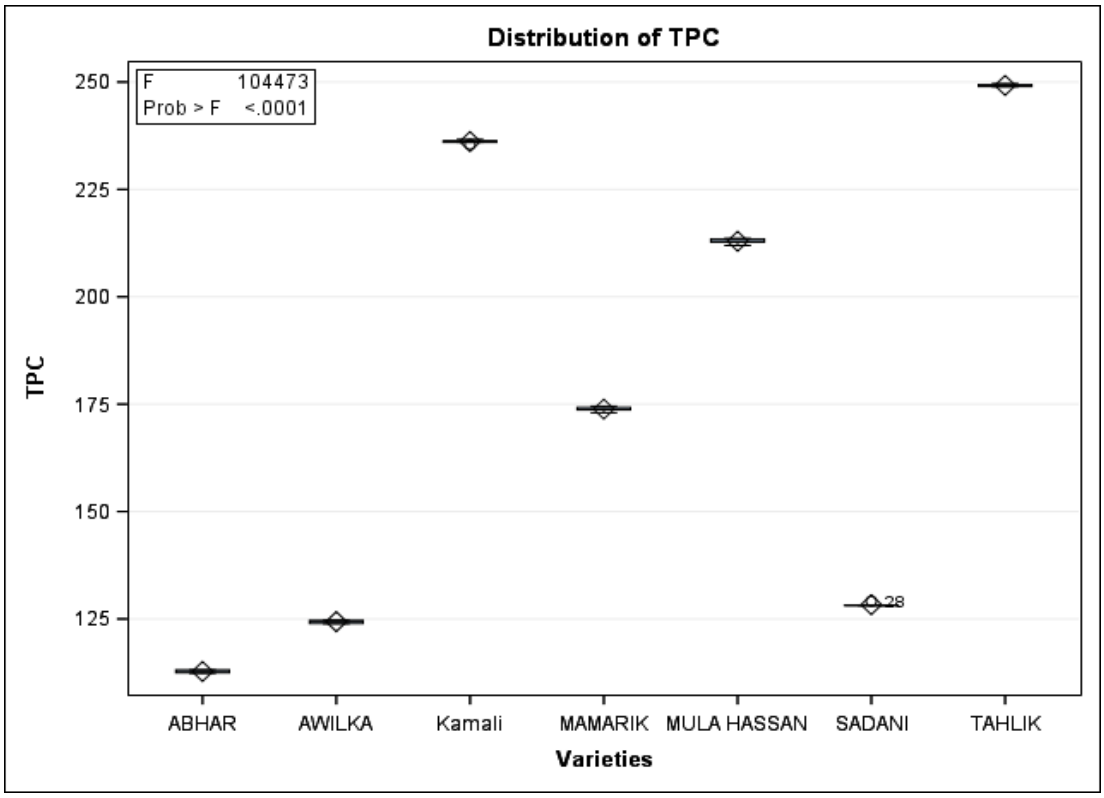

Figure 1 Distribution of total polyphenolic content.

As per picked up comes about, the polyphenols content (TPC) in all tried were fundamentally differenced relying upon grape cultivar and assortment.

Statistically significant highest value of total polyphenols was found in Tahlik black color and the lowest TPC content was found in Abhar red variety. According to the average contents of total polyphenols in fresh matter of grape, there is the following line showed in Table 1, Figure 1: Tahlik black color with (249.19 ±0.29 mg of Gallic acid equivalents/100g) > Kamaly red color (236.14 ±0.41) > Mula Hassan black color (212.91 ±0.59) > Mamarik black color (173.83 ±0.53) > Sadani red color (128.31 ±0.4) >Awilka black color (124.36 ±0.37) >Abhar red color (112.77 ±0.34) mg of Gallic acid equivalents /100g FW. The phenolic compounds composition of fruits depended on genotypes, environmental factors and postharvest processing conditions **(Benvenuti *et al.*, 2004; Kadir *et al.*, 2009)**. Many researchers have also been observed differences in the phenolic content among grape varieties **(Frankel et al., 1995; Simonetti et al., 1997; Burns et al., 2000)**. The composition and the quantity of phenolic compounds vary due to difference in varieties, species and maturity of the grapes, the place and location where the grapes are grown **(Burin et al., 2010)**.

Total flavonoid

The results as shown in Table 1, Figure 2 Tahlik variety obtained the highest total flavonoid content (584.23 ±0.07d mg rutin E/100 g FW) milligram rutin equivalents per 100 gram fresh weight grape followed by Kamali, Mula Hassan, Mamarik, Sadani, Awilka and Abhar. The difference might be due to variety grapes or the different factor: climate, cultivar, cultivation site and ecological factors, cultural practices or genetic factors influenced on phenolic and flavonoid content.

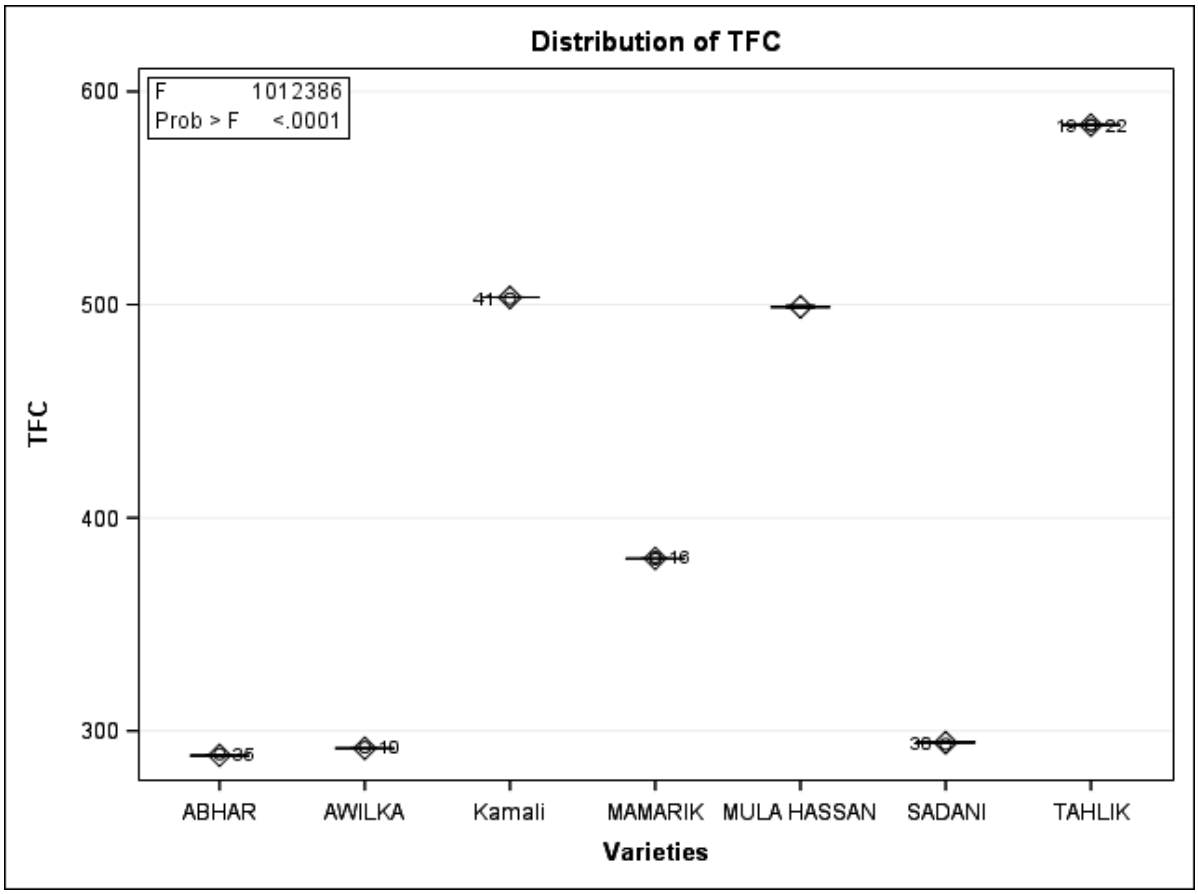

Figure 2 Distribution of total flavonoid content.

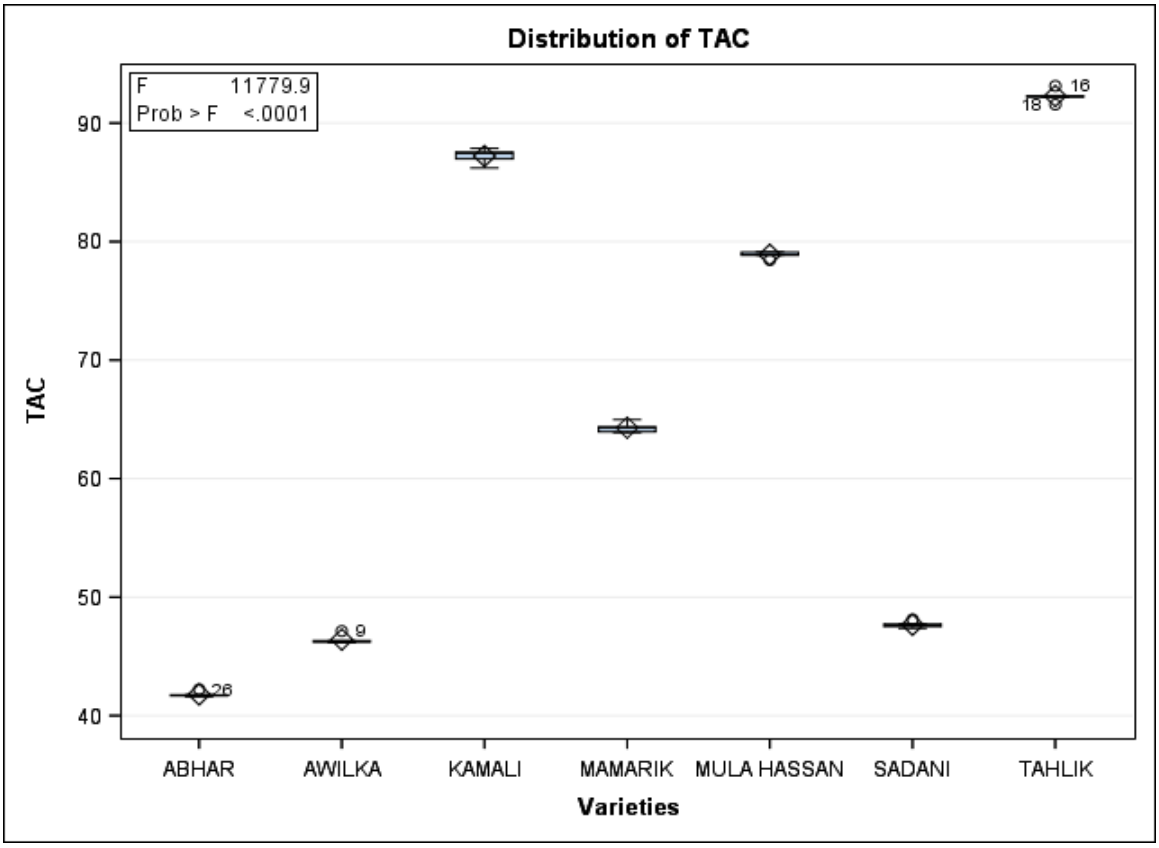

Figure 3 Distribution of antioxidant capacity.

DPPH ASSAY

The free radical rummaging exercises of concentrates rely upon the capacity of cancer prevention agent mixes (sample) to lose hydrogen atom and the basic compliance of parts **(Shimada et al., 1992)**. DPPH radical's has ability to bind hydrogen atom which has a radical scavenging property. The solution of DPPH prepared in methanol (CH_3OH) is converted into DPPH-H molecule in the presence of an antioxidant agent, as shown by the equation. Discoloration occurs due to the decreasing quantity of DPPH radicals in surroundings. The staining of DPPH in this manner mirrors the radical searching action of the investigated separate **(Guo et al., 2007)**. The strategy depends on the lessening of alcoholic DPPH• arrangements within the sight of a hydrogen giving cancer prevention agent (AH) to the non-radical frame DPPH-H.

DPPH• +A-H→DPPH-H + A•

The antioxidant capacity of the grapes samples as shown in Table 1. Figure 3.The antioxidant capacity values for seven varieties are significantly different and decreased by the order 92.30% Tahlik > 87.22 % Kamali > 78.89% Mula Hssan > 64.30 % Mamarik > 47.67 % Sadani > 46.41% Awilka > 41.7 9% Abhar. In present study the antioxidant activity of grape is influenced by their phenolic composition content **(Dávalo et al., 2005)**. The antioxidant capacity values ranged from 41.79 ±0.25 to 92.30 ±0.56 of the 7 grape samples, Tahlik black color from Duhok showed a higher value of antioxidant capacity and Abhar red color from Sulaimanya had a lowest antioxidant capacity.

Correlation between Antioxidant Capacity value and Total Polyphenols

Analysis of variance (ANOVA) was used for linear correlation coefficients to appraise (evaluate) the relationships between TAC with TPC, TFC. Our result optioned statically very strongly positive correlations (R =1; p <0.05) was found (Figure 4) between add up to cancer prevention agent limit esteems and aggregate polyphenol content and statically solid positive correlation

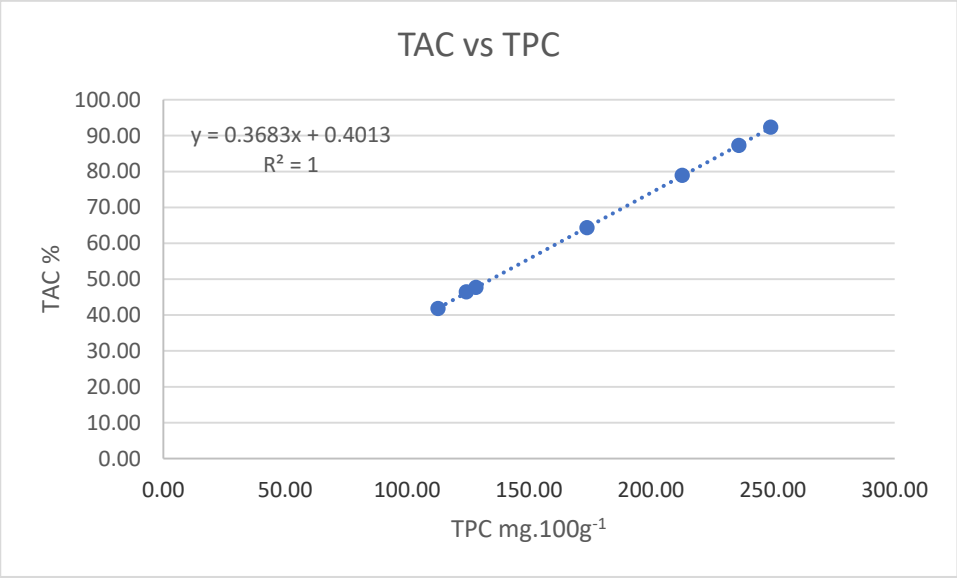

Figure 4 Correlation between Antioxidant Capacity value and Total polyphenolic content.

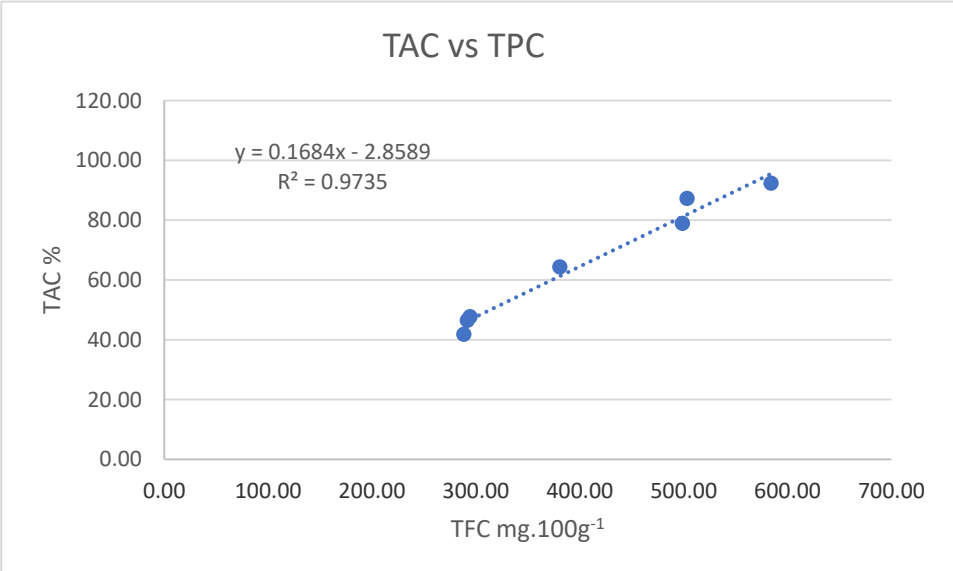

Figure 5 Correlation between Antioxidant Capacity value and Total Flavonoid content.

(R = 0.9735; p <0.05) between cell reinforcement limit esteem and aggregate flavonoid content was discovered (Figure 5). A positive relationship between's aggregate phenolic and cell reinforcement limit has likewise been discovered **(Hulya-Orak, 2007)**. In exhibit examine a high connection comes about among add up to phenolic substance and cancer prevention agent limit are in concurrence with the consequences of numerous specialists. (0.97 and 0.95, p <0.05) between cancer prevention agent limit and aggregate phenolic content (measured by the FRAP techniques). Numerous analysts found a high connection among cancer prevention agent limit, add up to phenols, flavonoid, anthocyanins content content **(Wang and Lin, 2000; Burns et al., 2000)**]. The outcomes exhibited here in gave significant information of cell reinforcement limit, phenolic substance and flavonoid content for seven grape assortments, the outcome demonstrated that there is a high relationship among expanded phenolic content and the expanded cancer prevention agent limit **(Susana et al., 2014)**.

CONCLUSION

The phenolic content, antioxidant activity, and the correlation between phenolic content and antioxidant capacity were studied in varieties grape. The comparison of seven grape varieties showed that Tahlik variety grape provided significantly highest total phenolic content (average value of 249.19 ±0.29 mg.100g^{-1} FW grape), total flavonoid content (584.23 ±0.07 mg of rutin equivalents/100gFw), as well as strong antioxidant capacity (92.30 ±0.56%). showing that there is a correlation between increased phenolic content and increased antioxidant activity. Strong correlations were found between total phenolic, total flavonoid contents and their antioxidant activities.

REFERENCES

Ahmadi, S. M., Ali Siahsar, B. 2011. Analogy of physicochemical attributes of two grape seeds cultivar. *Ciencia e Investigación Agraria*, vol. 38, no. 2, p. 291-301. https://doi.org/10.4067/S0718-16202011000200014

Benvenuti, S., Pellati, F., Melegari, M., Bertelli, D. 2004. Polyphenols, Anthocyanins, Ascorbic Acid and Radical Scavenging Activity of *Rubus*, *Ribes*, and Aronia. *J. Food Sci*. vol. 69, no. 3, p.164-169. https://doi.org/10.1111/j.1365-2621.2004.tb13352.x

Brand-Williams, W., Cuvelier, M. E., Berste, C. 2005. Use free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, vol. 28, no. 1, p. 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Bub, A., Watzl, B., Blockhaus, M., Briviba, K., Liegibel, U., Müller, H., Pool-Zobel, B., Rechkemmer, G. 2003. Fruit juice consumption modulates antioxidative status, immune status, and DNA damage. *Journal of Nutrition Biochemistry*, vo.14, no. 2, p. 90-98. https://doi.org/10.1016/S0955-2863(02)00255-3

Burin, V. M., Falcao, L. D., Gonzaga, L.V., Fett, R., Rosier, J. P., Bordignon-Luiz, M. T. 2010. Color, phenolic content and antioxidant activity of grape juice. *Cienc Tecnol Aliment*, vol. 30, no. 4, p.1027-1032. https://doi.org/10.1590/S0101-20612010000400030

Burns, J., Gardner, P. T., O'Neil, J., Crawford, S., Morecroft, I., McPhail, D. B., Lister, C., Matthews, D., MacLean, M. R., Lean, M. E., Duthie, G. G., Crozier, A. 2000. Relationship among Antioxidant Activity, Vasodilation Capacity, and Phenolic Content of Red Wines. *Journal of Agricultural and Food Chemistry*, vol. 48, no. 2, p. 220-230. https://doi.org/10.1021/jf9909757

Celep, G. S., Rastmanesh, R. 2013. Polyphenol consumption and metabolic diseases. *Journal of Nutrition Disorders and Therapy*, vol. 3, no. 1. https://doi.org/10.4172/2161-0509.1000e106

Darra, N. E. Tannous, J. Mouncef, P. B. Palge, J. Yaghi, J. Vorobiev, E. Louka, N. Maroun, R. G. 2012. A comparative study on antiradical and antimicrobial properties of red grapes extracts obtained from different *Vitis vinifera* varieties. *Food Nutr. Sci.*, vol. 3, no. 10, p. 1420-1432. https://doi.org/10.4236/fns.2012.310186

Dávalos, A. Bartolomé, B. Gómez-Cordovés, C. 2005. Antioxidant properties of commercial grape juice and vinegars. *Food Chem*., vol. 93, no. 2, p. 325-330. https://doi.org/10.1016/j.foodchem.2004.09.030

Dell Agli, M., Buscala, A. Bosisio, E. 2004. Vascular effects of wine polyphenols. *Cardiovasc. Res.*, vol. 63, no. 4, p. 593-602. https://doi.org/10.1016/j.cardiores.2004.03.019

FAO. Statistical Databases (Electronical Resource); FAO: Paris, France. 2010. Available online: http://faostat.fao.org (accessed on1 November 2012).

Forman, H. J. Maiorino, M. Ursini, F. 2010. Signaling functions of reactive oxygen species. *Biochemistry,* vol. 49, no. 5, p. 835-842. https://doi.org/10.1021/bi9020378

Frankel, E. N. Waterhouse, A. L., Teissedre, P. L. 1995. Principal Phenolic Phytochemicals in Selected California Wines and their Antioxidant Activity in Inhibiting Oxidation of Human Low-Density Lipoproteins. *J. Agr. Food Chem*., vol. 43, no. 4, p. 890-894. https://doi.org/10.1021/jf00052a008

Guo, X. Y. Wang, J. Wang, N. L. Kitanaka, S. Yao, X. S. 2007. 9,10-Dihydrophenanthrene derivatives from Pholidota yunnanensis and scavenging activity on DPPH free radical. *Journal of Asian Natural Products Research*, vol. 9, no. 2, p. 165-174. https://doi.org/10.1080/10286020500480522

Hegde, S. V., Adhikari, P., Mandini, M., D'souza, V. 2013. Effect of daily supplementation of fruits on oxidative stress indices and glycaemic status in Type 2 Diabetes Mellitus. *Complementary Therapies in Clinical Practice*, vol. 19, no. 2, p. 97-100 https://doi.org/10.1016/j.ctcp.2012.12.002

Hulya-Orak, H. 2007. Total Antioxidant Activities, Phenolics, Anthocyanins, Polyphenoloxidase Activities of Selected Red Grape Varieties and their Correlation. *Sci. Hort*., vol. 111, no. 3, p. 235-241. https://doi.org/10.1016/j.scienta.2006.10.019

Jia, Z. Tang, M. Wu, J. 1999. The determination of flavonoid contents in mulberry and their scavenging effetcs on superoxide racicals. *Food Chem.,* vol. 64, no. 4, p. 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2

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, no. 4, p. 142-147. https://doi.org/10.17221/3876-HORTSCI

Lachman, J., Hejtmánková, A., Dudjak, E. 2003. *Content polyphenolic antioxidants and phenol carboxylic acids in* selected parts of yacon. Vitamins 2003 – Přírodní antioxidanty a volné radikály. Pardubice: Univerzita Pardubice, 89-97 p. ISBN 80-7194-549-8 Available online: http://www.agriculturejournals.cz/publicFiles/51932.pdf.

Park, Y., K, Park, E., Kim, J. S., Kang. M. H. 2003. Daily grape juice consumption reduces oxidative DNA damage and

plasma free radical levels in healthy Koreans. *Mutation Research*, vol. 529, no. (1-2), p. 77-86.

Rautiainen, S. Larsson, S. Virtamo, J. and Wolk, A. 2012. Total antioxidant capacity of diet and risk of stroke a population-based prospective cohort of women. *Stroke,* vol. 43, no. 2, p. 335-340. https://doi.org/10.1161/STROKEAHA.111.635557

Ruiz, V. S. 2011. Avances em viticultura em el mundo. *Revista Brasileira de Fruticultur, vol. 33, no. Spe1, p.* 131-143. https://doi.org/10.1590/S0100-29452011000500016

Shimada, K. Fujikawa, K. Yahara, K. Nakamura, T. 1992. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. *Agricultural and Food Chemistry*. vo. 40, no. 6, p. 945-948. https://doi.org/10.1021/jf00018a005

Simonetti P., Pietta P., Testolin G. 1997. Polyphenolic content and total antioxidant potential of selected Italian wines. *Agric. Food Chem*., vol. 45, no. 4, p. 1152-1155. https://doi.org/10.1021/jf960705d

Susana Sério Maria D. Rivero-Pérez, Ana Cristina Correia, António Manuel Jordão, Maria L. González-San José. 2014. Analysis of commercial grape raisins: phenolic content, antioxidant capacity and radical scavenger activity. *Ciência téc. vitiv*. vol. 29, no. 1, p.1-8. https://doi.org/10.1051/ctv/20142901001

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. https://doi.org/10.1021/jf9908345

Wang, X., Ouyang, Y., Liu, J., Zhu, M., Zhao, G., Bao, W., Hu, F. B. 2014. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. *British Medical Journal*. vol. 349, https://doi.org/10.1136/bmj.g4490

Wrolsta, R. E., Durst, R. W., Lee, J. 2005. Tracking color and changes in anthocyanin products. *Trends in Food Science and Technology*, vol. 16, no. 9, p. 423-428. https://doi.org/10.1016/j.tifs.2005.03.019

Yang, J., Martinson, T. E., Liu, R. H. 2009. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chem*., vol.116, no. 1, p. 332-339. https://doi.org/10.1016/j.foodchem.2009.02.021

Yang, J., Martinson, T. E., Liu, R. H. 2009. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chem*., vol. 116, no. 1, p. 332-339. https://doi.org/10.1016/j.foodchem.2009.02.021

Yang, J., Martinson, T. E., Liu, R. H. 2009. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chem.*, vol. 116, no. 1, p. 332-339. http://dx.doi.org/10.1016/j.foodchem.2009.02.021

Yilmaz, K. U., Ercisli, S., Zengin, Y., Sengul, M., Kafkas, E. Y. 2009. Preliminary Characterization of Cornelian Cherry (*Cornus mas* L.) Genotypes for their Physicochemical Properties. *Food Chem*., vo. 114, no. 2. p. 408-412. https://doi.org/10.1016/j.foodchem.2008.09.055

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ALTERATION OF BIOCHEMICAL PARAMETERS AND MICROSTRUCTURE OF FAGOPYRUM ESCULENTUM MOENCH GRAIN IN PROCESS OF GERMINATION

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ABSTRACT

Biochemical parameters alteration of *Fagopyrum esculentum Moench* grain in process of germination was studied. It was found that during germination of *Fagopyrum esculentum Moench* grain within 24 hours the content of ascorbic acid, thiamine, nicotinic acids, pantothenic acids and routine was increased. The peptide composition of *Fagopyrum esculentum Moench* grain was studied by gel electrophoresis. The most significant alteration of reserve globulins structure are observed during germination phase from 20 till 24 hours. New low-molecular polypeptides appear during above mentioned period, that indicates embryonic awakening and synthesis of new protein compounds, mainly hydrolases. The process of proteolysis during germination of *Fagopyrum esculentum Moench* grain promotes a content increase of soluble fractions and sum of albumins and globulins. There is a significant decrease of insoluble protein residue during germination phase change. Chromatographic method was used to determine the change of carbohydrate composition of *Fagopyrum esculentum Moench* grains during germination. It was established that the content of total carbohydrates amount in grain extracts increases. Electron scanning microscopy revealed that after 12 hours germination of *Fagopyrum esculentum Moench* grain, swelling of starch grains and minor damage of their packaging in endosperm are observed. After 24 hours, endosperm of germinated grain significantly changed microstructure: starch grains and components of protein matrix had a vague outline, grain disintegration was observed. Evaluation of antioxidant activity of alcohol extract from *Fagopyrum esculentum Moench* grain germinated during 24 hours showed that percentage of DPPG free radicals inhibition increases with process prolongation. Thus, *Fagopyrum esculentum Moench* grain germinated within 24 hours is characterized by a high content of biologically active substances and can be used in food technologies for functional products development.

Keywords: *Fagopyrum esculentum Moench* grain; germination; vitamins; peptide composition; carbohydrate composition; microstructure; antioxidant activity

INTRODUCTION

The nutritional value of *Fagopyrum esculentum Moench* in terms of protein, vitamins, minerals, organic acids, amino acid and dietary fibers content similar to wheat and triticale, so *Fagopyrum esculentum Moench* is used for wheat substitution in bakery, pasta production and confectionery **(Wronkowska et al., 2010; Marijana et al., 2015)**. Whole-grain buckwheat flour has a positive effect on the physical, chemical and sensory properties of bread and enhances its functional and antioxidant properties **(Li and Zhang, 2001; Lin et al., 2009; Yıldız and Bilgiçli, 2012)**. The antioxidant activity of *Fagopyrum esculentum Moench* grain primarily associated with the content of polyphenols and tocopherols. Polyphenols in buckwheat flour exist in free and bound forms, concentration of free polyphenols can be from 48% up to 64%. Buckwheat flavonoids have positive effects in hypotension treatment, have an inflamotor and anti-allergic effect **(He et al., 1995; Kim et al., 2003; Kim et al., 2004; Wloch et al., 2016)**. Buckwheat proteins are characterized by a unique amino acid composition that helps lower blood cholesterol levels and improve patient's conditions with constipation and obesity **(Huff and Carroll, 1980; Ahmed et al., 2014; Sikder et al., 2014; Sakač et al., 2015)**. Proteins of *Fagopyrum esculentum Moench* grain are characterized by an overestimated amino acid content in terms of tryptophan **(Chao et al., 2002)**. Buckwheat is the best source of magnesium, potassium, phosphorus, zinc, manganese and cuprum than other cereals. Among the vitamins, pyridoxine is the most

prevalent in buckwheat, and presented phytosterols are useful to lower cholesterol level in the blood. Whole grains contain 7% of fiber. Outer layers of buckwheat grain contain main part of effective prophylactic compounds **(Danihelová and Šturdík 2012)**.

Buckwheat is a potentially safe source of gluten-free products for patients with celiac disease and chronic enteropathy **(Sedej et al., 2011; Katar et al., 2016)**. *Fragopyrum esculentum Moench* grain extracts have antibacterial and immunostimulating effects **(Čabarkapa et al., 2008; Świątecka et al., 2013)**. Germination of *Fagopyrum esculentum Moench* grain is considered as an effective method to improve nutritional properties. This is a complex process with significant changes in biochemical and sensory characteristics due to the activation of enzymes. Germinated grain or sprouts have bigger nutritional value than their original grain in terms of protein content and starch digestibility **(Nonogaki et al., 2010)**. A significant increase in total amount of phenolic compounds and a higher antioxidant activity are observed after 64 hours germination, while heating leads to decrease in total amount of phenolic compounds and antioxidant activity. Germinated buckwheat has a better nutritional value and antioxidant activity and it is an excellent natural source of flavonoids and phenolic compounds, especially rutin and C-glycosylflavones. Therefore, germinated *Fagopyrum esculentum Moench* grain can be used as a promising functional nutrition for health promotion **(Koyama et al., 2013; Zhang et al., 2015; Terpinc et al., 2016)**.

The study of buckwheat flour blending with wheat flour in different ratios for cookies production showed a significant change in the physico-chemical and functional properties of blended flour. The overall acceptability of cookies according to sensory analysis was at the highest level at 40% mixing. This study showed that buckwheat addition to wheat flour can not only improve the physicochemical and functional properties of mixed flour, but also increase the nutraceutical potential of product made of it **(Jan et al., 2015)**.

The target of the study was to study alteration of biochemical parameters and microstructure of *Fagopyrum esculentum Moench* grain during germination.

Scientific hypothesis

Germination process has a significant effect on biochemical parameters change of *Fagopyrum esculentum Moench* grain.

MATERIAL AND METHODOLOGY

The parameters of grain's technological qualities for 2 local varieties and 26 breeding varieties of different morphotype representing the main stages of selection of *Fagopyrum esculentum Moench* were analyzed. As a result of analysis *Fagopyrum esculentum Moench* variety Dikul (laboratory of cereal crops selection of the Institute of Leguminous and Cereal Cultures, Russian Federation) was selected for further research. *Fagopyrum esculentum Moench* grain was separated from various impurities and washed with a large amount of cold water. Then it was poured with water at a ratio of grain:water = 1 : 1, soaked for 12 hours at 25 °C, distributed in equal layer and germinated in incubation chamber at an air humidity of 60 – 70%, temperature 18 – 25 °C for 12 – 24 hours.

Vitamin content determination was carried out by HPLC on a Milichrom-5 device (NJSC Nauchpribor, Russia). An aqueous buckwheat grane extract (pH 3) was used, eluent of the composition was acetonitrile: an aqueous solution of sodium heptanesulfonate and potassium phosphate monosubstituted (pH 3.0, 20 : 80 ratio); mobile phase flow rate was 1 $cm^3.min^{-1}$; elution mode was isocratic, detection was carried out in wavelength range 200 – 400 nm, analysis time 12 – 25 min, sample volume 2 – 6 μL.

The polypeptide composition of the total buckwheat grain protein was determined by one-dimensional DDS-Na electrophoresis on gel plates with an acrylamide concentration gradient of 10 – 20% in resolving gel (pH 8.8) and 6% acrylamide in concentrated gel (pH 6.8). A Helicon camera was used for vertical electrophoresis with Elf-4 power supply unit (OOO Helikon Company, Russia).

The concentration of low-molecular carbohydrates in grain samples was determined by chromatographic method using electrochemical detection on an Agilent 1100 liquid chromatograph with an ESA Coulochem III electrochemical detector (Agilent Technologies, USA). Separation of the sugar mixture was carried out on an anion exchange column with a grafted aminophase followed by electrochemical detection.

Microstructural studies were carried out using an electronic scanning microscope ZEISS EVO LS (Carl Zeiss Industrielle Messtechnik GmbH, Germany) with an accelerated voltage of 15 kV.

The complex of phenolic compounds was determined by HPLC on a Milichrom-5 device (NJSC Nauchpribor, Russia). An alcohol extract of grain was used, eluent of the composition was acetonitrile: an aqueous solution of trifluoroacetic acid (pH 2.5, 15 : 85 ratio); elution mode was isocratic, analysis time is 12 – 25 minutes, sample volume was 2 – 6 μL.

Antioxidant activity was determined by spectrophotometric method in an alcohol extract described by **Silva et al. (2005)** based on percentage of inhibition of DPPH radical (2,2-diphenyl-1-picrylhydrazyl). We determined the optical density of solutions in the interaction DFPG with extractive substances of plants by spectrophotometer "Specord M40" (Carl Zeiss Industrielle Messtechnik GmbH, Германия) at a wavelength of 515 nm.

Statisic analysis

T-statistics (a two-sample t-test for independent samples) was used to assess reliability of test differences, Analysis were conducted to a significance level $p < 0.05$ using Statistica 7.0 software (StatSoft Inc., USA).

RESULTS AND DISCUSSION

The study of water-soluble vitamins accumulation process by *Fagopyrum esculentum Moench* grain was carried out during period of swelling and germination, which is characterized by especially intensive metabolism. During above mentioned period, the reserve substances are transformed into vital compounds used by sprout to form new tissues. The content of water-soluble vitamins in *Fagopyrum esculentum Moench* grain was determined by

HPLC before germination and after 36 hours of incubation. Chromatograms are shown on Figures 1 and 2.

Study results shows that after 24 hours of incubation concentration of ascorbic acid in seeds increased by 821%, thiamine by 664%, nicotinic acid by 571%, pantothenic acid by 574%, routine by 706%. According to published data, during *Fagopyrum esculentum Moench* grain germination, a gradual increase of vitamin B1 content is observed, which corelates with our data. The amount of vitamins B2 and B6 does not change while content of vitamin C decreases in the early stages of germination, and then on the third day of buckwheat grains germination increases dramatically **(Yiming et al., 2015)**. According to other researchers **(Kim et al., 2004)**, the content of vitamin C in buckwheat grains increases rapidly, as well as in our studies, and for B1 and B6, a relatively moderate increase is noticed.

During study of biochemical processes in germinating seeds used for food purposes, one of the tasks was to determine the technological characteristics of reserve proteins. During germination some proteins are synthesized using a series of biochemical reactions. Meanwhile, other proteins can be hydrolyzed by an activated protease. The protein content in the grain during germination is determined by both the effect of proteolysis and the rate of protein molecules synthesis. Therefore, a change of protein content during germination of grain is a dynamic process. In previous studies about effect of germination on proteins in cereals and legumes, conflicting data were obtained. **(Uppal and Bains, 2012; Zhang et al., 2015)** indicate an increase of protein content during grain germination. At the same time **(Yiming et al., 2015)** found that the protein content decreases during germination of buckwheat grain. In our case, a decrease of protein nitrogen content in *Fagopyrum esculentum Moench* grain during germination is also observed (Table 1). The contradictory nature of the data may be related with specific features of plants and germination conditions. Structural changes in the protein complex at germination stages were examined by gel electrophoresis of peptide composition of *Fagopyrum esculentum Moench* grain. The electrophoregrams are shown on Figure 3.

Three main groups of peptides with molecular weights of 20 – 25, 40 – 45 and 65 kDa have been allocated. Computer processing by program "Biotest-D" of obtained electrophoregrams by luminosity of peptides was carried out, which confirmed the changes in their quantitative content. The most significant changes in structure of reserve globulins were observed during the germination phase after 20 – 24 hours.

New low-molecular polypeptides appear in this period, which indicates the embryonic awakening of the embryo and the synthesis of new protein compounds. This group of protein includes mainly hydrolases (lipases, proteases, peptidases, amylases, phytases) aimed at hydrolysis of all reserve nutrients. The electrophoregram of *Fagopyrum esculentum Moench* grain proteins after 36 hours of germination indicates unrestricted soaking and dissolution of peptides. This is probably due to the deep hydrolysis and decomposition of reserve proteins.

Obtained electrophoregrams of buckwheat grain protein correspond to the published data. Proteins with molecular weights of 15 and 22 kDa were identified as the main allergic proteins **(Morita et al., 2006)**. According to other data, buckwheat grain with a molecular weight of 24, 19, 16 and 9 kDa are the main allergens **(Park et. Al., 2000)**. The results of our research show that low-molecular proteins of this fraction were destroyed during germination process and *Fagopyrum esculentum Moench* germinated grain can be used for the production of food with a low content of allergens.

For food technology, usage of sprouted buckwheat grain is most acceptable, due to peptides biological activity. Therefore, further studies of biochemical parameters were carried out for a germinated within 24 hours grain.

Table 1 shows the change in the composition of *Fagopyrum esculentum Moench* grain protein fractions during germination.

Proteolysis process during *Fagopyrum esculentum Moench* grain germination promotes an increase of soluble fractions content, sum of albumins and globulins: in soaked grain by 12.8% ($t = 4.37$, $p = 0.02$), in sprouted grain – by 27.2% ($t = 8.18$, $p = 0.001$). There is a significant reduction of insoluble protein residue during germination phases change from 45.5 ($t = 11.82$, $p = 0.001$) up to 56.0% ($t = 15.35$, $p = 0.001$).

Determination of carbohydrate composition change in *Fagopyrum esculentum Moench* grain during germination was made by chromatographic method. The results are shown in Table 2.

It was found that hydrolysis of glycosidic bonds in polysaccharide molecules occurred under the influence of own carbohydrases, and substances with low molecular weight and high solubility were formed. The content of carbohydrates in grain extracts increases in the process of germination. This indicates the biochemical processes occurring in the molecules of starch, due to activation of enzymes.

The content of carbohydrates in grain extracts increases during germination by 27.79% in soaked grains and by 38.97% in sprouted grain. Differences are significant and statistically significant $t = 3.92$, $p = 0.02$ for soaked grain and $t = 10.18$, $p = 0.001$ for sprouted.

Reducing of carbohydrates content in buckwheat grain during germination was observed **(Colmenares De Ruiz and Bressani, 1990)**. Germination of legumes increas digestibility of carbohydrates, this is associated with the degradation of starch into smaller fragments and the formation of reducing sugars **(Kelkar et al., 1996)**.

Alteration results of buckwheat grain carbohydrate composition in germination process are coordinated with the data of electron scanning microscopy. Figure 4 shows microphotographs of cut surface structure of dry buckwheat grain and sprouted for 12 and 24 hours.

In the process of *Fagopyrum esculentum Moench* grain germination, there is a soaking of grain starch and a slight damage to their packaging in endosperm after 12 hours.

In the process of *Fagopyrum esculentum Moench* grain germination, there is a soaking of grain starch and a slight damage to their packaging in endosperm after 12 hours. After 24 hours, endosperm of germinated grain underwent significant changes in microstructure: grain starch and components of protein matrix had a vague outline, grain disintegration was observed.

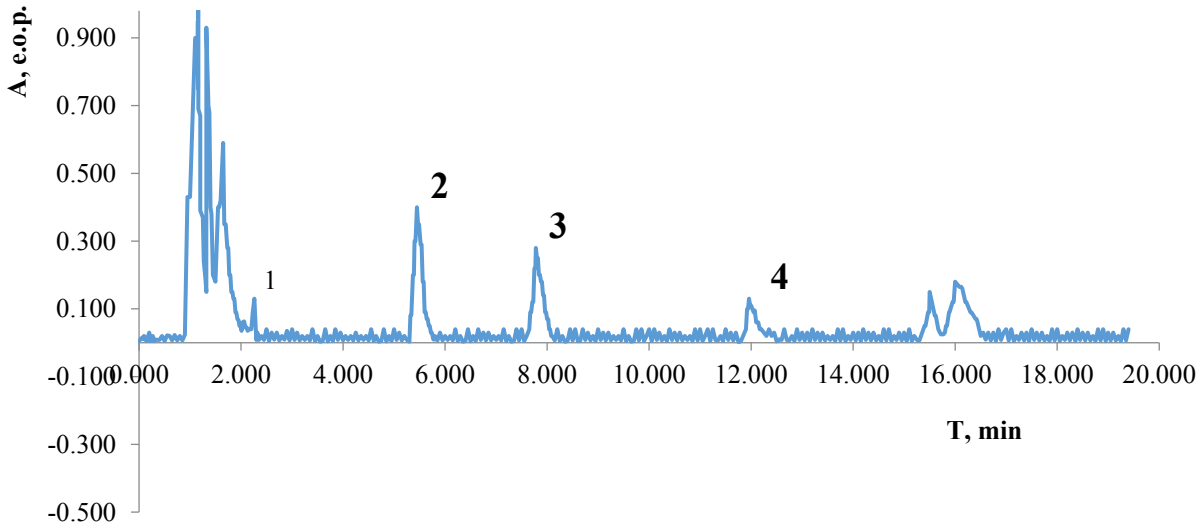


Figure 1 Chromatogram of extract from native buckwheat : 1 – ascorbic acid (C), 2 – thiamine (B_1), 3 – nicotinamide (PP), 4 – pantothenic acid (B_3).

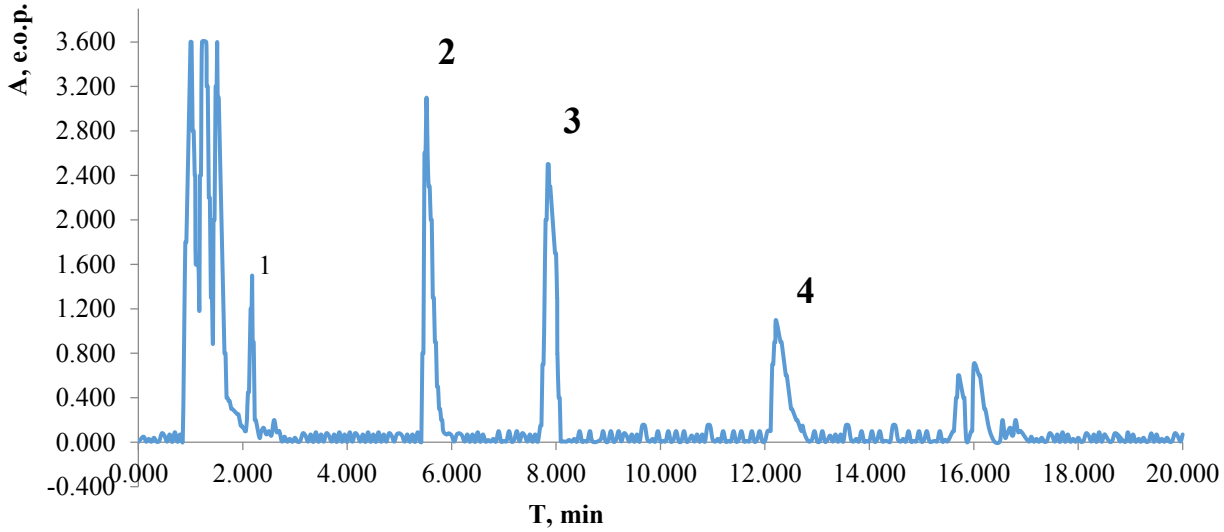


Figure 2 Chromatogram of extract from buckwheat after 24 hours of germination: 1 – ascorbic acid (C), 2 – thiamine (B_1), 3 – nicotinamide (PP), 4 – pantothenic acid (B_3).

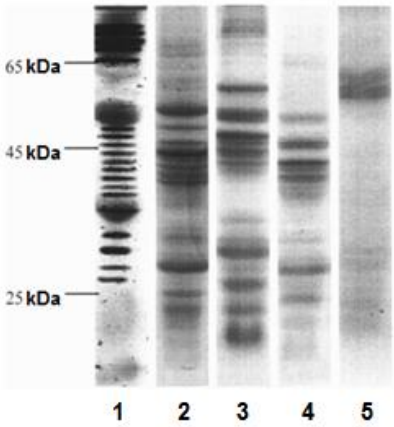


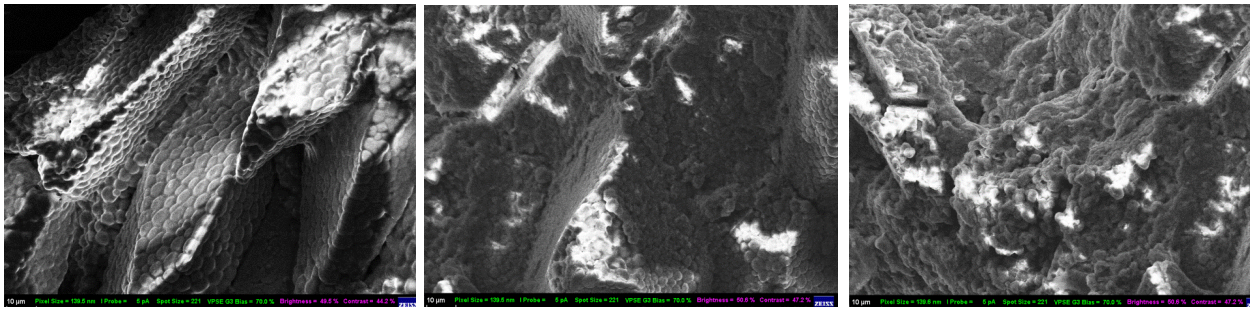
Figure 3 The electrophoregrams of buckwheat seeds protein (1 – standart, 2 – native grain; 3 – germinated (12 h); 4 – sprouting (24 h), 5 - sprouted (36 h).

Table 1 Fractional composition of *Fagopyrum esculentum Moench* grain protein during germination.

Indicators	Physiological state of grain		
	Resting	Swollen after 12h of germination	Sprouted after 24h of germination
Total nitrogen, %	2.42 ±0.42	2.12 ±0.18	2.15 ±0.20
Protein nitrogen, %	2.26 ±0.21	1.90 ±0.15	1.76 ±0.19
Fractions composition (compared to total extracted nitrogen in %):			
Albumins	22.12 ±0.80	24.50 ±0.98	27.83 ±1.10
Globulins	42.34 ±1.95	48.24 ±1.63	54.16 ±2.22
Glutelins	11.25 ±0.41	12.92 ±0.32	10.72 ±0.33
Insoluble residue	23.15 ±0.79	12.73 ±0.39	10.18 ±0.30

Table 2 Alteration of carbohydrate composition of *Fagopyrum esculentum Moench* grain during germination, $g.L^{-1}$.

Type of carbohydrate s	Sprouting time		
	0 h	12 h	24 h
Dextrins of the 2nd order	28.85 ±2.24	39.60 ±2.18	39.71 ±2.09
Dextrins of the 1nd order	5.23 ±0.42	7.62 ±0.51	13.26 ±0.81
Rafinose	2.17 ±0.30	2.98 ±0.38	2.09 ±0.25
Maltose	9.28 ±0.68	18.55 ±0.72	18.45 ±0.69
Glucose	17.85 ±0.72	23.29 ±0.98	27.50 ±0.90
Fructose	13.96 ±0.84	6.79 ±0.65	6.47 ±0.52
Total carbohydrates	77.34 ±2.88	98.83 ±4.66	107.48 ±0.69



1 2 3

Figure 4 Microstructure of cut surface of *Fagopyrum esculentum moench* grain (1 – dry grain, 2 – grain sprouted in 12 hours, 3 – grain, sprouted within 24 hours), x 2000 magnification. Photo: S. Motyleva, 2017.

Table 3 Flavonol content and antioxidant activity of *Fagopyrum esculentum Moench* seeds after germination.

Type of raw material	Total content of flavonols on basis of rutin, %	Antioxidant activity, inhibition % of DPPG radical
Dry buckwheat grains	0.26 ±0.02	29.0 ±0.90
Buckwheat grains after 12 hours of germination	0.55 ±0.04	47.0 ±0.72
Buckwheat grains after 24 hours of germination	0.80 ±0.04	63.0 ±0.94

A lot of works have been published based on definition of antioxidant activity of germinating grain of *Fagopyrum esculentum Moench*, and grain of other plants, **(Lan-Sook Lee et al., 2016; Bolnvar, Cevallos-Casals and Cisneros-Zevallos, 2010)**. These studies demonstrate increase of polyphenols content and antioxidant activity during grain germination. According to **(Jiang et al., 2007)**, the antioxidant activity of *Fagopyrum esculentum Moench* grain correlates with the total content of flavonoids and routine. Other authors did not observe significant correlations between flavonoids and the measured antioxidant effect **(Danihelová and Šturdík, 2013)**.

Determination of antioxidant activity (Table 3) of alcohol extract from buckwheat grain germinated for 24 hours showed that inhibition percentage of DPPG free radicals increases with duration of the process. The total content of flavonols in recalculation on routine increased by 2.11 ($t = 6.48$, $p = 0.01$) times in 12 hours of germination and in 3.07 ($t = 12.07$, $p = 0.001$) times in 24 hours. The obtained values of the total content of flavonols and antioxidant activity of grain *Fagopyrum esculentum Moench* correspond to those established by other authors **(Morishita et al., 2007)**.

Experimental data show that antioxidant activity of buckwheat grain increases with growth of total flavonols content during germination.

CONCLUSION

It is experimentally established that in the early stages of germination process of *Fagopyrum esculentum Moench* grain, its biochemical parameters change. The process of proteolysis during *Fagopyrum esculentum Moench* grain germination promotes an increase of soluble fractions content, the amount of albumins and globulins: in the soaked grain by 12.8%, in the germinated grain by 27.2%, the amount of insoluble protein decrease from 45.5 to 56.0%. During germination, the amount of low-molecular carbohydrates in grain extracts increases by 27.79% in soaked grain and by 38.97% in sprouted grain, and the total content of flavonols in terms of routine amounts increases in 2.11 and 3.07 times, respectively. Differences between measurements of the chemical composition in the germination process are statistically significant. It is shown that in the process of *Fagopyrum esculentum Moench* grain germination content of water-soluble vitamins C, B1, PP, B3 increases, the microstructure of the endosperm surface changes - the elements of packing of starch grains disintegrate and they lose clear outlines. During the germination of *Fagopyrum esculentum Moench* grain, antioxidant activity increases after 24 hours.

Thus, sprouted within 24 hours grain of *Fagopyrum esculentum Moench* is characterized by a high content of biologically active substances and has indicators of high nutritional value. In the sprouted grains of buckwheat, there are no major protein allergens. Such a grain can be used in food technology to create products with a functional purpose with a low content of allergens. Based on the sprouted grain of *Fagopyrum esculentum Moench*, we created a complex food supplement, which additionally includes the green mass of macrophyte *Lemna minor*, succinic acid and yeast *Saccharomyces boulardi*. This additive can be used in the production of bakery and confectionery products, as well as dairy products.

REFERENCES

Ahmed, A., Khalid, N., Ahmed, A., Abbasi, N. A., Latif, M. S. Z., Randhawa, M. A. 2014. Phytochemicals and biofunctional properties of buckwheat: a review. *Journal of Agricultural Science*, vol. 152, no. 3, p. 349-369. https://doi.org/10.1017/S0021859613000166

Butenko, L. I., Ligay, L. V. 2013. Study of the chemical composition of germinated seeds of buckwheat, oats, barley and wheat. *Fundamental research*, no. 4-5, p. 1128-1133.

Čabarkapa, I. S., Sedej, I. J., Sakać, M. B., Šarić, L. C., Plavšić, D. V. 2008. Antimicrobial activity of buckwheat (*Fagopyrum esculentum Moench*) hulls extract. *Food Processing Quality Safety*, vol. 35, no.4, p. 159-163.

Cevallos-Casals, B. A, Cisneros-Zevallos, L. 2010. Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. *Food Chemistry*, vol. 119, no. 4, p. 1485-1490. https://doi.org/10.1016/j.foodchem.2009.09.030

Chao, P. L., Hsiu, S., Hou, Y. 2002. Flavonoids in herbs: biological fates and potential interactions with xenobiotics. *Journal of Food and Drug Analysis*, vol. 10. p. 219-228.

Colmenares de Ruiz, A. S., Bressani, R. 1990. Effect of germination on the chemical composition and nutritive value of amaranth grain. Cereal Chem, vol. 67, no. 6, p. 519-522.

Danihelová, M., Šturdík, E. 2012. Nutritional and health benefits of buckwheat. *Potravinarstvo*, vol. 6, no. 3, p. 1-9. https://doi.org/10.5219/206

Danihelová, M., Šturdík, E. 2013. Antioxidant and antiproteinase effects of buckwheat hull extracts. *Potravinarstvo,* vol. 7, no. 1, p. 89-94. https://doi.org/10.5219/272

Fesenko, A., Kuznetsova, E., Polehina, N., Shipulin,O., Fesenko, N., Selifonova, N. 2015. Comparative analysis of grain quality of buckwheat varieties with different breeding history. *Technology and the study of merchandise of innovative foodstuffs,* vol. 4, no. 33, p. 76-86.

He, J., Klag, M. J., Whelton. P. K., Mo, J. P., Chen, J. Y., Qian, M. C., Mo, P. S., He, G. Q. 1995. Oats and buckwheat intakes and cardiovascular disease risk factors in an ethnic minority of China. *The American Journal of Clinical Nutrition*, vol. 61, no. 2, p. 366-372. https://doi.org/10.1093/ajcn/61.2.366 PMid:7840076

Huff, M. W., Carroll, K. K. 1980. Effects of dietary protein on turnover, oxidation, and absorption of cholesterol, and on steroid excretion in rabbits. *Journal of Lipid Research*, vol. 21, no. 5, p. 546-558. PMid:7400686

Jan, U., Gni, A., Ahmad, M., Shah, U., Baba, W. N., Masoodi, F. A., Magsood, S., Gani, A., Wani, I. A., Wani, S. M. 2015. Characterization of cookies made from wheat flour blended with buckwheat flour and effect on antioxidant properties. *Journal of Food Science and Technology-Mysore*, vol. 52, no. 10, p. 6334-6344. https://doi.org/10.1007/s13197-015-1773-8 PMid:26396378

Jiang, P., Burczynski, F., Campbell, C., Pierce, G., Austria, J. A., Briggs, C. J. 2007. Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum, F. tataricum*, and *F. homotropicum* and their protective effects against lipid peroxidation. *Food Research International*, vol. 40, no. 3, p. 356-364. https://doi.org/10.1016/j.foodres.2006.10.009

Katar, D., Olgun, M., Turan, M. 2016. Analysis of morphological and biochemical characteristics of buckwheat (*Fagopyrum esculentum Moench*) in comparison with cereals. *Cyta-journal of food*, vol. 14, no. 2, p. 176-185. https://doi.org/10.1080/19476337.2015.1076522

Kelkar, K, Shastri, P, Rao, B. Y. 1996. Effect of processing on *in vitro* carbohydrate digestibility of cereals and legumes. *Journal of Food Science and Technology – Mysore*, vol. 33, no. 6, p. 493-497.

Kim, C. D., Lee, W. K., No, K. O., Park, S. K., Lee, M. H., Lim, S. R., Roh, S. S. 2003. Anti-allergic action of buckwheat (*Fagopyrum esculentum Moench*) grain extract. *International Immunopharmacology*, vol. 3, no. 1, p. 129-136. https://doi.org/10.1016/S1567-5769(02)00261-8

Kim, S. L., Kim, S. K., Park, C. H. 2004. Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Research International*, vol. 37, no. 4, p. 319-327. https://doi.org/10.1016/j.foodres.2003.12.008

Koyama, M., Nakamura, C., Nakamura, K. 2013. Changes in phenols contents from buckwheat sprouts during growth stage. *Journal of Food Science Technology*, vol. 50, no. 1, p. 86-93. https://doi.org/10.1007/s13197-011-0316-1

Lee, L. S., Choi, E. J., Kim, C. H., Sung, J. M., Kim, Y. B., Seo, D. H., Choi, H. W., Choi, Y. S., Kum, J. S., Park, J. D. 2016. Contribution of flavonoids to the antioxidant properties of common and tartary buckwheat. *Journal of Cereal Science*, vol. 68, p. 181-186. https://doi.org/10.1016/j.jcs.2015.07.005

Li, S. Q., Zhang, Q. H. 2001. Advances in the development of functional foods from buckwheat. *Critical Reviews in Food Science and Nutrition*, vol. 41, no. 6, p. 451-464. https://doi.org/10.1080/20014091091887 PMid:11592684

Lin, L., Liu, H., Yu, Y., Lin, S., Mau, J. 2009. Quality and antioxidant property of buckwheat enhanced wheat bread. Food Chemistry, vol. 112, no. 4, p. 987-991. https://doi.org/10.1016/j.foodchem.2008.07.022

Morishita, T., Yamaguchi, H., Degi, K., 2007. The contribution of polyphenols to antioxidative activity in common buckwheat and tartary buckwheat grain. *Plant Production Science*, vol. 10, no. 1, p. 99-104. https://doi.org/10.1626/pps.10.99

Morita, N., Maeda, T., Sai, R., Miyake, K., Yoshioka, H., Urisu, A., Adachi, T. 2006. Studies on distribution of protein and allergen in graded flours prepared from whole buckwheat grains. *Food Research International*, vol. 39, no. 7, p. 782-790. https://doi.org/10.1016/j.foodres.2006.02.005

Nonogaki, H., Bassel, G. W., Bewley, J. D. 2010. Germination – still a mystery. *Plant Science*, vol. 179, no. 6, p. 574-581. https://doi.org/10.1016/j.plantsci.2010.02.010

Oomah, B. D., Mazza, G. 1996. Flavonoids and antioxidative activities in buckwheat. *Journal of Agricultural and Food Chemistry*, vol. 44, no. 7, p. 1746-1750. https://doi.org/10.1021/jf9508357

Park, J. W., Kang D. B., Ким, C. W., Ko, S. H., Yum, H. Y., Kim, K. E., Hong, C. C., Lee, K. Y. 2000. Identification and characterization of the major allergens of buckwheat. Allergy. *European Journal of Allergy and Clinical Immunology*, vol. 55, no. 11, p. 1035-1041.

Sakač, M. B., Sedej, I. J., Mandić, A. I., Mišan, A. Č. 2015. Antioksidativna svojstva brašna od heljde – Doprinos funkcionalnosti pekarskih, testeničarskih i brašneno-konditorskih proizvoda. *Hemijska Industrija*, vol. 69, no. 5, p.469-483.

Sedej, I., Sekač, 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. https://doi.org/10.1016/j.lwt.2010.11.010

Sikder, K., Kesh, S. B., Das, N., Manna, K., Dey, S. 2014. The high antioxidative power of quercetin (aglycone flavonoid) and its glycone (rutin) avert high cholesterol diet induced hepatotoxicity and inflammation in Swiss albino mice. *Food and Function*, vol. 5, no. 6, p. 1294-1303. https://doi.org/10.1039/c3fo60526d

Świątecka, D., Markiewicz, L. H., Wróblewska, B. 2013. *In vitro* evaluation of the effect of the buckwheat protein hydrolysate on bacterial adhesion, physiology and cytokine secretion of Caco-2 cells. *Central European Journal of Immunology*, vol. 38, no. 3, p. 317-327. https://doi.org/10.5114/ceji.2013.37753

Terpinc, P., Cigić, B., Polak, T., Hribar, J., Požrl, T. 2016. LC-MS analysis of phenolic compounds and antioxidant activity of buckwheat at different stages of malting. *Food Chemistry*, vol. 210, p. 9-17. https://doi.org/10.1016/j.foodchem.2016.04.030 PMid:27211614

Uppal, V., Bains, K. 2012. Effect of germination periods and hydrothermal treatments on *in vitro* protein and starch digestibility of germinated legumes. *Journal of Food Science Technology*, vol. 49, no. 2, p. 184-191. https://doi.org/10.1007/s13197-011-0273-8 PMid:23572840

Wloch, A., Strugala, P., Pruchnik, H., Żyłka, R., oszmiański, J., Kleszczyńska, H. 2016. Physical effects of buckwheat extract on biological membrane *in vitro* and its protective properties. *Journal of Membrane Biology*, vol. 249, no. 1-2, p. 155-170. https://doi.org/10.1007/s00232-015-9857-y

Wronkowska, M., Zielińska, D., Szawara-Nowak, D., Troszyńska, A., Soral-Śmietana, M. 2010. Antioxidative and reducing capacity, macroelements content and sensorial properties of buckwheat-enhanced gluten-free bread. *International Journal of Food Science and Technology*, vol. 45, p. 1993-2000. https://doi.org/10.1111/j.1365-2621.2010.02375.x

Yıldız, G., Bilgiçli, N. 2012. Effects of whole buckwheat flour on physical, chemical, and sensory properties of flat bread, lavaş. *Czech Journal of Food Science*, vol. 30, no. 6, p. 534-540. https://doi.org/10.17221/10/2012-CJFS

Yiming, Z., Hong, W., Linlin, C., Wen, T., Xinli, S. 2015. Evolution of nutrient ingredients in tartary buckwheat seeds during germination. Food Chemistry, vol. 186, p. 244-248. https://doi.org/10.1016/j.foodchem.2015.03.115 PMid:25976817

Zhang, G., Xu, Z., Gao, Y., Huang, X., Zou, Y., Yang, T. 2015. Effects of germination on the nutritional properties, phenolic profiles, and antioxidant activities of buckwheat. *Journal of Food Science*, vol. 80, no. 5. p. 1111-1119. https://doi.org/10.1111/1750-3841.12830 PMid:25858540

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CHEMICAL COMPOSITION OF CHICKEN MEAT AFTER APPLICATION OF HUMIC ACID AND PROBIOTIC *LACTOBACILLUS FERMENTUM*

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ABSTRACT

The aim of the present study was analysed and evaluated chemical parameters of chicken breast and thigh muscles after addition of humic acids and probiotic into diet for broiler chicken. A total of 200 pcs Ross 308 broiler chickens were divided into 4 groups (n=50). The control group of chickens was fed with complete feed mixtures without any additives. Chickens in experiment groups were fed a diet containing: P1 (1% of humic acid), P2 (1% of humic acid and probiotic supplement *Lactobacillus fermentum*) and P3 were fed with complete feed mixture containing combination of starter feed mixture (1. – 21. day) with coccidiostaticum Diclazuril and growth feed mixture (21. – 35. day) containing Salinomycinum sodium. Besides, the groups were kept under the same conditions. Fattening period lasted for 42 days. Chicken meat was analyzed for content of water, crude protein, fat and cholesterol. Based on the results, we can state that the application of humic acids or the combination of Humac Natur with probiotic did not affect the chemical composition of the breast muscle. In the breast muscle, the protein content in the experimental group P3 with the coccidiostat (22.98 g.100 g^{-1}) was reduced ($p \leq 0.05$) compared to control group (23.42 g.100 g^{-1}). In the case of thigh muscle was significantly higher content of fat and cholesterol ($p \leq 0.05$) in chickens feeding with addition of Humac Natur (fat – 9.08 g.100g^{-1}; cholesterol – 0.86 mg.100g^{-1}) and similar results were recorded in experimental group with combination of Humac Natur and probiotic (fat – 9.15 g.100g^{-1}; cholesterol – 0.86 mg.100g^{-1}) compared to control group (fat – 7.15 g.100g^{-1}; cholesterol – 0.70 mg.100g^{-1}). From a general point of view, we can recommend the application of Humac Natur, respectively combination Humac Natur with probiotics in feeding of broiler chickens Ross 308.

Keywords: chemical composition; chicken meat; humic acids; dietery supplemation

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)**. High product quality and food safety are key targets for the food industry, since they relate to customer satisfaction and ultimately to repeat purchase **(O'Sullivan, 2017)**. In addition, the aim of food researchers and producers is to increase the nutritional value of food without decreasing sensory quality or consumers' acceptability **(Miezeliene et al., 2011)**. Poultry meat represents an important component of human diet. Complete feed mixtures for broiler chickens are often enriched with various additives as vegetable oils, probiotic, prebiotic and enzyme preparations **(Lee et al., 2003, 2004; Shalmany and Shivazad, 2006)**. After the application of antibiotics as feed additives in order to enhance growth in production animals has lately been restricted **(Enberg et al., 2000),** researchers have looked for new feed additives that are not harmful to human health.

The importance of these alternative supplement consists mainly in the replacement of antibiotics and coccidiostats which were banned by the European Union since 2006 for poultry husbandry. Several additives have been tested as growth promoters to avoid the excessive use of antibiotics or at least reduce or substitute their inclusion in feeds, while maintaining an efficient animal production to obtain safe edible products **(Islam et al., 2005; Gomez et al., 2012)**. The use of most antibiotic growth promoters has been banned in many countries, because it is risky due to cross resistance amongst pathogens and residues in tissues. Humic acids, one of the potential substances alternatives to antibiotics in the diet of poultry, are formed from decayed plant matter with the aid of living bacteria in the soil **(Nagaraju et al., 2014)**.

The basic problems of poultry feeding consist not only of sufficient supply of the main feed materials **(Kluth and Rodehutscord, 2006; Gous, 2010),** but also of looking for safe and native food sources **(Corzo et al., 2005; Brenes and Roura, 2010)**. The humic substances are very common in nature as they originate from the decomposition of organic matter, and are normally present in the drinking water and soil **(Islam et al., 2005)** and humic acid shows antibacterial, antiviral, antithyroideal and antimicrobial effects in animal husbandry to improve the economy and ecology of animal production by increasing growth rate, decreasing feed expenditure per gain and diminishing the

risk of disease **(Eren et al., 2000; Kocabagli et al., 2002; Rath et al., 2006)**. Humic acid is known to be nontoxic and nonteratogenic and has been used as analgesic and antimicrobial agent in veterinary practices **(Yasar et al., 2002)** and humic acid based mixtures have the potential to be an alternative to antibiotic growth promoters in broiler diets **(Ceylan et al., 2003)**.

Humic acids are organic compounds naturally present in water and soil. They form three-dimensional structure molecules, containing aromatic nuclei with oxygen and nitrogen heterocycles. In the side chains, bound to an aromatic nucleus, hydroxyl, carbonyl, carboxyl, amine and sulfhydryl functional groups are present **(MacCarthy 2001; Zralý et al., 2008)**.

Ozturk et al. (2010) found that the humic acids had positive effect on growth, meat quality, carcass characteristics, selected parameters determined in the blood and in the gastrointestinal tract. Humic acids are naturally occurring decomposed organic constituents of soil and lignite that are complex mixtures of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains **(MacCarthy, 2001)**. The use of humic acid to replace antibiotics in poultry has gained widespread interest **(Mutus et al., 2006)**. It has been observed that humic acid included in the feed and water of poultry promote growth **(Kocabagli et al., 2002; Mirnawati and Marlida, 2013)**. The humic substances had positive effect on the growth of animals and feed conversion **(Kocabağli et al., 2002; El-Husseiny et al., 2008; Ozturk et al., 2012; Mirnawati and Marlida, 2013)**.

As diet is one of the most important factors affecting meat quality **(Tateo et al., 2013)**, various benefits in regard to meat quality characteristics can be gained by supplementing broiler diets, particularly using probiotics as feed additives **(Karaoglu et al., 2004)**. Among the possible alternatives, probiotics are considered a promising alternative to antibiotics, as well. Probiotic is defined as a live microbial feed supplement that beneficially affects the host animal by improving the intestinal microbial balance **(Alkhalf et al., 2010; Daneshmand et al., 2015)**. Probiotics cover a wide range of living microorganisms with supposed positive effects on gut flora and producing many substances supporting many different effects **(Bernardeau and Vernoux, 2013)**. Probiotics are live, non-pathogenic bacteria that contribute to the health and balance of the intestinal tract **(Giannenas et al., 2012; Bajaj et al., 2015; Uyeno et al., 2015)**. 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)**. 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)**. Various studies have reported a wide variety of health-promoting properties influencing the host intestinal balance **(Shim et al., 2012; Blajman et al., 2015)**, as well as quality of chicken eggs **(Zhang et al., 2012; Angelovičová et al., 2013)** and chicken meat **(Abdel-Latif et al., 2008; Bobko et al., 2015; Haščík et al., 2016; Wang et al., 2017)**.

Scientific hypothesis

This study was designed to investigate the effects of dietary addition of humic acids or combination of humic acids with probiotic preparation based on Lactobacillus fermentum on meat chemical composition of Ross 308 broiler chickens. We expect that addition of these preparations will have a positive affcet on chosen parameters of chemical composition of broiler meat.

MATERIAL AND METHODOLOGY

Animal and diets

The experiment was realized at the Department of Poultry Science and Small Farm Animals in the experimental poultry house on College farm in Kolíňany.

In every experiment a total 200 one-day-old Ross 308 meat hybrid chicken was included. Chickens were randomized into four groups, each containing 50 birds. Chickens in individual groups were stabled on deep budding, with a maximum occupation of the breeding areas 33 $kg.m^{-2}$. During the fattening period, the light regimen based on 23 h of light and 1 h of dark was used.

The temperature at the beginning of the experiment was 31 – 33 °C and decreased to 20 – 22 °C during the experiment. The temperature was maintained using electronic hen-like devices providing radiant heat.

The fattening lasted 42 days. The feeding program included three phases: starter (1^{st} – 21^{st} days of age), grower (22^{nd} – 35^{th} days of age), and finisher (36^{th} – 42^{nd} days of age). Feed and water were supplied *ad libitum*. Composition of complete feed mixtures is presented in Table 1.

In control group we used complete feed mixture without any additives. Group of chickens P1 was fed a diet containing 1% of preparation Humac Natur. The group marked as P2 was fed a diet containing 1% of preparation Humac Natur and probiotic supplement Propoul in water (0.03 g per pc) and group P3 containing combination of starter feed mixture with coccidiostaticum Diclazuril (each kg contains 5 g of Diclazuril) and growth feed mixture containing Salinomycinum sodium (each kg contains 120 g of Salinomycin).

In the experiment, the probiotic preparation "Propoul" based on *Lactobacillus fermentum* (1.10^9 CFU per 1 g of bearing medium) was used.

Humac Natur purchased from Humac s.r.o., Kosice is preparation of humic substances on base of oxihumolit contain min. 62% humic acids in dry matter, of this 48% free munic acids in dry matter, minerals and trace elements, carboxymethylcellulose complex with humic substances. Moisture was maximum 11%.

Slaughter and measurements

At 42 days of age, chickens were weighed and slaughtered at the experimental slaughterhouse of Slovak University of Agriculture in Nitra. The chemical analysis of chicken meat (breast muscle without skin, thigh muscle with skin and subcutaneous fat) for analyse of crude protein, fat, wate and cholesterol content, was performed using an Infratec 1265 Meat Analyzer.

Statisic analysis

A statistical analysis was computed using the ANOVA procedures of SAS software with using of Enterprise Guide.

4.2 application (version 9.3, SAS Institute Inc., USA, 2008). Data were reported as mean ± standard deviation. Statistical significance was calculated using t-test. Differences between the groups were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of experiment with Ross 308 broiler chickens after addition of humic acid and probiotic, which was aimed at analysed and evaluated chemical parameters, are presented as follows: the results of crude protein, fat, water and cholesterol content in breast and thigh muscle are given Table 2.

Humic acids stabilize the intestinal flora and thus ensure an improved utilization of nutrients in animal feed **(Islam et al., 2005)**.

The higher average value of crude protein content measured in fresh breast muscle after addition of supplements was in control group C without addition (23.42 g.100 g^{-1}) and the lowest value was measured in experimental group P2 with addition of humic acid and probiotic (22.49 g.100 g^{-1}). We have found statistically significant differences ($p \leq 0.05$) between control group C and experimental groups P2 and P3.

In the case of fresh thigh muscle was measured the lowest value of crude protein content 18.70 g.100 g^{-1} in experimental group P2 with combination of humic acid and probiotic *Lactobacillus fermentum* and the higher value in experimental group P3 containing combination of starter feed mixture with coccidiostaticum Diclazuril and growth feed mixture containing Salinomycinum sodium (19.93 g.100 g^{-1}). We have found statistically significant differences ($p \leq 0.05$) between experimental group P1 and experimental groups P2 and P3.

Ozturk et al. (2010) evaluated the chemical composition of breast and thigh muscles of broiler chickens Ross 308 after addition of humic substances (0.5; 1.0 and 1.5 %) into fed. Content of protein was the higher in control group 22.94% and the lowest in group with addtiotion 1% of humic substances (22.49%). In the thigh muscles, content of protein was 17.32% in cotrol group and 17.44% in group with 1% of humic substances.

Ozturk et al. (2011) reported that addition of 1% humic substance into fed for broiler chickens Ross 308 decreased the protein content of thigh meat in relation to control group and 1.5% humic substance, and the protein content of breast meat compared to control group ($p < 0.10$).

The effect of adding humic acids on the quality of the meat of broiler chickens Cobb 500 was monitored in their work **Reitznerová et al. (2016)**. They found out that content of protein in breast muscle was 23.36% compared to control group (23.52%) and in thigh muscle was 19.76% compared to 20.16% in control group.

Ondruška et al. (2012) evaluated the addition of combination Humac Natur (0.3%) and probiotic *L. fermentum* on rabbit meat quality. Content of total protein was in control group 22.07 g.100 g^{-1} compared to tested group 21.70 g.100 g^{-1}.

In evaluating of fat content in fresh breast muscle was found the lower value 0.84 g.100 g^{-1} in experimental group P1 with addition of humic acid and the higher average value 1.96 g.100 g^{-1} in group with addition of Humac Natur and probiotic (experimental group P2). In thigh muscle was found the higher value of fat content in experimental group P2 (9.15 g.100 g^{-1}) and the lower average value 7.15 g.100 g^{-1} in experimental group P1. We have found statistically significant differences ($p \leq 0.05$) in breast

Table 1 Composition of feed mixtures.

Ingredients (%)	Starter (HYD-01) (1st – 21st day of age)	Grower (HYD-02) (22nd – 35th day of age)	Finisher (HYD-03) (36th – 42nd day of age)
Wheat	35.00	35.00	36.82
Maize	35.00	40.00	37.00
Soybean meal (48% N)	21.30	18.70	20.00
Fish meal (71% N)	3.80	2.00	-
Dried blood	1.25	1.25	-
Ground limestone	1.00	1.05	1.10
Monocalcium phosphate	1.00	0.70	1.00
Fodder salt	0.10	0.15	0.20
Sodium bicarbonate	0.15	0.20	0.25
Lysine	0.05	0.07	0.29
Methionine	0.15	0.22	0.29
Palm kernel oil Bergafat	0.70	0.16	2.50
Premix Euromix BR 0.5%*	0.50	0.50	0.50
Nutrient composition (g.kg^{-1})			
Linoleic acid	13.51	14.19	149.1
Fibre	30.18	29.93	30.54
Crude protein	210.76	190.42	170.58
MEN (MJ.kg^{-1})	12.01	12.03	12.37
Ash	24.24	19.93	38.49
Ca	8.15	7.27	7.37
P	6.75	5.70	6.00
Na	1.70	1.77	1.73

Note: *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.

Table 2 Chemical composition of breast and thigh muscle meat (g.100 g^{-1}).

Parameter	Breast muscle			
	C	P1	P2	P3
Protein	23.42 ±0.21^{b}	23.24 ±0.48^{ab}	22.49 ±0.78^{a}	22.98 ±0.19^{a}
Fat	1.09 ±0.79^{ab}	0.84 ±0.29^{a}	1.96 ±1.06^{b}	1.17 ±0.33^{ab}
Water	74.32 ±0.75	76.13 ±2.51	74.34 ±0.57	74.09 ±0.24
Cholesterol	0.37 ±0.08	0.33 ±0.01	0.39 ±0.06	0.34 ±0.01
	Thigh muscle			
	C	**P1**	**P2**	**P3**
Protein	19.85 ±1.28^{ab}	19.44 ±0.28^{b}	18.70 ±0.29^{a}	19.93 ±0.34^{a}
Fat	7.15 ±1.66^{a}	9.08 ±1.19^{b}	9.15 ±0.14^{b}	7.67 ±2.05^{ab}
Water	71.73 ±1.47^{ab}	70.42 ±0.51^{a}	73.41 ±1.63^{b}	73.34 ±2.60^{b}
Cholesterol	0.70 ±0.05^{a}	0.86 ±0.05^{b}	0.86 ±0.05^{b}	0.78 ±0.15^{ab}

Note: C – control group; P1, P2, P3 – experimental groups; a, b – means with different superscripts within a line differ significantly ($p \leq 0.05$).

muscle between experimental groups P1 and P4 and in thigh muscle between control group C and experimental group P1 and P2.

The lowest average value of fat content measured in fresh breast muscle was in experimental group P1 with addition 0.6 % humic acid (0.84 g.100 g^{-1}) and the higher value was measured in experimental group P2 with addition of humic acid and probiotic (1.96 g.100 g^{-1}). We have found statistically significant differences ($p \leq 0.05$) between experimental groups P1 and P4.

In the case of fresh thigh muscle was measured the higher value of fat content 9.15 g.100 g^{-1} in experimental group P2 with addtion of humic acid and probiotic *Lactobacillus fermentum* and the lowes taverage value in control group C witout addition (7.15 g.100 g^{-1}). We have found statistically significant differences ($p \leq 0.05$) between control group C and experimental group P1 and P2.

The content of fat 2.75 g.100 g^{-1} in breast muscle and 11.98 g.100 g^{-1} in thigh muscle after addition 0.6% humic acids into feeding mixture for broiler chickens Cobb 500 describe in their work **Reitznerová et al. (2016)**.

Ozturk et al. (2010) found content of fat in breast and thigh muscle of broiler chickens Ross 308 after addition 1% humic substances 2.81 g.100 g^{-1} and 11.45 g.100 g^{-1}, respectively. In another work **Ozturk et al. (2011)** reported content of fat in breast and thigh muscle meat of broiler chickens Ross 308 2.67 g.100 g^{-1} in breast muscle and 11.43 g.100 g^{-1} in thigh muscle.

In the case of rabbit meat, **Ondruška et al. (2012**) reported content of fat 1.4 g.100 g^{-1} compared to control group 1.43 g.100 g^{-1} after addition Humac Natur into the feed in amount 0.3% and probiotic.

The water content of fresh breast muscle meat was in control group 74.32 g.100 g^{-1}, while the higher average valuae was measured in experimental group P1 with addition humic acids (76.13 g.100 g^{-1}) and the lowest value in experimental group with addition of coccidiostaticums (P3) 74.09 g.100 g^{-1}. We have not found statistically significant differences ($p \leq 0.05$) between tested groups.

In the thigh muscle was observed the lower value 70.42 g.100 g^{-1} in experimental group P1 and the higher value in experimental group P2 73.41 g.100 g^{-1}. We have found statistically significant differences ($p \leq 0.05$) between experimental group P1 and experimental group P2 and P3.

Ozturk et al. (2010) in their work stated, the content of dry matter in breast muecle of broiler chicken Ross 308 after addition 1% humic substances into fed 26.62 g.100 g^{-1} and in thigh muscle 30.52 g.100 g^{-1} compared to control group 26.86 g.100 g^{-1} and 29.40 g.100 g^{-1}, respectively.

The dry mater content after addition 0.6% humic acids into feed mixture for broiler chickens Cobb 500 was in the work **Reitznerová et al. (2016)** 28.12 g.100 g^{-1} in breast muscle and in the case thigh muscle 33.07 g.100 g^{-1}.

Ozturk et al. (2011) measured content of dry matter in breast muscle of broiler chicken Ross 308 26.55 g.100 g^{-1} and in thigh muscle 30.07 g.100 g^{-1} after addition of 1% humic substances.

In the experiment of **Ondruška et al. (2012)** with addition Humac Natur and probiotic into feed mixture for rabbit reported the total content of water 75.87 g.100 g^{-1} in experimental group and 75.53 g.100 g^{-1} in control group.

The cholesterol content in chicken breasts ranged from 0.33 g.100 g^{-1} (experimental group P1 with addition humic acids) to 0.39 g.100 g^{-1} (experimental group P2 with addition humic acids and probiotic) and in thigh muscle from 0.70 g.100 g^{-1} in control group C to 0.86 g.100 g^{-1} in experimental groups P1 and P2.

We have not found statistically significant differences ($p \leq 0.05$) in breast muscle between tested groups, but in the case of thigh muscle we have found statistically significant differences ($p \leq 0.05$) between control group and experimental group P1 and P2.

Haščík et al. (2016) found cholesterol content in breast muscle of broilers Ross 308 from 86.42 mg.100 g^{-1} in experimental group with propolis addition to 92.17 mg.100 g^{-1} in experimental group with probiotic and in thigh muscle was measured 113.08 mg.100 g^{-1} (probiotic), 118.68 mg.100 g^{-1} (propolis) and in control group 121.25 mg.100 g^{-1}.

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 muscle (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}). 63.14 mg.100 g^{-1} (thigh muscle pomegranate) compared with the control (65.78 mg.100 g^{-1}).

CONCLUSION

The results of our study have shown that the application of humic acids or combination of humic acids with probiotics did not effect the chemical composition of breast muscle. In the breast muscle was reducted ($p \leq 0.05$) content of protein in experimental group P3 with coccidiostaticums compared to control group. In thigh muscle was statisticaly higher ($p \leq 0.05$) content of fat and cholesterol in experimental group of chickens fed with Humac Natur or combination Humac Natur and probiotic compared to control group, as confirmed by the results of other authors, who also found a slight increase in fat content and therefore cholesterol in thigh muscle of broiler chicken after using various feed supplements. From a general point of view, we can recommend the application of Humac Natur, or its combination with probiotic into diet of broiler chickens Ross 308.

REFERENCES

Abdel-Latif, K. O., El-Husseiny, O. M., Abdallah, A. G. 2008. The Influence of Biological Feed Additives on Broiler Performance. *International Journal of Poultry Science*, vol. 7, no. 9, p. 862-871. https://doi.org/10.3923/ijps.2008.862.871

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. https://doi.org/10.1016/j.foodchem.2015.04.140

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. https://doi.org/10.1016/j.sjbs.2010.04.005

Angelovičová, M., Alfaig, E., Král, M., Tkáčová, J. 2013. The effect of the probiotics *Bacillus Subtilis* (PB6) on the selected indicators of the table eggs quality, fat and cholesterol. *Potravinarstvo*, vol. 7, no. 1, p. 80-84. https://doi.org/10.5219/271

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. https://doi.org/10.3382/ps.2012-02813

Bajaj, B., Claes, I., Lebeer, S. 2015. Functional mechanisms of probiotics. *Journal Of Microbiology, Biotechnology and Food Sciences*, vol. 4, no. 4, p. 321-327. https://doi.org/10.15414/jmbfs.2015.4.4.321-327

Bernardeau. M., Vernoux, J. 2013. Overview of differences between microbial feed additives and probiotics for food regarding regulation, growth promotion effects and health properties and consequences for extrapolation of farm animal results to humans. *Clinical Microbiology and Infection*, vol. 19, no. 4, p. 321-330. https://doi.org/10.1111/1469-0691.12130

Blajman, J., Gaziano, C., Zbrun, M. V., Soto, L., Astesana, D., Berisvil, A., Scharpen, Signorini, M., Frizzo, L. 2015. *In vitro* and *in vivo* screening of native lactic acid bacteria toward their selection as a probiotic in broiler chickens. *Research in Veterinary Science*, vol. 101, p. 50-56. https://doi.org/10.1016/j.rvsc.2015.05.017

Bobko, M., Haščík, P., Bobková, A., Pavelková, A., Tkáčová, J., Trembecká, L. 2015. 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. https://doi.org/10.5219/495

Brenes E., Roura A. 2010. Essential oils in poultry nutrition: Main effects and modes of action. *Animal Feed Science and Technology*. 2010. vol. 158, no. 1-2, p. 1-14. https://doi.org/10.1016/j.anifeedsci.2010.03.007

Ceylan, N., Ciftci, I., Ilhan, Z. 2003. Effects of Some Alternative Feed Additives for Antibiotic Growth Promoters on Performance and Gut Microflora of Broilers. *Turkish Journal of Veterinary and Animal Sciences*, vol. 27, no. 3, p. 727-733.

Corzo, A., Fritts, C. A., Kidd, M. T., Kerr, B. J. 2005. Response of broiler chicks to essential and nonessential amino acid supplementation of low crude protein diets. *Animal Feed Science and Technology*, vol. 118, no. 3-4, p. 319-327. https://doi.org/10.1016/j.anifeedsci.2004.11.007

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. https://doi.org/10.1016/j.livsci.2015.04.012

El-Husseiny, O. M., Abdallah, A. G., Abdel-Latif, K. O. 2008. The influence of biological feed additives on broiler performance. *International Journal of Poultry Science*, vol. 7, no. 9, p. 862-871. https://doi.org/10.3923/ijps.2008.862.871

Enberg, 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. https://doi.org/10.1093/ps/79.9.1311

Eren, M., Deniz, G., Gezen, S. S., Türkmen, I. I. 2000. Broyler yemlerine kat lan humatlar n besiperformans, serum mineral konsantrasyonu ve kemik külü üzerine etkileri. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, vol. 47, no. 3, p. 255-263. https://doi.org/10.1501/Vetfak_0000000463

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. https://doi.org/10.1016/j.vetpar.2012.02.017

Gomez, S., Angeles, M. L., Mojica, M. C., Jalukar, S. 2012. Combination of an enzymatically hydrolyzed yeast and yeast culture with a direct-fed microbial in the feeds of broiler chickens. *Asian-Australasian Journal of Animal Sciences*, vol. 25, no. 5, p. 665-673. https://doi.org/10.5713/ajas.2011.11316

Gous, R. M. 2010. Nutritional limitations on growth and development in poultry. *Livestock Science,* vol. 130, no. 1-3, p. 25-32. https://doi.org/10.1016/j.livsci.2010.02.007

Haščík, P., Trembecká, 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, 2016, no. 1, p. 223-231. https://doi.org/10.5219/581

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. https://doi.org/10.5219/429

Islam, K. M. S., Schuhmacher, A., Gropp, J. M. 2005. Humic acid substances in animal agriculture. *Pakistan Journal of Nutrition*, vol. 4, no. 3, p. 126-134. https://doi.org/10.3923/pjn.2005.126.134

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. 4, p. 828-836. https://doi.org/10.1016/j.meatsci.2013.04.044

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. https://doi.org/10.3923/ijps.2004.361.364

Karaoglu, M., Aksu, M. I., Esenbuga, N., Kaya, M., Macit, M., Durdag, H. 2004. Effect of dietary probiotic on the pH and colour characteristics of carcasses, breast fillets and drumsticks of broilers. *Animal Science*, vol. 78, no. 2, p. 253-259. https://doi.org/10.1080/10942910500267364

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. https://doi.org/10.5713/ajas.2005.1153

Kluth H., Rodehutscord M. 2006. The role of methodical aspects in the study of aminoacid subacute digestibility with poultry (Metodinių aspektų reikšmė amino rūgščių precekalinio virškinamumo tyrimuose su naminiais paukščiais). *Veterinarija ir zootechnika*, vol. 35, no. 57, p. 36-42.

Kocabağli, N., Alp, M., Acar, N, Kahraman, R. 2002. The effects of dietary humate supplemantation on broiler growth and carcass. *Poultry Science*, vol. 81, no. 2, p. 227-230. https://doi.org/10.1093/ps/81.2.227

Lee, K. W., Everts, H., Beynen, A. C. 2004. Essential oils in broiler nutrition. *International Journal Poultry Science,* vol. 3, no. 12, p. 738-752. https://doi.org/10.3923/ijps.2004.738.752

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. https://doi.org/10.1080/0007166031000085508

MacCarthy, P. 2001. The principles of humic substances. *Soil Science*, vol. 166, no. 11, p. 38-751. https://doi.org/10.1097/00010694-200111000-00003

Miezeliene, A., Alencikiene, G., Gruzauskas, R., Barstys, T. 2011. The effect of dietary selenium supplementation on meat quality of broiler chickens. *Biotechnology, Agronomy, Society and Environment*, vol. 15, no. S1, p. 61-69.

Mirnawati, Y. R., Marlida, Y. 2013. Effects of humic acid addition via drinking water on the performance of broilers fed diets containing fermented and non-fermented palm kernel cake. *Archiva Zootechnica*, vol. 16, no. 1, p. 41-53.

Mutus, R., Kocabagli, N., Alp, M., Acar, N., Eren, M., Gezen, S. S. 2006. The effect of dietary probiotic supplementation on tibial bone characteristics and strength in broilers. *Poultry Science*, vol. 85, no. 9, p. 1621-1625. https://doi.org/10.1093/ps/85.9.1621

Nagaraju, R., Reddy, B. S. V., Gloridoss, R., Suresh, B. N., Ramesh, C. 2014. Effect of dietary supplementation of humic acids on performance of broilers. *The Indian Journal of Animal Sciences*, vol. 84, no. 4, p. 447-452.

Ondruška, Ľ., Chrastinová, Ľ., Rafay, J., Pospíšilová, D., Parkányi, V. 2012. Effect of humic substances and probiotics on growth performance and meat quality of rabbits. *Potravinarstvo*, vol. 6, no. 2, p. 39-41. https://doi.org/10.5219/192

O'Sullivan, M. G. 2017. *A Handbook for Sensory and Consumer-Driven New Product Development.* Cambridge : Woodhead Publishing. 370 p. ISBN 978-0-08-100352-7.

Ozturk, E., Ocak, N., Coskun, I., Turhan, S., Erener, G. 2010. Effects of humic substances supplementation provided through drinking water on performance, carcass traits and meat quality of broilers. *Journal of Animal Physiology and Animal Nutrition*, vol. 94, no. 1, p. 78-85. https://doi.org/10.1111/j.1439-0396.2008.00886.x

Ozturk, E., Ocak, N., Turan, A., Erener, G., Altop, A., Cankaya, S. 2011. Performance, carcass, gastrointestinal tract and meat quality traits, and selected blood parameters of broilers fed diets supplemented with humic substances. *Journal of the Science of Food and Agriculture*, vol. 92, no. 1, p. 59-65. https://doi.org/10.1002/jsfa.4541

Rath, N. C., Huff, W. E., Huff, G. R. 2006. Effects of humic acid on broiler chickens. *Poultry Science*, 2006, vol. 85, no. 3, p. 410-414. https://doi.org/10.1093/ps/85.3.410

Shalmany, K. S., 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. https://doi.org/10.3923/ijps.2006.84.88

Reitznerová, A., Nagy, J., Marcinčák, S., Mačanga, J., Vaško, L., Vašková, J., Poláková, Z. 2016. The effect of humic acids on oxidative stability of frozen chicken. *Bezpečnosť a kontrola potravín : Zborník prác z XIII. Vedeckej konferencie s medzinárodnou účasťou*, Nitra : SPU, p. 182-185. ISBN 978-80-552-1481-8. (In Slovak)

Shim, Y. H., Ingale, S. L., Kim, J. S., Kim, K. H., Seo, D. K., Lee, S. C., Chae, B. J., Kwon, I. K. 2012. A multi-microbe probiotic formulation processed at low and high drying temperatures: effects on growth performance, nutrient retention and caecal microbiology of broilers. *British Poultry Science*, vol. 53, no. 4, p. 482-490. https://doi.org/10.1080/00071668.2012.690508

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. https://doi.org/10.4236/as.2012.31002

Tateo, A., De Palo, P., Maggiolino, A., Centoducati, P. 2013. Post-thawing colour changes in meat of foals as affected by feeding level and post-thawing time. *Archives Animal Breeding*, vol. 56, no. 29, p. 293-302. https://doi.org/10.7482/0003-9438-56-029

Uyeno, Y., Shigemori, S., & Shimosato, T. 2015. Effect of Probiotics/Prebiotics on Cattle Health and Productivity. *Microbes and Environments*, vol. 30, no. 2, p. 126-132. https://doi.org/10.1264/jsme2.ME14176

Wang, Y., Sun, J., Zhong, H., Li, N., Xu, H., Zhu, Q., Liu, Y. 2017. Effect of probiotics on the meat flavour and gut microbiota of chicken. *Scientific Reports*. vol. 7, no. 1, p. 6400. https://doi.org/10.1038/s41598-017-06677-z

Yasar, S., Gokcimen, A., Altuntas, I., Yonden, Z., Petekkaya, E. 2002. Performance and ileal histomorphology of rats treated with humic acid preparations. *Journal of Animal Physiology and Animal Nutrition*, vol. 86, no. 7-8, p. 257-264. https://doi.org/10.1046/j.1439-0396.2002.00383.x

Zhang, J. L., Xie, Q. M., Ji, J., Yang, W. H., Wu, Y. B., Li, C., Ma, J. Y., Bi, Y. Z. 2012. Different combinations of probiotics improve the production performance, egg quality, and immune response of layer hens. *Poultry Science*, vol. 91, no. 11, p. 2755-2760. https://doi.org/10.3382/ps.2012-02339

Zralý, Z., Písaříková, B. 2010. Effect of sodium humate on the content of trace elements in organs of weaned piglets. Acta *Veterinaria Brno*, vol. 79, no. 1, p. 73-79. https://doi.org/10.2754/avb201079010073

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CHARACTERIZATION OF TUNISIAN CASTOR BEAN GENOTYPES USING SDS-PAGE OF TOTAL SEED STORAGE PROTEINS

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ABSTRACT

The objectives of this work, were to find out the level of genetic variability present in 56 Tunisian castor germplasm by using the electrophoretic profiles of total seed proteins with different molecular weights through SDS-PAGE. Fifty-six castor (*Ricinus communis* L.) genotypes were used in the present study. Seeds of castor were obtained from the University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGREF), Regional Station of Gabès, Tunisia. Storage proteins were extracted from individual grains by the standard reference electrophoretic method by ISTA in the presence of sodium dodecyl sulfate (SDS-PAGE). Out of twentynine polypeptide bands, 5 (18%) were commonly present in all accessions and considered as monomorphic, while 24 (82%) showed variations and considered as polymorphic. The size of the protein bands obtained through SDS-PAGE ranged from 30 to 180 kDa. 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. The dendrogram tree demonstrated the relationship among the 56 Tunisian castor genotypes according to the similarity index, using UPGMA cluster analysis. The dendrogram was divided into three main clusters. 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 castor.

Keywords: castor; Tunisian genotypes; dendrogram; protein markers; SDS-PAGE

INTRODUCTION

Castor bean (*Ricinus communis* L.), also known as castor oil bean, mole bean and wonder tree, is a member of Spurge family (*Euphorbiaceae*) which is originated from tropical Africa and is currently cultivated as an oilseed crop and also grown as an ornamental plant in many countries of Asia, Central and North America, Africa and Europe **(Doan, 2004; Aslani et al., 2007)**.

Castor bean has recently been highly rated as a source of raw material (oil) for biodiesel production, because beyond its high oil content (25 – 55%), it is a culture of great social appeal in Brazil by intensive use of workmanship in the field and allows for intercropping with other crops as beans, groundnuts or maize **(Madail et al., 2007; Lacerda et al., 2014)**. The castor bean contains 40% oil, 1 – 5% ricin and 0.3 – 0.8% ricinin **(Johnson et al., 2005)**.

In mature castor seed, 90 – 95% of the total seed protein is in the endosperm. Castor seeds contain two toxins called ricin and *Ricinus communis* agglutinin **(Hartley and Lord, 2004)**. Ricin is a ribosome inactivating protein that is manufactured in the endosperm. It is a small dipeptide molecule (approx. 65 kDa) containing both an A chain (~32 kDa) and a B chain (~32 – 34 kDa) linked together by a disulphide bond **(Kumar et al., 2004)**. The A chain of ricin is a ribosome-inactivating protein **(Lord et al., 1994; Cheema et al., 2010)**.

The most common method used to determine the molecular size of a protein is SDS-PAGE analysis **(Cheema et al., 2010; Malook et al., 2016)**. In this analysis, proteins are denatured using SDS and then, moved across a polyacrylamide matrix on an electric field, known as electrophoresis. 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); Vivodík et al., (2018)**.

Scientific hypothesis

The objectives were to find out the level of genetic variability present in 56 Tunisian castor germplasm by using the electrophoretic profiles of total seed proteins with different molecular weights through SDS-PAGE.

MATERIAL AND METHODOLOGY

Fifty-six castor (*Ricinus communis* L.) genotypes were used in the present study. Seeds of castor were obtained from the University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGREF), Regional Station of Gabès, Tunisia. The ricin genotypes were obtained from 12 regions of Tunisia: S- Souassi (5 genotypes), BT- Bouthay (4 genotypes), GH- Ghomrassen (5 genotypes), BA- Sidi bou ali (5 genotypes), MT- Matmata (4 genotypes), AG- Mateur (5 genotypes),
N- Nefza (4 genotypes), MD- Mednine (5 genotypes), M- Mornag (5 genotypes), G- Gabes (4 genotypes), K- Kebili (5 genotypes), KJ- Ksar jedid (5 genotypes).

Storage proteins were extracted from individual grains by the standard reference electrophoretic method by ISTA in the presence of sodium dodecyl sulfate (SDS-PAGE) **(Wrigley, 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.

Statisic analysis

A dendrogram was constructed based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA).

RESULTS AND DISCUSSION

The number of total scorable protein bands was twentynine 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 56 accessions of castor were screened. Out of twentynine polypeptide bands, 5 (18%) were commonly present in all accessions and considered as monomorphic, while 24 (82%) showed variations and considered as polymorphic. The size of the protein bands obtained through SDS-PAGE ranged from 30 to 180 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 castor showed more diversity in seed storage proteins in minor bands in comparison to major bands. In zone A out of 13 protein bands, 3 were monomorphic and 10 were polymorphic. In zone B out of 10 protein bands, 2 was monomorphic and 8 was polymorphic and in zone C out of 6 protein bands, 1 were monomorphic whereas 5 polymorphic. By considering these facts zone A and B were more polymorphic.

The dendrogram tree (Figure 2) demonstrated the relationship among the 56 Tunisian castor genotypes according to the similarity index, using UPGMA cluster analysis. The dendrogram was divided into three main clusters. The first one contained eight genotypes from castor, while the second cluster contained the two genotypes of Tunisian castor (MD 1 and KJ 2). Cluster 3 was divided into 3 subclusters – 3A, 3B and 3C. Subcluster 3A contained three genotypes (BA 5, KJ 5 and G 1), subcluster 3B contained eight genotypes of Tunisian castor and subcluster 3C contained 35 Tunisian castor genotypes (Figure 2).

Similar results were detected by other authors **(Cheema et al., 2010; Arslan et al., 2012; Lacerda et al., 2014; Riaz and Farrukh, 2014; Schieltz et al., 2015; Brandon et al., 2016; Malook et al., 2016; Rao et al., 2017)** and these results presented a high level of polymorphism of Tunisian castor genotypes detected by SDS-PAGE.

The objective of **Lacerda et al. (2014)** was to study the parameters of an extraction process of protein from castor bean cake by solubilisation in alkaline medium. Initially, the castor bean cake was ground, sieved and submitted to chemical analyses in order to determine its composition. The present work of **Cheema et al. (2010)** was conducted to see the feasibility of electrophoresis for intra-specific characterization of castor bean on the basis of their total seed storage proteins. To facilitate the analysis of castor (*Ricinus communis* L.) seed fractions and germplasm for ricin content, **Brandon et al. (2016)** investigated the use of enzyme-linked immunosorbent assay (ELISA) methods to differentiate between ricin toxin and the related *Ricinus communis* agglutinin (RCA). **Schieltz et al. (2015)** used liquid chromatography and MRM-MS to determine rRNA Nglycosidase activity for each cultivar and the overall activity in these cultivars was compared to a purified ricin standard. **Riaz and Farrukh (2014)** analyzed sixteen different medicinal castor oil samples for contamination with ricin toxin. The classical and gel filtration methods for extraction of purified ricin were adopted. In the present study **Malook et al. (2016)** observed the biochemical and molecular characterization of castor bean (*Ricinus communis* L.) collected from different climatic zones of Pakistan (Lahore, Peshawar, Rawalpindi, Dera Ismail Khan, Swat and Kohat). The protein banding pattern of all 6 accessions was found same and no specific variation was noticed among the proteins of high molecular weight. **Arslan et al. (2012)** study the genetic diversity of wild castor bean genotypes collected from the eastern Mediterranean region of Turkey was evaluated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of seed storage proteins. Five distinct groups were identified from the cluster analysis of the castor bean genotypes studied at 0.80 coefficient level. **Rao et al. (2017)** analyzed six varieties, nine hybrids of castor and their parents1 on the basis of electrophoresis of total soluble seed proteins.

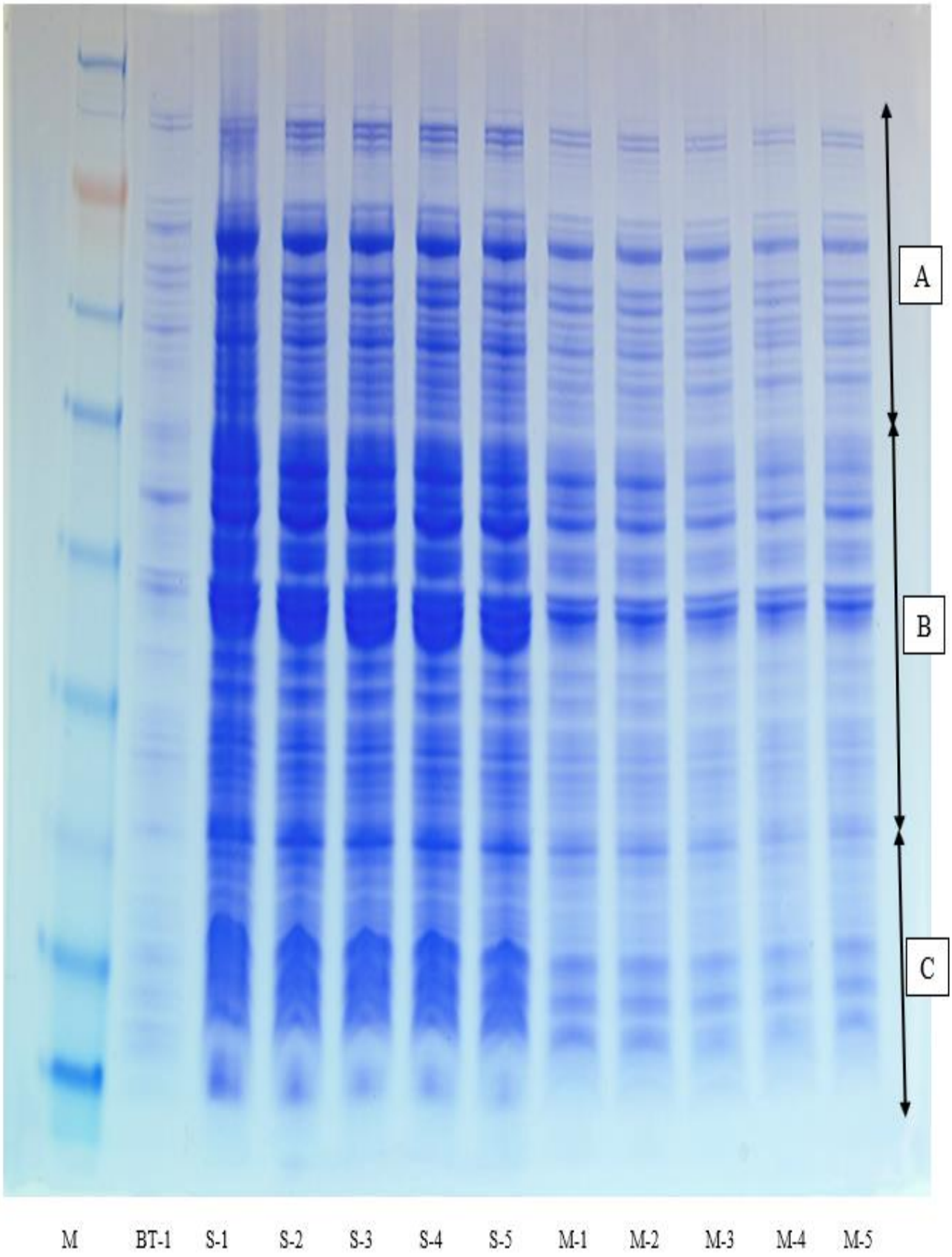

Figure 1 Protein profile showing total seed storage proteins in Tunisian castor genotypes as a result of SDS-PAGE.

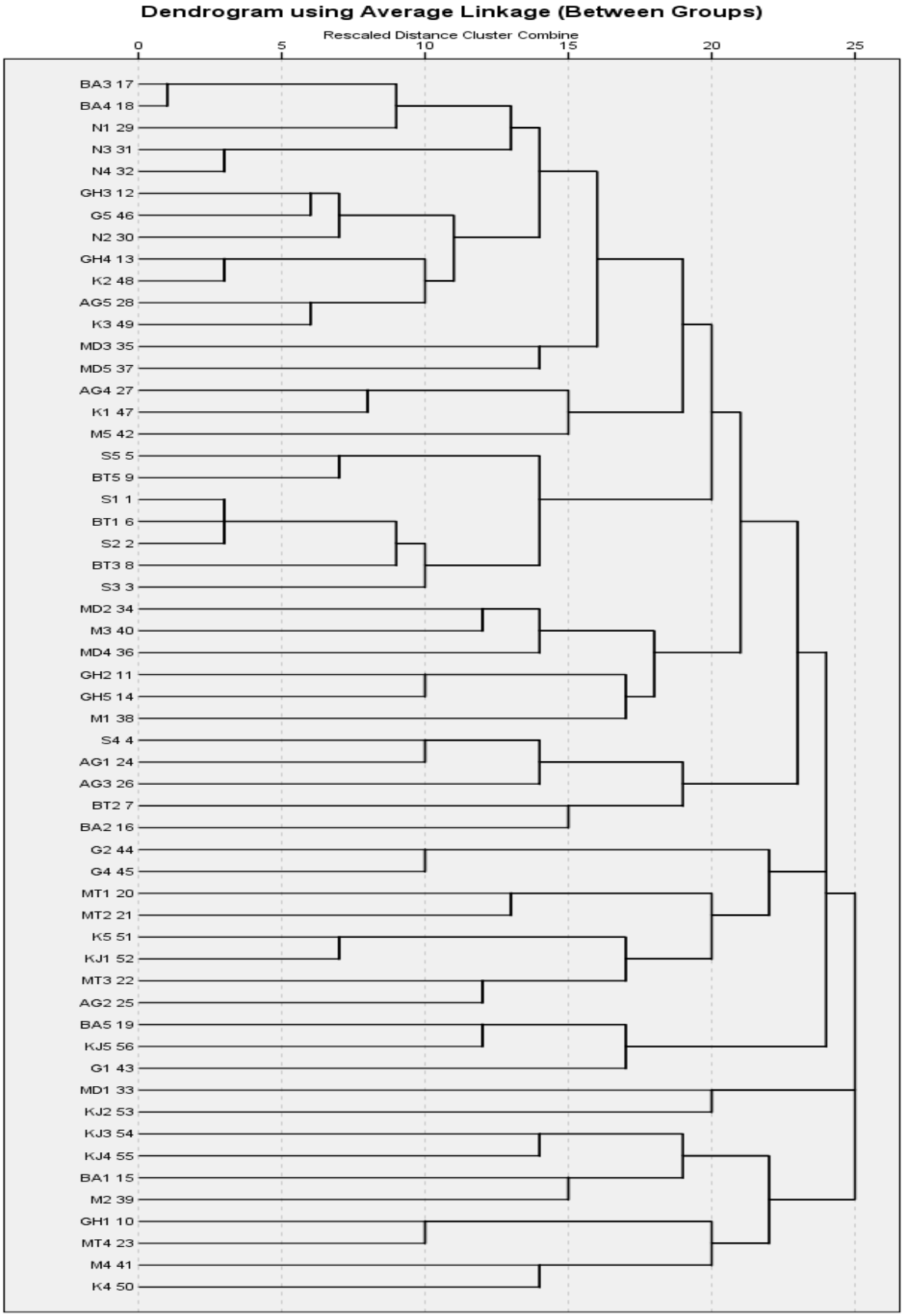

Figure 2 Dendrogram of 56 Tunisian castor genotypes prepared based on protein marker. Note: S- Souassi (5 genotypes), BT- Bouthay (4 genotypes), GH- Ghomrassen (5 genotypes), BA- Sidi bou ali (5 genotypes), MT- Matmata (4 genotypes), AG- Mateur (5 genotypes), N- Nefza (4 genotypes), MD- Mednine (5 genotypes), M- Mornag (5 genotypes), G- Gabes (4 genotypes), K- Kebili (5 genotypes), KJ- Ksar jedid (5 genotypes).

CONCLUSION

Seeds of castor were obtained from the University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGREF), Regional Station of Gabès, Tunisia. The dendrogram was divided into three main clusters. The first one contained eight genotypes from castor, while the second cluster contained the two genotypes of Tunisian castor (MD 1 and KJ 2). Cluster 3 was divided into 3 subclusters – 3A, 3B and 3C. Result from this study show that protein markers are powerful and efficient in characterising and identifying of castor 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. https://doi.org/10.1155/2015/874906

Arslan, M. 2012. Evaluation of genetic similarity and agronomic traits of Castor bean populations naturally grown in the Eastern Mediterranean region of Turkey. *Biotechnology & Biotechnological Equipment*, vol. 26, no. 4, p. 3089-3093. https://doi.org/10.5504/BBEQ.2012.0034

Aslani, M. R., Maleki, M., Mohri, M., Sharifi, K., Najjar-Nezhad, V., Afshari, E. 2007. Castor bean (*Ricinus communis*) toxicosis in a sheep flock. *Toxicon,* vol. 49, no. 3, p. 400-406. https://doi.org/10.1016/j.toxicon.2006.10.010

Brandon, D. L., McKeon, T. A., Patfield, S. A., Kong, Q., He, X. 2016. Analysis of Castor by ELISAs that distinguish Ricin and *Ricinus communis* agglutinin (RCA). *J Am Oil Chem Soc.,* vol. 93, p. 359–363. https://doi.org/10.1007/s11746-015-2773-6

Doan, L. G. 2004. Ricin: mechanism of toxicity, clinical manifestations, and vaccine development. *Review J. Toxicol*., vol. 42, p. 201-208. https://doi.org/10.1081/CLT-120030945

Gregova, E., Šliková, S., Hozlár, P. 2015. Seed protein electrophoresis for identification of oat registered cultivars. *Potravinarstvo*, vol. 9, no. 1, p. 411-416. https://doi.org/10.5219/513

Hartley, M. R. and Lord, J. M. 2004. Cytotoxic ribosome-inactivating lectins from plants. *Biochim. Biophys. Acta*, vol. 1701, p. 1-14. https://doi.org/10.1016/j.bbapap.2004.06.004

Cheema, N. M., Malik, M. A., Qadir, G., Rabbani, M. A. 2010. Characterization of castor bean genotypes under various environments using SDS-PAGE of total seed storage proteins. *Pak. J. Bot.*, vol. 42, no. 3, p. 1797-1805.

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., 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.

Johnson, R. C., Lemire, S. W., Woolfitt, A. R., Ospina, M., Preston, K. P., Olson, C. T., Barr, J. R. 2005. Quantification of ricinin in rat and human urine: a biomarker for ricin exposure. *J. Anal. Toxicol*., vol. 29, p. 149-155. https://doi.org/10.1093/jat/29.3.149

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. https://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.

Kumar, O., Nashikkar, A. B., Jayaraj, R., Vijayaraghavan, R., Prakash, A. O. 2004. Purification and biochemical characterisation of Ricin from castor seeds. *Defense Sci. J*., vol. 54, no. 3, p. 345-351. https://doi.org/10.14429/dsj.54.2048

Lacerda, R. S., Makishi, G. L. A., Chambib, H. N. M., Bittante, A. M. Q. B., Gomide, C. A., Costa, P. A., Sobral, P. J. A. 2014. Castor Bean (*Ricinus communis*) Cake Protein Extraction by Alkaline Solubilization: Definition of Process Parameters. *Chemical engineering transactions*, vol. 37, p. 775-780.

Lord, J. M., Roberts, L. M. and Robertus J. D. 1994. Ricin: structure, mode of action and some current applications. *FASEB J*., vol. 8, p. 201-208. https://doi.org/10.1096/fasebj.8.2.8119491

Madail, J. C. M., Belarmino, L. C., Neutzling, D. M. 2007. Aspectos Econômicos da mamona. Sistemas de Produção. Embrapa, 11. ISSN 1806-9207.

Malook, I., Jamil, M., Fayyaz, M., Rehman, H., Irshad, M., Ul Bashar, K., Khan, S., Akhter, N., Aslam, M. M., Rha, E. S. 2016. Biochemical and molecular characterization of Castor Bean (*Ricinus Communis* L.) collected from different climatic zones of Pakistan. *Journal of Bio-Molecular Sciences*, vol. 4, no. 1, p. 16-23.

Osman, G., Munshi, A., Altf, F., 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. https://doi.org/10.5897/AJB12.2644

Rao, P. S., Keshavulu, K., Reddy B. K., Reddy M. N., Ankaiah, R. 2017. Characterization of Castor Bean Genotypes Using SDS-Page of Total Soluble Seed Proteins, *Int. J. Pure App. Biosci.,*vol. 5, no. 6, p. 132-137. https://doi.org/10.18782/2320-7051.2994

Riaz, S., Farrukh, M. A. 2014. Toxicological analysis of Ricin in medicinal castor oil with evaluation of health hazards. *Asian Journal of Chemistry*, vol. 26, no. 2, p. 499-503. https://doi.org/10.14233/ajchem.2014.15596

Schieltz, D. M., McWilliams, L. G., Kuklenyik, Z., Prezioso, S. M., Carter, A. J., Williamson, Y. M., McGrath, S. C., Morse, S. A., John R. Barr, J. R. 2015. Quantification of ricin, RCA and comparison of enzymatic activity in 18 *Ricinus communis* cultivars by isotope dilution mass spectrometry, *Toxicon*, vol. 95, p. 72-83. https://doi.org/10.1016/j.toxicon.2015.01.003

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. https://doi.org/10.5219/540

Vivodík, M., Balážová, Ž., Gálová, Z. and Petrovičová, L. 2018. Study of polymorphism of maize using DNA and protein markers. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 157-165. https://doi.org/10.5219/874

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. https://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. https://doi.org/10.1007/978-3-662-01639-8_2

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MAPPING AND DEVELOPMENT STRATEGY OF PEMPEK — A SPECIALTY TRADITIONAL FOOD OF SOUTH SUMATRA, INDONESIA

Agus Supriadi, Daniel Saputra, Gatot Priyanto, Rindit Pambayun, Liniyanti Djanuri Oswari

ABSTRACT

Pempek, a specialty traditional food of South Sumatra, has not been developed toward industrialization. A development process should not negate the preference taste of the consumer who is used to the taste of the traditional food. The innovation in the development of pempek to overcome its limitation for improving the marketing access as well as leading toward its industrilization was needed. A SWOT and AHP analysis were used to select the criteria and priority of consumer toward pempek development followed by the PCA to cluster the criteria and the preference of consumer. The analysis of the samples shows that pempek development requires a change in packaging design that meets the aspects of convinient and the right size by doing the engineering process to suppress the influence of inconsistent fish raw material quality. The analysis also shows that the pempek samples collected from the city of Palembang could be classified into 4 classes. The first one was the pempek which have a higher value and similarities in elasticity, chewiness, and hardness. The second one was the pempek which have a higher value and similarities in stickiness, aroma, taste, and brittleness. The third one was the pempek which have a lower value and similarities in the value of smoothness, colour, and juiciness. And the fourth one was the pempek which are not related in any of the quality attribute of pempek. The main characteristic of Pempek which could be used as the control variable on the development and processing of Pempek were taste, brittleness, stickiness, and aroma. The variables which needed an attention due to negative contribution to the development of pempek were hardness, ease of chew, elasticity, smoothness, juiciness, and color. The development of pempek should suppresed the variable aroma especially fish aroma and while the taste and brittleness should be improved.

Keywords: Pempek; traditional food; SWOT; AHP; PCA

INTRODUCTION

Pempek is a specialty traditional food of South Sumatra, Indonesia, which was made from ground fish flesh, tapioka flour, spices, salt, and water. Pempek is very famous in Indonesia and has the important position of cultural, identity, and heritage of South Sumatra. Due to its position, pempek, has been granted a certificate of intangible cultural heritage by the Indonesian government. Pempek, different from fish sausage, has a relatively higher concentration of starch, some time up to 40% starch for a good quality one **(Amiza and Ng, 2015; Karneta, 2014)**.

Pempek, since its invention, has not been developed toward industrialization. Pempek, up untill now, is processed manually and in a small scale home industry which resulted in a relatively short shelf life and a limited marketing acceses **(Karneta, 2014)**. This limitation made pempek, eventhough has a high demand, could not be consumed anytime or exported fresh. Frozen pempek had a relatively long shelf life but need thawing and reheating before consumed.

The innovation in the development of pempek to overcome its limitation for improving the marketing access as well as leading toward its industrilization was needed because innovation is the key for the success of the product **(Galanakis, 2016).** The probable direction of product development is to design the food according to the consumer demand **(Celi and Rudkin, 2016)**. The consumer demand could be based on organoleptic attributes **(Bednářová et al., 2015)** such as taste **(Kozelova et al., 2015; Guziyi et al., 2017)**, texture **(Bobková et al., 2016; Pająk et al., 2010)** and culture **(Kozelová et al., 2011)**.

The devolepment process is started with the conception of an idea and product concept as a starting point and because of that the devolepment of pempek as a modern product should be started with the mapping and product development strategy. Maping and product development

strategy is ussually analyzed with the SWOT approach. SWOT is a common tool used to analyze situations, develop and implement appropriate strategies with internal and external factors **(Chang and Huang, 2006)**. However, SWOT result was expressed qualitatively which resulted in a qualitative list that is often incomplete for analyzing the internal and external factors **(Kangas et al., 2001)**. Another weaknesess of SWOT analysis is its factors often was not tested for consistency **(Chang and Huang, 2006)**.

Analytically, SWOT could not be used to determine the importance of factors which affected the process, because the loading of the factor was not calculated to determine the influence of each factor on the proposed alternative strategy. The SWOT framework, then, needs to be transformed into a hierarchical structure by integrating analysis using Analytical Hierarchy Process (AHP) whose calculations are based on eigenvalues **(Görener et al., 2012)**. This transformation would improve the qualitative of SWOT strategic planning into a quantitative information base to facilitate in making priority decisions strategic alternatives with high consistency **(Kurttila et al., 2000)**. AHP is a multi-criteria decision-making method involving structuring multiple selection criteria into the hierarchy, assessing the relative importance of the criteria, comparing alternatives for each criterion, determining the overall ranking of alternatives, choosing the optimal alternative by taking into account the relative preference of weighting criteria **(Yavuz and Baycan, 2013)**.

Preference mapping is a strategy to understand the position of the product (food) as a basis toward the direction of a new product development by manipulating the sensory properties to get the ideal product profile to get a desired position from the position of other similar products with the aim of increasing market share **(MacFie, 2007; Perrot et al., 2017)**. A method often used to construct product mapping of difference, disadvantages, advantages, and comparison of sensory profile data is Principal Component Analysis (PCA). Although PCA does not account for average score variants due to product variants but the result of PCA mapping was as good as the method of Canonical Analysis Variation (CVA) **(Peltier et al., 2015)**. The PCA method has been used very well in mapping the products of orange cake **(Volpini-Rapina et al., 2012)**, apple and raspberry juice **(Endrizzi et al., 2014)**, sausage **(Braghieri et al., 2016; Jakobsen et al., 2014; Pires et al., 2017; Zajác et al., 2015)**, honey **(Kalaycıoğlu et al., 2017)**, sensory characterization of ultra pasteurized milk **(Chapman et al., 2001)**, and differenciation of milk fatty acids **(Werteker et al., 2017)**.

Scientific hypothesis

The main hypothesis of this work is that the modern pempek could be developed accordingly to the cluster of quality attribute required by the consument.

MATERIAL AND METHODOLOGY

Identification toward the development and quality mapping of pempek was performed with the method of Focus Group Discussion (FGD). The participants for this FGD were a selected 19 expert people. The participant was selected from the academician, the business person, the government officers, and the consumers who were then facilitated by a facilitator.

All the participant at least has a BS degree and fond of pempek. At the FGD the participants were asked to discuss the level of importance of the SWOT factors while doing the sensoric grading by a description with scale. The expert panelis were asked to grade all the atributes of SWOT and sensories of 10 different sample, which was bought from 10 different famous pempek's vendor in Palembang. The sensoric grading of all the samples were performed using the AHP method. The sensoric grading was performed by filling up a description questioner which was arranged by scale. The data were later processed and the Principal Component Analysis (PCA) of the data were computed with the help of XLSTAT 2016© (Addinsoft) software.

Statisical analysis

Statistical analysis of data collected was analyzed with the help of Microsoft Excel version 2010 (Microsoft) to determine the attribute of SWOT and then sensoric grading of AHP. The PCA was performed with the help of XLSTAT 2016© software (Addinsoft) to the determine the Principal Factor which could describe the most variation of the data collected.

RESULTS AND DISCUSSION

Identification of the direction of the development

Based on the SWOT analysis performed by the experts in the FGD toward the ten sample of commercial pempek from Palembang then the internal and external factors of pempek were identified as shown on Table 1.

The level of importance of SWOT factors were then analyzed using the AHP method by means of Scale Pairwise Comparison **(Saaty, 2008)**. The comparison results were shown on Table 2. The pairwise comparison for each factor of SWOT were then calculated and its level of priority were computed. The result of pairwise comparison for all factors of SWOT were shown on Table 3.

Based on SWOT analysis, the percentage of each SWOT factors are strength (6%), weakness (21%), opportunity (21%) and threat (52%). The priority score for all the SWOT factors (Table 3) show that the highest score for, consecutively, the strength is the factor of nutrition value of pempek (42%); the weakness is the factor of inconsistent fish raw material quality (42%); the opportunity is the factor of consumer preference (48%); and the threat is the factor of packaging design which does not meet the aspect of ease and exact proportion (57%). Therefore, the alternative strategies that could be proposed is to take the advantage of high consumer preferences because of pempek high nutritional value which require a development in packaging design that meets the aspects of ease and the right size by doing the engineering process to suppress the influence of inconsistent fish raw material quality.

Table 1 SWOT matrix of Pempek.

Strength (S)	**Weakness (W)**
(S1). Intangibel Cultural Heritage	(W1). Relatively short shelf life
(S2). Wide product variations	(W2). Need a special handling on shipping
(S3). Accepted by most people	(W3). Fishy flavor
(S4). Nutritiouss	(W4). The quality of raw material (fish) is not consistent.
Opportunity (O)	**Threat (T)**
(O1). Could be furtherly developed	(T1). The main raw material (fish) is limited
(O2). Has a great market opportunity	(T2). Competition among gel products are on the rise
(O3). Consumer preferences are high	(T3). Improper and unhygienic presentation methods
(O4). Could become an alternative to basic food	(T4). The packaging design does not meet the aspect of ease and proper servings

Table 2 Pairwise comparisons factor of SWOT.

SWOT Groups	**S**	**W**	**O**	**T**	**Priority**
Strength (S)	1.00	0.14	0.33	0.20	0.06
Weakness (W)	6.00	1.00	0.50	0.33	0.21
Opportunity (O)	3.00	2.00	1.00	0.25	0.21
Threat (T)	5.00	3.00	4.00	1.00	0.52
CR = 0.092					

Table 3 Priority Score for all the SWOT factor.

SWOT groups	**Group priority**	**SWOT factor**	**Priority within Group**	**Overall Priority factor**
Strength	0.06	Intangibel Cultural Heritage	0.05	0.003
		Wide product variety	0.26	0.017
		Accepted by most people	0.28	0.018
		Nutritiouss	0.42	0.027
Weakness	0.21	Relatively short shelf life	0.05	0.010
		Need a special handling on shipping	0.28	0.058
		Fishy flavor	0.27	0.056
		The quality of raw material (fish) is not consistent	0.41	0.085
Opportunity	0.21	Could be furtherly developed	0.15	0.031
		Has a great market opportunity.	0.15	0.031
		Consumer preferences are high	0.48	0.100
		Could become an alternative to basic food	0.23	0.048
Threat	0.52	The main raw material (fish) is limited	0.14	0.074
		Competition among gel products are on the rise	0.09	0.045
		Improper and unhygienic presentation methods	0.20	0.101
		The packaging design does not meet the aspect of ease and proper servings	0.57	0.296

Quality Mapping

Sensory data from the ten pempek samples were then processed using Principle Component Analysis (PCA). PCA is a method that can explain the amount of variability from the largest to the smallest and also the hidden variability. The average value of each parameter was processed into the standard value (Z) and then the Z value with the help of XLSTAT® were converted into the eigenvalues, percentage of variation, and the cummulative of variation (Table 4). It was shown that the number of Principal Component or Factor (F) needed to describe the variability were nine components with the percentage explain by the eigenvalue range from 35.8% to 0.1%. The percentage of variability shows the variability that could

Table 4 Eigenvalues, percentage and cummulative variation of sensory data of pempek.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalue	3.58	2.82	1.22	0.93	0.72	0.49	0.19	0.06	0.01
Variability, %	35.8	28.2	12.1	9.3	7.2	4.9	1.9	0.6	0.1
Cumulative, %	35.8	64.0	76.1	85.4	92.5	97.5	99.3	99.9	100.0

Table 5 Eigenvector for Pempek sample.

Factor	PC1	PC2
Hardness	-0.031	0.521
Elasticity	-0.165	0.176
Brittleness	**0.422**	0.261
Stickiness	**0.402**	0.246
Ease of Chew	-0.160	0.495
Smoothness	0.346	-0.148
Juiciness	0.311	-0.394
Taste	**0.457**	0.126
Aroma	**0.384**	0.218
Color	0.181	-0.288

Table 6 The loading of factor of Pempek.

	PC1	PC2
Hardness	-0.059	0.874
Elasticity	-0.313	0.295
Brittleness	**0.800**	0.438
Stickiness	**0.762**	0.413
Ease of Chew	-0.302	0.831
Smoothness	0.656	-0.248
Juiciness	0.589	-0.661
Taste	**0.865**	0.212
Aroma	**0.726**	0.365
Color	0.343	-0.484

be described by each of the main component. The percentage of variability was found by the value of each eigenvalue divided by the total value of eigenvalue times 100%.

It was shown on Table 4 that the variability describe by PC1 was 35.8% which means the main component PC1 could explain the variability of data 35.8% from of all data. The lower the value of eigenvalue means the lower the variability that could be explained by the related component.

The main objective of using PCA was to describe the largest amount of variation of original data with the smallest number of main component. For that reason, some components (PC) were chosen to explain the largest variation of data. The number of component chosen were based on the eigenvalue which could describe the variability of the main component. Plot of the nine components and its variability was shown on Figure 1.

The number of main component needed for principal component analysis was based on the amount of variability which could be described by those components. The components chosen must be able to explain at least 60% to 70% of all the variability. The total variability which could be described by PC1 (35.8%) and PC2 (28.1%) was 63.9% which was adequate to explain the variability of Pempek. If PC3 was included the amount of variability would be 76.1%, however the contribution of PC3 was only 12.1%. The value of eigenvector for each factor for PC1 and PC2 were shown on Table 5. The eigenvector value could be used to determine the variable which characterized the Pempek quality. The high eigenvector value act as the main characteristics of Pempek which the taste is, the brittleness, the stickiness, and the aroma.

Besides eigenvector value there was also the loading of the factor which shown on Table 6. Factor loadings are the correlation between the original variables and the factors, and the key to understanding the underlying nature of a

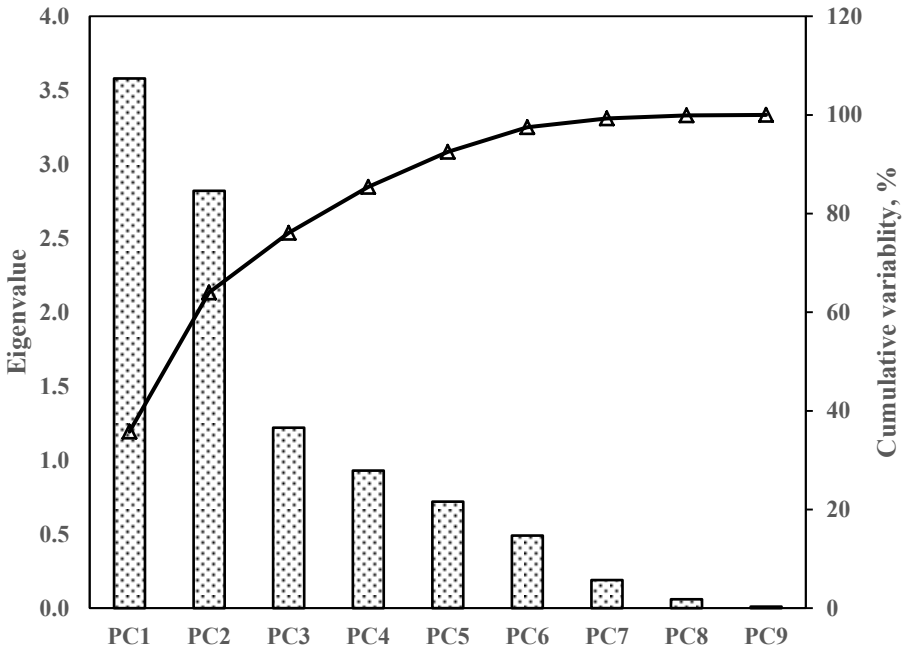

Figure 1 Plot of principal component of Pempek.

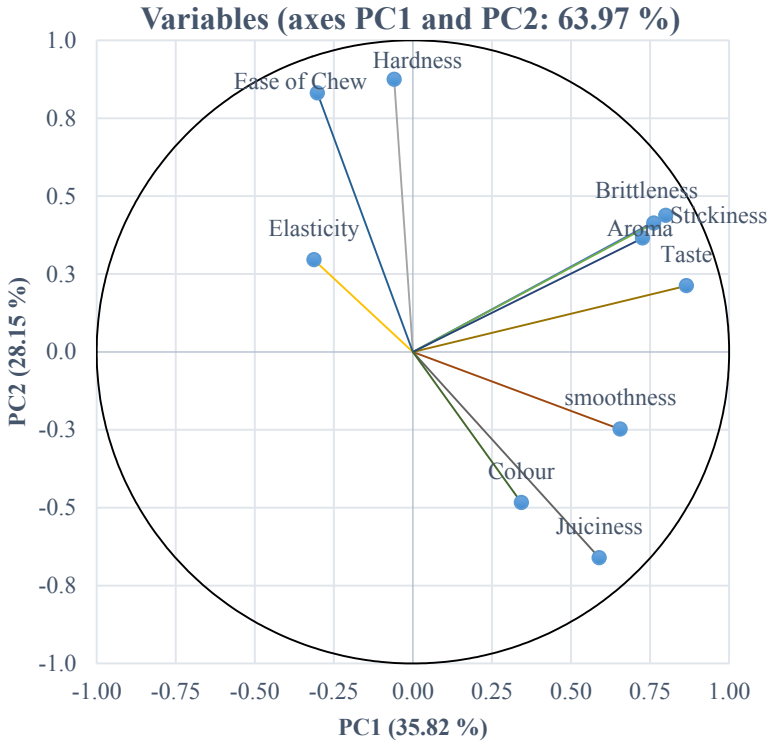

Figure 2 Plot of Pempek Loading factor PC1 versus PC2.

particular factor. Based on the two main componen of PC1 and PC2, the relationship of the two could be determined by taking the absolute value the vector as shown on Figure 2.

The line on Figure 2 shows the variable name of Pempek. The length of the line indicates the variability of each variable. The variables with a less variablity was shown by a shorter vector line while the variables with a highest variability was shown by the longest vector line. Figure 2 shows that the variable taste, brittleness, stickiness, and aroma have the highest variability. These four variables are in the First quadrant of Figure 2 which means these four variables are the main characteristics of Pempek which could be uses as the control variable in processing Pempek or in the product development of Pempek. Whilst

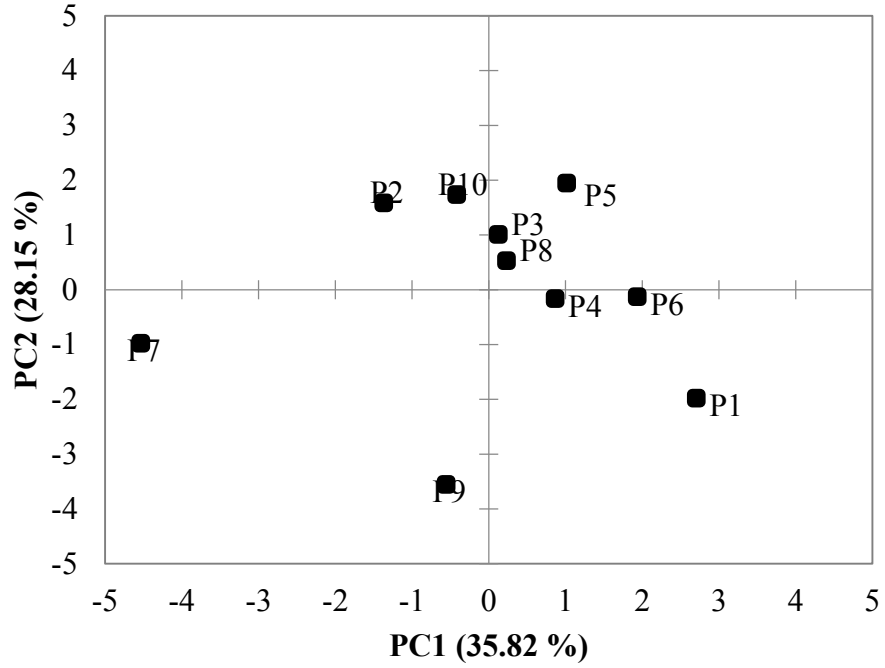

Figure 3 Plotting of Pempek's sample score on PC1 and PC2.

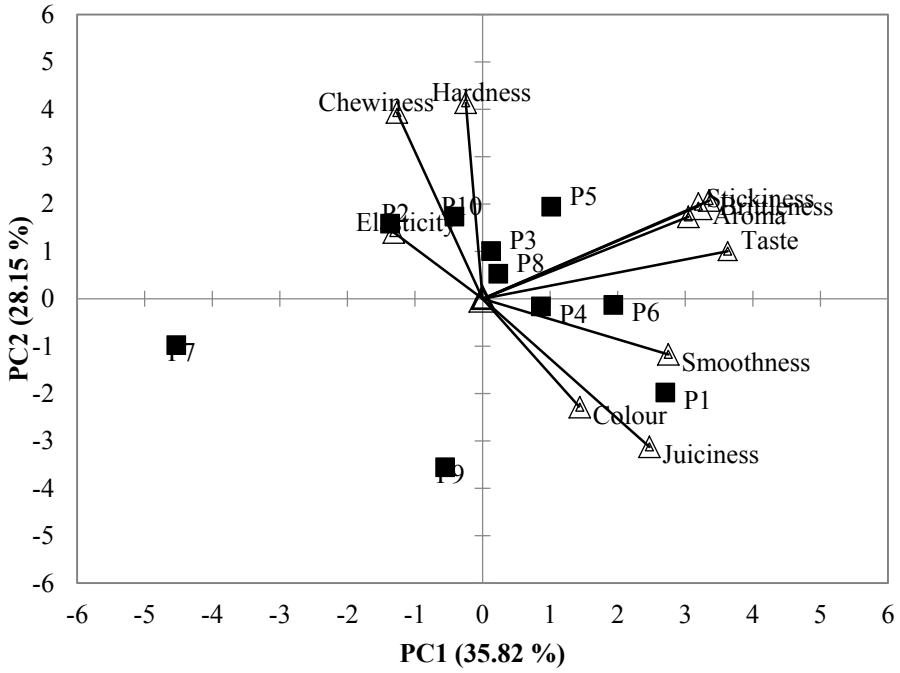

Figure 4 Bi-plot sample position and variable types of pempek.

these four variables had a positive impact to the development of Pempek, the other six variables needed a careful attention because of their negative values either in PC1 or PC2.

The variables hardness, ease of chew, and elasticity (Second quadrant) had a negative values on PC1; and the variables smoothness, juiciness, and color (Fourth quadrant) had a negative value on PC2. These negative values means that eventhough the main variables are taste, brittleness, stickiness and aroma, some attention should be given to the other variables because these other variables would give negative impression to the overall characteristics of Pempek.

The coordinate value of each Pempek sample tested on PC1 and PC2 was shown on Table 7. Each coordinate value will determine the position of the coordinate on the quadrant. The coordinate value of the first sample (P1) on PC1 is 2.7 (positive value) and on PC2 is -1.9 (negative value) then the first sample is in the positive and negative quadrant (Fourth quadrant).

Table 7 The ordinate value of sample and its main value on PC1 and PC2.

Sample (Pempek)	PC1	PC2
P1	2.705	-1.979
P2	-1.369	1.584
P3	0.124	1.008
P4	0.865	-0.162
P5	1.016	1.945
P6	1.936	-0.129
P7	-4.534	-0.979
P8	0.231	0.529
P9	-0.555	-3.554
P10	-0.418	1.736

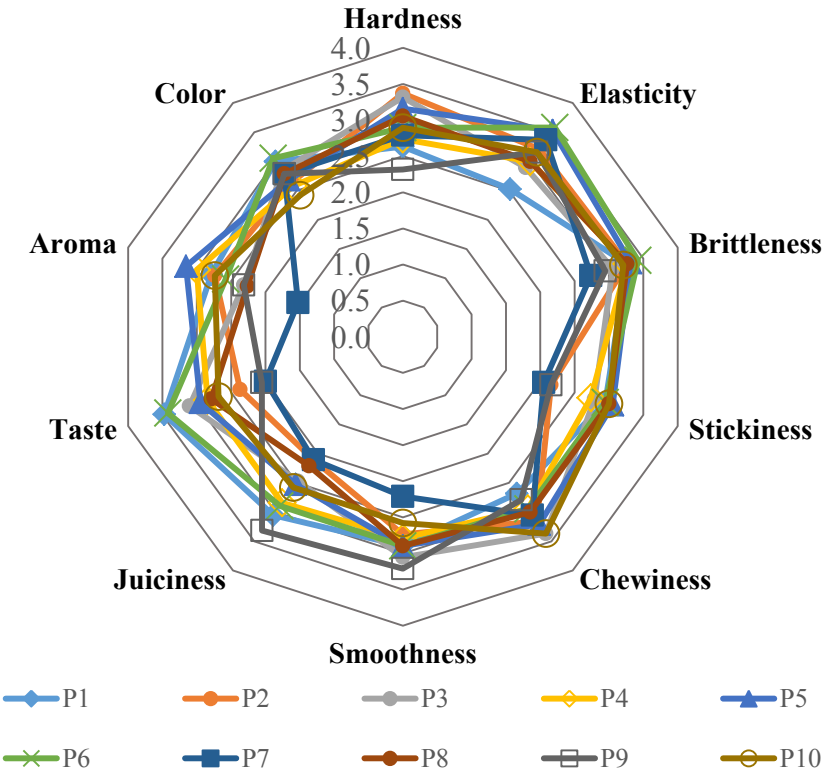

Figure 5 Diagram area of Pempek variable.

Score plots obtained from graphic ordinate between PC1 and PC2 could explain the relationship of similarity between samples. The adjacent samples had similar characteristics, whereas the samples that were located far apart had a different characteristic. Figure 3 shows that the sample spreads across all quadrants, samples in the same quadrant also have similarities. The Strength and weaknesses of the similarity of one variable to the other were determined by the closeness of the position among them in one quadrant. Sample P3, P5 and P8 are in the first quadrant, so that the three samples have similarity to one particular variable, but because the location of sample P3 is closer to P8 or vice versa than to P5 then P3 and P8 has a strong resemblance compared to the sample P5. In the third quadrant, although the sample P7 and P9 are in the same quadrant they are similar in one variable, but the resemblance is not strong due to its position that is far apart. Similarly, the samples P2 and P10 in the second quadrants, and the sampel P4, P6, P1 in the fourth quadrant.

The similarity among the variables of the sample in each quadrant could be explained through biplot image which is a combination of loading plot and score plot as shown on Figure 4.

Figure 4 show the position of the sample on the ordinate axis (solid box) and the type of determining variables of pempek which was shown by the line toward the center of the axis with triangle at each end. Some information could be drawn from this biplot. The first information that can be drawn from the biplot image is: on the first quadrant the samples P3, P8 and P5 have similarities in the variability of brittleness, stickiness, aroma and taste. While in the

second quadrant the samples P2 and P10 have similarities in the variable elasticity, chewiness and hardness. Sample P2 has a special similarity to the variable elasticity than can be said that P2 has a more specific characteristic of the elasticity. In the third quadrant there are samples P7 and P9, both samples have no similarity to any of the pempek variables. The sample P4, P6 and P1 in the fourth quadrant have the similarity in color, smoothness and juiciness.

The second information which could be drawn from Figure 4 is the variable value of a sample. If a sample that are located in the direction of a variable then that sample has a value above the average, on the other hand if a sample is located opposite to the line of the variable then it has a value below the average. Samples P7 and P9 have variable values below the average value of all variables. Sample P1 has a variable value below the average on the smoothness, samples P4 and P6 have a variable values above average on the variables value of smoothness, color and juiciness. While the samples P3, P5, P8 have a variable values above the average on the variable value of aroma, taste, stickiness and bittleness. The sample P10 has a variable value above the average on chewiness and elasticity while sample P2 has a variable value above the average on elasticity.

The third information that could be drawn from the biplot of Figure 4 is that the correlation between variables shown by biplot line direction. Positive correlation is indicated by direct line direction, the closer or narrower the angle of the variable line indicates the stronger the correlation. The variable of taste, aroma, brittlenes and stickiness (first quadrant) has a strong correlation, along with smoothness, color and moist (fourth quadrant) and hardness, elasticity, chewiness (second quadrant). Then between groups of variables that are in first, second and fourth quadrants have a strong negative correlation.

In the product development process there is one factor that should not be missed is the range of development, especially on the variables that become the main character of pempek that is taste, aroma, brittleness and stickiness. The magnitude of the value indicated from the size of the area on the four variables becomes the optimal range of the pempek product development process. The size of the development area for each variable is shown in Figure 5.

Based on the main characteristic of pempek development variables, it could be shown on Figure 5 that the upper limit of the development for taste and aroma is on sample P1 and the lower limit is on sample P7. While the development of stickiness the upper limit is on the sample P5 and the lower limit was on sample P7 and the variable of brittleness the upper limit was on sample P6 and the lower limit was on sample P7.

CONCLUSION

By utilizing the high consumer preferences on Pempek's nutrition value, Pempek could be developed by using new packaging design and shape with respect to the aspect of convinience and bite size.

Sample P3, P8 and P5 had the similarity on the variabel of brittleness, stickiness, aroma, and taste. Sample P2 and P10 had the similarities on the variable of elasticity (specifically on P2), chewyness, and hardness; while the sample of P1, P4 and P6 had the similarities on colour, smoothness, and moist; and P7 with P9 did not have the similarities on all the variable.

The main characteristic of Pempek which could be used as the control variable on the development and processing of Pempek were taste, brittleness, stickiness, and aroma.

The development of pempek should suppresed the variable aroma especially fish aroma and while the taste and brittleness should be improved.

The upper limit of Pempek's development variable for taste and aroma was found on the P1 sample; and its lower limit was found on sample P7. Meanwhile the development for the stickiness variable upper limit was on the sample P5 and its lower limit was on sample P7; and the brittleness upperlimit was on sample P6 and its lower limit on sample P7.

REFERENCES

Amiza, M. A., Ng, S. C. 2015. Effects of Surimi-to-Silver Catfish Ratio and Potato Starch Concentration on the Properties of Fish Sausage. *Journal of Aquatic Food Product Technology*, vol. 24, no. 3, p. 213-226. https://doi.org/10.1080/10498850.2013.766293

Bednářová, M., Oštádalová, M., Král, M., Tremlová, B. 2015. Comparison of selected sensory properties of wholemeal breads. *Potravinarstvo,* vol. 9, no. 1, p. 211-216. https://doi.org/10.5219/454

Bobková, A., Šnirc, M., Král, M., Oštádalová, M., Pokorná, J., Fekete, T., Belej, L. 2016. Texture quality of muskmelons (Cucumis melo L.) from different retailers during storage. *Potravinarstvo,* vol. 10, no. 1, p. 578-584. https://doi.org/10.5219/668

Braghieri, A., Piazzolla, N., Galgano, F., Condelli, N., De Rosa, G., Napolitano, F. 2016. Effect of preservative addition on sensory and dynamic profile of Lucanian dry-sausages as assessed by quantitative descriptive analysis and temporal dominance of sensations. *Meat Science,* vol. 122, p. 68-75. https://doi.org/10.1016/j.meatsci.2016.07.020

Celi, M., Rudkin, J. 2016. Drawing food trends: Design potential in shaping food future. *Futures*, vol. 83, p. 112-121. https://doi.org/10.1016/j.futures.2016.05.002

Endrizzi, I., Gasperia, F., Rødbotten, M., Næsb, T. 2014. Interpretation, validation and segmentation of preference mapping models. *Food Quality and Preference*, vol. 32, p. 198-209. https://doi.org/10.1016/j.foodqual.2013.10.002

Galanakis, C. M. 2016. *Innovation Strategies in the Food Industry Tools for Implementation* 1st ed., San Diego, California : Academic Press. 334 p. ISBN 9780128037935.

Görener, A., Toker, K., Uluçay, K. 2012. Application of Combined SWOT and AHP: A Case Study for a Manufacturing Firm. *Procedia - Social and Behavioral Sciences*, vol. 58, p. 1525-1534. https://doi.org/10.1016/j.sbspro.2012.09.1139

Guziy, S., Šedík, P., and Horská, E. 2017. Comparative Study Of Honey Consumption In Slovakia And Russia. *Potravinarstvo*, vol. 11, no. 1, p. 472-479. https://doi.org/10.5219/784

Chang, H. H., Huang, W. C. 2006. Application of a quantification SWOT analytical method. *Mathematical and Computer Modelling*, vol. 43, no. 1–2, p. 158-169. https://doi.org/10.1016/j.mcm.2005.08.016

Chapman, K. W., Lawless, H. T., Boor, K. J. 2001. Quantitative Descriptive Analysis and Principal Component Analysis for Sensory Characterization of Ultrapasteurized Milk. *Journal of Dairy Science*, vol. 84, no. 1, p. 12-20. https://doi.org/10.3168/jds.S0022-0302(01)74446-3

Jakobsen, L. M. A., Vuholm, S., Aaslyng, M. D., Kristensen, M., Sørensen, K. V., Raben, A., Kehlet, U. 2014. Sensory characteristics and consumer liking of sausages with 10% fat and added rye or wheat bran. *Food Science and Nutrition,* vol. 2, no. 5, p. 534–546. https://doi.org/10.1002/fsn3.126

Kalaycıoğlu, Z., Kaygusuz, H., Döker, S., Kolaylı, S., Erim, F. B. 2017. Characterization of Turkish honeybee pollens by principal component analysis based on their individual organic acids, sugars, minerals, and antioxidant activities. *LWT-Food Science and Technology,* vol. 84, p. 402-408. https://doi.org/10.1016/j.lwt.2017.06.003

Kangas, J., Pesonen, M., Kurttila, M., Kajanus, M. 2001. A'WOT : Integrating The AHP With SWOT Analysis. *In: 6th ISAHP. Berne, Switzerland*, p. 189-198. Available at: http://www.isahp.org/2001Proceedings/Papers/037-P.pdf

Karneta, R. 2014. The Characteristics of Pempek Lenjer: heat diffusivity, quality, and shelf life (in Indonesian) : doctoral thesis. Palembang, Indonesia : Universitas Sriwijaya.

Kozelová, D., Mura, L., Matejková, E., Lopašovský, Ľ., Vietoris, V., Mendelová, A., Bezáková, M., Chreneková, M. 2011. Organic Products, Consumer Behavior on Market and European Organic Product Market Situation. *Potravinarstvo,* vol. 5, no. 3, p. 20-26. https://doi.org/10.5219/96

Kozelová, D., Országhová, D., Fil'a, M., Čmiková, Z. 2015. Quantitative indicators of fruit and vegetable consumption. *Potravinarstvo,* vol. 9, no. 1, p. 487-493. https://doi.org/10.5219/570

Kurttila, M., Pesonen, M., Kangas, J., Kajanus, M. 2000. Utilizing the analytic hierarchy process (AHP) in SWOT analysis — a hybrid method and its application to a forest-certification case. *Forest Policy and Economics,* vol. 1, no. 1, p. 41-52. https://doi.org/10.1016/S1389-9341(99)00004-0

MacFie, H. 2007. Preference mapping and food product development. In: MacFie, H., Ed., *Consumer-Led Product Development.* Cambridge : Woodhead Publishing Limited, p. 551-592. ISBN 978-1-84569-072-4. https://doi.org/10.1533/9781845693381.3.551

Pająk, P., Fortuna, T., Tomasiak, I. B. 2010. Textural, Flow and Sensory Properties of Five "Fruzelina" With Sour Cherries. *Potravinarstvo*, vol. 4, no. 2, p. 55-58. https://doi.org/10.5219/53

Peltier, C., Visalli, M., Schlich, P. 2015. Comparison of Canonical Variate Analysis and Principal Component Analysis on 422 descriptive sensory studies. *Food Quality and Preference*, vol. 40, p. 326-333. https://doi.org/10.1016/j.foodqual.2014.05.005

Perrot, M., Pineau, N., Antille, N., Moser, M., Lepage, M., Thaler, T., Voirin, A., Rytz, A. 2018. Use of multi-market preference mapping to design efficient product portfolio. *Food Quality and Preference,* vol. 64, p. 238-244. https://doi.org/10.1016/j.foodqual.2017.08.008

Pires, M. A., Munekata, P. E. S., Baldin, J. C., Rocha, Y. J. P., Carvalho, L. T., Santos, I. R., Barros, J. C., Trindade, M.A. 2017. The effect of sodium reduction on the microstructure, texture and sensory acceptance of Bologna sausage. *Food Structure,* vol. 14, p. 1-7. https://doi.org/10.1016/j.foostr.2017.05.002

Saaty, T. L. 2008. Decision making with the analytic hierarchy process. *International Journal of Services Sciences*, vol. 1, no. 1, p. 83-98. https://doi.org/10.1504/IJSSCI.2008.017590

Volpini-Rapina, L. F., Sokei, F. R., Conti-Silva, A. C. 2012. Sensory profile and preference mapping of orange cakes with addition of prebiotics inulin and oligofructose. *LWT. Food Science and Technology*, vol. 48, no. 1, p. 37-42. https://doi.org/10.1016/j.lwt.2012.03.008

Werteker, M., Huber, S., Kuchling, S., Rossmann, B., Schreiner, M. 2017. Differentiation of milk by fatty acid spectra and principal component analysis. *Measurement*, vol. 98, p. 311-320. https://doi.org/10.1016/j.measurement.2016.10.059

Yavuz, F., Baycan, T. 2013. Use of Swot and Analytic Hierarchy Process Integration as a Participatory Decision Making Tool in Watershed Management. *Procedia Technology*, vol. 8, p. 134-143. https://doi.org/10.1016/j.protcy.2013.11.019

Zajác, P., Čurlej, J., Barnová, M., Čapla, J., 2015. Analysis of texturometric properties of selected traditional and commercial sausages. *Potravinarstvo*, vol. 9, no. 1, p. 458-467. https://doi.org/10.5219/473

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IS CURRENT SYSTEM OF DIRECT PAYMENTS SUITABLE FOR FARMERS IN SLOVAKIA?

Jana Kozáková, Mária Urbánová

ABSTRACT
Current system of direct payments in Slovakia can be described as per hectare payment. It is based on the logical assumption that the more land farmers cultivates, the more support they need. However, it seems like this principle works differently among EU member states. Historically, Slovakia is a country with the largest farms in EU 28. This extreme big physical farm size is here connected with the lowest output among EU 28 and simultaneously Slovak farms display also lowest efficiency. Paper examine generally accepted assumption that the more hectares farms utilize, the more subsidies they should receive, to help achieve more output. Research is based on the mutual pair combined correlation analysis, which examined relationship between utilized agricultural area, total output and total subsidies. Surprisingly just the relationship between total subsidies and total output was proved to be positive and in a moderate manner. Relation of total subsidies and utilised agricultural area, respectively total output and utilised agricultural area show the inverse relationship. In spite of the development in most European countries, Slovak outcomes indicates that the more subsidies farmers receive, the less output they achieve. This paradox can be caused by the actual Common Agricultural Policy system of subsidies remuneration which is not necessary suitable for whole EU 28 on the same level.

Keywords: utilized agricultural area; total output; subsidies; Slovakia; farm efficiency

INTRODUCTION
Farmers' support in the European Union (EU) is currently implemented through various instruments, including financial ones, which are applied through direct payments. According to **Jankacká and Lincényi (2013)**, the context of direct payments has created a space for farmers to focus more on demand and therefore on the consumer. In addition, one of the functions of support is also to regulate the volume of production of certain commodities linked to production quota, price or even non-production on land **(EC, 2014)**. Direct payments are therefore an effective tool of the European Commission (EC) to regulate the agri-food sector in the EU. From 2015, the principle of decoupling of payments from production is applied also in Slovakia, and direct payments are paid per hectare of the agricultural area in order to ensure a direct positive impact on the actual performance of farms **(Duricová, 2016)**. As summarised by **Gordon and Davodora (2004)**, the question of farms' productivity and efficiency in post-socialist countries is crucial to understand whether the countries could compete within the enlarged EU after their accession and how farm structures in these countries would evolve.

Agriculture and food production in Slovakia are one of the main pillars of the national economy. The sustainability of these industries is crucial for further economic development as well as for ensuring the country's food security and satisfying domestic demand. According to **Matoškova and Gálik (2013)** this can be achieved mainly by ensuring a sufficient supply of competitive, high-quality and affordable home-grown food, while making use of the benefits of international trade and all instruments of the Common Agricultural Policy for trade in agricultural and food products. Subsidies affects the total input, so it is very important to monitor the link between input and total output. The relationship between output and input may be asociated to farm efficiency. **Bakhshood and Thomson (2001)** define efficiency in terms of production as output maximization for a given set of inputs or outputs at a given output level using a minimum input level, or a mixture of both. Agricultural subsidies help to increase the performance and reduce world prices but on the other hand also disrupt international markets and reduce economic efficiency. According to **Adamišin et al. (2015)**, the direct impact on the performance and efficiency may have also effect on the management of the agricultural entity. This can create better conditions, which can contribute higher performance and can be also positive inspiration to other companies in the neighbourhood.

In 2003, European Council reformed Common Agricultural Policy (CAP) system which caused dramatic

changes in direct payment scheme through decoupling of the direct support. After this payments were no longer connected to the production and farmers received direct payments (single farm payments) conditional on certain cross-compliance requirements which based on the keeping the land in good agricultural and environmental condition, soil protection, preventing deterioration of habitats, and protection of water resources **(Blomquist and Nordin, 2017)**. New system was fully implemented in 2005 and Slovakia as one of EU new entrants was allowed to adopt (temporarily until 2010) simplified system of direct payments (SAPS – single area payment scheme) which is payed yearly on the hectare basis. SAPS is connect only to agriculture area and has no link with the amount of production **(MARD SR, 2018)**.

The level of agricultural production in Slovakia is close to two billion EUR per year, with the largest share of total production in the region of Western Slovakia, where an annual production exceed 1.3 billion EUR. Plant production amounted to 1.149 billion EUR and livestock production 861 million EUR. Of the total agricultural production, measured at current prices, up to 94% was agricultural production, and the remaining 6% on average represented the production of agricultural services **(SO SR, 2016)**.

Scientific hypothesis

Based on the previous research these indicators suggests that there is a link between total subsidies (TS) utilized agricultural area (UAA) and total output (TO). Therefore the assumptions were set and examined by the correlation analysis:

Assumption 1: There is a statistical relationship between total subsidies (TS) and total output (TO) on farm level.

Assumption 2: There is a statistical relationship between total subsidies (TS) and utilized agridultural area (UAA) on farm level.

Assumption 3: There is a statistical relationship between total output (TO) and utilized agricultural area (UAA) on farm level.

MATERIAL AND METHODOLOGY

Article is based on the Farm Accountancy Data Network (FADN) data from twelve year time period from 2004 to 2015. FADN database includes data of agricultural holdings surveyed by the Farm Structure Survey (FSS), carried out by the EU countries and managed by Eurostat. This set of farms consists of all agricultural holdings in the European Union of at least 1 hectare and those of less than 1 hectare provided the latter market a certain proportion of their output or produce more than a specified amount of output **(FADN, 2017)**. Analysis includes 28 EU member states: (BEL) Belgium, (BGR) Bulgaria, (CYP) Cyprus, (CZE) Czech Republic, (DAN) Denmark, (DEU) Germany, (ELL) Greece, (ESP) Spain, (EST) Estonia, (FRA) France, (HRV) Croatia, (HUN) Hungary, (IRE) Ireland, (ITA) Italy, (LTU) Lithuania, (LUX) Luxembourg, (LVA) Latvia, (MLT) Malta, (NED) Netherlands, (OST) Austria, (POL) Poland, (POR) Portugal, (ROU) Romania, (SUO) Finland, (SVE) Sweden, (SVK) Slovakia, (SVN) Slovenia, (UKI) United Kingdom. Considering the fact, that different member states entered EU in different time, BGR and ROU data starts in 2007 and HRV in 2013. In chosen period these indicators were examined: average utilised agricultural area (UAA) in ha.farm^{-1} in EU 28 (2004 – 2015), total subsidies – excluding on investments (EUR.farm^{-1}) in EU 28 (2004-2015), average total output (EUR.farm-1) and total input (EUR.farm^{-1}) in EU 28 (2004 – 2015), total output (EUR.farm^{-1}) and total input (EUR.farm^{-1}) ratio in EU 28 (2004 – 2015).

All displayed calculations, graphical views and statistical analyzes were implemented on software Microsoft Excel as a part of product Microsoft Office 2013 Professonal Plus.

Statisic analysis

From the methodological point the statistical methods for measurement of the dependence, resp. associations of observed variables were used. We assessed the statistical significance of relations **(Orsághová, et al., 2016)**. If there is a reversible dependency between variables, which means that the dependence of the variable X from the Y variable has also meaning, then we found correlation dependency **(Obtulovič, 2001)**. To interpret correlation coefficient which can arise from -1 to +1, certain ranges were used: almost perfect correlation (0.9 – 1), very large correlation (0.7 – 0.9), strong correlation (0.5 – 0.7), moderate correlation (0.3 – 0.5), small correlation (0.1 – 0.3) and trivial correlation rate (0.0 – 0.01) **(Munk, 2011).** These ranges can gain both positive and negative linear relationship.

RESULTS AND DISCUSSION

Utilised agricultural area (UAA) is the EU standardized unit which describes the area used for farming in hectares per farm. It includes **(Eurostat, 2018)** the following land categories: arable land, permanent grassland, permanent crops, other agricultural land such as kitchen gardens (even if they only represent small areas of total utilised agricultural area). The term does not include unused agricultural land, woodland and land occupied by buildings, farmyards, tracks, ponds, etc. This area varies in member states. The smallest farms in EU 28 are in Malta (2.84 ha.farm^{-1}), the most of other members has farms with utilised between 10 and 100 hectares. Farms with more than 100 hectares are in: Estonia (119.85 ha.farm^{-1}), United Kingdom (154.70 ha.farm^{-1}) and Czech Republic (218.41 ha.farm^{-1}). Unlike these usual values, Slovakia has the absolutely biggest farms in EU 28 with the UAA value of 556.15 ha.farm^{-1} (Figure 1). This extreme can be described by historical farm size in Slovakia after process of collectivization after World War II, when huge collective ownership was established **(Lančarič, et al., 2013)**. The UAA of farms remained mostly unchanged also after privatization and transition to private ownership.

According to the size of UAA, subsidies are remunerated on the basis of hectare area, which is projected into the change of total subsidies. This system was set by the Common Agricultural Policy (CAP) for the all EU members. This system should mean advantage for Slovak farmers who utilise huge acreage of land. In examining time period this means that single Slovak farm received averagely 136 775 EUR.farm^{-1} per year. This was more than double amount of EUR than the second CZE where farmers received averagely 69,827.92 EUR.farm^{-1} per year (Figure 2). However, there were 12 countries where farmers got less

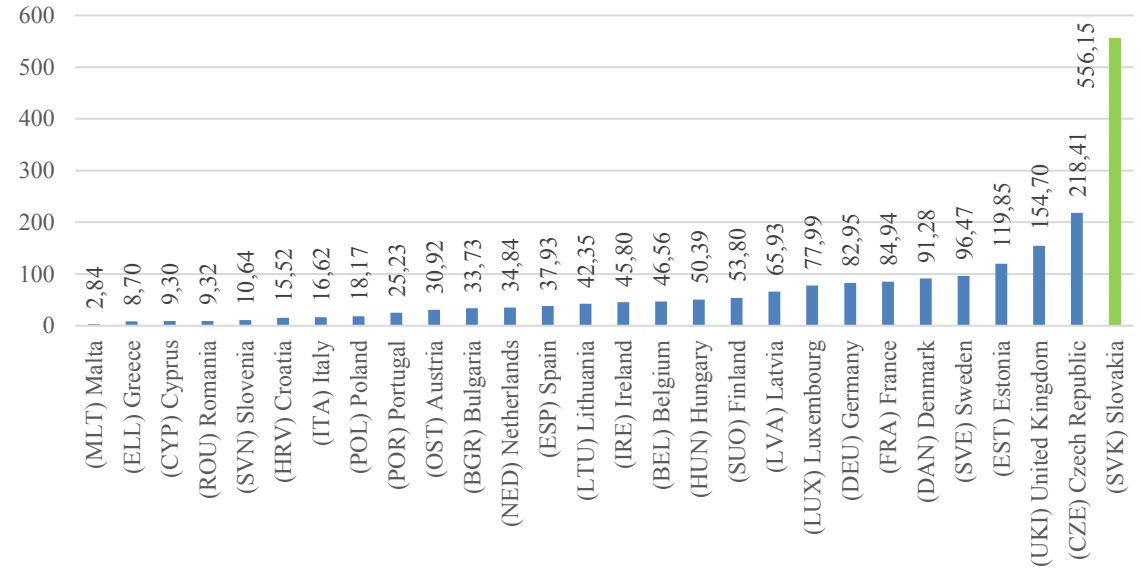

Figure 1 Average utilised agricultural area (ha.farm^{-1}) in EU 28 (2004 – 2015).
Source: own calculations based on FADN (2018) data.

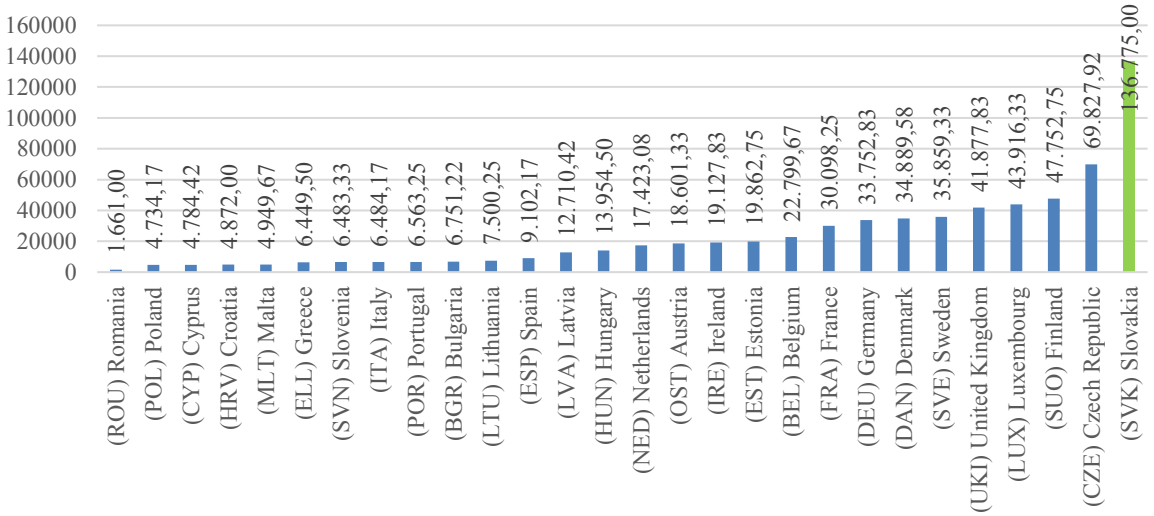

Figure 2 Total subsidies – excluding on investments (EUR.farm^{-1}) in EU 28 (2004 – 2015).
Source: own calculations based on FADN (2018) data.

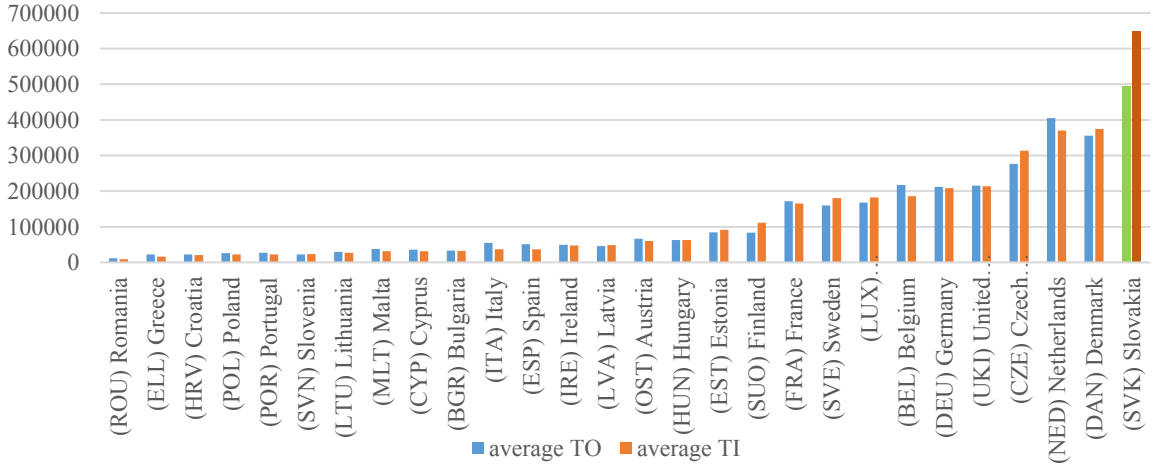

Figure 3 Average total output (EUR.farm^{-1}) and total input (EUR.farm^{-1}) in EU 28 (2004 – 2015).
Source: own calculations based on FADN (2018) data.

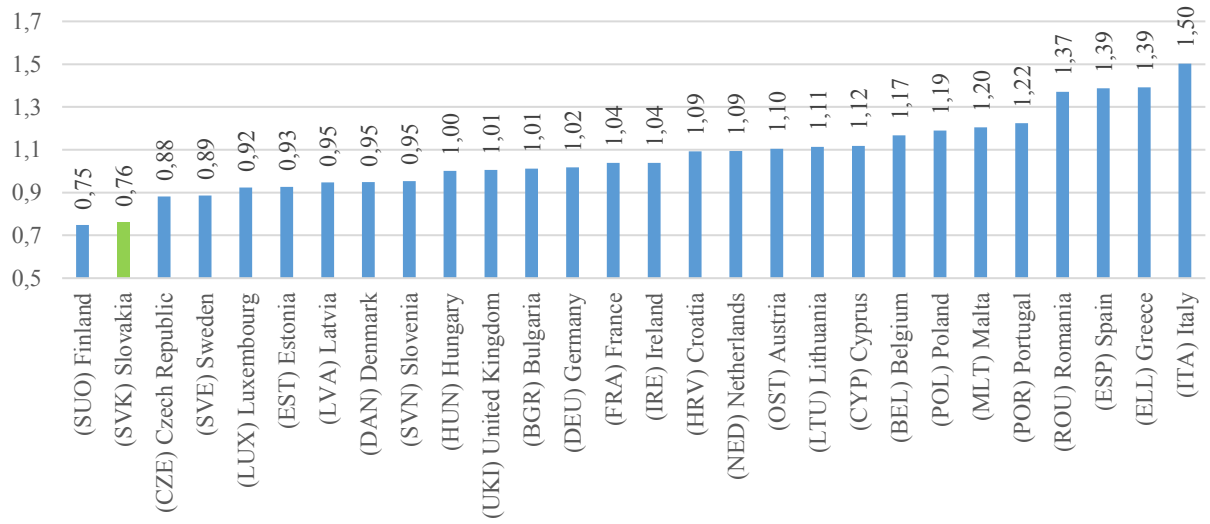

Figure 4 Total output (EUR.farm^{-1}) and total input (EUR.farm^{-1}) ratio in EU 28 (2004 – 2015).
Source: own calculations based on FADN (2018) data.

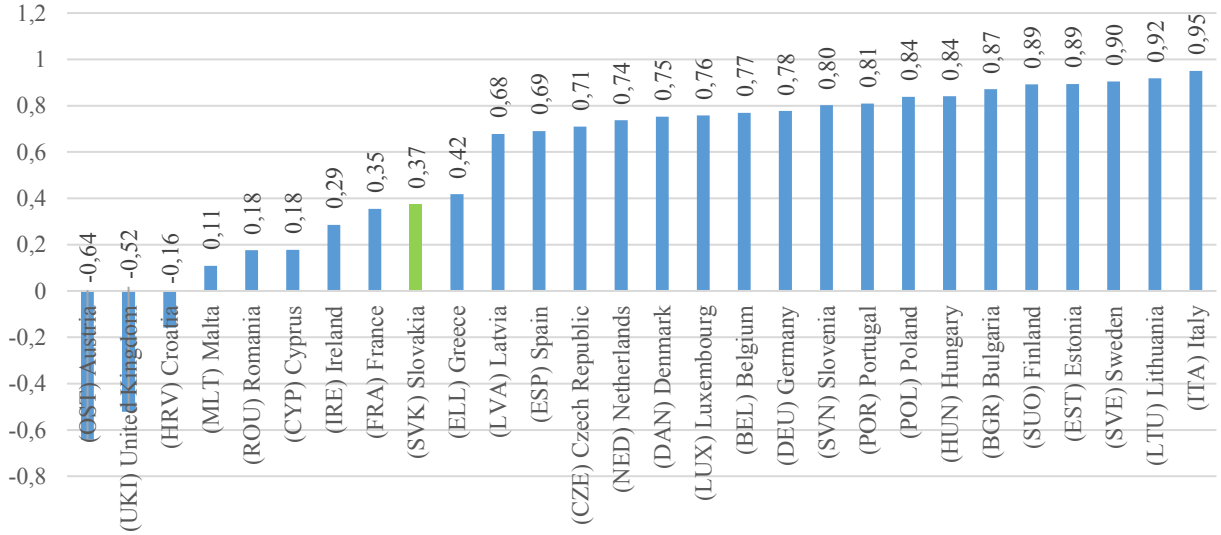

Figure 5 Correlation between total subsidies (EUR.farm^{-1}) and total output (EUR.farm^{-1}) in EU 28, (2004 – 2015).
Source: own calculations based on FADN (2018) data.

than 10,000 EUR.farm^{-1} yearly, with the lowest number in Romania 1,661 EUR.farm^{-1}.

It can be expected that immense acreage and simultaneously subsidies should be projected also in the great value of total output. This expectation can be proved in most of the examined EU countries, including SVK. But after closer view (Figure 3), it is important to compare the total amount of output with the amount of used input. When looking at the absolute values, Slovakia is leader in the number of total output and also input. However, the most important is to indicate the difference between these two variables in positive manner (TO – TI), which indicates the efficiency. Slovakia is leader also in the average amount of this difference, but surprisingly, in the negative manner (-155,076.33 EUR.farm^{-1}). Despite Slovakias input is the highest out of EU 28 (648,784.58 EUR.farm^{-1}), this brings much lower output (493,708.25 EUR.farm^{-1}), compared to other countries.

Ratio between input and output imply efficiency. These two indicators (measured in EUR per farm) have both the biggest value in Slovakia and also their ratio shows the biggest value between them which results in almost the lowest productivity (0.76). This indicator suggest that one euro used in Slovak agribusiness brings output in amount of just 0.76 EUR that is second lowest in EU 28 (Figure 4).

Low amount of output in Slovak agribusiness and high amount of subsidies at the same time indicates unusual attitude of Slovak farmers to primarily agricultural production. In addition when examining relationship between total subsidies and total output (Figure 5) the moderate positive relationship (0.37) can be found. This means that increase in total subsidy cause also an increase of total output.

In spite of fact, that there are several factors which affect total subsidies (state support system, the type of agricultural production, ect.), the current per hectare payment system indicates the acreage of utilise agricultural area as one of the most important. When examining correlation between utilised agricultural area and total subsidies (Figure 6) in Slovakia we can find strong negative correlation (-0.57). This relationship puts Slovakia in the position of leader again, since similar but not as strong relationship has been discovered in case of Czech Republic, United Kingdom and Romania. This surprisingly negative relation indicates

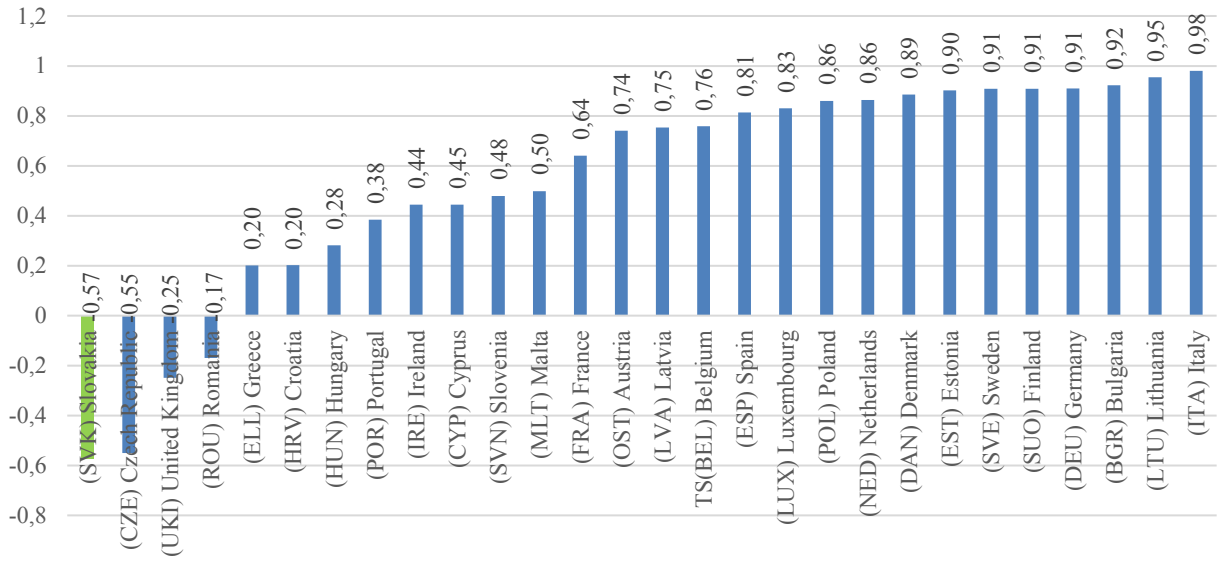

Figure 6 Correlation between utilised agricultural area (ha) and total subsidies (EUR.farm^{-1}) in EU 28 (2004 – 2015). Source: own calculations based on FADN (2018) data.

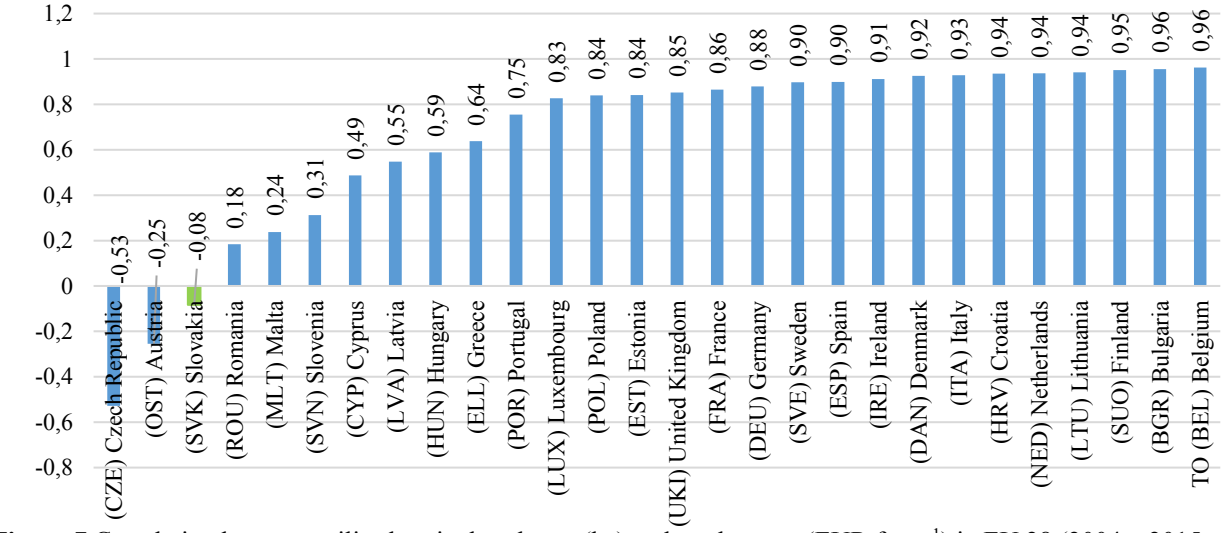

Figure 7 Correlation between utilised agricultural area (ha) and total output (EUR.farm^{-1}) in EU 28 (2004 – 2015 Source: own calculations based on FADN (2018) dat.

decreasing total subsidies when acreage of agricultural area increases or vice versa the more subsidies farmers get the less acreage they will utilise.

Productivity of farms can be represented by many indicators, for instance output, value added or revenue per hectare **(Ladvenicová and Miklovičová, 2015)**. The relationship between farm size and output is one of the basic questions in development economics which was already solved in many research studies. It is well known as the inverse relationship between farm productivity and farm size **(Ciaian, 2012)**. The inverse relationship can be also seen in the correlation of UAA and total output where the expectation of this relation is to be strong, but for namely Slovakia the correlation coefficient (Figure 7) shows trivial relationship with the value of (-0.083). On the other hand, the correlation does not strictly imply causation between two variables, thus these results can't be related explicitly with the subsidies. Therefore, it would be necessary to examine this problem along with the other factors affecting total output.

According to **Ladvenicovoá and Miklovičová (2015)** for Slovak farmers it would be better to operate on smaller size of farm than they do. The inverse relationship between farm productivity and farm size described by **Ciaian (2012)** states that Slovak farmers can profit from this size, since actually implemented CAP system is based on the per hectare support **(Tóth, et al., 2017).** Results of analysis of **Kravcakova, et al. (2016)**, also confirmed strong correlation between amount of gross agricultural production and the volume of subsidies granted in Slovakia.

Tangermann (2011) stated that the CAP after 2013 must move from the decoupling of direct payments to their connection to concrete goals and successes beneficial to society. In the future it would be worth considering the application of hybrid model which is successfully established, for example, in Sweden. Hybrid model **(Blomquist and Nordin, 2017)** is a combination of historical and the regional model, where direct payments are calculated according to the regional model, but with payments per hectare varying between different geographical regions. This approach would be more

suitable, since according to **OECD (2016)** Slovakia is on the 4th position out of 33 states with the biggest regional disparities.

CONCLUSION

Utilised agricultural area of farm in EU 28 varies from less than 3 hectares in Malta to more than 100 in Estonia, United Kingdom and Czech Republic. However, physical size of farm in Slovakia is more than twice bigger than mentioned. Slovakia has the absolutely biggest farms in EU 28 with the UAA value of 556.15 ha.farm^{-1}. This fact is considered by historical size of farm. Implemented CAP system is based on the support per hectare, which can be profitable for the countries with big UAA values as Slovakia. Therefore the implementation of the (CAP) system is bringing annually large amount of subsidies to Slovak agricultural sector, which greatly affects it and even deforms to some extent.

Slovakia has been the leader in the volume of average farm subsidies received over the two (yet finished) program periods and has surpassed all EU 28 countries. Surprisingly, Slovak records show much larger total farm input than farm output, with a difference of 155,076.33 EUR.farm^{-1}, which is the biggest difference among EU 28 countries. This discrepancy is visible also on the efficiency of Slovak farms which is second lowest in EU 28 with the value of 0.76 calculated as the ratio of total output and total input on the farm level. The inverse relationship between farm productivity and farm size was proven in the results of correlation between utilized agricultural area and total output, but for Slovakia with the trivial value of (-0.083). They indicate decreasing returns to scale, where each hectare of land leads to the decrease of production.

Slovakia's coefficient of the correlation between total output and total subsidies indicates moderate positive relationship with the number of 0.37, which means that when total subsidy increases, the value of total output increases proportionally. The strong negative correlation of total subsidies and utilised agricultural area (-0.57) showed inverse relationship what can be interpreted as the more subsidies farmers get, the less acreage will they utilize. Therefore, chosen model of CAP support seems to be not suitable for Slovak conditions and these facts indicate that the currently set subsidy system of CAP in Slovakia does not work entirely efficiently and should therefore be reformed in the forthcoming programming periods. The need to reform CAP system of farmers support in EU is strengthened by the existence of significant regional disparities in EU. Regarding to the generous production potential of individual areas, it is very difficult to select a suitable support system at Member State level.

REFERENCES

Adamisin, P., Kotulic, R. Kravcakova Vozarova, I., Vavrek, R. 2015. Natural climatic conditions as a determinant of productivity and economic efficiency of agricultural entities. *Agricultural Economics – Czech,* vol. 61, no. 6, p. 265-274.

Bakshoodeh, M., Thomson, K. J. 2001. Input and output technical efficiencies of wheat production in Kerman, Iran. *Agricultural Economics,* vol. 24, no. 3, p. 307-313. https://doi.org/10.1111/j.1574-0862.2001.tb00032.x

Blomquist, J., Nordin, M. 2017. Do the CAP subsidies increase employment in Sweden? Estimating the effects of government transfers using an exogenous change in the CAP. *Regional Science and Urban Economics*, vol. 63, p. 13-24. https://doi.org/10.1016/j.regsciurbeco.2016.12.001

Ciaian, P. 2012. *Productivity and farm size. Selective presentation in Slovak Agricultural University in Nitra*. Nitra : Slovak Agricultural University.

Duricová, I. 2016. Government of the Slovak Republic for the Monitoring of Agrarian Policies of the Member States of the OECD 2015 [online] s.a [cit. 2018-05-29] Available at: http://www.vuepp.sk/dokumenty/oecd/oecd2015.pdf.

European Commision. 2014. Direct payments for agriculture. Agriculture. EC, 2014 [online] s.a [cit. 2018-05-29] Available at: http://www.sppk.sk/clanok/239.

Eurostat, 2018. Utilised agricultural area by categories. Dataset details [online] s.a [cit. 2018-05-29] Available at: http://www.sppk.sk/clanok/239.

FADN. 2018. Farm Accountancy Data Network. Data Collection from Agricultural Holdings [online] s.a [cit. 2018-05-29] Available at: http://ec.europa.eu/agriculture/rica/collect_en.cfm.

Gordon, M., Davodora, S. 2004. Farm productivity and efficiency in the CEE applicant countries: a synthesis of results. *Agricultural Economics of Agricultural Economists*, vol. 30, no. 1, p. 1-16. https://doi.org/10.1111/j.1574-0862.2004.tb00172.x

Jankacká, K., Lincényi, M. 2013. Development of economic indicators of agricultural holdings. *Ekonomika poľnohospodárstva,* vol. 13, no. 4, p. 12.

Kao, C. 2017. *Network Data Envelopment Analysis*. Switzerland : Springer International Publishing. 443 p. ISBN 978-3-319-31716-8.

Kravcakova Vozarova, I., Kotulič, R. 2016. Quantification of the effect of subsidies on the production performance of the Slovak agriculture. *Procedia Economics and Finance,* vol. 39, p. 298-304. https://doi.org/10.1016/S2212-5671(16)30327-6

Ladvenicová, J., Miklovičová, S. 2015. The relationship between farm size and productivity in Slovakia. *Visegrad Journal on Bioeconomy and Sustainable Development,* vol. 4, no. 2, p. 46-50. https://doi.org/10.1515/vjbsd-2015-0011

Lančarič, D., Tóth, M., Savov, R. 2013. Which legal form of agricultural firm based on return on equity should be preferred? A panel data analysis of Slovak agricultural firms. *Studies in Agricultural Economics*, vol. 115, p. 172-173. https://doi.org/10.7896/j.1323

MARD SR. 2018. Single Area Payment Scheme – SAPS. Direct payments and cross- compliance. Ministry of Agriculture and Rural Development of the Slovak Republic, 2018. [online] s.a [cit. 2018-05-29] Available at: http://www.mpsr.sk/index.php?navID=47&sID=43&navID2=587.

Matošková, D., Gálik, J. 2013. *Ther Competitiveness of Slovak products of animal origin*. Bratislava, Slovalia : VUEPP, 111 p., ISBN 978-80-8058-594-5.

Munk, M. 2011. *PC data analysis*. Nitra : UKF. 361 p. ISBN 978-80-8094-895-5. (In Slovak)

Obtulovič, P. 2001. *Biostatistics.* Nitra : Slovak Agricultural University in Nitra. 90 p. ISBN 80-7137-901-8. (In Slovak)

OECD. 2016. OECD *Regions at a glance 2016*. Paris, France : OECD Publishing. 184 p. ISBN 978-92-64-25679-8.

Orsághová, D., Kozelová, D., Chládok, P. 2016. Evaluation of consumers' purchase behavior by means of mathematical and statistical methods. *MERAA – Mathematics in Education, Research and Applications*, vol. 2, no. 2, p. 75-81.

SO SR, 2016. Time series database. Slovstat 2016. Statistical Office of the SR [online] s.a [cit. 2018-05-29] Available at: https://slovak.statistics.sk/wps/portal/ext/Databases/slovstat/!ut/p/b1/04_Sj9CPykssy0x

Tangermann, S. 2011. Direct Payments in the CAP Post 2013. Note for the European Parliament's Committee on Agriculture and Rural Development. Directorate-General for Internal Policies [online] s.a [cit. 2018-05-29] Available at: http://www.europarl.europa.eu/RegData/etudes/note/join/2011/438624/IPOL-AGRI_NT(2011)438624_EN.pdf

Tóth, M., Boháčiková, A., Palkovič, J. 2017. Effects of CAP on acess to credit in Slovak Farms. In *Agrarian Perspectives XXVI. Competitiveness of European Agriculture and Food Sectors*. Prague, Czech Republic : Czech University of Life Science, p. 410-416. ISBN 978-80-213-2787-0.

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EVALUATION OF THE FUNCTIONAL STATE OF PEACH VARIETIES (*PRUNUS PERSICA* Mill.) WHEN EXPOSED HYDROTHERMAL STRESS TO PLANTS

Juliya Abilfazova, Oksana Belous

ABSTRACT

The article presents data on the change in the ratio of pigments and fluorescence of chlorophyll in peach leaves in the optimal period of vegetation and under hydrothermal stress. When determining the resistance of a peach to unfavorable environmental factors, methods based on laboratory, fixed changes in the physiological and biochemical processes occurring in plants are used. In the period of inadequate water availability, the water deficit in peach leaves increased to an average of 15%, while less stable – an increase in the parameter to 18% was observed. It is shown that the xeromorphic character of the leaf apparatus is associated with a slight change in the anatomical characteristics of the leaf (the thickness of the leaf blade before and after the drought), which determines the permanence of the turgor. In this case, in the leaves of resistant varieties, the loss of turgor is insignificant (LSD ($p \leq 0.05$) = 7.24); the thickness of the leaf fluctuates within 0.20 mm. On the background of stress impact, a clear varietal difference was shown, which allowed us to develop a scale for a comparative assessment of the resistance of peach varieties and clones. During the active growth of the assimilation surface, an increase in the amount of green pigments in the leaves of experimental plants was noted. Perspective varieties of peach contain significantly higher amounts of chlorophylls compared to other varieties (LSD ($p \leq 0.05$) = 0.30). Under unfavorable conditions, in these varieties the ratio of the sum of chlorophylls to carotenoids is higher, which is confirmed by their more developed adaptive potential. Reorganization of the pigmentary apparatus during the period of hydrothermal stress is accompanied by an increase in the coefficient of photosynthetic activity (Kf_n) and a decrease in the fluorescence level (F_T) of chlorophyll. Thus, the water deficit, pigment composition and fluorescence of chlorophyll make it possible to identify the resistance of peach varieties and clones to the action of hydrothermal stressors. Based on the results of the studies, the most resistant varieties and clones of peaches have been identified for the humid subtropics of Russia (Larisa, Early bloy, Medin red, Slavutich, Donetskij zheltij, Vanity and Form 1).

Keywords: peach; humid subtropics; hydrothermal stress; pigments; fluorescence; water deficit; viability; sustainability

INTRODUCTION

Peach (*Prunus persica* Mill.) – is a perennial deciduous plant from East Asia, belonging to the family of rosaceous (*Rosaceae* Juss). The height of the tree is up to 5 – 8 m, the diameter of the trunk is up to 30 cm. Crown, depending on the variety, is broad-spread or back-pyramidal. Peach distinguishes leaf and flower buds, which are located in the axils of the leaves. Leaves are alternate, narrow-lanceolate with jagged edges. Flowering is abundant, lasts from 5 to 20 days, flowers – pink or campanulate **(Shaitan et al., 1989)**. In conditions of humid subtropics of Russia, a productive period with a fairly stable crop can last from 15 to 20 years. The silvicultural care of forest plantations for culture includes the use of systems for the formation of crowns, taking into account the age and variety, the use of fertilizers and phyto-processing, which contributes to the fruitfulness of the peach (in the amount of more than 25 c.ha^{-1}) from the third year of life **(Eremin, 2006; DeJong et al., 2004; Tworkoski and Takeda, 2007; Zec et al., 2013; Ryndin et al., 2016)**. Fruit ripening depending on the variety occurs from July to September, which makes it possible to provide the population with fresh fruits for a long period of time (**Eremin, 2006; Insausti and Gorjon, 2016**).

Peach is one of the leading drupaceous fruit crops, which, because of its high rate of fertility, is the most economically viable **(Cociu et al., 1985; Vietoris et al., 2014; Divis et al., 2017; Fikselova et al., 2018)**. The popularity of peach is great all over the world, it is grown in almost all European countries (Austria, France, Germany, Italy, Croatia, etc.), Asian countries (Afghanistan, Pakistan, Nepal, India, etc.), America (Argentine, Brazil, Bolivia, Peru, Venezuela, etc.), in the United States and Canada, in African countries (Kenya,

Zimbabwe, Ethiopia, Egypt, etc.) and in the post-Soviet space (Armenia, Azerbaijan, Kazakhstan, Moldova, Tajikistan etc.). **(FAOSTAT, 2018; DeJong et al., 2004; Eremin and Eremina, 2014; Ryndin et al., 2016)**. In Russia it is grown (in Transcaucasia, Krasnodar Krai, Crimea and other regions), not only for domestic use, but also exported (on average 253 tons) to such countries as Mongolia, Belarus, Ukraine, etc. Demand for fruits peach is always quite high, so, only in 2017, in addition to its products, 185 thousand tons of fruits were imported to Russia, mainly from China and Serbia (on average, 28 – 29% of imports) **(Export and import of Russia by goods and countries, 2018)**.

Crops are high in vitamins, sugars, pectins and organic acids, a fairly large list of chemical elements (potassium, magnesium, calcium, zinc, manganese, etc.). The market annually offers new, more advanced varieties for resistance to abiotic and biotic stressors. It is no exception that complex studies conducted at the All-Russian Scientific Research Institute of Floriculture and Subtropical Crops (Sochi) aimed at identifying the most adaptive plants to biotic and abiotic factors with the output for obtaining stable-yielding, high-quality varieties (**Abilfazova, 2014; Abilfazova, 2016; Abilfazova, 2018; Ryndin et al., 2011; Smagin, 2014; Besedina et al., 2017)**. At this given moment, the collection of the Institute includes 58 varieties.

In humid subtropics of Russia, soil and climatic conditions are the limiting factors for this culture: the spring is cold and rainy, in summer there is air and soil drought against the background of high solar activity, air temperature up to 30 °C and above, relative humidity over 80% **(Abilfazova, 2014)**. These adverse conditions contribute to the weakening of the adaptive potential of the crop, which leads to a decrease in yield, a deterioration in the quality of products, and often also to plant death.

Scientific hypothesis

The most resistant varieties have a loss of turgor insignificant; higher amounts of chlorophylls; the ratio of the sum of chlorophylls to carotenoids is higher increase; and higher the coefficient of photosynthetic activity (Kf_n). The water deficit, pigment composition and fluorescence of chlorophyll make it possible to identify the resistance of peach varieties and clones to the action of hydrothermal stressors.

MATERIAL AND METHODOLOGY

Experimental varieties and clones were selected: Red Heaven, Larisa, Early red, Earley blow, Slavutich, Donetskij zheltij, Donetskij belij, Medinas red, Pamjat Grishko, Vanity, Osennij sjurpriz, Form 1 and Form 2. Plant was planting 2004 – 2008. Under the scheme of 5 x 2 m grown on the plantation of the experimental and technological department of the sector of fruit crops Institute. The stock of the AP – 1 ("Kuban" – 86).

The site soil – brown forest, residual-carbonate, depth 78 – 100 cm, humus content 1.39 – 2.95%, pH = 6.49 – 7.86. Agrotechnics common for cultivation of peach culture in conditions of humid subtropics of Russia provides V-shaped pruning with short annual pruning for fruiting **(Eremin, 2006)** and introduction of a fertilizer complex N120P90K90.

The selection of leaves for laboratory tests was carried out from june to september. As an indicator organ used physiologically mature leaves, which were the 7^{th} or 9^{th} leaf from the base of the sprout. To diagnose the varietal stability of the peach against unfavorable environmental conditions, the index of water deficiency, the content of photosynthetic pigments and an estimation of the functional state of plants by the parameters of slow induction of fluorescence of chlorophyll were used using the following methods:

- Water deficiency by comparing the water content in plant tissue with the amount of it in the same tissue in a state of complete turgor **(Gol`d et al., 2008)**;
- The content of chlorophyll a, b and carotenoids in leaves by spectrophotometric method (spectrophotometer PE-5400 VI, Russia) in acetone extracts (absorption 662, 644 and 440.5 nm, respectively) **(Rogozhin, 2013)**;
- Slow induction of fluorescence of chlorophyll – on LPT-3CF / RT-Df (Russia) instrument with subsequent processing of information in the computer program "SIA Interface", developers Budagovskaya ON, Budagovsky AV and Budagovsky IA **(Budagovsky et al., 2010)**.

STATISTICAL ANALYSIS

Statistical processing of the experimental data was carried out using the ANOVA package in STATGRAPHICS Centurion XV, version 15.1.02 and MS Excel 2007.

Statistical analysis included univariate analysis of variance (method of comparing averages using variance analysis, t-test) and variance analysis (ANOVA). The significance of the difference between the means of the least significant difference (LSD) with p-values ≤ 0.05 was also assessed. For establish the dependence of parameters on abiotic factors, correlation analysis with calculation of pair correlation coefficient was used.

RESULTS AND DISCUSSION

During the year, the average air temperature in the humid subtropical of Russia is 15.2 °C. The coldest month is January with an average temperature of 6.3 °C, and the warmest in August (an average of about 26.1 °C). However, during periods of active plant vegetation, meteorological conditions are often critical. A significant factor contributing to crop losses and deterioration in the quality of peach fruits is the increased instability and stress of weather conditions, as a result of which plants are exposed to a complex of unfavorable factors of nutrient and abiogenic nature **(Smagin, 2014; Tsipouridis et al., 2005)**. For more complete diagnosis of the functional state of plants in modern studies, one or two analyzes are not enough, complex ecological and physiological studies are needed to identify promising varieties and forms that are resistant to changing environmental conditions **(Ryndin et al., 2014)**. Lack of moisture is one of the often occurring adverse environmental factors affecting plants. The greatest harm it inflicts in the spring and summer, when the formation of generative organs of plants. In conditions of humid subtropics of Russia, repeated long droughts in combination with high temperature and solar insolation lead to the inhibition of plants, considerable shedding of generative formations and loss of yield. Violation of the water regime of plants is reflected in all physiological

functions, and the resulting wilting of plants is accompanied by the appearance of water deficiency.

That is why the analysis of the functional state of plants under conditions of drought is carried out not only on indicators with an assessment of the state of the photosynthetic apparatus, but also with the analysis of water deficiency in leaves.

Analysis of the weather in the years of observation showed that in 2016 **(Weather data of Sochi, 2016-2017)** from May to August 575 mm of precipitation, which is almost equal to the average multi-year (566 mm). At the same time, in June and August a dry period was observed - only 50% of the norm fell out. The year 2017 was also characterized by a dry period during the active vegetation period: if in May the rainfall norm was closed – 184 mm (110 mm norm), in June 82% fell, in July – 53% of the average annual rate, in August it was only 26%. The years of observation were characterized by lower temperatures; the deviation from long-term values in 2016 was -7.3 °C, in 2017: -5.4°C. This led to a delay in vegetation on average by two weeks. However, during the growing season (June-September), the maximum air temperature exceeded the long-term norm and rose to 31.6 – 34.1 °C, which caused the early decay of growth processes on the background of the drought, affected the quality and quantity of the crop harvest. Determination of the level of water deficiency in peach leaves showed that in a favorable period this figure did not exceed 12%. In the period of inadequate water availability (July-August), in all varieties the water deficit rose to an average of 15%, in less stable - an increase in the parameter was observed to 18% (Figure 1), which correlated with high air temperature (r = +0.77). The most resistant was the grade Earley blow in which the water deficit in the leaves was significantly lower than the control Red Heaven in both optimal and stressful periods (LSD ($p \leq 0.05$) = 5.46).

Varieties Vanity and Osenniy surpis are later ripening, during the active fruiting period (July-August) they get into unfavorable conditions when the air temperature reaches +35 °C and higher against the background of a long absence of precipitation. However, low resistance to stressors of humid subtropics of Russia does not entreat the value of these varieties for use as quality indicators donors.

The increase in the indicator "water deficiency" was accompanied by a decrease in the leaf turgor and a change in its anatomical characteristics. Measurement of the thickness of leaf blades before and after a drought showed that in leaves of resistant peach varieties the loss of turgor was insignificant (LSD ($p \leq 0.05$) = 7.24), the thickness of the leaf fluctuated within 0.20 mm.

Against the backdrop of stress, a clear varietal difference was shown, which allowed us to develop a scale for a comparative assessment of the resistance of peach varieties and clones (Table 1).

During the impact of stressors in the most resistant to drought varieties and clones, the water deficit rises to 15%, the largest loss of turgor is observed because of the reduction in the thickness of the leaf blade to 0.14 mm. For medium-resistant varieties and clones, the water deficit is 18%, the thickness of the leaf blade is 0.12 mm. In low-resistant varieties, the water deficit is more than 18%, the thickness of the leaf is less than 0.10 mm. When studying the water regime of a peach, it was found that drought-resistant varieties are distinguished by an increase in water-holding capacity during a period of stress of hydrothermal conditions and a decrease in leaf turgor in comparison with unstable plants. Judging by the loss of water by the leaves of the plants (within 24 hours), Red Heaven, Medin red and Larisa varieties (65 – 72%) were distinguished by a high water-holding capacity, which is one of the signs of their better adaptation to unfavorable external conditions.

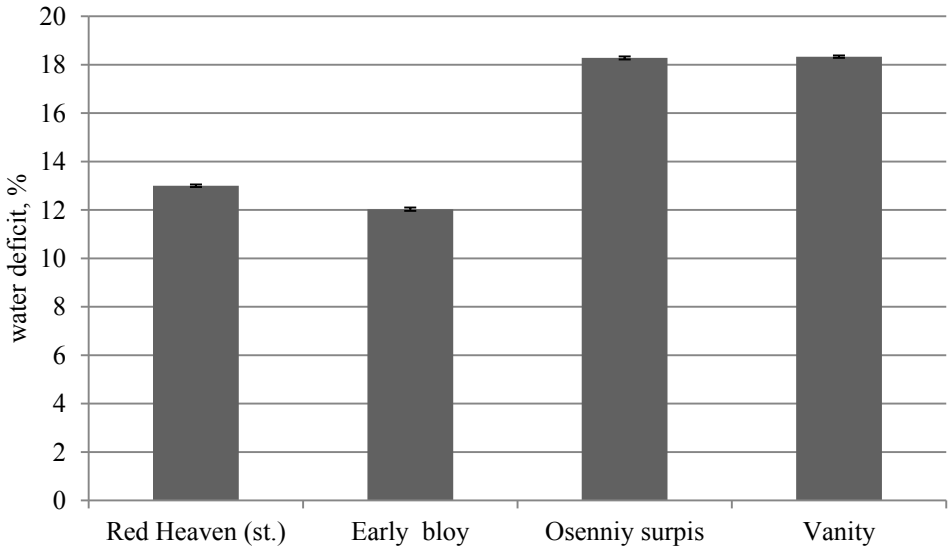

Figure 1 Water deficiency of leaves of resistant and unstable peach varieties, (LSD ($p \leq 0.05$) = 5.46.

Table 1 The scale of changes in the parameters of the water regime of leaves for a comparative assessment of the drought resistance of peach varieties and clones.

Terms of assessment	Parameter	Degrees of resistance of varieties		
		high	mid	low
Optimal conditions	Water deficit, %	10 – 12	12 – 15	>15
	Leaf thickness, mm	0.17 – 0.22	0.15 – 0.17	<0.15
Drought period	Water deficit, %	10 – 15	15 – 18	>18
	Leaf thickness, mm	0.13 – 0.15	0.10 – 0.13	<0.10

Table 2 The content of photosynthetic pigments in the leaves of experimental varieties and peach clones (mg.100 g^{-1} wet weight).

Cultivars/clones	Chlorophyll			
	a	b	a+b	a/b
Red Heaven (st.)	2.03 ±0.03	1.31 ±0.01	3.34 ±0.05	1.56 ±0.01
Larisa	2.27 ±0.01	1.60 ±0.01	3.87 ±0.03	1.41 ±0.01
Early red	2.08 ±0.01	1.26 ±0.02	3.34 ±0.03	1.65 ±0.02
Early bloy	1.92 ±0.02	1.10 ±0.02	3.06 ±0.04	1.83 ±0.01
Slavutich	2.28 ±0.03	1.67 ±0.01	3.95 ±0.02	1.38 ±0.01
Donetskij zheltij	2.22 ±0.03	1.58 ±0.01	3.80 ±0.06	1.41 ±0.03
Donetskij belij	2.19 ±0.04	1.29 ±0.01	3.48 ±0.01	1.79 ±0.01
Medin red	1.99 ±0.01	1.24 ±0.03	3.23 ±0.01	1.62 ±0.03
Pamjat Grishko	1.79 ±0.01	1.07 ±0.01	2.86 ±0.02	1.69 ±0.03
Vanity	2.26 ±0.02	1.56 ±0.04	3.82 ±0.04	1.45 ±0.02
Osenniy surpis	2.17 ±0.01	1.35 ±0.01	3.52 ±0.03	1.64 ±0.01
Form 1	1.92 ±0.01	1.09 ±0.01	3.01 ±0.02	1.77 ±0.01
Form 2	1.81 ±0.01	0.99 ±0.01	2.80 ±0.02	1.82 ±0.02

As one of the main criteria for assessing the functional state of plants under adverse conditions is the state of the photosynthetic apparatus, which is very sensitive to external influences. High air temperatures (above +30 °C) and insufficient water supply lead to a disruption of the synthesis of green pigments with an increase in the amount of carotenoids.

During the research in the optimal period of vegetation during the active growth of the assimilation surface, we noted an increase in the amount of green pigments in the leaves of experimental plants (Table 2). Peach varieties such as Larisa, Donetskij zheltij, Slavutich and Vanity contain significantly higher amounts of chlorophylls than other varieties (LSD ($p \leq 0.05$) = 0.30). In the same varieties, a low Ca/Cb ratio is also noted, which is an indicator of plant resistance to stressful conditions.

As the hydrothermal stress intensifies, the ratio of the sum of chlorophylls to carotenoids in all varieties and peach clones increases, which indicates the inclusion of adaptation mechanisms (Figure 2). At the same time, in the varieties Larisa, Donetskij zheltij, Slavutich and Vanity, this indicator is higher, which confirms their more developed adaptive potential.

For the diagnosis of the physiological state of plants, in particular, the study of the influence of abiotic factors on the photosynthetic productivity of plants, the fluorescence method **(Budagovskaya et al., 2010; Budagovskaya, 2013)**, which fixes the slow induction of chlorophyll fluorescence (SICF or Kautsky effect) is increasingly used. This method allows evaluating the efficiency of photosynthetic transformation of the energy absorbed by the leaf. Devices developed for this purpose A. V. Budagovskiy, O. N. Budagovskoy and I. A. Budagovskii, automatically register the SICF curve and calculate its main parameters **(Budagovsky et al., 2010)**. Determined by the effect of Kautsky (the change in the fluorescence intensity of chlorophyll, which occurs under the action of sufficiently bright light, reflects the work of the photosynthetic apparatus of the cell), the kinetics of IFC shows the state of the photosynthetic apparatus of the cell and serves as an important diagnostic indicator **(Budagovskaya, 2013)**.

As a result of the conducted studies it was found that high temperatures, affecting the functional state of plants, in particular, on the pigment composition of the leaf, led to a decrease in the viability level (F_m/F_T) of a number of varieties (Table 3).

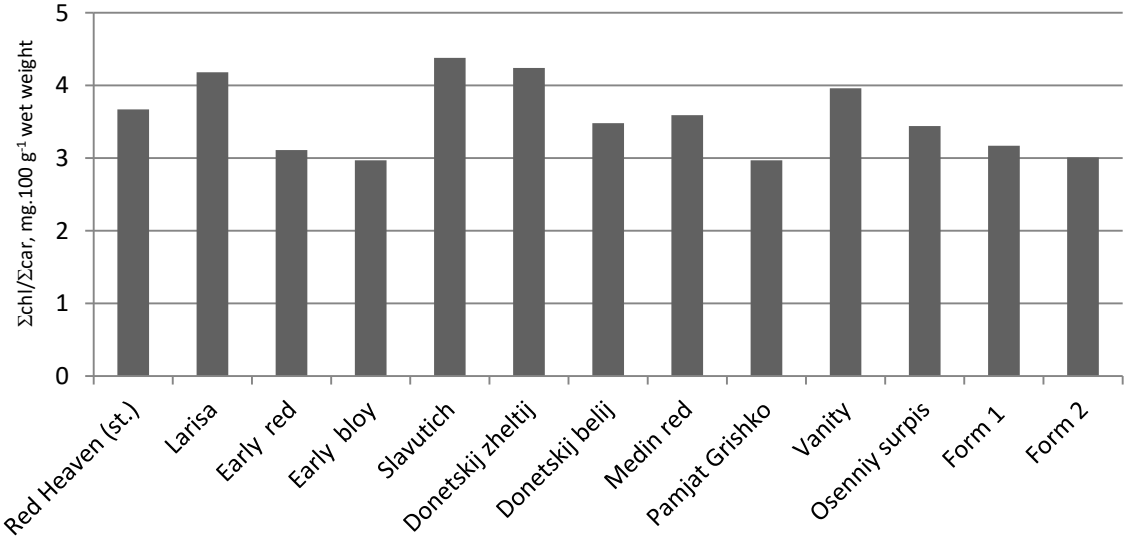

Figure 2 The ratio of the amount of chlorophylls to carotenoids in the critical period of vegetation LSD ($p \leq 0.05$) = 1.05.

Table 3 Influence of hydrothermal stressors on the fluorescence of peach leaf chlorophyll

Cultivars/clones	Fm*	$F_{\_T}$*	Kf_n*	Fm/F_T*
Red Heaven (st.)	124.11	37.13	0.46	3.52
Early red	130.96	40.54	0.46	3.24
Early bloy	115.16	18.90	0.58	6.18
Donetskij zheltij	125.45	29.43	0.52	4.43
Donetskij belij	131.66	35.11	0.49	3.83
Medin red	122.02	30.12	0.52	4.45
Pamjat Grishko	126.80	31.66	0.49	4.16
Osenniy surpis	135.07	46.80	0.45	3.11
Form 1	123.13	30.42	0.51	4.50
Form 2	128.28	36.76	0.46	3.52

Note: * Fm is the fluorescence maximum; $F_{\_T}$ – stationary level of fluorescence; Kf_n is the coefficient of photosynthetic activity; F_m/F_T - viability index.

In stable varieties (Early bloy, Medin red, Donetskij zheltij, and Form 1), this process was characterized by an increase in the coefficient of photosynthetic activity (Kf_n) and a decrease in the fluorescence level ($F_{\_T}$) of chlorophyll.

CONCLUSION

Thus, when determining the resistance of peach plants to unfavorable environmental factors, methods based on laboratory, fixing changes in physiological and biochemical processes occurring in plants are used. Such indicators as water deficit, pigment composition and fluorescence of chlorophyll allow revealing the resistance of varieties and clones of peach to the action of hydrothermal stressors. Thus, it was found that the water deficit in the peach leaves during unfavorable for water availability did not exceed 15%, which is due to the xeromorphic nature of the leaf device. Simultaneously, an increase in the viability index and the coefficient of photosynthetic activity was observed in a number of varieties (Early bloy, Medin red, Donetskij belij, Form 1). According to the results of the research, the varieties and clones of peach that are the most resistant to the stressful conditions of the humid subtropics of Russia are noted (Larisa, Early bloy, Medin red, Slavutich, Donetskij zheltij, Vanity and Form 1).

REFERENCES

Abilfazova, Yu. S. 2014. Physiological and biochemical indicators of peach stability in relation to weather conditions in Sochi. *Horticulture and viticulture,* vol. 2004. no. 4, p. 42-44.

Abilfazova, Yu. S. 2016. Persistence of peach to stress factors of humid subtropics of Russia. *Vestnik of the Russian agricultural science*, [S.l.], no 6, p. 40-42. Available at: http://www.vestnik-rsn.ru/vrsn/article/view/336.

Abilfazova, Yu. S. 2018. Correlation dependence of indicators of the water status of *Persica vulgaris* (Mill.) On hydrothermal stressors of humid subtropics of Russia. *New technologies,* vol. 2018, no 1, p. 100-105.

Besedina, T. D., Smagin, N. E., Dobezhina, S. V. 2017. Adaptive potential of varieties of peach cultivated in humid subtropics of Russia. *APK Stavropol Newsletter*, vol. 1, no. 25, p. 123-129.

Budagovskaya, O. N. 2013. *Laser-optical methods and technical means of multiparameter diagnostics of plants and fruits*: Thesis of doctor of Technical Sciences. Moscow, Russia. 39. p. Available at: https://dlib.rsl.ru/viewer/01005532090

Budagovskaya, O. N., Budagovskii, A. V., Budagovskii, I. A. 2010. *Laser diagnostics of plant*s. *Methodical. recommendations*. Michurinsk : Publishing House "Michurinsk". 54 p. ISBN 978-5-98429-064-7.

Budagovsky, A. V., Budagovskaya, O. N., Budagovskiy, I. A. 2010. Paradoxes of the optical properties of green cells and their practical application. *Photonics*, vol. 6, p. 22-28.

Cociu, V., Hough, L. F., Ionescu, P. R., Topor, E. 1985. Results on breeding new very early and early ripening peach and nectarine varieties. *Acta Horticulture,* vol. 173, p. 25-30. https://doi.org/10.17660/ActaHortic.1985.173.3

DeJong, T. M., Johnson, R. S., Doyle, J. F., Weibel, A., Solari, L., Marsal, J., Basile, B., Ramming, D., Bryła, D. 2004. Growth, yield and physiological behaviour of size-controlling peach rootstocks developed in California. Acta Horticulture, vol. 658, p. 449-455. https://doi.org/10.17660/ActaHortic.2004.658.66

Diviš, P., Šťávová, E., Pořízka, J., Drábiková, J. 2017. Determination of tin, chromium, cadmium and lead in canned fruits from the czech market. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 564-570. https://doi.org/10.5219/749

Eremin, V. G. 2006. New technologies of peach cultivation in the Krasnodar region. Horticulture and viticulture, no. 6, p. 7-8.

Eremin, V. G., Eremina, O. V. 2014. Peach assortment for Krasnodar region. Contemporary horticulture, vol. 2, p. 6-12. Available at: http://journal.vniispk.ru

Export and import of Russia by goods and countries. 2018. [online] s.a. [cit.2018-08-07] Available at: http://ru-stat.com/date.

FAOSTAT. 2018. [online] s.a. [cit.2018-08-14] Available at: http://www.fao.org/faostat.

Fikselová, M., Czako, P., Gažo, J., Mendelová, A., Mellen, M. 2018. Sauvignon wine quality as affected by its processing and storage. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 299-303. https://doi.org/10.5219/885

Gol`d, V. M., Gaevsky, N. A., Golovanova, T. I., Belonog, N. P., Gorbaneva, T. B. 2008. *Plant physiology : method. instructions on the lab. work*s. Krasnoyarsk, Russia : IPK SFU, 61 p. ISBN 978-5-7638-1275-6.

Insausti, P., Gorjon, S. 2016. Floods affect physiological and growth variables of peach trees (*Prunus persica* (L.) Batsch), as well as the postharvest behavior of fruits. *Scientia Horticulturae*, vol. 152, p. 56-60. https://doi.org/10.1016/j.scienta.2013.01.005

Rogozhin, V. V. 2013. *Training manual on plants physiology and biochemistry*. Saint-Petersburg, Russia: GIORD. 350 p. ISBN: 978-5-98879-151-5.

Ryndin, A. V., Belous, O. G., Malyarovskaya, V. I., Pritula, Z. V., Abilfazova, Yu. S., Kozhevnikova, A. M. 2014. The use of physiological and biochemical methods for identifying the mechanisms of adaptation of subtropical, southern fruit and ornamental crops in the subtropical environment of Russia. *Agricultural Biology*, no 3, p. 40-48. https://doi.org/10.15389/agrobiology.2014.3.40rus

Ryndin, A. V., Lyakh, V. M., Smagin, N. E. 2016. Peach culture in different countries of the world. *Subtropical and decorative horticulture*, vol. 57, p. 9-24.

Ryndin, A. V., Smagin, N. E., Abilfazova, Yu. S. 2011. Perspective varieties of peach for humid subtropics of Russia (Sochi). *Subtropical and decorative horticulture*, vol. 44, p. 119-123. ISSN: 2225-3068.

Shaitan, I. M., Chuprina, L. M., Anpilogova, V. A. 1989. Biological features and cultivation of peach, apricot and cherry plum. Kyiv, Ukraine : Naukova Dumka. 154 p. ISBN 5-12-00082.

Smagin, N. E. 2014. The impact of extreme weather conditions on the productivity of peach in subtropical regions of Russia. Material conference. Sochi, p. 335-340. Available at: https://elibrary.ru/item.asp?id=35014590.

Tsipouridis, C., Thomidis, T., Michailides, Z., 2005. Influence of some external factors on the rooting of GF677, peach and nectarine shoot hardwood cuttings. *Aust. J. Exp. Agric*., vol. 45, p. 107-113. https://doi.org/10.1071/EA03120

Tworkoski, T., Takeda, F. 2007. Rooting response of shoot cuttings from three peach growth habits. *Scientia Horticulturae*, vol. 115, p. 98-100. https://doi.org/10.1016/j.scienta.2007.08.004

Vietoris, V., Czako, P., Mendelová, A., Remeňová, Z., Závracký, M. 2014. Relations between must clarification and organoleptic attributes of wine varieties. *Potravinarstvo*, vol. 8, no. 1, p. 155-160. https://doi.org/10.5219/359

Weather data of Sochi. Hydrometeorological center. 2016-2017. [online] s.a. [cit.2018-09-03] Available at: http://www.pogodasochi.ru/inf.

Zec, G., Fotirić Akšić, M., Čolić, S., Vulić, T., Nikolić, D., Oparnica, Č., Janković, Z. 2013. Influence of vineyard peach selections on vigour and initial yield in peach and nectarine. *Genetika*, vol. 45, no.1, p. 11-20. https://doi.org/10.2298/GENSR1301011Z

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Influence of feeding colored wheat varieties on selected quality parameters of broiler chicken's meat

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ABSTRACT

The effect of feeding colored wheat varieties (PS Karkulka and Skorpion) on chicken´s performance and texture, color and sensory characteristics of broiler chicken´s meat were evaluated in this study. The experiment was performed with 66 of Ross 308 cockerels. Cockerels were divided into three equal groups. The two experimental groups received feed mixtures containing 40% of wheats with different grain colour: groups PS Karkulka (n = 22) with PS Karkulka wheat cultivar and Skorpion (n = 22) with Skorpion cultivar. The third group (n = 22) had 40% of common wheat Vánek cultivar (Control). The live weight of broilers between all three groups was not significant different, as well as carcass yield and chemical composition of breast and thigh meat of chickens. In the parameter Razor Shear Force was found statistically significant higher breast meat tenderness in PS Karkulka against Control and Skorpion groups. In parameters L* and b* of colour of the meat samples was found statistically significant higher value in L* parameter in Skorpion group and b* parameter was higher in Control group. The total colour change was 2.25 and 2.53 for PS Karkulka and Skorpion group, respectively. In sensory analysis of broilers breast muscle was found statistically significant differences in odour, colour, fibreness, chewiness, juiciness and flavour parameter. The fatty taste parameter was non-significant. The odour parameter of chicken´s breast muscle was significantly lower in Skorpion group against PS Karkulka and Control groups. The significantly most intense colour of breast muscle was found in Control group versus Skorpion and PS Karkulka groups (91.71 mm, 79.71 mm and 71.15 mm, respectively). The fibreness parameter was significantly higher for Control group, as well. Significantly higher chewiness of breast meat was in Control (68.49 mm) than PS Karkulka (52.02 mm) and Skorpion (43.32 mm) group. The feeding of wheat cultivars with different grain pigmentation had no effect on performance parameters of broiler chicken´s as well as to it´s body and chemical composition of breast and thigh meat in this study.

Keywords: poultry nutrition, purple pericarp, blue aleurone, meat quality, sensory traits

INTRODUCTION

Anthocyanins are found in plants in glycosylated forms, generally linked with glucose, galactose, arabinose, rhamnose, xylose and fructose **(Choi et al., 2007; Hosseinian and Beta, 2007).** Cyanidin is the most common anthocyanidin (aglycone) followed by delphinidin, peonidin, pelargonidin, petunidin and malvidin **(Oomah and Mazza, 1999)**. Anthocyanins have evoked the interest of many researchers. Anthocyanins are common constituents in colored fruits and vegetables. These substances can act as antioxidants and help in prevention of cardiovascular diseases **(Kris-Etherton et al., 2004)**, diabetes **(Patel et al., 2013)**, inflammation, cancer **(Arts and Hollman, 2005)**, obesity **(Tsuda et al., 2003)** and aging **(Chen et al., 2013)**.

The colour in wheat grain is mainly due to natural pigments (such as anthocyanins). These substances accumulate in the aleurone layer or pericarp of wheat and provide the blue, purple and red colours of the grain **(Ficco et al., 2014)**. Common wheat cultivars across the world are white (amber) in color. The colored wheat, rich in anthocyanin is quite uncommon. Black wheat resulted by the combination of genes for both purple and blue colors. Colored wheat has attracted the attention of many breeders across the world but these lines exhibit low yield compared to conventional varieties **(Martinek et al., 2014)**.

Modern bird hybrids grow very fast due to genetic selection, improved nutrition and efficient production systems. Broiler chicken´s meat have high breast meat yields due to the high demand for breast meat. Selection for fast growth and high yield chickens may have negative impact to the sensory and functional parameters of the meat **(Dransfield and Sosnicki, 1999**; **Fanatico et al., 2007)**. A range of natural substances such as propolis may positively affects the sensory quality of Ross 308 chickens

meat, which is one of the most important parts for use in human food chain **(Haščík et al., 2012)**. Chickens meat from our previous study with colored wheat varieties was characterized by steady overall quality between Konini wheat and UC66049 wheat. The effect of various wheat feeding did not affect meat quality of broiler chicken´s **(Šťastník et al., 2017)**. In the previous study were evaluated a genetic resources but not a registered varieties in the Czech Republic. This study already carried out on registered varieties, thereby increasing the relevance of the study for practice.

The effect of feeding colored wheat varieties (PS Karkulka and Skorpion) on chicken´s performance and texture, color and sensory characteristics of broiler chicken´s meat were evaluated in this study.

Scientific hypothesis

The inclusion of non-traditional colored wheat varieties containing anthocyanins will influence selected quality parameters of chicken´s meat.

MATERIAL AND METHODOLOGY

The animal procedures were reviewed and approved by the Animal Care Committee of the Mendel University in Brno.

Animals and nutrition

The experiment was performed with 66 of Ross 308 cockerels. Chickens were fed with starter feed mixture until seventh day of age. From the seventh to the tenth day of the chickens a preparatory period was carried out. Chickens were fed with experimental and control feed mixtures in this period. The trial was performed from day 10 to day 36 of chick's age. Cockerels were divided into three equal groups. The two experimental groups received feed mixtures containing 40% of wheats with different grain colour: groups PS Karkulka (n = 22) with PS Karkulka wheat cultivar and Skorpion (n = 22) with Skorpion cultivar, respectively. The third group (n = 22) had 40% of common wheat Vánek cultivar (Control). Table 1 shows proximate analyses of used wheat varieties and Table 2 shows nutrient composition of experimental diets. The rations were calculated according to the Broiler nutrition specifications **(Aviagen group, 2014a)**. The chickens were fed *ad-libitum*. Table 3 shows chemical composition of used feed mixtures.

The conventional deep litter system with wood shavings were used. Room temperature, humidity and lighting regime were controlled according to requirement for actual age of chickens in **Aviagen group (2014b)**. Health status was evaluated daily and live weight measured every week during the trial.

The chemical composition of nutrient content of diets were determined for dry matter, crude protein, ether extract, crude fibre, and ash according to Commission Regulation (EC) **(Commission Regulation, 152/2009)**. The total content of anthocyanins was measured by previous published methods **Varga et al. (2013)** and

Table 1 Chemical analysis of used wheat varieties (in dry matter).

	Control	PS Karkulka	Skorpion
Crude Protein (%)	13.40	13.68	16.76
Ether extract (%)	1.43	1.57	1.60
Crude fibre (%)	2.88	2.92	2.44
Ash (%)	1.46	1.79	1.81
Cyanidin 3-glucoside ($mg.kg^{-1}$)	3.64	37.24	37.93

Table 2 Nutrient composition of diets ($g.kg^{-1}$).

Component	Control	PS Karkulka	Skorpion
Wheat	400	400	400
Soybean meal	260	260	260
Maize	205	207.5	223.7
Rapeseed oil	40.5	40.5	40.5
Wheat gluten	31	28	12.2
Premix*	30	30	30
Maize starch	20	20	20
Monocalciumphosphate	7.5	7.5	7.5
$CaCO_3$	3	3	3
L-Lysine	1.5	2.1	1.7
DL-Methionine	1.5	1.5	1.5

Note: *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; phylloquinone 50 mg; thiamine 140 mg; riboflavin 230 mg; cobalamin 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.

Table 3 Proximate analysis of used diets in dry matter.

	Control	PS Karkulka	Skorpion
AME (MJ.kg^{-1})*	12.61	12.61	12.62
Crude Protein (%)	23.57	23.22	23.55
Ether extract (%)	6.66	6.37	6.41
Crude fibre (%)	5.31	5.17	5.38
Ash (%)	6.41	6.23	6.10

Note: *Apparent Metabolisable Energy, calculated value.

expressed as the cyanidine-3-glucoside content.

Meat samples preparation

At the end of the experiment fifteen birds were selected randomly from each group, weighed and slaughtered. Feathers were removed, and chickens were eviscerated.

Table 4 Live weight (g) of broilers at 36th day of age.

Group	n	Mean ±standard error
Control	22	2,218 ±42.95
PS Karkulka	21	2,048 ±78.15
Skorpion	22	2,033 ±48.40

Note: Differences between groups are not statisticaly significant (p >0.05).

Carcass yield was calculated. Breast and leg meats without skin were separated from carcasses after cooling. All visible external fat was removed from sample meats. The breast and leg meat were weighed, and their percentage of live body weight was calculated.

The left part of breast and the left of thigh were pack up in aluminium foil, marked and stored at -20 °C until sensory analyses. Meat from the right half of breast and deboned right thigh meat were milled (in machine Moulinex Moulinette; France). Dry matter content of meat was determined by a method with sea sand and the total nitrogen according to Kjeldahl using OPSIS Liquid Line (KjelROC Analyser; KD 310-A-1015; Sweden). The crude protein content was calculated using the factor 6.0 (N*6) pertinent to meat. The content of total fat was determined gravimetrically after extraction with diethylether under reflux for 6 hours.

Texture and colour of meat

The tenderness of the raw fillets (breast muscle) was determined through the application of the Meullenet–Owens razor shear (MORS) test, using a texture analyser TIRATEST 27025 (TIRA Maschinenbau GmbH, Germany) as described by **Meullenet et al. (2004)** and **Cavitt et al. (2005)** during which Razor Shear Force (N) was recorded. Tests using the MORS blade are conducted on whole intact right fillets with 5 replicates 1-hour post mortem. The sharp blade was replaced every 80 measurements for optimum shearing performance. Test Settings: test speed 10 mm.s^{-1}, penetration depth was 20mm.

Colour measurement was performed by CIE L*a*b* colour space. L* (lightness), a* (redness) and b* (yellowness) values from the breast meat sample surface on the dorsal side were measured using a Spectrophotometer CM-3500d (Konica Minolta Sensing Inc., Japan) in SCE mode (specular component excluded), angle 8°, 8 mm slot. 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)**:

$$\Delta E^*_{ab} = \sqrt{(L^*_{control} - L^*_{group})^2 + (a^*_{control} - a^*_{group})^2 + (b^*_{control} - b^*_{group})^2}$$

Sensory analysis

Sensory analysis of breast (n = 6) and leg meat (n = 6)

Table 5 Body composition of chickens.

Group	n	Carcass (%)	Breast meat (%)	Thigh meat (%)
		Mean ±standard error		
Control	15	69.4 ±0.40	21.5 ±0.55	14.7 ±0.28
PS Karkulka	15	68.0 ±0.69	20.0 ±0.69	15.1 ±0.32
Skorpion	15	66.5 ±0.31	18.6 ±0.56	14.6 ±0.22

Note: Differences between the groups are not significant (p >0.05).

Table 6 Chemical analysis of breast and thigh meat (%) of broilers chickens.

		n	Control	PS Karkulka	Skorpion
			Mean ±standard error		
Dry matter	Breast meat	6	24.44 ±0.40	24.31 ±0.59	24.90 ±0.26
	Thigh meat	6	24.12 ±0.33	23.42 ±0.31	24.45 ±0.16
Crude protein	Breast meat	6	22.10 ±0.61	21.89 ±0.52	21.83 ±0.29
	Thigh meat	6	17.86 ±0.33	17.65 ±0.20	17.38 ±0.21
Total fat	Breast meat	6	1.19 ±0.12	1.13 ±0.17	0.95 ±0.12
	Thigh meat	6	4.93 ±0.32	4.62 ±0.23	5.16 ±0.27

Note: Differences between the groups are not significant (p >0.05).

samples were evaluated by 10 panellists in special sensory laboratory (Department of Food Technology, Mendel University) according to **ISO 8589**. Each sample (breast and thigh) was packed into plastic case and frozen (-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, 0 = the worst, 100 = the best) to compare experimental group of descriptors (odour, colour, fibriness, chewiness, juiciness, flavour, fatty taste) with control group.

Statisic analysis

Data were processed by Microsoft Excel (USA) and Statistica version 12.0 (USA). One-way analysis (ANOVA) was used. To ensure evidential differences Scheffe´s test was applied and p <0.05 was regarded as statistically significant difference.

RESULTS AND DISCUSSION

Performance and body composition of chickens

Live weight of chickens was without statistically significant differences (p >0.05) among groups at the end of experiment (Table 4). This is consistent with results in chickens live weight in another study **(Šťastník et al., 2017)**.

Table 5 presents mean percentage of chicken´s body composition. There was not observed any significant differences (p >0.05). Our results of average crude protein content in breast muscle was found 22.10% for control group. No statistically significant differences (p >0.05) were found in the chemical analysis of the breast and thigh muscles (Table 6).

Table 7 Effect of feeding wheat varieties with different grain pigmentation on texture and colour of breast meat (Mean ±standard error).

Parameter	n	Control	PS Karkulka	Skorpion
Razor Shear Force (N)	30	5.91 ±0.17^{a}	7.26 ±0.26^{b}	6.03 ±0.24^{a}
L*	12	65.89 ±0.54^{ab}	64.97 ±1.05^{a}	67.87 ±0.65^{b}
a*	12	5.46 ±0.26^{a}	4.53 ±0.44^{a}	4.40 ±0.22^{a}
b*	12	12.01 ±0.58^{b}	10.18 ±0.47^{a}	10.85 ±0.22^{ab}
ΔE^*_{ab}		0	2.25	2.53

Note: ΔE^*_{ab} is compared with control group. ^{a,b} Means in a row within effect with no common superscript differ significantly (p <0.05).

Table 8 Sensory analysis of breast meat of chickens (mm in 100 mm scale).

Group		Control	PS Karkulka	Skorpion
		Mean ±standard error		
Sensory trait	n	60	60	60
Odour		90.49 ±1.12^{b}	88.62 ±0.95^{b}	84.81 ±0.92^{a}
Colour		91.71 ±0.54^{c}	71.15 ±1.61^{a}	79.71 ±1.49^{b}
Fibriness		91.54 ±0.86^{b}	78.87 ±1.16^{a}	80.00 ±1.13^{a}
Chewiness		68.49 ±2.10^{c}	52.02 ±1.42^{b}	43.32 ±1.25^{a}
Juiciness		43.20 ±1.44^{ab}	47.33 ±1.20^{b}	38.75 ±1.19^{a}
Flavour		88.07 ±1.35^{b}	82.70 ±1.26^{a}	81.80 ±1.08^{a}
Fatty taste		98.38 ±0.89^{a}	99.88 ±0.08^{a}	99.92 ±0.06^{a}

Note: $^{a, b, c}$ – different letters in one line means statistically significant differences (p <0.05).

Table 9 Sensory analysis of broilers thigh meat (mm in 100 mm scale).

Group		Control	PS Karkulka	Skorpion
		Mean ±standard error		
Sensory trait	n	60	60	60
Odour		88.18 ±1.30^{c}	74.78 ±1.46^{a}	82.00 ±1.31^{b}
Colour		89.21 ±0.87^{b}	80.77 ±1.16^{a}	85.52 ±1.20^{b}
Fibriness		88.12 ±1.33^{c}	80.52 ±0.96^{b}	74.62 ±1.37^{a}
Chewiness		79.42 ±1.64^{b}	69.20 ±1.34^{a}	69.30 ±1.69^{a}
Juiciness		71.06 ±1.80^{a}	68.52 ±1.36^{a}	66.44 ±1.61^{a}
Flavour		87.00 ±1.16^{b}	80.48 ±0.90^{a}	85.28 ±1.10^{b}
Fatty taste		97.66 ±0.78^{a}	99.65 ±0.17^{b}	99.37 ±0.26^{b}

Note: $^{a, b, c}$ – different letters in one line - statistically significant differences (p <0.05).

Texture and colour of chicken´s meat

Table 7 presents effect of feeding different wheat varieties with different grain pigmentation on texture and colour of chicken´s breast meat. In the parameter Razor Shear Force (N) was found statistically significant differences (p <0.05). It was found higher breast meat tenderness in PS Karkulka against Control and Skorpion groups. Between Control and Skorpion groups was not found significant differences (p >0.05).

The meat color is one of the first characteristics noticed by consumers when buying meat products **(Fanatico et al., 2007)**. In parameters L* and b* of colour of the meat samples was found statistically significant differences (p <0.05). L* parameter was higher value in Skorpion group and b* parameter was higher in Control group. Parameter a* was not statistically different (p >0.05) among experimental groups (Table 7). The total colour change was 2.25 and 2.53 for PS Karkulka and Skorpion group, respectively.

Sensory analysis

In sensory analysis of broilers breast muscle was found statistically significant differences (p <0.05) in odour, colour, fibreness, chewiness, juiciness and flavour parameter. The fatty taste parameter was non-significant (p >0.05). The odour parameter of chicken´s breast muscle was significantly lower in Skorpion group against PS Karkulka and Control groups. The significantly most intense colour of breast muscle was found in Control group versus Skorpion and PS Karkulka groups (91.71 mm, 79.71 mm and 71.15 mm, respectively). The fibreness parameter was significantly higher for Control group, as well.

Significantly higher chewiness of breast meat was in Control (68.49 mm) than PS Karkulka (52.02 mm) and Skorpion (43.32 mm) group. Results of higher chewiness of Control group breast was confirmed with the content of total fat in chicken breast meat. The amount of total fat in chicken breast influenced the sensory analysis especially odour and flavour these chicken breast. Breast meat was better evaluated with the higher content of total fat.

The lowest Razor Shear Force (N) was measured in a sample of chicken's breast from the Control group (5.91 N), which corresponds to a sensory analysis (0 = the worst, 100 = the best), especially color (91.71 mm) and fibriness (91.54 mm) of chicken breasts. The higher Razor Shear Force (N) was measured in the Skorpion group (6.03 N) and the highest in the PS Karkulka group (7.26 N). With the higher Razor Shear Force was chicken breast evaluated as the breast with the worse color and coarse fiberness. The PS Karkulka group was significantly (p <0.05) rated as the best juiciness parameter against Skorpion group. Differences in this parameter in Control group was not significant against PS Karkulka and Skorpion groups. In flavor parameter of breast meat was found significantly better value for Control group against PS Karkulka and Skorpion group. Among PS Karkulka and Skorpion groups was non-significant differences.

The results of sensory analysis of chicken´s thigh meat shows statistically significant differences (p <0.05) in almost all monitored parameters except juiciness parameter. Odour parameter of broilers thigh meat was significantly rated as the best in Control group versus Skorpion and PS Karkulka groups. The thigh meat of PS Karkulka group was rated significantly lower for colour parameter beside Skorpion and Control groups. On the basis of evaluators, the Control group was significantly higher colour of thigh meat. The thigh muscle fibreness parameter was significantly higher in Control group against other experimental groups. In chewiness parameter was found significantly higher value for Control group. The lowest value of flavour parameter was rated in PS Karkulka thigh meat (p <0.05) against Control and Skorpion groups. In thigh meat of broiler chicken´s was found significantly lower fatty taste in the Control group versus PS Karkulka and Skorpion groups.

CONCLUSION

The feeding of wheat cultivars with different grain pigmentation had no effect on performance parameters of broiler chicken´s as well as to it´s body and chemical composition of breast and thigh meat in this study.

REFERENCES

Arts, I. C., Hollman P. C. 2005. Polyphenols and disease risk in epidemiologic studies. *The American Journal of Clinical Nutrition*, vol. 81, no. 1, p. 317-325. https://doi.org/10.1093/ajcn/81.1.317S

Aviagen Group, 2014a. Broiler nutrition specifications [online] s.a. [cit. 2018-08-09]. Available at: http://en.aviagen.com/ross-308.

Aviagen Group, 2014b. Technological procedure for broiler Ross [online] s.a. [cit. 2018-08-08]. Available at: http://en.aviagen.com/ross-308.

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 Meat Foods*, vol. 16, no. 3, p. 223-242. https://doi.org/10.1111/j.1745-4573.2005.00001.x

Chen, C. D., Sloane, J. A., Li, H., Aytan, N., Giannaris, E. L., Zeldich, E., Luebke, J. I. 2013. The antiaging protein Klotho enhances oligodendrocyte maturation and myelination of the CNS. *Journal of Neuroscience*, vol. 33, no. 5, p. 1927-1939. https://doi.org/10.1523/JNEUROSCI.2080-12.2013

Choi, Y., Jeong, H. S., Lee, J. 2007. Antioxidant activity of methanolic extracts from some grains consumed in Korea, *Food Chemistry*, vol. 103, no. 1, p. 130-138. https://doi.org/10.1016/j.foodchem.2006.08.004

CIE. 2007. Colorimetry, part 4: CIE 1976 L*a*b* Colour space. CIE Central Bureau, Vienna, CIE DS 014-4.3/E:2007, p.8 Available at: http://www.unife.it/scienze/astro-fisica/insegnamenti/ottica-applicata/materiale-didattico/colorimetria/CIE%20DS%20014-4.3.pdf

Commission regulation (EC) 152/2009. 2009. Laying down the methods of sampling and analysis for the official control of feed. Brussels: The commission of the European Communities.

Dransfield, E., Sosnicki, A. A. 1999. Relationship between muscle growth and poultry meat quality. *Poultry Science*, vol. 78, no. 5, p. 743-746. https://doi.org/10.1093/ps/78.5.743

Fanatico, A. C., Pillai, P. B., Emmert, J. L., Owens, C. M. 2007. Meat quality of slow-and fast-growing chicken genotypes fed low-nutrient or standard diets and raised indoors or with outdoor access. *Poultry Science*, vol. 86, no. 10, p. 2245-2255. https://doi.org/10.1093/ps/86.10.2245

Ficco, D. B. M., Mastrangelo, A. M., Trono, D., Borrelli, G. M., Vita, P., Fares, C., Beleggia, R., Platani, C., Papa, R. 2014. The colours of durum wheat: a review. *Crop and Pasture Science Journal*, vol. 65, no. 1, p. 1-15. https://doi.org/10.1071/CP13293

Haščík, P., Kačániová, M., Čuboň, J., Mellen, M., Mihok, M., Eliman, I. O. E. 2012. Sensory evaluation of meat chickens ross 308 after application of propolis in their nutrition. *Potravinarstvo*, vol. 6, no. 1, p. 14-20. https://doi.org/10.5219/158

Hosseinian, F. S., Beta, T. 2007. Saskatoon and wild blueberries have higher anthocyanin contents than other Manitoba berries. *Journal of Agricultural and Food Chemistry*, vol. 55, no. 26, p. 10832-10838. https://doi.org/10.1021/jf072529m

ISO 8586-1. 2015. Sensory analysis – General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors (in Czech).

ISO 8589. 1993. Sensory analysis. General guidelines for the organization of a sensory workplace (in Czech).

Kris-Etherton, P. M., Beecher, G. R., Etherton, T. D., Gross, M. D., Keen, C. L., Lefevre, M. 2004. Bioactive compounds in nutrition and health-research methodologies for establishing biological function: The antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. *Annual Reviews of Nutrition*, vol. 24, no. 1, p. 511-538. https://doi.org/10.1146/annurev.nutr.23.011702.073237

Martinek, P., Jirsa, O., Vaculova, K., Chrpova, J., Watanabe, N., Burešová, V., Kopecký, D., Štiasna, K., Vyhnánek, T., Trojan, V. 2014. Use of wheat gene resources with different grain colour in breeding. In Proceeding from Conference: *Tagungsband der 64. Jahrestagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs*, vol. 1, p. 75-78. ISBN-13:978-3-902849-00-7.

Meullenet, J. F., Jonville, E., Grezes, D., Owens, C. M. 2004. Prediction of the texture of cooked poultry pectoralis major meats by near-infrared reflectance analysis of raw meat. *Journal of Texture Studies*, vol. 35, no. 6, p. 573-585. https://doi.org/10.1111/j.1538-7836.2004.01165.x-i1

Oomah, B. D., Mazza, G. 1999. Health benefits of phytochemicals from selected Canadian crops, *Trends in Food Science & Technology*, vol. 10, p. 193-198 https://doi.org/10.1016/j.cj.2015.04.004

Patel, K. K., Jain, A., Patel, D. K. 2013. Medicinal significance, pharmacological activities, and analytical aspects of anthocyanidins 'delphinidin': A concise report. *Journal of Acute Disease*, vol. 2, no. 3, p. 169-178. https://doi.org/10.1016/S2221-6189(13)60123-7

Šťastník, O., Karásek, F., Jůzl, M., Mrkvicová, E., Pavlata, L., Nedomová, Š., Trojan, V., Vyhnánek, T., Doležal, P., Martinek, P. 2017. The effect of feeding wheat varieties with different grain pigmentation on growth performance, texture, colour and meat sensory traits of broiler chickens. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 145-150. https://doi.org/10.5219/753

Tsuda, T., Aoki, H., Horio, F., Osawa, T., Uchida, K. 2003. Dietary cyanidin 3-O-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia. *Journal of Nutrition*, vol. 133, no. 7, p. 2125-2130. https://doi.org/10.1093/jn/133.7.2125

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. https://doi.org/10.1016/j.meatsci.2008.07.009

Varga, M., Bánhidy, J., Cseuz, L., Matuz, J. 2013. The anthocyanin content of blue and purple coloured wheat cultivars and their hybrid generations. *Cereal Research Communications*, vol. 41, no. 2, p. 284-292. https://doi.org/10.1556/CRC.41.2013.2.10

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ASSESSMENT OF ANTIOXIDANT PROPERTIES OF GRAIN CONCENTRATE AND OXIDANT-ANTIOXIDANT STATUS PIGS AFTER ITS INCLUSION IN RATION FEEDING

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ABSTRACT

A grain concentrate was developed for use in bread baking based on whole-ground fermented wheat grain, to enhance that the beneficial properties have fermented wholegrain buckwheat grains in an amount of 20% by weight of the fermented wheat. For the fermentation of grain used dry complex enzyme preparation comprising cellulose, β-glucanase and xylanase (producing Penicillin canescens), dissolved in a buffer based on succinic acid. Under the action of the drug, the micro structure surface of grain was changed. It is established that the character of the change in surface micro structure of wheat and buckwheat grain is the same. The results of the study of the content of vitamin E, flavonoids and antioxidant activity in wheat grains, buckwheat and grain concentrate are obtained by different technologies. The results show that grain concentrates from wheat grain with the addition of 20% buckwheat grains prepared using a solution of enzyme preparation of cellulolytic action in a buffer, based on succinic acid has a high antioxidant activity. As a biological model for studying changes oxidant-antioxidant status of the organism under stress when included in a diet designed grain concentrate, used pigs, that are under stress, caused by weaning them from sows and transportation. Investigated the following parameters oxidant-antioxidant status of the organism pigs: the level of malondialdehyde, ceruloplasmin, vitamins A, E and C in the blood of animals. It is concluded that, to improve the oxidative status of the piglets after weaning period recommended addition of concentrate fodder ration of grain wheat and buckwheat prepared using a solution of an enzyme preparation buffered cellulolytic action on the basis of succinic acid. The developed grain concentrate can be used for making the manufacture of cereal products, including grain bread included in the diet of people who live in conditions of oxidative stress.

Keywords: fermented grain; wheat; buckwheat; cereal concentrate; antioxidant activity; piglets, stress; oxidant-antioxidant status

INTRODUCTION

Studies show that whole grains of cereals can protect from obesity, diabetes, cardiovascular diseases and cancer. Nutritionists recommend cereal products to provide a diet, with food fibers, proteins, vitamins and minerals, located mainly in the shells of grain **(Buddrick et al., 2014; Vitaglione et al., 2008)**. Phenolic acids are widely distributed in grains and are present in high concentrations in whole grains. Ferulic acid is the main, and the most abundant phenolic acid is in wheat grains. Lesser concentrations of caffeine, p-coumaric, synapic acid, and other phenolic acids are also observed in wheat **(Verma et al., 2009)**. They are considered one of the important compound groups that are responsible for health **(Slavin et al., 2000; Truswell, 2002)**.

Buckwheat has a high level of antioxidant activity due to the content of flavonoid compounds in it **(Holasova et al., 2002)**. Over the past of few decades, the following flavonoid compounds have been identified in the buckwheat grains: rutin, quercetin and flavone C-glycosides (which include orientin, vitexin and isovitinexin) **(Zielińska et al., 2012)**.

It was found that in the wheat bread with buckwheat flour the total antioxidant activity increased with the increase in the percentage of buckwheat flour, and the level of routine in such bread ranged from 7.76 to 26.90 $mg.kg^{-1}$ **(Lin et al., 2009; Bojňanská et al., 2009; Brindzová et al., 2009)**.

Succinic acid is used as a growth stimulator of organisms and an antimicrobial agent for the prevention of bacterial pathogens, **(Ng et al., 2015; Kumar et al., 2015)**. It can

inhibit necrosis and apoptosis **(Tang et al., 2013)**, has anticonvulsant and antiepileptic action **(Cong et al., 2009)**, inhibits the skin allergic reaction and reduces the formation of serum antibodies IgE **(Ke et al., 1983)**. Oral administration of succinic acid to experimental animals after resuscitation helped normalize the formation of free radicals in the brain and serum **(Gurvitch et al., 1997)**. The introduction of succinic acid into the diet of mice exposed to hyperbaric oxygen, led to an increase in the level of γ-aminobutyric acid in the brain and the activity of the enzyme catalyzing his synthesis **(Schatz and Lal, 1980)**. Treatment with a complex preparation containing pantogs, succinic acid and chitosan normalizes the concentration of reduced glutathione and the activity of glutathione peroxidase, glutathione reductase in animals with experimental hypoxia/reperfusion of the brain. These results are explained by the suppression of free radical oxidation and the normalization of the antioxidant system associated with the neuroprotective, antihypoxic and antioxidant properties of these substances, their involvement in the regulation of cellular metabolism in pathological conditions accompanied by oxidative stress **(Safonova et al., 2015)**. Derivatives of succinic acid showed promising antifungal and antibacterial activity against various test microorganisms and possess antioxidant activity **(Raghavendra et al., 2017)**. The introduction of succinic acid derivatives in combination with plant polyphenols showed cytoprotective properties in rats exposed to histotoxic hypoxia, compared with the use of only a group of polyphenols plant **(Zadnipryany et al., 2016)**.

Thus, it is known that grains of buckwheat and wheat contain a complex of biologically active compounds, and succinic acid has an antioxidant effect. But in the literature, there is no information on how these components in combination, affect on the antioxidant activity of the grain product and the antioxidant status of the experimental animals.

Scientific hypothesis

The use of the wheat grain and buckwheat fermentation process in the presence of succinic acid for the production of grain concentrate has a significant effect on the modification of the antioxidant activity of the concentrate and on the oxidant-antioxidant status of the organism of experimental animals (pigs) under stress.

MATERIAL AND METHODOLOGY

The concentrate cereal was developed for use in bakery on the basis of whole grains fermented variety of winter wheat Moscow139. In the concentrate, to increase useful properties, fermented buckwheat grains in the open ground of the Bashkir variety of red wines were added at a rate of 20% of the weight of the fermented wheat grain. For the study was taking grain of wheat and buckwheat harvest in 2017 grown in the Orel region of the Russian Federation. A dry complex enzymatic preparation was used to ferment the grain, including cellulase, β-glucanase and xylanase (producer of *Penicillium canescens*). The enzymes have the following activities: cellulase-58 711 nkat.g^{-1}, xylanase-12 135 nkat.g^{-1}, β-glucanase-51 317 nkat.g^{-1}, provided by the Physico-Chemical Polymer Transformation Laboratory of the Faculty of Chemistry in Moscow university M.V. Lomonosov **(Sinitsyna et al., 2003)**. Fermentation of grain of wheat and buckwheat was carried out separately. Succinic acid was added as part of the buffer solution. The enzymatic preparation in powder form is mixed with a magnetic stirrer with a succinic acid buffer (pH 4.6) for 0.5 hours at a concentration of 0.6 g.L^{-1} for wheat grains and 0.4 g.L^{-1} for buckwheat kernels, then the grain was placed in the solution. As a reference sample, a solution of the enzymatic preparation in water was used. Whole grains of wheat and buckwheat were stored in a solution of the enzyme preparation in a grain ratio of 1:1.5 for 8 hours at 50° C in a thermostat. The hydrolysis regimes (t = 50° C, pH 4.6) are optimal for the action of their enzymes. After incubation, enzymatic inactivation was not performed. After the enzymatic hydrolysis time, the grain was washed with running water at t = 18 – 20 °C for 5 – 10 minutes. The wheat and buckwheat grain thus fermented were subjected to drying at a temperature not exceeding 50 – 60° C to a moisture content of at most 11 – 14% and then grinding to a size of particles not exceeding 0.08 mm. The vitamin content was determined by HPLC on a Milichrome-5 instrument (ZAO Nauchpribor, Russia). Analytical chromatographic column separon-SGX-C18 with the internal diameter of 2 mm, length of 70 mm for reversed-phase HPLC and software UniChrom (ZAO Nauchpribor, Russia). An aqueous extract of buckwheat grain (pH 3) was used: the eluent of the composition - acetonitrile: aqueous solution of sodium heptanesulfonate and monosubstituted potassium phosphate (pH 3.0, ratio 20/80), flow rate mobile phase 1 cm^3.min^{-1}; the elution mode is isocratic, the detection was carried out in the 200 - 400 nm wavelength range, the analysis time 12 – 25 min, the sample volume 2 – 6 μL. Microstructural studies were performed using a ZEISS EVO LS scanning electron microscope (Carl Zeiss Industrial Messerechnik GmbH, Germany). The investigation was conducted at an accelerated voltage of 15 kV. The complex of phenolic compounds was determined by HPLC on a Milichrome-5 instrument (ZAO Nauchpribor, Russia). An alcoholic extract of buckwheat grains was used, the eluent of the composition was acetonitrile: an aqueous solution of trifluoroacetic acid (pH 2.5, in the ratio 15/85); the mode of elution is isocratic, the analysis time is 12 to 25 minutes, the volume of the sample is 2 to 6 μL. Antioxidant activity was determined by the spectrophotometric method in an alcohol extract described by **Silva et al. 2005** based on percent inhibition of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. We have been determined by the optical density of the solutions in the interaction, Specord M40 (Carl Zeiss Industriel Messtechnik GmbH, Germany) at a wavelength of 515 nm. All chemical reagents were used by Merk (Germany).

To study the effect of grain concentrates on the oxidant-antioxidant status of the animal body, an experiment was conducted on piglets under stress due to weaning of sows and transport. Pigs immediately after weaning 28-day-old sows were transported by motorized transport from the breeding farm to the cropping site (a distance of approximately 220 km). The loading time of the animals in the car before placement was 6 hours. On the day of arrival, four groups of 25 piglets each were formed from newly arrived pigs on the basis of analogues. Piglets in the control group received a ration in the form of mixed feed,

of which the evening portion for 14 days after weaning and transport was partially replaced (by 20% by weight) with native wheat flour. In the ration of the animals in the first test group, during the 14 days after weaning and transport, the evening portion of the compound feed was replaced by 20% by weight of a grain concentrate containing only wheat grain. wheat fermented with a succinic acid buffer. Piglets 2nd experimental group received a ration within 14 days of weaning and transport, the evening portion of mixed feed being replaced by 20% fermented wheat grain, the transformed enzyme preparation being dissolved in water. Succinic acid was not administered to the piglets of the 2nd test group. Animals third test group within 14 days after weaning and transport regime established in which the portion of a load portion at 20% by weight was replaced by concentrate of cereals prepared according to the developed wheat technology fermented and buckwheat using a succinic acid buffer to dissolve the enzyme preparation. Grain concentrate test samples were mixed with the mixed feed just before feeding the animals. The dose of succinic acid introduced with the feed corresponded to the recommended daily standard for piglets and was 30 $mg.kg^{-1}$ body weight.

Blood samples for laboratory tests were taken from five animals in each group prior to the experimental feed grain concentrate samples (weaning and transport days), and on day 3 and day 15 of the experiment. As indicators of the antioxidant status of pigs in the blood serum of their blood, the secondary product content of lipid peroxidation - malonic dialdehyde - was determined by reaction with thiobarbituric acid **(Korobeynikova, 1989)**, the level of antioxidant ceruloplasmin - express method according to E.V. Tan **(Ravin, 1961)**.

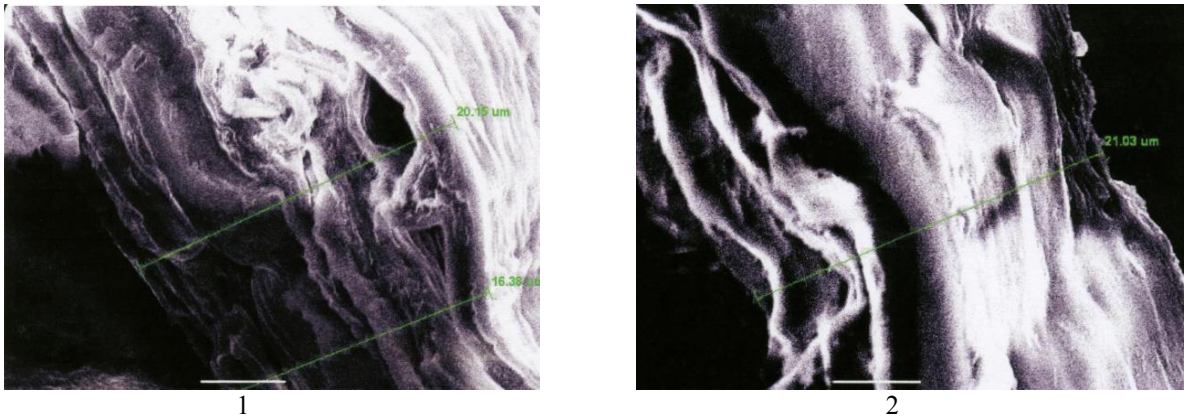
To evaluate the reliability of the test differences, t-statistics (a two-sample t-test for independent samples) were used. The tests were conducted at a level of significance p <0.05 using the Statistica 7.0 software (StatSoft Inc., USA). To confirm the linear relationship between the quantitative indicators, the Pearson correlation criterion (coefficient) was used, the paired linear regression method.

Statisic analysis

The results were evaluated statistically using the Analysis of Variance. Procedure compares the data in six varieties. The results assays were expressed as mean ±SD of eight repeated samples. To evaluate the reliability of the test differences, t-statistics (a two-sample t-test for independent samples) were used. The p -value used to test the null hypothesis in order to quantify the idea of statistical significance have to be provided. The tests were conducted at a level of significance p <0.05 using the Statistica 7.0 software (StatSoft Inc., USA). To confirm the linear relationship between the quantitative indicators, the Pearson correlation criterion (coefficient) was used, the paired linear regression method.

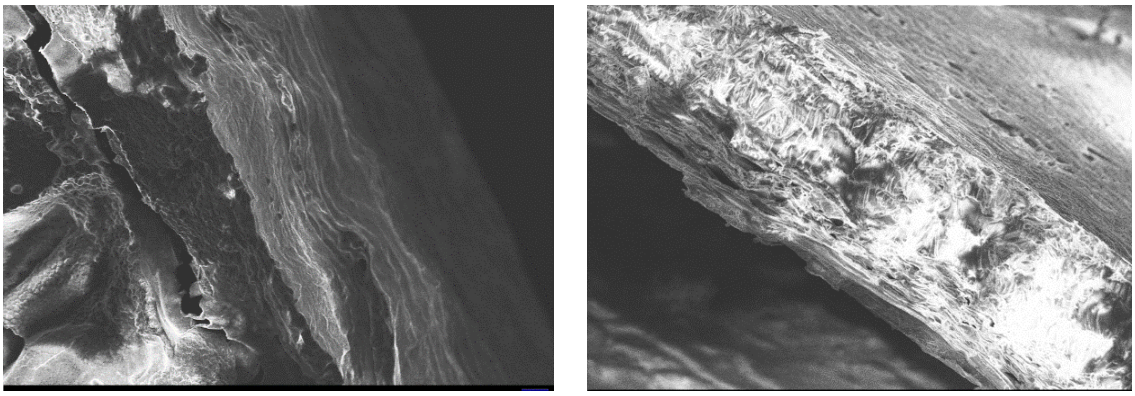
RESULTS AND DISCUSSION

Bioactive compounds in grain of wheat and buckwheat are mainly found in shells and bran. The peripheral parts of the grain have higher antioxidant activity, polyphenols, phytic acid, vitamins and minerals are concentrated here **(Vitaglione et al., 2008; Li et al., 2013; Higuchi, 2014)**. The largest proportion (80%) of thiamine is found in the outer layers of wheat **(Batifoulier et al., 2006)**. To modify the shell structure of wheat and buckwheat fruits and seeds in order to increase the yield of biologically active substances and to facilitate grain milling, a complex cellulase-based enzymatic preparation was used. Figure 1 shows microphotographs of the surface of the peripheral parts of the wheat grain in a cross-section. The photos were taken with a scanning electron microscope with an increase of 4000x. Figure 2 – microphotographs of the surface of the peripheral parts of the buckwheat kernels (increase of 800x). Under the action of cellulase - based biocatalysts in the fruit shells of the grain, longitudinal breaks are formed, bare strands of polysaccharides are found at the ends of the fibers – fibrillation. The fibers are folded, the outer layers of the adjacent microfibrils fibers are destroyed. The nature of the modification of the microstructure of the surface of fruit shells in wheat and buckwheat is identical.



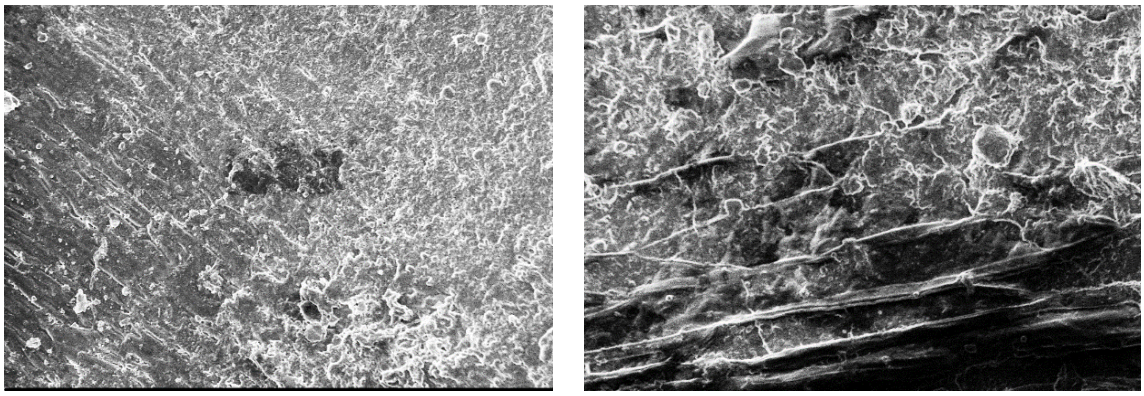
1 2

Figure 1 Microphotographs of the superficial structure of the peripheral parts of buckwheat grains on a cross – section. Note: 1 - control without enzymes; 2 - complex enzyme preparation. Microscope: ZEISS EVO LS, Software: SmartSEM 5.06, magnification 4000x (Photo: S. Motyleva, 2017).



1 2

Figure 2 Microphotographs of the structure of the surface of peripheral parts of buckwheat grains on a transverse section. Note: 1 - control without enzymes, 2 - complex enzyme preparation, magnification). Microscope: ZEISS EVO LS, Software: SmartSEM 5.06, magnification 800x (Photo: S. Motyleva, 2017).



1 2

Figure 3 Microphotographs of the surface structure of buckwheat grains. Note: 1 - control without enzymes, 2 - complex enzyme preparation. Microscope: ZEISS EVO LS, Software: SmartSEM 5.06, magnification 800x (Photo: S. Motyleva, 2017).

Table 1 - The content of vitamins, flavonoids in wheat grains, buckwheat and grain concentrate and their antioxidant activity (AOA).

Option of experience	**The content of biologically active substances (t/P) ***		**AOA, % inhibition of DPPH**
	Vitamin E, mg.100g^{-1}	**Mass fraction of flavonoids, %**	
Wheat grain	3.62 ±0.12	0.16 ±0.01	16.2 ±0.5
Wheat grain fermented	4.23 ±0.14 (3.31/0.05)	0.20 ±0.01 (2.83/0.05)	28.4 ±1.1 (10.01/0.001)
Buckwheat Grain	0.82 ±0.02	0.33 ±0.02	25.5 ±1.0
Buckwheat grains are fermented	0.91 ±0.01 (4.02/0.02)	0.45 ±0.01 (3.81/0.05)	40.8 ±1.3 (9.33/0.001)
Grain concentrate without succinic acid (the enzyme preparation was dissolved in water)	3.78 ±0.14	0.21 ±0.02	30.5 ±0.9
Grain concentrate with succinic acid (the enzyme preparation was dissolved in buffer based on succinic acid)	4.35 ±0.11 (3.20/0.05)	0.28 ±0.02 (3.13/0.05)	38.1 ±1.6 (4.14/0.02)

Note: * (t - Student's test, P - significance level).

In the works **(Kuznetsova et al., 2016)**, a change in the surface microstructure of cereal grains of wheat, rye and triticale under the action of complex enzymatic preparations based on cellulases is presented. The native grain surface has a characteristic first-order relief, showing parallel strands of cellulose fibrils of various thicknesses and tortuosities, coated with epidermal derivatives of polysaccharide matrix components.

Under the influence of a solution of cellulase-based biocatalysts, the surface relief of the grain has been modified, expressed in the form of bundles of long and virtually intact fibers, and interfibrillary cross-links constructed from hemicellulose molecules disintegrated. A similar picture can be seen on the micrographs of the surface of buckwheat grains (Figure 3).

The microphotographs show that the non-starch polymers of the grain blankets are destructuring, which can lead to the release of bound forms of biologically active compounds, for example flavonoids, and increase the antioxidant activity of the cereal product.

There was a statistically significant a relation between total flavonoid content, vitamin E, and antioxidant activity in the plant material studied. These data are consistent with the data **(Jiang et al., 2007)**. **Holasova et al. (2002)** found no correlation between tocopherol content and antioxidant activity. Differences in the results of different authors' experiments may be due to the difference between the objects used and the methods of analysis.

The results obtained show that the grain concentrate prepared using a solution of the cellulolytic enzymatic preparation in succinic acid buffer has a high antioxidant activity. This is due to the presence of succinic acid in the grain concentrate. In addition, it is known that flavonoids are located in associated forms in the cell walls of the seed, and their extraction requires alkaline, acidic or enzymatic treatment **(Hung and Morita, 2008; Ragaee et al., 2011)**. Most of the grain's antioxidants is linked by ester bonds to cell wall components by arabinoxylans **(Liyana-Pathirana and Shadidi, 2006)**. The increase in flavonoid and vitamin E content in wheat and buckwheat grains after fermentation was due to the decomposition of the complexes of these compounds with the cell wall polysaccharides after their modification, and also to the awakening of the embryo. The germination process is accelerated in the presence of succinic acid and cellulase biocatalysts. Under the conditions of germination in the grain, enzymatic systems are activated, the biologically active compounds are synthesized, including vitamin E, responsible for the antioxidant properties of biological systems. The combined use of grain in cellulolytic enzyme preparation stage preparation technology and succinic acid leads to an intensification of enzymatic hydrolysis of non-starch polysaccharides of the cell wall, and increased pore sizes in the cells. membranes mezhfibrillyarnyh intervals, the intensive penetration of the solvent in the caryopsis and acceleration of the synthesis of substances with antioxidant properties. The use of enzyme preparations in a succinic acid buffer solution increased the vitamin E content from 10.9 to 16.9%, 25.0 – 36.3% of the flavonoids, and AOA of 24.91 – 75.3% in the wheat concentrate. Differences in values of reliability values $p \leq 0.05$ in all investigated indicators. The value of the Pearson correlation coefficient was 0.8871 for antioxidant activity, 0.9992 for vitamin E content and 0.9996 for flavonoid content in grain raw material before and after enzymatic treatment. Using the Cheddock table, it can be concluded that it is advisable to use the cellulase enzyme preparation in a succinic acid buffer solution to pretreat the wheat and buckwheat kernels in order to increase their antioxidant activity.

Bojňanská et al. (2009) conducted clinical studies on a group of volunteers who consumed wheat bread enriched with 30% buckwheat for 4 weeks. The total antioxidant status of the participants in the experiment was found to increase significantly from 1.135 to 1.46 mmol.dm^{-3}. The porcine organism is similar to the human organism in many morphophysiological indices and these animals are therefore often used as a biological model to study the physiology of stress, obesity, cardiovascular disease and others **(Gorelov et al., 2002; Kapanadze, 2006)**. The elimination of piglets from sows and transporters is an important stressor that causes stress in young pigs. The development of a state of stress in animals is accompanied by an activation of lipid peroxidation processes, which is manifested by an increase in the content of these biochemical reactions, including malonic dialdehyde, in their blood **(Zhu et al., 2012)**. The imbalance between oxidative and antioxidant systems in pigs is also a concentrate of vitamins A, E, C in the blood **(Buchet et al., 2017**). Indicators of the antioxidant-oxidant status of the piglet organism, which received grain concentrate from fermented wheat and buckwheat as part of the diet, are presented in Table 2.

The results showed that the level of malonic dialdehyde increases on the third day after weaning and piglet transport, while the number of chemical compounds responsible for maintaining the antioxidant state - ceruloplasmin, vitamins A, E and C – is significantly reduced in the blood of animals. These data indicate a reduced ability of the body to generate reactive oxygen species. Similar results were obtained by **Yin et al. (2014); Buchet et al. (2017)**, who note that imbalance can be restored when the antioxidant system develops. The diet of the developed grain concentrates had a positive effect on the oxidant-antioxidant state of the body stressed animals manifest a decrease in the serum of the oxidative stress index in the blood – malondialdehyde levels and the increase antioxidants – ceruloplasmin, vitamins A, E and C, compared to the control. The best results were obtained with the use of the grain concentrate, the technology of which used an enzymatic preparation of the cellulolytic action, dissolved in a succinic acid buffer. Thus, on the fifteenth day from the beginning of the experiment, the level of malonic dialdehyde in piglets 1, 2 and PEP was lower than that of the control at 22.4; 7.1 and 27.7%. Depending on the blood serum content of ceruloplasmin, the animals of the 1st, 2nd and 3rd more detailed studies on analogues of the control group were evaluated at 11.7; 7.6 and 15.2%, in vitamin A content - 23.5; 11.8 and 33.3%, vitamin E - 5.5; 4.8 and 7.6%, vitamin C - 10.8; 4.3 and 12.9% of the total body weight. The differences in oxidant-antioxidant status values of the piglets obtained are significant, $p \leq 0.05$ in all the indicators studied. These results coincide with the findings that to improve the oxidative status of pigs after weaning, it is recommended that vitamin E be added to the diet **(Rey et al., 2017)**.

Table 2 - Indicators of Oxidative-Antioxidant Status of Piglets Included in their Diet of Fermented Wheat Bean Concentrate and Buckwheat Grain

Indicators	Groups piglets	Indicators of oxidant-antioxidant status (t/P) *		
		research dates		
		on the day of weaning and transport	after weaning and transportation	
			on the 3rd day	on the 15th day
Malondialdehyde μmoL.L^{-1}	control	0.52 ±0.002	0.98 ±0.007	0.60 ±0.003
	1st experience	0.54 ±0.003 (5.54/0.01)	0.95 ±0.005 (3.49/0.05)	0.49 ±0.029 (3.77/0.05)
	2nd experience	0.53 ±0.001 (4.4/0.02)	0.96 ±0.001 (2.83/0.05)	0.56 ±0.009 (4.21/0.02)
	3rd experience	0.53 ±0.002 (3.54/0,05)	0.94 ±0,005 (4.65/0.02)	0.47 ±0.022 (5.85/0.01)
Ceruloplasmin, μmoL.L^{-1}	control	2.27 ±0.005	1.56 ±0.006	1.71 ±0.023
	1st experience	2.25 ±0.004 (3.12/0.05)	1.58 ±0.002 (3.16/0.05)	1.91 ±0.045 (3.96/0.02)
	2nd experience	2.26 ±0.001 (3.2/0.05)	1.58 ±0.001 (3.29/0.05)	1.84 ±0.017 (4.55/0.02)
	3rd experience	2.25 ±0.002 (3.71/0.05)	1.59 ±0.004 (4.16/0.02)	1.97 ±0.051 (4.65/0.02)
Vitamin A, μmoL.L^{-1}	control	0,67 ±0,007	0.42 ±0.004	0.51 ±0.02
	1st experience	0.65 ±0.005 (3.25/0.005)	0.44 ±0.004 (3.54/0.005)	0.63 ±0.03 (3.32/0.05)
	2nd experience	0.64 ±0.006 (3.32/0.05)	0.44 ±0.005 (3.12/0.05)	0.59 ±0.01 (3.58/0.05)
	3rd experience	0.65 ±0.001 (2.82/0.05)	0.45 ±0.006 (4.16/0.02)	0.68 ±0.03 (4.71/0.02)
Vitamin E, μmoL.L^{-1}	control	8.14 ±0.009	6.32 ± 0.005	6.93 ±0.10
	1st experience	8.09 ±0.003 (5.27/0.01)	6.37 ±0.002 (9.28/0.001)	7.31 ±0.06 (3.26/0.05)
	2nd experience	8.06 ±0.007 (7.01/0.01)	6.35 ±0.013 (5.14/0.01)	7.26 ±0.02 (3.24/0.05)
	3rd experience	8.11 ±0.002 (3.25/0.05)	6.40 ±0.016 (4.77/0.02)	7.46 ±0.09 (3.99/0.02)
Vitamin C, μmoL.L^{-1}	control	19.53 ±0.026	14.65 ±0.04	16.48 ±0.15
	1st experience	19.38 ±0.012 (5.24/0.01)	14.81 ±0.03 (3.2/0.05)	18.26 ±0.48 (3.54/0.05)
	2nd experience	19.34 ±0.019 (5.9/0.01)	14.78 ±0.01 (3.15/0.05)	17.19 ±0.13 (3.58/0.05)
	3rd experience	19.40 ±0.017 (4.18/0.02)	14.87 ±0.05 (3.44/0.05)	18.61 ±0.27 (6.9/0.01)

It has also been established that the addition of a mixture of plant polyphenols improves the antioxidant status of piglets after weaning and helps counteract some negative effects **(Jiang et al., 2014)**. Thus, experimental data show that the antioxidant activity of the grain concentrate increases after grain fermentation and that the feeding of the grain concentrates to piglets after weaning and transport results in an improvement of their oxidant-antioxidant status.

CONCLUSION

Thus, it was experimentally established, that the use of the process of fermentation of grain of wheat and buckwheat under the action of a complex enzyme preparation of celluloses ,dissolved in a buffer based on succinic acid, leads to a change in the microstructure of the grain surface.

Microphotographs show that the destructuring of non-starch polymers of grain covers is observed, which can lead to the release of bound forms of biologically active compounds, for example, flavonoids, and to increase the antioxidant activity of the grain product. It is established that the nature of the change in the microstructure of the surface of wheat and buckwheat grains is identical. There was a statistically significant relationship between the total content of flavonoids, vitamin E, and antioxidant activity in the plant material under study. The use of enzyme preparations in a solution of the buffer based on succinic acid allowed to increase the content of vitamin E by 10.9 – 16.9%, flavonoids by 25.0 – 36.3%, and AOA by 24.91 – 75.3% in processed wheat, buckwheat and grain concentrate. The statistical treatment of the results shows the expediency of using an enzyme preparation of cellulases in a buffer solution based on succinic acid for pretreating wheat and buckwheat grains to increase their antioxidant activity. To study the change in the oxidant-antioxidant status of an organism in a stressful state, when pigs were developed in the developed grain concentrate,

they were used in conditions of stress caused by their weaning from sows and transportation. The parameters of the oxidant-antioxidant status of the body of piglets were studied: the level of malonic dialdehyde, ceruloplasmin, vitamins A, E and C in the blood of animals. The results show that on the third day after weaning and transporting the pigs, the level of malonic dialdehyde is multiplied by 1.8, while the number of chemical compounds responsible for maintaining the antioxidant state – ceruloplasmin, vitamins A, E and C - is greatly reduced. (68 to 76%) in the blood of animals. These data indicate a reduced ability of piglets to bind to active forms of oxygen. On the 15th day after the start of the experiment, the level of malonic dialdehyde in the piglets was lower than that of the control. Depending on the blood content of ceruloplasmin blood and the animal vitamins studied, the experimental groups were higher than those in the control group. Thus, the addition of wheat grain concentrates, and buckwheat concentrates in the diet, prepared with a cellulase enzyme preparation solution in a succinic acid-based buffer, significantly influences the oxidative-antioxidant status of the diet. The organism of experimental animals (piglets) was subjected to stress.

The developed grain concentrate can be used to make grain products, including cereal bread, included in the diets of people living in oxidative stress.

REFERENCES

Batifoulier, F., Verny, M. A., Chanliaud, E., Demigne C. 2006. Variability of B vitamin concentrations in wheat grain, milling fractions and bread products. *European Journal of Agronomy*, vol. 25, no. 2, p. 163-169. https://doi.org/10.1016/j.eja.2006.04.009

Bojňanská, T., Frančáková, H., Chlebo, P., Vollmannová, A. 2009. Rutin content in buckwheat enriched bread and influence of its consumption on plasma total antioxidant status. *Czech Journal of Food Sciences*, vol. 27, p. 236-240. https://doi.org/10.17221/967-CJFS

Brindzová, L., Mikusova, L., Takacsova M. 2009. Antioxidant effect of wheat bakery products supplemented with buckwheat, oat and barley beta-D-glucan and their nutritional and sensory evaluation. *Proceedings of the 5th International Congress Flour-Bread '09. 7th Croatian Congress of Cereal Technologists, Opatija, Croatia, 21–23 October*, p. 485-491.

Buddrick, O., Jones, O. A. N., Cornell, H. J., Small, D. M. 2014. The influence offermentation processes and cereal grains in wholegrain bread on reducing phytate content. *Journal of Cereal Science*, vol. 59, p. 3-8. https://doi.org/10.1016/j.jcs.2013.11.006

Buchet, A., Belloc, C., Leblanc-Maridor M., Merlot, E. 2017. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. *PLoS ONE*, vol. 12, no. 5, p. e0178487. https://doi.org/10.1371/journal.pone.0178487

Cong, H.Q., Yue, W., Yang, Z. H., Qian, Q. H., Chi, H. F. 2009. Effects of succinic acid on the presynaptic GABA release in hippocampal CA1 field. *Chinese J. Neuroanatimy*, vol. 25, p. 6-10. ISSN: 1000-7547.

Gorelov, I. G., Savina, M. A., Novikov, A. A., Yudina, O. P., Ermolaev, V. I. 2002. Pigs as model for biomedical research. *Agrarnaya Rossiya*, vol. 5, p. 45-49. ISSN: 2500-1396.

Gurvitch, A. M., Mutuskina, E. A., Zarzhetsky, Y. V., Trubina, I. E., Avruschenko, M. S., Pylova, S. I., Volkov, A. V., Lazareva, N. A., Stepanichev, M. Y., Onufrievb, M. V., Gulyaeva, N. V. 1997. Prophylaxis of encephalopathies and risk factors of atherogenesis development in the postresuscitation period in rats by means of succinic acid. *Resuscitation*, vol. 35, p. 165-170. https://doi.org/10.1016/S0300-9572(97)00045-2

Higuchi, M., 2014. Chapter 15 - Antioxidant Properties of Wheat Bran against Oxidative Stress. In *Wheat and Rice in Disease Prevention and Health*. United States : Academic Press, p. 181-199. ISBN: 978-0-12-401716-0. https://doi.org/10.1016/B978-0-12-401716-0.00015-5

Holasova, M., Fiedlerova, V., Smrcinova, H., Orsak, M., Lachman, J., Vavreinova, S. 2002. Buckwheat - the source of antioxidant activity in functional foods. *Food Research International*, vol. 35, p. 207-211. https://doi.org/10.1016/S0963-9969(01)00185-5

Hung, P. V., Morita, N. 2008. Distribution of phenolic compounds in the graded flours milled from whole buckwheat grains and their antioxidant capacities. *Food Chem*., vol. 109, p. 325-331.

Jiang, P., Burczynski, F., Campbell, C., Pierce, G., Austria, J. A., Briggs, C. J. 2007. Rutin and flavonoid contents in three buckwheat species Fagopyrum esculentum, F. tataricum, and F. homotropicum and their protective effects against lipid peroxidation. *Food Research International*, vol. 40, p. 356-364. https://doi.org/10.1016/j.foodres.2006.10.009

Jiang, X. R., Zhang, H. J., Mantovani, G., Alborali, G. L., Caputo, J. M., Savoini, G., Dell'Orto, V., Bontempo, V. 2014. The effect of plant polyphenols on the antioxidant defence system of weaned piglets subjected to an Escherichia coli challenge. *Journal of Animal and Feed Sciences*, vol. 23, p. 324-330. https://doi.org/10.22358/jafs/65668/2014

Kapanadze, G. D. 2006. The use of miniature pigs in biomedical experiments. *Biomedicina*, vol. 1, p. 40-51.

Ke, J., Zhang, G. Q., Wong, S. A., Li, L. B., Yang, Y. H., Wang, J. K. 1983. Effects of succinic acid on immune function. *J. Zhengzhou Univ*, vol. 3, p. 20-24.

Korobeynikova, E. N. 1989. Modification of determination of lipid peroxidation products in reaction with thiobarbituric acid. *Laboratory work*, vol. 7. p. 8-10. ISSN: 0023-6748.

Kumar, M. S., Karthikeyan, S., Ramprasad, C., Aruna, P. R., Mathivanan, N., Velmurugan, D., Ganesan, S. 2015. Investigation of phloroglucinol succinic acid dendrimer as antimicrobial agent against Staphylococcus aureus, Escherichia coli and Candida albicans. *Nano Biomed. Eng*, vol. 7, no. 2, p. 62-74. https://doi.org/10.5101/nbe.v7i2.p62-74

Kuznetsova E., Cherepnina, L., Motyleva, S., Brindza, J. 2016. Redistribution of mineral elements in wheat grain when applying the complex enzyme preparations based on phytase. *Potravinarstvo*, vol. 10, no. 1, p. 47-53. https://doi.org/10.5219/413

Kuznetsova, E., Motyleva, S., Mertvischeva, M., Zomitev, V., Brindza, J. 2016. Composition and microstructure alteration of triticale grain surface after processing by enzymes of cellulose complex. *Potravinarstvo*, vol. 10, p. 23-29. https://doi.org/10.5219/411

Li, F. H., Ya, Y., Yang, X. L., Tao, S. Y., Ming, J. 2013. Phenolic profiles and antioxidant activity of buckwheat (Fagopyrum esculentum Möenchand Fagopyrum tartaricum L. Gaerth) hulls, brans and flours. *J. Integr. Agric*, vol. 12, p. 1684-1693. https://doi.org/10.1016/S2095-3119(13)60371-8

Lin, L. Y., Liu, H. M., Yu, Y. W., Lin, S. D., Mau, J. L. 2009. Quality and antioxidant property of buckwheat enhanced wheat bread. *Food Chemistry*, vol. 112, p. 987-991. https://doi.org/10.1016/j.foodchem.2008.07.022

Liyana-Pathirana, C. M., Shadidi, F. 2006. Importance of insoluble-bound phenolics to antioxidant properties of wheat. *J. Agric. Food Chem*., vol. 54, p. 1256-1264. https://doi.org/10.1021/jf052556h

Ng, W. K., Koh, C. B., Teoh, C. Y., Romano, N. 2015. Farm-raised tiger shrimp, Penaeus monodon, fed commercial feeds with added organic acids showed enhanced nutrient utilization, immune response and resistance to vibrio harveyi challenge. *Aquaculture*, vol. 449, p. 69-77. https://doi.org/10.1016/j.aquaculture.2015.02.006

Ragaee, S., Guzar, I., Dhull N., Seetharaman, K. 2011. Seetharaman Effects of fiber addition on antioxidant capacity and nutritional quality of wheat bread. *LWT – Food Science and Technology*, vol. 44, p. 2147-2153.

Raghavendra, K. R., Renuka, N., Kumar, K. A., Shashikanth S. 2017. An Accessible Route for the Synthesis of Novel Lignan Derivatives and Their Biological Evaluation. *Pharmaceutikal chemistry journal* vol. 51, no. 8, p. 661-669. https://doi.org/10.1007/s11094-017-1671-7

Ravin, H.A. 1961. Improved colometric enzymatic ceruloplasmin assay. *Journal of laboratory and clinical medicine*, vol. 58, p. 161-168.

Rey, A. I., López-Bote, C. J., Litta, G. 2017. Effects of dietary vitamin E (DL-α-tocopheryl acetate) and vitamin C combination on piglets oxidative status and immune response at weaning. *Journal of Animal and Feed Sciences*, vol. 26, no. 3, p. 226-235. https://doi.org/10.22358/jafs/76595/2017

Safonova, O. A., Popova, T. N., Slivkin, A. I., Talmi, Y. 2015. Effects of a Preparation Containing Pantogam, Succinic Acid, and Chitosan on Activities of the Glutathione System and NADPH-Generating Enzymes in Rat Tissues under Conditions of Cerebral Ischemia/Reperfusion. *Bulletin of Experimental Biology and Medicine*, vol. 159, no. 2, p. 221-224. https://doi.org/10.1007/s10517-015-2927-4

Schatz, R. A., Lal, H.. 1980. Protection against hyperbaric oxygen toxicity by pargyline, succinic acid and ascorbic acid: Role of brain GABA and brain ammonia. *Brain Research Bulletin*, vol. 5, no. 2, p. 781-788. https://doi.org/10.1016/0361-9230(80)90130-6

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. https://doi.org/10.1016/j.foodchem.2004.03.049

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

Slavin, J., Marquart, L., Jacobs, D. 2000. Consumption of whole-grain foods and decreased risk of cancer: proposed mechanisms. *Cereal Food World*, vol. 45, p. 54-58.

Tang, X. L., Liu, J. X., LI, P., Dong, W., Lei, L., Zheng, Y. Q., Hou J. C. 2013. Protective effect of succinic acid on primary cardiomyocyte hypoxia/reoxygenation injury. *China J. Chinese Material Med*, vol. 38, no. 21, p. 41-45. https://doi.org/10.4268/cjcmm20132127

Truswell, A. S. 2002. Cereal grains and coronary heart disease. *Eur. J. Clin. Nutr*., vol. 56, no. 1, p. 11-14. https://doi.org/10.1038/sj.ejcn.1601283

Verma, B., Hucl, P., Chibbar, R. N. 2009. Phenolic acid composition and antioxidant capacity of acid and alkali hydrolyzed wheat bran fractions. *Food Chem*, vol. 116, p. 947-954. https://doi.org/10.1016/j.foodchem.2009.03.060

Vitaglione, P., Napolitano, A., Fogliano, V. 2008. Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science and Technology*, vol. 19, no. 9, p. 451-463. https://doi.org/10.1016/j.tifs.2008.02.005

Yin, J., Wu, M. M., Xiao R. W. K., Duan J. L., Yang, G., Li, T. J., Yin, Y. L. 2014. Development of an antioxidant system after early weaning in piglets. *Journal of Animal Science*, vol. 92, no. 9, p. 612-619. https://doi.org/10.2527/jas.2013-6986

Zadnipryany, I. V., Tretiakova, O. S., Kubyshkin, A. V., Sataieva, T. P. 2016. Cardioprotective effect of native antihypoxants in experimental cobalt cardiomyopathy. *Bulletin of Siberian medicine*, vol. 15, no. 3, p. 33-40. https://doi.org/10.20538/1682-0363-2016-3-33-40

Zhu, L. H., Zhao, K. L., Chen, X. L., Xu, J. X. 2012. Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs. *Journal of Animal Science*, vol. 90, no. 8, p. 2581-2589. https://doi.org/10.2527/jas.2012-4444

Zielińska, D., Turemko, M., Kwiatkowski, J., Zieliński, H. 2012. Evaluation of flavonoid contents and antioxidant capacity of the aerial parts of common and tartary buckwheat plants. *Molecules*, vol. 17, no. 8, p. 9668-9682. https://doi.org/10.3390/molecules17089668

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EFFECT OF INFRARED RADIATION ON CHEMICAL AND PHYSICAL PROPERTIES ON DUKU'S PEEL

Laila Rahmawati, Daniel Saputra, Kaprawi Sahim, Gatot Priyanto

ABSTRACT

Infrared radiation has a potential for drying agricultural commodities such as the peel of duku. Drying of duku's peel in a whole duku using infrared radiation could become an effective method to eliminate the water on the peel but not in the flesh and could increase the shelf life of duku. The objective of this study was to investigate the potential of using infrared radiation for drying the peel of duku which would increase the shelf life of duku during storage. Duku's peel drying process was achieved by means of heating duku using a pairs of electric infrared emitters (IRE) facing each other with the emitter distance of 6 cm and 10 cm for a relatively short heating time of 50, 60, 70 and 80 seconds and after that stored at a cool room at the temperature of 15 °C for the length of one month. During storage, the physical and chemical changes of duku were then evaluated. It was found that the weight loss, fruit firmness, and total soluble solid of duku dried by means of exposing to Infra Red Emitter (IRE) were significantly affected by the distance of IRE, the temperature of IRE and the time exposed to IRE. However the titratable acidity only affected significantly by the distance of IRE. There were no significantly changes of browning index on duku during drying by exposing to IRE and while stored up to 25th day of storage. Drying duku by exposing it to IRE show a slightly better shelf life than the previous work.

Keywords: infrared radiation; duku; shelf life; drying

INTRODUCTION

Lansium domesticum corr. is a tropical fruit of South East Asia with its member known as langsat, longkong and duku. Duku is one of the most favourite fruit in Indonesia because the mature and ripe one has a unique aroma and pleasant taste. Duku has a thicker peel and no latex while langsat has a peel that contains a milky sticky sap. There are 4 to 12 fruits per bunches for duku and 15 to 25 fruits for langsat. Both of them have five separate segments, with one to five seeds in it **(Paull, 2014)**. The fruit is easily peeled from the stem end and the flesh is commonly eaten out-of-hand or served as a dessert. Peel color (green for the immature to yellow for the mature one) and the lack of latex could be used as an indicator for the level of fruit maturity. All the bunches of one tree could not be harvested at the same time because of the non-uniformity of the fruit maturity. The fresh peel contains 0.2% of a light yellow, volatile oil, a brown resin and reducing acids. A dark, semi-liquid oleoresin composed of 0.17% volatile oil and 22% resin could be extracted from the dried peel. The dried peel could be burned to create an aromatic smoke serving as a mosquito repellant **(Phantumas, 1998)**. The extract of *Lansium domesticum* fruit was found to have an antioxidant as evaluated by the DPPH free radical assay and the dried hydro-ethanol extract of *Lansium domesticum* fruit could be used as cosmetic **(Tilaar et al., 2008)**.

Duku, at room temperature, has a limited post-harvest life of about 3 – 7 days. The deteroriation of quality which took place was in the form of pericarp browning, changes in texture, appearance and off-flavour after harvest **(Lichanporn et al., 2009; Venkatachalam and Meenune, 2012a)**. Post-harvest damage on duku could be controlled by several methods: chemical soaking, low temperature storing, modified atmosphere packaging and drying. The most commonly drying method is using hot air. This method is chosen because it is the easiest and the lowest cost **(Diamante et al., 2010)**. However, these treatments cause degradation of the product's components which leads to censorial loss of dry product. The food drying method that's commonly developed nowadays is using the low humidity-low temperature method **(Ondier et al., 2010)** and using high radiation frequency such as microwave, radio frequency, and infrared **(Contreras et al., 2008; Kassem, 2011)**.

The infrared radiation had been implemented in food processing to reduce the water content, lowering energy consumption and time spent in the process, securing and

ensuring the quality of foodstuffs processed **(Pan et al., 2011)**. Infrared also has been used to predicted quality of foods and agricultural by near infrared radiation due to the simple and minimal sample preparation **(Liguori et al., 2016)**. Some drying studies had been performed using infrared radiation systems to dry banana **(Pan et al., 2008; Pekke et al., 2013)**, tomato peeling **(Li et al., 2014; Li and Pan, 2014a; Li and Pan, 2014b; Pan et al., 2009; Pan et al., 2011; Pan et al., 2015)**, apple **(Nowak and Lewicki, 2004; Togrul, 2005)**, longan **(Nathakaranakule et al., 2010)**, celery **(Jezek et al., 2008)**, carrot **(Botelho et al., 2011; Grdzelishvili and Hoffman, 2012; Kocabiyik and Tezer, 2009)**, onion **(Mongpraneet et al., 2002; Pathare and Sharma, 2006; Sharma et al., 2005)**, potato **(Afzal and Abe, 1998; Doymaz, 2012; Grdzelishvili and Hoffman, 2012)**, red pepper **(Nasiroglu and Kocabiyik, 2007)**, peach **(Jun and Sheng, 2006)**, and spices **(Rachmat et al., 2010)**. The use of radiation in the food sector is not entirely beneficial for the food process. The previous research has suggested that the use of UV-C radiation negatively affects the sensory properties of grape juice because it has a significant change in the smell and taste of wine **(Czako et al., 2018)**. Infrared radiation had been used to inactivate lipoxygenase, an enzyme in soybean, with the result of 95.5% inactivation within 60 seconds **(Kouzeh-Kanani et al., 1982)**. Certain enzyme reactions involving lipase and amylase might be affected by the infrared heating **(Kohashi et al., 1992; Sawai et al., 2003)**. Infrared radiation could also inactivate pathogens in the material. Infrared radiation could inactivate bacteria, spore, yeast, and mold by controlling some of the influential parameters such as power on infrared heater **(Hamanaka et al., 2000)**, sample temperature **(Sawai et al., 2003)**, wavelength and the target wave in a wide range **(Krishnamurthy et al., 2008)**, sample thickness **(Sawai et al., 2000)**, and sample water content **(Hamanaka et al., 2006)**. The increased supply of radiant heat coud also be the result of tools design **(Zverev and Sesikashvili, 2018)**. The unique characteristics possessed by infrared radiation was it could only heat up the surface of the material in a short time without raising the temperature of the inside material **(Li and Pan, 2014a)**.

The unique characteristics of infrared radiation could be used to dry the outside surface of duku peel which would result in a shell like that would protect duku flesh from the outside air and from the attack of microbes. The microorganisms could be originated from storage. The controlled storage (the refrigerator and the showcase) could kept the product still fresh and it most suitable for most of foods and fruits **(Bubelová et al., 2017)**. Currently, there was no previous report found on the literature about the usage of infrared radiation for drying the whole duku's peel. Therefore, the objective of this research was to develop a new drying method for the whole peel of duku by means of infrared radiation.

Scientific hypothesis

The hypothesis of this research was that the infra red radiation could dry the skin of duku which would extend its shelf life.

MATERIAL AND METHODOLOGY

Sample of duku

Duku (Lansium domesticum) used in this study were obtained from a local farm in South Sumatera, Indonesia. The diameter of duku was measure using a Vernier Calliper that has a precision of 0.01 mm to determine the size of duku. The weight of the fruit was measured with electronic balance with a precision of 0.001 g. Duku fruits with a diameter of 2.5 to 3.5 cm were selected, checked and visually inspected and a defected ones were eliminated before drying test.

Infrared radiation heating system

A laboratory scale of IR-emitter heating consisted of two ceramic emitters (245 mm x 60 mm size) which had capacity of 1000 W for each emitter were installed facing each other. The vertical distance between the infrared heaters in the heating position could be adjusted by tightening and loosening of the nut moving on the screws provided on the space bar and it has cover to prevent the loss of temperature. The heating chamber was placed inside an enclosure with a door that opens upward. The fruit basket was placed horizontally on a frame with the wall cover the same size as the wall of the heating chamber. The fruit basket would accomodate three dukus, horizontally, with the diameter of 2.5 to 3.5 cm. The fruit basket was connected by means of a horizontal shaft which would rotate automatically with the speed of 30 rpm upon operating by means of sliding it into the heating chamber.

Infrared heating procedure

Initially, before the heating process, the fruit basket with its frame system was placed outside of the heating chamber at a distance of 1 m. The distance of the infrared emitter was adjusted according the distance treatment of 6 cm and 10 cm (treatment A). The infrared emitters were then turned on and were let to reach the treatment temperature of 200 °C, 300 °C and 400 °C respectively (treatment B). The temperature was monitored by a laboratory thermostat ($\pm$10 °C) and was shown by the LED termperature display (Extech 445815: Hygro-Thermometer Humidity with basic accurancy ±4% RH; ±1.8 °F, ±1 °C). The temperature was also confirmed by means of using laser radiation thermometer (Wintact-WT900-EN00). Upon reaching the desired temperature the heating system was let idle for 5 minutes to stabilized the heating system. Then the covered door was open upward and the empty fruit basket system, which was rotating at the speed of 30 rpm, was pushed into the heating chamber and then the door was closed down. The empty basket system was let to work for 60 seconds and then it was pulled out and the heating system door was closed down. This simulation was performed to let the whole system to adjust to the operating temperature. The fruit basket system was then let to cool down for 5 minutes and then 3 dukus with the diameter size of about 3 cm, arranged parallel, was placed into the fruit basket and locked to make sure the fruit would stay inside the basket during the treatment. The basket was then let to rotate for 60 seconds and the heating chamber door was open upward and the fruit basket system was slide pushed into the heating chamber and the door closed down. The fruit basket was

let inside the heating chamber for the lenght of 50, 60, 70 and 80 seconds according to the treatment length of exposure (treatment C). After the fruits exposed for the determined time the heating chamber door was open upward, fruit basket system was then slide pulled outward, the door closed down and the fruits were let to cool down to room temperature by means of placing it at room temperature for 30 minutes. After cooling down the fruits were stored in controlled temperature case of 15 °C ±2 °C and then the physical and chemical characteristics were measured every two days up to 20 days and everyday between the 20th to 30th day by means of destructive measurement. So there were time series of physical and chemical properties measurement. The determination of time series of duku were performed completely randomized. All the experiment was replicated three times.

The physical-chemical properties of the fruits that were measured periodically in this research were: weight of duku, fruit firmness, total soluble solids (TTS), titratable acidity (TA) and browning index.

At the initial of the experiment each duku was marked with a permanent marker and its weight measured. The initial physical and chemical properties of duku were measured by means of taking randomly 5 fruits and its physical and chemical properties measured and averaged and was determined as the initial physical and chemical properties of duku. The distance of the emitter was made fix until all the treatment of temperature and time exposed were completed; and then it was adjusted to other distance. The distance of the emitter was determined randomly and the temperature and time exposed treatment were completely randomized.

Physical quality changes

Physical quality changes measured were weight loss and fruit firmness. The weight loss (%) of duku were measured by taking the percentage ratio (%) of the different of initial weight to the measured weight of fruit at the deteremined time measured to the initial weight. Fruit firmness was measured by means of using a portable Penetrometer (Andilogs CNR-FT&V, precision of 0.25% FS) at room temperature (27 °C). The fruit firmness of duku were performed destructively at three point (Figure 1) which was at the top, middle and bottom of duku. The fruit firmness was digitally display in Newton and then the average of the three measurement was taken as the fruit firmness for one fruit.

Chemical quality changes

The chemical properties measured were the acidity by means of titratable acidity (TA), sugar content by means of total soluble solid (TSS) and browning index. The TA measurement was performed by means of titration method which is taking 5 gram of ground duku flesh into a beaker glass and then diluted with 100 mL distilled water by stirring it. The dilution was then filtered by using Whatman paper no. 42. The titration was performed by taking 20 mL of filtrate, adding two drop of the indicator PP and then titrated using standard solution (NaOH, 0.1 M).

The sugar content of duku on this experiment was measured by measuring the total soluble solid (TSS). The

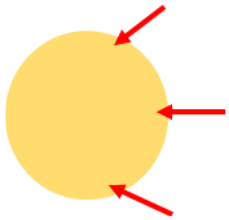

Figure 1 The Point of measurement for fruit firmness.

TSS was measured by grinding the duku flesh and then filtered by using the Whatman paper no. 42. The filtrate was then drop, by means of pippeting the filtrate, into a glass window of a digital refractometer and the TSS was shown on the display as a °brix (Atago Digital Refractometer Model DBX-55A range 0.0 – 55.0 brix, accuracy ±0.1%).

The Browning index was determined by means of spectrophotometric method **(Cohen et al., 1994)**.

The measurement was performed by initially making a standard solution to determine the wavelenght, λ_{max}, of duku solution. The λ_{max} for this measurement was found on 417 nm with absorbancy of 0.193 (Spectrophotometer Vis Thermo Scientific-Genesys 20; wavelength 325 – 1100 nm, accuracy 2.0 nm). The browning index was measured by taking 20 mL filtrate of duku as performed for the TA measurement and then putting it into a cuvette which was then inserted into the spectrometer. The browning index was then shown on the digital display of the spectrometer.

Statisic analysis

The statistical design used for this research was a split-split plot design with the main factor the distance of IR-emitter, while the sub factor was temperature of the IR-emitter, and the sub-sub factor was exposure time. The analysis of Variance (ANOVA) for the dependent variables were calculated with the help of Statistical Package (SAS version 9.4, 2014). The response of the dependent variables and its significancy were analysed by means of the F-value.

RESULTS AND DISCUSSION

Effect of parameters on physical and chemical properties on duku's peel

The effect of parameters on physical and chemical properties of duku's peel after exposing to IR-Emitter (IRE) was shown on Table 1. The IRE distance had a very significant effect (p <0.0001) to the weight loss, fruit firmness, titratable acidity, total soluble solid, and browning index. The IRE temperature of 200, 300 and 400 °C had a significant effect (p <0,05) to the weight loss, fruit firmness, TA and TSS but not to the browning index. While the exposure time of 50, 60, 70 and 80 second only significant (p <0.0001) to the weight loss, fruit firmness, TSS, and browning index. In general, the IRE treatment only had a significant effect to the weight loss, fruit firmness and TSS.

Table 1 Statistical Result of ANOVA.

Source	Weight loss		Fruit Firmness		Titratable Acidity		Total Soluble Solid		Browning Index	
	F Value	*p* >F	F Value	*p*>F	F Value	*p* >F	F Value	*p* >F	F Value	*p* >F
D	29.24	<.0001	605.25	<.0001	46.06	<.0001	151.81	<.0001	40.85	<.0001
T	57.89	<.0001	10.33	<.0001	4.00	0.0085	13.02	<.0001	2.01	0.79027
D*T	17.68	0.0004	17.65	<.0001	1.73	0.1631	5.83	0.0008	1.82	1.00555
E	19.98	<.0001	12.01	<.0001	1.90	0.1119	6.29	<.0001	11.44	<.0001
D*E	9.79	<.0001	41.92	<.0001	2.31	0.0591	2.43	0.0492	10.55	<.0001
T*E	0.000	1	4.07	0.0015	5.15	0.0002	0.96	0.4461	0.00	1
D*T*E	0.000	1	4.32	0.0009	2.31	0.0459	4.24	0.0011	0.31	6.28263

Note: D = distance of IR-emitter (cm), T = Temperature of IR-emitter (°C), E = exposure time to the IR-emitter (s).

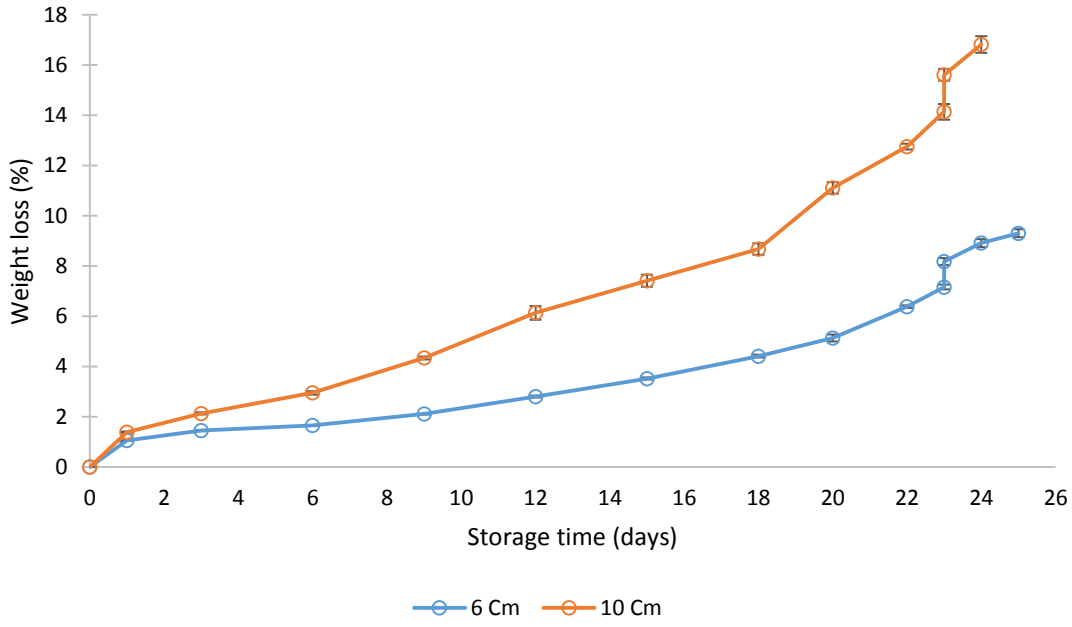

Figure 2 The weight loss of duku during storage time exposed to IRE distance of 6 cm and 10 cm.

Weight Loss

The weight loss of duku during storage occurs due to the influence of IRE distance, temperature and exposure time. The IRE distance of 6 cm and 10 cm has has a significant effect (p <0.0001) on weight loss during storage (Figure 2). The largest weight loss occurs in duku exposed to IRE radiation with a distance of 10 cm. This result was paralled to the results of fruit firmness at a distance of 10 cm. This condition could happen because the skin of duku was not receiving energy as much as the IRE distance of 6 cm which make duku's skin of 10 cm distance was not as dried as the 6 cm distance. The drier the duku's skin the firmer the skin and the more the skin would protect the duku's flesh and the less moisture could evaporate from the duku which would make the moisture loss of the duku exposed to IRE distance of 10 cm consistently had higher weight loss than the 6 cm distance (Figure 2).

The temperature of the IRE also had a significant effect (p <0.0001) on the weight loss during the storage time. Figure 3 shows that at a temperature of 400 °C (IRE distance of 6 cm) had the greatest weight loss compared to the temperatures of 200 °C and 300 °C. The higher the IRE temperature, the greater the heat energy radiated from the IRE which would cause the difference between the heating medium and the fruit layer. The temperature difference between the heating medium and the material causes transfer of heat into the material and the faster the transfer of water vapor from the material to the environment. The faster the vapor evaporates from the material the higher the weight loss **(Brackmann et al., 2014)**. Some studies reported that the higher the weight loss or mass loss on fruit skin the higher the postharvest quality due to the the gas exchange **(Brackmann et al., 2007)**. This also might be attributed to the fact that the

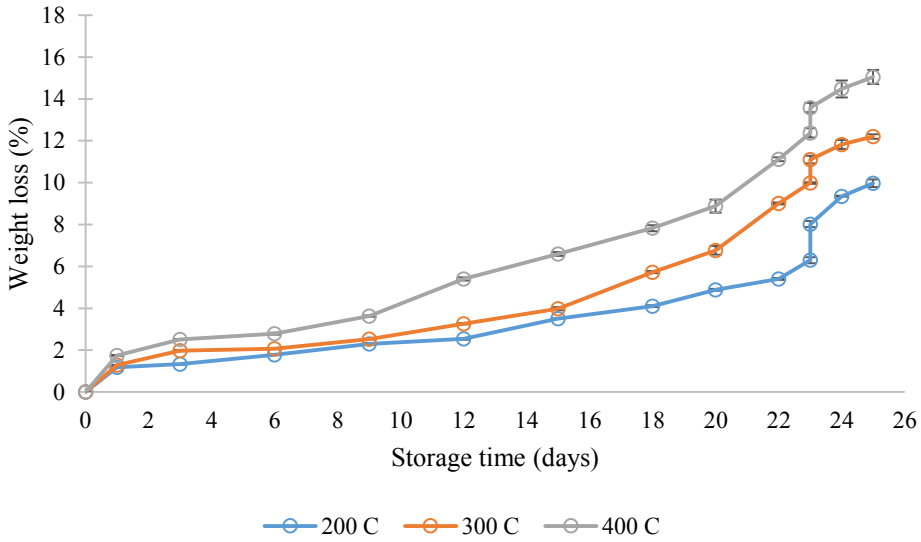

Figure 3 The weight loss of duku during storage time exposed to the IRE distance of 6 cm and temperatur of 200 °C, 300 °C and 400 °C.

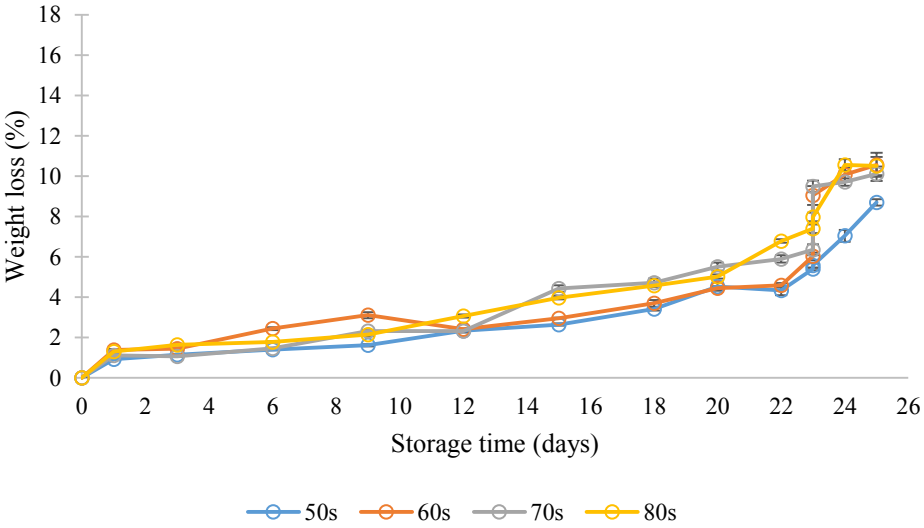

Figure 4 The weight loss of duku during storage time exposed to temperature IRE of 400 %or exposure time of 50, 60, 70 and 80 seconds.

increase of exposure time and temperature of IRE may increase the weight loss because of the accumulation of heat and evaporation of water vapor on fruit surface and flesh fruit during the process **(Li and Pan, 2014a)**, which is in agreement with the significant result shown on Table 1 that the exposure time was significant (p <0.0001) to the weight loss of fruit (Figure 4). The temperature of duku surface immediately rose up when the IR drying time was increase, which was mainly due to the enhanced radiative heat transfer to the fruit surface **(Ding et al., 2015)**.

Fruit Firmness

The fruit firmness was affected significantly by the IRE distance, temperature, and exposure time (p < 0.0001). Duku exposed to the IRE distance of 6 cm showing the increase of skin texture with the storage time which was parallel the weight loss shown on the Figure 2. Duku's skin exposed to the IRE with the distance of 10 cm was not as dried as the distance of 6 cm which make duku's skin less firmer and had a tendency of higher moisture content. The effect of IRE distance of 6 cm and 10 cm had the intensity of the emission on the surface of the fruit skin which is inversely proportional to the square of the distance from the radiation source. The closer the emitter is to the object (duku fruit), the greater the amount of radiation to the object (duku fruit) the harder the skin of duku with the storage time (Figure 5).

The changes in fruit firmness were also significant (p <0.0001) due to temperature and exposure time (Table 1). The higher the temperature used in the drying process, the drier the duku's skin and the firmer the skin which make the fruit firmnes increasing with storage time (Figure 6). During the exposure of duku to the IRE, the infra-red

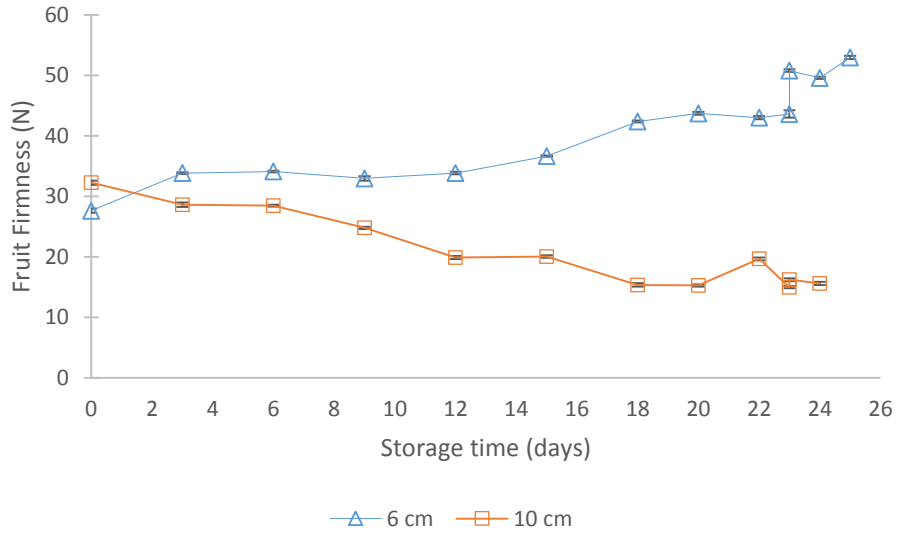

Figure 5 Fruit firmness during storage time in 6 cm and 10 cm distance of IRE.

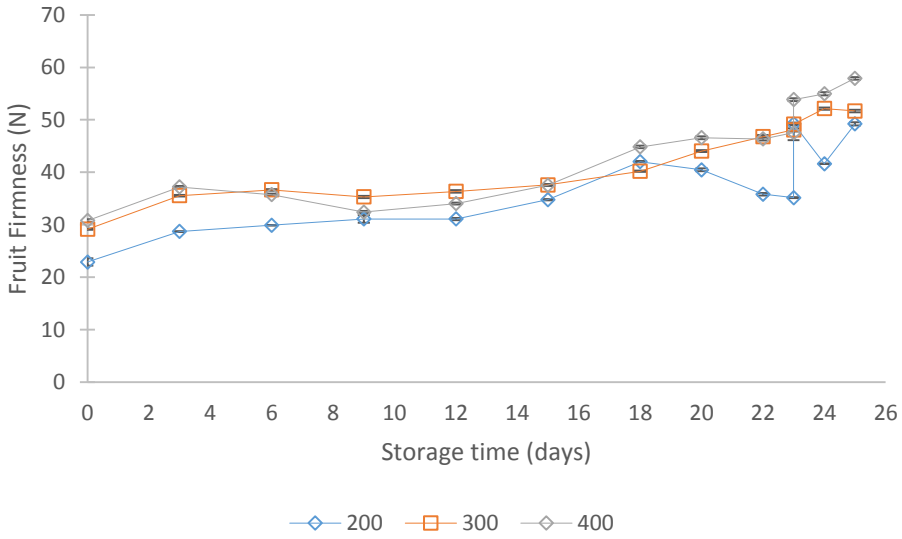

Figure 6 Fruit firmness during storage time at 200 °C, 300 °C and 400 °C in 6 cm distance of IRE.

beaming would hit the skin of duku then the heat would penetrate the inner tissue through radiation heat transfer. This thermal energy would cause sudden temperature increase which would cause cell wall damage, cell's water vapor evaporation, pectin breaks, mid-lamella cell damage, and polysaccharides cell wall degradation. The changes of firmness on duku also affected by the duration of radiation exposure and storage time after drying. A longer exposure time of infra red might result in the degradation of tissue's skin and the reduction of adhesiveness to the fruit flesh **(Li and Pan, 2014b)**. It could be seen from statistical analyze that exposure time has significant effect (p <0.0001) to the firmness of duku skin (Figure 7). Further analysis show that there is no significancy found on the firmness of skin between the duku exposure time of 50 and 60 second; and also between the 70 and 80 second exposure time. However there was a significancy occurs between the groups of 50, 60 second exposure with the groups of 70 and 80 second exposure time. The fruit firmness also reduced gradually during storage might be caused by polygalacturonase (PG) and pectin methyl esterase (PME). PG is a texture related fruit enzyme that catalyzes the hydrolysis of the linear α-1, 4, D-galacturonan backbone of pectic polysaccharides **(Poovaiah and Nukuya, 1979)**, and PME is one of the enzyme types that could hidrolyze pectin.

Titratable Acidity

Although the sweetness in one of the important component of fruit but organic acid is also has a role in the overall fruit flavor. The acid-sugar balance on fruit is essential for producing a pleasant taste in fruit, especially in duku. The IRE distance on this experiment as shown on Table 1 has a significant effect to the titratable acidity of duku (p <0,0001). The titratable acidity of duku exposed to the IR-emitter distance of 6 cm and 10 cm were going

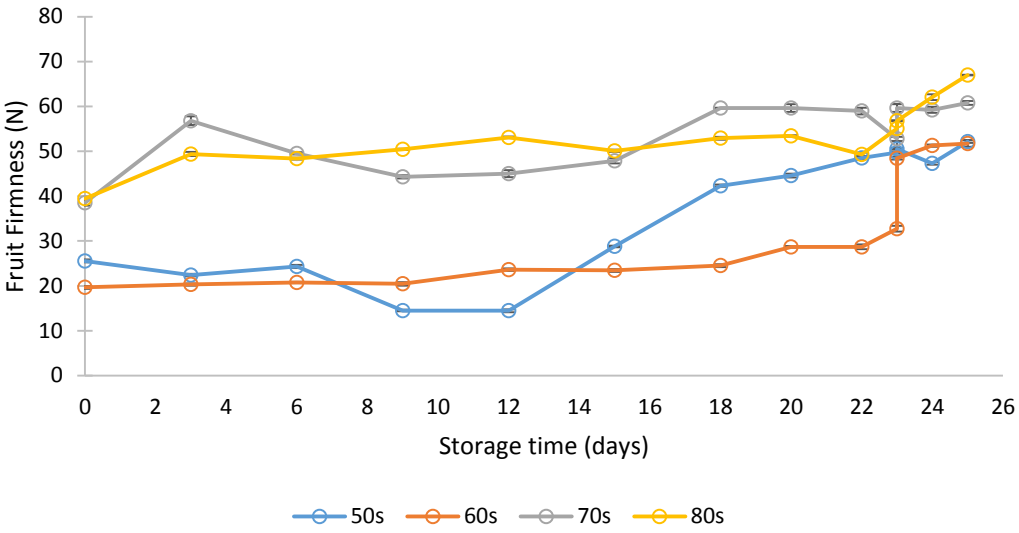

Figure 7 Fruit firmness during storage time at 50 s, 60 s, 70 s and 80 s in 400 °C temperature of IRE.

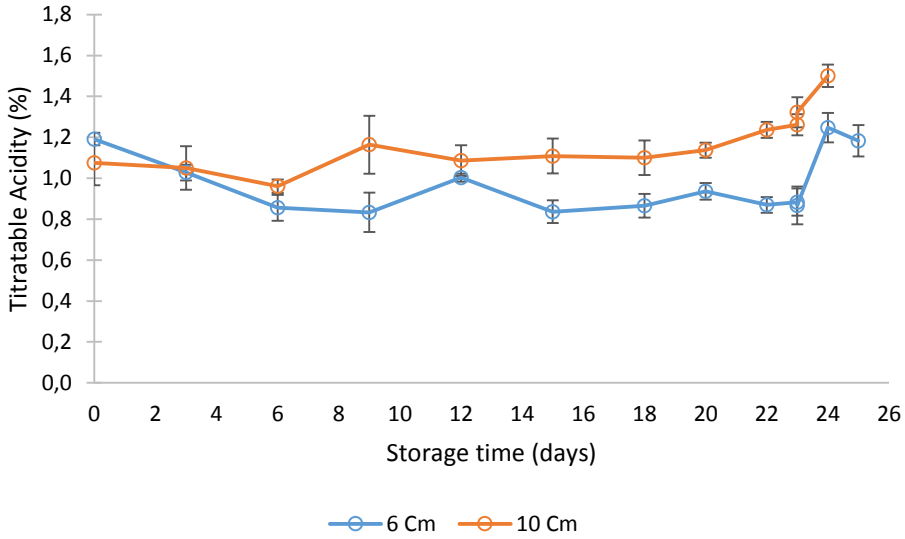

Figure 8 Titratable acidity (%) during storage time in 6 cm and 10 cm distance of IRE.

down slight up to the 6th days of storage and then split up. Duku exposed to the IRE distance of 6 cm stayed relatively the same around the 0.8% while the IRE distance of 10 cm went up and stayed relatively the same around the 1.15% until the end of the observation.

The changes of titratable acidity up to the 10th storage day affected by the IRE distance paralel to the increase and also the decrease of sugar (total soluble solid) which indicates that the changes of titratable acidity was the result of sugar changes. The organic acid would degrade into fructose and glucose (Figure 9) until the 10th storage days which then slowly increases until the last day of storage. The titratable acidity of duku after the 10th day of storage was relatively flat but the titratable acidity of duku exposed to IRE with the distance of 10 cm is always higher than duku exposed to IR-emitter distance of 6 cm. Duku's skin, after exposing to the IRE, had a tendency to have a firmer texture and a lower moisture content that would make the duku skin to have a shell like skin. This condition would create an armor to the fruit flesh that result in a semi-modified atmosphere to the duku's flesh which reduce the transport of CO_2 and O_2 from the duku's flesh to the environment. This semi-modified atmosphere condition would prevent the conversion of acid into sugar and would make duku flesh fell into a slower metabolism and respiration which would reduce the usage of energy. This phenomenon was shown by duku exposed to the IR-emitter with the distance of 6 cm but not for the one exposed to the 10 cm distance. Duku´s exposed to IRE of 10 cm has a softer skin compare to the 6 cm (Figure 5) which make its skin would transfer more O_2 to the flesh and produce more acid compare to the duku exposed to the IRE of 6 cm.

The reduction of O_2 and CO_2 on the 12th to the 22nd storage days impaired the function of Krebs cycle **(Davies, 1980)**. The reduction of O_2 resulting in pyruvate would

undergo homolactic fermentation or alcohol fermentation. The experiment conducted for duku did not resulting in the alcohol fermentation but resulting in relatively the same level of titratable acidity (Figure 8) and only increase significantly after the 22nd day due to the decaying of duku.

Contrary to the effect of the effect of IR-emitter distance which have a very significant effect to the titratable acidity, the temperature of the IR-emitter only have a significant difference in the level of 5% (p =0.0085) while the exposure time to the IR-emitter did not have a significant effect to the titratable acidity. This result show that the IRE distance has a more significant effect to the shell like skin which preventing the organic acid of fruit converted to other substance due to the semi-modified atmosphere condition within the duku.

Total Soluble Solid

The sugar content of duku on this experiment was measured by means of using refractometer and shown as a total soluble solid (TSS). TSS in duku was significantly affected by the IRE distance (Table 1). TSS in duku exposed to the IRE radiation, for the first 10th day of storage were influenced by the changing of titratable acidity (TA) but after that decrease gradually because it was converted to monosaccharide due to the respiration process (Figure 9). The increase of TSS after the infrared radiation exposure and storage was due to the conversion of carbohydrate to glucose. The carbohydrate conversion to glucose could be caused by the respiration in storing period. Starch can be converted back to glucose by the least three different enzymes namely, α-amylase, β-amylase and starch phosphorylase **(Suárez-Dieguez et al., 2009)**. In non-climacteric fruit, the accumulation of sugar is associated with the development of optimum eating quality, although the sugar may be derived from sap imported into the fruit rather than from the fruit breakdown of starch reserves **(Nascimento et al., 2006)**.

Duku's skin which became firmer for the IRE distance of 6 cm after the 10th day of storage preventing the transport

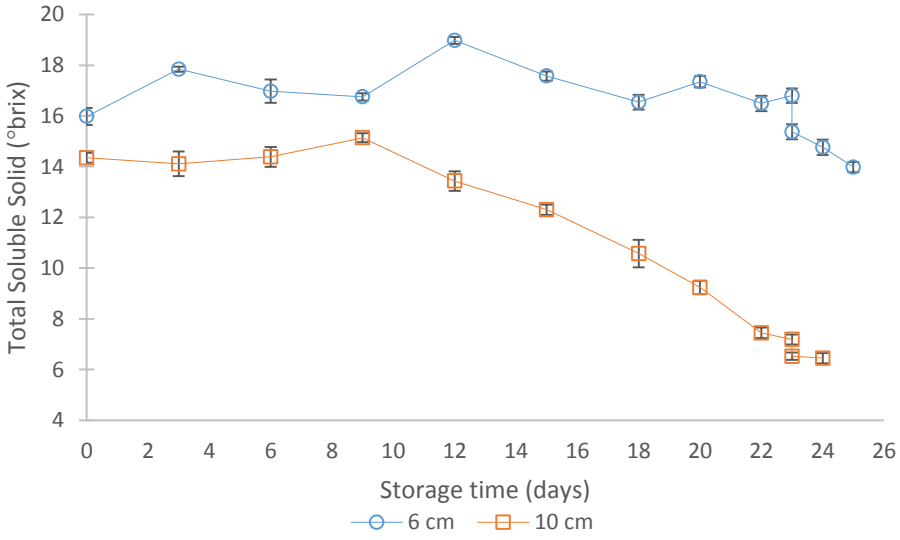

Figure 9 Total soluble solid (brix) during storage time in 6 cm and 10 cm distance of IRE.

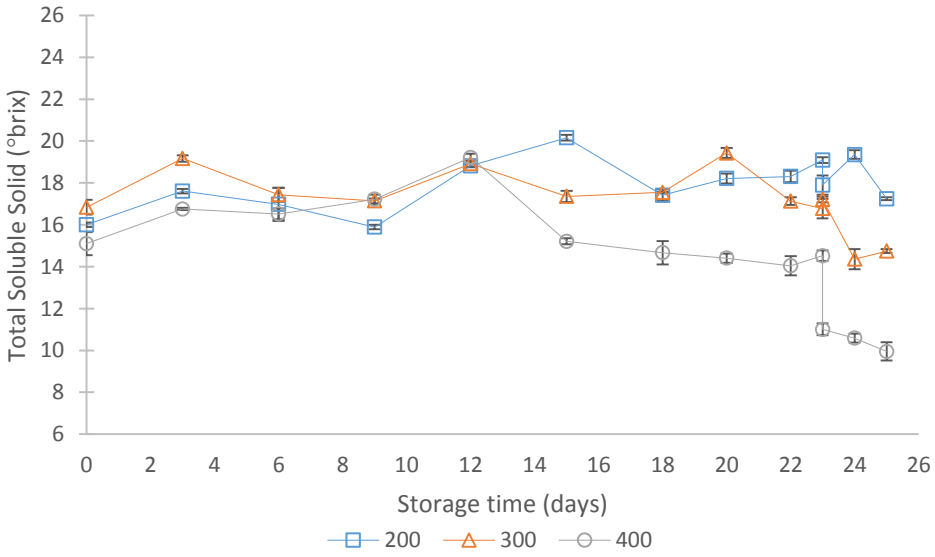

Figure 10 Total soluble solid (brix) during storage time at 200 °C, 300 °C and 400 °C in 6 cm IRE distance.

of O_2 and CO_2. However this condition was different for the TSS exposed to IRE distance of 10 cm which was softer than the 6 cm distance that would facilitate the gas exchange. The gas exhange would facilitate the conversion of acid into sugar which was happen to duku exposed to the IRE distance of 10 cm, however, since the requirement of energy for metabolism and respirtion relatively higher for duku with less modified atmosphere would consume the sugar in the flesh and resulted in lower concentration of TSS for duku exposed to IRE distance of 10 cm. However, when TA had decreased (Figure 8), TSS has increased significantly during storage. The increase in TSS was probably due to the solubilization of neutral sugar from carbohydrate polymer and resulted in the TSS/TA ratio and led to the sweetness of duku **(Sapii et al., 2000).**

The temperature and expose time of IRE, both had the significant effect to the TSS but the significancy mostly happened after the 10th day storage. This differences were affected by the IRE distance to the TSS which was very significantly different after the 10th day of storage. The TSS of duku after that time period significantly lower for the one exposed to the IRE distance of 10 cm. In this condition, the effect and the interaction between IR radiation (temperature and exposure time) and component of TSS (Fructose, Glucose, and Sucrose) should be further investigated.

Browning Index

Many tropical fruits are susceptible to browning and therefore might cause the economic losses to the agriculturist. The browning on fruit caused by browning enzyme was catalized by the polyphenol oxidase (PPO) enzyme and peroxidase enzyme. Peroxidase and phenylalanine ammonia lyase could also increase the activity of browning **(Kincal et al., 2006; Zocca et al., 2008)**. Browning might happened due to the membrane damage that was caused by the heating process using infrared radiation. The membrane damage would also damage the cell and in turn resulted in the lysis of plasma from the cell and increase the permeability of fruit cells and facilitated the mixing of enzyme and substrate which produce the browning reaction on the fruit **(Lichanporn et al., 2008; Venkatachalam and Meenune, 2012b)**. Browning reactions on duku could also occur due to fruit storage condition. The lipoxygenase, polyphenol oxidase, phenylalanine ammonia lyase, peroxidase enzymes are activated if the chilling injury occurs on storage time. The operating conditions such as IRE distance had a significant effect to the Browning index while temperature, and exposure time had no-significant effect to the Browning index of this research (Table 1). The browning index measurement for this research was found, for almost all the measurement performed on this research, to have value under or around the sensitivity of the instrument used (0.2 Abs) except after the 20th day of storage which was caused by the decaying duku (Figure 11). Due to the value of Browning index very close to the sensitivity of the instrument it was decided that the IRE treatment did not have a significant effect to the Browning index of duku after exposing duku to IRE and during storage.

Shelf life

The effect of infra red drying to the shelf life of duku by considering the weight loss, fruit firmness, titratable acidity, total soluble solid, and browning index during storage show a slightly better result than the previous work **(Saputra and Pratama, 2013; Saputra et al., 2014)**. This conculsion was drawn by considering the limiting factor which is the fruit firmness and total soluble solid. The fruit firmnes of duku expose to the IRE distance of 10 cm after 14 days of storage had came to the level of 50% of the fruit firmnes of day zero which was to low for a good duku. Besides that the level of total soluble solid for duku exposed to IRE distance of 10 cm after 14 days of storage also had came to a low concentration which eventhough still acceptable but its firmness made it un-acceptable.

Different from the result found for the duku exposed to the IRE distance of 10 cm, the one exposed to IRE distance of 6 cm show a different result. Eventhough the weight loss, fruit firmness, titratable acidity, and total soluble solid did not show a significant changes compare to the original value but from visual inspection it was found that some mold had been growing on duku's skin on some of the duku at the 20th day of storage which would be

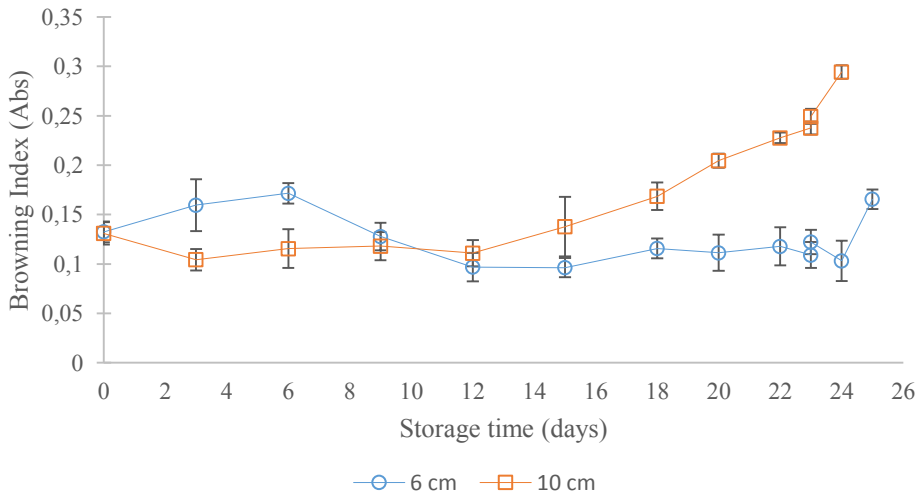

Figure 11 Browning index during storage of duku which was affected by the distance of IRE.

un-acceptable to the consumer. Based on that the shelf life of duku at the 20th storage day and the safety of consumer it was decided that the shelf of duku exposed to the IRE was 16 days.

CONCLUSION

It was found that the weight loss, fruit firmness, and total soluble solid of duku dried by means of exposing to Infra Red Emitter (IRE) were significantly affected by the distance of IRE, the temperature of IRE and the time exposed to IRE. However the titratable acidity only affected significantly by the distance of IRE. There were no significantly changes of browing index on duku during drying by exposing to IRE and while stored up to 25th day of storage. Drying duku by exposing it to IRE show a slightly better shelf life than the previous work.

REFERENCES

Afzal, T. M., Abe, T. 1998. Diffusion in Potato During Far Infrared Radiation Drying. *Journal of Food Engineering*, vol. 37, p. 353-365. https://doi.org/10.1016/S0260-8774(98)00111-3

Botelho, F. M., Corrêa, P. C., Goneli, A. L. D., Martins, M. A., Magalhães, F. E. A., Campos, S. C. 2011. Periods of constant and falling-rate for infrared drying of carrot slices. *Revista Brasileira de Engenharia Agricola e Ambiental*, vol. 15, no. 8, p. 845-852. https://doi.org/10.1590/S1415-43662011000800012

Brackmann, A., Pinto, J. A. V., Weber, A., Neuwald, D. A., Steffens, C. 2007. Indução da perda de massa fresca e a ocorrência de distúrbio. *Revista Brasileira de Armazenamento*, vol. 32, p. 87-92.

Brackmann, A., Thewes, F. R., Anese, R. de O., Both, V., Gasperin, A. R. 2014. Respiration rate and its effect on mass loss and chemical qualities of "Fuyu" persimmon fruit stored in controlled atmosphere. *Ciência Rural*, vol. 44, no. 4. p. 612-615. https://doi.org/10.1590/S0103-84782014000400006

Bubelová, Z., Cerníková, M., Bunková, L., Talár, J., Zajícek, V., Foltin, P., Bunka, F. 2017. Quality changes of long-life foods during three-month storage at different temperatures. *Potravinarstvo Slovak Journal of Food Sciences,* vol. 11, no. 1, p. 43-51. https://doi.org/10.5219/688

Cohen, E. L. I., Birk, Y., Mannheim, C. H., Saguy, I. S. 1994. Kinetics parameter esFcohentimation for quality change during continuous thermal processing of grapefruit juice. *Journal of Food* Science, vol. 59, no. 1, 155 p. https://doi.org/10.1111/j.1365-2621.1994.tb06922.x

Contreras, C., Martín-Esparza, M. E., Chiralt, A., Martínez-Navarrete, N. 2008. Influence of microwave application on convective drying: Effects on drying kinetics, and optical and mechanical properties of apple and strawberry. *Journal of Food Engineering*, vol. 88, no. 1, p. 55-64. https://doi.org/10.1016/j.jfoodeng.2008.01.014

Czako, P., Zajác, P., Čapla, J., Vietoris, V., Maršálková, L., Čurlej, J., Belej, L., Golian, J., Benešová, L., Martišová, P. 2018. The effect of UV-C irradiation on grape juice turbidity, sensoric properties and microbial count. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 1-10. https://doi.org/10.5219/856

Davies, D. D. 1980. Anaerobic metabolism and the production of organic acid. *The Biochemistry of Plants*, New York, p. 581-611. https://doi.org/10.1016/B978-0-12-675402-5.50020-9

Diamante, L. M., Ihns, R., Savage, G. P., Vanhanen, L. 2010. A new mathematical model for thin layer drying of fruits. *International Journal of Food Science and Technology*, vol. 45, p. 1956-1962. https://doi.org/10.1111/j.1365-2621.2010.02345.x Ding, C., Khir, R., Pan, Z., Zhang, J., Tu, K., El-Mashad, H. 2015. Effect of Infrared and Conventional Drying Methods on Physicochemical Characteristics of Stored White Rice. *Cereal Chemistry*, vol. 92, no. 5, p. 441-448. https://doi.org/10.1094/CCHEM-11-14-0232-R Doymaz, İ. 2012. Infrared drying of sweet potato (Ipomoea batatas L.) slices. *Journal of Food Science and Technology*, vol. 49, p. 760-766. https://doi.org/10.1007/s13197-010-0217-8

Directorate General of Intelectual Property Ministry of Law and Human Rights Republic of Indonesia. *Flexible plastic bag for the storage of fruits and vegetables*. Patent inventor: Saputra, D., Pratama, F., Halimi, E. S. 2014. Indonesian Patent IDP0035665. IPC: B 65 D 30/00.

Grdzelishvili, G., Hoffman, P. 2012. Infrared drying of food products. *Czech Technical University in Prague*, Department of Process Engineering. 11 p. Available at: http://chps.fsid.cvut.cz/pt/2012/pdf/2511.pdf.

Hamanaka, D., Dokan, S., Yasunaga, E., Kuroki, S., Uchino, T., Akimoto, K. 2000. The Sterilization Effects of Infrared Ray on The Agricultural Products Spoilage Microorganisms (part I). *ASAE Annual Meeting Presentation*. Paper No. 006090, p. 1-9. Available at: https://www.researchgate.net/publication/286575964_The_sterilization_effects_of_infrared_ray_on_the_agricultural_products_spoirage_microorganisms.

Hamanaka, D., Uchino, T., Furuse, N., Han, W., Tanaka, S. ichiro. 2006. Effect of the wavelength of infrared heaters on the inactivation of bacterial spores at various water activities. *International Journal of Food Microbiology*, vol. 108, p. 281-285. https://doi.org/10.1016/j.ijfoodmicro.2005.11.019

Jezek, D., Tripalo, B., Brncic, M., Karlovi, D., Brncic, S., Rimac.,V. T., Drazen., Karlovic, S. 2008. Dehydration of Celery by Infrared Drying. *Croatica Chemica Acta*, vol. 81, p. 325-331.

Jun, W., Sheng, K. 2006. Far-infrared and microwave drying of peach. *LWT - Food Science and Technology*, vol 39, p. 247-255. https://doi.org/10.1016/j.lwt.2005.02.001

Kassem, A. S. 2011. Comparison of drying characteristics of Thompson seedless grapes using combined microwave oven and hot air drying. *Journal of the Saudi Society of Agricultural Sciences,* vol. 10, no.1, p. 33-40. https://doi.org/10.1016/j.jssas.2010.05.001

Kincal, D., Hill, W. S., Balaban, M., Portier, K. M., Sims, C. A., Wei, C. I., Marshall, M. R. 2006. A continuous high-pressure carbon dioxide system for cloud and quality retention in orange juice. *Journal of Food Science*, vol. 71, no. 6, p. 338-344. https://doi.org/10.1111/j.1750-3841.2006.00087.x

Kocabiyik, H., Tezer, D. 2009. Drying of carrot slices using infrared radiation. *International Journal of Food Science and Technology*, vol. 44, no. 5, p. 953-959. https://doi.org/10.1111/j.1365-2621.2008.01767.x

Kohashi, M., Akao, K., Watanabe, T. 1992. Non-thermal Effects of a Ceramics Heater on Xanthie Oxidase Activity. *Bioscience, Biotechnology, and Biochemistry, Biotech, Biochem*, vol. 57, no. 12, p. 1999-2004. https://doi.org/10.1271/bbb.57.1999

Kouzeh-Kanani, M., Zuilichem D. J. van, Roozen, J. P., Pilnik, W. 1982. A Modified Procedure for Low Temperature Infrared Radiation of Soybeans. II. Inactivation of Lipoxygenase and Keeping Quality of Full Fat Flour. *Lebensmittel-Wissenschaft und -Technologie*, vol. 15, no. 3, p. 139-142. ISSN 0023-6438.

Krishnamurthy, K., Jun, S., Irudayaraj, J., Demirci, A. 2008. Efficacy of infrared heat treatment for inactivation of staphylococcus aureus in milk. *Journal of Food Process Engineering*, vol. 31, no. 6, p. 798-816. https://doi.org/10.1111/j.1745-4530.2007.00191.x

Li, X., Pan, Z. 2014a. Dry-peeling of Tomato by Infrared Radiative Heating: Part I. Model Development. *Food and Bioprocess Technology*, vol. 7, p. 1996-2004. https://doi.org/10.1007/s11947-013-1203-8

Li, X., Pan, Z. 2014b. Dry Peeling of Tomato by Infrared Radiative Heating: Part II. Model Validation and Sensitivity Analysis. *Food and Bioprocess Technology,* vol. 7, p. 2005-2013. https://doi.org/10.1007/s11947-013-1188-3

Li, X., Pan, Z., Atungulu, G. G., Wood, D., McHugh, T. 2014. Peeling mechanism of tomato under infrared heating: Peel loosening and cracking. *Journal of Food Engineering*, vol. 128, p. 79-87. https://doi.org/10.1016/j.jfoodeng.2013.12.020

Liguori, G., Sortino, G., Inglese, P., Farina, V. 2016. Quality changes during postharvest life in white fleshed peach (*Prunus Persica* L. Batsch) fruits: Preliminary observations. *Bulgarian Journal of Agricultural Science*, vol. 22, no. 3, 497-504. Available at: https://www.agrojournal.org/22/03-25.pdf.

Lichanporn, I., Srilaong, V., Wongs-Aree, C., Kanlayanarat, S. 2008. External quality and physiological changes in longkong fruit (Aglaia dookkoo Griff) during storage at various relative humidity. *Acta horticulturae*, vol. 804, p. 373-378. https://doi.org/10.17660/ActaHortic.2008.804.53

Lichanporn, I., Srilaong, V., Wongs-Aree, C., Kanlayanarat, S. 2009. Postharvest physiology and browning of longkong (*Aglaia dookkoo* Griff.) fruit under ambient conditions. *Postharvest Biology and Technology*, vol. 52, p. 294-299. https://doi.org/10.1016/j.postharvbio.2009.01.003

Mongpraneet, S., Abe, T., Tsurusaki, T. 2002. Accelerated drying of welsh onion by far infrared radiation under vacuum conditions. *Journal of Food Engineering*, vol. 55, no. 2, p. 147-156. https://doi.org/10.1016/S0260-8774(02)00058-4

Nascimento, J. R. O., Júnior, A. V., Bassinello, P. Z., Cordenunsi, B. R., Mainardi, J. A., Purgatto, E., Lajolo, F. M. 2006. Beta-amylase expression and starch degradation during banana ripening. *Postharvest Biology and Technology*, vol. 40, p. 41-47. https://doi.org/10.1016/j.postharvbio.2005.11.008

Nasiroglu, S., Kocabiyik, H. 2007. Thin-layer infrared radiation drying of red pepper slices. *Journal of Food Process Engineering*, vol. 32, no. 1, p. 1-16. https://doi.org/10.1111/j.1745-4530.2007.00195.x

Nathakaranakule, A., Jaiboon, P., Soponronnarit, S. 2010. Far-infrared radiation assisted drying of longan fruit. *Journal of Food Engineering*, vol. 100, no. 4, p. 662-668. https://doi.org/10.1016/j.jfoodeng.2010.05.016

Nowak, D., Lewicki, P. P. 2004. Infrared drying of apple slices. *Innovative Food Science and Engineering Technologies,* vol. 5, no. 3, p. 353-360. https://doi.org/10.1016/j.ifset.2004.03.003

Ondier, G. O., Siebenmorgen, T. J., Mauromoustakos, A. 2010. Low-temperature, low-relative humidity drying of rough rice. *Journal of Food Engineering*, vol. 100, no. 3, p. 545-550. https://doi.org/10.1016/j.jfoodeng.2010.05.004

Pan, Z., Li, X., Bingol, G., McHugh, T. H., Atungulu, G. G. 2009. Development of infrared radiation heating method for sustainable tomato peeling. *Applied Engineering in Agriculture,* vol. 25, no. 6, p. 935-941. https://doi.org/10.13031/2013.29227

Pan, Z., Li, X., Khir, R., El-Mashad, H. M., Atungulu, G. G., McHugh, T. H., Delwiche, M. 2015. A pilot scale electrical infrared dry-peeling system for tomatoes: Design and performance evaluation. *Biosystems Engineering*, vol. 137, p. 1-8. https://doi.org/10.1016/j.biosystemseng.2015.06.003

Pan, Z., Li, X., Yong, W., Atungulu, G. 2011. Development of infrared heating technology for tomato peeling. *The 11th International Congress on Engineering and Food*, May 22-2. Athens, Greece. p. 795-96.

Pan, Z., Shih, C., McHugh, T. H., Hirschberg, E. 2008. Study of banana dehydration using sequential infrared radiation heating and freeze-drying. *LWT - Food Science and Technology*, vol. 41, no. 10, p. 1944-1951. https://doi.org/10.1016/j.lwt.2008.01.019

Pathare, P. B., Sharma, G. P. 2006. Effective Moisture Diffusivity of Onion Slices undergoing Infrared Convective Drying. *Biosystem Engineering*, vol. 93, no. 3, p. 285-291. https://doi.org/10.1016/j.biosystemseng.2005.12.010

Paull, R. E. 2014. Longkong, Duku, and Langsat: Postharvest Quality-Maintenance Guidelines. *College of Tropical Agriculture and Human Resources*. University of Hawai'i at Manoa. Available at: https://www.ctahr.hawaii.edu/oc/freepubs/pdf/F_N-42.pdf.

Pekke, M. A., Pan, Z. L., Atungulu, G. G., Smith, G., Thompson, J. F. 2013. Drying characteristics and quality of bananas under infrared radiation heating. *International Journal of Agricultural and Biological Engineering*, vol 6, no. 3, p. 58-70. https://doi.org/10.3965/j.ijabe.20130603.008

Phantumas, A. 1998. Longkong Planting. *The Encyclopedia of Fruit & Nuts.* London, UK : CABI International, 954 p. ISBN: 9780851996387.

Poovaiah, B. W., Nukuya, A. 1979. Polygalacturonase and cellulase enzymes in the normal Rutgers and mutantrin tomato fruits and their relationship to the respiratory climateric. *Plant Physiology*, vol. 64, p. 534-537. https://doi.org/10.1104/pp.64.4.534

Rachmat, R., Hadipernata, M., Sumangat, D. 2010. Pemanfaatan Teknologi Far Infra Red (FIR) Pada Pengeringan Rempah. *Balai Besar Penelitian dan Pengembangan Pascapanen Pertanian,* vol. 22, no. 1, p. 31-37.

Sapii, A. T., Yunus, N., Muda, P., Lin, T. S. 2000. Postharvest Quality Changes in Dokong (*Lansium domesticum* Corr.) Harvested at Different Stages of Ripeness. Quality Assurance in Agricultural Produce In: *ACIAR Proceedings 100*. Available at: https://www.aciar.gov.au/node/7836.

Saputra, D., Pratama, F. 2013. Quality Changes of Exotic Tropical Fruits during Storage in Semi- Passive Modified Atmosphere. *Acta Horticulturae* (ISHS), vol. 1011, p. 243-249. https://doi.org/10.17660/ActaHortic.2013.1011.29

Sawai, J., Sagara, K., Hashimoto, A., Igarashi, H., Shimizu, M. 2003. Inactivation characteristics shown by enzymes and bacteria treated with far-infrared radiative heating. *Journal International Microbiology Biotechnology*, vol. 38, no. 6, p. 661-667. https://doi.org/10.1046/j.1365-2621.2003.00717.x

Sawai, J., Sagara, K., Kasai, S., Igarashi, H., Hashimoto, A., Kokugan, T., Shimizu, M., Kojima, H. 2000. Far-infrared irradiation-induced injuries to Escherichia coli at below the lethal temperature. *Journal of Industrial Microbiology and Biotechnology*, vol. 24, p. 19-24. https://doi.org/10.1038/sj.jim.2900772

Sharma, G. P., Verma, R. R., Pathare, P. P. 2005. Thin-layer infrared radiation drying of onion slices. *Journal of Food*

Engineering, vol. 67, no. 3, p. 361-366. https://doi.org/10.1016/j.jfoodeng.2004.05.002

Suárez-Dieguez, T., Soriano-García, M., Anaya-Sosa, I., Cruz y Victoria, M. T. 2009. Comparative studies of two α-amylases acting on two Sorghum hybrids starches (Montecillos hybrid 2 and 3) and their significant differences in their catalytic activities. *Carbohydrate Polymers,* vol. 75, no. 3, p. 538-540. https://doi.org/10.1016/j.carbpol.2008.09.015

Tilaar, M., Wih, W. L., Ranti, A. S., Wasitaatmadja., S. M., Suryaningsih, F. D. J. Maily. 2008. Review of Lansium domesticum Corrêa and its use in cosmetics. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, vol. 7, no. 4, 183-189. ISSN: 0717-7917.

Togrul, H. 2005. Simple modeling of infrared drying of fresh apple slices. *Journal of Food Engineering*, vol. 71, no. 3, p. 311-323. https://doi.org/10.1016/j.jfoodeng.2005.03.031

Venkatachalam, K., Meenune, M. 2012a. Physical and chemical quality changes of longkong (*Aglaia dookkoo* Griff.) During passive modified atmospheric storage. *International Food Research Journal*, vol. 19, no. 3, 795-800.

Venkatachalam, K., Meenune, M. 2012b. Changes in physiochemical quality and browning related enzyme activity of longkong fruit during four different weeks of on-tree maturation. Food Chemistry, vol. 131, no. 4, p. 1437-1442. https://doi.org/10.1016/j.foodchem.2011.10.022

Zocca, F., Lomolino, G., Lante, A. 2008. 3,4-Dihydroxyphenylalanine gel diffusion assay for polyphenol oxidase quantification. Analytical Biochemistry, vol. 383, no. 2, p. 335-336. https://doi.org/10.1016/j.ab.2008.09.001

Zverev, S., Sesikashvili, O. 2018. Heating and Dehydration of Grain and Cereals at a Combined Energy Supply. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, p. 79-90. https://doi.org/10.5219/840

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STUDIES ON RELATIONSHIP BETWEEN BODY LENGTH, WEIGHT AND ELEMENTS CONTENTS IN FISH *CHIROCENTRUS NUDUS* SWAINSON (1839) IN IRAN

Ali Aberoumand, Farideh Baesi

ABSTRACT

Determination of toxic elements in seafood was important for the health of humans and marine organisms. This study was carried out on effects of total length and weight on concentration of toxic and non-toxic elements in fish of *Chirocentrus nudus* of Persian Gulf, Iran. For this purpose, 20 pieces of fish in two different fish sizes were purchased from Behbahan fish market and transferred to laboratory of Khatam-al-Anbia University of Technology, Behbahan, Iran. The fish muscle after the preparation, which contains washing, biometrics and abdominal discharges, was separated. Finally, the samples elements were analyzed after extraction and digestion processes using an atomic absorption apparatus. The obtained results were compared with the World Food Standards. The concentration of elements in different amounts in fish Kharo were as follows: Fe >Zn >Pb >Cd >Ni. Results showed that with increase fish length and weight, the amount of toxic and non-toxic elements such as Ni, Pb, Fe and Zn, except Cd, were increased significantly. It can be concluded that, although the concentration of elements found in the fish in both sizes were lower than the standard levels proposed for humans, but excessive consumption fish caught from contaminated areas, can be dangerous for human health.

Keywords: *Chirocentrus nudus*; elements; fish muscle; fish length and weight

INTRODUCTION

Fish is a suitable indicator for measuring the concentration of toxic elements in water systems, which are different in size and age. **(Burger et al., 2002; Lukáčová et.al., 2014)**. At the same time, fish in many parts of the world are consumed by humans as food, and fish can damage human health. The importance of measuring toxic elements in fish and seafood is related to food management and human health **(Romeoa et al., 1999; Jordao et al., 2002)**. Since marine food resources are one of the most important ways of transmitting toxic elements to humans, assessing and controlling the amount of different marine food contamination and identifying contaminating sources, modifying or eliminating, has a significant impact on human health and life. The effect of these elements on humans and marine foods depends on the type of element and its concentration. The biological effects of lead in fish include reduced fetal growth, preventing reproduction and preventing growth, increasing levels of gas, vascular problems, and inappropriate kidneys. Also, lead in the human body is transmitted through the blood to various organs of the body, and in particular, it binds to the bones and causes calcium intake and calcium deficiency in humans. This element, especially in children, causes brain damage and depression in the nervous system **(Jalali and Meshgi, 2007)**. Several studies have been conducted to compare the length, age, and weight of fish with toxic minerals, with different results **(Katsuhisa et al., 2014; Yi and Zhang, 2012)**. This shows that the exposure time of this element is an important factor for its condensation in tissue fish. The concentration of different toxic elements in fish species is different, but in this study, muscle of fish was important because of the important role in human nutrition and the need to ensure its health. Studied sample was *Chirocentrus nudus*. Its weight varies from 250 g to 5 kg. The maximum length of this fish was 1 m and its standard sizes were 45 cm. Although the fish from point of quality in the Persian Gulf was low-level fish, but it was including delicious fishes obtained from in Persian Gulf. Due to the high marketability of this fish, we reviewed on the concentration of toxic elements found in the Kharu fish to determine the health status of the fish in different sizes and valuable information for the fish consumption provided in

Figure 1 White fin Kharo fish.

different sizes.

Studies have been conducted to investigate the concentration of toxic elements in fishes of Iran Persian Gulf **(Shahriari, 2005; Hashim et al., 1996; Agha et al., 2010)**. However, few studies have been conducted in terms of these elements in different sizes of the fishes.

Barletta et al. (2012) Showed that the condensation of toxic elements to critical parts of the fish like the muscle depends on the environmental variables. Muscle and liver in fish, has been widely studied for toxic elements. **Al-weher (2008)**, studied on the levels of toxic elements Cu, Cd, and Zn in three fish species found in the North Sea of Jordan. Also, **Pourang et al. (2005)** showed the concentration of toxic elements in the tissues of five species Sturgeon in Southern Black Sea (Caspian Sea). **Agusa et al. (2004)**, studied on the elements in the muscle tissue of the five species of Javier fish in the different countries of the Caspian Sea. **Zhelyazkov et al. (2014)** examined the presence of some toxic elements in the *Alburnus alburnus* and *Rutilus rutilus* in the Zarishchev Lake in Bulgaria. **Fuentes-Gandara et al. (2016)** examined the biological resources of Hg, Cd, Zn, Cr and Pb in the muscle, liver and trap tissues. **Zheng et al. (2008)**, studied on toxic concentration of muscle in 19 species of Yangtze River fish. The results showed high levels of toxic elements except Zn in intestine compared with fish muscle.

Scientific hypothesis

Aim of this study was to determination of elements of cadmium (Cd), nickel (Ni), lead (Pb), iron (Fe) and zinc (Zn) in fish kharkh tissue in two different sizes and compare it with world standards and investigation on effect of the fish length of the weight on the concentration of toxic elements found in the fish tissue. The fish's length and weight do not have much effect on the toxic elements stored in the fish, unless the environment of the fish is contaminated.

MATERIAL AND METHODOLOGY

Sampling station

This research was carried out in the harbor of Bushehr port, Southern Iran. This port is one of the most important fishing and fishing areas in the Persian Gulf, Iran and there are many fish in various harbors, the most important of which are shardai, poetry, white halvah, Shank, Sangasar etc.

Preparation of samples

Twenty fishes of Khuro were bought in two different sizes from the Behbahan city market in the spring 2018 and they were clean completely were placed in plastic packages. After transferring samples to Fisheries Laboratory of Natural Resources Faculty of Khatam-al-Anbia University of Technology in Behbahan, total length and weight all samples were recorded. Then, the samples were rinsed with distilled water to exit the viscous layer and foreign particles of toxic elements absorbent from the body surface. First, all the laboratory dishes were washed with detergent and then the dishes were put for 24 h in nitric acid so that completely cleaned. Finally, it was washed with distilled water and then dried. The head, tail and internal organs of the body first separated the fish by knife, and then the muscle tissue was separated and transferred to the pre-cleaned containers.

Fish Biometric

Before the preparation of the samples for measuring the concentration of toxic elements in the fillet, the fish total length and weight of the samples were calculated as shown in the below Table 1.

Table 1 Average total length and weight of fish *Churocentrus nudus*.

Samples	Weight (g)	Total length (cm)
Big kharo fish	973.33 ±30.55	63.66 ±0.57
Small kharo fish	746.66 ±15.27	56 ±1.00

Measuring the elements

The samples were placed in an oven for 18 h at a temperature of 80 °C and, after leaving the oven it was completely powdered in a Chinese dish **(**Model Apha, Awwa, Wef, 1992). The dry method was used for digestion of the samples. First, 1 g of the prepared sample transferred to dish and 10 mL of concentrated nitric acid was added to digest the contents of the dishes, and the samples were placed at room temperature for 30 min to initially digest. The samples were then heated to a temperature of 90 °C in under a hood with a steam system to dry. After cooling and reaching the ambient temperature, the sample was transferred from a 45 mm Watten filter paper and transferred to a Balloon Jogege 25 mL and reached to a volume of 25 mL. Finally, the samples were transferred into the polyethylene containers for injection into the apparatus **(**Model, Moopam, 1999). For measuring the elements contents in all samples, atomic absorption apparatus was used. Apparatus: ICP - OES: Simultaneous ICP - OES(Varian 735 - ES) equipped with charge coupled device (CCD) detector and SPS3 Autosampler (Varian) has been used for the determination of elements. Instrumental and operating conditions for ICE - AES measurements. Power (K W), Plasma flow rate ($L.Min^{-1}$), Auxiliary flow rate ($L.Min^{-1}$), Nebulizer flow rate ($L.Min^{-1}$), Viewing configuration, Viewing height (mm), Replicate read time (s). Apparatus: ICP- OES: Simultaneous ICP- OES(Varian 735- ES) equipped with charge coupled device (CCD) detector and SPS3 Autosampler (Varian) has been used for the determination of elements. Instrumental and operating conditions for ICE- AES measurements. Power (K W), Plasma flow rate ($L.Min^{-1}$), Auxiliary flow rate ($L.Min^{-1}$), Nebulizer flow rate ($L.Min^{-1}$), Viewing configuration, Viewing height (mm), Replicate read time (s).

Statisic analysis

The obtained data in this study was analyzed for significant differences with the help of analysis of variance (ANOVA) conducted using SPSS 16.0 software (IBM Corporation).

RESULTS AND DISCUSSION

Table 2, the amounts of toxic and non-toxic elements in *Chirocentrus nudus* were shown in two small and medium sizes. The results of this experiment showed that the amounts of toxic elements of cadmium, nickel, lead, iron and zinc in different tissues were different. In fact, exept cadmium, with increase the size of fish, other elements also showed a significant increase. The concentration of iron in the tissue of the fish was higher than other toxic elements. Non-similar letters in each column showed a significant difference $p > 0.05$.

According to the Figure 2, the toxic metal content of cadmium in the tissue of Khoro, with small size was 1.87 ±0.01 ppb, and in fish greater was 1.60 ±0.06 ppb was reported, that showed significant different between the two experimental groups. In fact, with the increase in the size of the fish, the amount of of cadmium in the tissue of the fish kharo has decreased.

Table 2 The elements contents found in *Chirocentrus nudus* muscle in different sizes.

Samples	Cd (ppb)	Ni (ppb)	Pb (ppb)	Fe (ppm)	Zn (ppm)
Big kharo fish	1.60 ±0.06[a]	1.78 ±0.03[a]	3.82 ±0.08[a]	61.55 ±2.03[a]	2.05 ±0.05[a]
Small kharo fish	1.87 ±0.01[b]	1.47 ±0.11[b]	2.33 ±0.12[b]	46.10 ±0.40[b]	1.46 ±0.01[b]

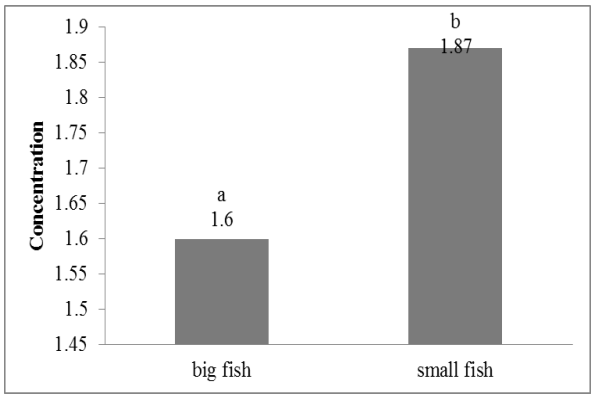

Figure 2 The Average of concentration of Cd in the fish muscle *Chirocentrus nudus* in two different sizes.

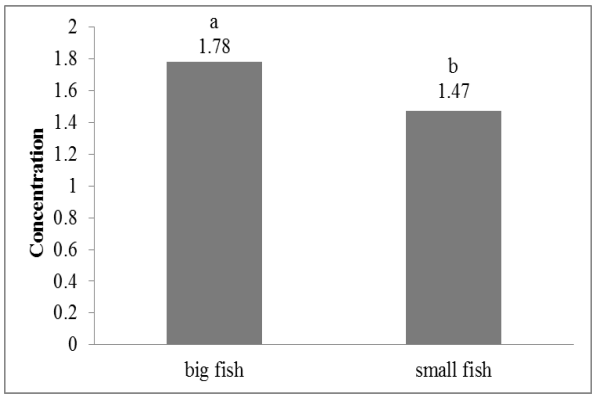

Figure 3 The Average of concentration of Ni in the fish muscle *Chirocentrus nudus* in two different sizes.

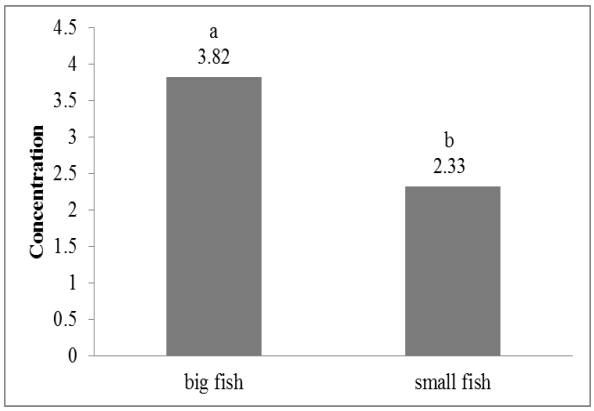

Figure 4 The Average of concentration of Pb in the fish muscle *Chirocentrus nudus* in two different sizes.

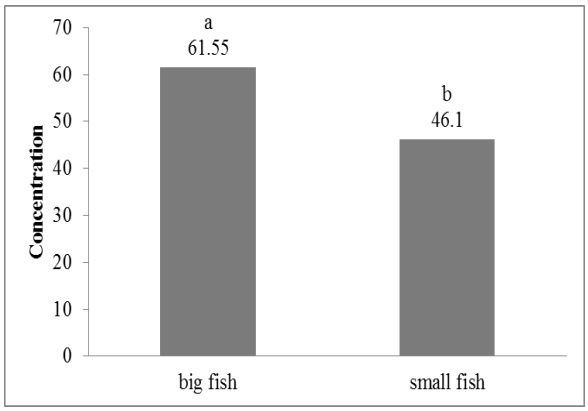

Figure 5 The Average of concentration of Fe in the fish muscle *Chirocentrus nudus* in two different sizes.

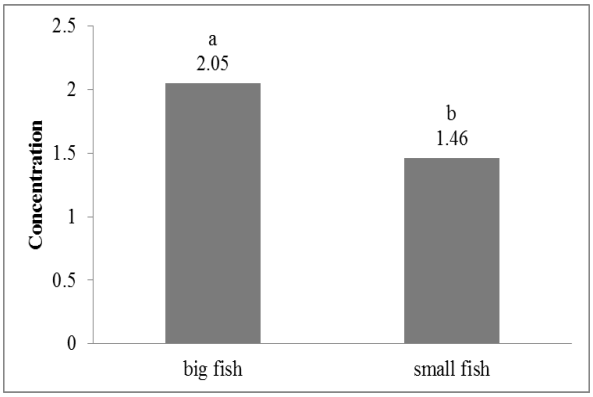

Figure 6 The Average of concentration of Zn in the fish muscle *Chirocentrus nudus* in two different sizes.

The Figure 3 results of this experiment showed that there is a significant difference in the amount of nickel in the tissue of fish Kharo in the two experimental groups, so that its value in fish larger found 1.78 ±0.03 ppb and in fish smaller was 1.47 ±0.11 ppb reported.

Lead was measured another metal in this study, which was 3.82 ±0.88 ppb in fish big and 2.33 ±0.12 ppb in small fish. There was a significant difference between the two groups, which results shown in Figure 4.

As shown in Figure 5, the amount of iron found in the fish big was higher than that of the smaller fish and found a significant difference. The amount of iron found in fish big found 61.55 ±2.03 ppm and 46.10 ±0.40 ppm in the fish smaller was recorded.

This study showed that the toxic metal content of zinc in the large fish filet was higher than the fish smaller, with a significant difference. The average concentrations of zinc for large fish were reported 2.05 ±0.05 ppm and 1.46 ±0.01 ppm for small fish as shown in Figure 6.

In recent decades, due to the nutritional characteristics of fish and its role in preventing certain diseases, the tendency to fish consumption has increased. Fish and other aquatic foods are rich in nutrients such as protein, unsaturated fatty acids, vitamins and iron, zinc, calcium, iodine, etc. But because toxic elements are present in the tissue and are transmitted to the human body through the food chain, fish should be prevented from contaminating the environment **(Agusa et al., 2004)**. Over the past few decades, the increase in toxic elements in the industry has caused significant pollution through sewage. Because of their toxicity and persistence in water, these elements can cause many problems for aquatic animals. Fish is an important source of human nutrition and is an important part of many natural food chains. Therefore, determining the level of pollutants in fish is important because it is present in the fish that feeds it. **(Burger and Gochfeld, 2005)**. Heavy metal adsorbent tissues are organs that are very variable and dependent on various factors such as metals concentration, age, size, physiological status, type of biology, nutritional behavior and fish growth rate **(Chapman et al., 1996)**. Based on the present study, the change size of fish Kharo, the concentration of elements in fish tissues also changed, and these changes were significantly different. In other words, with the exception of cadmium (Cd), the longer the water is exposed to the pollutants in the aquatic environment, the more pollutants accumulate in fish body. Iron element found the highest concentration among other elements in the fish tissue, while nickel showed lowest concentration in the tissue. These data were agreed with the data of other researchers, but iron was the highest concentration in different organs of the fish **(Mahboob et al., 2016; Alinoor and Obiji, 2010).** In this research, it was observed that the amounts of Cd, Ni, Pb elements in the fish fillet was 1.60, 1.78, 2.33, 46.10 and 1.46 ppb respectively, and in fish with smaller size, 1.87, 1.47, 3.82, 61.55 and 2.05 ppb respectively; and the concentration of iron and zinc in the large fish was 46.10 and 1.46 ppm, respectively, in the small fish 61.55 and 2.05 ppm reported. The results of this experiment were in many items agreed with other researcher's results. **Yi and Zhang (2012)** reported there is a positive relationship between fish size and level of elements in most of the tested samples, and the mercury and chromium contents present in yellow catfish head and found a negative relationship and its amount decreased. **Katsuhisa et al. (2014)** A similar study on the effects of length, weight, and age on the concentration of toxic elements in dolphin muscle, which according to their reports was found a positive correlation between the concentrations of the cadmium, nickel, lead, iron and the mercury in muscle, liver and kidney dolphin and body length, weight, and age, but a negative correlation was found for such elements as zinc, magnesium and copper. **Karadede et al. (2004)**, found a positive correlation between concentration of mercury, zinc, copper and cadmium in the *Lethrinus lentjan* species with the length and weight of fish. A different assessment has been made in relation to toxic elements polluting the marine environment in the Persian Gulf and Oman Sea. In most of these studies, the concentration of toxic elements, especially mercury, has increased with increase fish age and size **(De Mora et al., 2004)**. It has been showed that for *Branchiostoma belcheri* there was a significant positive relationship between the amounts of copper and zinc with the length of the fish, and between concentration of mercury and cadmium with the length and weight of the fish, but there was not found, however, no significant relationship between fish length and weight and the amount of lead. But in this the study, cadmium content showed a significant decrease with increase fish size, and with fish size, lead content found a significant increase. The results of the two studies may be related to species differences, nutritional behavior, and immigration issues. The lead concentration obtained from the present experiment was reported 3.82 ppb in large fish, which was less than the other world standards. But in the present study, the concentration of this metal was 1.87 ppb in smaller fish and 1.60 ppb in larger fish, which was below the world standard levels. Absorption of 3 – 330 $mg.day^{-1}$ of cadmium is toxic and 1.5 – 9 $mg.day^{-1}$ will be deadly for humans. This metal infects on kidneys. Chronic symptoms of cadmium contamination include renal dysfunction, reduced fertility, weakness, tumor, and liver function disorders **(Waalkes, 2000)**. According to the World Health Organization, the daily tolerable and safe intake of nickel metal is 0.005 $mg.kg^{-1}$ body weight **(Samali Sari et al., 2007)**. According to the results of this study, the amount of nickel in the white muscle of the fish in both sizes was lower than the world standard, so there is no problem for consumption and human health. The zinc content recorded in this study was 2.05 ppm in larg fish and 1.46 ppm in the smaller fish. There is no the acceptable level of zinc in the muscle of the fish. Our study average values found lower compared to Chinese food standards (50 $mg.kg^{-1}$), Canadian food standards (100 $\mu g.g^{-1}$), Hungarian standards (150 $\mu g.g^{-1}$) and a range of international standards (40 – 100 $\mu g.g^{-1}$) **(Pourang et al., 2005).** Therefore, it can be concluded that this metal is not dangerous for the consumption of the fish. It has been reported that toxicity due to excessive consumption of zinc lead to electrolyte imbalance, nausea, anemia, and lethargy **(Prasad, 1984)**. **FAO and WHO (1976)** determined limit toxic elements consumption based on body weight. For an adult (60 kg body weight), the standard daily intake of lead, copper and zinc was 214 µg, 3 and 60 mg for muscle, respectively, which were much higher compared the values obtained from this report.

CONCLUSION

Concentration of toxic elements near to safe levels found limitations for human consumption and should be reported to populations that continuously use contaminated water. Toxic elements that accumulate in fish were dangerous for the environment as well as for human health. Despite the fact that there was no high level of toxic elements were found in the studied fish, there are potential risks to the future due to the development of industries and the expansion of agriculture around aquatic environments. The findings from this study also showed that people should be careful about regular use of fish caught from infected environments, especially larger fishes, because according to the results, although the concentration of the tested elements was lower than world standards. However, with increase fish size, the concentration of toxic and non toxic elements in fish muscle also were increased significantly.

REFERENCES

Agha, H., Leermakers, M., Gao, Y., Fatemi, S. M. R., Mohseni Katal, M., Baeyens, W., Elskens, M. 2010. Mercury concentration in fish species from the Persian Gulf and in human hair from fishermen. *Environment Monitoring and Assessment*, vol. 169, no. 1-4, p. 203-216. https://doi.org/10.1007/s10661-009-1162-8

Agusa, T., Kainito, T., Tanabe, S., Pourkazemi, M., Aubrey, D. G. 2004. Concentrations of trace elements in muscle of sturgeonsin the Caspian Sea. *Marine Pollution Bulletin,* vol. 49, no. 9-10, p. 789-800. https://doi.org/10.1016/j.marpolbul.2004.06.008

Alinoor, I. J., Obiji, A. 2010. Assessment of trace metal composition in fish samples from Nworie River. *Pakistan Journal of Nutrition,* vol. 9, no. 1, p. 81-85. https://doi.org/10.3923/pjn.2010.81.85

Al-Weher, S. M. 2008. Levels of toxic metal Cd, Cu and Zn in three fish species collected from the Northern Jordan Valley, Jordan. *Jordan Journal of Biology Science*, vol. 1, no. 1, p 41-46.

Arantes, F. P., Savassi, L. A., Santos, H. B., Gomes, M. V. T., Bazzoli, N. 2016. Bioaccumulation of mercury, cadmium, zinc, chromium, and lead in muscle, liver, and spleen tissues of a large commercially valuable catfish species from Brazil. *An. Acad. Bras. Cienc.*, vol. 88, no. 1, p. 137-47. https://doi.org/10.1590/0001-3765201620140434

Barletta, A. M., Lucena, L. R. R., Costa, M. F., Cysneiros, F. J. A. 2012. The interaction rainfall vs. weight as determinant of total mercury concentration in fish from atropical estuary. *Environment Pollution,* vol. 167, p. 1-6. https://doi.org/10.1016/j.envpol.2012.03.033

Burger, J., Gaines, K. F., Shane, B. C., Stephens, W. L., Snodgrass, J., Dixon, C., Mcmahon, M., Shukla, S., Shukla, T., Gochfeld, M. 2002. Metal levels in fish from the Savannah River: potential hazards to fish and other receptors. *Environment Research*, vol. 89, no. 1, p. 85-97. https://doi.org/10.1006/enrs.2002.4330

Burger, J., Gochfeld, M. 2005. Toxic metals incommercial fish in New Jersey. *Environment Research,* vol. 99, p. 403-412. https://doi.org/10.1016/j.envres.2005.02.001

De Mora, S., Fowler, S. W., Wyse, E., Azemard S. 2004. Distribution of toxic metals in marine bivalves, fish and coastal sediments in the Gulf and Gulf of Oman. *Marine Pollution Bullutan,* vol. 49, no. 5-6, p. 410-24. https://doi.org/10.1016/j.marpolbul.2004.02.029

FAO/WHO. 1976. List of maximum levels recommended for con-taminants by the Joint FAO/WHO codex alimentarius com-mission. New York : Unipub, 9 p. ISBN 9251006938.

Fuentes-Gandara, F., Pinedo-Hernández, J., Marrugo-Negrete, J., Dıéz, S. 2016. Human health impacts of exposure to metals through extreme consumption of fish from the Colombian Caribbean Sea. *Environ Geochem Health*, vol. 40, no. 1, p. 229-242. https://doi.org/10.1007/s10653-016-9896-z

Guo, J., Yue, T., Li, X. Yuan, Y. 2016. Heavy metal levels in kiwifruit orchard soils and trees and its potential health risk assessment in Shaanxi, China. *Environmental Science and Pollution Research*, vol. 23, no. 14, p.14560-14566. https://doi.org/10.1007/s11356-016-6620-6

Hashim, A. A., Jalal, A., Ismail, M. M., Dhabia, A. 1996. Toxic metals in the grouper fish Epinephelus coioides from the coast of Bahrain: an assessment of monthly and spatial trends. *International Journal of Environment Studies*, vol. 50, no. 3-4, p. 237-246. https://doi.org/10.1080/00207239608711060

Chapman, P. M., Allen, H. E., Godtfredsen, K., Graggen, M. N. 1996. Evaluation of bioaccumulation factors in regulating metals. *Environment Science Technology*, vol. 30, no. 10, p. 448A-452A. https://doi.org/10.1021/es962436d

Jalali, J. B., Aghazadeh Meshgi, M. 2007. *Fish poisoning due to toxic metals in water and its importance in public health.* Tehran, Iran : Manan Book Publishing. 134 p.

Jordao, C. P., Pereira, M. G., Bellato, C. R., Pereira, J. L., Matos, A. T. 2002. Assessment of water systems for contaminants from domestic and industrial sewages. *Journal of Environment Monitoring and Assessment*, vol. 79, no. 1, p. 75-100. https://doi.org/10.1023/A:102008581

Karadede, H., Oymak, S. A., Unlu, E. 2004. Heavy metals in mullet, Liza abu, and catfish, *Silurus triostegus*, from the Atatürk Dam Lake (Euphrates) Turkey. *Environment International,* vol. 30, no. 2, p. 183-8. https://doi.org/10.1016/S0160-4120(03)00169-7

Katsuhisa, H., Ryo, T., Kazuomi, I., Nobuyuki, M., Toraya, F. 2014. Heavy metal concentrations in muscle, liverand kidney tissue of striped dolphin, stenellacoeruleoalba, and their variations with body length, weight, age and sex. *Agricultural and biology chemistry*, vol. 47, no. 6, p. 1219-1228. https://doi.org/10.1271/bbb1961.47.1219

Lukáčová, A., Golian, J., Massanyi, P., Formicki, G. 2014. L ead concentration in meat an meat products of different origin. *Potravinarstvo*, vol. 8, no. 1, p. 43-47. https://doi.org/10.5219/334

Mahboob, S. H., Shazia, M., Farhat, K., Sultana, J., Sultana, S., Al-Ghanim, T., Bilal, K. A., Fahad, H., Al, M., Ahmed, Z. 2016. Effect of toxic metals on liver, kidney, gills and muscles of *Cyprinus carpio* and *Wallago attu* inhabited in the Indus. *Brazilian archives of biology and technology*, vol. 59, p. 1-10. https://doi.org/10.1590/1678-4324-2016150275

Minareci, O., Öztürk,M., Özözen,G., Minareci, E. 2009. Determination of heavy metals in fish, water and sediments of Avsar Dam Lake in Turkey, *Iran. J. Environ. Health. Sci. Eng*., vol. 6, no. 2, p. 73-80.

Pourang, N., Tanabe, S., Rezvani, S., Dennis, J. H. 2005. Trace elements concentration in edible tissues of five sturgeon species from the Caspian Sea. *Environment Monitoring and Assessment,* vol. 100, no. 1-3, p. 89-108. https://doi.org/10.1007/s10661-005-7054-7

Prasad, A. S. 1984. Discovery and importance of zinc in human nutrition. *Federation Proceedings*. vol. 43, no. 13. p. 2829-2834. Available at: https://www.ncbi.nlm.nih.gov/pubmed/6383875.

Romeoa, M., Siaub, Y., Sidoumou, Z., Gnassia- Barelli, M. 1999. Toxic metal distribution in different fish species from the Mauritania coast. *Science Total Envionment*, vol. 232, no. 3, p. 169-75.

Samali Sari, S., Nouri Sari, A. Ismaili Sari, H. 2007. *Mercury in the Environment.* 1st ed. Rasht : Bazargan Publication. 226 p. ISBN: 9780520271630.

Shahriari, A. 2005. Determination of cadmium, chromium, lead and nickel in edible tissues of Tiger-Toothed Croaker and Russels snapper from Persian Gulf in 1382. *Journal of Gorgan University of Medicine Science,* vol. 2, no. 7, p. 65-67.

Waalkes, M. P. 2000. Cadmium carcinogenesis in review. *Journal of Inorganic Biochemistry,* vol. 79, no. 1-4, p. 241-244. https://doi.org/10.1016/S0162-0134(00)00009-X

Yi, Y. J., Zhang, S. H. 2012. The relationships between fish toxic metal concentrations and fish size in the upper and middle reach of Yangtze River. *Dia Environment Science,* vol. 13, p. 1699-1707. https://doi.org/10.1016/j.proenv.2012.01.163

Zhelyazkov, G. I., Georgiev, D. M., Dospatliev, L. K., Staykov, Y. S. 2014. Determination of toxic metals in Roach (*Rutilus rutilus*) and Bleak (*Alburnus alburnus*) in Zhrebchevo Dam Lake. *Journal of Ecology Balkani*, vol. 5, no. 1, p. 15-20.

Zheng, N., Wang, Q. C., Liang, Z. Z., Dongmei, Z. 2008. Characterization of toxic metal concentration in the sediments of three fresh water rivers in Huludao city. *Northeast China. Environtment Pollution,* vol. 154, no. 1, p. 135-142. https://doi.org/10.1016/j.envpol.2008.01.001

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COMPARISON OF TWO METHODS OF PROTEIN QUALITY EVALUATION IN RICE, RYE AND BARLEY AS FOOD PROTEIN SOURCES IN HUMAN NUTRITION

Soňa Nitrayová, Matej Brestenský, Peter Patráš

ABSTRACT

Different foods differ in their protein quality, which is characterized by the content and digestibility of individual amino acids. The Food and Agriculture Organisation has recommended replacing the method for protein quality evaluation of foods called protein digestibility corrected amino acid score (PDCAAS) with the new method - digestible indispensable amino acid score (DIAAS), in which the values of ileal amino acid digestibility obtained in pigs are used. However, the information about DIAAS values of foods are limited. Therefore, the study on growing pigs was conducted to determine true fecal protein digestibility and standardized ileal amino acid digestibility of rice, rye and barley. Using these values, the PDCAAS and DIAAS were calculated and compared. A total of 18 gilts with a T-cannula inserted in the terminal ileum were allotted to 3 diets with six replicate pigs per diet. Three semi-purified diets were formulated to contain the tested cereal grains (rice, rye, barley) as the sole nitrogen source. Chromic oxide was used as indigestible marker. Each experimental period comprised of a 7-d adaptation period followed by 24 h collection of feces and ileal digesta. The content of nitrogen, dry matter and chromic oxide was analyzed in samples of diets, feces and ileal digesta. Moreover, in the samples of diets and ileal digesta the content of amino acids was determined. Calculated ratio of crude protein to lysine was greatest in rice (4.50) followed by rye (3.65) and the lowest one in barley (3.35). True fecal protein digestibility was greater when compared with ileal amino acid digestibility for all tested samples, thus suggesting an overestimation of protein quality determined by PDCAAS. Calculated PDCAAS values for rice, rye and barley (81, 65 and 61%) were generally greater than the DIAAS values (79, 56 and 55%), especially for the poorer quality protein sources such as rye and barley in comparison with rice. The lysine was the first limiting amino acid in all tested cereal grains. Based on the DIAAS evaluation, rice is better protein source in human nutrition in comparison with rye or barley.

Keywords: PDCAAS; DIAAS; protein quality; amino acid; ileal

INTRODUCTION

Protein quality describes the nutritive value of proteins. A precise assessment of the ability of a dietary protein source to match the body's needs for individual amino acids (AA) will allow their better use **(FAO, 2013)**.

The quality of dietary protein is a function of its individual constituent AA. The FAO/WHO Expert Consultation on Protein Quality Evaluation recommended the use of the Protein Digestibility Corrected AA Score (PDCAAS) as suitable method for protein quality evaluation **(FAO, 1991)**. Using this method, PDCAAS is calculated by multiplying the limiting AA score (i.e. the ratio of the first-limiting AA to the same AA of the reference protein) by true fecal protein digestibility. However, the PDCAAS has limitations - the main is that fecal protein digestibility as a measure of AA availability is inaccurate due to metabolic transformations of dietary and endogenous proteins by microbial population of the large intestine **(Darragh and Hodgkinson, 2000; Gilani, 2012; Schaafsma, 2012)**.

Considering the number of critical reviews on this subject **(Moughan, 2003; Fuller and Tomé, 2005; Hendriks et al., 2012)** a new protein quality measure called digestible indispensable AA score (DIAAS) is now recommended to replace the PDCAAS for evaluating protein quality in human nutrition **(FAO, 2013)**.

The main difference between PDCAAS and DIAAS is that dietary AA is treated as individual nutrients and their digestibility is used in calculations. The AA are absorbed only from the small intestine and their digestibility is measured as ileal digestibility (a difference between dietary AA and those appearing in terminal ileum) which is more accurate assessment of how much of the protein consumed is available to the body **(Columbus and de**

Lange, 2012). The apparent ileal digestibility (AID) of AA is defined as the net disappearance of ingested dietary AA from the digestive tract proximal to the distal ileum. When AID is corrected for the basal endogenous losses in pigs, the resulting value is termed standardized ileal digestibility (SID), which can be used to calculate approximate DIAAS values in humans **(Stein et al., 2007)**. Using the DIAAS method, researchers are now able to differentiate protein sources by their ability to supply AA for use by the body **(Brestenský et al., 2018)**.

There is no non-invasive method of ileal digesta collection applicable in humans and therefore, the number of relevant data is very limited. The pig has been recognized as a good animal model for estimating crude protein (CP) and AA digestibility in humans **(Rowan et al., 1994; Deglaire et al., 2009)**. However, in this time there are only several studies dealing with protein quality of cereal grains or different protein sources in terms of DIAAS quality evaluation **(Cervantes-Pahm et al., 2014; Mathai et al., 2017; Abellila et al., 2018)**.

Furthermore, that the cereal grains are the major source of energy, they can be also a good source of protein. The aim of the present study was to compare PDCAAS and DIAAS values of rice, rye and barley calculated using digestibility coefficients obtained in a series of pig experiments.

Scientific hypothesis

We tested the protein quality of different cereal grains for human nutrition by PDCAAS and DIAAS methodology. Due to the fact, that the DIAAS method is new and both methods are difficult, there is little studies which would evaluate different food sources in human nutrition from the point of view of their protein quality.

MATERIAL AND METHODOLOGY

Animals and experimental design

The experimental study was perfomed in Laboratory of pig nutrition at National Agricultural and Food Center, Research Institute of Animal Production Nitra. All experimental procedures were reviewed and approved by the Animal Care Committee of the Research Institute of Animal Production Nitra (Slovakia).

A total of 18 Large white gilts (BW, 50 ±3.5 kg) fitted with ileal T-cannulas were used throughout the study. They were allotted to 3 diets - six replicate pigs per diet. After a 14-d recovery period, an experimental period, consisting of a 7-d adaptation period followed by a 1-d (24-h) collection of ileal digesta and feces, was started.

Three semi-purified diets (Table 1) were formulated to contain the tested cereal grains (rice, rye, barley) as the sole nitrogen (N) source. Chromic oxide was added to the diets as an indigestible marker. All diets were fed twice daily at 07:00 and 16:00 h in 2 equal meals at a daily rate of 80 g.kg $^{-0.75}$. Water was available *ad libitum*.

Chemical analysis

The diets, feces and ileal digesta were analyzed for dry matter (DM) and total nitrogen (N) **(AOAC, 1990)**. Chromic oxide was measured by atomic absorption spectrometry **(Williams et al., 1962)**. The content of AA, in diets and ileal digesta, after acid hydrolysis with 6 M HCl and methionine and cysteine after oxidative hydrolysis were determined using an automatic AA analyzer (AAA 400, Ingos, Prague, Czech Republic).

Calculations

Coefficients of true fecal protein digestibility (TD) or standardized ileal AA digestibilities (SID) were calculated using the following formula:

$$TD, SID\ (\%) = 100 \times [(1 - N_{ex}/N_d \times Cr_d/Cr_{ex}) + N_{end}/N_d],$$

where N_{ex} is concentration of the nutrient in feces or ileal digesta, N_d is concentration of the nutrient in the diet, Cr_d is concentration of chromic oxide in the diet, Cr_{ex} is concentration of chromic oxide in feces or ileal digesta (all values in g.kg^{-1} DM) and N_{end} is the endogenous loss of the nutrient expressed as g.kg DM^{-1} intake.

Table 1 Composition of experimental diets.

Ingredient	Protein source		
	Rice	Rye	Barley
Rice (g.kg^{-1} air-dry basis)	964.0	-	-
Rye (g.kg^{-1} air-dry basis)	-	958.0	-
Barley (g.kg^{-1} air-dry basis)	-	-	972.0
Sunflower oil (g.kg^{-1} air-dry basis)	-	9.0	-
Limestone (g.kg^{-1} air-dry basis)	6.0	13.0	11.8
Monocalcium phosphate (g.kg^{-1} air-dry basis)	21.0	11.0	6.2
Salt (g.kg^{-1} air-dry basis)	3.0	3.0	4.0
Premix (g.kg^{-1} air-dry basis) [1]	3.0	3.0	3.0
Chromic oxide (g.kg^{-1} air-dry basis)	3.0	3.0	3.0

Note: [1]Provided the following per kg of diet: retinol 1.2 mg; cholekalciferol 25 mg; α-tocopherol 10 mg; menadione 0.2 mg; riboflavin 4 mg; pyridoxine 2.5 mg; d-pantothenic acid 10 mg; niacin 20 mg; folic acid 0.5 mg; biotin 0.1 mg; cyanocobalamin 30 μg; choline 500 mg; Fe 92 mg; Zn 103 mg; Mn 40 mg; Cu 19 mg; Co 0.5 mg; Se 0.16 mg.

The values of endogenous N losses in feces were taken from the study by **Whiting and Bezeau (1957)** and the endogenous AA losses in ileal digesta from the study of **Jansman et al. (2002)** which were also suggested by **Stein et al. (2007)**. For the calculation of PDCAAS and DIAAS,

indispensable AA reference pattern (reference protein) for adult humans, as defined by the FAO Expert consultation was used **(FAO, 2013)**.

Statisic analysis

Experimental data were analyzed by General Linear Model of Statgraphics Plus package (version 3.1, Statistical Graphic Corp., Rockville, MD, USA). When the analysis of variance indicated a significant (p <0.05) p-value for treatment means, the differences between means were assessed by Tukey HSD test. True fecal digestibility values were compared with weighted means of ileal digestibility of all indispensable AA by a two-sample comparison method using Student's t-test.

RESULTS AND DISCUSSION

The contents of CP in rye and barley were approximately two times greater than in rice, whereas the calculated ratio Lys : CP was greatest in rice (Table 2). Compared with other indispensable AA, all cereal grains contained relatively high amounts of leucine similarly as reported **Cervantes-Pahm et al. (2014)**.

Mean data on true faecal protein digestibility as well as standardized ileal digestibility of AA are summarized in Table 3. Significant differences (p <0.05) in both protein and AA digestibility among the rice and other cereals (rye and barley) were found. The lowest values of both protein and AA digestibility among the tested protein sources were found in rye which was due to the ability of arabinoxylans to form highly viscous solutions, interfering with digestion or absorption along the alimentary tract **(Jondreville et al., 2001)**. A similar effect has been attributed to mixed-linked beta-glucans of barley **(Graham et al., 1989)**. The comparison of true fecal protein digestibility with the mean ileal AA digestibility (Table 3) showed that the fecal digestibility is not a good predictor of ileal digestibility, because the estimated values were greater than those of ileal digestibility which suggesting an overestimation of protein quality determined by means of PDCAAS. Similar results were reported also by other authors **(Moughan and Donkoh, 1991; Darragh and Hodgkinson, 2000)**.

The calculations of PDCAAS and DIAAS values are shown in Table 4. In both cases, quite large differences in protein quality measures were found. Calculated PDCAAS values were generally greater than the DIAAS values, especially for the poorer quality proteins of rye and barley in comparison with rice. These findings were due primarily to the degree of deficiency of the first-limiting AA, which was lysine, in all tested samples and similarly its various ileal digestibility in rice, rye and barley (94.1, 73.2 and 79.4%, respectively).

Table 2 Determined crude protein and amino acid composition of rice, rye and barley.

Item	Protein source		
	Rice	**Rye**	**Barley**
Crude protein (g.kg^{-1} DM)	76.88	153.13	141.25
Cysteine (mg.g^{-1} CP)	14.9	13.6	17.0
Histidine (mg.g^{-1} CP)	31.2	22.5	23.7
Isoleucine (mg.g^{-1} CP)	44.3	28.5	30.3
Leucine (mg.g^{-1} CP)	93.5	65.1	64.5
Lysine (mg.g^{-1} CP)	40.5	36.5	33.5
Methionine (mg.g^{-1} CP)	23.1	11.5	16.7
Phenylalanine (mg.g^{-1} CP)	54.6	44.5	45.9
Tryptophan (mg.g^{-1} CP)	11.0	6.6	8.5
Threonine (mg.g^{-1} CP)	36.7	33.1	34.0
Tyrosine (mg.g^{-1} CP)	29.5	23.0	33.6
Valine (mg.g^{-1} CP)	62.8	42.1	43.9
Calculated value Lys : CP (%)	4.50	3.65	3.35

Table 3 Coefficients of true fecal digestibility of crude protein and standardized ileal digestibility of AA in pigs fed diets containing various protein sources.

Item	Protein source		
	Rice[1]	Rye[1]	Barley[1]
True fecal digestibility:			
Crude Protein (%)	95.5 ±3.2[b]	86.0 ±3.3[a]	86.5 ±2.6[a]
Standardized ileal digestibility:			
Weighted AA mean (%)	92.9 ±2.1[b]	75.7 ±3.4[a]	80.7 ±3.6[a]
Significance of fecal protein digestibility vs. mean ileal AA digestibility:			
***p* -value[2]**	0.119	<0.001	0.010

Note: [1]Values are expressed as means ±SD; [a,b] Means within a row not sharing a common superscript were significantly different; [2]Tukey HSD test, $p < 0.05$.

Table 4 Calculation of protein digestibility-corrected amino acid score and digestible indispensable amino acid score of protein sources.

Item	Amino acid									PDCAAS (%)	DIAAS (%)
	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val		
Reference pattern[1]	15	30	61	48	23	41	25	6.6	40		
Protein digestibility-corrected reference amino acid ratios[2]											
Rice	1.98	1.41	1.46	0.81	1.57	1.96	1.40	1.65	1.50	81	
Rye	1.29	0.82	0.92	0.65	0.94	1.42	1.14	0.87	0.91	65	
Barley	1.37	0.87	0.91	0.60	1.27	1.68	1.18	1.12	0.95	61	
Digestible amino acid reference ratios[3]											
Rice	1.93	1.41	1.45	0.79	1.55	1.81	1.35	1.33	1.49		79
Rye	1.12	0.71	0.85	0.56	0.83	1.24	0.95	0.76	0.79		56
Barley	1.25	0.86	0.88	0.55	1.25	1.52	0.98	1.02	0.92		55

Note: [1]Reference amino acid pattern for adults, $mg.g^{-1}$ protein **(FAO, 2013)**; [2]Ratios of amino acids ($mg.g^{-1}$ crude protein) corrected for true fecal digestibility to **FAO (2013)** reference pattern; [3]Ratios of amino acids ($mg.g^{-1}$ crude protein) corrected for ileal digestibility to **FAO (2013)** reference pattern; SAA - sulfur amino acids; AAA - aromatic amino acids.

The comparison of PDCAAS and DIAAS values showed that both methods gave the same results as for ranking proteins in terms of their quality. However, the absolute values differed. The values of DIAAS were considerably lower than the PDCAAS values. Differences between them tended to increase with decreasing ileal AA digestibility. These results suggest that protein quality evaluation based on the ileal digestibility of AA - DIAAS are more reasonable estimates of their bioavailability than PDCAAS.

CONCLUSION

The values of PDCAAS were generally greater than that of DIAAS, especially for the poorer quality proteins in rye and barley in comparison with rice. All tested parameters were greatest in rice and therefore based on the results from the present study we can conclude that rice is better protein source in human nutrition than rye or barley.

REFERENCES

Abelilla, J. J., Liu, Y., Stein, H. H. 2018. Digestible indispensable amino acid score (DIAAS) and protein digestibility corrected amino acid score (PDCAAS) in oat protein concentrate measured in 20 to 30 kilogram pigs. *Journal of Science Food and Agriculture*, vol. 98, no. 1, p. 410-414. https://doi.org/10.1002/jsfa.8457

Association of Official Analytical Chemists (AOAC). 1990. *Official Methods of Analysis*. 15th ed. AOAC, Arlington, VA.

Brestenský, M., Nitrayová, S., Patráš, P., Nitray, J. 2018. Dietary requirements for protein and amino acids in human nutrition. *Current Nutrition and Food Science*, vol. 14, p. 1-8. https://doi.org/10.2174/1573401314666180507123506

Cervantes-Pahm, S. K., Liu, Y., Stein, H. H. 2014. Digestible indispensable amino acid score and digestible

amino acids in eight cereal grains. *British Journal of Nutrition*, vol. 111, no. 9, p. 1663-1672. https://doi.org/10.1017/S0007114513004273

Columbus, D., de Lange, C. F. M. 2012. Evidence for validity of ileal digestibility coefficients in monogastrics. *British Journal of Nutrition*, vol. 108 (Suppl S2), p. 264-72. https://doi.org/10.1017/S0007114512002334

Darragh, A. J., Hodgkinson, S. M. 2000. Quantifying the digestibility of dietary protein. *Journal of Nutrition*, vol. 130, no. 7, p. 1850-1856. https://doi.org/10.1093/jn/130.7.1850S

Deglaire, A., Bos, C., Tomé, D., Moughan, P. 2009. Ileal digestibility of dietary protein in the growing pig and adult human. *British Journal of Nutrition*, vol 102, no. 12, p. 1752-1759. https://doi.org/10.1017/S0007114509991267

FAO. 1991. *Protein quality evaluation*. Report of Joint FAO/WHO Expert Consultation. FAO Food and nutrition Paper 51, Food and Agriculture Organization, Rome.

FAO. 2013. *Dietary protein quality evaluation in human nutrition.* Report of an FAO Expert Consultation. FAO Food and Nutrition Paper 92, Food and Agriculture Organization, Rome.

Fuller, M. F., Tomé, D. 2005. In *vivo* determination of amino acid bioavailability in humans and model animals. *Journal of AOAC International*, vol. 88, no. 3, p. 923-934.

Gilani, G. S. 2012. Background on international activities on protein quality assessment of foods. *British Journal of Nutrition*, vol. 108 (Suppl S2), p. 168-182. https://doi.org/10.1017/S0007114512002383

Graham, H., Fadel, J. G., Newman, C. W., Newman, R. K. 1989. Effect of pelleting and beta-glucanase supplementation on the ileal and fecal digestibility of a barley-based diet in the pig. *Journal of Animal Science*, vol. 67, no. 5, p. 1293-1298. https://doi.org/10.2527/jas1989.6751293x

Hendriks, W. H., van Baal, J., Bosch, G. 2012. Ileal and faecal protein digestibility measurement in humans and other non-ruminants - a comparative species view. *British Journal of Nutrition*, vol. 108, (Suppl S2), p. 247-257. https://doi.org/10.1017/S0007114512002395

Jansman, A. J. M., Smink, W., Leeuwen, P., Rademacher, M. 2002. Evaluation through literature data of the amount and amino acid composition of basal endogenous crude protein at the terminal ileum of pigs. *Animal Feed Science and Technology*, vol. 98, no. 1-2, p. 49-60. https://doi.org/10.1016/S0377-8401(02)00015-9

Jondreville, C., van den Broecke, J., Gatel, F., Grosjean, F., van Cauwenberghe, S., Sève, B. 2001. Ileal digestibility of amino acids and estimates of endogenous amino acid losses in pigs fed wheat, triticale, rye, barley, maize and sorghum. *Animal Research*, vol. 50, no. 2, p. 119-134. https://doi.org/10.1051/animres:2001120

Mathai, J. K., Liu, Y., Stein, H. H. 2017. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). *Britisch Journal of Nutrition*, vol. 117, no. 4, p. 490-499. https://doi.org/10.1017/S0007114517000125

Moughan, P. J. 2003. Amino acid availability – aspects of chemical analysis and bioassay methodology. *Nutrition Research Reviews*, vol. 16, no. 2, p. 127-141. https://doi.org/10.1079/NRR200365

Moughan, P. J., Donkoh, A. 1991. Amino acid digestibility in non-ruminants: a review. *Recent Advances in Animal Nutrition - Australia*, p. 172-184.

Rowan, A. M, Moughan, P. J., Wilson, M. N., Maher, K., Tasman-Jones, C. 1994. Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model animal for digestion studies in man. *British Journal of Nutrition*, vol. 71, no. 1. p. 29-42. https://doi.org/10.1079/BJN19940108

Schaafsma, G. 2012. Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. *British Journal of Nutrition*, vol. 108, no. 2, 333-336. https://doi.org/10.1017/S0007114512002541

Stein, H. H., Seve, B., Fuller, M. F., Moughan, P. J., de Lange, C. F. M. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. *Journal of Animal Science*, vol 85, no. 1, p. 172-180. https://doi.org/10.2527/jas.2005-742

Whiting, F., Bezeau, L. M. 1957. The metabolic fecal nitrogen excretion of the pig as influenced by the amount of fibre in the ration and body weight. *Canadian Journal of Animal Science*, vol. 37, no. 1, p. 95-105. https://doi.org/10.4141/cjas57-014

Williams, C. H., David, D. J., Lismaa, O. 1962. The determination of chromic oxide in fecal samples by atomic absorption spectrophotometry. *Journal Agricultural Science*, vol. 59, no. 3, p. 381-385. https://doi.org/10.1017/S002185960001546X

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NUTRITION MARKETING OF HONEY: CHEMICAL, MICROBIOLOGICAL, ANTIOXIDANT AND ANTIMICROBIAL PROFILE

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ABSTRACT

Honey and all bee products have very good biological and chemical properties. They have been used in medicine for a long time. In our study we evaluated twenty polyfloral honey samples, the first ten were commercial honeys from a selected supermarkets with country of origin indicated "blend of EU and non-EU honeys" and represented imported honey. The second ten honeys were from a local beekeepers (Nitra region) and represented the Slovak origin. The aim of the study were to analyze chemical, microbiological, antioxidant, antimicrobial profile of honey and recommend marketing strategies for honey producers by applying nutrition marketing. From chemical point of view, the study examined mineral profile of honeys, antioxidant properties as antioxidant activity, total polyphenols, flavonoid and phenolic acid content and from microbiological view the study evaluated a total count bacteria, coliform bacteria and microscopic filamentous fungi. Results of minerals showed that the most dominant element in commercial honeys is sodium (30 mg.100g^{-1}) followed by calcium, potassium, magnesium and phosphorus. Iron, arsenic and selenium are present only in trace amounts. In local honeys the most dominant element is potassium (84.181 mg.100g^{-1}) followed by calcium, phosphorus, sulfur and magnesium. The presence of hazardous heavy metals (cadmium, lead and chrome) was not detected in either of the samples. Moreover, antioxidant activity determined by the DPPH method was slightly higher in local polyfloral honeys and vice versa the content of total polyphenol, flavonoid and the phenolic acid content was slightly higher in commercial polyfloral honeys. From the microbiological point of view, the total count of bacteria was found only in commercial polyfloral honeys while local honeys were without detectable microorganisms. The best antimicrobial activity was found against gram-negative bacteria such as *Escherichia coli* in both concentrations of honeys, and the local honeys reached better antimicrobial activity. All in all, honey has very good biological properties and mineral composition which opens opportunities for beekeepers to apply nutrition marketing and target new segments of consumers, e.g. sportsmen, people in convalescence, consumers following healthier lifestyle or seeking functional food. Moreover, educating consumers from a nutritional point of view will foster daily intake of honey and will increase annual consumption of honey in the future.

Keywords: nutrition marketing; honey; microorganisms; polyphenolic content; mineral profile

INTRODUCTION

Nutrition marketing can be defined as a marketing which provides nutrition and health information about certain food or beverages as well as the health claims for these products. It may involve any form of marketing communication starting with advertisement in TV, radio, press or product labelling **(Colby et al., 2010)**. According to **Gulevska and Martinovski (2018)** it is a concept of creating a platform for emphasizing product qualities and components which either enables consumers to improve or strengthen their health. Besides well-known marketing mix (product, price, place and promotion), the nutritive marketing recognises 5N principles (nutritive qualities, nutritive quality, nutritive benefits, nutritive strategy and nutritive integration). Furthermore, this type of marketing could attract consumers´ attention and influence their perception and preferences for certain product, its healthiness and nutritional qualities by applying health claims and nutrition facts **(Dunay et al., 2015; Royo-Bordonada et al., 2016; Zou, Li, and Liu, 2018; Pulker, Scott and Pollard, 2018)**. Health claims are defined as statements which indicate a certain relationship of health related issues and substance in the product and include phrases like reducing the risk of heart attacks **(Schaefer, Hooker and Stanton, 2016; Grunert, 2017)**. In general, nutrition marketing provides consumers the necessary information to make healthy food choices in the purchasing process **(Schermel, 2013)** and it opens new opportunities towards functional consumers and segment of health and well-being of consumers **(Paluchová, 2017)**. Consumers usually perceive the environment in shops by all their senses and prefer to buy products with high quality **(Berčík et al., 2016; Nagyová et**

al., 2018; Paluchová, 2012) as well as with concerns towards the food origin which depends on educational attainment **(Golian et al., 2018)**. Moreover, consumers of different age categories perceive the surroundings differently
and have different purchasing habits **(Géci, Nagyová and Rybanská, 2017)**. Quality of food products may be assessed in microbial aspects as well and the related measurements may reduce food risk **(Tóth et al., 2018)**. According to a consumer study in Slovakia, consumer´s age plays a crucial role in honey consumption patterns and preferences for this product **(Guziy, Šedík and Horská, 2017)**. According to a Romanian consumer research, honey is mostly perceived as natural, healthy food from a local beekeeper **(Pocol, and Bolboaca, 2013; Pocol and Ilea, 2011)**. Another study conducted in Poland highlights the need for consumer education in terms of nutritional knowledge which will increase overall consumption and the attractiveness of honey **(Kowalczuk, Jeżewska-Zychowicz and Trafiałek, 2017)**.

The aim of the paper is to analyse chemical, microbiological, antioxidant, antimicrobial profile of honeys and recommend marketing strategies for honey producers by applying nutrition marketing.

Scientific hypothesis

The scientific hypothesis of this study was to examine the differences between commercial honeys and honeys from local beekeepers in chemical composition, polyphenolic profile, microbiological spoilage of microorganisms and antibacterial activity.

MATERIAL AND METHODOLOGY

Samples

For microbiological properties, antimicrobial activity, antioxidant activity and chemical properties twenty honey samples were tested. First ten samples were commercial polyfloral honey purchased from selected supermarkets with country of origin marked as "blend of EU and non-EU honeys" and represent imported honey. The second ten samples were polyfloral honey directly from the local beekeepers (Nitra region) and represent the Slovak origin.

Sample preparation for antioxidant activity

An amount of 0.5 g of sample was extracted with 20 mL of 80% ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min., the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids, phenolic acids). Extraction was carried out in triplicate.

Radical scavenging activity – DPPH method

The radical scavenging activity of the extract was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(Sánchéz-Moreno et al., 1998)**. The sample (0.4 mL) was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL methanol). Absorbance of the reaction mixture 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 $mg.L^{-1}$; $R^2 = 0.989$) was used as the standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Total polyphenol content

The total polyphenol content extract was measured by the method of **Singleton and Rossi (1965)** using Folin-Ciocalteu reagent. 0.1 mL of each sample 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 min in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25 – 300 $mg.L^{-1}$; $R^2 = 0.998$) was used as the standard and the results were expressed in $mg.g^{-1}$ gallic acid equivalents.

Total flavonoid content

The total flavonoids were determined using the modified method of **Willett (2002)**. 0.5 mL of sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M potassium acetate and 4.3 mL of distilled water. After 30 min in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.5 - 20 $mg.L^{-1}$; $R^2 = 0.989$) was used as the standard and the results were expressed in $mg.g^{-1}$ quercetin equivalents.

Total phenolic acid content

Total phenolic acid 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 $mg.L^{-1}$, $R^2 = 0.999$) was used as a standard and the results were expressed in $mg.g^{-1}$ caffeic acid equivalents.

Mineral compounds

The samples of honey were subjected to mineralization under high pressure, in HNO_3 65%, super pure. 0.2 gram samples were weighed and placed in Teflon vessels which were then filled with 8 mL of nitric acid and sealed tightly. For each group of nine samples, during the microwave dissolution process, the rotor of the digestion system was additionally filled with a blank sample comprising 8 mL of nitric acid alone. The samples were digested for one hour, with the applied algorithm of temperature increase as specified for biological samples, without exceeding 200 ºC. This was carried out using Ethos One microwave digestion system from Milestone. The vessels were opened after the mineralization process had been completed and the samples with acid had been brought to room temperature. The samples were cooled down to room temperature and supplemented with water to the volume of 50 mL. The obtained detection threshold for each element was not lower than 0.01 $mg.kg^{-1}$ (with the assumed detection capacity of the measuring apparatus at a level exceeding 1 ppb). The measurements were performed by ICP-OES spectrometer, Thermo iCAP Dual 6500 with horizontal plasma, and the capacity of detection along and across the plasma flame (Radial and Axial). Before measuring each batch of 2 samples the method was calibrated with the use of certified Merck models, with concentrations of 10000 ppm for Ca, Fe, K, Mg, P and 1000 ppm for Al, Ba, Cd, Cu, Na, Pb. The

measurement result for each element was compensated to account for the measurement of elements in the blank sample. In each case a 3-point calibration curve was used for each element, with optics correction applying the method of internal models, in the form of yttrium and ytterbium ions, at the concentrations of 2 $mg.L^{-1}$ and 5 $mg.L^{-1}$, respectively. The analytic methods were validated with two independent tests.

Microbiology of honey

Determination of cfu counts

The plate diluting method was applied for quantitative cfu count determination of the respective groups of microorganisms in 1 g of honey. Into Petri dishes, 1 mL of honey dilution was inoculated and poured by gelatinous nutritive substrate (Table 1).

Dilution of the samples

Basic dilution (10^{-1}) was prepared as follows: 5 g of honey content was added to the test tube containing 45 mL of distilled water.

Antimicrobial activity of honey

Honey solutions were prepared in two fractions: 50 and 25% (by mass per volume). The samples of each honey (10 g) and sterile water were stored at 37 °C for 30 min. before mixing, to facilitate homogenization. The 50% (by mass per volume) solutions thus prepared were diluted to 25%. The samples were assayed immediately after dilution. Six strains of microorganisms were tested in this study, including three Gram-negative bacteria (*Escherichia coli* CCM 3988, *Klebsiela pneumoniae* CCM 2318, *Salmonella enterica* subs. *enterica* CCM 3807), three Gram-positive bacteria (*Bacillus cereus* CCM 2010, *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* subs. *aureus* CCM 4223). All tested strains were collected from the Czech Collection of microorganisms (Brno, Czech Republic). The bacterial suspensions were cultured in the nutrient broth (Oxoid, Basingstoke, United Kingdom) at 37 °C. The antimicrobial effect of the natural honey was tested using the agar well diffusion method. Overnight microbial cultures were used for surface inoculation of Petri dishes containing 15 mL of Muller Hinton agar (MHA). Each Petri dish was spread on with 100 µL of strain inoculum streaked thoroughly all over the surface of the MHA. Subsequently, four equidistant wells 6 mm in diameter each were punched into the inoculated medium with sterile glass Pasteur pipettes and were filled up with 250 µL of honey using a precise eppendorph.

All plates were incubated at 37 °C and inhibition zones were measured after 24 hours. Six different strains of bacteria species were tested in sets of plates, which were simultaneously processed for each strain. All the experiments were repeated triplicate, including control with plain 40% phenol every time. After incubation the zones of inhibition of the growth of the bacteria around the disks were measured. The mean values of the three trials were calculated.

Statistical analysis

All experiments were carried out in triplicate and the results reported are the results of those replicate determinations with standard deviations. In addition, t-test for Equality of Means was applied in SPSS Statistics 25 to examine differences among honey samples.

RESULTS AND DISCUSSION

Antioxidant activity

Antioxidant activity determined by the DPPH method was slightly higher in polyfloral honeys from local beekeepers in comparison to commercial honeys (Table 2). In antioxidant activity, statistically significant differences were found between commercial honey samples and local honey samples from beekeepers ($p \leq 0.001$). Generally, bee honey is rich in compounds with antioxidant activity. According to **Khalil et al. (2010)** antioxidant properties have peroxides, glucose, vitamin C, glucose oxydase enzymes, phenol compounds and catalase. Moreover, carotenoids and flavonoids are present in honey as well. Antioxidants in honey are ensured by the high number of these indicators which depends on locality, type of honey and agro ecological condition. **Wilczyńska (2010)** determined antioxidant activity of different Polish honey and found the best activity by the DPPH method in heather (100%) and honeydew honey (83.51%), which was higher compared to polyfloral honey (42.53%). **Mellen et al. (2015)** found the best activity evaluated by the ABTS method in buckwheat honey from Poland (4.63 mmol $TEAC.kg^{-1}$) and also in frost honey from Serbia (2.71 mmol $TEAC.kg^{-1}$). These authors also measured polyfloral honey from Slovakia (1.47 mmol $TEAC.kg^{-1}$) which had higher activity compared to polyfloral honey from Poland (1.11 mmol $TEAC.kg^{-1}$). Their results confirmed that the antioxidant activity of honey is very strongly influenced by origin and locality.

In addition, **Juszczak et al. (2015)** determined significantly higher antioxidant activity in honey enriched with propolis, bee pollen and royal jelly with compared to pure polyfloral honey as well as **Džugan et al. (2017)** tested honey enriched with dried herbs and determined significantly higher antioxidant activity in this honey compared to pure polyfloral honey. Nowadays, the attractivity of enriched honey is increasing among consumers and these facts can be an effective tool for

Table 1 Applied nutritive substrates.

Microbial groups	Medium	Length of incubations (h)	Cultivation method	Temperature (°C)
Total count of bacteria (TCB)	Plate count agar	48	aerobic	30
Coliform bacteria (CB)	Violet red bile agar	24	aerobic	37
Moulds (M)	Malt extract agar	120	aerobic	25

nutrition marketing to strengthen the position of these new products.

Total polyphenol, flavonoid and phenol acid content

The content of total polyphenol, flavonoid and phenolic acid content was slightly higher in commercial polyfloral honey in comparison to local polyfloral honey (Table 2). Statistically significant differences of total polyphenol, flavonoid and acid content were found between commercial honeys and local honeys of beekeepers ($p \leq 0.001$). **Sime et al. (2015)** tested several polyfloral honeys from Ethiopia and reported that honey is rich in biologically active compounds especially from phenolic groups. The sample had from 3.3 to 6.1 mg GAE.g^{-1} of total polyphenol content. The results of total polyphenol content in the current study are comparable with results of **Al-Mamary et al. (2002)** which tested different types of honey and amount of total polyphenols ranged from 0.56 to 2.46 mg CE.g^{-1} (catechin equivalent). Total polyphenol content was higher than flavonoid content. The similar results were reported **Sime et al. (2015)** where study determined the total flavonoid content in honey from Ethiopia was from 0.18 to 0.42 mg CE.g^{-1} (catechin equivalent). These authors also reported that amount of flavonoids is attributable to the differences in the type of honey samples, floral origin and season of collection. Darker honeys use to have higher flavonoids in comparison to lighter ones. In general, honey contains approximately 0.02 mg.g^{-1} of the flavonoid concentration **(Ayoub et al., 2009)**. This value is in agreement with current samples. In addition, **Özkök et al. (2010)** determined total phenolic acid content in Turkish pine honeydew honey and results ranged from
0.035 to 0.36 mg GAE.g^{-1} (gallic acid equivalent).

Mineral composition in analyzed honeys

In general, minerals in honey originate from nectar, honeydew or pollen grains and their content is influenced by soil composition, geographical and botanical origin **(Madejczyk and Baralkiewicz, 2008).** Mineral content of honey range between 0.04% to 0.20% and usual variation

Table 2 The total polyphenol, flavonoid and phenolic acid content in analyzed samples.

Parameter	Commercial honeys	Local honeys from beekeepers
DPPH [mg TEAC.g^{-1}]	0.273 ±0.015	0.313 ±0.018
TPC [mg GAE.g^{-1}]	1.656 ±0.022	1.212 ±0.011
TFC [mg QE.g^{-1}]	0.087 ±0.012	0.033 ±0.013
TPAC [mg CAE.g^{-1}]	0.110 ±0.019	0.084 ±0.022

Note: DPPH – radical scavenging activity; TEAC – trolox equivalent antioxidant capacity; GAE – gallic acid equivalent; QE – quercetin equivalent; CAE – caffeic acid equivalent; mean (n = 10) ±standard deviationthe possition near to the top or bottom of the page.

Table 3 Mineral composition in analyzed samples.

Parameter [mg.100g^{-1}]	Commercial honeys	Local honeys from beekeepers
Aluminum (Al)	nd	0.004 ±0.002
Arsenic (As)	0.018 ±0.002	0.019 ±0.002
Calcium (Ca)	2.626 ±0.066	3.309 ±0.098
Zinc (Zn)	nd	nd
Cadmium (Cd)	nd	nd
Chrome (Cr)	nd	nd
Copper (Cu)	nd	nd
Iron (Fe)	0.009 ±0.002	nd
Selenium (Se)	0.033 ±0.003	0.032 ±0.003
Potassium (K)	2.397 ±0.037	84.181 ±0.501
Magnesium (Mg)	2.367 ±0.012	1.181 ±0.015
Sodium (Na)	30.000 ±0.525	0.059 ±0.002
Phosphorus (P)	0.979 ±0.074	3.193 ±0.044
Lead (Pb)	nd	nd
Sulfur (S)	nd	1.642 ±0.087
Manganese (Mn)	nd	nd

Note: mean (n = 10) ±standard deviation; nd – not detected.

of macroelement and microelement minerals are represented by potassium, sodium, calcium, magnesium, phosphorus, iron, manganese, arsenic, selenium and etc. **(Alqarni, Owayss and Mahmoud, 2012).**

As shown in Table 3, in commercial polyfloral honey was dominant sodium (30 mg.100g^{-1}) following by calcium, potassium, magnesium and phosphorus. Iron, arsenic and selenium were present only in trace amounts. Presence of hazardous mineral compounds was not detected (aluminum, cadmium, lead and chrome). In polyfloral honeys from local beekeepers was dominant potassium (84.181 mg.100g^{-1}) followed by calcium, phosphorus, sulfur and magnesium. The presence of sulfur can be linked to soil composition **(Fermo et al., 2013)**. Selenium, sodium, arsenic, and aluminum were present only in trace amounts. For example, selenium plays a critical role in reproduction, hormone metabolism or DNA synthesis **(Altun et al., 2017)**. The presence of heavy metals was not detected (cadmium, lead and chrome). Commercial honeys contained higher content of sodium, which is typical for coastal regions rather than inlands **(Kováčik et al., 2016)** and supports the facts that commercial honey with country of origin indicated as "blend of EU and non-EU honeys" represents imported honey and includes coastal countries. Local honeys contained higher amount of potassium which is the most abundant mineral in honey and in general the highest amounts are usually found in linden honeys **(Jovetić et al., 2017)**. The similar results of trace elements were obtained by **Cantarelli et al. (2008)** in honeys from Argentina. The samples contained potassium, calcium, sodium and phosphorus. Moreover, the authors compared Argentinian honeys with honeys from Spain, Turkey, Italy and Egypt. The significantly higher content of iron and zinc was present in honeys from Egypt. Another study analyzed trace elements in honey from different regions in Rio de Janeiro. Results showed the dominance of calcium and potassium,

while copper, zinc and selenium was present in trace amounts **(Ribeiro et al., 2014)**.

Mineral composition in honey could be used for identification of its geographical and botanical origin. Other trace elements (Pb, Cd, Hg, Cu, Mn, Zn, Ag), belonging to the heavy metals, play important roles as bio-indicators for environmental pollution **(Solayman et al., 2015)**. In our study was not detected presence of hazardous heavy metals. Statistically significant dufferences between commercial and local honeys were found in case of Ca ($p \leq 0.001$), K ($p \leq 0.001$), Na ($p \leq 0.001$), Mg ($p \leq 0.001$) and P ($p \leq 0.001$).

Microbiology of honey

The number of microorganisms found in honey samples is shown in Table 4. Polyfloral honey from local beekeepers was without any detectable microorganisms. In commercial honey were found only total count of bacteria (1.50 ±0.07 log cfu.g^{-1}). Total viable count of aerobic bacteria did not exceed 2.00 log cfu.g^{-1} in any sample. **Kňazovická et al. (2011)** reported a mean value of 1.4 x 10^2 cfu.g^{-1} of the bacteria. Several authors indicate, that total aerobic viable count in honey range between zero and tens of thousands per gram **(Kačániová et al., 2009)**.

A good secondary manufacturing practise controls secondary sources of contamination. A usual microbiological examination may include several different assays. General information is provided by a standard plate count. Furthermore, the useful information is provided by count of yeasts and an assay for bacterial spore-formers.

Bacteria are not able to replicate in honey, thus the high amount of vegetative bacteria may be the result of secondary contamination. Nevertheless, some vegetative microbes are able to survive at cool temperatures in honey for several years. Due to antimicrobial properties, the persistence and growth of many microorganisms are being discouraged. In general, the low number of microbes and its limited variation is expected in honey **(Kačániová et al., 2007)**.

Table 4 Indicience of microorganisms in analyzed samples (in log cfu.g^{-1}).

Parameter	Commercial honeys	Local honeys from beekeepers
TCB	1.50 ±0.07	nd
CB	nd	nd
M	nd	nd

Note: mean (n=10) ±standard deviation, nd – not detected.

Antimicrobial activity

Honey has been found to possess antimicrobial activity which has been attributed to specific chemicals in the honey **(Kačániová et al., 2008)**. The antibacterial activity of honey is shown in Table 5. The best antibacterial activity of commercial polyfloral honey was found against Gram negative bacteria *Escherichia coli* >*Klebsiella pneumoniae* >*Salmonella enterica*. The lower antimicrobial activity of commercial honey was found against Gram positive bacteria. Statistically significant differences were found between commercial honeys and local honeys from beekeepers in case of all microorganisms ($p \leq 0.001$, $p \leq 0.004$ and $p \leq 0.006$).

Polyfloral honeys from local beekeepers have the best antimicrobial activity similar to commercial honeys against *E. coli*. The bactericidal activity of the honeys on *Pseudomonas aeruginosa, Salmonella typhi* and *E. coli* was found to be between 50 and 100% concentration of honey sample from Dembia, Debark and Gondar Zuria **(Ahmed et al., 2014)**.

The honeys showed bactericidal activities against the tested organisms up to the dilutions of 50%. This is similar to those reported by **Nzeako and Hamdi (2000)** who studied on six commercial honeys found inhibition in an agar diffusion of *S. aureus, E. coli* and *P. aeruginosa*. The well documented antibacterial properties of honey are mainly caused by the hydrogen peroxide which is a potent antimicrobial agent mostly produced during oxidation of glucose catalysed by bee enzymes added by the bees during nectar harvest. The rate of hydrogen peroxide production, glucose oxidase and its destruction by catalases determine its concentration in honey. The amount of hydrogen peroxide differs from honey to honey. Another study re-examined the importance of this component for antibacterial activity of honey **(Bizerra et al., 2012)**.

CONCLUSION

The study evaluated chemical, biological, microbiological and antibacterial properties of twenty different polyfloral honey samples. The first ten were commercial honeys from a selected supermarket with country of origin indicated as "blend of EU and non-EU honeys" while second ten were honeys from local beekepers (Nitra region) representing the domestic origin. Results showed that all samples have antioxidant activity, antimicrobial activity and possess polyphenol, flavonoid phenolic acid and mineral substances. Local honeys obtained better results in

Table 5 Antimicrobial activity of analyzed samples (mm).

Microorganisms	Commercial honeys 50%	Commercial honeys 25%	Local honeys from beekeepers 50%	Local honeys from beekeepers 25%
E. coli	7.45 ±0.49	4.28 ±0.26	8.33 ±0.26	5.17 ±0.37
K. pneumoniae	7.00 ±0.41	3.69 ±0.11	8.12 ±0.34	4.33 ±0.26
S. enterica	5.81 ±0.55	3.35 ±0.15	7.77 ±0.35	4.74 ±0.25
B. cereus	4.71 ±0.29	3.19 ±0.48	7.150 ±0.40	3.83 ±0.44
L. monocytogenes	4.86 ±0.24	2.86 ±0.31	6.41 ±0.22	3.56 ±0.38
S. aureus	4.93 ±0.32	2.50 ±0.39	5.69 ±0.39	3.00 ±0.26

Note: mean (n=10) ±standard deviation.

antioxidant activity, antimicrobial activity and possess no detectable microorganisms. Commercial honeys have slightly higher content of total polyphenol, flavonoid, phenolic acid and few microorganisms. Regarding the mineral composition, the differences were in sodium and potassium content comparing of commercial and local honey. Commercial honeys contained sodium (30 $mg.100g^{-1}$) following by calcium, potassium, magnesium and phosphorus, iron and selenium. Local honeys contained higher amount of potassium (84.181 $mg.100g^{-1}$) followed by calcium, phosphorus, magnesium, sulfur, sodium and selenium. Heavy metals (cadmium, lead and chrome) were not detected in either of the samples..

In conclusion, honey has very good biological properties and mineral composition which offer many opportunities for applying nutrition marketing. Beekeepers should educate their consumers not only about positive healing effects of honey, but also include nutritional point of view. By emphasizing the benefits of consuming honey on a daily basis will increase consumers´annual consumption in the future. Furthermore, there is a space for targeting new segments such as sportsmen, people in convalescence or consumers following the healthy lifestyle.

REFERENCES

Ahmed, M., Sahile, S., Subramanian, C. 2014. Evaluation of antibacterial potential of honey against some common human pathogens in north gondar zone of ethiopia. *International Journal of Pure and Applied* Zoology, vol. 2, no. 4, p. 286-295.

Al-Mamary, M., Al-Meeri, A., Al-Habor, M. 2002. Antioxidant activity and total phenolic of different types of honey. *Nutrition Research*, vol. 9, p. 1041-1047. https://doi.org/10.1016/S0271-5317(02)00406-2

Alqarni A. S., Owayss A. A., Mahmoud A. A. 2012. Mineral content and physical properties of local and imported honeys in Saudi Arabia. *Journal of Saudi Chemical Society*, vol. 18, no. 5, p. 618-625. https://doi.org/10.1016/j.jscs.2012.11.009

Altun, S. K., Dinç, H., Paksoy, N., Temamoğulları, F. K., Savrunlu, M. 2017. Analyses of Mineral Content and Heavy Metal of Honey Samples from South and East Region of Turkey by Using ICP-MS,". *International Journal of Analytical Chemistry*, vol. 2017, p. 6. https://doi.org/10.1155/2017/6391454

Ayoub, H., Zein, S., Makawi, A., Gadkariem, E. Mohamed, S. 2009. Determination of antioxidant flavonoids in Sudanese honey samples by solid phase extraction and high performance liquid chromatography. *European Journal of Chemistry*, vol. 6, p. 429-437. https://doi.org/10.1155/2009/382504

Berčík, J., Palúchová, J., Vietoris, V., Horská, E. 2016. Placing of aroma compounds by food sales promotion in chosen services business. *Potravinarstvo*, vol. 10, no. 1, p. 672-679. https://doi.org/10.5219/666

Bizerra, F. C., Da Silva Jr. P. I., Hayashi, M. A. F. 2012. Exploring the antibacterial properties of honey and its potential. *Frontiers in Microbiology*, vol. 3, p. 398. https://doi.org/10.3389/fmicb.2012.00398

Cantarelli, M. A., Pellerano, R. G., Marchevsky, E. J., Camina, J. M. 2008. Quality of honey from Argentina: study of chemical composition and trace elements. *The Journal of the Argentine Chemical Society*, vol. 96, p. 33-41.

Colby S. E., Johnson L., Scheett A., Hoverson B. 2010. Nutrition marketing on food labels. *Journal of Nutrition Education and Behavior*, vol. 42, no. 2, p. 92-98. https://doi.org/10.1016/j.jneb.2008.11.002

Dunay, A., Bittsánszky, A., Tóth, A., Illés, B. Cs. 2015. Minőség az iskolai étkeztetésben. (Quality in School Catering Service.) *The Hungarian Journal of Food Nutrition and Marketing*, vol. 11, no. 1-2, p. 17-22. (In Hungarian)

Džugan, M., Sowa, P., Kwasniewska, M., Weselowska, M., Czernicka, M. 2017. Physicochemical parameters and antioxidant activity of bee honey enriched with herbs. *Plant Foods for Human Nutrition*, vol. 72, p. 74-81. https://doi.org/10.1007/s11130-016-0593-y

Farmakopea Polska, 1999. The Polish Farmaceutical Society [online] s.a. [cit.2017-01-25] Available at: http://www.ptfarm.pl/?pid=1&language=en.

Fermo, P., Beretta, G., Facino, R. M., Gelmini, F., Piazzalunga, A. 2013. Ionic profile of honey as a potential indicator of botanical origin and global environmental pollution. *Environmental Pollution*, vol. 178, p. 173-181. https://doi.org/10.1016/j.envpol.2013.03.029

Géci, A., Nagyová, Ľ., Rybanská, J. 2017. Impact of sensory marketing on consumer´s buying behaviour. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 709-717. https://doi.org/10.5219/835

Golian, J., Nagyová, Ľ., Andocsová, A., Zajác, P., Palkovič, J. 2018. Food safety from consumer perspective: health safety. *Potravinarstvo Slovak Journal of Food Sciences,* vol. 12, no. 1, p. 313-322. https://doi.org/10.5219/917

Grunert, K. G. 2017. The health trend. In Grunert, K. G. *Consumer trends and new product opportunities in the food sector*. Wageningen, Netherlands : Wageningen Academic Publishers, p. 15-31. ISBN: 978-90-8686-307-5.

Gulevska, F., Martinovski, S. 2018. Nutritive marketing and analysis of consumption behaviour for honey. *Journal of Hygienic Engineering and Design*, vol. 23, p. 72-80.

Guziy, S., Šedík, P., Horská, E. 2017. Comparative study of honey consumption in Slovakia and Russia. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 472-479. https://doi.org/10.5219/784

Jovetić M., Trifković J., Stanković D., Manojlović D., Milojković-Opsenica D. 2017. Mineral Content as a Tool for the Assessment of Honey Authenticity. *Journal of AOAC International*, vol. 100, no. 4, p. 862-870 https://doi.org/10.5740/jaoacint.17-0145

Juszczak, L., Galkowska, D., Ostrowska, M., Socha, R. 2015. Antioxidant activity of honey supplemented with bee products. *Natural Product Research*, vol. 30, p. 1436-1439. https://doi.org/10.1080/14786419.2015.1057582

Kačaniová M., Melich M., Kňazovická V., Haščik P., Sudzinová J., Pavličová S., Čuboň J. 2009. The indicator microorganisms value in relation to primary contamination of honey. *Lucrări stiintifice Zootehnie şi Biotehnologii* vol. 42, no. 2, p. 159-166. ISSN 2344-4576.

Kačániová, M., Fikselová, M., Felšöciová, S., Kňazovická, V., Fatrcová-Šramková, K., Nôžková, J., Ubrežiová, I. 2008. Evaluation of antioxidant and antimicrobial activities of natural honeys. *Chemické listy*, vol. 102, no. 15, p. 680-681.

Kačániová, M., Sudzina, M., Sudzinová, J., Fikselová, M., Čuboň, J., Haščík, P. 2007. Microbiological and physicochemical quality of honey collected from different Slovak habitats. *Slovak Journal of Animal Sciences*, vol. 40, no. 1, p. 38-43.

Khalil, M. I., Sulaiman, S. A., Boukraa, L. 2010. A properties of honey and its role in preventing health disorder. *The Open Nutraceuticals Journal*, vol. 3, p. 6-16. https://doi.org/10.2174/1876396001003010006

Kňazovická, V., Kačaniová, M., Dovičičova, M., Melich, M., Kadási-Horáková, M., Barborákova Z., Mareček J. 2011. Microbial quality of honey mixture with pollen.

Potravinarstvo, vol. 5, no. 1, p. 27-32. https://doi.org/10.5219/110

Kováčik, J., Grúz, J., Biba, O. Hedbavny, J. 2016. Content of metals and metabolites in honey originated from the vicinity of industrial town Košice (eastern Slovakia). *Environmental Science and Pollution Research*, vol. 23, no. 5, p. 4531-4540. https://doi.org/10.1007/s11356-015-5627-8

Kowalczuk, I., Jeżewska-Zychowicz, M., Trafiałek, J. 2017. Conditions of honey consumption in selected regions of Poland. *Acta Scientiarum Polonorum Technologia Alimentaria*, vol. 16, no. 1, p. 101-112.

Madejczyk, M., Baralkiewicz, D. 2008. Characterization of Polish rape and honeydew honey according to their mineral contents using ICP-MS and F-AAS/AES. *Analytica Chimica Acta*, vol. 617, p. 11-17. https://doi.org/10.1016/j.aca.2008.01.038

Mellen, M., Fikselová, M., Mendelová, A., Haščík, P. 2015. Antioxidant effect of natural honeys affected by their source and origin. *Polish Journal of Food and Nutrition Sciences*, vol. 65, p. 81-85. https://doi.org/10.1515/pjfns-2015-0020

Nagyová, Ľ., Golian, J., Géci, A., Palkovič, J., Čapla, J., Kádeková, Z. 2018. Food safety from a consumers´ point of view: food quality *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 355-363. https://doi.org/10.5219/918

Nzeako, B., Hamdi, J. 2000. Antimicrobial potential of honey on some microbial isolates. *Sultan Qaboos University Medical Journal*, vol. 2, no. 2, p. 75-79. ISSN 2075-0528.

Özök, A., D´arcy, B., Sorkun, K. 2010. Total phenolic acid and total flavonoid content of Turkish pine honeydew honey. *Journal of ApiProduct and ApiMedical Science*, vol. 2, p. 65-71. https://doi.org/10.3896/IBRA.4.02.2.01

Paluchová, J. 2012. The new aproach on food quality: an important factor on consumer behaviour and trends in food quality. *Polityki europejskie,* vol. 57, no. 8, p. 355-365.

Paluchová, J. 2017. Consumer segments in an international context. In Grunert, K. G. *Consumer trends and new product opportunities in the food sector.* Wageningern, Netherlands : Wageningen Academic Publishers, p. 173-197. ISBN: 978-90-8686-307-5.

Pocol, C. B., Bolboaca, S. D. 2013. Perception and trends related to the consumption of honey: A cse study of North-West Romania. *International Journal of Consumer Studies*, vol. 37. p. 642-649. https://doi.org/10.1111/ijcs.12046

Pocol, C. B., Ilea, M. 2011. The development of local products in Romania: A case study of honey. *Lucrări Științifice, Universitatea de Științe Agricole Și Medicină Veterinară a Banatului, Timisoara, Seria I, Management Agricol*, vol. 13, no. 2, p. 153-160.

Pulker C. E., Scott J. A., Pollard C. M. 2018. Ultra-processed family foods in Australia: nutrition claims, health claims and marketing techniques. *Public Health Nutrition*, vol. 21, no. 1, p. 38-48. https://doi.org/10.1017/S1368980017001148

Ribeiro, R. O., Marsico, E. T., DeJesus, E. F., DaSilva, C. C., Junior, C. A., DeAlmeida, E., Filho, F. 2014. Determination of trace elements in honey from different regions in Rio de Janeiro state (Brazil) by total reflection X-ray fluorescence. *Journal Of Food Science*, vol. 79, p. 1738-1742. https://doi.org/10.1111/1750-3841.12363

Royo-Bordonada, M. A., Bosqued-Estefanía, M. J., Damián, J., López-Jurado, L., Moya-Geromini, M. A. 2016. Nutrition and health claims in products directed at children via television in Spain in 2012 *Gaceta Sanitaria*, vol. 30, no. 3, p. 221-226, https://doi.org/10.1016/j.gaceta.2016.01.004

Sánchés-Moreno, C., Larrauri, A., Saura-Calixto, F. 1998. A procedure to measure the antioxidant afficiency of polyphenols. *Journal of Science and Food Agriculture*, vol. 76, p. 270-276. https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9

Schaefer D., Hooker N. H., Stanton J. L. 2016. Are Front of Pack Claims Indicators of Nutrition Quality? Evidence from 2 Product Categories. *Journal of Food Science*, vol. 81, no.1, p. 223-34. https://doi.org/10.1111/1750-3841.13150

Schermel, A., Emrich, T. E., Arcand, J. A., Wong, CH. L., L'Abbé, M. R. 2013. Nutrition marketing on processed food packages in Canada : 2010 Food Label Information Program. *Applied Physiology, Nutrition, and Metabolism*, vol. 38, no. 6, p. 666-672. https://doi.org/10.1139/apnm-2012-0386

Sime, D., Atlabachen, M., Redi-Abshiro, M., Zende, T. 2015. Antioxidant activities of natural honeys and propolis collected from different geographical regions of Ethiopia. *Bulletin of the Chemical Society of Ethiopia*, vol. 29, p. 163-172.

Singleton, V. L., Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic. phosphotungstic acid reagents. *American Journal of Enology and Agricultural*, p. 144-158.

Solayman, M., Islam, A. M., Paul, S., Ali, Y., Khalil, M. I., Alam, N., Gan, S. H. 2015. Physicochemical properties, minerals, trace elements and heavy metals in honey of different origins: A comprehensive review. *Comprehensive reviews in Food Science and Food Safety*, vol. 15, p. 219-233. https://doi.org/10.1111/1541-4337.12182

Tóth, A. J., Szakmár, K., Dunay, A., Illés, B. C., Bittsánszky, A. 2018. Hygiene assessments of school kitchens based on the microbiological status of served food. *Acta Scientiarum Polonorum Technologia Alimentaria*, vol. 17, no. 2, p. 159-168. https://doi.org/10.17306/J.AFS.2018.0564

Wilczyńska, A. 2010. Phenolic content and antioxidant activity of different types of polish honey- A short report. *Polish Journal of Food and Nutrition Sciences*, vol. 60, p. 309-313.

Willett, W. C. 2002. Balancing life-style and genomic research for disease prevention. *Science*, vol. 292, p. 695-698. https://doi.org/10.1126/science.1071055

Zou, P., Li, Y., Liu, Y. 2018. The Impact of Nutrition Label Regulation on Firm Marketing Action and Performance in China. *Journal of Public Policy & Marketing: Spring,* vol. 37, no. 1, p. 119-130. https://doi.org/10.1509/jppm.16.169

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DO REDUCED VAT RATES ON FOODSTUFFS IN EU AFFECT CONSUMERS?

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ABSTRACT

Value added tax represents an important fiscal policy instrument, mainly because of the stable source of tax revenues of state budgets. At the level of EU member states, the value-added tax is most harmonized, individual member states have always an option to implement more types of VAT rates on selected goods. In order to keep prices of foodstuffs and basic necessity products at the lowest possible level and with the highest availability for consumers, EU member states apply reduced VAT rates on these goods. The main objective of the paper is to research the impact on reduced VAT rates on foodstuffs in relation to consumers. The complexity of legislative adjustments on taxation of foodstuffs should be progressively simplified because reduced VAT rates on foodstuffs become ineffective and increase the burden on households compared to incomes of households. This paper is dedicated to the observation whether the application of the reduced VAT rate on foodstuffs affects the change in total expenditures of households. Based on the panel regression model with fixed effects on states, the standard VAT rate at the chosen significance level plays a significant factor, which affects total expenditures on foodstuffs of households and thus affects consumers. In the case of the reduced VAT rate on foodstuffs, this fact has not been confirmed.

Keywords: value added tax; reduced tax rate on foodstuffs; household consumption

INTRODUCTION

In 2010**, Mirrlees et al. (2010)** have published that value-added tax (VAT) represents unquestionably the most successful fiscal innovation of the last half-century, perhaps the most economically efficient way in which countries can raise significant tax revenues. What additional benefits does the existence of VAT have? Does the reduced VAT rate on foodstuffs in Europe affect the behaviour of consumers and affect their total consumption of foodstuffs?

The tax system should be neutral, simple, fair, and effective. The neutrality, simplicity, and uniformity of VAT have been supported since 2007 by EC legislation in the form of **Council Directive 2006/112/EC** on a common system of VAT in the member states with the aim of neutral economic competition and the harmonization of VAT legislation, including approaching rates. The standard VAT rate in the EU-28 reaches an average level of 21.5%. When observing existing VAT rates, special rates are applied on selected goods and services within EU member states.

Lowering the level of taxation can lead to an increase in consumption of private households. **Alm and El-Ganainy (2013)** researched the effect of the standard VAT rate on consumption of households and concluded that a 1% increase in the VAT rate negatively affects the consumption of households by approximately 1%. Households with a higher percentage of consumption of goods and services with reduced VAT rates will gain from reduced rate more than other households. This fact is considered to be the main advantage of having the reduced VAT rate on foodstuffs. Moreover, the existence of a negative correlation between the level of the tax rate and the consumption of households supports this importance.

Low-income states dispose of higher percentage of total expenditures spent on foodstuffs compared to high-income states. In order to keep prices of foodstuffs and basic necessity products at the lowest possible level and with the highest possible availability for consumers, EU member states apply reduced VAT rates on these goods. According to the current Directive, the reduced VAT rate level on foodstuffs cannot be lower than 5%. The reduced VAT rate on foodstuffs reaches an average at 11%. The effectiveness and fairness of the tax in the context of the reduced VAT rate are observed only if the tax really affects a decline in consumer prices. Assuming that the decline in the tax rate does not appear in the price of foodstuffs, it is an increase in deadweight loss and a decrease in availability of goods for low-income citizens. The effectiveness of reduced tax rates use is particularly supported in states where fiscal policy disposes of lower number of tools that deal with the issue of income differentiation and poverty in state **(Ebrill et al., 2001)**.

The main objective of the paper is the ex-post research of reduced VAT rates on foodstuffs in EU states and to assess their impact on total expenditures on foodstuffs of households and thus on the behaviour of consumers.

Back in 1939, **Brown (1939)** stated that in a perfectly competitive market, any tax burden on consumption taxes is being moved to the input price of production factors. **Fullerton and Metcalf (2002)** examined how the burden of a particular tax is transfered to consumers, whether through higher commodity prices, transfered to work - through changes in wages, or transfered to capital - through its return. The tax burden depends on demand elasticity and supply elasticity. Given the perfectly competitive market and low-price elasticity of demand, neoclassical economics assumes that a change in the tax rate will be reflected almost exclusively in the price of the goods. According to several empirical studies **(Bernal, 2018; Bernal, 2014; Cornelsen et al., 2015)**, the price elasticity of demand for basic foodstuffs is low, therefore the transfer of tax burden on the consumer is close to one. In the opposition, for example, **Attanasio and Weber (2010)**, who state that if unexpected changes in incomes of households do not occur, changes in tax rates should be a significant factor in change of their consumption.

The existence of reduced VAT rates on foodstuffs is according to **Mirrlees et al. (2010)** important for three reasons. Firstly, reduced tax rates will be reflected in the prices of basic necessity foodstuffs, and they become more affordable for a wider range of consumers. Secondly, their impact is significant in the effective and fair distribution of pensions. It allows the low-income groups of the population to maintain a reasonable level of income. Thirdly, through the observation of households spending, these reduced tax rates help to detect consumers' other specific needs.

Empirical studies that focus on transfering the tax burden from VAT implemented on foodstuffs and subsequently burdening consumption of households and behaviour of consumers are unique and differ from one another. Often, the results of the investigated impact of the tax rate growth are different from the results of the investigated impact of the tax rate fall. **David (2012)** analyses the transfer of tax burden under influence of tax rate reduction on agricultural products within the Czech Republic. The increase in prices for these products is, according to the author, precisely influenced by an increase in the tax rate. He notes that the level of tax burden is doubled in prices of products. In case of a reduction of the tax rate on these products, the author does not record such a significant change. Similar conclusions are provided by **Benkovskis and Fadajeva (2013)**. **Bahl, Bird and Walker (2003)** offer an empirical case study conducted within Ireland, where they examine the impacts of reduced excise tax rate on soft drinks. The authors estimate that 30% of the tax income from these goods is the result of VAT applied. VAT tax rate reduction is not fully captured in lowering prices. When the level of rate falls, the authors do not record any evidence of the Laffer effect. The empirical study on tax incidence **Politi and Mattos (2011)** suggest that in case the ad-volarem tax rate is reduced, the price of goods will be underestimated over a short period of time (up to four months). **Miki (2011)** states that consumers decide according to notifications of change in the VAT level about their consumption. In case of reduced VAT on certain products, it can be assumed that the consumer will postpone the consumption of these products to the future. The range of effect depends on the income and price elasticity of demand. **Hayashi (1985)** states that consumption of households is sensitive to the current level of income of households.

The asymmetry in the effect of level changes in VAT rates is influenced by several facts. Firstly, the increase in rates is reflected in the price of the goods, while their decline does not lead to an adequate drop in prices. This is the result of a weak motivation for enterprises to lower the prices of these goods, as the expected decline would not be caused by pressure of consumers. Secondly, considering the character of the basic foodstuffs and goods, consumers do not consider their price to be decisive and, more importantly, from time perspective they do not consider and compare them in the long term. Recurring patterns of purchases of consumers increase the feeling of security **(Anon, 2009)**.

Consumers respond differently to price cutting than to escalation of prices **(Hardie, Johnson and Fader, 1993; Kalyanaram and Winer, 1995)**. Transfer of tax burden as well as the change in the behaviour of consumers in the context of their total expenditures are affected by the range between standard and reduced VAT rate. **David (2016)** states that the average transfer of tax burden on consumers is by 14% lower with the standard rate than from goods with a reduced rate.

In addition to the factors influenced by the reduced VAT rate, consumption of households is affected by the set of macroeconomic variables and maturity of the economy in each state. These factors need to be sensitively perceived while examining the consumption of household. When considering the implementation of a reduced VAT rate, it is necessary to take into consideration the phase of the political cycle and the phase of the economic cycle **(Miki, 2011)**.

Other important factors are the unemployment rate, other income taxes in the state, the level of disposable income of households, and other consumption indicators **(Alm and El-Ganainy, 2013)**.

Scientific hypothesis

Based on the main objective of the paper, the following hypothesis is formulated: "Applying a reduced VAT rate on foodstuffs affects the change in expenditures of households".

MATERIAL AND METHODOLOGY

According to the hypothesis, the analysis of the paper is divided into two parts.

Governments that reduced VAT rates on foodstuff in order to influence foodstuffs prices must follow all changes in tax rates, both standard and reduced. The partial analysis examines the change in the reduced VAT rate on foodstuffs compared to the change in the total expenditures of households on foodstuffs in relation to GDP and compared to the change in the standard VAT rate. The dataset (*VAT rates, reduced VAT rates on foodstuffs, final consumption expenditure of households on foodstuffs in relation to GDP*) consists of the member

states of the European Union in the period from 2007 to 2017 obtained from **Eurostat (2018)**. MS Office Excel version 2013, from the company Microsoft for the operating system Microsoft Windows and Macintosh (Microsoft Corporation, USA), was used as the statistical analysis software.

Statisic analysis

The second part of the analysis examines whether a reduced VAT rate on foodstuffs, at the chosen level of significance, represents an important determinant of the total expenditures of households on foodstuffs using a panel regression model of fixed effects for states. The monitored variables include *tax rates* in the form of a reduced VAT rate on foodstuffs and a standard VAT rate and *other monitored variables* which examined the influence of VAT and other macroeconomic indicators on household consumption. The regression model is expressed by the following relation.

$$\frac{EXP}{GDP_{i,T}} = \beta_0 + \sum_{k=1}^{l} \beta_k VAT_{k_{i,T}} + \sum_{m=l+1}^{n} \beta_m Cyclical_{m_{i,T}} + \sum_{r=m+1}^{s} \beta_r Other_{r_{i,T}} + \mu_T + \varepsilon_{i,T} \quad (1)$$

where, $\frac{EXP}{HDP\ i,T}$ are the final consumption expenditures of households by consumption of foodstuffs in relation to GDP, in % (*Consumption*), $VAT_{i,T}$ represents a set of tax variables, specifically the standard VAT rate in % (*Standard VAT rate*) and the reduced VAT rate on foodstuffs in % (*Reduced VAT rate*), $Cyclical_{i,T}$ represents a set of cyclical variables that affect final spending of households in the form of unemployment rate in % (*Unemployment)* and inflation rate in % (*Inflation*), $Other_{i,T}$ represent a set of other macroeconomic variables that affect development of final expenditures of households on foodstuffs, in the form of natural logarithm GDP per capita (*Ln(GDP per capita)*), GDP growth rate in the state compared to other EU-28 states in % (*GDP Relative*) and the existence of the crisis recorded in 2008 - 2011 in the form of a binary variable (*Factor (Crisis)*). Furthermore, β represents individual regression coefficients, μ_T are annual effects, $\varepsilon_{i,T}$ represents a random independent error, states are labeled with i index, and the time is labelled with T index.

The dataset consists of the member states of the European Union in the period from 2007 to 2017 obtained from **Eurostat (2018)**. The output variable is examined through the mathematical and statistical methods in software R Studio Version No. 0.99.491 **(RStudio Team, 2015)** by using the packages *tseries*, *plm*, and *aTSA*.

Prior to modelling, the stacionarity of time series data of explanatory variables based on the KPSS test and on the extended DF test over the period of time 2007 - 2017 is tested. The significance of the selected variables entering the models is verified by the Kaiser-Mayer-Olkin criterion. Criteria values below significance level (0.5) are considered as insufficient and therefore are excluded from the models.

RESULTS AND DISCUSSION

VAT belongs to the group of taxes burdening consumption. Average income from excise taxes is at the level of 28.5% of total income tax **(Eurostat, 2018)**. Some member states tend to maintain this income tax at the increased level (BG, HR, LV, EE, RO, HU, and CY above 40%), mainly due to the existence of low below the average pensions or lower tax ethics (BG, EE, HR, and HU).

In the EU-28 states, the standard VAT rate ranges from 17% (LU) to 27% (HU). The average rate is at the level of 21.5% and it seems like its level has not significantly changed in several EU states since 2014. The necessity for unifying VAT rates prefer and emphasise the new member states. The very number and the level of VAT rates themselves are extensive and it is difficult to create their clear comprehensive overview and comparison. Within the member states, average expenditures of households on foodstuffs and non-alcoholic beverages represent 12.2% out of total expenditures of households **(Eurostat, 2018)**. The range of these expenditures varies between states from 8.2% in the UK to 27.8% in RO.

Taxing foodstuffs at reduced rates changes relative prices, leading consumers to buy relatively more foodstuffs and producers, including farmers, to choose food production over other lines of production **(Cnossen, 2017).** By qualitative research of tax legislation, we observe that foodstuffs are taxed in several member states by several rates. The reduced VAT rate for foodstuffs is applied between 2% and 18% (HU) in 2018. In addition to the reduced VAT rate on foodstuffs, we are experiencing super-reduced VAT rates (less than 5%) valid in IE (4.8%), ES (4%), FR (2.1%), IT (4%), and LU (3%). These states most often implement this rate in livestock and live horses - use in the preparation of foodstuffs. The exception is LU, which applies the reduced VAT rate on all foodstuffs. Some states use two tax rates not just one reduced VAT rate on foodstuffs applied in EC, LT, SK, HU, CY, FR, DE, SE, and the UK. Currently, three VAT rates on foodstuffs are applied in PL, BE, and PT. Reduced VAT rates can be divided into three categories according to their scope of application:

1) to *"all foodstuffs"* apply a reduced VAT rate mainly old member states (FI, SE, AT, RO, DE, LU, EL, IT, NL, and SI). In these states, their application is justified by social and production reasons. The reduced tax rate on foodstuffs (the minimum value for the state) reaches an average of 8.75% in 2017 in the cluster. Average final consumption expenditure of households on foodstuffs in relation to GDP reaches level of 6.99% of GDP.

2) to *"selected foodstuffs"* apply a reduced VAT rate mainly a group of new member states (BE, CZ, PL, SK, IE, HR, LV, HU, and PT). While deciding about the determination of foodstuffs, the impact of taxation on the most basic living needs in relation to the development of social conditions in the state is carefully considered. States apply a reduced rate only on selected foodstuffs, otherwise, they use a standard VAT rate on foodstuffs. Examples of special foodstuffs are essential baby smooth

puree in CZ (10%), in IE animals intended for food-producing (4.8%), bread, butter, milk in HR (5%). Application of established rates is justified by the necessity of ensuring a stable budget income in state. The reduced tax rate on foodstuffs (minimum value) reaches an average at the level of 6.31% in 2017. Average final consumption expenditure of households on foodstuffs in relation to GDP reaches level of 7.76% of GDP. Reduction of VAT on selected goods does not bring the promised effect probably due to unsuitable choice of foodstuffs with the reduced tax rate. Nationwide reduction of VAT on foodstuffs would increase consumption and significantly help not only the food producers but also the trade sector. An interesting approach to analyse the effect of introducing second reduced rate of VAT on consumer purchase behaviour with gluten‑free food in the Czech Republic suggest **Šálková, Kučera and Moravec (2017)**. Authors prove that the respondents' experience says, the introduction of the second reduced VAT rate of 10% has not significantly affected the prices for the final consumers. The reason for that may be the fact that the second reduced VAT rate applies mostly to ingredients used in gluten‑free production and only a few final products.

3) DK, BG, EE, and LT do not apply reduced VAT rate to foodstuffs. The average VAT rate on foodstuff in 2017 reaches level of 21.5%. In the same time period, an average final consumption expenditure of households on foodstuffs in relation to GDP reaches level of 9.48% of GDP in these states.

The range of these expenditures varies from 8.2% in the UK to 27.8% in RO. In the observed time period, the change in the reduced VAT rate on foodstuffs, the standard VAT rate combined with the change in the final expenditures on foodstuffs of households in relation to GDP, has four possible situations:

1) No change in the reduced VAT rate on foodstuffs, nor the change in the standard VAT rate, and the final expenditures on foodstuffs of households show fluctuating trend: BE, BG, DK, DE, MT, AT, and SE.

2) No change in the reduced VAT rate on foodstuffs and the increase in the standard VAT rate in the combination with an increase of expenditures on foodstuffs of households: CZ, CY, EE, HU, NL, RO, FI, UK, and PT.

No change in the reduced VAT rate on foodstuffs and an increase in the standard VAT rate in the combination with a decline of expenditures on foodstuffs of households: IE, FR, and IT.

3) Increase of reduced VAT rate on foodstuffs, no change in standard VAT rate in the combination with an increase of expenditures on foodstuffs of households: EL.

4) Increase in both the reduced and standard VAT rate on foodstuffs in the combination with an increase of expenditures on foodstuffs of households: EE, EL, and LV.

Increase in both the reduced and standard VAT rate on foodstuffs in the combination with a decrease of expenditures on foodstuffs of households: CZ, LT, PT, SI.

The time period of implementation of changes in the standard VAT rates respectively changes in the level of reduced VAT rates can be divided into three phases. The most quantitative changes occurred between 2007 and 2010 when states implemented an increase in both tax rates. Since 2014, no significant changes have been observed in the development of rates. A decline in the standard VAT rate was identified only for RO in 2015 when the state dropped it from 24% to 20% and consequently to 19% in 2017. A decline in the rate was accompanied by a decline in total expenditures on foodstuffs of households despite the fact that the level of the reduced VAT rate did not change. In all cases, the overall increase in the reduced VAT rate on foodstuffs is observed. Such a change, whether alone or in conjunction with an increase in the standard VAT rate, simultaneously leads to an increase in expenditures on foodstuffs of households.

Table 1 VAT rates on foodstuffs in the EU member states (in 2018, %).

State	Reduced Rate	Standard Rate
BE	6 | 12	21
BG	20	20
CZ	10 | 15	21
DK		25
DE	7	19
EE		20
IE	4.8 | 9 | 13.5	23
EL	13	24
ES	4 |10	21
FR	2.1 | 5.5 | 10	20
HR	5 | 13	25
IT	4 | 5 | 10	22
CY	5	19
LV	5 | 12	21
LT		21
LU	3	17
HU	5 | 18	27
MT	0 | 5	18
NL	6	21
AT	10	20
PL	5 | 8	23
PT	6 | 13	23
RO	9	19
SI	9.5	22
SK	10	20
FI	14	24
SE	12	25
UK	0	20

Note: Source: Authors' elaboration based on **Eurostat (2018)**.

Table 2 Changes identified in the development of VAT rates in the EU member states (in 2007 - 2018).

	2007 - 2009	2012 - 2014	2015 - 2018
Synchronously Δ SR and Δ RR	EE, LV, LT, CZ, EL, ES, PL HU, HR, PT, RO, SK, FI, UK	ES, SI CZ, IE, FR, IT, CY, HU, NL, FI	
Only Δ SR			EL, LU, RO
Only Δ RR		HR	FI

Note: *SR – Standard VAT rate, RR – Reduced VAT Rate on foodstuffs.* Source: Authors' elaboration based on **Eurostat (2018)**.

The European Parliament **(Næss-Schmidt, Mekonnen Ali and Nieto Arias, 2012)** points out that individual legislative adjustments to VAT taxation should be gradually simplified and should prevent further increases in standard tax rates. Compared to incomes of households, reduced VAT rates on foodstuffs are becoming ineffective and they increase the burden of households in all states.

Bettendorf and Cnossen (2015) and **Cnossen (2017)** calculate the VAT burden distribution. Authors assume, that disposable income is usually taken as the denominator. Total consumer spending net of VAT is a better alternative because it varies less than income over the life cycle of the individual. Therefore, consumption expenditures are a more stable denominator than income is. The panel regression model is compiled from a set of explanatory variables that affect total expenditures on foodstuffs of households in relation to GDP within the EU-28 states. EL is omitted from the model due to the unavailability of the data. Regression is firstly realised by pooling model. Using the Hausman test, a fixed model with effects for states is selected. Based on the results, it can be stated that the variables chosen in the models are statistically significant determinants which can affect the level of final consumption (Table 3).

Models are based on the assumption that the price of the final goods and expenses intended for the purchase of these foodstuffs increases with the increase of taxes. The most important factor in this model has a direct impact on expenditures of households. The positive sign with the regression coefficient β within the *Standard VAT rate* confirms conclusion of the economic theory. With increasing levels of tax rates, growth of consumption is expected. In case of a perfectly competitive market, neoclassical economics assumes that the change in the level of tax rates burdening goods where demand is relatively inelastic is influenced by change in prices that directly determine the behavior of consumers.

The results of *Reduced VAT rate* are in contrast with these assertions. **Široký et al. (2016)** based on the analytic-synthetic methods determine how are the changes between the rates of VAT in reference to the share of commodity consumption in Slovak and Czech Republic. Authors show a different situation in the Slovak and Czech Republic. In Slovakia, the amount of household expenditures on commodities taxed at the reduced VAT rate declined. Authors state that the impact of changes in VAT as a consumption tax is, in principle, differentiated.

Pestel and Sommer (2015) carry out microsimulations of several revenue-neutral policy scenarios. Authors simulate a step-wise increase of the standard VAT rate accompanied by a reduction in personal income taxes or social security contributions. A tax shift might be favourable with respect to employment as a consequence of lower marginal tax rates on labor income, implying higher incentives to take up work. Higher consumption taxes are often associated with lower tax progressivity and higher levels of inequality. However, employment increases from a tax shift may outweigh adverse distributional impacts. Authors demonstrate the negligible redistributive impact of the reduced VAT rate.

Incorporating cyclical factors such as rate of unemployment (*Unemployment*) and inflation rate (*Inflation*), the second model reaffirms that a low level of inflation rate may lead to the generation of additional incomes of households. In case of its growth, the positive value of the regression coefficient β determines an increase in expenditures on foodstuff of households. With the growth of unemployment rate, a decline in expenditures on foodstuffs of households occurs.

Table 3 Selected determinants of the final consumption of households on foodstuffs in relation to GDP.

Final consumption of individuals on foodstuffs in relation to GDP					
Independent variables	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
Standard VAT rate	1.2738e-01			1.0397e-01	0.103778
	(3.571e-06) ***			2.602e-05) ***	(2.423e-05) ***
Reduced VAT rate	-2.7758e-06			-2.8318e-06	
	(0.806)			(0.722712)	
Unemployment		-0.096426		-2.3619e-02	-0.027375
		(<2.2e-16) ***		(0.091776) .	(0.037208) *
Inflation		0.030102		9.6480e-03	
		(0.02298) *		(0.465321)	
Ln (GDP per capita)			-6.519530	-6.8360e+00	-6.902335
			(<2.2e-16) ***	(<2.2e-16) ***	(<2.2e-16) ***
GDP Relative			0.305297	3.3638e-01	0.326305
			(0.05165) .	(0.028170) *	(0.032047) *
Factor (Crisis)			-0.270170	-2.0747e-01	-0.187018
			(4.14e-07) ***	(0.001414) **	(0.001295) **
Number of observations	n = 27, T = 9, N = 243	n = 27, T = 9, N = 243	n = 27, T = 9, N = 243	n = 27, T = 9, N = 243	n = 27, T = 9, N = 243
p-value of F-statistic	2.1078e-05	1.8228e-15	8.5648e-14	<2.22e-16	<2.22e-16
R^2	0.095731	0.2718	0.52377	0.56402	0.56258
Adjusted R^2	-0.022584	0.17652	0.45893	0.49518	0.49831

Note: *The table shows the values of the coefficients for the individual models and the p-values of the t-statistics in brackets. The significance of the model is indicated at the significance level of 0.001 '***', at the significance level of 0.01 '**' at the significance level of 0.05 '*', at the significance level of 0.1'.'.* Source: Authors' elaboration in software RStudio [Version 0.99.491] based on **Eurostat (2018)**.

Within the all EU Member States **Martinková and Bánociová (2016)** identify 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. In case the size of the corporate sector can be expressed by the size of the *Ln (GDP per capita)*, then with the growth of this variable, we expect the growth of the corporate sector that influences the growth of the explained variable. Statistically significant determinant is a relative share of state's GDP in relation to total GDP of the EU-28 states sample in % (*Relative*).

The existence of a crisis (*Factor (Crisis)*) is associated with a decline in GDP growth, with a decline in production, and a decline in consumption of households. The regression coefficient β take the negative value. In case of crisis existence, a decline in expenditures of households on foodstuff is expected.

Summary models represent a connection of all significant determinants affecting the final expenditures on foodstuffs of households. As in previous models, the significance of the *Reduced VAT rate* variable has not been confirmed at the chosen significance level.

If it is unclear how the market will respond to changes in taxation, or more precisely to changes in the level of *Reduced VAT rate*, it is difficult to design an effective tax policy. Knowledge of the consequences of a small reduction of *Reduced VAT rate* can help states rationally decide on tax rates in the future. Based on the main objective of the paper, the assumptions of the established hypothesis were not confirmed. The lack of evidence that the *Reduced VAT rate* on foodstuffs really affects expenditures of households is the nature of using this tax rate subdued. It needs to be pointed out that any change is visible only if it has a certain minimum size **(Niesiobędzka, 2013)**.

The reasons for inadequate reduction of prices, respectively no expression of changes of rates in the development of expenditures of households may be several - there may occur only reductions in VAT rates for foodstuff of a certain type which is not reflected in the consumer basket; the change of the rate can be implemented only for low-priced foodstuffs which is not reflected in the total change in expenditures of households; within the implemented change of the rate may re-qualification of goods which are liable to rate occur. According to **Crossley et al. (2009)**, consumers are aware of a possible increase in the future when deciding about the implementation of reduced VAT rates, so they adapt their current consumption to that.

In a market economy, the government has a limited impact on prices. Sometimes VAT affects prices for consumers in ways that are not in accordance with tax authorities' intentions. Tax reductions are often used to help households' secure basic goods but do not guarantee low prices. Knowledge of the factors that influence range and timing of tax transferring is crucial for application an effective tax policy. If VAT is used to create justice, the government should avoid a temporary increase in tax rates due to necessity. In this case, it is important to use additional income tax revenues from higher tax rates to support households living in poverty than establishing small reductions in tax rates which may not lead to lower prices.

The effect of reduced VAT rates on foodstuffs which should determine the final consumption of foodstuffs and on the behaviour of consumers can also be affected by the fact that in individual time periods establishing, respectively adjusting reduced VAT rates is not the only modification in fiscal policy and tax policy but may be affected by changes in standard VAT rates that have a significant impact on prices of foodstuffs.

CONCLUSION

The importance and the roles of value added tax are constantly increasing, which is supported not only by fair economic competition, achieving the neutrality, uniformity, simplicity, and efficiency of this tax but also by the fact that value-added tax itself represents one of the major sources of state budget revenues. At EU member states level, this tax is harmonized the most, despite that legislation of individual states, allow adjustments in the level of VAT rates. Based on the theoretical assumptions that reducing the level of taxation may lead to an increase in private consumption, EU governments are introducing reduced VAT rates on foodstuffs, trying to keep prices of foodstuffs and basic necessity products at the lowest possible level and with the highest availability for consumers. Changes in the levels of reduced VAT rates for foodstuffs follow different state strategies, either by introducing higher reduced VAT rates on all foodstuffs or by implementing several reduced VAT rates on different types of foodstuffs. Based on the empirical literature under review and the quantitative analysis carried out, the significant determinants of total expenditures on foodstuffs of households and factors that influence the behaviour of consumers are the standard VAT rate, unemployment rate, economic growth in the state, and GDP growth compared to other member states. As a significant determinant is the existence of a crisis period considered. The assumptions of the tested hypothesis that "Applying a reduced VAT rate on foodstuffs affects the change in expenditures of households" at the chosen level of significance have not been confirmed. This fact can be influenced by the fact that in individual periods of time insertion, respectively modification of reduced VAT rates is not the only adjustment in tax policy. In addition to the listed factors influencing the reduced VAT rate, consumption of households is affected by the set of macroeconomic variables and maturity of the economy in each state.

REFERENCES

Alm, J., El-Ganainy, A. 2013. Value-added taxation and consumption. *International Tax and Public Finance*, vol. 20, no. 1, p. 105-128. https://doi.org/10.1007/s10797-012-9217-0

Attanasio, O. P., Weber, G. 2010. Consumption and Saving: Models of Intertemporal Allocation and Their Implications for Public Policy. *Journal of Economic Literature*, vol. 48, no. 3, p. 693-751. https://doi.org/10.2307/20778765

Bahl, R., Bird, R., Walker, M. B. 2003. The Uneasy Case Against Discriminatory Excise Taxation: Soft Drink Taxes in Ireland. *Public Finance Review*, vol. 31, no. 5, p. 510-533. https://doi.org/10.1177/1091142103253753

Benkovskis, K., Fadejeva, L. 2013. *The Effect of VAT Rate on Price Setting Behaviour in Latvia, Evidence from CPI*

Micro Data. Working Paper no. 2013/01. 48 p. Riga : Latvijas Banka.

Bernal, A. 2014. Shifting Value Added Tax on Labor: The Case of the Book Market in Central and Eastern European Countries. *Vadyba*, vol. 25, no. 2, p. 89-95.

Bernal, A. 2018. Do Small Value-Added Tax Rate Decreases on Groceries Imply Lower Prices for Consumers? *Eastern European Economics*, vol. 56, no. 1, p. 81-98. https://doi.org/10.1080/00128775.2017.1412265

Bettendorf, L., Cnossen, S. 2015. The long shadow of the European VAT, exemplified by the Dutch experience. *FinanzArchiv Public Finance Analysis,* vol. 71, no. 1, p. 118-139. https://doi.org/10.1628/001522115X14180276055734

Brown, H. G. 1939. The incidence of general output or a general sales tax. *Journal of Political Economy*, vol. 47, no. 2, p. 254-262.

Cornelsen, L., Green, R., Turner, R., Dangour, A. D., Shankar, B., Mazzocchi, M., Smith, R. D. 2015. What Happens to Patterns of Food Consumption When Food Prices Change? Evidence from a Systematic Review and Meta-Analysis of Food Price Elasticities Globally. *Health Economics*, vol. 24, no. 12, p. 1548-1559. https://doi.org/10.1002/hec.3107

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.

Crossley, T. F., Phillips, D., Wakefield, M. 2009. Value Added Tax. In Chote, R., Emmerson, C., Miles, D., Shaw, J. (Eds.). *The IFS Green Budget 2009*. London : The Institute for Fiscal Studies, p. 194-212. ISBN 978-1-903274-58-3.

David, P. 2012. Distribution of the increased tax burden for agricultural products and food in the Czech Republic. *Zemědělská ekonomika*, vol. 58, no. 5, p. 239-248. https://doi.org/10.17221/58/2011-AGRICECON

David, P. 2016. The effects of increased VAT rates applied in the EU member states in 2014. *Society and Economy*, vol. 38, no. 3, p. 331-340. https://doi.org/10.1556/204.2016.38.3.3

Ebrill, L., Keen, M., Bodin, J.-P., Summers, V. (Eds.) 2001. *The Modern VAT*. Washington DC, USA : International Monetary Fund. 223 p. ISBN 1-58906-026-1.

Eurostat, 2018. Database [online] s.a. [2018-11-11] Available at: https://ec.europa.eu/eurostat.

Fullerton, D., Metcalf, G. E. 2002. Tax Incidence. In: Auerbach, A. J., Feldstein, M. (Eds.) *Handbook of Public Economics*. Amsterdam, Netherland : Elsevier. p. 1787-1872. ISBN 978-0-444-82315-1.

Hardie, B. G., Johnson, S. J., Fader, P. S. 1993. Modeling Loss Aversion and Reference Dependence Effects on Brand Choice. *Marketing Science*, vol. 12, no. 4, p. 378-394. https://doi.org/10.1287/mksc.12.4.378

Hayashi, F. 1985. The permanent income hypotheses and consumption durability: analysis based on Japanese panel data. *Quarterly Journal of Economics*, vol. 100, no. 4, p. 1083-1113. https://doi.org/10.2307/1885676

Kalyanaram, G., Winer, R. S. 1995. Empirical Generalizations from Reference Price Research. *Marketing Science*, vol. 14, no. 3, p. 161-170.

Martinková, S., Bánociová, A. 2016. The Economic Impact of Reduced Value Added Tax Rates for Groceries. *Potravinarstvo*, vol. 10, no. 1, p. 656-662. https://doi.org/10.5219/679

Miki, B. 2011. The effect of the VAT rate change on aggregate consumption and economic growth. *Columbia University Academic Commons*. 31 p. https://doi.org/doi.org/10.7916/D8862QBH

Mirrlees, J., Adam, S., Besley, T., Blundell, R., Bond, S., Chote, R., Gammie, M., Johnson, P., Myles, G., Poterba, J. (eds.), 2010. *Dimensions of Tax Design – The Mirrlees Review*. Oxford, UK : Oxford University Press. 1397 p. ISBN 978-0-19-955375-4.

Næss-Schmidt, H. S., Mekonnen Ali, D., Nieto Arias, M. 2012. *Simplifying and modernising VAT in the digital single market for e-Commerce*. Brussels : European Union. 96 p. Available at: http://www.europarl.europa.eu/document/activities/cont/201209/20120924ATT52130/20120924ATT52130EN.pdf.

Niesiobędzka, M. 2013. *Dlaczego nie płacimy podatków? Psychologiczna analiza uchylania się od opodatkowania*. Warsaw, Poland : Wydawnictwo Naukowe Scholar. 183 p. ISBN 978-83-7383-658-7.

Pestel, N., Sommer, E. 2017. Shifting Taxes from Labor to Consumption: More Employment and More Inequality? *Review of Income and Wealth*, vol. 63, no. 3, p. 542-563.

Politi, R. B., Mattos, E. 2011. Ad-valorem tax incidence and after-tax price adjustments: evidence from Brazilian basic basket food. *Canadian Journal of Economics*, vol. 44, no. 4, p. 1438-1470. https://doi.org/10.1111/j.1540-5982.2011.01680.x

RStudio Team. 2015. RStudio: Integrated Development for R (Version 0.99.491) [Computer Software. Boston, MA : RStudio, Inc. Available at: http://www.rstudio.com.

Simon, H., Fassnacht, M. 2016. *Preismanagement: Strategie-Analyse–Entscheidung-Umsetzung*. Wiesbaden, Germany : Springer Gabler. 616 p. ISBN 978-3-658-11871-6. https://doi.org/10.1007/978-3-658-11871-6

Šálková, D., Kučera, P., Moravec, L. 2017. Effect of Introducing Second Reduced Rate of VAT on Consumer Purchase Behaviour with Gluten‑Free Food. *Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis*, vol. 65, no. 3, 2017, p. 1045-1053. https://doi.org/10.11118/actaun201765031045

Široký, J., Střílková, R., Bánociová, A., Zlaczká, V. 2014. Reflection of the Change in VAT Rates on Selected Household Expenditures in the Czech Republic and the Slovak Republic (2007 - 2013). *Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis*, vol. 62, no. 6, p. 1465-1474. https://doi.org/10.11118/actaun201462061465

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VARIATION IN MORPHOMETRIC TRAITS OF FRUITS OF *MESPILUS GERMANICA* L.

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ABSTRACT

According to the International Program for the Botanic Gardens Conservation, botanic gardens contribute to the conservation plant species, which are of great socio-economic importance, and develop and implement a policy to use herbal products derived from sustainably developing sources. The aim of this study was to determine morphometric parameters of fruits of seven genotypes of *Mespilus germanica* L., which are growing in the Forest-Steppe of Ukraine in M. M. Gryshko National Botanical Garden of NAS of Ukraine (Kyiv). Their morphometric parameters were following: fruit weight from 35.29 to 39.12 g, fruit length from 27.69 to 42.29 mm, fruit diameter from 24.98 to 44.75 mm, length of calyx basin from 17.55 to 32.46 mm. The shape index of the fruits was found in the range of 0.79 to 1.23. It was found that fruit diameter positively correlated with traits such as fruit weight and calyx basin length. Fruit weight was also highly correlated with calyx basin length and fruit length. Using the cluster analysis with Euclidian distances allowed to establish the relationships among the fruits *Mespilus germanica* germplasm and arranged the genotypes into five relatively homogenous clusters. Hence, the introduction population characterized by quite variability and contains plants with almost all types of fruits. Obtained data can be used for breeding programs and introducing of cultivars in *Mespilus germanica*.

Keywords: *Mespilus germanica*; fruits; genotypes; morphometric characteristics; variability; Forest-Steppe of Ukraine

INTRODUCTION

Recently, the increasing importance attached to the consumption of healthy food. Attention is attracted not by traditional fruit crops, but by wild plants with smaller fruits, which, however, contain natural vitamins and antioxidants: cornelian cherry, mountain ash, sea buckthorn, rose hip, service tree, elderberry, bilberry, mulberry, jujube. The *Mespilus germanica* L. (medlar) is also included in this group of popular fruit trees. This species belonging to the family Rosaceae Juss. was introduced to Greece around 700 BC, and to Rome about 200 BC **(Baird and Thieret, 1989)**. Until the seventeenth century, the medlar was the most important fruit crop. However, interest in it gradually faded away, and later it was replaced by other, more productive and undemanding crops. Currently, medlar is grown quite rarely and, mainly, in botanical gardens or in small farms. *Mespilus germanica* is indigenous to southwest Asia and possibly also southeastern Europe – from northern Turkey (some occurrence in Greece and on the Crimea) to the Caucasus and Transcaucasus and the north-eastern part of Iran **(Lim, 2012)**.

Studies found ripe *Mespilus germanica* fruit to be rich in potassium (740-841 mg.100 g^{-1}), calcium (67-80 mg.100 g^{-1}), phosphorus (30-48 mg.100 g^{-1}), magnesium (50-62 mg.100 g^{-1}) **(Ercisli et al., 2012)**. Medlar fruit was found to have dry matter content 27.0%, protein 11.4%, fibre 3.71%, energy 16.5 kcal.g^{-1}, and ash 1.96%, acidity 0.28%, pH 4.26 **(Haciseferogullari et al. 2005)**, fructose 2230 mg.g^{-1}, glucose 845.2 mg.g^{-1}, sucrose 228.4 mg.g^{-1} **(Glew et al., 2003b)**, high content of amino acids **(Glew et al., 2003a)**, fatty acids **(Ayaz et al., 2002a; Ayaz et al., 2002b)**, volatile components **(Pourmortazavia and Ghadirib, 2005)**, polyphenols and antioxidants **(Gruz et al., 2011; Gülçin et al., 2011; Nabavi et al., 2011; Rop et al., 2011)**. The *Mespilus germanica* fruit used as a treatment for constipation, as a diuretic, and to rid the kidney and bladder of stones **(Haciseferogullari et al., 2005; Glew et al., 2003a)**.

Morphological characterization continues to be the first step for germplasm description and classification **(Badenes et al., 2000; Nazari et al., 2012)**. The existence of a large variability in fruits has been demonstrated in different species such as *Prunus persica* (L.) Batsch **(Scorza, 1984)**, *Cornus mas* L. **(Brindza et al., 2007)**, *Prunus* spp. **(Zhang et al., 2008; Perez-Sanchez et al., 2008; Nazari et al., 2012)**, *Malus* sp. **(Mratinić and Akšić, 2012)**, *Diospyros* spp. **(Grygorieva et al., 2011)**, *Pseudocydonia sinensis* Schneid. **(Monka et al., 2014)**, *Vitis vinifera* L. **(Lamine et al., 2014)**, *Ziziphus*

jujuba Mill. (**Grygorieva et al., 2014; Ivanišová et al., 2017**), *Castanea sativa* Mill. **(Poljak et al., 2016; Grygorieva et al., 2017b)**. For the *Mespilus germanica* breeders or plant biologists, the description of the fruit morphology is of significant importance for phenomics studies.

Scientific hypothesis

In our experiment, we have been supporting that fruit phenotyping variability of evaluated genotypes collection *Mespilus germanica* not predominate only cultivation conditions but also genetical features.

MATERIAL AND METHODOLOGY

Locating trees and data collection

The objects of the research were 10-year-old plants of *Mespilus germanica* from seed origin, which are growing in Forest-Steppe of Ukraine in M. M. Gryshko National Botanical Garden of NAS of Ukraine (NBG). The study was conducted 2018 year. We have described 7 genotypes of *Mespilus germanica*. Fruits were harvested at commercial maturity stage (skin brownish, pulp white, fruit hard).

Morphometric characteristics

30 fruits from each genotype were used immediately after harvest for phenotypic measurements such as fruit weight, (FW), in g, fruit length (FL), in mm, fruit diameter (FD), in mm, calyx basin length (CL), in mm. Fruit mass was measured by using a digital balance with a sensitivity of 0.01 g (PS6000/C/1). Linear dimensions of fruits as length and diameter were measured by using a digital calliper gauge with a sensitivity of 0.01 mm than shape index was calculated by using length/width ratio.

The measurements were made in each nut element as shown in Figure 1.

Statistical analyses

Basic statistical analyses were performed using PAST 2.17; hierarchical cluster analyses of similarity between phenotypes were computed on the basis of the Bray-Curtis similarity index; multi-dimensional scaling (MDS) analyses were performed in PRIMER **(Clarke and Gorley, 2006)**. Variability of all these parameters was evaluated using descriptive statistics. Level of variability determined by **Stehlíková (1998)**.

RESULTS AND DISCUSSION

In this study was selected certain fruit parameters, which can be interesting for commercial use.

The fruit weight, fruit length, fruit diameter, calyx basin length, and shape index of fruits in seven *Mespilus germanica* genotypes are shown in Tab. 1.

The data in the Table indicate the high variability of fruits, especially their mass. Thus, some genotypes can be used further in the selection process. The images of *Mespilus germanica* fruits of various genotypes are shown in Figure 2 and 3.

The highest fruit weight was обтаинед in genotype MG-05 as 35.29 g, and followed by MG-04 as 39.12 g (Figure 4).

The fruit weight was determined in the range from 2.59 g by **Nezhadghan and Hassanpou (2018)** to 40.80 g by **Bostan and Islam (2007)**. Our fruit weight results are in accordance within the range of the values reported literature (Tab. 2).

Table 1 The variability of some morphometric parameters of fruits for the collection of *Mespilus germanica* L. genotypes.

Characteristics	Unit	*n*	*min*	*max*	*mean*	*V%*
Fruit weight	**g**	210	5.70	54.20	24.14	47.56
Fruit length	**mm**	210	23.54	46.33	34.30	15.46
Fruit diameter	**mm**	210	20.26	51.17	35.11	21.02
Calyx basin length	**mm**	210	12.13	42.19	21.69	31.40
Shape Index		210	0.65	1.67	1.00	18.09

Note: n – number of measurements; min, max – minimal and maximal measured values; mean – arithmetic mean; V – coefficient of variation (%).

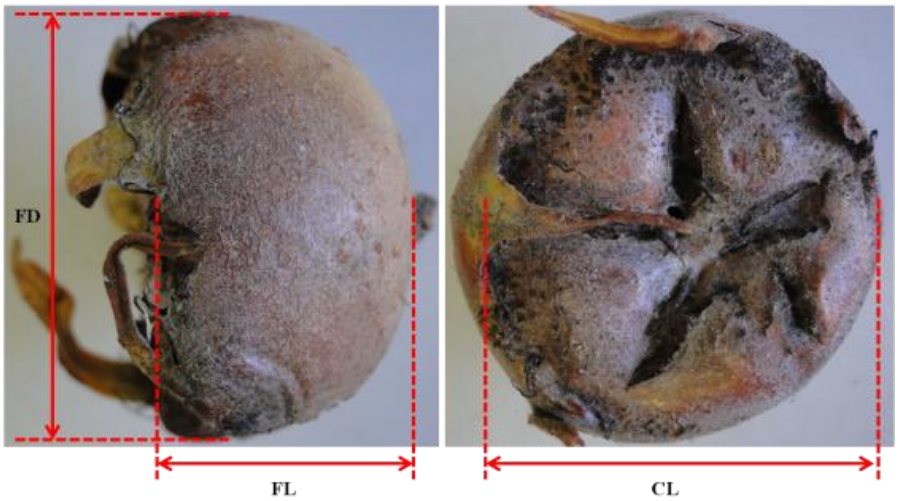

Figure 1 Variability in the shape of genotypes *Mespilus germanica* L. fruits.

Table 2 Variability of some morphometric characteristics on *Mespilus germanica* L. fruits according to the authors from different countries.

Authors	Weight (g)	Length (mm)	Diameter (mm)	Calyx basin length (mm)	Shape Index
Ozkan et al. (1997)	16.51 – 32.98	–*	–*	14.93 – 27.71	–*
Bostan and Islam (2007)	9.46 – 40.80	–*	–*	13.54 – 31.84	–*
Ercisli et al. (2012)	11.21 – 33.24	27.45 – 38.88	28.44 – 42.51	13.92 – 26.48	0.81 – 1.09
Aygun and Tasci (2013)	6.32 – 36.42	21.80 – 40.10	20.60 – 42.70	8.30 – 23.30	–*
Miko and Gažo (2014)	6.36 – 40.53	23.02 – 42.83	27.20 – 42.83	13.46 – 33.80	0.73 – 1.12
Akbulut et al. (2016)	12.30 – 23.60	–*	–*	13.80 – 22.10	0.87 – 1.04
Sulusoglu and Unver (2016)	9.69 – 24.45	21.00 – 33.30	21.20 – 33.60	13.20 – 17.60	–*
Nezhadghan and Hassanpou (2018)	2.59 – 10.95	18.50 – 29.00	16.00 – 28.20	–*	0.78 – 1.51
Yilmaz et al. (2016)	15.99 – 37.54	14.96 – 38.27	17.49 – 43.63	–*	–*

Figure 2 Variability in the shape of genotypes *Mespilus germanica* L. fruits.

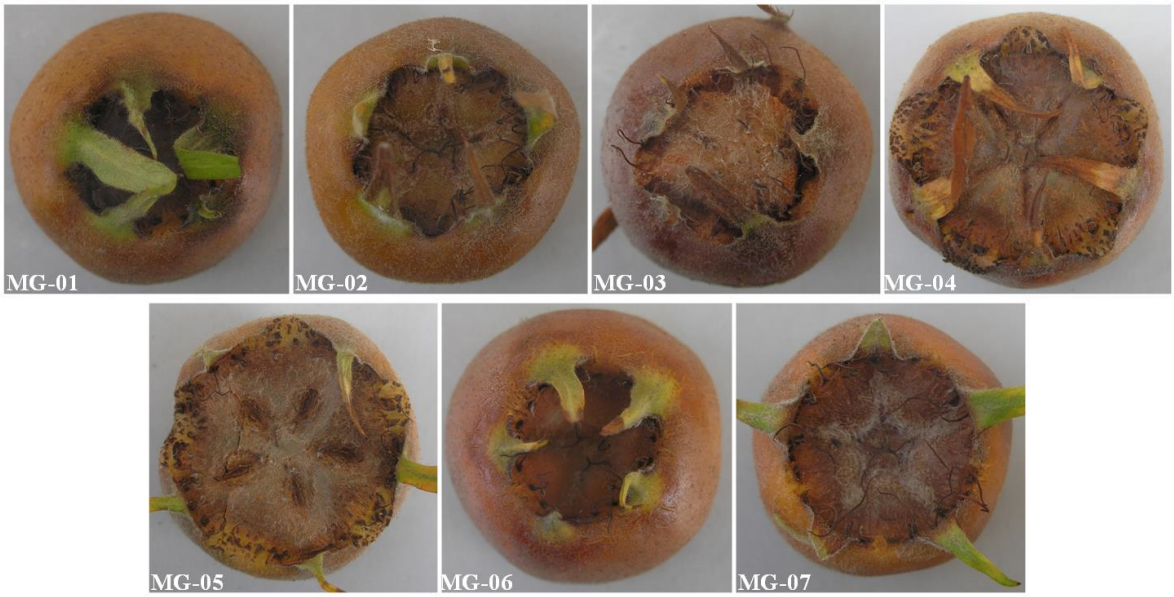

Figure 3 Variability in the shape of genotypes *Mespilus germanica* L. fruits.

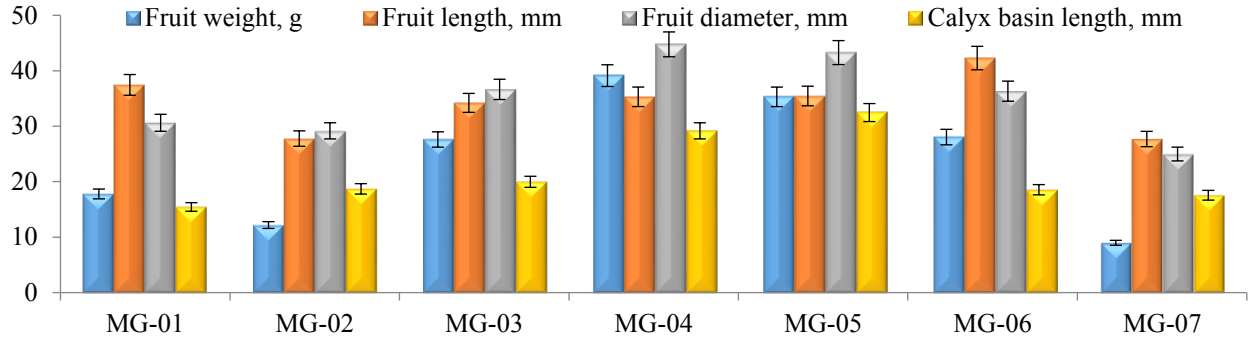

Figure 4 Mean values for various morphometric parameters of fruits and seeds of *Mespilus germanica* L. genotypes.

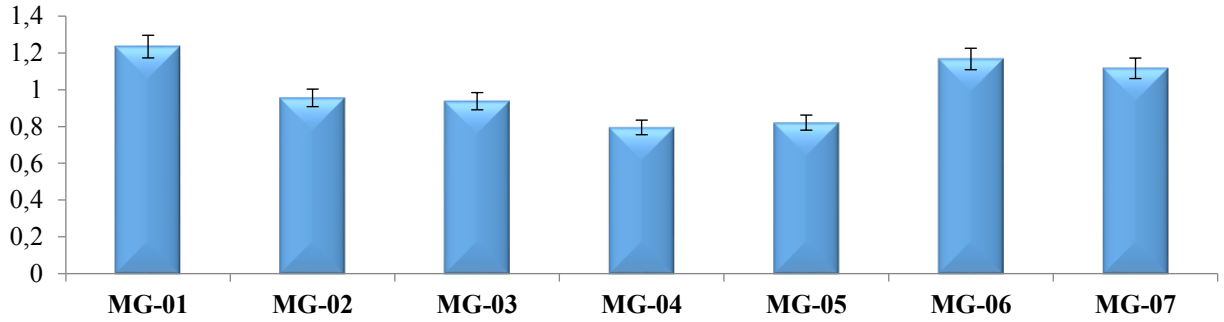

Figure 5 Comparison of the tested *Mespilus germanica* L. genotypes in the shape index of fruit.

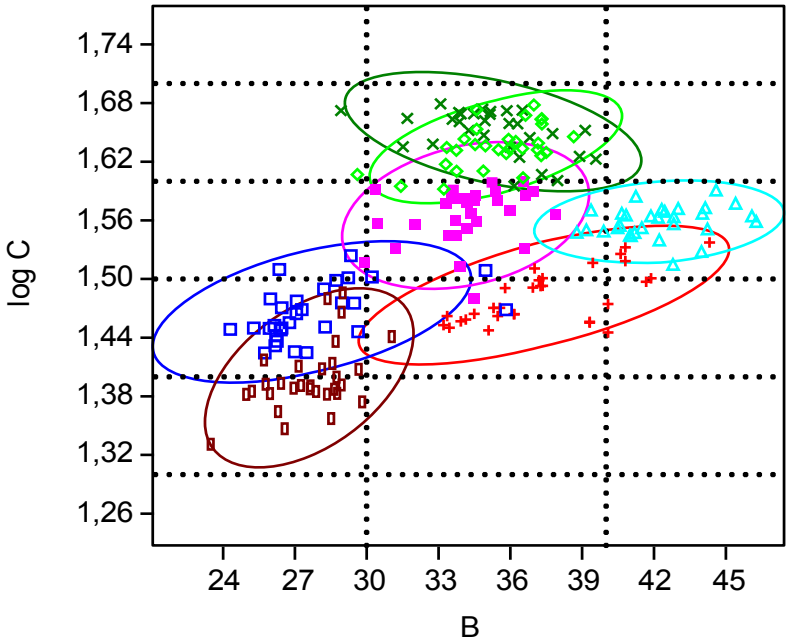

Figure 6 MDS plot of the similarity illustrating the length (C) and diameter (B) of fruits for studying genotypes of *Mespilus germanica* L.

The highest values in terms of fruit lengths were found in the genotype MG-06 (42.29 mm). The lowest values were determined in the genotype MG-07 (27.69 mm) and MG-02 (27.77 mm). In previous studies, it was indicated that the fruit lengths ranged from 14.96 to 40.10 mm in the genotypes selected **(Ercisli et al., 2012; Aygun and Tasci, 2013; Akbulut et al., 2016; Nezhadghan and Hassanpou, 2018; Sulusoglu and Unver, 2016; Yilmaz et al., 2016)**.

The fruit diameters of analyzed genotypes ranged from 24.98 (MG-07) to 44.75 (MG-04) mm. In the literature, it was stated that the fruit diameters of identified genotypes varied from 16.00 to 42.51 mm **(Ercisli et al., 2012; Aygun and Tasci, 2013; Sulusoglu and Unver, 2016; Yilmaz et al., 2016; Nezhadghan and Hassanpou, 2018).**

The length of calyx basin increased with the coarsening of fruit. For the length of the calyx, the basin was found between 17.55 (MG-07) and 32.46 (MG-05) mm. Studies in the literature carried showed that the values of length of

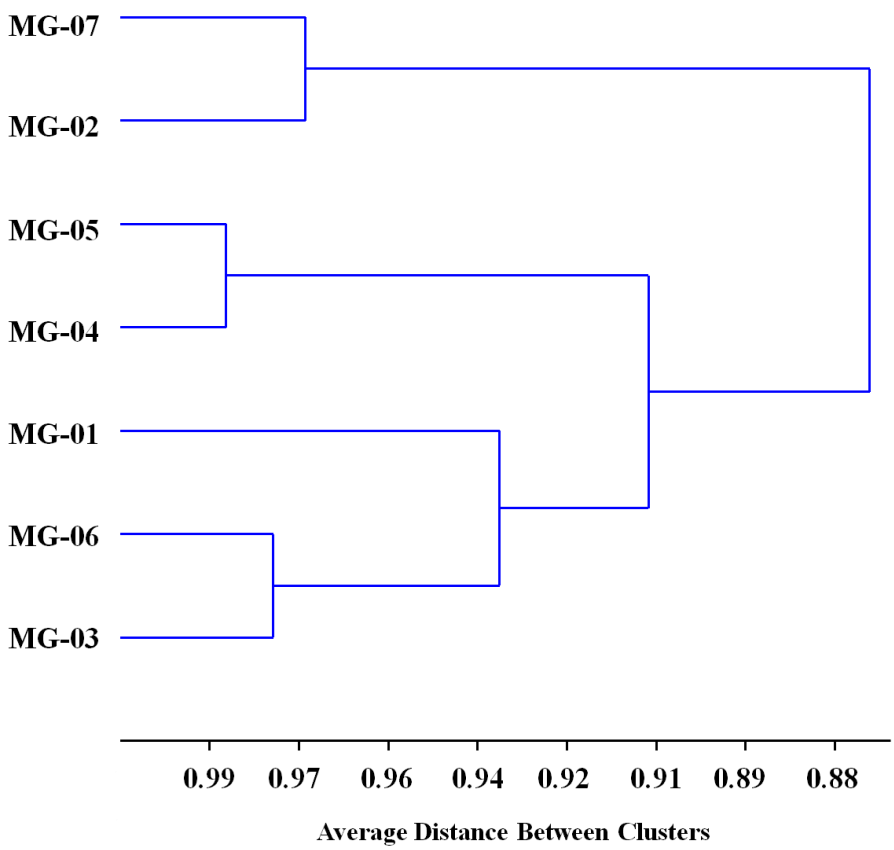

Figure 7 Dendrogram of 7 genotypes of *Mespilus germanica* L. based on morphometric characteristics of fruits.

calyx basin ranged from 8.30 g **(Aygun and Tasci, 2013)** to 33.80 g **(Miko and Gažo, 2014)**.

Shape index was found between 0.79 (MG-04) and 1.23 (MG-01) (Figure 5). According to shape index results, the genotypes MG-01, MG-06 and MG-07 have pearshaped and the others are apple-shaped fruit form. **Ercisli et al. (2012)** reported a shape index between 0.81 and 1.09. **Miko and Gažo (2014)** identified the shape index from 0.73 to 1.12. **Akbulut et al. (2016)** found a shape index between 0.87 and 1.04. In addition, **Nezhadghan and Hassanpou (2018)** identified the shape index between 0.78 and 1.51. Our result on shape index could be comparable with those studies.

The highest coefficient variability (Table 1) identified was for fruit weight (47.56%), followed by calyx basin length (31.40%).

It was found that fruit diameter positively correlated with traits such as fruit weight (r = 0.98) and calyx basin length (0.85). Fruit weight was also highly correlated with calyx basin length (0.79) and fruit length (0.60).

Multidimensional scaling (MDS) is to detect meaningful underlying dimensions that allow the researcher to explain observed similarities or dissimilarities (distances) between the investigated objects. Results of multidimensional scaling are shown in Figure 6.

In Figure, it is possible to see the visual distribution the size of the fruits of the studied genotypes. The genotypes MG-04 (green ellipse) and MG-05 (light green ellipse) with the largest fruit size and the genotypes MG-01 (red ellipse), MG-02 (blue ellipse) and MG-07 (brown ellipse) with the smallest fruit size differ each another with the probability of 95% (the ellipses in the figure do not overlap).

We applied cluster analysis to evaluate the differences in the morphological features of the *Mespilus germanica* fruits. This technique has been carried out earlier for studying the genetic variability of some other plant species **(Rakonjac et al., 2014; Blazakis et al., 2017; Grygorieva et al., 2017a; Metougui et al., 2017; Vinogradova et al., 2017)**.

Cluster analysis allowed the assessment of similarity or dissimilarity and clarified some of the relationships among wild cherry accessions.

Based on the cluster analysis of all 7 studied fruit's characteristics, a dendrogram for the genotypes of *Mespilus germanica* was made (Figure 7). On the dendrogram, you can see that the samples Mg-04 and MG-05 is really separated from the other samples. They differ from other genotypes in all studied parameters.

CONCLUSION

The introduction population of *Mespilus germanica* at M. M. Gryshko National Botanical Garden of NAS of Ukraine is quite variable and contains plants with almost all types of fruits. Evaluating of 7 genotypes of *Mespilus germanica* determined the weight of the fruits in the range from 39.12 to 35.29 g, fruit length from 27.69 to 42.29 mm, fruit diameter from 24.98 to 44.75 mm, length of calyx basin from 17.55 to 32.46 mm. The shape index of the fruits was found in the range of 0.79 to 1.23. This study carried revealed that the genotypes MG-04 and MG-05 was promising in terms of the characteristics evaluated

in variety development. The presence of a broadly variable population provides the conservation *Mespilus germanica*, which is of potentially great socio-economic importance, and will help in the future to use herbal products derived from sustainably developing sources.

REFERENCES

Akbulut, M., Ercisli, S., Jurikova, T., Mlcek, J., Gozlekci, S. 2016. Phenotypic and bioactive diversity on medlar fruits (*Mespilus germanica* L.). *Erwerbs-Obstbau*, vol. 3, p. 185-191. https://doi.org/10.1007/s10341-016-0272-z

Ayaz, F. A., Glew, R. H., Huang, H. S., Chuang, L. T., VanderJagt, D. J., Strnad, M. 2002a. Evolution of fatty acids in medlar (*Mespilus germanica* L.) mesocarp at different stages of ripening. *Grasas y Aceites*, vol 53, no. 3, p. 352-356. https://doi.org/10.3989/gya.2002.v53.i3.328

Ayaz, F. A., Huang, H. S., Chuang, L. T., Vanderjagt, D. J., Glew, R. H. 2002b. Fatty acid composition of medlar (*Mespilus germanica* L.) fruit at different stages of development. *Italian Journal of Food Science*, vol. 14, no. 4, p. 439-446.

Aygun, A., Tasci, A. R. 2013. Some fruit characteristics of medlar (*Mespilus germanica* L.) genotypes grown in Ordu, Turkey. *Sci. Pap. Ser. B. Hortic.*, vol. 57, p. 149-152.

Badenes, M. L., Martinez-Calvo, J., Llacer, G. 2000. Analysis of a germplasm collection of loquat (*Eriobotrya japonica* Lindl.). *Euphytica*, vol. 114, no. 3, p. 187-194. https://doi.org/10.1023/A:1003950215426

Baird, J., Thieret, J. 1989. The Medlar (*Mespilus germanica*, Rosaceae) from antiquity to obscurity. *Econ. Bot.*, vol. 43, no. 4, p. 328-372. https://doi.org/10.1007/BF02858732

Blazakis, K. N., Kosma, M., Kostelenos, G., Baldoni, L., Bufacchi, M., Kalaitzis, P. 2017. Description of olive morphological parameters by using open access software. *Plant Methods*, vol. 13, no. 111, p. 1-15. https://doi.org/10.1186/s13007-017-0261-8

Bostan, S. Z., Islam, A. 2007. A research on breeding by selection of medlar (*Mespilus germanica* L.) types in Eastern Black sea region of Turkey. *Proceedings of 5th National Horticultural Congress*, p. 330-337.

Brindza, P., Brindza, J., Tóth, D., Klimenko, S. V., Grigorieva, O. 2007. Slovakian cornelian cherry (*Cornus mas* L.) : potential for cultivation. *Acta Hortic.*, vol. 760, p. 433-437. https://doi.org/10.17660/ActaHortic.2007.760.59

Clarke, K., Gorley, R. 2006. PRIMER v6 : User Manual/Tutorial. Plymouth, UK : Primer-E, Ltd. 192 p.

Ercisli, S., Sengul, M., Yildiz, H., Sener, D., Duralija, B., Voca, S., Purgar, D. D. 2012. Phytochemical and antioxidant characteristics of medlar fruits (*Mespilus germanica* L.). *Journal of Applied Botany and Food Quality*, vol. 85, no. 1, p. 86-90.

Glew, R. H., Ayaz, F. A., Sanz, C., VanderJagt, D. J., Huang, H. S., Chuang, L. T., Strnad, M. 2003a. Effect of postharvest period on sugars, organic acids and fatty acids composition in commercially sold medlar (*Mespilus germanica* "Dutch") fruit. *Eur Food Res Technol.*, vol. 216, p. 390-394. https://doi.org/10.1007/s00217-002-0654-3

Glew, R. H., Ayaz, F. A., Sanz, C., VanderJagt, D. J., Huang, H. S., Chuang, L. T., Strnad, M. 2003b. Changes in sugars, organic acids and amino acids in medlar (*Mespilus germanica* L.) during fruit development and maturation. *Food Chemistry*, vol. 83, p. 363-369. https://doi.org/10.1016/S0308-8146(03)00097-9

Gruz, J., Ayaz, F. A., Torun, H., Strnad, M. 2011. Phenolic acid content and radical scavenging activity of extracts from medlar (*Mespilus germanica* L.) fruit at different stages of ripening. *Food Chem.*, vol. 124, no. 1, p. 271-277. https://doi.org/10.1016/j.foodchem.2010.06.030

Grygorieva, O., Abrahamová, V., Karnatovská, M., Bleha, R., Brindza, J. 2014. Morphological characteristic of fruit, drupes and seeds genotypes of *Ziziphus jujuba* Mill. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 8, no. 1, p. 306-314. https://doi.org/10.5219/414

Grygorieva, O., Brindza, J., Vietoris, V., Kucelová, L., Tóth, D., Abrahamova, V., Hricová, M. 2011. Morphological and organoleptic fruit properties of various persimmon species (*Diospyros* spp.). *Potravinarstvo Slovak Journal of Food Sciences*, vol. 5, no. 3, p. 11-19. https://doi.org/10.5219/150

Grygorieva, O., Klymenko, S., Brindza, J., Schubertová, Z., Nikolaieva, N., Šimková, J. 2017a. Morphometric characteristics of sweet chestnut (*Castanea sativa* Mill.) fruits. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 288-295. https://doi.org/10.5219/684

Grygorieva, O., Klymenko, S., Vergun, O., Hudz, N., Nikolaieva, N., Schubertová, Z., Palamarchuk, O., Brindza, J. 2017b. Morphological characteristics and determination of volatile organic compounds of *Diospyros virginiana* L. genotypes fruits. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 612-622. https://doi.org/10.5219/808

Gülçin, L., Topal, F., S. Sarıkaya, B. Ö., Bursal, E., Bilsel, G., Gören, A. C. 2011. Polyphenol contents and antioxidant properties of medlar (*Mespilus germanica* L.). *Rec. Nat. Prod.*, vol. 5, no. 3, p. 158-175.

Haciseferogullari, H., Ozcan, M., Sonmete, M. H., Ozbek, O. 2005. Some physical and chemical parameters of wild medlar (*Mespilus germanica* L.) fruit grown in Turkey. *Journal of Food Engineering*, vol. 69, p. 1-7. https://doi.org/10.1016/j.jfoodeng.2004.07.004

Ivanišová, E., Grygorieva, O., Abrahamová, V., Schubertova, Z., Terentjeva, M., Brindza, J. 2017. Characterization of morphological parameters and biological activity of jujube fruit (*Ziziphus jujuba* Mill.). *Journal of Berry Research*, vol. 7, no. 4, p. 249-260. https://doi.org/10.3233/JBR-170162

Lamine, M., Zemni, H., Ziadi, S., Chabaane, A., Melki, I., Mejri, S., Zoghlami, N. 2014. Multivariate analysis and clustering reveal high morphological diversity in Tunisian autochthonous grapes (*Vitis vinifera*) : insights into characterization, conservation and commercialization. *Journal international des sciences de la vigne et du vin Received*, vol 48, no. 2, p. 111-122. https://doi.org/10.20870/oeno-one.2014.48.2.1565

Lim, T. K. 2012. *Mespilus germanica*. *Edible Medicinal And Non-Medicinal Plants*. Dordrecht, Netherland, Springer. https://doi.org/10.1007/978-94-007-4053-2_50

Metougui, M. L., Mokhtari, M., Maughan, P. J., Jellen, E. N., Benlhabib, O. 2017. Morphological variability, heritability and correlation studies within an argan tree population (*Argania spinosa* (L.) Skeels) preserved in situ. International *Journal of Agriculture and Forestry*, vol. 7, no. 2, p. 42-51. https://doi.org/10.5923/j.ijaf.20170702.02

Miko, M., Gažo, J. 2014. *Biologické vlastnosti a hodnotenie genofondu mišpule obyčajnej (Mespilus germanica L.) Biological properties and evaluation of the genus of the moss (Mespilus germanica L.)*. 1st ed. Nitra, Slovakia : Slovak University of Agriculture, 97 p. ISBN 978-80-552-1261-6. (In Slovak)

Monka, A., Grygorieva, O., Chlebo, P., Brindza, J. 2014. Morphological and antioxidant characteristics of quince

(*Cydonia oblonga* Mill.) and chinese quince fruit (*Pseudocydonia sinensis* Schneid). *Potravinarstvo Slovak Journal of Food Sciences*, vol. 8, no. 1, p. 333-340. https://doi.org/10.5219/415

Mratinić, E., Akšić, M. F. 2012. Phenotypic diversity of apple (*Malus* sp.) germplasm in South Serbia. *Braz. Arch. Biol. Technol*., vol. 55, no. 3, p. 49-358. https://doi.org/10.1590/S1516-89132012000300004

Nabavi, S. F., Nabavi, S. M., Ebrahimzadeh, M. A., Asgarirad, H. 2011. The antioxidant activity of wild medlar (*Mespilus germanica* L.) fruit, stem bark and leaf. *Afr. J. Biotechnol.*, vol. 10, no. 2, p. 283-289.

Nazari, S. A., Zamani, Z., Fatahi, M. R., Sofla, H. S. 2012. Morphological characterization of *Prunus incana* Pall. by multivariate analysis. *Plant Syst. Evol.*, vol. 298, p. 1805-1814. https://doi.org/10.1007/s00606-012-0681-x

Nezhadghan, B., Hassanpou, H. 2018. Investigation of physicochemical characterizations of some Medlar (*Mespilus germanica* L.) genotypes in East Azerbaijan province. *Iranian Journal of Horticultural Science*, vol. 49, no. 1, p. 157-169. https://doi.org/10.22059/ijhs.2017.214449.1069

Ozkan, Y., Gercekcioglu, R., Polat, M. 1997. A study on the determination of fruit properties of medlar types (*Mespilus germanica* L.) from Tokat region. *Proceedings of National Pome Fruit Symposium*, p. 123-129. (In Turkish)

Perez-Sanchez, R., Gomez-Sanchez, M. A., Morales-Corts, R. 2008. Agromorphological characterization of traditional Spanish sweet cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L.) and duke cherry (*Prunus gondouinii* Rehd.) cultivars. *Span. J. Agric. Res.*, vol. 6, no. 1, p. 42-55. https://doi.org/10.5424/sjar/2008061-293

Poljak, I., Vahčić, N., Gačić, M., Idžojtić, M. 2016. Morphological characterization and chemical composition of fruits of the traditional croatian chestnut variety 'Lovran Marron'. *Food Technol Biotechnol*., vol. 54, no. 2, p. 189-199. https://doi.org/10.17113/ftb.54.02.16.4319

Pourmortazavia, S. M., Ghadirib, M., Hajimirsadegh, S. S. 2005. Supercritical fluid extraction of volatile components from *Bunium persicum* Boiss. (Black cumin) and *Mespilus germanica* L. (medlar) seeds. *J. Food Comp. Anal.*, vol. 18, no. 5, p. 439-446. https://doi.org/10.1016/j.jfca.2004.01.003

Rakonjac, V., Mratinic, E., Jovkovic, R., Akšic, M. F. 2014. Analysis of morphological variability in wild cherry (*Prunus avium* L.) genetic resources from central Serbia. *J. Agr. Sci. Tech.*, vol. 16, p. 151-162.

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. https://doi.org/10.3390/molecules16010074

Scorza, R. 1984. Characterization of four distinct peach tree growth types. *J. Am. Soc. Hort. Sci.,* vol. 109, p. 455-457.

Stehlíková, B. 1998. *Basics of biostatiscics (Biodiversity protection 51)*. Nitra, Slovakia : Slovak University of Agriculture in Nitra, 79 p. ISBN 80-7137-539-X.

Sulusoglu, M., Unver, H. 2016. Morphological and chemical properties of medlar (*Mespilus germanica* L.) fruits and changes in quality during ripening. *AGROFOR International Journal*, vol. 1, no. 2, p. 133-140. https://doi.org/10.7251/AGRENG1602133S

Vinogradova, Yu., Grygorieva, O., Vergun, O., Brindza, J. 2017. Morphological characteristics for fruits of *Aronia mitschurinii* A. K. Skvortsov & Maitul. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 754-760. https://doi.org/10.5219/845

Yilmaz, A., Gerçekcioğlu, R., Özatasever, Ö. 2016. Determination of pomological and chemical properties of some medlar (*Mespilus germanica* L.) genotypes. *Journal of New Results in Science*, vol. 5, no. 11, p. 118-124.

Zhang, Q., Yan, G., Dai, H., Zhang, X., Li, C., Zhang, Z. 2008. Characterization of tomentosa cherry (*Prunus tomentosa* Thunb.) genotypes using SSR markers and morphological traits. *Sci Hortic.*, vol. 118, no. 1, p. 39-47. https://doi.org/10.1016/j.scienta.2008.05.022

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CLUSTER ANALYSIS OF BEEF PRODUCTION DISTRIBUTION IN EUROPE

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ABSTRACT

Fragmentation and poor connection within the beef production industry affects its positive contribution to the economy, land management, and development of rural areas. Despite the third place in world beef production European countries have achieved one of the best results in environmental management of cattle breeding worldwide. On the other side there is a huge variability of beef and veal production on national and regional level, reflecting the varied geographical, economic and social requirements of different European regions. Even in case of moderate beef consumption (16 kg per capita per year) in the European Union, meat as the source of proteins of animal origin is connected to higher value added, higher employment, profit and incomes in agriculture comparing to crop production. On the other side it also requires higher investments and represents a greater risk. Different levels of agrarian subsidies and the efficiency of their use exacerbate the differences in the production of beef and veal in the countries of the European Union. In submitted paper we investigated beef production distribution similarity of selected countries in Europe. Quantitative approach was applied using cluster analysis in accordance with the Ward's minimum variance method with previous computation of similarity of the territories through the Euclidean distance. Three clusters representing the beef production similarity among the explored countries were visualised by dendrograms within observed steps in the year 2008 and the year 2017. Order of similarity and dissimilarity in beef production according to the Euclidean distance values of all the possible pairs of the districts from the whole data set in observed countries was processed for examined period of time. Finally, the heat maps were constructed to demonstrate the similaritity between each pair of the comprised countries. Obtained results could serve as a valuable resource for meat producers to understand the time dynamics impact and differences in level of beef production in European countries.

Keywords: bovine; meat production; cluster analysis; Euclidean distance

INTRODUCTION

Production of food of animal origin is an important part of securing nutrition of the growing population. The impacts of volatility of agricultural production alleviate the food trade internationalization. New societal challenges such as population growth, urbanization, climate changes, innovations, changes in the demographic structure of the population bring about changes that have a significant impact on the agricultural economy and rural life **(Paraschiv, 2016; Kowal et al., 2016; Mura and Mazák, 2018)**.

The World Summit on Food Security named the four pillars of food security: availability, access, utilization and stability **(World Summit on Food Security, 2009)**. The latest research on characteristics of signs of increasing food insecurity showed the urgent need for considerable additional work to ensure we "leave no one behind" on the road towards achieving the goals on food security and nutrition **(Food and Agriculture Organization of the United Nations et al., 2018)**. In response to these facts in the member states of the European Union national and European policies and strategies were developed, in addition to the common agricultural policy and the Europe 2020 Strategy, in particular, the medium and long-term strategy 2020–2030 for agri-food sector.

Products of animal origin are no longer considered only in terms of quality (ex. flavour) but also safety, nutritive value, sustainability of production methods and animal welfare standards are becoming increasingly important. In these conditions, cluster analysis is a very useful complex statistical method that allows to investigate consumers' behaviour more precisely then using traditional methods **(Gábor et al., 2010; Tekień et al., 2018)**.

The demand for beef as a protein source is increasing worldwide, but in the European Union, consumption of beef declined since 25 kg in 1985 to nowadays 16 kg equivalent **(Hocquette et al., 2018)**. The sustainability of beef production has different meanings in the various geographical and socio-economic regions of the world. Natural resources including land mass and uses, rainfall

and access to livestock feed, and the robustness of the economy are major determinants of the perception of beef sustainability **(Smith et al., 2018; Mura and Gasparikova, 2010)**. **C**ountry of origin as regional aspect of animal production, denoted by labelling, become more important lately **(Sepulveda et al., 2011)**. Also, the other credence attributes associated with cattle production – production system, feeding, animal welfare, slaughtering, traceability, among others – have acquired importance for meat products in developed countries, representing information that must be included on label **(Schnettler et al., 2009)**.

Environmental impact of beef production

Various approaches have been carried out to extrapolate environmental assesments of animal production **(Avadi et al., 2016)**. Increasing volume of animal production due to growing food demand and needs is connected to serious environmental problems. Cattle emits the highest, about 65% of the livestock production emissions of greenhouse gases **(Fiore et al., 2018)**. The emission intensity of beef from specialised beef herds is almost fourfold that produced from dairy herds. On the other side, in Europe, about 80 % of the beef is produced from dairy animals, surplus calves and culled cows, resulting in lower emission intensities, which are the most efficient and least polluting in the world **(Gerber et al., 2013; Hocquette et al., 2018)**. The diverse nature of beef production was captured by establishing a farm typology using principal component analysis and cluster analysis. The typology not only provided a strategy by which the beef cattle industry could be characterised, but also improved understanding of the diversity of farm management practices to help develop policies and beneficial management practices **(Alemu et al., 2016; Jasińska-Biliczak and Sitkowska, 2014)**.

Beef origin and consumer preferences

Beef consumption is generally associated with developed countries and with high levels of total meat and poultry consumption **(Cottle and Kahn, 2014)**. Understanding consumers segment preferences towards food products of animal origin plays crucial role in food research **(Tekień et al., 2018)**. Meat consumption diversification for many reasons is influenced by cultural preferences or economic status of the households. This phenomenon is also indicated by the magnitude of positive cross price elasticity between beef and mutton, beef and poultry meat, and between poultry meat and fish. Therefore, every effort to push higher consumption of one meat type, will reduce the participation rate of others **(Soedjana, 2013)**. Consumers did not prefer the same type of meat within the same country and it is possible that there are individual preferences that could lead to the concept of market segmentation being based on taste preferences. It would appear that Uruguayan beef would be very acceptable in Germany and to a lesser extent in Britain and Spain **(Oliver et al., 2006)**. Results of the Spanish study of consumers' preferences is of great interest in the beef sector, where the bovine spongiform encephalopathy crisis generated deep changes in the basic conditions of demand for meat and in the behaviour of consumers. Results showed that the origin of the product is the most important attribute for the choice of beef, followed by quality labelling, production system and price **(Mesias et al., 2005)**. Market research results confirmed connection of certain consumer segments preference of beef consumption to the brand and area of origin. Branded beef produced under high production standards enjoys a higher level of trust and consumers are willing to pay comparatively higher prices for such products **(Hochuli et al., 2018)**. The most important factor explaining the differences among consumer responses relates to consumers' perceptions of the importance of meat attributes related to production practices – for instance use of antibiotics, hormones and environmentally friendly grazing. Interestingly, the consumer segments that are willing to pay a significantly higher premium for natural, local beef are motivated by different aspects of the meat and its intrinsic production attributes **(Thilmany et al., 2006)**.

Beef production systems diversity

Comparison of the beef production systems to establish the main technical, socio-economic and productive aspects of the beef farms showed their differences in term of orientation market type, intensification level, dimensionality and economic performance **(Perea et al., 2014)**. The intensification process of the livestock sector has been characterised in recent decades by increasing output of product per hectare, increasing stocking rate, including more concentrated feed in the diet, and improving the genetic merit of the breeds. Clusters of farms characterised by different levels of production intensity showed similar environmental performances on product basis, despite important differences in terms of intensification level, management, and structural characteristics. Considering the environmental burden on a local perspective, the impacts per hectare were positively associated with the intensification level **(Bava et al., 2014)**. Clustering of livestock system based on the production intensity showed that the intensive systems had larger herds, modern structures and equipment, and were strongly production oriented, whereas the extensive systems had smaller herds and productivity, with often traditional or obsolete structures and equipment, but showed a tendency to diversify production or mixed farming of different livestock categories eventually. Livestock systems differ not only in production practices but also in the ability to maintain landscape, which is generally higher in the extensive or even marginal systems **(Sturaro et al., 2009; Kordoš, 2015; Stasiak-Betlejewska, 2015)**.

Beef production in Europe

Heterogeneity of livestock numbers distribution and study of dynamics of its change were found fundamental to the identification of drivers that shaped the various intensification trajectories and led to these different states, as well as to the prediction of future changes **(Domingues et al., 2018)**. Investigation of production volatility by species showed the highest variation coefficient for the production of live weight meat in beef, followed by poultry meat and mutton and goat meat **(Grodea, 2016)**.

The European Union is the world's third largest producer of beef after the United States of America and Brazil with almost 8.0 million tons of carcasses in 2018 **(Hocquette et al., 2018)**.

The number of cattle in the Slovak Republic reached 446.1 thousand in 2016, of which the number of cows was 194.2 thousand heads **(Ministrstvo pôdohospodártsva a rozvoja vidieka Slovenskej republiky, 2017)**. In 2017, 44.063 tonnes of carcass weight of beef cattle were sold in the Slovak Republic as well as 1.316 tonnes of calves. At the Slovak slaughterhouses, 29.3 thousand cattle heads were slaughtered with a carcass weight of 7.8 thousand tons. Domestic consumption of beef is estimated to be 26.400 tonnes in 2017, which is 4.9 kg per capita per year **(Gálik, 2018)**. Due to regional differences in terms of climate and pasture availability, and also in terms of livestock practices and fattening farm characteristics, the productivity and incomes of beef producers vary widely across European countries and regions, being regularly among the lowest of the agricultural systems **(Smith et al., 2018)**. The heterogeneity of the European Union cattle sector at the regional level is substantial. Pronounced differences exist between regions in western and eastern, as well as between regions in northern and southern European Union member states **(Ihle et al., 2017)**.

Aim of the article was to provide the cluster analysis of beef production within the member countries of the European Union which will allow to understand their similar behaviour, livestock practices, as well as different environmental policies and their future scenarios.

Scientific hypothesis

The fundamental goal of the analysis is to construct a potential platform to be ready to prepare the common directives creating a policy framework aimed at a set of the mutual rules providing a better support in the process of regulation of the appropriate markets where the analysed fragment of the beef production is traded.

MATERIAL AND METHODOLOGY

The methodology selected to carry out the analysis is adapted to the data obtained from the database. Animal production statistics cover three main sub-domains based on three pieces of relevant legislation and related gentlemen's agreements.

Data

The data comes from the database of the Statistical Office of the European Union (Eurostat). It contains the tables from the database "Meat production and foreign trade" marked apro_mt_pann **(Eurostat, 2018a)** and the database "Cattle population" marked apro_mt_lscatl **(Eurostat, 2018b)**.

According to the metadata manual of the Eurostat Animal Production Statistics, bovine animal is domestic animal of the *Bos taurus* species, which covers cattle, and the *Bubalus bubalis* sp., covering water buffalo, respectively domestic Asian water buffalo, including hybrids like *Beefalo* **(Eurostat, 2017)**. This integration is done due to clarification of the implementation of buffaloes and hybrids into this category.

There is to note that census of bovine population is due only once a year for the member states of the European Union where its size is below a one and a half million level when counting heads. A statistics accuracy is determined by the European Commision regulation in a way that the sampling error for the results of each member state of the European Union has not to exceed 1% of the total number of bovine animals in a case of the members whose population is above and equal to one million head and 5% in a case where the population is below one million head with a confidence interval of 68% **(European Commission, 2008)**.

The data source may come out from sample survey or census. Nevertheless, administrative source may represent a basis for obtaining the requested result in order to limit burden on the respondents. This is especially the case for bovine livestock according to the database manual.

The dimensions of the analysis cover a territorial angle of a view an area of the countries whose data is provided by the Eurostat and from a time perspective a time span from 2008 to 2017 is involved. The data is comprised in an annual way, which is the most often provided time interval for this data. Regarding to its characteristics, it is a suitable and common time interval.

An observed set of the area involved in the analysis consists of the following countries: Albania (AL), Austria (AT), Belgium (BE), Bosnia and Herzegovina (BA), Bulgaria (BG), Croatia (HR), Cyprus (CY), Czechia (CZ), Denmark (DK), Estonia (EE), Finland (FI), France (FR), Germany (DE), Greece (GR), Hungary (HU), Iceland (IS), Ireland (IE), Italy (IT), Kosovo (XK), Latvia (LV), Lithuania (LV), Latvia (LT), Luxembourg (LU), Malta (MT), Montenegro (ME), the Netherlands (NL), Poland (PL), Portugal (PT), Romania (RO), Serbia (RS), Slovakia (SK), Slovenia (SI), Spain (ES), Sweden (SE), Switzerland (CH), Turkey (TR), United Kingdom (GB). There is only to note minorly that Kosovo uses the temporary code XK until it will be assigned the final code. The mentioned countries are ordered alphabetically according to their colloquial alternative name. They are called by the alternative names in the further text of the paper. These abbreviations are determined by the International Organization for Standardization 3166-1 standard that is part of the the International Organization for Standardization 3166 norm **(International Organization for Standardization, 2013)**. Especially, the two-letter entry of the mentioned standard is applied in the results section of the paper.

There is to note that not all the countries have provided the data for the whole analysed period. Therefore, the mean data are applied to carry out the cluster analysis for a whole time span case.

Methodology

There are several quantitative methods applied in the given analysis. The main approach is the cluster analysis. Firstly, the normalisation of the data is applied too in order to get it to be compared mutually.

Secondly, the similarity of the territories is computed through the Euclidean distance:

$$D(c_1, c_2) = \sqrt{\left(c_{1_x} - c_{2_x}\right)^2 + \left(c_{2_y} - c_{2_y}\right)^2}$$

where the involved variables mean:
- c_1 – the first country;
- c_2 – the second country;
- $D(c_1, c_2)$ – the mutual Euclidean distance of c_1 the country and the c_2 country;
- c_{1_x} – the x coordinate of the c_1 country;
- c_{2_x} – the x coordinate of the c_2 country;
- c_{1_y} – the y coordinate of the c_1 country;
- c_{2_y} – the y coordinate of the c_2 country.

Thirdly, a number of clusters is determined according to the following methods:
- the Ball-Hall index **(Ball and Hall, 1965)**;
- the McClain-Rao index **(McClain and Rao, 1974)**;
- the point-biserial correlation coefficient **(Milligan, 1981)**.

Successively, the cluster analysis is carried out itself. This means a construction of the clusters in accordance with the Ward's minimum variance method.

The final step of the analysis is a creation of the heat maps displaying the similarities of the individual pairs of the explored countries. This graphical output supplements the dendrograms appropriately.

Statistic analysis

The whole analysis is executed in the R statistical environment through the programming language R **(R Core Team, 2018)** using the three packages: *NbClust* **(Charrad et al., 2014; Charrad et al., 2015)**, *shape* **(Soetaert, 2018)** and *gplots* **(Warnes et al., 2016)**.

RESULTS AND DISCUSSION

Firstly, a number of clusters is determined by means of the described methods in the previous chapter. The more detailed information is shown in the Table 1.

According to the selected approaches, a number of clusters representing the beef production similarity among the explored countries is determined to three. The situation looks like as follows at the beginning of the explored time span. The first cluster consists of a majority of the involved countries where Luxembourg, Cyprus, Malta, Bulgaria, Estonia, Slovakia, Hungary, Lithuania, Slovenia, Portugal, Croatia, Greece, Denmark, Sweden, Latvia, Czechia, Finland, Belgium, Austria, Romania, Ireland, and Poland belong. This cluster encompasses the 22 countries. The second cluster is created by the four countries. It involves Spain, United Kingdom, Germany, and Italy. The third cluster consists of only the two countries: France and the Netherlands. The mentioned countries are ordered according their position in the dendrogram.

There are visible some changes in the distribution of the clusters visualising similarity of the analysed beef production situation related to the end of the explored time span in 2017. The biggest first cluster involves 28 countries, where Croatia, Lithuania, Serbia, Latvia, Hungary, Slovenia, Cyprus, Iceland, Malta, Bulgaria, Slovakia, Estonia, Luxembourg, Bosnia and Herzegovina, Montenegro, Albania, Kosovo, Portugal, Greece, Romania, Sweden, Finland, Czechia, Turkey, Belgium, Austria, Denmark, and Switzerland. The second cluster is created only by the sole country, Spain. All the remaining coutries participating in the second cluster before: Germany, United Kingdom, and Italy are assigned to the third cluster in succession. They are followed by Ireland, Poland, France and the Netherlands. Poland and Ireland are only new countries in this cluster, as they are assigned to the osculant tail of the first cluster at the beginning of the explored period in 2008.

The mean situation, as it could be called, is constructed according to the mean Euclidean distances between the individual countries throughout the whole observed period. This illustrates the countries, which are similar in a field of the beef production for the whole observed period. It is partially different than the intiating point and the terminating point of the explored time span. The substantial structure from an angle of view of number of the involved countries is very similar, as the first cluster comprises a big majority of the elaborated entities:

Table 1 The numbers of clusters of the observed countries according to beef production distribution.

Method	Statistic	Statistic value	Number of clusters
Ball-Hall index	barycentre mean dispersion	503.5025	3
McClain-Rao index	denominator	0.0828	3
point-biserial correlation coefficient	correlation	0.8822	3

Table 2 The most similar and the most dissimilar countries according to beef production distribution.

Year	The nearest pair of the countries			The outermost pair of the countries		
	Distance	Country 1	Country 2	Distance	Country 1	Country 2
2008	0.01995	CZ	FI	11.33987	FR	MT
2009	0.01891	EE	LU	11.43255	FR	MT
2010	0.01493	EE	LU	11.67193	BG	ES
2011	0.01522	EE	LU	11.51365	FR	MT
2012	0.00989	EE	SK	11.63335	FR	MT
2013	0.00816	EE	SK	11.60456	FR	MT
2014	0.01077	CY	IS	11.80299	FR	MT
2015	0.00998	LU	SK	12.52704	FR	MT
2016	0.00944	LU	SK	11.41347	FR	TR
2017	0.01026	BG	SK	12.52704	FR	SK

Sweden, Czechia, Finland, Iceland, Malta, Slovakia, Estonia, Luxembourg, Montenegro, Bulgaria, Cyprus, Albania, Bosnia and Herzegovina, Lithuania, Hungary, Slovenia, Latvia, Serbia, Denmark, Portugal, Kosovo, Croatia, Greece, Romania, Ireland, Poland, Austria, Turkey, Belgium, and Switzerland. The second cluster consists of only the two countries and it has the same content as the third cluster from the beginning of the explored period in 2008: its members are France and the Netherlands. Finally, the third cluster encompasses the four countries, which are the same countries that are included in the second cluster at the beginning of the observed time span: these are Spain, Italy, Germany, and United Kingdom.

It is an interesting alteration of the intitating situation, because this expresses a considerable move of these four

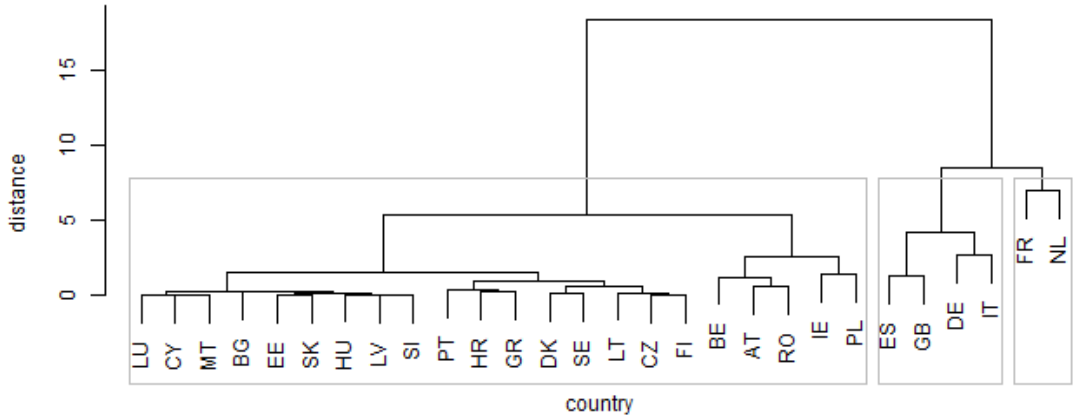

Figure 1 The dendrogram of the beef production distribution similarity according to the explored countries for the year 2008.

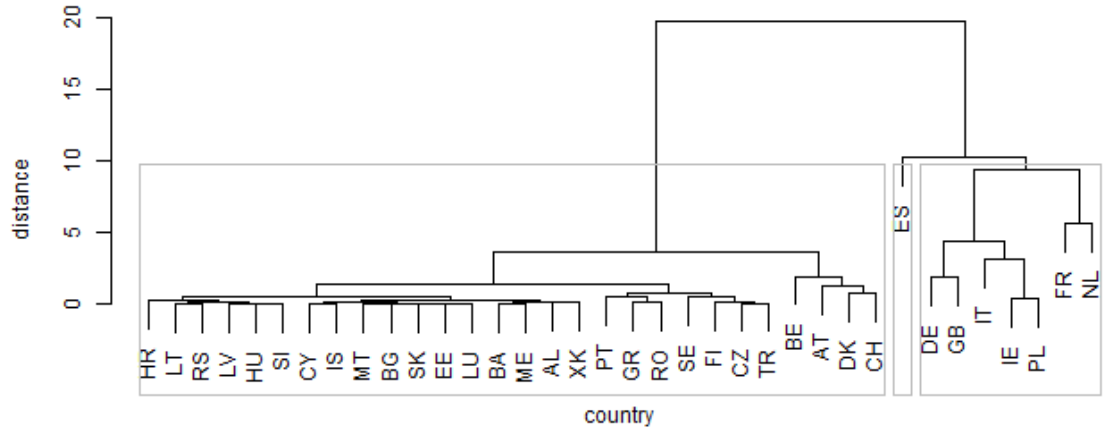

Figure 2 The dendrogram of the beef production distribution similarity according to the explored countries for the year 2017.

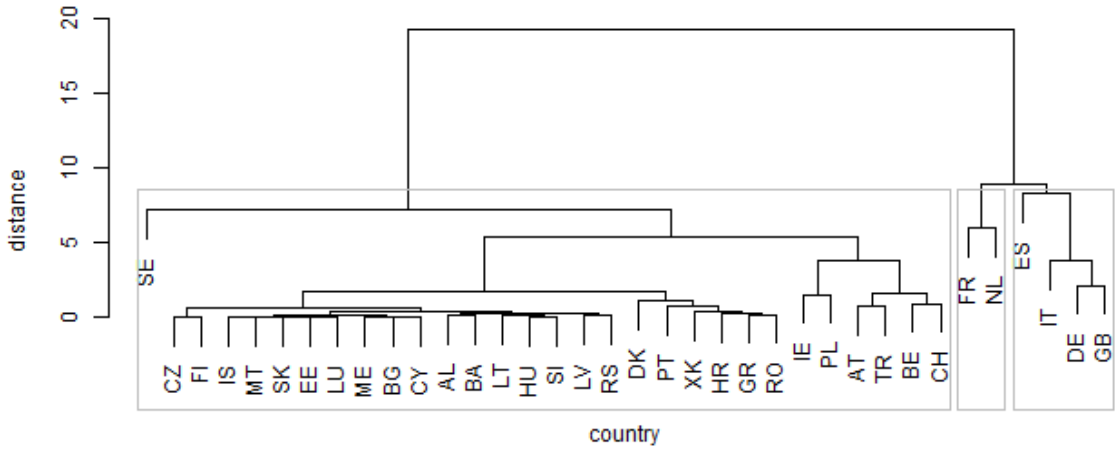

Figure 3 The dendrogram of the beef production distribution similarity according to the explored countries for the whole observed period.

countries further from a remaining majority represented by the first cluster.

Table 2 shows the most extreme similarities according to the Euclidean distance values of all the possible pairs of the districts from the whole data set. The displayed values are rounded to five decimal places. As it is seen, the most similar pairs are created by the eight countries, which Bulgaria, Cyprus, Czechia, Estonia, Finland, Iceland, Luxembourg, and Slovakia belong among.

The most mentioned countries are Estonia, Luxembourg and Slovakia, all three entities five times. Each one of the remaining countries is represented only once. The most similar pair, the nearest one of the whole explored time span is created by Estonia and Slovakia in 2008 with the Euclidean distance value of 0.00816.

On the other hand, the most dissimilar pairs of the countries are represented the six various countries throughout the whole analysed time span, which Bulgaria, Estonia, France, Malta, Slovakia, and Turkey belong among. France is set all, but one years and Malta is located here seven times. The remaining four countries are represented only once. The absolutely biggest disparity during the whole explored period at a level of 12.52704 is found twice: between France and Malta in 2015 and between France and Slovakia in 2017. There is to note for curiosity, Slovakia appears in the both sides: several times it creates the nearest pair and once it creates the outermost pair. Such a result can be expectable because Malta and Slovakia dispose an absolutely different composition of the cattle livestock holdings.

The following heat maps demonstrate the similaritity between each pair of the comprised countries. Each cell is assigned the particular shade of gray: the darker colour is placed, the more distant pair of the countries there is. It means such countries have more similar situation in beef production. The first heat map shows a situation at the beginning of the explored period in 2008.

As it can be seen from the previous heat map on Figure 4, there is clearly visible that there are the three countries which stand out among the other countries. This triplet consists of France with the average mutual distance to all the other countries at a level of 9.84472, Italy with a distance of 6.46702, and the Netherlands with a distance of 6.21732. Successively, the United Kingdom with a distance of 4.88268 and Spain with a distance of 4.09161 are visibly more distant from the remaining group of the involved countries.

The second heat map visualises a situation at the end of the explored period in 2017.

Figure 5 demonstrates the final situation in the analysed field. France with the average mutual distance to all the remaining countries in the data set at a level of 9.42508 is the outermost entity. It is followed by Spain with a distance of 8.85159, the Netherlands with a distance of 7.27699, Germany with a distance of 6.34874, the United Kingdom with a distance of 5.22425, Italy with a distance of 5.05540, Poland with a distance of 4.61316 and finally, Ireland with a distance of 3.82692.

The third heat map visualises a situation according to the mean values of the observed countries for the whole explored period from 2008 to 2017.

The final heat map on Figure 6 demonstrates the average situation of the whole analysed time span. The outermost

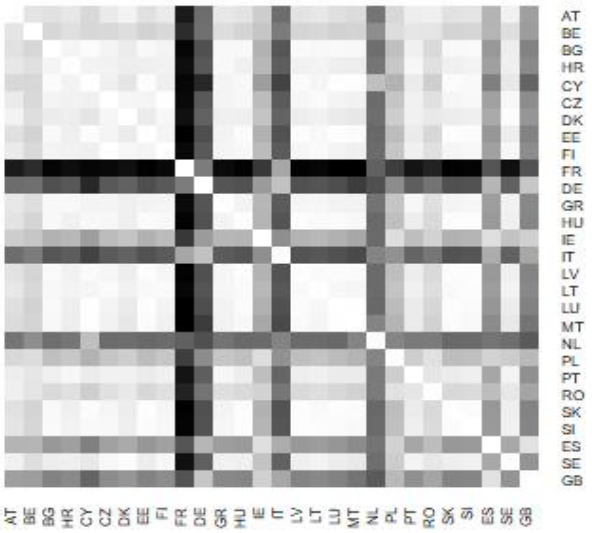

Figure 4 The heat map of the beef production similarity according to the explored countries for the year 2008.

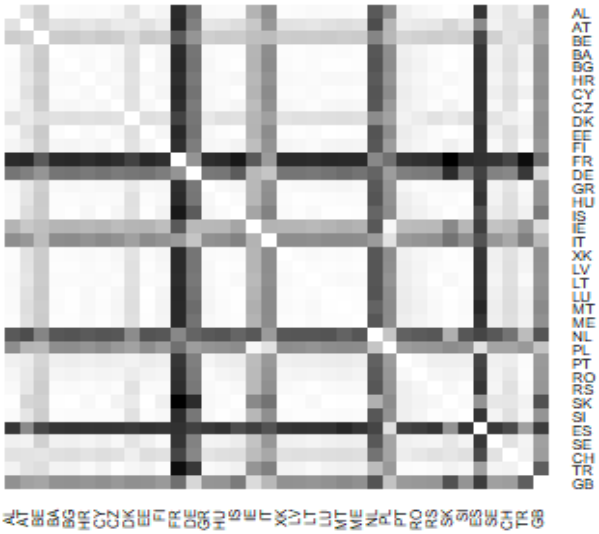

Figure 5 The heat map of the beef production similarity according to the explored countries for the year 2017.

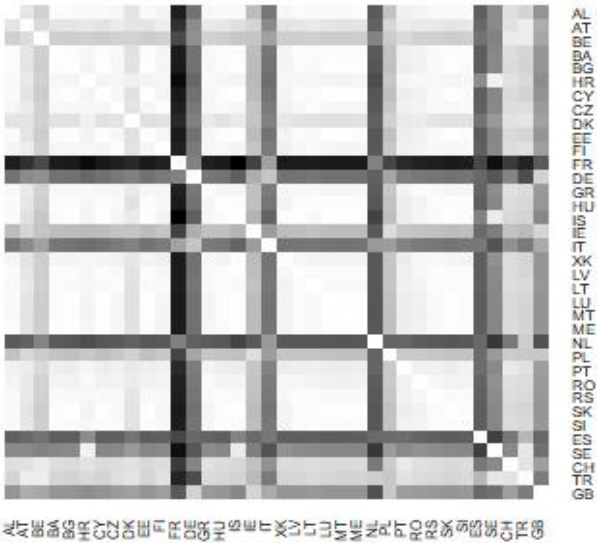

Figure 6 The heat map of the beef production similarity according to the explored countries for the whole observed period.

country is France again. It lies the most distantly: at a level of 10.03562 Euclidean units. It is followed by the Netherlands with a value of 7.58086, Spain with a value of 7.27290, Germany with a value of 6.30685, Italy with a value of 6.06541 and, finally the United Kingdom with a value of 5.00537.

Obtained results of the mean situation of clustering, namely the third cluster containing Germany, the United Kingdom, Italy and Spain, showed the same order of beef production as the results of **Hocquette et al. (2018)**. The mean distance of the second cluster, represented in our results by France and the Netherlands are much higher in their results in both total cattle numbers in 2014 and beef production in 2016. Also, the results of **Ihle et al. (2017)** confirm that Germany, France, the United Kingdom and Italy account for half of the gross production value of the EU cattle sector. They also stated that the EU cattle herd is concentrated in and around the Benelux, the Alps, eastern Poland, north-western France and Ireland, and confirm the substantial regional differences of the EU cattle sector in western and eastern, as well as northern and southern member states. **Mauracher and Valentini (2006)** in their work clustered Europe into four regions based on level of meat consumption with high consuming cluster containing Austria, Finland, France, Ireland, Switzerland and the United Kingdom, and the low income and low meat consumption cluster containing Albania, Bosnia and Herzegovina, Cratia, Moldova, Romania and the Former Yugoslav Republic of Macedonia.

The results of **Smith et al. (2018)** focused to future prospect of global beef production mentioned unprecedented challenges of European beef industry related to animal welfare, environmental impact, origin and authenticity of beef, nutritional benefits, and consistency of eating quality.

CONCLUSION

Evolution of food consumption and associated meat production in European countries has been analysed with focus to general picture as well as on the level of specific regions. Significant regional differences within the European Union member countries reflect the geographical, economic and social requierements of different European regions. Provided cluster analysis showed grouping of countries based on the similarity of the territories through the Euclidean distance and its time dynamics within observed periods 2008 and 2017. Created heat maps displayed the similarities of the individual pairs of the surveyed countries. Obtained results can serve as background for preparation of common directives of policy framework of beef production, as well as to understand the future changes within the beef production industry.

REFERENCES

Alemu, A. W., Amiro, B. D., Bittman, S., MacDonald, D., Ominski, K. H. 2016. A typological characterization of Canadian beef cattle farms based on a producer survey. *Canadian Journal of Animal Science*, vol. 96, no. 2, p. 187-202. https://doi.org/10.1139/cjas-2015-0060

Avadi, A., Nitschelm, L., Corson, M., Vertes, F. 2016. Data strategy for environmental assessment of agricultural regions via LCA: Case study of a French catchment. *International Journal of Life Cycle Assessment*, vol. 21, no. 4, p. 476-491. https://doi.org/10.1007/s11367-016-1036-6

Ball, G. H., Hall, D. J. 1965. Isodata, a Novel Method of Data Analysis and Pattern Classification [online]. Menlo Park, United States of America : Stanford Research Institute. [cit.2018-12-02] Available at: http://www.dtic.mil/dtic/tr/fulltext/u2/699616.pdf.

Bava, L., Sandrucci, A., Zucali, M., Guerci, M., Tamburini, A. 2014. How can farming intensification affect the environmental impact of milk production? *Journal of Dairy Science*, vol. 97, no. 7, p. 4579-4593. https://doi.org/10.3168/jds.2013-7530

Cottle, D. J., Kahn, L. 2014. *Beef Cattle Production and Trade*. Collingwood, Australia : Csiro Publishing, 574 p. ISBN: 9780643109889.

Domingues, J. P., Ryschawy, J., Bonaudo, T., Gabrielle, B., Tichit, M. 2018. Unravelling the physical, technological and economic factors driving the intensification trajectories of livestock systems. *Animal*, vol. 12, no. 8, p. 1652-1661. https://doi.org/10.1017/S1751731117003123

Eurostat. 2017. *Animal Production Statistics* [online]. Luxembourg, Luxembourg : Eurostat. Available at: https://ec.europa.eu/eurostat/cache/metadata/en/apro_mt_esms.htm.

Eurostat. 2018. *Bovine population: Annual data* [online]. Luxembourg, Luxembourg : Eurostat. Available at: http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=apro_mt_pann&lang=en.

Eurostat. 2018. *Slaughtering in slaughterhouses: Annual data* [online]. Luxembourg, Luxembourg : Eurostat. Available at: http://appsso.eurostat.ec.europa.eu/nui/show.do?dataset=apro_mt_pann&lang=en.

Food and Agriculture Organization of the United Nations, International Fund for Agricultural Development, United Nations International Children's Emergency Fund, World Food Programme, World Health Organization. 2018. The State of Food Security and Nutrition in the World 2018 [online]. In *Building climate resilience for food security and nutrition*. Rome : Food and Agriculture Organization of the United Nations, 202 p. ISBN 978-92-5-130571-3. Available at: http://www.fao.org/3/I9553EN/i9553en.pdf.

Fiore, M., Spada, A., Conte, F., Pellegrini, G. 2018. GHG and cattle farming: CO-assessing the emissions and economic performances in Italy. *Journal of Cleaner Production*, vol. 172, p. 3704-3712. https://doi.org/10.1016/j.jclepro.2017.07.167

Gábor, M., Trakovická, A., Miluchová, M., Moravčíková, N. 2010. Genetic markers as one of tools for production of tenderness meat in cattle. *Potravinarstvo*, vol. 10, no. 4, p. 16-21. https://doi.org/10.5219/102

Gálik, J. 2018. Jatočný hovädzí dobytok a jatočné teľatá, Situačná a výhľadová správa k 31.12.2017 [online]. *NPPC-VÚEPP Bratislava*, vol. 26, no. 1, 67 p. Available at: http://www.vuepp.sk/dokumenty/komodity/2018/Hd04_18.pdf.

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., Tempio, G. 2013. *Tackling climate change through livestock - A global assessment of emissions and mitigation opportunities* [online]. Rome, Italy : Food and Agriculture Organization of the United Nations (FAO), 115 p. ISBN 978-92-5-107920-1. Available at: http://www.fao.org/3/a-i3437e.pdf.

Grodea, M. 2016. Milk and Beef Production Volatility in Romania-Domestic Supply Stability Factor. *Scientific Papers-*

Series Management Economic Engineering in Agriculture and Rural Development, vol. 16, no. 1, p. 193-196.

Hocquette, J. F., Ellies-Oury, M. P., Lherm, M., Pineau, Ch., Deblitz, C., Farmer, L. 2018. Current situation and future prospects for beef production in Europe – A review. *Asian-Australasian Journal of Animal Science*, vol. 31, no. 7, p. 1017-1035. https://doi.org/10.5713/ajas.18.0196

Hochuli, A., Raemy, D., Hofer, H., Fluckiger, D. 2018. Swiss private-label branded beef: brand credibility and consumer trust. *Agrarforschung Schweiz*, vol. 9, no. 9, p. 288-295. Available at: https://www.agrarforschungschweiz.ch/artikel/download.php?filename=2018_09_2409.pdf.

Charrad, M., Ghazzali, N., Boiteau, V., Niknafs, A. 2014. NbClust: An R Package for Determining the Relevant Number of Clusters in a Data Set. *Journal of Statistical Software*, vol. 6, no. 6, p. 1-36. https://doi.org/10.18637/jss.v061.i06

Charrad, M., Ghazzali, N., Boiteau, V., Niknafs, A. 2015. *Package NbClust*. Vienna, Austria : R Foundation for Statistical Computing. Available at: https://cran.r-project.org/web/packages/NbClust/NbClust.pdf.

Ihle R., Dries L., Jongeneel R., Venus T., Wesseler J. 2017. *Research for Agri-Committee-The EU Cattle Sector: Challenges and Opportunities-Milk and Meat* [online]. Brussels, Belgium : European Parliament, DG for Internal Policies, 169 p. ISBN: 978-92-846-0601-6. Available at: http://www.europarl.europa.eu/RegData/etudes/STUD/2017/585911/IPOL_STU(2017)585911_EN.pdf.

International Organization for Standardization 3166. 2013. *International Organization for Standardization 3166 – International Standard for country codes and codes for their subdivisions*.

Jasińska-Biliczak, A., Sitkowska, R. 2014. Influence of small and medium enterprises sector at the change of innovation potential of Polish regions. *Grant Journal*, vol. 3, no. 1, p. 57-61. Available at: http://www.grantjournal.com/issue/0301/PDF/0301sitkowska.pdf.

Kordoš, M. 2015. The role and significance of clusters within the EU industry policy. *Acta Oeconomica Universitatis Selye*, vol. 4, no. 1, p. 53-63.

Kowal, J., Jasińska-Biliczak, A., Hafner, J. 2016. Innovative Capacity in Small Regional Enterprises in Transition Economies: An Exploratory Study in Poland. In *22nd Americas Conference on Information Systems (AMCIS)*. San Diego, United States of America : Association for Information Systems. ISBN 978-0-9966831-2-8.

Mauracher, Ch., Valentini, M. 2006. Tipicità e convergenza alimentare dei PECO. *Economia Agro-Alimentare*, vol. 3, no. 22, p. 115-136.

McClain, J. O., Rao, V. R. 1975. Clustisz: A Program to Test for the Quality of Clustering of a Set of Objects. *Journal of Marketing Research*, vol. 12, no. 4, p. 456-460. Available at: https://www.jstor.org/stable/3151097.

Mesias, F. J., Escribano, M., de Ledesma, A. R., Pulido, F. 2005. Consumers' preferences for beef in the Spanish region of Extremadura: a study using conjoint analysis. *Journal of the Science of Food and Agriculture*, vol. 85, no. 14, p. 2487-2494. https://doi.org/10.1002/jsfa.2283

Milligan, G. W. 1981. A Monte Carlo Study of Thirty Internal Criterion Measures for Cluster Analysis. *Psychometrika*, vol. 46, no. 2, p. 187-199. https://doi.org/10.1007/BF02293899

Ministrstvo pôdohospodártsva a rozvoja vidieka Slovenskej republiky. 2017. *Správa o poľnohospodárstve a potravinárstve v Slovenskej republike za rok 2016 (Zelená správa) Report on Agriculture and Food Industry in the Slovak Republic for 2016 (Green report)*. Bratislava, Slovakia : Ministry of Agriculture and Rural Development of the Slovak Republic, 325 p. Available at: http://www.vuepp.sk/dokumenty/zelena/zelena2017.pdf. (In Slovak)

Mura, L., Gasparikova, V. 2010. Penetration of small and medium sized food companies on foreign markets. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 58, no. 3, p. 157-163. https://doi.org/10.11118/actaun201058030157

Mura, L., Mazák, M. 2018. Innovative activities of family SMEs: case study of the Slovak regions. *On-line Journal Modelling the New Europe*, vol. 27, p. 132-147. https://doi.org/10.24193/OJMNE.2018.27.06

Oliver, M. A., Nute, G. R., Furnols, M. F. I., San Julian, R., Campo, M. M., Sanudo, C., Caneque, V., Guerrero, L., Alvarez, I., Diaz, M. T., Branscheid, W., Wicke, M., Montossi, F. 2006. Eating quality of beef, from different production systems, assessed by German, Spanish and British consumers. *Meat Science*, vol. 74, no. 3, p. 435-442. https://doi.org/10.1016/j.meatsci.2006.03.010

Paraschiv, A. 2016. Agrifood Sector Development Strategy for Medium and Long Term Horizon 2020-2030. *Journal of Young Scientist*, vol. 4, p. 73-76. Available at: http://journalofyoungscientist.usamv.ro/pdf/vol_IV_2016/art13.pdf.

Perea, J., Blanco-Penedo, I., Barba, C., Angon, E., Garcia, A. 2014. Organic Beef Farming in Spain: Typology According to Livestock Management and Economic Variables. *Revista Cientifica-Facultad de Ciencias Veterinarias*, vol. 24, no. 4, p. 347-354.

R Core Team. 2018. *R: Language and Environment for Statistical Computing* [online]. Vienna, Austria : R Foundation for Statistical Computing. Available at: https://www.R-project.org.

Regulation (EC) No 1165/2008 of the European Parliament and of the Council of 19 November 2008 concerning livestock and meat statistics and repealing Council Directives 93/23/EEC, 93/24/EEC and 93/25/EEC [online]. *Official Journal of the European Union* L, no. 321, 1. 12. 2018, p. 1-13. Available at: https://eur-lex.europa.eu/eli/reg/2008/1165/oj.

Sepulveda, W. S., Maza, M. T., Pardos, L. 2011. Aspects of quality related to the consumption and production of lamb meat. Consumers versus producers. *Meat Science*, vol. 87, no. 4, p. 366-372. https://doi.org/10.1016/j.meatsci.2010.11.013

Schnettler, B., Silva, R., Sepulveda, N. 2009. Utility to Consumers and Consumer Acceptance of Information on Beef Labels in Southern Chile. *Chilean Journal of Agricultural Research*, vol. 69, no. 3, p. 373-382. https://doi.org/10.4067/S0718-58392009000300010

Smith, S. B., Gotoh, Tk., Greenwood, P. L. 2018. Current situation and future prospects for global beef production: overview of special issue. *Asian-Australasian Journal of Animal Sciences*, vol. 31, no. 7, p. 927-932. https://doi.org/10.5713/ajas.18.0405

Soedjana, T. D. 2013. Participation Rate as a Basis for Measuring Food Security Status of Meat. *Wartazoa-Buletin Ilmu Peternakan Dan Kesehatan Hewan Indonesia*, vol. 23, no. 4, p. 166-175.

Soetaert, K. 2018. *Package shape* [online]. Vienna, Austria : R Foundation for Statistical Computing. Available at: https://cran.r-project.org/web/packages/shape/shape.pdf.

Stasiak-Betlejewska, R. 2015. Clusters as the element of eco-innovations promoting in the European construction on the Polish example. *Acta Oeconomica Universitatis Selye*, vol. 4, no. 2, p. 182-197.

Sturaro, E., Cocca, G., Gallo, L., Mrad, M., Ramanzin, M. 2009. Livestock systems and farming styles in Eastern Italian Alps: an on-farm survey. *Italian Journal of Animal Science*, vol. 8, no. 4, p. 541-554. https://doi.org/10.4081/ijas.2009.541

Tekień, A., Gutkowska, K., Żakowska-Biemans, S., Jóźwik, A., Krotki, M. 2018. Using cluster analysis and choice-based conjoint in research on consumers preferences towards animal origin food products. Theoretical review, results and recommendations. *Animal Science Papers & Reports*, vol. 36, no. 2, p. 171-184.

Thilmany, D. D., Umberger, W. J., Ziehl, A. R. 2006. Strategic market planning for value-added natural beef products: A cluster analysis of Colorado consumers. *Renewable Agriculture and Food Systems*, vol. 21, no. 3, p. 192-203. https://doi.org/10.1079/RAF2005143

Warnes, G. R., Bolker, B., Bonebakker, L., Gentleman, R., Liaw, W. H. A., Lumley, T., Maechler, M., Magnusson, A., Moeller, S., Schwartz, M., Venables, B. 2016. Package gplots [online]. Vienna, Austria : R Foundation for Statistical Computing. Available at: https://cran.r-project.org/web/packages/gplots/gplots.pdf.

World Summit on Food Security. 2009. *Declaration of the World Summit on Food Security* [online]. Rome, Italy : WSFS. 7 p. [cit.2018-12-02]. Available at: http://www.fao.org/fileadmin/templates/wsfs/Summit/Docs/Final_Declaration/WSFS09_Declaration.pdf.

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MILK PRODUCTION RELATED TO PRICE OF RAW COW'S MILK IN SELECTED EUROPEAN COUNTRIES

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ABSTRACT

Dairy industry and its production contributes to the economies of many regions and countries worldwide. Except the milk production there is also number of other impacts such as the human nutrition, landscape creation and environment among the others. The European dairy sector undergoes numerous changes a period of crises and regulations in last few decades. After abolition of milk quota system, the European milk producing countries started to be exposed to the milk prices of the world market. In the submitted article, the impact of five explanatory variables, which cow's milk, butter, milk powder, cheese, and farm milk production belong among, is analysed to the explained variable the price of raw cow's milk coming from the countries whose data is available in the Eurostat database; that is, Austria, Belgium, Croatia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom. Regression analysis of panel data with territorial and time dimensions is applied using three techniques, which the pooling, the random, and the between approach belong to. Supplementary analytical approach represented by the cluster analysis resulted into triplet of clusters, selected for the further modelling process. Results of the regression analysis showed no influence of butter production to the level of raw cow's milk. The visualised outcome signifies the distribution of the individual countries among the examined clusters. It underlines the fact that the cheaper raw cow's milk price causes a concentration on the specific part of the production that is easier to produce. It is important to realise that the coefficient of determination of the regression models reveal their statistical significance as a whole. Obtained results can serve as the background for further analysis of impact of other milk products as the factors influencing the raw cow's milk prices.

Keywords: milk; milk product; dairy; price; regression analysis

INTRODUCTION

Dairy represents important industry in many European countries, not only for production of milk and milk products, but also for its contribution to the landscape creation and environment.

Growing consumption of dairy and other livestock products is bringing important nutritional benefits to large segments of the population worldwide. However, the rapid growth in production and consumption of livestock products also presents risks to human and animal health, the environment and the economic viability of many poor smallholders, but may also offer opportunities for small- and medium-scale dairy industries **(Muehlhoff et al., 2013; Mura and Gasparikova, 2010; Jasińska-Biliczak and Sitkowska, 2014; Stasiak-Betlejewska, 2015; Kowal et al., 2016; Mura and Mazák, 2018)**. For the last fifty years, the dairy sector in most developed countries has shifted towards bigger herd size and significantly higher annual milk production per cow. The driving force in this development has been the farmers' ability to increase incomes through higher productivity, adopting the many technological innovations which often require high capital and therefore bigger herds to be profitable **(Gerosa and Skoet, 2012)**.

The European dairy sector is characterised by 600,000 dairy farms, 12,000 processing facilities and 300,000 jobs. It produces 15% of all agricultural revenues of the European Union. This production creates a quarter of the world´s milk production and its dairy products are also exported all over the world but 87% of all dairy production is consumed by european households. This European Union sector has many strengths. The first and the most important strength is the capacity to supply milk of a consistent quality with very slight year-on-year variations in supply volumes. Milk production in the European Union is the only agricultural output that can boast this stability **(Lemoine, 2016)**. The vast majority of milk produced on farms (96.8%) located in the European Union comes from cows, although in a number of the southern member sates significant quantity of milk is also produced by sheep, goats and buffaloes. The European Union milk sector is highly varied, something which can blur the measured changes. Specialised farms had on national average between 3 and 141 dairy cows. Milk is

used either on farms or processed in dairies **(Marquer, 2015)**. Milk in the European Union is used for fabrication of cheese (37%), butter (30%), cream (13%), drinking milk (11%), acidified milk (4%), powser products (3%), and other products (2%). Majority of milk (96.8%) is processed and known as the whole milk, remaining part (3.2%) is non-processed milk, which is delivered to the national nondairy industry, returned to farms or lost **(Eurostat, 2016)**. Milk from other milk producing species is usually more expensive and thus many times a subject to fraudulent activities like many other high-priced foodstuffs **(Velioglu et al., 2017)**.

Agricultural products price volatility is influenced by crop production; the more dispersed and volatile crop production is, the higher the volatility of agricultural prices; in the case of cow milk, market stability is higher, compared to the sheep milk market **(Grodea, 2011)**. Dairy products, in particular, have higher income elasticities of demand than most other food items, including meat and fish. In other words, as incomes increase, expenditures on dairy products will grow more rapidly in percentage terms than most other food items **(Muehlhoff et al., 2013)**.

Milk production regulations and dairy crisis

In a well-functioning and free market, firms which cannot keep up with competitors are forced to reduce their market share or even cease their market participation, freeing the resources bound by their production activity and making them available for production by more productive firms. This process contributes to a more efficient production at the sector level, that is, aggregate productivity). Market regulation, however, is suspected to hinder this resource flow by keeping firms with low productivity in the market **(Frick and Sauer, 2016; 2018)**. The European milk quota system was introduced in 1984 and has put limit on the amount of milk EU dairy farmers produce each year. Under the quota system, if a farmer delivered more milk than his quota in any one year he was penalised financially. This involved paying a „superlevy“ on the over-quota amount **(European Commission, 2006)**. The purpose of the milk quotas was the control of structural surpluses resulting from imbalances between supply and demand for milk encouraged by subsidies to the sector **(Costa-Font and Revoredo-Giha, 2018)**. The quotas were originally introduced as temporary instrument for five years, but their use was prolonged several times.

The European Union’s dairy market seems to be slowly emerging from its recent “dairy crisis”, when EU farmers were faced with overproduction and the lowest commodity prices since 2009. However, most of the subsequent recovery and price stabilisation has been due to the stabilisation of global dairy prices due to decreased world and European Union production rather than any EU-led interventions **(Polet and Kuypers, 2017)**. Changes to the European Union's common agricultural policy with subsequent shift to greater market orientation for the European Union dairy industry caused sharp increase of the volatility of European Union dairy commodity. Price variability has become a serious problem for farmers, processors and consumers, which prefer stable prices because they provide increased planning security. Prices for European Union butter increased from 209 € per 100 kg in January 2009 to a high of 424 € in July 2011 before falling back to 241 € in May 2012. After this trough, butter prices started to rise again with a peak of 421 € in September 2013, followed by a trough of 283 € in December 2015 **(Bergmann et al., 2016)**.

After the European dairy quota abolition on the 1st of April 2015, the declining trend in domestic production followed in many countries and exposure to free European market significantly affected the competitiveness of domestic production. European dairy farmers become more dependent on the milk price of the world market **(Schullte and Musshoff, 2018)**. Coincidence of Russian embargo on European food products led the well-subsidised European Union farmers to export to the new markets, especially into West Africa. Analyses of the prospects of Croatian dairy industry under certain conditions of the common agricultural policy and the projections simulation showed that in 2025 in line with the common agricultural policy implementation there might be a decrease of dairy cows number, the raw milk price increase and the collected cow's milk amount increase compared to the five-year average of the 2008 to 2012 period. The positive effect was noted in productivity increase, which consequently may lead to increased deliveries to dairies **(Zrakic et al., 2015)**. In the context of increasing milk production in European Union and overproduction in the Czech Republic, compared to degree of so called self-sufficiency, and difficulties to market the raw milk due to the degree of market demand for milk and milk products but also due to the market position of dairy processors, it is necessary to adopt measures in order to achieve as high quality parameters as possible together with stability of those parameters **(Kovarova and Prochazkova, 2017)**. In some countries ownership concentration of fresh milk processing sector, together with a considerable dispersion and fragmentation of the primary production of raw cow's milk can led to insufficient supply and lack of basic dairy products on the market. The shortage phenomena are manifested in the circumstances of depressed and economically unsustainable low prices of production inputs: raw milk, and quantity decrease, accompanied by changes in the structure of the milk products final production **(Draskovic and Rajkovic, 2010)**.

The new common agricultural policy for the period from 2014 to 2020 for the milk sector, which will have as main component the removal of milk quotas after 2014, represents both a challenge and a threat for the farmers, whose raw milk prices may decrease, resulting in great losses. In order to adapt to the competition on the European Single Market, the dairy industry needs to get supported through investments, in the conditions in which there is a global conjuncture favourable to the consumption of dairy products, in which their world prices are expected to go up, on the basis of the increasing demand of the developing regions **(Grodea, 2014)**.

Environmental aspects of milk production

In the recent years, climate change has become one of the most discussed topic and therefore the environmental impact of livestock production is also more discussed because it is known to have a great impact on the environment **(Steinfeld et al., 2006)**. All food production has an environmental impact and therefore it is critical to

produce sufficient high-quality food from a finite resource supply while minimizing effects upon the environment **(Capper et al., 2009)**. The dairy sector, and agriculture in general, faces three key challenges: the need to produce more in order to feed a growing world population, to produce something different to adjust to consumer demands for food and new services and, last but not least, to produce better in respect of the environment, ecology and efficient resource use **(de Jong, 2013)**. Livestock industry, with dairy sector as one of the fastest growing, largely contributes to the atmospheric and soil pollution and greenhouse gases emissions on the global scale, that is, methane, carbon dioxide, and nitrous oxide. In order to successfully respond to the increasing global demand for raw milk and milk products, the dairy industry will have to mitigate future negative impacts on the environment, modifying the current production systems, and maintain at the same time high quality of final products at an economic priceacceptable for the consumers **(Bosnjak et al., 2018)**. Peculiarities of the implementation of the environmental component of the economic security of the enterprises of the dairy industry and the main aspects of state regulation of milk processing enterprises were investigated also in Ukraine **(Lysenko, 2014)**. To reduce the environmental impact of a product efficiently, it is crucial to consider the entire value chain of the product; that is, to apply life cycle thinking, to avoid suboptimisation and identify the areas where the largest potential improvements can be made. Carbon footprint of butter and dairy blend products, with the focus on fat content and size and type of packaging, including product waste at the consumer level, were investigated. The greatest share of greenhouse gas emissions associated with butter production occurred at the farm level; thus, minimizing product losses in the whole value, chain from cow to consumer, is essential for efficient production **(Flysjo, 2011)**.

Milk production quality and safety

Milk price is influenced by milk quality **(Hanus et al., 2008)** and milk safety. Regulation of food systems exists to ensure safety and enhance consumer confidence in the food which they purchase and consume **(Kendall et al., 2019)**. Farmers‘ production practices such as basic production environment and hygienic condition, disease prevention, and source and use of feed all contribute to the food safety of raw milk **(Yu et al., 2018)**. The likelihood of milk safety being important was two times higher in large farms compared to small-scale farms **(Paraffin et al., 2018)**. Improvement of milk safety can be achieved through good management practices by dairy farmers, market incentives, and increased efforts of various stakeholders and the adoption of best practices **(Lemma et al, 2018)**. Current market shares for premium welfare products are small in Europe **(de Graaf et al., 2016)**. Comparison of organic and convetionally produced milk quality showed, that the factors influencing milk composition, for instance diet, breed, and stage of lactation, have been studied individually, whereas interactions between multiple factors have been largely ignored. Lack of research on interactions between several influential factors and differences in trial complexity and consistency between studies, for instance sampling period, sample size, reporting of experimental conditions, complicate data interpretation and prevent us from making unequivocal conclusions **(Schwendel et al., 2015)**.

Scientific hypothesis

The primary aim of the paper is to prepare a prospective platform with a possible objective of its further future expansion into a regulatory policy intended to arrange for simplification of the controlling mechanisms of the market competition not only in a field of a price determination, but also for the other influencing aspects related to this process.

MATERIAL AND METHODOLOGY

The applied scientific methods correspond with the data examined by the analysis. They bear the specific aims which this paper deals with.

Data

The data comes from the database of the Statistical Office of the European Union (Eurostat). It contains the tables from the database “Selling prices of animal products (absolute prices): annual price” marked apri_ap_anouta **(Eurostat, 2018a)** and the database “Milk collection (all milks) and dairy products obtained: annual data” marked apro_mk_pobta **(Eurostat, 2018b)**. The explored time period covers the time period beginning in the year 2006 and ending in the year 2017.

The explained variable is represented by a price of raw cow’s milk. This analysed value is understood as a price of raw cow’s milk with fat content at a level of 3.7% coming from the agricultural holdings that are covered by the Eurostat data collection. It is stated in the euro currency.

On the other hand, there are the five explanatory variables, where cow’s milk production (CM), butter production (B), milk powder production (MP), cheese production (C), and farm milk production (FM) belong. Cow’s milk production describes amount of the whole output of the explored holdings expressed in tonnes. The remaining dimensions represent production of the appropriate products by the agricultural holdings expressed in tonnes too.

The data set covers all the countries whose data are available in the Eurostat database. The following countries are involved: Austria, Belgium, Croatia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom. The mentioned countries are ordered alphabetically according to their colloquial alternative name. They are called by the alternative names in the further text of the paper.

There is to remind that not all the countries have provided the data collected for the whole explored period. Therefore, the mean data are computed from the available values during the analysed time span.

Methodology

The substantial methodological approach applied in the paper is the sensitivity analysis in a form of the regression analysis. The data set entering the modelling process bears

a form of the panel data meaning there are two dimensions: a territorial dimension and a time dimension.

There are the three approaches of the panel linear regression employed in the analysis: the pooling approach, the random approach and the between approach. The pooling regression model represents a standard form of the panel linear regression model, whilst the random regression model has a strong informative value in a case of the models which random effects are present at. Also, the between regression model performes as a model, which is calculated with a concentration on time factor and that is why, it discards the information present due to the intragroup variability by means of the involved dimensions. Such a procedure is selected due to a demonstration of robustness of the source data and also to have a platform to review the obtained results and a possibility to compare them mutually. All the regression model types are executed also with a presence of a constant value.

The sequential elimination method is selected as the main modelling technique for the regression analysis. This means the worst variable is excluded from the further modelling process. The elimination factor is represented by the *p*-value of the appropriate independent variable. Hence, the variable with highest *p*-value is omitted in the successive regression model. There is to note that the sequential elimination is related to the elementary altogether model for a whole of the countries. This implies the cluster regression models aimed at the particular clusters are adapted to the elementary model. That is why, it involves variables in the final model of the modelling row has not to fulfil the requirement of the statistical significance.

The supplementary analytical approach is represented by a trivial way of the cluster analysis in a form of the interval division. Because of a number of the involved countries, a triplet of the clusters is selected for the further modelling process. This means the first cluster encompasses eight countries, the second one nine countries and the third one eight countries again. This dissection is done according to the dependent variable that is explained by the regression models. So, after taking into account the raw cow's milk price, all the explored countries are ordered according to this value and thus, they are divided into the three clusters. The first cluster contains the countries with the lowest price of the raw cow's milk, the second cluster involves the countries with the middle price values and the third one with the highest prices of the raw cow's milk. As this price the mean price of the raw cow's milk throughout the whole explored time span is considered. Because some of the values are not available, the mean price is calculated by the available figures.

The final step of the analytical process is to compute and to describe the values of a ratio of the regression coefficients related ot the particular variables involved in the regression models meaning quantitative relation between the same independent variable of the altogether regression model and the models assigned to the three individual clusters. Such a procedure demonstrates how many times the particular analysed variable influences the modelled raw cow's milk price in the cluster than in a whole analysed set of the countries.

Statisic analysis

The whole analysis is executed in the R statistical environment through its own programming language **(R Core Team, 2018)** with supplementary help of the *plm* package **(Croissant and Milo, 2008; Croissant et al., 2017)**. There is to remind that the absolutely best statistical significance is demonstrated by p-value at a level of or lower than a value of 2.2×10^{-16}. Such a state means *p*-value can be considered to be equal to zero.

RESULTS AND DISCUSSION

The regression analysis result reveals the interesting relations between the individual observed dimensions. They are described in more detail in the subsequent paragraphs.

The following tables demonstrate the outcome of the regression analysis. Table 1 visualises the regression coefficients of the variables involved in the pooling regression models together with their *p*-values. The first data column shows the estimated coefficients of the altoghether model, the second column relates to the first cluster model, the third column to the second cluster model and finally, the fourth column to the third cluster model. Table 2 is assigned to the pooling model with a constant value, the third table to the model with a concentration on the random effects and finally, the fourth one to the model with concentration on the time factor. A subsequent foursome of the tables from Table 5 to Table 8 make evident the found ratios of the regression coefficients assigned to the individual clusters. A comparison with the original altogether regression models proceeds in a same manner as it is applied in the first four tables. Table 9 demonstrates the overall statistical significance of the produced regression models by means of displaying the coefficient of determination R^2 together with its adjusted version.

The first remarkable fact is that one of the explored variables appers in no final regression model. An only such variable that is not significant in any of the regression models is the butter production. This implies the fact that changes in butter production does not have statistically significant influence on a level of the raw cow's milk at all. It is true even for all the employed panel data regression approaches.

As it is seen in Table 1, the statistically significant dimensions of the pooling regression model are the cow's milk production, the milk powder production, the cheese production, and the farm milk production. Table 2 confirms this result with a supplement of a constant value to the regression model. On the other hand, Table 3 shows that the regression model concentrated on the random effects considers the cow's milk production and the farm milk production with a constant value statistically significant. On the contrary, the time-oriented regression model contemplates the cheese production and the farm milk production with a constant value statistically significant.

The subsequent tables, Table 5 to Table 8, expose the desired coefficient ratios. The visualised outcome signifies the distribution of the individual countries among the examined clusters. It underlines the fact that the cheaper raw cow's milk price causes a concentration on the specific part of the production that is easier to produce.

There is to note some of the coefficient ratios bear high values in consideration of the other ones: this is caused by their statistical insignificance. It is demonstrated by the *p*-values visualised in the first four tables.

The coefficient of determination of the regression models reveal their statistical significance as a whole. Some present values mean absolute insignificance because of the employed methodology: the cluster-aimed regression models are constructed according to the altogether regression model. Hence, for instance, negative values come out. Regarding this approach, it is not unnecessary to consider it not suitable. Such an approach can be understood methodically too. It suggests avoiding possibly this procedure.

Table 1 The pooling panel linear regression models without a constant value.

Regressor	Value	Altogether	Cluster 1	Cluster 2	Cluster 3
CM	coefficient	-5.1990×10^{-9}	6.0214×10^{-9}	-1.3003×10^{-8}	-1.3258×10^{-8}
	p-value	0.0499	0.1372	0.0396	0.0924
MP	coefficient	5.6777×10^{-8}	2.6373×10^{-8}	3.3222×10^{-7}	3.2675×10^{-7}
	p-value	0.0476	0.2686	5.017×10^{-5}	0.0003
C	coefficient	-3.4969×10^{-8}	-1.3169×10^{-8}	-5.9182×10^{-8}	6.8076×10^{-8}
	p-value	0.0243	0.4826	0.0053	0.3083
FM	coefficient	9.0227×10^{-9}	-1.2838×10^{-9}	1.6613×10^{-8}	1.8747×10^{-8}
	p-value	0.0002	0.6603	0.0041	0.0567

Table 2 The pooling panel linear regression models with a constant value.

Regressor	Value	Altogether	Cluster 1	Cluster 2	Cluster 3
constant value	coefficient	3.5183×10^{-2}	3.2072×10^{-2}	4.0474×10^{-2}	2.0300×10^{-2}
	p-value	2.2×10^{-16}	2.2×10^{-16}	2.2×10^{-16}	1.493×10^{-9}
CM	coefficient	1.5899×10^{-9}	2.3390×10^{-9}	3.0842×10^{-9}	-9.0066×10^{-9}
	p-value	0.0054	0.0037	0.0183	0.0517
MP	coefficient	-1.0385×10^{-8}	-7.3714×10^{-9}	-4.5982×10^{-8}	2.2634×10^{-7}
	p-value	0.0872	0.1110	0.0146	4.630×10^{-5}
C	coefficient	1.2412×10^{-8}	1.2593×10^{-8}	1.4656×10^{-8}	3.2861×10^{-8}
	p-value	0.0003	0.0014	0.0019	0.3977
FM	coefficient	-2.4673×10^{-9}	-2.8082×10^{-9}	-4.1077×10^{-9}	1.0159×10^{-8}
	p-value	1.077×10^{-5}	1.482×10^{-5}	0.0016	0.0787

Table 3 The random panel linear regression models with a constant value.

Regressor	Value	Altogether	Cluster 1	Cluster 2	Cluster 3
constant value	coefficient	3.2742×10^{-2}	2.9874×10^{-2}	3.4881×10^{-2}	2.9856×10^{-2}
	p-value	2.2×10^{-16}	2.2×10^{-16}	2.2×10^{-16}	2.2×10^{-16}
CM	coefficient	1.7687×10^{-9}	2.4295×10^{-9}	2.2179×10^{-9}	-3.9127×10^{-9}
	p-value	0.0290	0.0056	0.2806	0.1743
FM	coefficient	-1.6414×10^{-9}	-1.7607×10^{-9}	-2.2138×10^{-9}	4.3724×10^{-9}
	p-value	0.0378	0.0216	0.2703	0.1358

Table 4 The between panel linear regression models with a constant value.

Regressor	Value	Altogether	Cluster 1	Cluster 2	Cluster 3
constant value	coefficient	3.2823×10^{-2}	3.1783×10^{-2}	3.4275×10^{-2}	3.2236×10^{-2}
	p-value	2.2×10^{-16}	1.24×10^{-5}	1.034×10^{-5}	8.175×10^{-5}
C	coefficient	1.0795×10^{-8}	1.9384×10^{-8}	5.3594×10^{-9}	2.0777×10^{-8}
	p-value	0.0633	0.0350	0.5619	0.5609
FM	coefficient	-6.8792×10^{-10}	-1.2669×10^{-9}	-4.1043×10^{-10}	-9.7976×10^{-10}
	p-value	0.0911	0.0784	0.5278	0.6703

Table 5 The coefficient ratios of the pooling panel linear regression models without a constant value.

Regressor	Cluster 1	Cluster 2	Cluster 3
CM	-1.158182	2.501020	2.550129
MP	0.464509	5.851331	5.755038
C	0.376600	1.692393	-1.946730
FM	-0.142285	1.841202	2.077740

Table 6 The coefficient ratios of the pooling panel linear regression models with a constant value.

Regressor	Cluster 1	Cluster 2	Cluster 3
Constant value	0.894540	1.128878	0.566189
CM	1.471146	1.939878	-5.664858
MP	0.709832	4.427843	-21.795754
C	1.014575	1.180769	2.647442
FM	1.138180	1.664878	-4.117367

Table 7 The coefficient ratios of the random panel linear regression models with a constant value.

Regressor	Cluster 1	Cluster 2	Cluster 3
Constant value	0.912398	1.065335	0.911851
CM	1.373566	1.253971	-2.212157
FM	1.072683	1.348731	-2.663761

Table 8 The coefficient ratios of the between panel linear regression models with a constant value.

Regressor	Cluster 1	Cluster 2	Cluster 3
Constant value	0.968330	1.044241	0.982140
C	1.795545	0.496445	1.924596
FM	1.841604	0.596625	1.424231

Table 9 Statistical significance of the models.

Model	Type	R^2	Adjusted r^2
Pooling-without a constant value	altogether	0.13006	0.10633
	cluster 1	0.00105	-0.10229
	cluster 2	0.35898	0.30967
	cluster 3	0.54295	0.50263
Pooling-with a constant value	altogether	0.22469	0.19624
	cluster 1	0.61234	0.55696
	cluster 2	0.44463	0.38617
	cluster 3	0.60762	0.56006
Random-with a constant value	altogether	0.10001	0.09306
	cluster 1	0.1829	0.16272
	cluster 2	0.1429	0.12342
	cluster 3	0.08547	0.06370
Between-with a constant value	altogether	0.15165	0.07453
	cluster 1	0.64156	0.49818
	cluster 2	0.07149	-0.23802
	cluster 3	0.13474	-0.21136

The difference of earlier observed market situations with high price levels is that it is unilaterally based on the fat component of the milk. Changes of milk lipid composition in term of its enrichment are doable by the manipulation of the composition of animal diets or by the genetic engineering techniques **(Świątkiewicz et al., 2015)**. The contrast to the milkfat situation are the markets of the nonfat components. Large public stocks of skim milk powder are the major obstacle that prices might stabilise at higher levels, and therefore volatility in this sector will be limited. Skim milk powder has nutritional benefits and functional properties, including high calcium and potassium, a low-fat content, excellent gelation, emulsification and foaming properties **(Burke et al., 2018)**. The returns from the different dairy products adjust with some delay to the mix of prices for milk fat, which are mainly depending on butter, and the prices realised in the nonfat part which will mainly depend on the situation in the skim milk powder market.

More cheese and more whole milk powder would also absorb larger volumes of milkfat which are not available for butter and cream. The trade in fresh products like liquid milk, yogurt, cream and other items is developing with strong rates, but modest in terms of milk equivalents when compared to milk powders, butter and cheese **(Richarts, 2018)**.

CONCLUSION

In the submitted article regression analysis was used to verify the impact of five factors, where cow's milk, butter, milk powder, cheese, and farm milk production belong, to the price of raw cow's milk. Regression analysis of panel data claiming territorial and time dimensions coming from the countries whose data is available in the Eurostat database was applied using three techniques, which the pooling, the random and the between approach are. The complete data is accessible for Austria, Belgium, Croatia, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom. Supplementary analytical approach represented by the cluster analysis resulted into three clusters

(containing eight; nine; and eight countries respectively), selected for the further modelling process.

Results of the regression analysis showed no influence of butter production to the level of raw cow's milk. The obtained outcome from the analysis validates the desired aim of the paper in a way that it prepares a potential platform for the further research by demonstrating the relations between each individual pair of the explored variables. The illustrated coefficient ratios reveal the possible succession of the further steps to construct a regulatory policy.

REFERENCES

Bergmann, D., O'Connor, D., Thümmel, A. 2016. An analysis of price and volatility transmission in butter, palm oil and crude oil markets. *Agricultural and Food Economics*, vol. 23, no. 4, p. 1-23. https://doi.org/10.1186/s40100-016-0067-4

Bosnjak, R., Prpic, Z., Ugarkovic, N. K., Konjacic, M., Vnucec, I. 2018. Possibilities for mitigating the environmental footprint of dairy ruminants. *Mljekarstvo*, vol. 68, no. 3, p. 155-168. https://doi.org/10.15567/mljekarstvo.2018.0301

Burke, N., Southern, M., Ryan, A., Adley, C. C. 2018. Investigating the current skim milk powder inspection strategies for improvements in process optimisation, *Food Control*, vol. 94, p. 17-21.

Capper, J. L., Cady, R. A., Bauman, D. E. 2009. The environmental impact of dairy production: 1944 compared with 2007. *Journal of Animal Science*, vol. 878, no. 6, p. 2160-2167. https://doi.org/10.2527/jas.2009-1781

Costa-Font, M., Revoredo-Giha, C. 2018. An empirical analysis of UK milk contract prices 2004-2016. *Agribusiness*, vol. 34, no. 1, p. 112-141. https://doi.org/10.1002/agr.21531

Croissant, Y., Millo, G. 2008. Panel Data Econometrics in R: The plm Package. *Journal of Statistical Software*, vol. 27, no. 2, p. 1-43. https://doi.org/10.18637/jss.v027.i02

Croissant, Y., Millo, G., Tappe, K., Toomet, O., Kleiber, C., Zeileis, A., Henningsen, A., Andronic, L., Schoenfelder, N. 2017. *Package plm* [online]. Vienna, Austria : R Foundation for Statistical Computing. Available at: https://cran.r-project.org/web/packages/plm/plm.pdf.

de Graaf, S., Van Loo, E. J., Bijttebier, J., Vanhonacker, F., Lauwers, L., Tuyttens, F. A. M., Verbeke, W. 2016. Determinants of consumer intention to purchase animal-friendly milk. *Journal of Dairy Science*, vol. 99, no. 10, p. 8304-8313. https://doi.org/10.3168/jds.2016-10886

de Jong, P. 2013. *Sustainable Dairy Production*. 1st ed. Ede, Netherlands : John Wiley & Sons, Ltd. 266 p. ISBN 978-0-470-65584-9.

Draskovic, B., Rajkovic, Z. 2010. Monopoly, government policy and milk shortage in Serbia. *Ekonomika Poljoprivreda-Economics of Agriculture*, vol. 57, no. 2, p. 478-485.

European Commission. 2006. *Milk and Milk Products in the European Union-August 2007* [online]. Brussels, Belgium : European Commission, DG Agriculture and Rural Development. 27 p., ISBN 92-79-02199-0. Available at: https://ec.europa.eu/agriculture/publi/fact/milk/2007_en.pdf.

Eurostat. 2016. Utilisation of milk, EU-28 [online]. In *Eurostat database apro_mk_pobta*. Available at: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=File:Utilisation_of_milk,_EU-28,_2016.png.

Eurostat. 2018a. Selling prices of animal products (absolute prices) – annual price (from 2000 onwards). In *Database apri_ap_anouta*. Available at: https://data.europa.eu/euodp/data/dataset/sohBGjreMpODNZl9N6UW2Q.

Eurostat. 2018b. Milk collection (all milks) and dairy products obtained: annual data. In *Database apro_mk_pobta*. Available at: https://data.europa.eu/euodp/data/dataset/I0YHcLXN2PLoaJknlOEtew.

Flysjo, A. 2011. Potential for improving the carbon footprint of butter and blend products. *Journal of Dairy Science*, vol. 94, no. 12, p. 5833-5841. https://doi.org/10.3168/jds.2011-4545

Frick, F., Sauer, J. 2016. Deregulation and Productivity-Empirical Evidence on Dairy Production. *American Journal of Agricultural Economics*, vol. 100, no. 1, p. 354/378. https://doi.org/10.1093/ajae/aax074

Frick, F., Sauer, J. 2018. Deregulation and Productivity: Empirical Evidence on Dairy Production. *American Journal of Agricultural Economics*, vol. 100, no. 1, p. 354-378. https://doi.org/10.1093/ajae/aax074

Gerosa, S., Skoet, J. 2012. Milk availability Trends in production and demand and medium-term outlook [online]. *Agricultural Development Economics Working Papers*, no. 12-01. Rome, Italy : Food and Agriculture Organization of the United Nations. 39 p. Available at: http://www.fao.org/docrep/015/an450e/an450e00.pdf.

Grodea, M. 2011. Milk market and its volatility a regional approach. *Scientific Papers-Series Management Economic Engineering in Agriculture and Rural Development*, vol. 11, no. 2, p. 69-72.

Grodea, M. 2014. The critical milk evolution after quota removal, globalization and intercultural dialogue: multidisciplinary perspectives-economy and management. In *2nd International Conference on Globalization, Intercultural Dialogue and National Identity*. Târgu Mureș, Romania : Arhipelag XXI Press. p. 744-754. ISBN 978-606-93691-3-5.

Hanus, O., Vegricht, J., Frelich, J., Macek, A., Bjelka, M., Louda, F., Janu, L. 2008. Analysis of raw cow milk quality according to free fatty acid contents in the Czech Republic. *Czech Journal of Animal Science*, vol. 53, no. 1, p. 17-30. https://doi.org/10.17221/2717-CJAS

Jasińska-Biliczak, A., Sitkowska, R. 2014. Influence of small and medium enterprises sector at the change of innovation potential of Polish regions. *Grant Journal*, vol. 3, no. 1, p. 57-61. Available at: http://www.grantjournal.com/issue/0301/PDF/0301sitkowska.pdf.

Kendall, H., Kuznesof, S., Dean, M., Chan, M. Y., Clark, B., Home, R., Stolz, H., Zhong, Q. D., Liu, Ch., Brereton, P., Frewer, L. 2019. Chinese consumer's attitudes, perceptions and behavioural responses towards food fraud. *Food Control*, vol. 95, p. 339-351. https://doi.org/10.1016/j.foodcont.2018.08.006

Kovarova, J., Prochazkova, K. 2017. Influence of the season progress of the quality characteristics on to the purchase prices of milk in context of the market situation. *Deturope-The Central European Journal of Regional Development and Tourism*, vol. 9, no. 1, p. 35-46.

Kowal, J., Jasińska-Biliczak, A., Hafner, J. 2016. Innovative Capacity in Small Regional Enterprises in Transition Economies: An Exploratory Study in Poland. In *22nd Americas Conference on Information Systems*. San Diego, United States of America : Association for Information Systems. ISBN 978-0-9966831-2-8.

Lemma, H., Mengistu, A., Kuma, T., Kuma B. 2018. Improving milk safety at farm-level in an intensive dairy

production system: relevance to smallholder dairy producers. *Food Quality and Safety,* vol. 2, no. 3, p. 135-143. https://doi.org/10.1093/fqsafe/fyy009

Lemoine, R. 2016. European dairy sector rising to all challenges. *Revue Laitière Française*, no. 767, p. 2-3. Available at: https://www.rlf.fr/reussir/fichiers/107/JYE21Y0F_1.pdf.

Lysenko, M. 2014. The problem of ensuring the economic security of dairy industry in Ukraine. *Economics & Sociology*, vol. 7, no. 2, p. 160-171.

Marquer, P. 2015. *Portrait of the EU milk production sector*. Available at: https://ec.europa.eu/eurostat/statistics-explained/index.php/Archive:Milk_and_dairy_production_statistics.

Muehlhoff, E., Bennett, A. McMahon, D. 2013. *Milk and Dairy Products in Human Nutrition*. Rome, Italy : Food and Agriculture Organization of the United Nations. 376 p. ISBN 978-92-5-107863-1. Available at: http://www.fao.org/docrep/018/i3396e/i3396e.pdf.

Mura, L., Gasparikova, V. 2010. Penetration of small and medium sized food companies on foreign markets. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 58, no. 3, p. 157-163. https://doi.org/10.11118/actaun201058030157

Mura, L., Mazák, M. 2018. Innovative activities of family SMEs: case study of the Slovak regions. *On-line Journal Modelling the New Europe*, no. 27, p. 132-147. https://doi.org/10.24193/OJMNE.2018.27.06

Paraffin, A. S., Zindove, T. J., Chimonyo, M. 2018. Perceptions of Factors Affecting Milk Quality and Safety among Large- and Small-Scale Dairy Farmers in Zimbabwe. *Journal of Food Quality*, vol. 2018, 7 p. https://doi.org/10.1155/2018/5345874

Polet, Y., Kuypers, K. 2017. *EU Dairy Markets Still Looking for Equilibrium after Dairy Crisis* [online]. Global Agricultural Information Network Report, no. E 17022. Belgium : United States Department of Agriculture Foreign Agricultural Service. Available at: https://gain.fas.usda.gov/.

R Core Team. 2018. R: Language and Environment for Statistical Computing [online]. Vienna, Austria : R Foundation for Statistical Computing. Available at: https://www.R-project.org.

Richarts, E. 2018. *EU Dairy Markets, Situation and Outlook January-June 2018* [online]. Modena, Italy : Daily Economic Condulting Firm. Available at: https://www.clal.it/downloads/outlook/ForecastH12018.pdf.

Schullte, H., Musshoff, O. 2018. Insurance prospects after the European dairy quota abolition. *Agricultural Finance Review*, vol. 78, no. 2, p. 223-232. https://doi.org/10.1108/AFR-06-2017-0047

Schwendel, B. H., Wester, T. J., Morel, P. C. H., Tavendale, M. H., Deadman, C., Shadbolt, N. M., Otter, D. E. 2015. Invited review: Organic and conventionally produced milk: An evaluation of factors influencing milk composition. *Journal of Dairy Science*, vol. 98, no. 2, p. 721-746. https://doi.org/10.3168/jds.2014-8389

Stasiak-Betlejewska, R. 2015. Clusters as the element of eco-innovations promoting in the European construction on the Polish example. *Acta Oeconomica Universitatis Selye*, vol. 4, no. 2, p. 182-197.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., de Haan, C. 2006. *Livestock's Long Shadow. Environmental Issues and Options*. 1st ed. Rome, Italy : Food and Agriculture Organization of the United Nations, 390 p. ISBN 978-92-5-105571-7.

Świątkiewicz, S., Świątkiewicz, M., Arczewska-Włosek, A., Józefiak, D. 2015. The use of genetic engineering techniques to improve the lipid composition in meat, milk and fish products: A Review. *Animal*, vol. 9, no. 4, p. 696-706. https://doi.org/10.1017/S1751731114003012

Velioglu, S., D., Ercioglu, E., Bozaci, I. H. 2017. Rapid discrimination between buffalo and cow milk and detection of adulteration of buffalo milk with cow milk using synchronous fluorescence spectroscopy in combination with multivariate methods. *Journal of Dairy Research*, vol. 84, no. 2, p. 214-219. https://doi.org/10.1017/S0022029917000073

Yu, H. L., Wang, H. H., Li, B. L. 2018. Production system innovation to ensure raw milk safety in small holder economies: the case of dairy complex in China. *Agricultural Economics*, vol. 49, no. 6, p. 787-797. https://doi.org/10.1111/agec.12460

Zrakic, M., Salputra, G., Levak, V. 2015. Potential impact of EU Common Agriculture Policy on Croatian dairysector-modelling results. *Mljekarstvo*, vol. 65, no. 3. https://doi.org/10.15567/mljekarstvo.2015.0306

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STUDYING THE IMPACT OF NON-TRADITINAL SUPPLEMENTS ON THE QUALITY OF THE MINCED RABBIT MEAT PRODUCTS

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ABSTRACT

The topical challenge of our era is the development of such compositions of innovative polyfunctional products, which take into account, to the maximum degree, the effect of mutual enrichment of animal and vegetable products, physiological substances, the capacity of dietary fibers to remove harmful substances from the body and the capacity of probiotics to maintain micro-ecological balance in the gastro-intestinal tract. One of the priority areas of the implementation of these issues the development of technologies of rabbit meat products fortified with plant raw materials and prebiotic ingredients. The purpose of the work is to study the impact of vegetable supplements and lactulose on the quality of the minced meat products. When performing this work, there were used the modern, standard, commonly accepted methods of research, which are in compliance with solving the set objectives. Statistical processing of the results obtained and the evaluation of the reliability of data were carried out by the mathematical statistics methods using the IBM SPSS Statistics for Windows. There have been studied the chemical compositions of the brush rabbit meat bred in Georgia, as well as of haricot bean, pea and amaranth. It is reasonable to use the above-mentioned plant raw material and lactulose use as bioactive supplemented in the production of animal products. There has been justified the appropriateness of mentioned plant raw materials and lactulose as the bio-correcting supplements in the technology for producing animal food products. In order to control and identify the functionality of products, there have been identified the critical control points, such as: preparing and dosing functional raw materials, the introduction stages and an equal distribution of formula components. There have been established the hydration conditions and modes of haricot bean, pea and amaranth flours. There are shown the advantages of their hydration in mineral water, the optimal irrigation modulus has been determined. Also, there have been determined the maximum permissible levels of vegetable supplements and lactulose to be added into the minced rabbit meat, which have a positive impact on the functional-technological properties of semi-finished and finished products, in particular, improve the water binding and water holding capacities of the minced meat, increase the yield of product, as well as improve organoleptic indices of its quality, in comparison with a reference sample. Based on the study of the microbiological characteristics of developed products, it has been demonstrated that the number of mesophilic-aerobic and facultative-anaerobic microorganisms does not exceed the sanitary norms and standards, no *E. coli* group bacteria and pathogenic microorganisms were detected, including *Salmonella* that is in line with the microbiological safety hygienic requirements and norms. The obtained data set indicates the appropriateness of using a new type of product in a functional, dietetic and preventive nutrition.

Keywords: minced rabbit meat; plant raw materials; lactulose; semi-finished product; functional-technological properties

INTRODUCTION

Nutrition makes part of the factors determining health, potential and the development perspectives of the nation. Against the background of the less mobile modern lifestyle and related low energy cost, there is an acute lack of proteins, especially of animal origin in the population's diet. The consumption of animal fats and easily digested carbohydrates rose, and there were discovered high share of saturated fatty acids and cholesterol and a lack of unsaturated fatty acids. At the same time, the deficiency of vitamins, micro-and macro-elements was revealed. These deviations cause the abnormality of the immune status, reducing resistance of human body to infections and other negative environmental factors.

The most realistic way to solve this problem is to develop such compositions of innovative functional products, which take into account, to the maximum degree, the effect of mutual enrichment of animal and vegetable proteins, vitamin provision and optimal ratio, the range of actions of mineral and other physiological substances, the capacity of

dietary fibers to remove harmful substances from the body and the capacity of probiotics to maintain micro-ecological balance in the gastro-intestinal tract **(Arihara, 2006; Dalle Zotte and Szendrő, 2011; Decker and Park, 2010; Cavani et al., 2009; Shenderov, 2001; Tutelyan, 2003; Wambui et al., 2016; Weiss et al., 2010)**.

One of the priority areas of the implementation of these issues is the management of the process of delivering biologically active substances to the human body through the enrichment of meat products. In particular, the inclusion of such bio-correcting plant raw materials or preparations in stuffed semi-finished meat product formulas, which have a certain physiological effect **(Gorodok, 2009; Fomenko, 2011; Kidyaev, Litvinova and Jamalov, 2017; Kolenik et al., 2014; Maksimov et al., 2013; Okara et al. 2008; Samchenko and Mishina, 2013; Sharipova, 2014; Shtakhova, 2008; Vaytanis, 2012; Yartseva and Dolganova, 2010; Zinina, 2016)**.

Accordingly, the development of technologies of rabbit meat products fortified with plant raw materials and prebiotic ingredients is of practical interest.

Rabbit meat is distinguished by a high content of complete animal proteins and a moderate content of fats, and its morphological characteristics, technological properties, nutritional and biological value are much higher compared to beef, pork and poultry, the percentage of flesh is quite higher, the percentage of connective tissue is significantly lower, and the level of cholesterol, purine compounds and sodium is lower, and the meat consistency itself is fine-fibred and tender. Due to these qualities, rabbit meat is considered to be a dietetic food intended for a functional nutrition, which has no contraindications towards various diseases **(Antipova and Vasilenko, 2003; Dalle Zotte, 2004; Hernández and Gondret, 2006; Nistor et al., 2013; Pla, Pascual and Ariño, 2004; Tavdidishvili, Khutsidze and Tsagareishvili, 2018; Volkova et al, 2009)**.

Of plant raw materials, special mention should be made of leguminous crops and amaranth **(Erashova, Pavlova and Kashkarova, 2010; Sharipova, 2014; Shelepina, 2016; Shtakhova, 2008; Tavdidisvili and Lipatova, 2018; Vasneva and Bakumenko. 2010; Zharkova and Miroshnichenko, 2012)**.

Leguminous crops are distinguished by a high content of vegetable proteins, which, on account of their content, are close to animal proteins, and are characterized by substantial content of B-group vitamins, mineral substances and dietary fibers having preventive and nutritional properties.

As a functional supplement, greater attention should be given to the amaranth. It contains a significant amount of proteins and other biologically active substances. Along with high nutritional value, the amaranth has the antioxidant properties and a hypoallergenic potential. Special mention should be made of the fact that it contains substance called squalene, which has antimicrobial, anti-carcinogenic, fungicidal activity and the toxin absorbing capacity.

One of the ways to create food supplements having the prebiotic properties, is the introduction of lactulose into the minced meat, which can ensure high bifidogenic activity under conditions of a lower dosage. Along with the bifidogenic properties, it also has a salutary and curative effect: stimulates the growth of useful microflora and the intestinal peristalsis, suppresses the vitality of pathogenic microflora, lowers blood cholesterol level, reduces the risk of atherosclerosis development, protects from diarrhea, and increases the calcium absorbing capacity **(Dolgova, Khramova and Proskurina, 2013; Grigoriev and Yakovenko, 2000; Khramova, 2011; Leonidov, 2013; Zinina, 2013; Hernández and Gondret, 2006)**.

Thus, the studies aimed at producing high-quality food products having a positive impact on the human body are of great relevance and social importance. The purpose of the work is to study the impact of vegetable supplements and lactulose on the quality of the minced meat products.

Scientific hypothesis

Addition of non-traditional bio-correcting ingredients to the minced rabbit meat improves water binding capacity and water-holding capacity, as well as its qualitative and organoleptic indices.

MATERIAL AND METHODOLOGY

The studies were carried out in the laboratories of the Department of Food Technologies of Akaki Tsereteli State University. As targets for the studies, there have been selected: the brush rabbit meat haricot bean, pea, amaranth and flour produced from them; lactulose syrup; mineral water “Lugela”, model meat and vegetable semi-finished and finished products.

The chemical composition of plant raw materials was determined in accordance with the following indicators in mean sample of raw materials: moisture, protein, fat, and ash contents. Moisture content was determined at a temperature of 105 °C by method of drying weight sample to constant weight **(GOST ISO 24557-2015)**; total nitrogen was determined by modified Kjeldahl method **(GOST 25011-81)**; protein content was determined in accordance with the amount of nitrogen considering the conversion coefficient (6.25); fat content was determined Soxhlet method **(GOST 23042-86)**; ash content was determined by method of ashing through pre drying **(ISO 2171:2007)**. The functional-technological properties of the minced meat, such as: water binding capacity - by pressing with method of Grau and Hamm, the water holding capacity - by the difference between the moisture content existing in stuffing and the amount of moisture released during the thermal treatment **(Antipova, Glotova and Rogov, 2001)**.

Organoleptic indices were determined on a scale of 1 to 9 according to the following characteristics: appearance, color, smell, taste, consistency and succulence.

During the microbiological analysis, the number of the mesophilic-aerobic and the facultative-anaerobic microorganisms in samples were determined by **State Standard GOST 10444.15-94** "Food products. Methods for determining the amounts of the mesophilic aerobic the facultative anaerobic microorganisms"; the number of the *E. coli* group bacteria was determined by **State Standard GOST 50454-92** "Meat and meat products. Detection and registration of possible coliform bacteria and *Escherichia coli*”, and the amount of *Salmonella* - by **State Standard GOST P 50455-92** "Meat and meat products. *Salmonella* detection".

Producers of chemicals and instruments used for analyses are: Stavropol plant of chemicals (Russia), Chemical reagent (Tbilisi, Georgia); Alphalab (Tbilisi, Georgia), Oxjen import (Tbilisi, Georgia).

Table 1 The brush rabbit meat's chemical composition and energy cost.

Indicator	Content	Indicator	Content
Moisture, %	71.2	Ratio of fat: protein	0.34
Protein, %	21.1	Energy value, kJ	538.4
Fat, %	7.1	Water binding capacity, %	65.09
Ash, %	1.22	pH	5.71

Table 2 Chemical compositions of haricot bean, pea and amaranth.

Indicator	Haricot bean	Pea	Amaranth
		content in 100 g grain	
Moisture, %	13.7 ±0.32	14.1 ±0.27	12.6 ±0.30
Protein, %	28.5 ±0.36	29.2 ±0.25	18.1 ±0.28
Fat , %	1.8 ±0.26	2.1 ±0.30	3.6 ±0.26
Ash, %	3.6 ±0.28	3.9 ±0.27	0.8 ±0.18
Carbohydrates, g	57.3 ±0.34	55.8 ±0.31	61.3 ±0.32
Starch, g	47.3 ±0.38	63.1 ±0.18	53.2 ±0.32
Food fibers, g	10.2 ±0.34	10.6 ±0.29	0.9 ±0.27

Table 3 Quality indicators of the haricot bean, pea and amaranth flours.

Flour samples	Flour color	Crushing thickness – the mass remained on a 35 sieve, %	Taste	Aroma
Red haricot bean	Gray, with red layer particles	1.3	Specific, characteristic of leguminous crops	Normal, characteristic of leguminous crops
Yellow pea	Yellow	1.3	Specific, characteristic of leguminous crops	Normal, characteristic of leguminous crops
Amaranth	Dark gray	1.2	Characteristic pleasant taste of peanut	Characteristic pleasant aroma of peanut

Statistic analysis

To analyse the functional-technological and quality indicators of minced rabbit meat product is conducted a statistical analysis of the obtained data, the reliability of the obtained data was evaluated by the mathematical statistics methods using the Windows IBM SPSS Statistics software program (version 20.0). We calculated the arithmetic average of the measured value. Then, we computed the error of each measurement and calculated the squared errors in order to compute the absolute measurement error. We selected the value of reliability P = 0.95. Based on the number of measurements and the value of reliability, Student's coefficient equals t = 3.77 (Figures 2 and Figure 3) **(Romanov and Komarov, 2002).** Graphical interpretation of the results was made by using Microsoft Excel. In Tables and Figure, there are presented the data of typical tests, and each value is an average of at least ten determinations

RESULTS AND DISCUSSION

In conformity with the goal to be reached, using the principles of healthy eating theory, at the first stage of the work we chose the main formula ingredients of new types of pro haricot bean, pea and amaranth flour, as well as mineral water and lactulose.

The brush rabbit meat was selected for a study. Its chemical composition and some physical-chemical characteristics are presented in Table 1. The Table shows that rabbit meat contains a substantial amount of proteins, but the fat content is quite low, indicating that rabbit meat as healthy low-calorie.

The Table 2 shows that haricot bean, pea and amaranth are distinguished by a high content of proteins, carbohydrates and dietary fibers. In addition, the contents of proteins are almost the same in haricot bean and pea, and relatively lower in amaranth (by 36%). As for dietary fibers, their largest amounts are contained in haricot bean and pea, and their content is by 28 - 29% lower in amaranth.

The content of proteins and dietary fibers in studied plant raw materials justified expediency of using the functional bio-correcting supplements in the technologies for producing animal food products, raw material can be used in dietetic and preventive nutrition.

The obtained data on the chemical composition of rabbit meat are in conformity with available in the literature and the similar data that we obtained. For example, according to data of **Volkova (2009)**, the protein content in rabbit meat is 22.06%, and according to data of **Nistor et al. (2013)** - 21.2%, according to data of **Zhidik (2017)** - 18.97%, according to data of **Sautkin (2010)** - 22.3%, according to **Tavdidishvili, Khutsidze and Tsagareishvili (2018)** -

20%. According to data of these authors, the fat content is 4.85%, 9.2%, 4.69% and 3.9%, respectively, and the ash content - 1.29%, 1.1%, 1.68% and 1.06%, respectively as well.

Thus, the nutritional value of rabbit meat confirms the relevance of its in the production of functional-purpose meat-plant stuffed semi-finished products.

We have also studied the chemical compositions of haricot bean, pea and amaranth (Table 2). We took haricot bean, pea and amaranth as follows: the grains were cleared of the grain and rubbish impurities, we removed the damaged ones, washed and dried up to no more than 14% of moisture content. The dried grains were crushed to obtain particles, which pass through the hole with a diameter of 2.5 - 3 mm. Then, on a laboratory mill, we crushed it the powdery state and sifted through the #35 sieve. Qualitative indicators of the obtained haricot bean, pea and amaranth flour samples are shown in Table 3.

At the next stage of research, in order to develop the scientifically justified technologies, as well as to control and identify the functionality of products, there have been identified the critical control points, such as: preparing and dosing functional raw materials, the introduction stages, and an equal distribution of formula components.

It has been revealed that the flour in the dry form was unevenly distributed in the minced meat, while the supplementation in the hydrated form had a positive effect on the organoleptic indices of the minced meat - it was distributed equally in the entire mass.

For the hydration, we used tap water and mineral water "Lugela". The use of the latter is justified by the fact that "Lugela" water is a natural 9.5 percent concentration of $CaCl_2$, in which $CaCl_2$ is represented in the ionized form (Ca^{2+}, Cl^-), which facilitates its bioavailability **(Ardia and Janelidze, 1999; Georgian Soviet Encyclopedia, 1983)**.

We have determined that the hydration is 20 - 30 minutes for haricot bean and pea flours, for amaranth 40 - 45 minutes. The hydration was carried out at a temperature of 18 - 200 °C. The obtained results are shown in Figure 1.

Figure 1 illustrates that the process of hydration is more intensive in mineral water than in usual water, and besides, the haricot bean and pea flours have higher hydration capacities (133 and 120%, respectively), and the amaranth flour has lower hydration capacity (96%). Therefore, we have considered it worthwhile to use mineral water for the hydration of haricot bean, pea and amaranth flours.

We have also determined the time of swelling of flour samples, for which we have studied the process of their swelling. The most intensive swelling occurred in the case of the haricot bean and pea flours during the first 20 minutes, and then the intensity of water absorption was reduced, but in the case with the amaranth flour - during the 40 minutes, so the duration of swelling was determined at 30 minutes for the haricot bean and pea flours, and at 40 minutes - for the amaranth flour.

At the next stage of the study, we determined the optimal number of supplements to be added into the minced rabbit meat - the hydrated flours and lactulose. It was understood that by these supplements we replaced the same amount of the minced meat.

As a reference sample, we have taken a natural stuffing cooked through the traditional technology. On its basis, we prepared 3 types of semi-finished products with the following main ingredients: 1 - rabbit meat, haricot bean flour, lactulose, Georgian spices; 2 - rabbit meat, pea flour, lactulose, Georgian spices; 3 - rabbit meat, amaranth flour, lactulose, Georgian spices.

The amount of flours of the hydrated be haricot bean, pea and amaranth to be added into the minced meat varied from 15 to 30% with the irrigation modulus ratios of 1:2; 13 and 1:4.

As a result of organoleptic estimation of semi-finished products, we have chosen the optimal irrigation modulus ratio for haricot bean or pea flour is 1:3, for amaranth flour 1:2, because with the higher irrigation modulus ratio, the minced meat was significantly fluidized.

We have studied the impact of the hydrated supplements on the functional-technological properties of the minced meat - the water binding capacity and water-holding capacity. As is known, the higher these indicators, the less moisture is lost during heat treatment, the higher the quality, the yield of finished products and their organoleptic characteristics: tenderness, juiciness and taste properties.

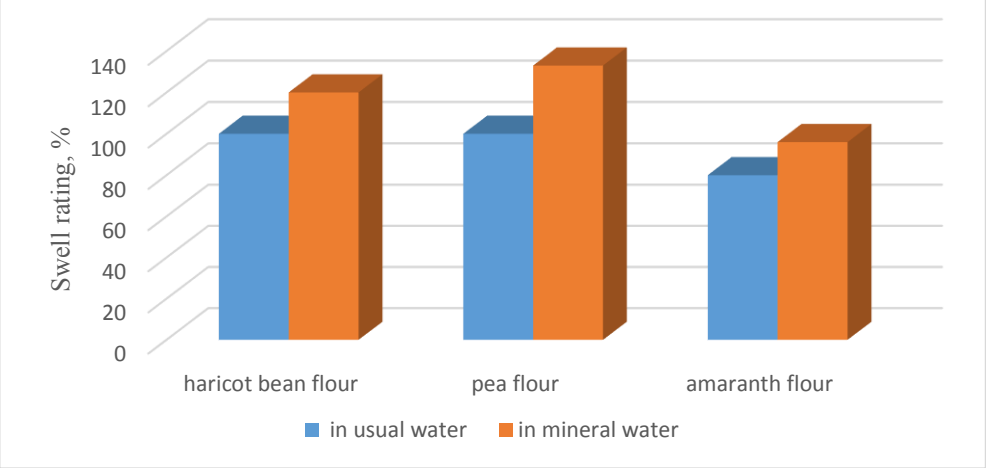

Figure 1 Swell rating of haricot bean, pea and amaranth flours in usual water and in mineral water.

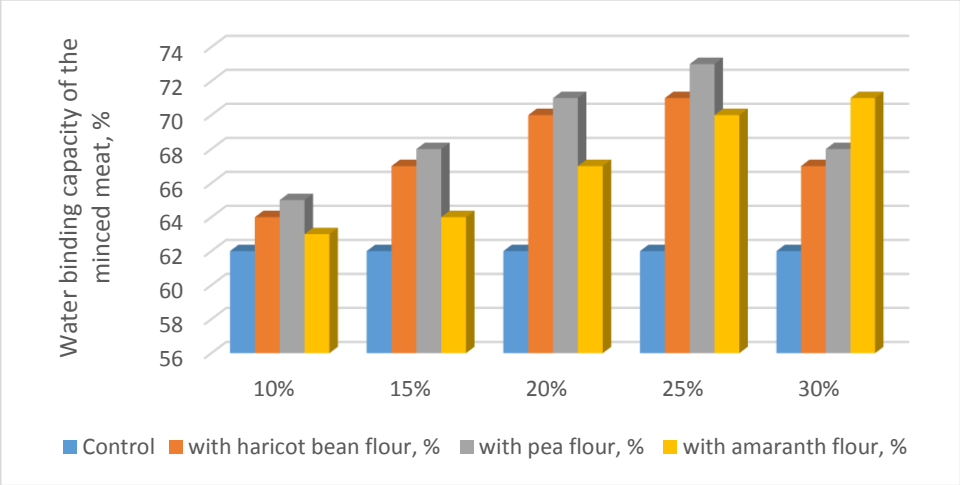

Figure 2 Water binding capacity of the minced meat (p <0.05).

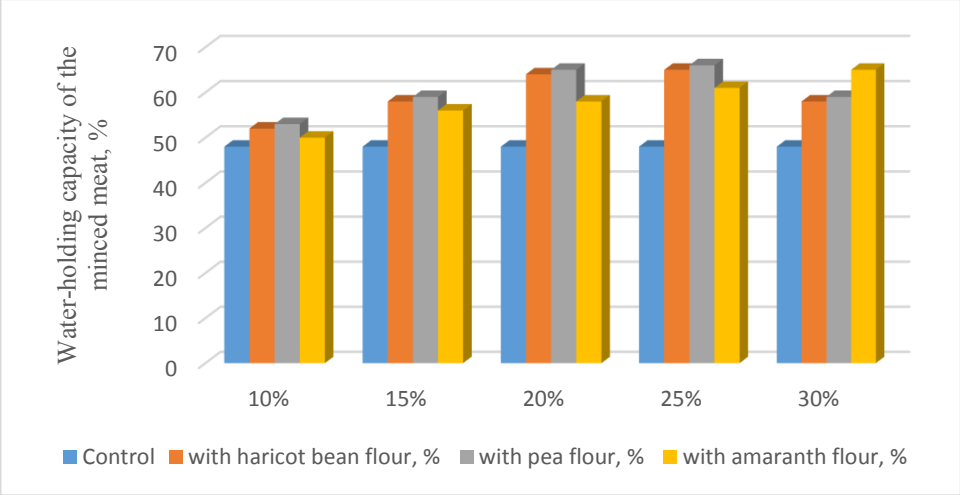

Figure 3 Water-holding capacity of the minced meat (p <0.05).

The obtained results are shown in Figures 2 and Figure 3. The diagrams illustrate that the increase in the dosage of all kinds of vegetable supplements leads to increasing water binding capacity and water holding capacity, and this can be explained by the fact that water is held due to significant amount of starch and cellulose existing in the supplements, while the relatively high water binding capacity is typical of the pea flour, and then of the haricot bean and amaranth flours.

Adding of 15 and 30% of the hydrated haricot bean flour increases the water binding capacity of the minced meat by 7.5 - 11.4% in comparison with a reference sample, and the water holding capacity in the same model minced meat was increased by 17.2 - 18.8%, in comparison with a reference sample.

We have obtained almost the same results for pea, and as for the amaranth, there is a slightly different picture: adding of 15 - 30% of the hydrated amaranth flour increases the water binding capacity and the water holding capacity by 3.2 - 12.7% and 14.3 - 26.2%, respectively, in comparison with a reference sample.

We have obtained almost the same results for pea, and as for the amaranth, there is a slightly different picture: adding of 15 - 30% of the hydrated amaranth flour increases the water binding capacity and the water holding capacity by 3.2 - 12.7% and 14.3 - 26.2%, respectively, in comparison with a reference sample.

The results we obtained confirm the similar data available in the literature on the positive impact of plant raw materials on the functional-technological properties of the minced meat, and in some cases, they even exceed them. For instance, according to **Shtakhova (2008)**, adding of the pea flour increases the water holding capacity of the minced meat by 6.9% - 8.4%, and the water binding capacity by 5.7% - 12.5%, and according to **Kidyayev, Litvinova and Jamalov (2017)**, adding of 9% of the hydrated amaranth flour into sausage products with the irrigation modulus ratio of 1:1.8 increased by the water holding capacity of the minced meat by 4%, in comparison with a reference sample according to **Gorodok (2009)**, by adding the amaranth flour into the minced chicken meat semi-finished products, the water holding capacity was increased by 10.46%; according to **Samchenko and Mihina (2013)**, it has been established that the increase in the dosage of the flax meal supplements leads to the increased water binding and water holding capacities of the minced meat**.**

The amount of lactulose to be added into the minced rabbit meat together with the vegetable supplements was determined with account for its daily consumption norms and organoleptic indices of product. We have established that Lactulose dosage was 1.5 - 2% of minced meat mass.

This concentration of lactulose does not have a negative impact on the functional-technological properties of semi-finished and finished products enriched with the plant supplements, as well as on organoleptic indices, in contrast, it increases slightly the water binding and water holding capacities, which is also confirmed by similar data available in the literature that lactulose and the biologically active supplements containing it, by improving the functional characteristics of stuffed products, give them the probiotic properties and promote the prevention of gastrointestinal diseases.

In particular, adding the lactulose-containing biologically active supplement “KUMELAK” into sausage products increases the water binding and water holding capacities of the minced meat from 3 to 7% **(Dolgova, Khramova and Proskurina, 2013)**; adding of up to 3% of lactulose into the minced fish impacts positively on the organoleptic and functional-technological properties of stuffing: the yield of product and water holding capacity are increased **(Yartseva and Dolganova, 2010)**; adding of 2.5 - 3% of the biologically active supplement "LACTUSAN" into culets with "Khabarovsk lactulose", increases the pH value and water binding capacity, and reduces the losses during the thermal treatment **(Okara et al., 2008)**.

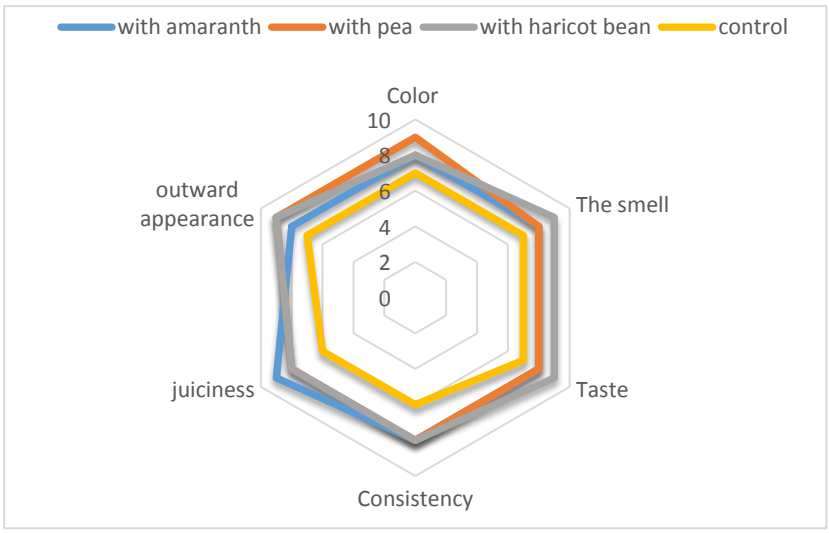

Figure 4 Organoleptic estimation of product.

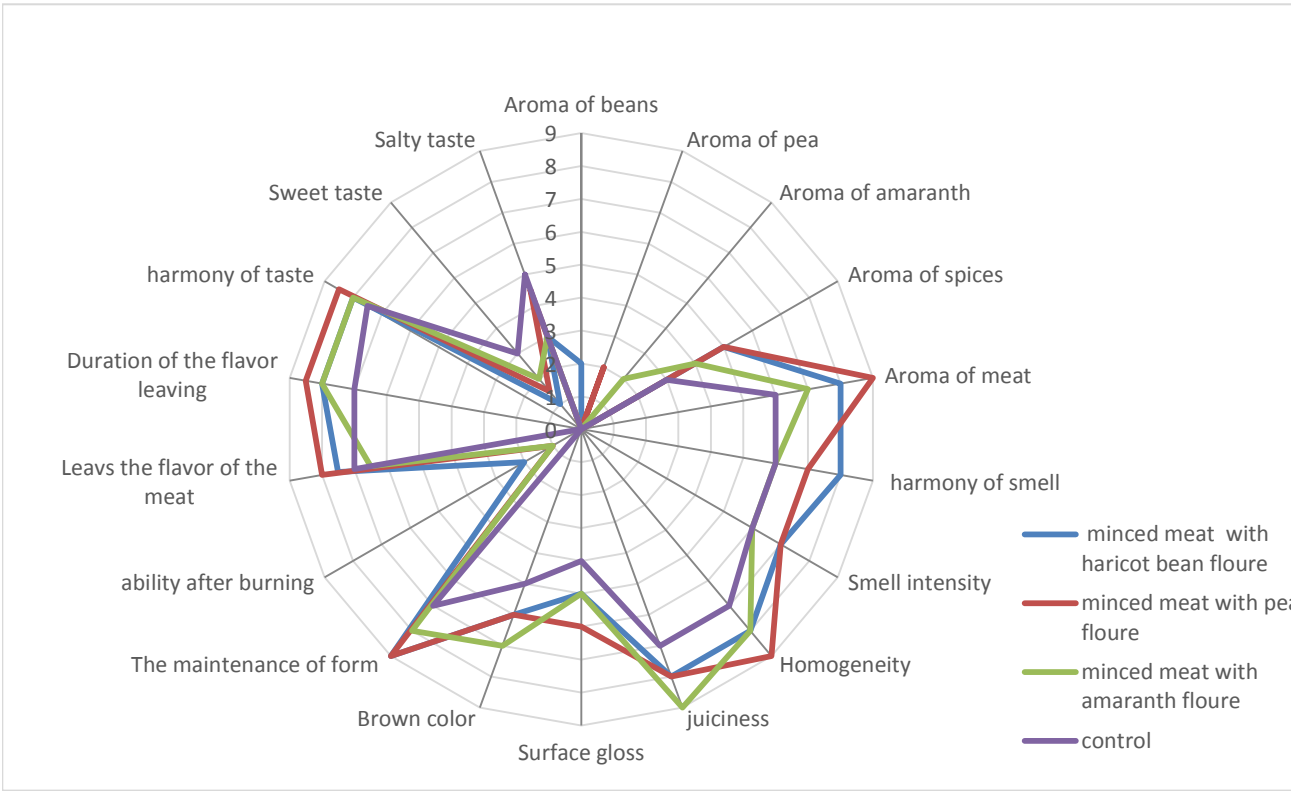

Figure 5 Profilogram of the taste-aroma characteristics of product.

We have made organoleptic estimation of the quality of model product enriched with vegetable supplements and lactulose, which has shown that with the same contents of, lactulose in the minced meat, adding of 10 - 15% of the haricot bean and pea flours did not affect organoleptic indices of stuffing, in comparison with a reference sample; the addition of 20% has improved organoleptic indices of stuffing, and by adding 25% of the plant supplements there have been improved the taste, aroma and colour of finished product, as well as its tenderness and succulence. Increasing the amount of haricot bean and pea flours to 30% led to the weakening of the taste and aroma of meat against the background of strengthening the aroma of leguminous crops, moreover, the colour of product has become darker and its consistency - denser.

As to the amaranth, we have obtained the similar results, when the number of supplements exceeded 30%.

Thus, the optimal amount of non-traditional bio-correcting ingredients, which improve the functional-technological and qualitative characteristics of finished products make up for haricot bean or pea 25% of the minced meat mass, for the amaranth flour 30%, and for lactulose 1.5 - 2%.

In order to neutralize specific taste of rabbit meat and to give product taste and aroma characteristic of Georgian cuisine, we added a set of Georgian spices.

"Khmeli suneli" into the minced meat. Its composition includes the spices (dried crushed herbal spices - coriander, dill, basil, bitter red pepper, crocus, marjoram, blue fenugreek, savoury, parsley, celery, bay leaf, etc.), which are known for their antioxidant and medical-preventive effect.

At the next stage of the work, we have studied the effect of used supplements on the mass loss during the thermal treatment. It has been established that replacing the part of the minced rabbit meat by the haricot bean, pea or amaranth flours results in a reduction in the mass loss of semi-finished products, by 7.5%, when adding the haricot bean flour, 7.2%, when adding the pea flour and by 8.1%, when adding the amaranth flour.

Organoleptic estimation of the quality of developed product was made on a scale of 1 to 9. The appropriate profilogram of the reference and model minced meats is shown in Figure 4, and their taste-aroma characteristics in Figure 5.

The profilogram shows that organoleptic indices in model product are better in comparison with a reference sample, in particular, the consistency, coloration, tenderness, succulence, aroma strength, surface smoothness, shape retention capacity during frying, meat aroma, taste harmonicity, no supplement taste.

The carried-out studies indicate that the non-traditional supplements we studied - haricot bean, pea or amaranth in conjunction with lactulose can be considered to BeBio-correcting ingredients, and they can be used in the technology for producing animal products.

Thus, the technological scheme of the minced rabbit meat product that we developed offers: making stuffing; the hydration of the haricot bean, pea or amaranth flours in mineral water, its binding with lactulose; mixing stuffing with the supplements; blending with spices and salt; the formation of semi-finished products and proper thermal treatment.

We have studied microbiological indicators of a new type of the minced rabbit meat product. The analysis was carried out on the existence of mesophilic-aerobic and facultative-anaerobic microorganisms, *Salmonella* and the *E. coli* group bacteria (Table 4).

The Table 4 shows that the number of mesophilic-aerobic and facultative-anaerobic microorganisms in the experimental samples was from that in the sample samples the number of mesophilic aerobic and facultative anaerobic microorganisms varied from 2.6×10^{-3} to 3.2×10^{-3} cfu.g^{-1} (colony-forming unit per gram), which does not exceed the values established by sanitary norms and regulations, the *E. coli* group bacteria have not been found in a 0.01 g sample, and it was in line with the microbiological safety hygienic requirements, and pathogenic microorganisms, including *Salmonella,* have not been identified in a 25 g sample, which also meets the microbiological safety norms and points to the safety of product.

The obtained data set indicates the appropriateness of using a new type of product in a functional, dietetic and preventive nutrition.

Table 4 Microbiological indicators of the minced rabbit meat product.

Indicators	Normalized value	Control	Experimental samples		
			with 25% haricot bean flour	with 25% pea flour	with 30% amaranth flour
Number of mesophilic-aerobic and facultative-anaerobic microorganisms, cfu.g^{-1}, (colony-forming unit per gram), less than	5×10^6	1.8×10^3	2.6×10^3	2.9×10^3	3.2×10^3
E. coli group bacteria, in a 0.01 g sample	Not allowed in 0.01g	have not been identified	have not been identified	have not been identified	have not been identified
Mould, cfu.g^{-1}, (colony-forming unit per gram), less than	500	have not been identified	have not been identified	have not been identified	have not been identified
Pathogenic microorganisms, including *Salmonella,* in a 25g sample	Not allowed in 25g	have not been identified	have not been identified	have not been identified	have not been identified

CONCLUSION

There has been justified the appropriateness of using plant raw materials and lactulose in the production of the minced rabbit meat products.

There have been established the hydration conditions and modes of vegetable supplements - haricot bean, pea and amaranth flours: the hydration in mineral water containing the ionized calcium chloride, the irrigation modulus ratio for haricot bean and pea is 1:3, the hydration duration 30 - 40 minutes; for amaranth - the irrigation modulus ratio is 1:2 and the hydration duration 50 - 60 minutes.

There have been determined the optimal amounts of the hydrated vegetable supplements and lactulose to be added into the minced rabbit meat, which is 25% for haricot bean and pea, 30% for amaranth, and 1.5 - 2% for lactulose.

The set of the developed supplements improves the functional-technological characteristics of semi-finished and finished products, in particular, the water binding capacity – for haricot bean or pea flours by 11.4%, the water holding capacity by 18.8%. For amaranth flour, accordingly by 12.7 and 26.2%. Also, it improves the quality organoleptic indices.

The study of microbiological indicators of the developed product points to its safety. The obtained data set indicates the appropriateness of using a new type of product in a functional, dietetic and preventive nutrition.

REFERENCES

Antipova L. V., Glotova I. A., Rogov I. A. 2001. *Methods for studying meat and meat products.* Moscow, Russia : Kolos. 376 p. ISBN: 5-9532-0187-7.

Antipova L.V., Vasilenko O.A., 2003. Histomorphological, structural-mechanical and functional- technological properties of rabbit meat. *International Scientific and Practical Conference : proceedings. "Actual directions of development of environmentally safe technologies for production, storage and processing of agricultural products."* Voronezh. p. 176-180.

Ardia, N., Janelidze, Ch., 1999. Samegrelo (Geographical Applications). Tbilisi - Zugdidi.

Arihara K. 2006. Strategies for designing novel functional meat products. *Meat Sci*., vol. 74, no. 1, p. 219-229. https://doi.org/10.1016/j.meatsci.2006.04.028

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, no. 2, p. 741-750 https://doi.org/10.4081/ijas.2009.s2.741

Dalle Zotte A. 2004. Dietary advantages: Rabbit must tame consumers. *Viandes ET Produits Carnés*, vol. 23, p. 161-167.

Dalle Zotte, A., Szendrő, Z. 2011. The role of rabbit meat as functional food. *Meat Science*, vol. 88, no. 3, p. 319-331. https://doi.org/10.1016/j.meatsci.2011.02.017

Decker E. A., Park Y. 2010. Healthier meat products as functional foods. *Meat Sci*., vol. 86, no. 1, p. 49-55. https://doi.org/10.1016/j.meatsci.2010.04.021

Dolgova, V. A., Khramova, V. N., Proskurina, O. Y. 2013. Development of meat products of functional appointment with the use of prebiotics. *Journal Nizhnevolzhsky Agroun complex.*, vol. 2, no. 30, p.168-171.

Erashova, L. D., Pavlova, G. N., Kashkarova, K. K. 2010. Foodstuffs based on grain beans. *Food industry,* vol. 2, no. 88, p. 48-49.

Fomenko O. S. 2011. *Development of recipes of minced semi-finished products from chikens with complex additive* : dissertation theses. Saratov : Saratov State University. 20 p. Available at: http://www.mgutm.ru/files/graduates-and-doctors/avtoreferat_fomenko_os.pdf.

Georgian Soviet Encyclopedia. 1983. vol. 6, p. 302, Tbilisi : Macmillan.

Gorodok, O. A. 2009. *Development and research of consumer properties of semi-finished products from the meat of chickens using amaranth flour* : dissertation theses. Kemerovo. 19 p.

Grigoriev, P. Y., Yakovenko, E. P. 2000. Lactulose in the treatment of diseases of the digestive organs. *Russian Gastroenterological Journal*, vol. 1, no. 2. Available at: http://medi.ru/doc/6700213.htm.

Hernández P., Gondret F. 2006. Rabbit meat quality. In Maertens L., Coudert P. *Recent Advances in Rabbit Sciences*. Merelbeke, Belgium : ILVO. p. 269-290. Available at: https://www.lybrate.com/medicine/lactulose.

Khramova, V. N. 2011. The effectiveness of the introduction of lactulose-containing dietary supplements in the formulation of boiled sausage products. In Khramova, V. N.; Dolgova, V. A.; Proskurina, O. Y. *Meat series*, vol. 1, no. 4. p. 50-51.

Kidyaev, S. N. Litvinova, E.V., Jamalov, N. K. 2017. Amaranth as an unconventional source of protein for meat products. *Meat technologies*, vol. 11, no. 79, p. 34-37. Available at: http://www.mgupp.ru/obuchayushchimsya/instituty-i-kafedry/download.php?id=80D1000C299AE95511E7DC1D98EA6918&t=ResearchArticles&name=Амарант%20как%20нетрадиционный%20источник%20белка%20для%20мясных%20продуктов.

Kolenik, T. K., Vershinina, A. G., Samchenko, O. N., Kravchenko M. V. 2014. Creating combined minced semi-finished products with the addition of non-traditional plant materials. *Food Product merchendise*, vol. 1, no. 1. p. 25-30.

Leonidov, D. S. 2013. Lactulose in food - back to the future. *Milk processing,* vol. 1, no. 2. p. 46-47. Available at: http://40.ru/upload/pererabotka_02-13.pdf.

Maksimov, I. V., Kurchayeva, E. E., Manzhesov, V. I., Lysenko, Y. V., Lyutikova, A. O. 2013. Development of functional meat products using complex additives of prebiotic-sorption orientation. *Young Scientist,* vol. 1, no. 10. p. 251-256. Available at: https://moluch.ru/archive/57/7889.

Nistor E., Bampidis V. A., Păcală N., Pentea M., Tozer J., Prundeanu H. 2013. Nutrient Content of Rabbit Meat as Compared to Chicken, Beef and Pork Meat. *Journal of animal production Advances*, vol. 3, no. 4, p. 172-176. https://doi.org/10.5455/japa.20130411110313

Okara A. I. et al. 2008. Meat-containing semi-finished products of functional purpose cutlets "Khabarovsk with lactulose", specifications TU 9214–006–02067994–08, Patent No 23984.

Pla, M., Pascual, M., Ariño B. 2004. Protein, fat and moisture content of retail cuts of rabbit meat evaluated with the NIRS methodology. *World Rabbit Sci*., vol. 12, p.149-158. Available at: https://riunet.upv.es/bitstream/handle/10251/9784/574-077-1-SM.pdf.

Romanov, V. N., Komarov, V. V. 2002. Analysis and processing of experimental data. Saint Petersburg, Russia. 113 p.

Samchenko, O. N, Mishina, E. D. 2013. Development of recipes and technology of combined meat products. *The current state and prospects of development of the food industry and catering*, vol. 2, p. 72-76. Available at: http://www.cnshb.ru/jour/j_g.asp?id=5147.

Sautkin, A. V. 2010. *Veterinary and sanitary assessment of rabbit meat when using "Emil" in their diet* : dissertation theses. Moscow, Russia. 137 p.

Sharipova, T. V. 2014. *Research and development of technology of meat-vegetable semi-finished products for elderly nutrition* : dissertation theses. Kemerovo. 20 p. Available at: http://www.dissercat.com/content/razrabotka-tekhnologii-myasorastitelnogo-produkta-dlya-gerodieticheskogo-pitaniya.

Shelepina, N. V. 2016. Use of pea grain processing products in food technologies. *Applied chemistry and biotechnology,* vol. 6, no. 4, p. 110-118. https://doi.org/10.21285/2227-2925-2016-6-4-110-118

Shenderov, B. A. 2001. Medical microbial ecology and functional nutrition. Russia : Probiotics and functional nutrition. Publishing house "Grant". 288 p. http://gastroportal.ru/php/content.php?id=111371.

Shtakhova, T.A. 2008. *Application of flour of legumes in technology of minced meat semi-finished products with increased biological value* : dissertation theses. Moscow. 24 p. Available at: http://www.dslib.net/texnologia-mjasa/primenenie-muki-bobovyh-kultur-v-tehnologii-mjasnyh-rublenyh-polufabrikatov.html.

ISO 2171:2007. *Cereals, legumes and their products. Determination of ash content during combustion*.

State Standard 10444.15-94. *"Food products. Methods for determining the amount of mesophilic aerobic and facultative-anaerobic microorganisms "*.

GOST 30518-97. *"Food products. Methods for the detection and determination of the number of bacteria of the Escherichia coli group (coli form bacteria)*.

GOST 30519-97. *"Food products. Methods for the detection of bacteria of the genus Salmonella.*

GOST 23042-86. *Meat and meat products. Methods of fat determination*.

GOST 25011-81. *Meat and meat products. Methods of protein determination*.

GOST ISO 24557-2015. *Grain legumes. Determination of moisture content. Air-heat drying method*.

GOST P 50454-92 (ISO 3811-79*). Meat and meat products. Detection and enumeration of presumptive coliform bacteria and presumptive Escherichia coli (Reference method)*.

GOST P 50455-92. *Meat and meat product. Detection of salmonellae (Reference method)*.

Tavdidishvili, D., Khutsidze, T., Tsagareishvili, D. 2018. A study of the quality and biological value of meat of different breeds of rabbit bread in Georgia. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 563-559. https://doi.org/10.5219/934

Tavdidisvili, D. R., Lipatova, L. P. 2018. Use of germinated beans in the production of culinary products of functional purpose. *Innovations: Perspectives, Challenges, Achievements* [online]. Berlin : West-Ost-Publishing House. p. 199-206. Available at: https://www.rea.ru/ru/org/cathedries/pitkaf/Documents/Наука/5%20СБОРНИК%20ДЕКАБРЬ%202018.pdf.

Tutelyan, V. A. 2003. Optimal nutrition as a new medical technology for extending and improving the quality of life. *Nutrition issues*, vol. 1. 22 p.

Vasneva, I. V., Bakumenko, O. M. 2010. Lentils - a valuable product of functional nutrition. *Bakery products,* vol. 11, p. 39-40.

Vaytanis, M. A. 2012. Anhancement of minced meat with vegetable raw materials. *Polesunskaya bulletin*, vol. 2, no. 2 p. 216-220. Available at: http://elib.altstu.ru/journals/Files/pv2012_02_2/pdf/215vaitanis.pdf.

Volkova, O. V., Inerbaeva, A. T., Motovilov, K. Y. 2009. Morphological composition and biological value of rabbit meat. *Siberian Herald of Agricultural Science*, vol. 8, p. 97

Wambui, J. M., Karuri, E. G. and Wanyoike, M. M. 2016. Interaction among Nutritive, Textural, and Sensory Properties of Rabbit Sausages. *Journal of Food Processing*, Vol. 2016, 6 p. https://doi.org/10.1155/2016/4059023

Weiss, J., Gibis, M., Schuh, V., Salminen, H. 2010. Advances in ingredient and processing systems for meat and meat products. *Meat Sci*., vol. 86, p. 196-213. https://doi.org/10.1016/j.meatsci.2010.05.008

Yartseva, N. V., Dolganova, N. V. 2010. Study of the organoleptic and technological properties of minced fish cutlets with the addition of lactulose. *ASTU Bulletin, Fishery Series,* vol. 2. p. 125-129. Available at: https://cyberleninka.ru/article/n/izuchenie-organolepticheskih-i-tehnologicheskih-svoystv-kotlet-iz-rybnyh-farshey-s-dobavleniem-laktulozy.

Zharkova, I. M., Miroshnichenko, L. A. 2012. Amaranth flour - an effective tool for the production of healthy food. *Bakery products,* vol. 12. p. 55-57. Available at: https://elibrary.ru/item.asp?id=18258391

Zhidik, I. Y. 2017. V*eterinary-sanitary evaluation of rabbit meat by the use of mineral additive "Zeolite Natural" of Kholinsky deposit* : dissertation theses. Russia : UFA. 21p.

Zinina, O. V. 2016. Half-finished minced meat products with the protein additive. *News of universities. Applied chemistry and biotechnology*, vol. 6, no.3, p.107-112.

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ATTENTION ANALYSIS OF HONEY JAR LABELS USING EYE-TRACKING TECHNIQUES

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ABSTRACT

Honey represents not just a specific product of animal origin, which's major part are plant products, but also the oldest sweetener of human kind. It is actually a sweet substance produced by beetles from nectar plants or from insects excreted on plants. Because of many different reasons (e.g. for trading, handling, storage), in the history of mankind, there have been introduced different forms of packaging and protective means through which this precious product could be protected. The present paper addresses consumer behaviour, focusing on the influence of packaging and labelling on young consumers aged from 20 to 35 years, especially when choosing honey. The realization of the main aim is conditional on meeting the following partial objectives - to identify the basic elements appearing on two samples of honey packaging and their impact on consumer perception, respectively to identify the differences in the perception of individual elements of the packaging, based on the respondents' gender. In the present paper, there are used different marketing research techniques, specifically the eye-tracking observation. The experiment involved exactly 12 samples of honey and finally 35 participants (18 women and 17 men). Based on the results of the authors' own work, it can be stated that the most eye-catching aspects of honey packaging are the producer's brand, as well as the variety description and name given to the honey. The least noticed aspects are the weight details of the packaging and the graphic design.

Keywords: consumer behaviour; labelling; packaging; honey; eye-tracking

INTRODUCTION

Product packaging is one of the key tools of marketing. Its significance varies with the type of consumer product, particularly when it comes to food. Manufacturers are investing in packaging technology, traditional packaging is replaced by modern ecological packaging, with designs that are attractive and emotionally engaging for consumers. The packaging can certainly also grab attention, evoke emotions, carry information, and thereby be a decisive factor in the purchasing decision.

In the case of packaging of honey, the most common form is the glass respectively the plastic packaging. Differently shaped, embossed or smooth packaging shapes do not belong to the only types of packaging into which the bee products are packaged. For several decades, honey has also been packaged in blown plastic packaging, of which the most popular forms are the bear-shaped or jar-wrapper forms. The range of honey packs on the Czech and Slovak markets is supplemented with crucibles, capsules, stick packs or laminate tubes.

Several authors, e.g. **Lehmann and Winer (2005)**, respectively **Kollár (1999)** and **Vysekalová (2004)** have in their works and researches focused on the influence of packaging on the consumer decision making at the time of purchase of goods. At the same time, they have tried to categorize packaging features from a marketing point of view.

The first work in this area was the study by **Hansen (2005)**, who has highlighted the impact of packaging on consumer purchasing behaviour, primarily through communication, rational and environmental functions. **Bech-Larsen (1996)** has on the other hand, in the cultural context of Denmark, shown that the ecological function has no influence on the consumer's decision to buy the product. **Velčovská (2009)** points to the same situation in the Czech Republic, where there is a lack of motivation of consumers to sort the waste, what affects their attitude to the ecological function of the packaging. The situation in Slovakia is similar to that in the Czech Republic. Based on **Minárová and Kubicová (2008)** research, only four consumers out of 240 consider the recycling of packaging to be important.

Kotler et al. (2007) argues that packaging, in addition to the core function of protecting a product, goes beyond these areas and can become an important marketing tool.

The quality, style, function, and design of the product are considered to be the intrinsic property of the product. **Keller (2007)** encompasses the packaging between the basic elements of the brand and he perceives it as a very effective way to build brand value. In addition to the logistic and protective features of the packaging, he also indicates the

packaging's ability to communicate the brand towards the consumer.

Rigaux-Bricmont (1982) has been interested in the influence of the name of the brand and the packaging on the consumers already in the year 1982. An important moment in buying goods is the consumer decision-making process at the point of sale, where the communication function of packaging is used, especially in the fast moving market segment. Consumers can make their purchasing decisions based on the visual information, which they get.

Pieters and Warlop (1999) have, on the other hand, investigated the effect of visual information on the perception of the brand under the time pressure by the eye-tracking method. They have focused on the knowledge or ignorance of the brand of goods and tracked the amount of time, which the consumers spend on them. Consumers naturally spend more time exploring the preferred product. However, under the time pressure, the process of reading the visual elements changes - they are passing low-value visual characters to high-value characters. Getting the attention of consumers is therefore the main role of the packaging, especialy at the point of sale. Customers, who are unable to determine the quality of the goods based on the packaging, choose the product according to its ability to attract their attention. This only works when the packagingis sufficiently attractive and interesting for them.

Visual perception deals with the ability of consumers to interpret the visual elements of the packaging. This issue is not thoroughly dealt with in marketing theory, although in recent years there has been a noticeable increase in its interest. The authors, who addressed the impact of visual images in marketing communication, have examined the processing process of visual information.

Perception in the process of buying behaviour can be characterized as getting the consumer's attention through visual means of expression.

Visual attention is often the only way for consumers to obtain the necessary brand and product information in the intentions of consumer decision-making. Anchoring visual attention can be found in the area of behavioural psychology that refers to the natural curiosity of human individuals who use their gaze to find something new and interesting. In general, one uses eyesight to search for stimuli, and if he finds something interesting, he concentrates his attention on the object. If these claims are transferred to the area of visual attention given to the packaging, consumers are searching for visual stimuli that are substitute symbols expressing goods that they seek for to satisfy their needs.

Simply explained, the purpose of the packaging is to attract the consumer's visual attention, to bring them to examine the specifics of the product **(Koprda and Košková, 2015)**.

Clement (2007), based on the knowledge from behavioural and cognitive science, has come to the conclusion that consumers are selectively using some of the principles of attention in their purchasing behaviour. Using eye-tracking, the author has identified six phases of visual perception that are going on during the buying process.

Despite the fact that, up to the results of some researches, it can be concluded, that regarding the purchasing criteria, the most important for consumers are the quality, taste and country of origin while the least important is the design of packaging **(Guziy et al., 2017; Šedík et al., 2018)**; results of the research done by **Grunert et al. (2001)** have confirmed that packaging plays an important role in purchasing, especially when it can form the consumer opinion on product quality.

Consumer-friendly packaging is a competitive advantage for the producer or seller. Taking the definition offered by **Jurášková and Horňák (2012)** *'packaging is a protective and promotional product facility at the point of sale.'* Some authors, such as **Křížek and Crha (2012)** emphasize predominantly the contents as communicated on the packaging, and the packaging as a marketing tool. The packaging content list substitutes for the vendor, the content and design seeks to resonate with the consumer's lifestyle. Not to be overlooked is the legal definition of packaging - in the Czech Republic by **Act No. 66/2006,** which defines packaging as a product made of material of any nature, intended for the containment, protection, handling, delivery or presentation of consumer products, including a definition of the method of labelling, and the foodstuff information. In the Slovak Republic the packaging functions are defined by **Act No 79/2015** for a well-circumscribed product, such as honey, there are numerous other stipulations, listed by **Titěra and Vořechovská (2013)** in a summary of legal regulations.

Given the subject matter of the present paper - the packaging label - we need to encompass the mandatory information: the producer's (bee-keeper's) name, the minimum durability date, the packaged size/weight of the product, the honey variety (flower, honeydew). Mass-producers are required to state the production lot reference. The label can list additional information, such as the way the honey has been processed, its place of origin, the botanical source the honey comes from etc. European legislation prohibits certain expressions being used on the label, such as 'natural', 'genuine', 'unadulterated', 'from honeybees', 'without additives and preservatives' etc.

The aim of the paper is to identify elements on the packaging of the product - honey, how captivating they are, for how long the given element captures attention, and ultimately, the impact of these aspects on the purchasing decisions of consumers. The realization of the main aim is conditional on meeting the partial objectives - to identify the basic elements appearing on two samples of honey packaging (glass and plastic packaging) and their impact on consumer perception and to identify the differences in the perception of individual elements of the packaging by the gender.

Scientific hypothesis

Before the experiment itself, four assumptions were formulated:

1. There are gender differences in the tracking of information on the honey packaging label.
2. Significant elements on the packaging (brand and type of honey) attract more attention.
3. Traditional glass packaging is preferred by customers in comparison with plastic packaging.
4. Traditional packaging is preferred to colorful and modern packaging.

MATERIAL AND METHODOLOGY

To meet the paper's investigative aims - whether and how much the packaging and label affects the purchase of the honey - experiments were carried out in the laboratory of the Department of Marketing and Trade at Mendel University in Brno. In the experiment 12 samples of honey were used, purchased randomly throughout the market, from a variety of vendors. Represented were hypermarkets and supermarkets, stall-holder sellers at farmers' markets and e-shops. Due to the limited range of the paper, 2 out of 12 samples of honey were selected - one in glass and the other in a plastic packaging. The respondents were young people aged 20-35 years old, a total of 42 of them, selected at random. The implementation phase was done at the eye-tracking laboratory, having professionally briefed all respondents and verified that they meet the prerequisites for using the technical equipment. The final number of respondents who took part in the experiment was 35 (18 women and 17 men).

The important part of the technical preparation of research was taking pictures of selected samples, editing pictures, conducting the experiment in the SMI Experiment CenterTM, realisation of pretests and verifying the functionality of research.

During in-depth interviews, respondents answered questions that matched the assumptions. These were questions like: „Does the gender influence the choice of honey? "and „Does the packaging and label of honey influence the buying behaviour?

During the buying of honey, the attention was paid to different types of honey, to different graphic processing of packaging, colours, lid shape, packaging material, packaging size and origin of honey (Czech Republic, EU and outside the EU). The pictures of each sample were taken by the high resolution digital SLR. Pictures were taken in the natural light in the improvised photo-tent. Subsequently, the pictures were edited in GIMP software and saved in the regular jpg format. Edited pictures were used when creating the graphical background in the SMI Experiment CenterTM. Then the picture called the "intentional cross "was used. This cross is important for focusing the eye exactly on the center of the screen. The cross was projected to respondents for the one second time. Then the mere pictures of honey followed. Each picture was projected for the twelve seconds time. An interview was held between projecting of individual pictures. Afterwards, the intentional cross was projected again to focus attention on the center. At the end of the experiment, short identification

Figure 1 Scan paths example. Note: Own processing, output from SMI BeGazeTM.

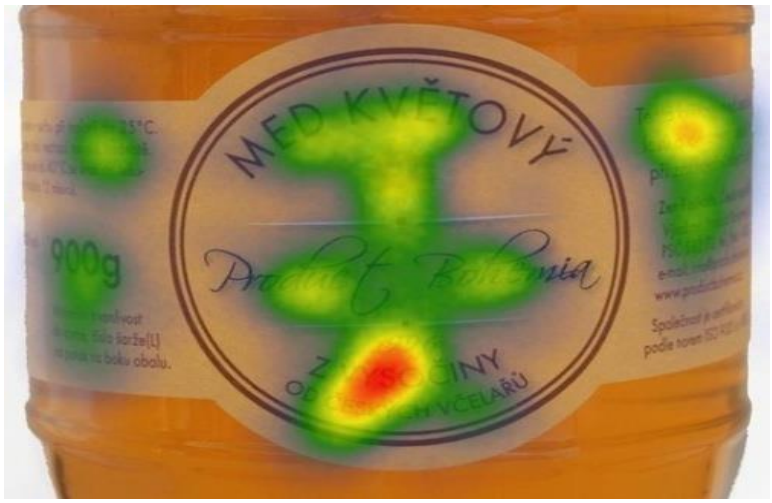

Figure 2 Heat map example. Note: Own processing, output from SMI BeGazeTM.

questions were asked again. The stationary SMI RED 250 eyetracker, which consists of two infrared cameras and high-resolution sensors. The device allows you to monitor eye movements. The software automatically tracks and records movement of the pupils.

The eyetracker works at the frequency up to 250 frames per second and accurately detects eye movement through dioptric lenses and contact lenses. The specialized software SMI BeGazeTM, which uses specific metrics, was used to interpret the data obtained from eyetracking research. Scan paths are used to find observations of individual stimuli, heat maps for identification of the exact areas of interest (AOI).

Statisic analysis

The technique of eye-tracking records the respondent's reaction to a change of stimulus (various labels). The device tracks eye movement over quite a wide viewing angle and at the same time automatically records this movement. Each respondent's gaze motion is calibrated before the measuring itself begins. The software used was SMI Be Gaze TM, with Scan path metrics. Scan paths as a metric show the movement of the eye pupil as it tracks the stimulus while also recording the time **(Holmqvist et al., 2011)**. Circles and straight lines were used to render the gaze pathways. The diameter of the circle reflects the time of fixation, in direct correlation. Lines show the movement of the pupil to the next point of focus (saccade) **Nielsen and Pernice (2010)**.

Another technique used in the experiment are eye-tracking heat maps. This is a visualization of the eye-tracking monitoring findings. The heat maps are used to show the AOI metric. This is the most widely used tool that allows exploring the relationships between areas (aspects) of interest. Areas of interest are analysed according to KPIs. A description of each KPI metric is shown in Table 1.

RESULTS AND DISCUSSION

The results of the eye-tracking survey of packaging on 12 samples of honey with different designs and different information are illustrated by two samples of honey that best show the packaging fulfilling its function, "med květový z Vysočiny" ('Flower honey from the Highlands') and "Medokomerc med luční" ('Medokomerc meadow honey').

Flower honey from the Highlands

The mentioned sample of honey is a lighter colour, which suggests that it is a flower honey, which in its name states not just the type of plants that are its basic raw material, but also the region from which it comes from. This type of honey, is offered to customers in a classic, smooth glass package, with a slight moulding in the top and bottom of the package. In the middle of the packaging is the label with a gold-coloured matte finish, with all the information required by law. The cover is closed with a metal capcovered with a decorative matching skirt. The weight of honey is 900 grams.

Up to the results of the research, it can be stated that all of the examined respondents (100%) have expressed their satisfaction with the packaging and 80% of respondents would buy honey. They have also positively evaluated the nature of the nearby design that evokes honey and the meadow with flowers.

In addition to the packaging and design, respondents have positively evaluated the simple graphic elements of the packaging, font and paper skirt. Most of the respondents were also very perceived by the region of its origin.

The heat map of the sample indicates that respondents focused mainly on the central circle of the label, containing the honey designation, the honey brand and the region. Less attention was paid to the informative data and the weight. For KPI analysis, this sample was ascribed 9 areas of interest.

Table 1 KPI metrics described.

KPI name	Unit	Characteristics
Sequence	count	The order of visits to the AOI based on entry time. Shorter times make better sequencing.
Entry time	ms	The average time to the first fixation of the selected AOI.
Dwell time	ms and %	The sum of all the fixation times and saccades of the given AOI. The total time spent on the selected AOI. The most important metric
Hit ratio	count and %	The number of respondents who looked at least once at the selected AIO.
Revisits	count	Specifies the number of times respondents returned gaze to the selected AOI on average.
Revisitors	count	The number of respondents whose gaze returned to the selected AOI.
Averagefixation	ms	The averaged sum of average fixations of the selected AOI.
First fixation	ms	The averaged sum of the first fixation of the selected AOI.
Fixation count	count	The total number of all fixations of the selected AOI.

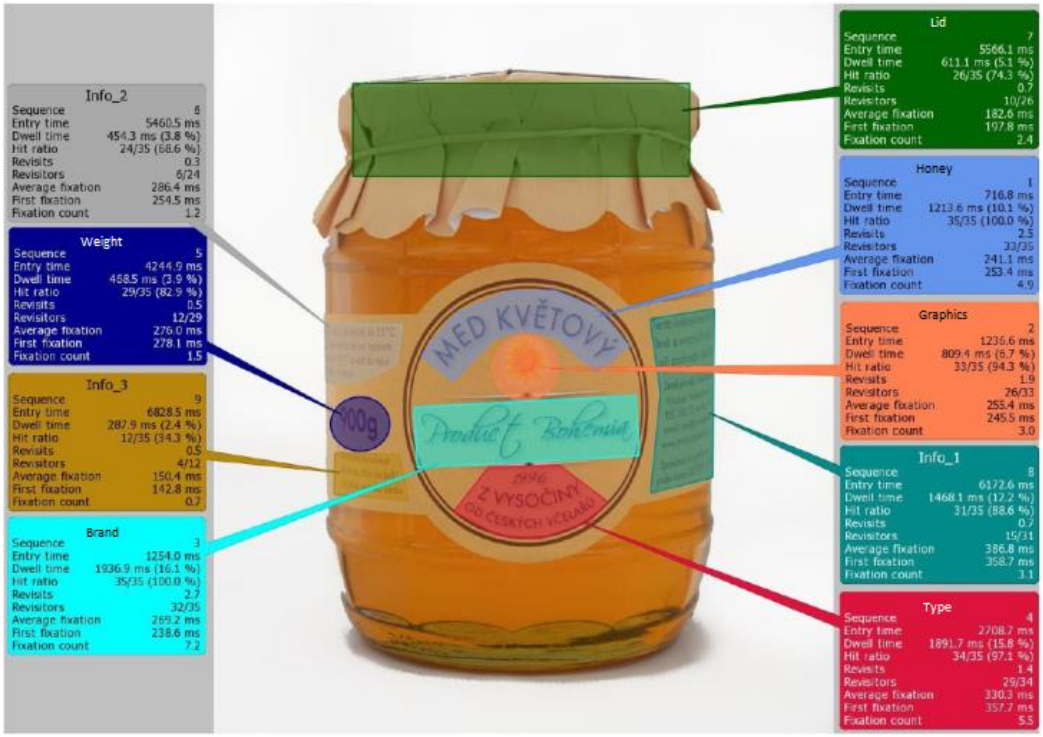

Figure 3 KPI analysis of the Flower honey from the Highlands.

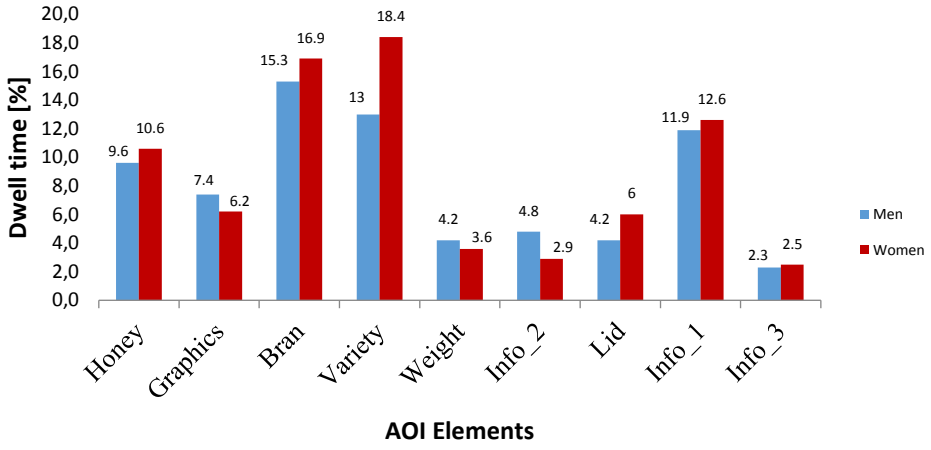

Figure 4 Relative expression of the time spent on the selected AOIs using the Flower honey from the Highlands sample. Note: Own statistical processing with SMI BeGazeTM.

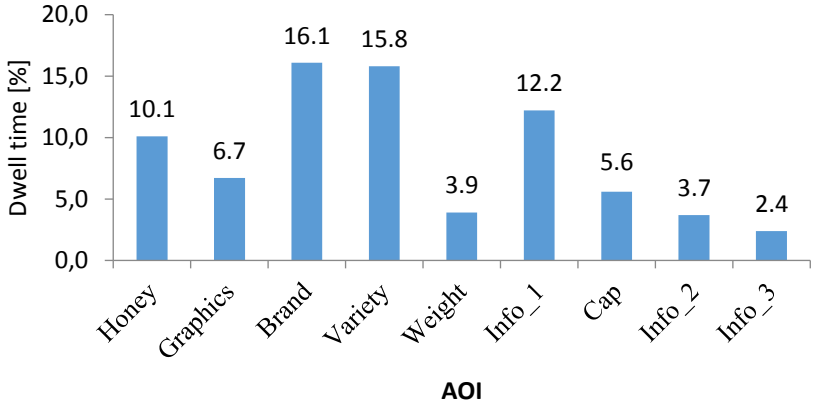

Figure 5 Dwell time on each label area.

An important AOI is the zone of the brand (16.1% of the time), variety (15.8% of the time), designation of the honey (10.1% of the time), graphics (6.7% of the time) and Info_1 (12.2% of the time). Other areas of interest were less significant and include the weight, the lid, Info_2 and Info_3. We can surmise that overall the attention paid to the central circle of the label took 48.7% of the respondents' time. The format of a central circle with fewer elements seems to be very useful for attracting attention. Also of interest is the different attention paid to the information located in three different places, and of different design. Attention was paid to that information section which differed significantly in colour and design.

Attention rates vary within sex. Men on one hand have at first paid their attention to the central elements like honey, graph, mark and species. They began to look up and continued smoothly at the bottom of the circle. Later they have focused on the elements shown on the left side (in the weight sequence), Info_3, Info_2, and just then they have looked to the top and finally looked at Info_1. The tracks of women's attention, on the other hand, was a bit different - their attention was paid to central elements such as honey, brand, graphics and species. Then they have focused their attention on the weight, which is shown on the left, the lid on the top, the Info_2 again on the left, the Info_1 on the right and finally the Info_3, which are shown on the other side of the packaging.

Medokomerc meadow honey

The analysed sample of honey is very light in colour and corresponds to the bee honey. Its packaging is made of plastic with a ribbed surface. The label on the packaging is simple, informative, without distinctive graphic elements. The packaging weight is 300 grams.

Results of the research show, that both, women and men, engaged in research have almost the same views on the appearance of honey. The question whether this honey would be bought by them was answered by almost 83% of respondents' negatively. The reason for the negative evaluation was the plastic packaging that respondents considered to be aesthetically unsuitable for this product.

On the other hand, they have positively evaluated the information on the nutritional values of the product and the practical use of this package when traveling.

Analysis of the heat map shows that most attention is given to the central part of the cover and the top of the label where all the reference elements are located. KPI analysis in this honey consisted of 8 AOIs. The most observed areas were Info_1 (26.5% of the time), Info_2 (23.1% of the time), the honey designation (9.7% of the time), the brand (7.6% of the time) and the other four areas of interest were unremarkable, their values being only in only a few units of % of the time.

Both genders have at first focused their attention on the middle of the etiquette - as the first element they have watched the honey, then the area Info_1 and the species area. Later, the results of the research have differed - men have slightly shifted their attention down to the Info_2, then completely up to the label and finally, they have looked at the lower part of the label, where the code, Info_4 and Info_3 are situated. Women, as the fourth element, have seen the label, which is shown at the top of the etiquette, then moved to the Info_2 and finally to the bottom at the code, Info_3 and Info_4.

From the results of the investigation we can surmise that unless the respondent's gaze is caught at first glance by an element they consider of interest such as the variety, brand, producer, or if it is not captivated by the design, the respondent will focus on text-based information and look there for what they consider crucial and have not yet ascertained.

As it can be seen, results of the research revealed the extent up to which the packaging, the material from which the packaging is made, the label, the packaging information, the design and the graphics, the colour of the packaging and the etiquette, and last but not least the product itself, affects the consumer in his purchasing decision.

Packaging is a part of the marketing communication between the consumer and the manufacturer. It represents one of the most important elements of modern marketing, while its essential role in protecting the quality of goods and distribution is complemented by an important communication function aimed at the consumer.

Results of some previously done researches have shown, that up to 70% of consumers make their daily decision to buy food directly in the shop; 85% of consumers' shop without gripping alternative goods in their hands; and even 90% of consumers buy only based on the front of the packaging without looking at the product itself. This was also confirmed by the results of authors' own research.

The mentioned shows and highlights the fact, that the visualized packaging incentives are important in the buying process. Consumers buy more and more on the basis of visual stimuli and make their decisions based on their decisions **(Urbany et al., 1996)**.

In particular, the packaging is a means of transporting and distributing goods from the manufacturer to the consumer, prolonging the durability of the goods, protecting them from the effects of the environment, allowing efficient storage and handling and, last but not least, communicating the properties of the packaged product to the consumer. This means that the packaging performs several functions at the same time, from the production of the goods to the purchase by the consumer.

As it was already indicated, **Rigaux-Bricmont (1982)** has demonstrated that the packaging and the brand of goods affect the perceived quality of the product but at the same time, they are not considered to be the only properties that are interconnected.

The results of the authors' own research have also shown the validity of the results of researches carried out more than 30 years ago. Respondents' opinions on the packaging versus the product packaged in it only confirmed the unanimous view that honey, which is considered to be a high-quality organic product packed in a plastic wrap, induces an association of consumers different from that of its producer. The negative rating of the plastic packaging was, on the other hand, mildly contradicted by positives - suchas its easy handling during traveling and the disclosure of required information by respondents on the etiquette.

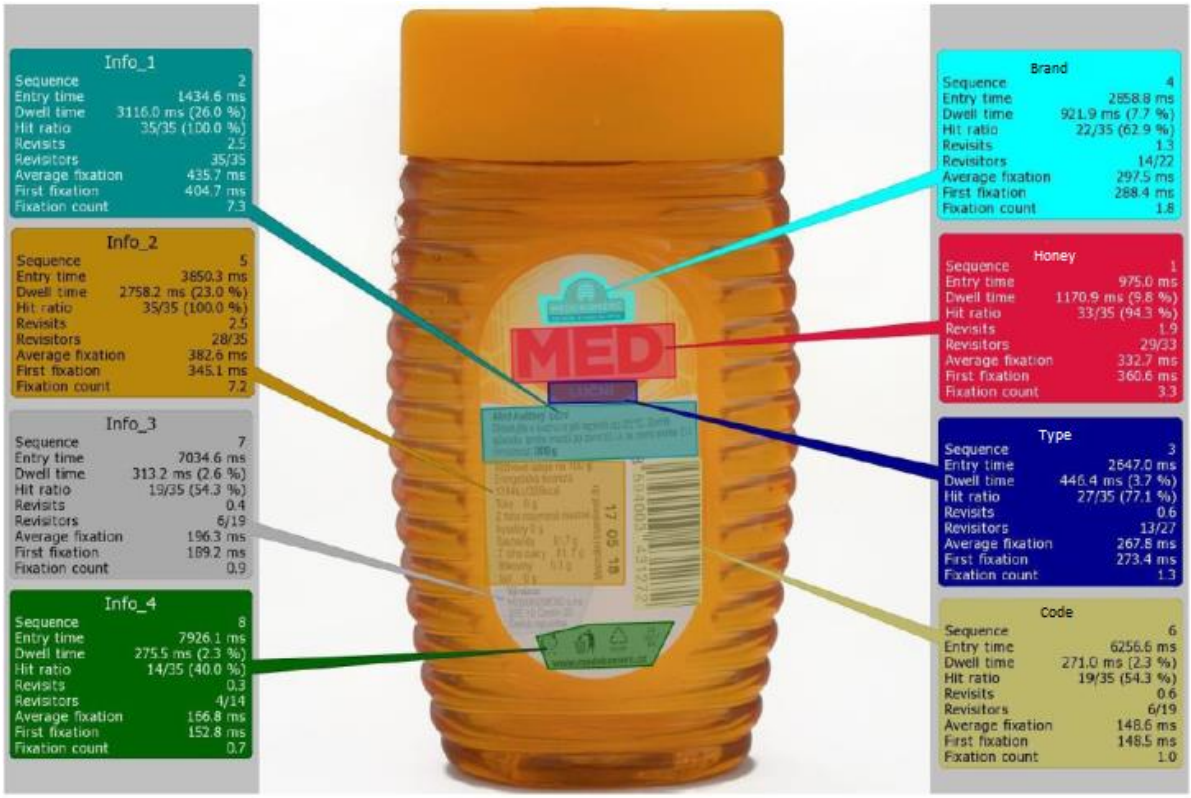

Figure 6 KPI analysis of the the Medokomerc meadow honey sample.

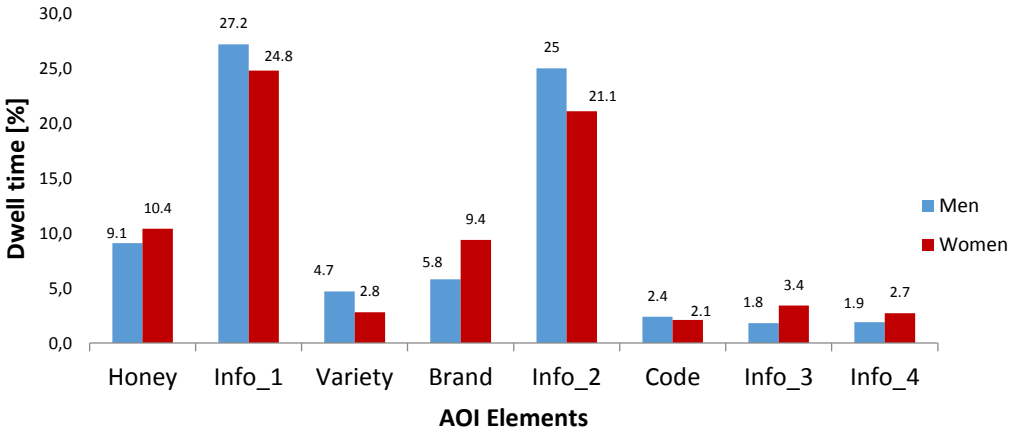

Figure 7 Relative expression of the time spent on the selected AOIs using the Medokomerc meadow honey sample. Note: Own statistical processing with SMI BeGazeTM.

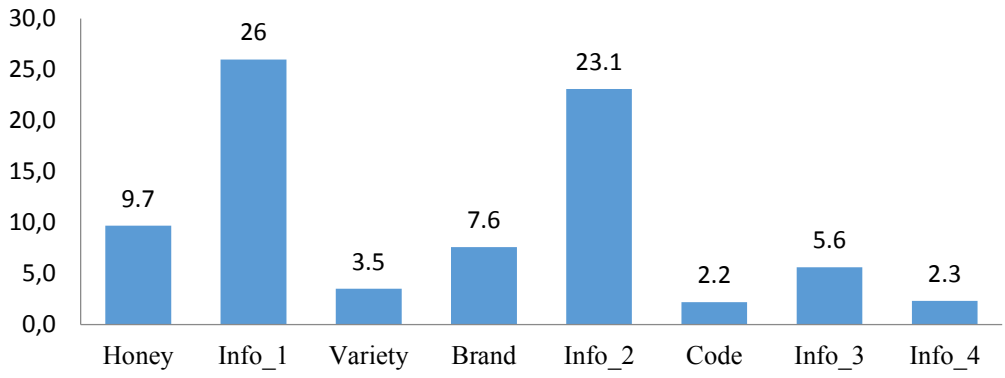

Figure 8 Dwell time on each label area.

CONCLUSION

Summing up the results of the KPI analyses of eye-tracking experiments carried out, we can express the relative time spent attending to specific AOI elements.

From the above Figure 9 we see that the greatest attention is drawn by the producer brand, the honey variety and the honey designation. Attention paid gradually falls away when it comes to the declaration of being Czech honey, additional information on the label and the lid. The least noticed aspects are the weight details of the packaging and the graphic design.

From the results of the eye-tracking experiments on 12 samples of honey and the generated heat maps of their labels we can surmise that the larger graphic elements on the packaging hold the gaze for longer, i.e. attract more consumer interest. Equally we can surmise that unconventional and bold colours attract more attention. The times spent tracking individual areas of the label do not differ significantly between men and women, neither is there any significant difference in the order of tracking the individual label elements.

The KPI analyses carried out lead us to several findings and enrich the subject area of utilizing marketing tools represented by the product, its packaging and the label on it, for a product as specific as honey. Honey has a variety of beneficial and healing properties, is an antiseptic, and has antioxidant and pro-biotic effects. Indeed, these are the reasons why it is consumed as an alternative sweetener, particularly instead of beet sugar. Hence the consumer pays attention to these aspects preferentially when deciding about purchase. This can also be documented by the finding that if the consumer is not captivated by the variety, brand or design, their attention turns to information about the product as such. Honey is therefore perceived by the consumer not only as a staple foodstuff, but a food with specific characteristics, which leads to the distinctive meaning and importance of the prime purchasing decision factors, compared with other consumer goods.

REFERENCES

Act no. 66/2006 Coll. Czech Republic of 1 February 2006 amending Act No. 477/2001 Coll. On Packaging and Amendment to Certain Acts (the Packaging Act), as amended. 15.02.2006. 1-16 p.

Act no. 79/2015 Coll. Czech Republic Waste Act and amendments to certain laws. 01.08.2018. 1-183 p.

Bech-Larsen, T. 1996. Danish consumers attitudes to the functional and enviromental charecteristic of food packaging. *Journal of Consumer Policy*, vol. 19, no. 3, p. 339-363. https://doi.org/10.1007/BF00411413

Clement, J. 2007. Visual influence on in-store buying decisions: aneye-track experiment on the visual influence of packaging design. *Journal of Marketing Management*, vol. 23, no. 9-10, p. 917-928. https://doi.org/10.1362/026725707X250395

Grunert, K. G., Juhl, H. J., Poulsen, C. S. 2001. Perception de la qualité en alimantaire etrôle des labels. *Revue Française du Marketing*, vol. 183-184, no. 3-4, p. 181-196.

Guziy, S., Šedík, P., Horská, E. 2017. Comparative study of honey consumption in Slovakia and Russia. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 472-479. https://doi.org/10.5219/784

Hansen, T. 2005. Perspectives on consumer decision making: An integrated approach. *Journal of Consumer Behavoir*, vol. 4, no. 6, p. 420-437. https://doi.org/10.1002/cb.33

Holmqvist, K. et al. 2011. Eye Tracking: A comprehensive guide to methods and measures. 1st edition. Oxford : Oxford University Press, 537 p. ISBN 9780070682160.

Juráškova, O., Horňák, O. 2012. *Velký slovník marketingových komunikací. (A big dictionary of marketing communication)*. 1st edition. Prague, Czech Republic : Grada Publishing. 260 p. ISBN 978-80-247-4354-7.

Keller, K. L. 2007. *Strategické řízení značky (Strategic management of a brand)*. Prgue, Czech Republic : Grada. 796 p. ISBN 80-247-1481-3.

Kollár, V. 1999. *Systém a špecifikácia produktovej politiky (The system and specification of a product policy)*. Bratislava, Slovakia : Sprintvfra, 385 p. ISBN 978-80-8884-843-1.

Koprda, T., Košková, M. 2015. *Vizuálna komunikácia v marketingovom prostredí (Visual communication in the marketing environment)*. Nitra, Slovakia : UKF, 43 p. ISBN 978-80-558-0895-6.

Kotler, P. Wong, V., Saunders, J., Armstrong, G. 2007. *Moderní marketing (Modern marketing)*. Prague, Czech Republic : Grada. 1041 p. ISBN 80-247-1545-2.

Křížek, Z., Crha, I. 2012. *Jak psát reklamní text (How to copywrite)*. 4th updated edition. Prague, Czech Republic : Grada. 224 p. ISBN 978-80-247-4061-4.

Lehmann, D. R., Winer, R. S. 2005. *Product Management*. US : McGraw-HillPrimis. 494 p. ISBN 978-0071238328.

Minárová, M., Kubicová, Ľ. 2008. Obal a jeho význam s pohľadu spotrebiteľa u potravinárskych výrobkov. (Product packaging and its importance from the point of view of the consumer of foodproducts). *Obchod, jakost a financev podnicich – determinanty konkurenceschopnosti VI*. Prague, Czech Republic : Česka zemědělska univerzita. p. 139-142. ISBN 978-80- 213-1774-1.

Nielsen, J., Pernice, K. 2010. *Eyetracking web usability*. Berkeley, US : New Riders. 437 p. ISBN 978-0-321-49836-6.

Pieters, R., Warlop, L. 1999. Visual attention during brand choice: The impact of time pressure and task motivation. *International Journal of Research in Marketing*, vol. 16, p. 1-16. https://doi.org/10.1016/S0167-8116(98)00022-6

Rigaux-Bricmont, B. 1982. Influences of Brand Name and Packaging on Perceived Quality. *Advances in Consumer Research*, vol. 9, no. 1, p. 472-477.

Šedík, P., Kňazovická, V., Horská, E., Kačániová, M. 2018. Consumer sensory evaluation of honey across age cohorts in Slovakia. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 673-679. https://doi.org/10.5219/938

Titěra, D., Voběchovská, M. 2013. *Etikety na medu. (Labels on honey)* [online] s.a. [cit. 2016-07-11]. Available at: http://1url.cz/itQ2P.

Urbany, J. E., Dickson, P. A., Kalapurakal, R. 1996. Price search in the retail grocery. *Journal of Marketing*,vol. 60, no. 2, p. 91-104. https://doi.org/10.2307/1251933

Velčovská, Š. 2009. Postoje spotřebitelů k obalůma obalovým materiálům na trhu potravinařských výrobků. (Consumers´ attitudes to packaging and packaging materials on the foodproducts market). Bratislava : EKONÓM. 234 p. ISBN 978-80-225-2746-0.

Vysekalová, J. 2004. *Psychologie spotřebitele. (Consumer psychology)*. Praha : Grada, 284 p. ISBN 80-2470-393-9.

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FEATURES OF HEAT TRANSFER IN THE ENVIRONMENT WHEN IT IS SPRAYED WITH ROTARY ROLLERS

Igor Stadnyk, Volodymyr Piddubnuy, Olena Eremeeva, Halyna Karpyk

ABSTRACT
The analytical analysis of roller impact on the medium and its behaviour at deformation influences are carried out, ways of choosing an optimal variant of the process for providing the maximum or minimum value of parameters (criterion) are proposed. The physical essence of the equation of energy flows of the intensity of deformation of the mass of the medium, which depends on the method of applying mechanical forces, the degree of its previous dispersion (recipe) and its physical and mechanical properties, is considered. For a more visual view and understanding of the overall performance of the research, a scheme of causal relationships between the medium and the roll, which determine the temperature change of the dough injection process, is proposed. It is noted that the determination of the influence of the temperature of deformation processes during the passage of the process of injection of the medium by roller working bodies plays an important role for calculations of the design of molding, roll-over equipment. The influence of thermal circulatory chaotic streams is considered, the character of which also leads to violations of the general heat circulation in the medium during the injection. At the same time, the level of such violations may be sufficiently deep with changes in the directions in their contours that influence the process, according to each particular period of the deformation stage in the injection site of the molding machine. Partly revealed methods for determining the change in temperature fluxes in the dough and on the surface of the roll. On the basis of which the change of the energy potential in the interaction of the viscous medium with rotating roller working bodies in the molding machine is considered. An exchange of energy between moving particles is discovered due to collisions of molecules of a more heated part of the body with a greater energy and the transfer of the energy particle to adjacent particles with less energy. It is noted that the definition of temperature fluxes during the process of injection of the medium by roller working bodies plays an important role in calculating the design of molding, roll-over, mixing equipment. The obtained data answer a number of questions about the possibility of thermoregulation of the process of the work of the working bodies into the environment.A set of measures for measuring the temperature during the use of devices was carried out. On the basis of their data, a real temperature change in the roller unit of the molding machine is considered, with a comparative analysis of existing ones with a newly designed design. The change of the energy potential in the interaction of the viscous medium with rotating roller working bodies in the molding machine is considered. An exchange of energy between moving particles is discovered due to collisions of molecules of a more heated part of the body with a greater energy and the transfer of the energy particle to adjacent particles with less energy. It is noted that the definition of temperature fluxes during the process of injection of the medium by roller working bodies plays an important role in calculating the design of molding, roll-over, mixing equipment. The obtained data answer a number of questions about the possibility of thermoregulation of the process of the working bodies into the environment. The essence of formation of temperature fluxes is revealed and the method of their determination in a roller assembly of a molding machine with a comparative analysis of existing ones with a newly designed design is proposed.

Keywords: dough; injection; heat conduction; heat propagation; heat flux; roll; phase; medium

INTRODUCTION
Among the heat processes used in production, the main place is the process of transfer of heat from its sources to the treated material. The exchange of energy between moving particles occurs as a result of their direct collisions. In this case, the molecules of the more heated part of the body, which have more energy, transmit the energy share to adjacent particles with less energy. In gases, the energy transfer is carried out by diffusion of molecules and atoms, in liquids and solid dielectrics, by elastic waves. In metals, the transport of energy is carried out mainly by the diffusion of free electrons.

The generated heat circulation flows are more often chaotic, which also leads to violations of the overall heat circulation in the environment. At the same time, the level of such violations can be sufficiently deep with changes in the directions in its contours. Thus, the hydrodynamic modes in the injection node of the molding machine are

determined by the heat flux formed in the dough when it interacts with the surfaces of roller working bodies.

Since all phenomena (processes) **(Drozdziel, 2016)** in nature are described by analogous kinetic equations, they also have a similar nature of the course in time. The speed of any process and, consequently, the rate of transfer of substance after the onset of the action of roller working bodies due to the inertia of the systems changes gradually: first increases to a certain maximum value, holds for this time for this value, and then, as the equilibrium state approaches, gradually slows down to zero (stopping process). Between the environment and the working body there is always an additional higher temperature. There is a difference in temperature potentials $\Delta t = t_1 - t_2$, as a result of which heat passes from a more heated point to a less heated one. The difference in the temperature potentials Δt in the points under consideration is a measure of the deviation of their state from equilibrium between them. This difference causes the heat to move from a heated to a less (colder) point of the space of the medium, which is in the space between the rolls. Thus, this characterizes the difference in the potential of the surface of the roll and the medium itself (dough).

Analysis of model approximations of medium types.

Determination of the temperature fluxes during the process of injection of the medium by roller working bodies plays an important role in calculating the design of molding, roll-over, mixing equipment. However, the research **(Singh et al., 1997)**, the process of compression of the dough between the rolls only the kinetics of its injection, in isolation from the very change in its structure, does not allow to determine the optimal parameters of the process. The change in the physico-chemical properties of the dough under the influence of the working body is considered in the work **(Khamis, 2009)**, but the method for determining the temperature is not shown. In the study of authors **(Bloksma and Niemann, 1975**), the sequence and mechanism of influence of temperature change on the structure at the injection of yeast dough is not completely considered. In their rheological studies, the authors **(Muratova and Smolihina, 2015)** reveal the value of temperature for changing the structure of the environment, but the method of determining the temperature field of the process is absent. More revealingly, the disclosure of temperature effects in the work **(Stadnyk, 2013)**. It led to the modeling of the viscous medium in the gap between the rollers and the temperature distribution was reflected.

Without an explanation of the formation and definition of heat flows in the yeast dough, it is impossible to substantiate optimal regimes and methods of controlling this process. Therefore, in addition to the rational mechanical action of rolls on the environment, it is necessary to maintain an optimal temperature, which should ensure the flow of nutrients to bacterial cells and the required moisture and gas exchange.

Data analysis gives a number of questions about the possibility of thermoregulation of the process of the work of the working bodies into the environment. Therefore, we propose methods for determining the temperature variations in the roller unit of the molding machine in comparison with the new structure **(Stadnyk, 2018)**.

At present, such an estimate is made by the value of the energy coefficient proposed by the well-known scientist V. Kirpichov. This coefficient is defined as the ratio of the amount of heat transmitted through the surface of the heat transfer to the amount of work spent on overcoming the hydraulic resistance when moving the medium. In practice, the energy factor is used in the form of:

$$E = \alpha / N_0$$

Where:

α - coefficient of heat transfer on the surface under given conditions of interaction, W/(m^{2}K); N0 is the energy expended for 1 sec on the movement of the medium, assigned to 1 m^2 surface, W/m2, the corresponding energy is determined by the expression:

$$N = \frac{G\Delta p}{\rho F_b}$$

Where:

G - mass flow rate of the medium, kg.s^{-1}; ΔP - hydraulic resistance of the channel, Pa; ρ - is the density of the medium, kg.m^{-3}; Fv - the working area of the channel, m^2.

Forced convective heat transfer allows you to align the temperature field in the environment (Non-Newtonian fluid) to create the same conditions in any zone of the working chamber. This makes it possible to assert that the temperature at any point over a certain period of time converges, reaching the average temperature of the medium at the beginning and end of the injection process. However, we have established **(Derkach, 2017)**, that when the medium is injected, there is a significant difference - the temperature inside the medium is approaching not the temperature of the roller working body formed on the surface, but to a temperature close to the initial 240.

Consequently, the injection process after a while becomes a character that can be considered practically as a regular mode of thermal conductivity. The extreme temperature to which the temperature is directed in the medium is the temperature at a given injection pressure produced by the action of rotating roller working bodies. It affects the nature of the temperature change and the duration of the stage of the injection discrete mode. Also, the temperature of the surrounding environment is influenced by the temperature field in the middle of the medium.

In fact, these phenomena occur simultaneously and, of course, affect one another. Convection, for example, is often accompanied by thermal radiation and thermal radiation - by heat conduction and convection.

When passing discrete deformation on the environment by rollers, the heat at the boundary section is approximately uniform. The propagation cycle is rather short, and heat loss on radiation and convection is negligible. Therefore, it can be assumed that the side surfaces of the roll are in the adiabatic limit state and that the temperature distribution along the plane parallel to the surface of the friction is uniform. Thus, the temperature in

this plane is approximated by one value at the intersection at one-dimensional analysis by finite difference method.

The rolls together with the dough are a multilayer cylindrical wall, which represents only ¼ mutual contact (Figure 1). In this case, the thermal resistance of a multilayered cylindrical wall is equal to the amount of resistance of individual layers. The first layer is the roll wall, and the next layer is the processed medium. This environment can be divided into several layers.

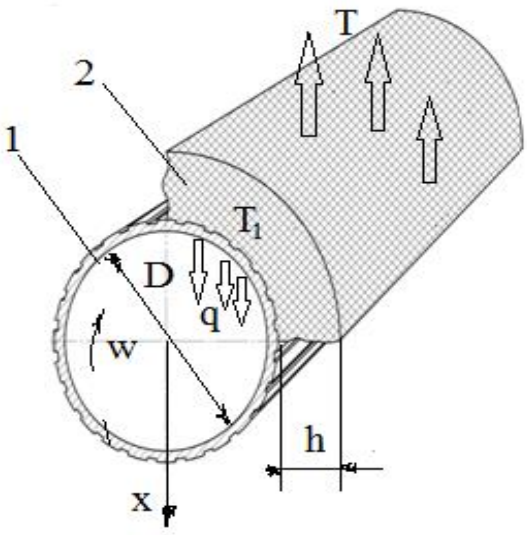

Figure 1 Scheme of impulse friction contact.
Note: 1 - roll working body, mm; 2 - medium (dough); T - constant temperature; T_1 - is the temperature on the surface of the frictional contact.

Scientific hypothesis

At present, scientists have carried out several hypotheses analytically describing the formation and nature of the change in heat transfer, which occured at the dough pieces during deformation. The energy consumption and the process total duration, namely energy level and time of the heat-mass transfer is highly dependent on heat flow movement level. The kinetic energy of deformation characterizes the local heat dynamics, and hence local heat and mass transfer throughout the working volume of the dough. However, the results obtained do not provide a well-founded definition, since the mathematical models that were used are relatively simplified and do not reflect the complexity of the overall heat transfer pattern. Due to the complexity of the solution of such models and the requirment to determine the parameters experimentally, they have not become widespread.

Consequently, the sequence and mechanism of changes formation of heat transfer in the homogeneous structure of the dough, taking into account inherent peculiarities, have been partially studied. Without clarification of the mechanism of formation, that is proceeding in a homogeneous structure during deformation, it is impossible to determine the optimal regimes and methods of control of this process. Analysis of the changes in heat transfer during dough formation and the effect of the working bodies on the finished dough during the formation are absent. Therefore, experimental research methods are more credible. Our research is aimed at overcoming the existing theories and disclosing a new vision of the essence of the formed temperature changes.

Spatial isotropy and temporal unevenness of distribution contribute to the implementation of different heat and mass transfer conditions for each local area of the working volume of the injection unit, and, accordingly, for each individual semi-finished product, which leads to a change in the quality of finished products with the same time of baking. So, due mainly to viscous friction in the dough layers and its partial sliding along the surface of the roller assisted by convection, the transfer of heat into the mass of the dough occurs.

The velocity fields' study of the heat flux was made in order to conduct analytically and practically comparative characteristic of the influence of roller working bodies of different design on the change in the temperature of the dough and to conduct their analysis, by verified statistical methods.

MATERIAL AND METHODOLOGY

Investigation of the process of injection and rollout of the dough was carried out on the molding machine B-54 of the confectionery factory (Ternopil) and physical models, created on the department of OCHT (TNTU named after Ivan Puluj). To determine the heat release on the surface of the friction (the boundary of the roll of the dough), the construction of the reverse heat conduction model is made. By calculation, the temperature of the heating of the dough was determined in the contact area with the rolls and on the basis of the obtained data the axial flow of the flow was determined. Since the temperature gradient in a solid is determined by experimental measurements, then the heat flux can be calculated as the product of the heat conductivity coefficient of the solid on the temperature gradient on the surface. In the inverse of the heat conduction, the method of finite difference is used to estimate the heat flux q (t) at the boundary of the friction section, provided that the known values of the transition temperature on the surface of the roll.

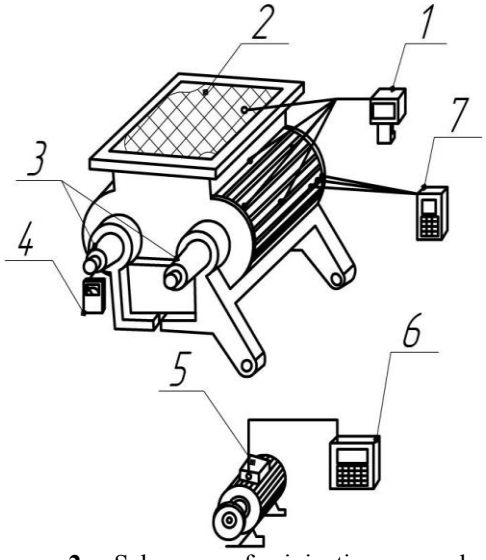

Figure 2 Scheme of injection nozzle for determination of temperature fluxes and power.
Note: 1 - thermal imager; 2 - medium (dough); 3 - roller working bodies; 4 - tachometer; 5 - electric motor; 6 - watt meter; 7 - potentiometer.

Instruments and eguipment

In the work the method of complex determination of the effective thermophysical characteristics of the bubble dough is used and experimentally determine the dependence of the thermal conductivity λ, the volumetric heat capacity cρ, the temperature-conductivity a on the temperature at the stages of its discrete injection of all the mixed mass. Calculations are carried out in the case of injection: the temperature of the environment Tc - 180 °C; the temperature of the test -25 - 280 °C; the thickness of the dough layer on the roll is accepted 20 - 22 mm; average dough velocity - 2.0 $m.s^{-1}$, density $\rho = 1165\ kg.m^{-3}$.

Experimental data on determining the heating temperature of the roller working body during the injection process were obtained using thermocouples located in the interaction zone (Figure 2). A hot copper-constantan junction thermocouple with a corresponding graduated table has been used. At complex temperature measurements simultaneously in a few coordinates of the object a battery of differential microthermopares, in which the number of single junctions is six, is used. The cold junction of the battery is common (Figure 3). Hot jumps in batteries are equal to the number of points in the measuring instrument.

The temperature change was fixed by three thermocouples fixed to the surface of the roll, respectively, at a distance of 5; 15 and 25 mm from the end of the roll. The data are used to calculate the heat flux on the viscous friction surface.

Emerging thermo-EDC thermocouples are proportional to the difference in temperature between hot and cold junctions, which is measured by compensating type devices - potentiometers or millivoltmeter. The compensation type device: DC potentiometer PP-63, class 0.05, (Priborpostavka) was used for researches.

Thermocouple calibration: The reliability of the results at temperature measurements with the aid of thermocouples was obtained by preliminary calibration of the produced thermocouples directly under laboratory conditions. The thermocouple calibration consists in determining the potentials of the thermocouples made by the battery and the corresponding indicators of the reference thermometer (Figure 3).

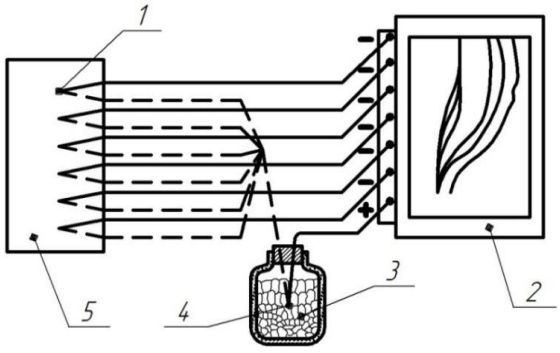

Figure 3 Schematic diagram of the device for measuring temperatures using microtherm battery.
Note: 1 - hot jig, 2 - multipoint electron potentiometer, 3 - Dewar dish, 4 - total cold junction, 5 - investigated object.

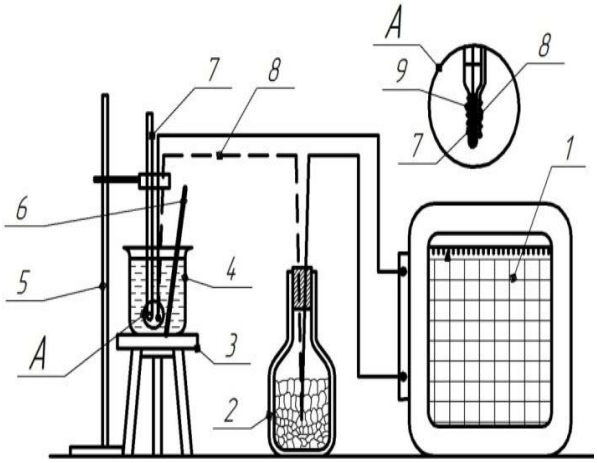

Figure 4 Scheme of installation for calibration of thermocouples.
Note: 1 - measuring self-recording device, 2 - vessel Dewar with ice, 3 - electric heater, 4 - capacity with working fluid, 5 - tripod for fastening the thermometer and thermocouples; 6 - glass rod for mixing; 7 - a mercury thermometer, 8 - a single copper-constantan thermocouple; 9 - a thread for fixing a hot junction thermocouple.

For direct measurement of the heat flow density, that cross-cutting the dough surface, we used the sensor, that was developed by Food Technology Chair of Ternopil Ivan Pul'uj National Technical University which is a several microthermocouple located close to each other. This apparatus uses, the measuring self-recording single-channel device RP-160-00 with a thermoelectric converter, (Priborpostavka) and the technical thermometer of the WT-1 type (Lviv) with a scale from 0 to 100 °C and the precision of 0.1 °C.

Construction of the temperature scale.

After calibrating the thermocouples according to the measurements, a table and the calibration curve of the thermocouple E in millivolts were constructed. E = f (t) is the dependence of the thermo-EDF developed by the thermocouple (in terms of millivoltmeter), on the temperature of its hot junction (in terms of the mercury thermometer).

Metrological analysis of measurable means

Checking the replication of experiments on an experimental installation. When checking the reproduction of pouring penny rolls from the flour of the highest grade of the same lot of grinding and the same baking properties. Initially, they found the average value of yj, then the difference (yj-yj) and the square of the difference $\Delta y_j^2 = |y_j - y_j|^2$ .The results of the measured parameters in the experiments of each series (yj (1), yj (2), yj (3)) of their deviations from the mean values $(y_j - y_j)$, The squares of deviations and dispersion for each series of experiments Si2 were summed up in a table.

The dispersion estimation for each series of experiments uses the formula:

$$S_i^2 = \frac{1}{k-1}\sum_{j-1}^{k}(yj - yj)^2$$

With the number of experiments in the series K = 3:

$$S_i^2 = \frac{1}{2}\sum_{j-1}^{k}(yj - yj)^2$$

Assessment of experiments was carried out according to the Cochran criterion:

$$Gp = \frac{(S_i^2)max}{\sum_{i=1}^{k} S_i^2}$$

For the temperature of the upper surface of the roll:

$$Gp = \frac{7}{19.34} = 0.0036$$

The calculated values of the Coherent Gp criterion are compared with the table values for N and F = K-1.

Determination of threshold of sensitivity of measuring devices

Acceptable threshold of sensitivity is given in the parts of the main error of the device (25 - 50%).

Determination of the metrological index

The basis is the rule of three sigms of the metrological index A for the measuring instruments used:

$$A = \frac{3\delta}{\bar{y}_j}$$

Where:

(δ - mean square deviation with repeated measurements,

$$\delta = \sqrt{\frac{\sum_{j=1}^{n}(y_j - \bar{y}_j)^2}{n-1}}$$

$\overline{y}_j$ - average value of the measuring parameter,

$$\overline{y}_j = \frac{\sum_{j=1}^{n} y_j}{n}$$

n - the number of repetitions of measurements.

The condition of sufficient accuracy for experimental studies is the value of A = 0.1:

$$A = \frac{3\sqrt{\frac{\sum_{j=1}^{kn}(y_j - \bar{y}_j)^2}{n-1}}}{\frac{\sum_{j=1}^{n} y_j}{n}}$$

For the investigated parameter / $t_{вк}$ / y_j / 1series calculated value A:

$$A = \frac{3\sqrt{\frac{38.68}{11}}}{16.1} = 0.035$$

Since, for measuring temperature values, the values of the metrological index A do not exceed 0.1, they are sufficiently accurate in measuring instruments.

On the basis of metrological analysis, the mean square error of measurement is determined.

Statisic analysis

For an unbounded plate (roll surface) with an appropriate thickness δ heated by a heat flux of constant density $g_п$ differentiation of non-stationary thermal conductivity is used:

$$\frac{\partial t}{\partial \tau} = a\frac{\partial^2 t}{\partial x^2}$$

with initial conditions t (x.0) = t_0 = const.

Heat transfer by heat conduction occurs due to the energy of the motility transfer:

$$g = -\lambda gradt + \sum_{1}^{n} i_k \gamma_k$$

Where:

g - density of the heat flux is superficial, W/m^2; IC-enthalpy of water or vapor, J.kg^{-1}, carried in the presence of a gradient temperature grad t with intensity (m^2.s^{-1}).$\gamma_к$, k, kg.

Boundary conditions of the second kind:

$$\frac{\partial t(\delta,\tau)}{\partial x} = -\frac{g(t)}{\lambda}.$$
$$\frac{\partial t(0,\tau)}{\partial x} = 0$$

The solution of this equation at given boundary conditions allows us to determine the temperature field t (x, t) and the field of heat fluxes in the plate (surface) of the roll.

The definition of λ was carried out on the basis of a generalized equation which was obtained on the basis of the second kind of regime and the solution of other equations for determining the temperature field:

$$\lambda = h\left[\frac{F(u^{-1}(\Delta t_1^{11} + \Delta t_2^{11}) - u^{-11}(\Delta t_1^1 + \Delta t_2^1))}{\overline{u^1 - u^{11}}} - R\right]^{-1}$$

Where:

F is the Fourier number, the dimensionless process time F = aτ / δ^2; u - tempo of heating the surface of the test, K / s, u = a / dt; Δt is the temperature difference on the surfaces of the test, K in (1) and (11) in different thermal modes; h - thickness of the dough layer, m; R-ballast thermal resistance, m^2 K / W, equal to h / λ.

In experiments, direct measurements were performed, ie the desired values were obtained directly as a result of the experiment. A number of repeated direct measurements of the same magnitude were processed mathematically, namely, the application of the theory of probabilities and statistical methods. To analyze the errors of the result for a large and small number of measurements, the law of distribution of random mistakes of Student was applied. In order to achieve the specified accuracy, the definition of gross errors used criterion Student.

To plan a physical experiment, the Seidel-Gauss method was used, the completion of which is a multifactor correlation dependence on the basis of the obtained Protoedaconov function. Applying it for processing the results of chemical research allowed to obtain adequate results

$$Y_{\Pi} = \frac{\prod_{i=1}^{k} y_i}{y_{cp}^{k-1}},$$

Where:
y_i - partial functions defined using the least squares method; k - number of factors; y_{cp} - the average value of all the results of the experiment that was taken into account.

The experiments were carried out to the plan with corresponding matrices of planning the experiment with the specified numbers of experiments and the boundaries of the change of factors. As the optimization parameter, the temperature that occurs in the dough during the movement is used. It depends on the input parameters that can be represented in the form:

$$T = f(P, L, h),$$

Where:
T - temperature, P - pressure, L - distance between thermocouples, h - thickness of the layer of mass of the dough.

During the planning of experiments to determine the functional change of the dough temperature, a standard symmetric planar matrix of the three-factor experiment was used at three levels of variation of factors for the total number of experiments of one repetition.

$$N = p^k = 3^3 = 27$$

The experiments were performed in a triple repetition for each numbered line of the matrix. The sequence of the first and subsequent experiments was established in accordance with the numbered order of the randomized matrix of the trifactorial experiment. The levels of factor variations and the results of the implementation of the plan matrix, or the experimental array of data obtained during the experiments.

The experimental data array was processed using the computer application package (Statistica-12). The approximating response function, or the optimization parameter, that is dough temperature T, determined experimentally, was calculated with a help of a mathematical model of the complete polynomial of the second power:

$$T = b_0 + b_1x_1 + b_2x_2 + b_{12}x_{12} + b_{11}x_1^2 + b_{22}x_2^2,$$

Where:
x_1, x_2, x_3 - respectively, the coded designation of the dough pressure *P*, the diameter of the rollers *d*, the gap between the rollers h; $b_0, b_1, b_2, b_{12}, b_{11}, b_{22}$ – free term and coefficients of the values of the corresponding factor x_i and their interaction.

At the probability value p = 0.95 and the t-alpha criterion value of 2.053, the following statistics data was obtained. The coefficient of multiple determination D = 0.962; coefficient of multiple correlation R = 0.981; estimate standard deviation s = 0.637; Fisher's F-criterion equal 467.71. Coefficient D is significant with a probability of P = 0.9999.

The evaluation of the reproducibility of the experiments was carried out according to the Cochran's criterion with significance level of 5%. The coefficients of the regression or approximation function equation, which is written as $T = f_T(x_1; x_2; x_3)$ under the condition of orthogonality and symmetry of the plan matrix of the planned factor experiment, were determined according to the standard method by known dependencies.

After evaluating the statistical significance of the coefficients of the regression equation according to the - Student criterion and verifying the relevance of the approximation model according to Fisher's F-criterion and the transition from coded indicators of factors to natural, a regression equation is obtained that characterizes the functional change in the dough temperature T depending on the change $200 \leq P \leq 700$ H, $15 \leq h \leq 35$ mm, $155 \leq d \leq 165$ mm.

$$T = 36{,}4 + 4{,}2 \cdot 10^{-3} P - 1{,}7d + 0{,}06h + 2{,}510^{-4} Pd - 6{,}8 \cdot 10^{-5} Ph + \\ + 8{,}3 \cdot 10^{-3} dh + 5{,}8 \cdot 10^{-6} P^2 + 0{,}01d^2 - 8{,}0 \cdot 10^{-4} h^2$$

The graphic representation of the dough temperature changes according to the experimental data (Figure 5), meaning the response surface of the dough temperature functional change as a function $T = f_T(h; d)$

Measurements of the temperature of the dough in the working chamber, the surface of rolls, the environment were carried out with the use of thermal imaging with the help of the thermal imager Fluke Ti25. The thermal imager manufacturer is "Flukosorpocen" Everett, USA. The software was developed under the software version (PLAST-002).

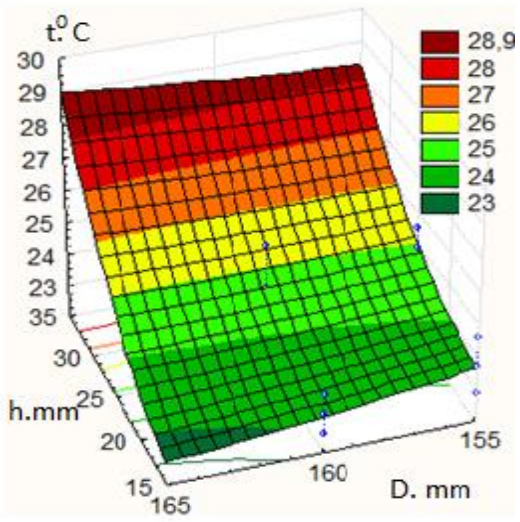

Figure 5 Response surface.

RESULTS AND DISCUSSION

Consider the process of motion of the medium between rotating rolls (Figure 6). After compression of the medium by rolls zone-2, it enters the main zone-3 injection, which, in accordance with Figure 3 can be conventionally divided into three components - the zone of intense heat generation - 111, the transition - 11, and the heat recovery zone - 1. For this part, in most of the constructions of the injection unit, a convective heat regime is implemented that involves the simultaneous transfer of heat by radiation and convection.

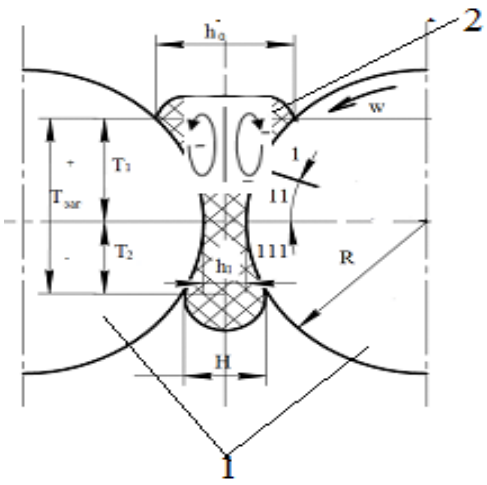

Figure 6 Scheme of temperature distribution at injection.

Note: 1 - rotary rollers; 2 - dough work environment.

The generated thermal circulation flows are more often of a chaotic nature, which also leads to violations of the general heat circulation in the middle case. At the same time, the level of such violations can be sufficiently deep with changes in the directions in the dough contours.

The authors of the works **(Lisovenko, 1983)** note that the change of the dough temperature occurs due to the structural parameters of the working bodies and the working chamber, which create hydrodynamic modes. Created modes determine the intensity of the dough deformation and the expended energy, which is distributed in two directions. The energy expended on the macromolecular structure of the dough gives a positive technological effect, and its expenditures associated with internal friction should be considered beneficial. The expenditures on external friction of the working bodies and the walls of the working chamber, accompanied by the heat output, adversely affect the technological process. In this regard, the mechanical action on the dough should adhere to the investigated limits.

Thus, the hydrodynamic modes in the injection node of the molding machine are determined by two reasons. The first one relates to the heat flux formed on the surfaces of the roll. The second reason concerns the formation of streams involving the gas phase.

The plastic and adhesion strength, form index, and flow index stated in paper **(Obolkina, 2016)** as the dominant factor in the formation of the structure of semifinished products for confectionery products, that must be created taking into account the design features of the equipment. By analogy with the theory of stability of disperse systems, he proposed a theoretical model of aggregative stability of a combined system consisting of semi-finished products with a different structure, taking into account kinematic, hydrodynamic and thermodynamic factors.

Table 1 Empirical coefficients for determining the heat loss in the environment.

Surface type	$\overline{c}$	$\overline{n}$
Vertical	1.4	1.33
Horizontal top	1.7	1.33
Horizontal lower	0.64	1.25

Obviously, each of these reasons is characterized by its driving forces. For the first reason, such a factor is the difference in temperature of the medium and the roll working body of the coolant. In the second case, the motive factor is the presence of the dispersed gas phase. The value of the temperature flux depends on the velocity of the medium and the discreteness of the roller working body, the mass of the yeast wheat dough in the working chamber and the loading hopper, which leads to partial fermentation of sugar in it.

According to **(Shurchkov U., 2013)**, the value of optimal control is referred to the specific energy consumption. It is proved that there is a critical value of velocity and energy consumption. However, in our opinion, false technical decisions are often adopted.

At the same time, a number of researchers **(Kozlov G. F., 1984)** believe that the magnitudes of specific work with varying strength do not give a sufficient view on the technological aspects of the process. As to their and our opinion, the mechanical impact of the working bodies during the deformation with the specific energy consumption – is imperfect.

The method of optimal control of energy load when frictional interaction of friction pairs of dough with rollers under different injection modes allows to set the summed heat to its working surfaces. The management of thermal processes is necessary for the following reasons: to limit the amount of heat accumulated by rollers, in order to reduce the thermal stresses; to lower the surface temperatures in the dough below the permissible ones for it in order to prevent changes in structural and mechanical properties; to provide the work of the injection unit with an acceptable energy load in order to increase the wear and friction properties of the surface layer of the roll; establish the relationship between the rate of change in the temperature of the surface layers and the temperature gradient both on its surface and in thickness.

With the help of a special device that regulates the change of the rheological properties of solid-liquid bodies, Nikolaev investigated the influence of mechanical processing on the structural and mechanical properties of the dough **(Nikolaev B. A., 1976)**. It was found that, with additional mechanical treatment dough viscosity and elastic modulus are reduced.

In mentioned paper, the authors' reveal the reason for dough temperature change, but neither detailed analysis of the phenomena that arise while working bodies operation,

nor the methods for identifying these changes are provided.

Consequently, in the investigated medium of the injection unit, the primary energy source is present in the form of chemical energy of hydrocarbons, which must be transformed into carbon dioxide. In this case, the formation of carbon dioxide has a double thermodynamic manifestation, which is accompanied by the release of thermal energy and the formation of a dispersed gas phase CO_2.The heat energy allocated in combination with the thermal energy of viscous friction is realized in a form that can be considered hypothetically as a system for converting thermal energy into mechanical work of partial mixing (reverse motion) of the medium. The mode of circulation is influenced by the structural parameters, the structure of the dough (the presence of gas) and the gravitational field. However, in large volumes of the medium and the discrete action on it, the regime is violated and leads to limited values of efficiency **(Stadnyk, 2018).**

The instability of the value is due to the fact that the temperature in the circulation part of the liquid phase decreases with the increase of the value h_0 (the dough layer in the chamber). The temperature has its greatest value when the dough is released from the gap of rolls. The coefficient of heat transfer from the medium to the wall of the roll depends on the physical and chemical parameters of the medium, the velocity of the rolls, their design parameters; speed upgrade liquid phase in; the rate of renewal of the liquid phase in transverse planes and the area of contact of the liquid phase in the roll gap (compression). The latter should be a complete reaction of the system to change the capacity of the gas phase.

Consequently, taking into account the complexity of the theoretical determination of the value of the convective heat flow $\dot{Q}_k$, the concept of thermal flow from compression of the dough $\dot{Q}_{cm}$, which already includes the value of the specific heat of absorption of the convective heat flow, is used in calculations. Determination of the specific heat of absorption q_A is based on the general laws of this mass transfer process in the injection node.Taking into account all the thermodynamic processes that occur in the environment under the influence of deformation, we have proposed one of the methods for determining the heat fluxes.

Calculation of thermal absorption flow.

The heat flux transferred to or from the liquid phase of the working medium can be calculated by the equation **(Sokolenko, 2012):**

$$Q_A = q_Д \cdot m_A = \frac{q_Д \cdot m_{X1} \cdot \left(x_к - x_н\right)}{\mu_X} \quad (3)$$

Where:

$q_Д$ - differential heat of gas dissolution (gas component); $x_к$, $x_н$ – final and initial relative molar particles of the absorbed gas in the liquid phase of the working medium; μ_X - molar mass of liquid; m_A - mass flow of absorbed gas (gas component).

Since there are technological requirements for the porous structure of the bagel, therefore, an equilibrium concentration in the gas phase should be established. Accordingly, this is described by Henry's law **(Sokolenko, 2008):**

$$x_i = \frac{1}{E} \cdot p_i \quad (4)$$

Where:

x_i - molar concentration of the absorbed gas in the dough, which is in the thermodynamic equilibrium with the gas phase, in which the partial pressure of the absorbed component is equal; p_i; E - Henry's constant, determined from the dependence:

$$\ln E = -\frac{q_Д}{R \cdot T} + \text{const} \quad (5)$$

For boundary conditions, for example, under atmospheric pressure, we have:

$$\ln\left(\frac{E}{E_{атм}}\right) = \frac{q_Д}{R} \cdot \left(\frac{1}{T_s} - \frac{1}{T}\right) \quad (6)$$

In the calculation, the final concentration of the absorbed gas in the douth $\overline{x}_к$ can be equal to the concentration at equilibrium. Then, on the basis of the above, the heat flux transferred to or from the dough during the injection process will be:

$$Q_a = \frac{m_x p R \mu}{\frac{1}{T_2} - \frac{1}{T_1}}$$

That is why in the general theory of calculation of the injection site it is necessary to take into account the processes of absorption in the working chamber, which in theory at this time are not described at all. Perhaps this is hampered by the considerable complexity of processes.

In this method of calculating the consumption of absorbed gas, as the operating temperature, can be taken by the average temperature of the gas-liquid phase $t = 0{,}5 \cdot \left(t_X + t_Y\right)$ and pressure $p = 0{,}5 \cdot \left(p_{Y1} + p_{Y2}\right)$.

The general heat exchange of the surface of the roller working body with the environment, due to the insignificance of the difference in temperature $T_X - T_{н.с}$, the specific heat flux $\tilde{q}_{н.с}$ can be considered as:

$$q_{н.с} = \frac{c \cdot \left(T_{cm} - T_{н.с}\right)^n \cdot F_{cm}}{m_{Y1}} \quad (7)$$

Where:

T_{cm} – the temperature of the outer wall of the roll, which does not interact with the medium; F_{cm} – the design surface of the roller working body; c, n – empirical coefficients, are selected according to table. 1 for different types of surfaces.

Calculation of the temperature of the roll.

When calculating the temperature of a roller working organ during all stages (tightening, compression, injection), one can use the heat transfer equation as a plane plate with a two-way heat output **(Derkach,, 2017)**.

$$Vc\rho\delta(\frac{-\partial T}{\partial x})dx = n\alpha(T - T_c)dx$$

Where:

V- the velocity of the roller working body; c - specific heat capacity of the roller working body; ρ - specific gravity of the material of the roller working body; δ is the thickness of the cylindrical wall of the roller working body; x - the distance passed by the roller working body with the environment from the previous stage; n - coefficient, which takes into account the cooling circuit of the roller working body (with two-sided return of heat п=2, and with one-sided-1); α - coefficient of heat transfer; T_c is the ambient temperature (cooler).

After integrating with the temperature of the roller working body at the exit fromthe $T_{вих}$ section, we obtain the equation:

$$T = T_0 + (T_{вих} - T_c)exp(\frac{-nx\alpha}{\rho cK_1V}) \quad (8)$$

Where:

K_1 is the correction factor characterizing the fraction of the length of the roller working body with the medium (non-Newtonian fluid) on its surface.

To determine the temperature of the medium after the discrete action of the roller working body on it, as well as the cooling temperature at the exit from the gap, the heat conduction problem was used for an unbounded flat wall.

$$\frac{\partial T(x,\tau)}{\partial \tau} = \frac{\lambda}{\rho}\frac{\partial^2 T(x,\tau)}{\partial x^2}$$

Under conditions τ>0; h/2<x<h/2;

$$T(x,0) = f(x)$$

$$\lambda\frac{\partial T(h/2\tau)}{\partial x} = \alpha_в\left[T_0 - T(h/2\tau)\right]$$

$$\lambda\frac{\partial T(h/2\tau)}{\partial x} = \alpha_н\left[T_0 - T(-h/2\tau)\right]$$

Where:

h - the thickness of the media band on the roll working body; $\alpha_в$ and $\alpha_н$- coefficient of heat transfer on the upper (external) and lower (internal) band of themedium; T_0 - ambient temperature (room).

Given that the cooling takes place in seconds in open space before applying to the formation, we use the formula:

$$\alpha = \alpha_a(\frac{T^{0.04}}{29} + 1.2\upsilon^{0.1})$$

Where:

α_a - correction factor; v - velocity of the medium, $m.s^{-1}$.

The Pep's application fopmly shows the discharge of the current from the roll by the radiation source, the second one - due to the convection.

In the course of the calculation of the current cost of the work of the roller working body as the basic values of the coefficient of capacity of the pipe was used for the weathering of the weathering system - 150 kkal /m^2h.°C.

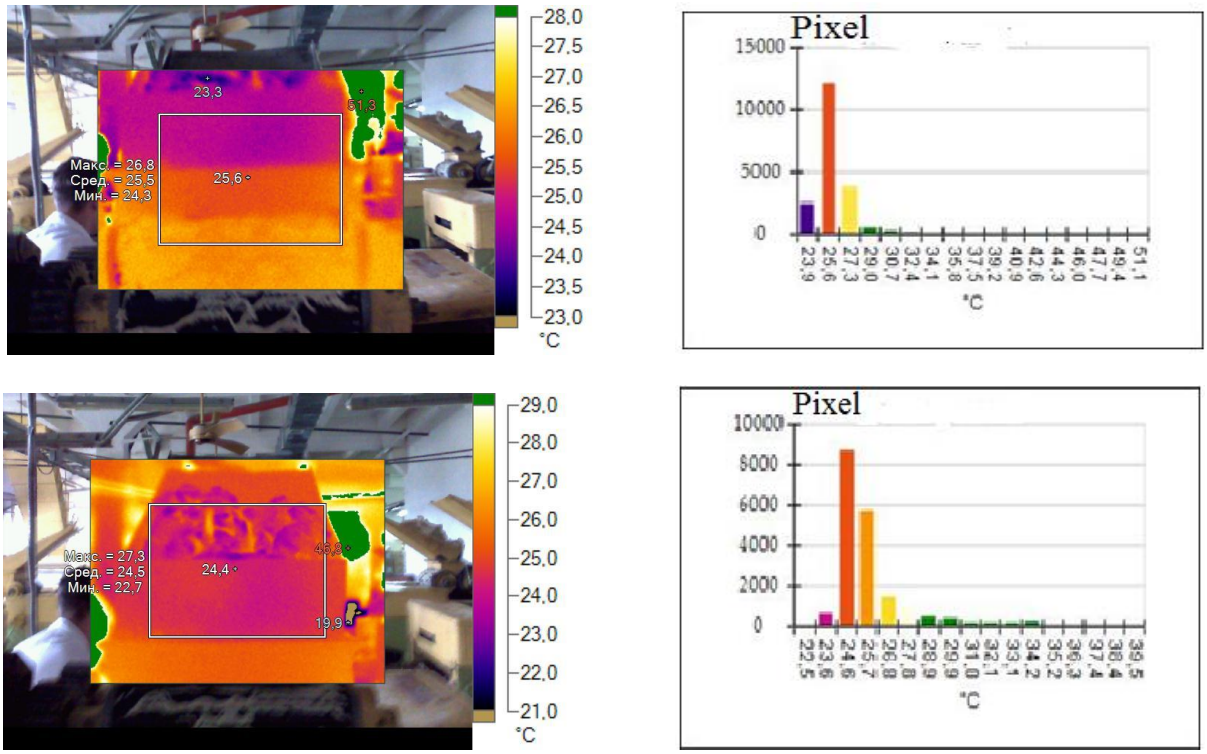

Figure 7 Thermal imaging of the injection unit. Note: a) a new roll construction; b) the existing roll construction.

The K_1 coefficient for the quantifiable value can be determined by comparing the optimal and quantitative data. In a trial, it can be kept by measuring the temperature of the roll working organ at the beginning and end of the discrete process. However, such measurements may not be measurable from the nonobacterial point of the tooth. The only one of the definitions of K_1 is the comparison of the calculated and factual temperature of the roller working body, known in the environment of the known action.

The proposed ways of determining the heat flows are quite complex and limited to the necessary information, since the yeast dough constantly changes over time with its structural and mechanical properties in its deformation. There are other calculation ways to determine the heat fluxes that are based on the processes of heat transfer (convective, heat conduction, radiant, heat transfer), which will be considered in subsequent work. Taking into account the complexity, we give a generalized method of determination.

Determination of the temperature of roll and dough devices.

In determining the coefficients included in equation (8), it is necessary to take into account the fact that at the compression region the value of the temperature pressure at the angle of rotation changes all the time, and the value of the coefficient of heat transfer can be determined rather approximate, since when determining it, it is necessary to take into account the presence of the site, which affects heat transfer. You also need to know the speed of the working environment in the area of rotating rolls. These values can be determined experimentally, but research on this subject has not yet been carried out.

In order to qualitatively evaluate the change in temperature in the dough and heat losses in the environment, which are known to depend on the magnitude of its deformation and biological processes, a thermal imaging of the test facility as a whole was carried out, the results of which are shown in Figure 6. Thermophysical survey was carried out for a number of old and new roller working bodies at given technological modes of work of the molding machine.

The calculations for calculating the temperature of the roller working body from the ground of 08 kp at the injection are shown in the form of cyphilic oxidation in Figure 7.

The shaft working bodies, according to the process, were evacuated by a natural clutch in a quiet air environment in the workshop. Starting from 18 - 27 s the process of compression and injection (curve -1) for the roll of the old cooling design has xapaktype increase in temperature, both external and internal surfaces at 25 - 28°C. At the same time, curves 2 of the new roll have a more smooth transition of temperatures with a high quality impact on the medium. This process lasts 5s less with coil roll to 260c, when the old - 280s. This phenomenon is due to the prolongation of the viscous flow of the medium and the friction of the surface of the roll. Under new designs, there is no extra heat.

With the corresponding deformation resistance, the temperature calculation is closely related to phase transformations in the medium. Certain thermophysical parameters, such as the thermal conductivity and the coefficient of thermal conductivity α, are determined by the time required for the determination of the temperature.

The change in the temperature of the roller working body in the zone of reflux and cooling (period of idling) was calculated by fopmyl (8). Calculations are carried out in the case of injection: the temperature of the environment Tc – 180°C; temperature of the mass of the medium – 28°C; the thickness of the medium layer on the roller working body is adopted – 25 - 35 mm; the average velocity of the medium is 2.0 m / s, the density ρ = 1165kg /m^3. The thermophysical dependencies of properties (bubble dough) on temperature are determined by the formulas λ (w /m^{2}K), c (j / kgK):

$$\lambda = 1.153(0.278 + 0.0045T)$$
$$C = 4167.4(0.58 + 0.0011T)$$

Table 2 shows the thermophysical properties of a dough processed by an old roll, and table 3 new rolls: specific (mass) heat capacity, thermal conductivity, density.

It is clear from the analysis of the tables that the thermophysical properties of the dough under the influence of the deformation of the new roll construction almost remain at one level.

The calculations of temperature are made for two roll structures at given technological pressure of injection (Figure 8).

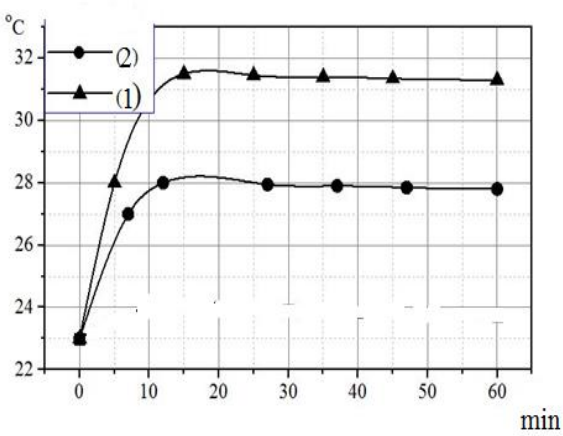

Figure 8. Influence of the stage of the injection process on the temperature of the roller working body.
Note: 1- for the old construction; 2nd for new

In Figure 8 shows dependencies illustrating the thermal efficiency of rolls of the old and new structures after each discrete injection. In the coordinate system, these dependencies have the form of straight lines with almost the same angle of inclination, but different values.

It is possible to note a rather significant difference between the effective accumulation and the return of the resulting temperature by roll when interacting with the medium. Simultaneously from the figure it follows that after the third cycle of injection, the temperatures have the same value. This indicates the stability of the temperature in the subsequent injection of the medium.

Taking into account the mathematical model of the process and experimental data, plotting dependencies

Table 2 The thermophysical properties of wheat dough.

Stage of injection bagel dough	Condition of the dough after the technological process						
	t, ^{0}C	Density ρ, kg/m^3		Heat capacity C, J/(kg^{o}K)		Thermal conductivity λ, W/(m^{o}K)	
	After injection	After injection	After the formation	After injection	After the formation	After injection	After the formation
Mixed up	26.5	1190	1180	0.648	0.680	0.355	0.360
After 60 minutes deformations	28	1160	1153	0.695	0.705	0.362	0.372
After 100 minutes deformations	32	1090	1030	0.708	0.718	0.380	0.4

Table 3 The thermophysical properties of wheat dough when sprayed with a new roll.

Stage of injection bagel dough	Condition of the dough after the technological process						
	t, ^{0}C	Density ρ, kg/m^3		Heat capacity C, J/(kg^{o}K)		Thermal conductivity λ, W/(m^{o}K)	
	After injection	After injection	After the formation	After injection	After the formation	After injection	After the formation
Mixed up	25.5	1190	1188	0.648	0.65	0.355	0.36
After 60 minutes deformations	25.7	1190	1189	0.65	0.65	0.357	0.352
After 100 minutes deformations	26	1179	1189	0.66	0.673	0.36	0.365

T = f (h, P) were constructed at distances between the thermocouples L = const. The graphic image clearly captures the change in temperature in the plane, which varies within the appropriate range for different rolls.

Analyzing the following results of measurements and calculations, one can make the following general rationale for the effect of rotating rolls on the environment:

- the temperature of the working medium in the radial direction increases with the approach to the working roll 4 - 7°C with a difference in at speeds u=(0.4-1) M/C and the relative radius of the medium on the roll and the roll r_2/r_1=1.2; the magnitude of this difference depends on the magnitude of the degree of compression increase;
- the temperature of the working medium at the compression and injection area is practically changed at the angle of rotation of the roll, indicating that it is intensively transported and partly stirred;
- the temperature of the surface of the body of the roll t_{no6} is evenly distributed at the angle of its rotation, with increasing pressure the injection increases linearly and repeats the increase in the temperature at the exit with a difference of about 7°C;
- the temperature of the working medium and the roller surface in the axial direction is evenly distributed in the new designs. In determining the temperature in the axial direction, no changes were found.

CONCLUSION

From the general justified actions of the rotating roller worker on the environment: we can conclude:

- to intensify the injection process possible by ensuring temperature constancy;
- increase of heat transfer surface;
- making rolls from a material having the maximum thermal conductivity;
- intensification of the process of heat conduction through the wall of the roll and heat transfer from the wall of the roll to the inner hollow cylindrical surface;
- increase of heat loss in the environment (return of heat by a roller surface that does not come into contact with the dough).

REFERENCES

Bloksma, A. Niemann, W. 1975. *The effects of temperature on some rheological propertits of wheat flour doughs. Journal of Texture studies*, vol. 6, no. 3, p. 343-361. https://doi.org/10.1111/j.1745-4603.1975.tb01130.x

Derkach, A., Stadnyk, I., Sukhenko, B., Vasiliev, V. 2017. Methodology determination of temperature flows in the zone of action of valveworking swaths. *Food Industry of Agriculture*, vol. 1-44, no. 3. p. 19-23.

Derkach, A., Stadnyk I., Vitenko, T. 2016. Simulation of components mixing in order to determine rational parameters of working bodies. *Advances in Science and*

Technology Research Journal, vol. 9., p. 130-138. https://doi.org/10.12913/22998624/64068

Khamis, M. 2014. *Characterization and Evaluation Of Heat Treated Wheat Flours:* dissertation theses. Manhattan, Kansas: Kansas State University. 160 p. Available at: https://core.ac.uk/download/pdf/33353737.pdf

Kozlov, G., Brazhnik, M., Pshenishnjuk, G. 1984. Influence of the dough deformation while kneading on its rheological properties. *Izvestiya Vuzov, Food Technology,* vol. 3. p. 39-41.

Lytovchenko, I., Lisovenko, O. 1983. Determination of energy consumption for heating the dough while kneading. *Bakery and Confectionery Industry*, vol. 8. p. 28-30.

Muratova, E., Smolihina, P. 2015. *Reologiya konditerskih mass*: monografiya: Russia, Orel University, vol. 6, p. 188.

Nikolaev, B. A. 1976. Measuring of structural and mechanical properties of flour dough. Moscow : Pishchevaya Promyshlennost (Food Industry), 246 p.

Obolkina, V., Skrypko, A., Kyianytsia, S., 2016. The scientific approach to the creation of technologies pastry recreational purposes using flour with malt oats. Food industry, vol. 19, p. 73-78. Available at: http://nbuv.gov.ua/UJRN/Khp_2016_19_16

Kenneth, J. V., Rotstein, E., Singh, R. P. 1997. *Handbook of food engineering practice*. United States : CRC Press. 736 p. ISBN: 9780-8493-86947.

Shurchukova, U., Radchenko, O. 2013. Theoretical study of hydrodynamic and heat transfer processes occurring in the channel of the pre-matrix and matrix zones of the extruder. *Scientific Wworks of Odessa National Academy of Food Technologies*. vol. 43, no. 2, p.7-10. Available at: http://nbuv.gov.ua/UJRN/Np_2013_43%282%29__4

Sokolenko, A., Mazaraki, O., Piddubnuy, V., et al. 2012. *Energy transformations and energy saving in food technologies.*monograph. Kiev, Ukraine : Phoenix, 484 p.

Sokolenko, A., Shevchenko, O., Piddubnuy, V., 2008. *Intensification of mass-exchange processes in food technologies:* Kiev, Ukraine : Luxar, 443 p.

Stadnyk, I., Novak, L, Matenchuk, L. 2018, Global rheological approach tothe quality of medium injected by the rollers. *Potravinarstvo Slovak Journal for Food Sciences*, vol. l1, no. 1, p. 234-245. https://doi.org/10.5219/867

Stadnyk I., 2013. *Scientific and technological bases of processes and development of equipment for loosely mixing of dough:* dissertation theses. Kyiv, Ukraine: National University of Food Technologies, 40 p. Available at: www.elartu.tntu.edu.ua/bitstream/lib/20799/1/Monohrafiya_Stadnyk.pdf

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[abstract] doi: https://doi.org/10.5219/878 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 116-122**
[abstract] doi: https://doi.org/10.5219/900 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 123-128**
[abstract] doi: https://doi.org/10.5219/876 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 129-134**
[abstract] doi: https://doi.org/10.5219/872 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 135-142**
[abstract] doi: https://doi.org/10.5219/881[fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 143-149**
[abstract] doi: https://doi.org/10.5219/873 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 150-156**
[abstract] doi: https://doi.org/10.5219/901 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 157-165**
[abstract] doi: https://doi.org/10.5219/874 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 166-172**
[abstract] doi: https://doi.org/10.5219/889 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 173-179**
[abstract] doi: https://doi.org/10.5219/883 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 180-185**
[abstract] doi: https://doi.org/10.5219/899 [fulltext]

EFFECT OF ADDITIVES TO MICROBIOLOGICAL QUALITY OF YOGURTS
Roman Pytel, Olga Cwiková, Sylvie Ondrušíková, Šárka Nedomová, Vojtěch Kumbár
***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 186-194**
[abstract] doi: https://doi.org/10.5219/886 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 195-201**
[abstract] doi: https://doi.org/10.5219/887 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 202-208**
[abstract] doi: https://doi.org/10.5219/888 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 209-215**
[abstract] doi: https://doi.org/10.5219/891 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 216-225**
[abstract] doi: https://doi.org/10.5219/894 [fulltext]

A THAUMATIN-LIKE GENOMIC SEQUENCE IDENTIFICATION IN VITIS VINIFERA L., STORMY WINES AND MUSTS BASED ON DIRECT PCR
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 226-232**
[abstract] doi: https://doi.org/10.5219/892 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 233-240**
[abstract] doi: https://doi.org/10.5219/898 [fulltext]

CONTENT OF ENDOGENOUS SULFUR DIOXIDE IN WINES
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 241-247**
[abstract] doi: https://doi.org/10.5219/854 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 248-253**
[abstract] doi: https://doi.org/10.5219/910 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 254-261**
[abstract] doi: https://doi.org/10.5219/902 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 262-266**
[abstract] doi: https://doi.org/10.5219/906 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 267-272**
[abstract] doi: https://doi.org/10.5219/895 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 273-278**
[abstract] doi: https://doi.org/10.5219/904 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 279-284**
[abstract] doi: https://doi.org/10.5219/908 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 285-291**
[abstract] doi: https://doi.org/10.5219/905 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 292-298**
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 299-303**
[abstract] doi: https://doi.org/10.5219/885 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 304-312**
[abstract] doi: https://doi.org/10.5219/897 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 313-322**
[abstract] doi: https://doi.org/10.5219/917 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 323-329**
[abstract] doi: https://doi.org/10.5219/829 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 330-336**
[abstract] doi: https://doi.org/10.5219/863 [fulltext]

INHIBITORY EFFECT OF AQUEOUS EXTRACTS OF RAW AND ROASTED SESAMUM INDICUM L. SEEDS ON KEY ENZYMES LINKED TO TYPE-2 DIABETES (α-AMYLASE AND α-GLUCOSIDASE) AND ALZHEIMER'S DISEASE (ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE)
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 337-346**
[abstract] doi: https://doi.org/10.5219/866 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 347-354**
[abstract] doi: https://doi.org/10.5219/869 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 355-363**
[abstract] doi: https://doi.org/10.5219/918 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 364-371**
[abstract] doi: https://doi.org/10.5219/909 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 372-378**
[abstract] doi: https://doi.org/10.5219/871 [fulltext]

FUNGAL DIVERSITY IN THE GRAPES-TO-WINES CHAIN WITH EMPHASIS ON PENICILLIUM SPECIES
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 379-386**
[abstract] doi: https://doi.org/10.5219/882 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 387-396**
[abstract] doi: https://doi.org/10.5219/870 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 397-404**
[abstract] doi: https://doi.org/10.5219/867 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 405-412**
[abstract] doi: https://doi.org/10.5219/830 [fulltext]

IN VITRO ANTIOXIDANT POTENTIAL AND INHIBITORY EFFECT OF HYDRO-ETHANOLIC EXTRACT FROM AFRICAN BLACK VELVET TAMARIND (_DIALIUM INDIUM_) PULP ON TYPE 2 DIABETES LINKED ENZYMES
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 413-421**
[abstract] doi: https://doi.org/10.5219/911 [fulltext]

ANTIOXIDANT, COMPOSITIONAL EVALUATION AND BLOOD PRESSURE MODULATING POTENTIALS OF _BRYOPHYLLUM PINNATUM_ (LAM.), _VISCUM ALBUM_ (L.) AND _ARTOCARPUS ALTILIS_ (PARKINSON) LEAVE EXTRACTS
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 422-430**
[abstract] doi: https://doi.org/10.5219/912 [fulltext]

ACCEPTABILITY AND SENSORY EVALUATION OF ENERGY BARS AND PROTEIN BARS ENRICHED WITH EDIBLE INSECT
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 431-437**
[abstract] doi: https://doi.org/10.5219/925 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 438-443**
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THE MORPHOLOGICAL AND ANTIOXIDANT CHARACTERISTICS OF INFLORESCENCES WITHIN WILD-GROWING GENOTYPES OF ELDERBERRY (_SAMBUCUS NIGRA_ L.)
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 444-453**
[abstract] doi: https://doi.org/10.5219/919 [fulltext]

OCCURRENCE OF SELECTED METALS IN FEED AND SHEEP´S MILK FROM AREAS WITH DIFFERENT ENVIRONMENTAL BURDEN
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 454-460**
[abstract] doi: https://doi.org/10.5219/920 [fulltext]

VICIA VILLOSA PROTEIN ISOLATE: A NEW SOURCE OF PROTEIN TO MAKE A BIODEGRADABLE FILM
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 461-471**
[abstract] doi: https://doi.org/10.5219/913 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 472-486**
[abstract] doi: https://doi.org/10.5219/865 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 487-490**
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EFFECT OF ESSENTIAL OILS OF LAMIACEAE PLANTS ON THE *RHIZOPUS* SPP.
Dana Tančinová, Zuzana Mašková, Denisa Foltinová, Jana Štefániková, Július Árvay
***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 491-498**
[abstract] doi: https://doi.org/10.5219/921 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 499-511**
[abstract] doi: https://doi.org/10.5219/915 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 512-519**
[abstract] doi: https://doi.org/10.5219/940 [fulltext]

DETERMINATION OF THE CARROT (*DAUCUS CAROTA* L.) YIELDS PARAMETERS BY VERMICOMPOST AND EARTHWORMS (*EISENIA FOETIDA*)
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 520-526**
[abstract] doi: https://doi.org/10.5219/946 [fulltext]

VARIATION OF FRUITS MORPHOMETRIC PARAMETERS OF ELAEAGNUS MULTIFLORA THUNB. GERMPLASM COLLECTION
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 527-532**
[abstract] doi: https://doi.org/10.5219/922 [fulltext]

BIOCHEMICAL COMPOSITION OF SWEET CHERRY (*PRYNUS AVIUM* L.) FRUIT DEPENDING ON THE SCION-STOCK COMBINATIONS
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 533-538**
[abstract] doi: https://doi.org/10.5219/923 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 539-545**
[abstract] doi: https://doi.org/10.5219/937 [fulltext]

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A STUDY OF THE QUALITY AND BIOLOGICAL VALUE OF MEAT OF DIFFERENT BREEDS OF RABBIT BRED IN GEORGIA
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 553-559**
[abstract] doi: https://doi.org/10.5219/934 [fulltext]

EFFECT OF DIFFERENT STORAGE TIMES ON JAPANESE QUAIL EGG QUALITY CHARACTERISTICS
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 560-565**
[abstract] doi: https://doi.org/10.5219/949 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 566-569**
[abstract] doi: https://doi.org/10.5219/959 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 570-577**
[abstract] doi: https://doi.org/10.5219/944 [fulltext]

THE EFFECT OF EXPLANT, BAP AND 2,4-D ON CALLUS INDUCTION OF *TRACHYSPERMUM AMMI*
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 578-586**
[abstract] doi: https://doi.org/10.5219/953 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 587-599**
[abstract] doi: https://doi.org/10.5219/952 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 600-606**
[abstract] doi: https://doi.org/10.5219/964 [fulltext]

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Andri Cahyo Kumoro, Jefri Pandu Hidayat
***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 607-614**
[abstract] doi: https://doi.org/10.5219/965 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 615-621**
[abstract] doi: https://doi.org/10.5219/969 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 622-627**
[abstract] doi: https://doi.org/10.5219/958 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 628-633**
[abstract] doi: https://doi.org/10.5219/971 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 634-643**
[abstract] doi: https://doi.org/10.5219/928 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 644-649**
[abstract] doi: https://doi.org/10.5219/957 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 650-656**
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 657-666**
[abstract] doi: https://doi.org/10.5219/960 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 667-672**
[abstract] doi: https://doi.org/10.5219/976 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 673-679**
[abstract] doi: https://doi.org/10.5219/938 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 680-686**
[abstract] doi: https://doi.org/10.5219/979 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 687-693**
[abstract] doi: https://doi.org/10.5219/932 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 694-700**
[abstract] doi: https://doi.org/10.5219/943 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 701-706**
[abstract] doi: https://doi.org/10.5219/973 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 707-715**
[abstract] doi: https://doi.org/10.5219/975 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 716-722**
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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 723-728**
[abstract] doi: https://doi.org/10.5219/974 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 729-734**
[abstract] doi: https://doi.org/10.5219/986 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 735-743**
[abstract] doi: https://doi.org/10.5219/981 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 744-755**
[abstract] doi: https://doi.org/10.5219/985 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 756-761**
[abstract] doi: https://doi.org/10.5219/987 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 762-766**
[abstract] doi: https://doi.org/10.5219/991 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 767-774**
[abstract] doi: https://doi.org/10.5219/988 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 775-781**
[abstract] doi: https://doi.org/10.5219/992 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 782-788**
[abstract] doi: https://doi.org/10.5219/999 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 789-797**
[abstract] doi: https://doi.org/10.5219/1001 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 798-805**
[abstract] doi: https://doi.org/10.5219/1002 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 806-814**
[abstract] doi: https://doi.org/10.5219/982 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 815-823**
[abstract] doi: https://doi.org/10.5219/1005 [fulltext]

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***Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, 2018, p. 824-835**
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