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CONTENTS

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Shrinkage effect on diffusion coefficient during carrot drying

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Abstract. Many studies have been previously carried out on the carrot drying and the undergoing processes. The developed mathematical models provide an opportunity to gain an understanding of this complex process and its dynamics. But they are simplified and based on a number of assumptions, including calculation of diffusion coefficient values. In one of the previous studies, the authors of this study determined that the diffusion coefficient is linearly dependent on the moisture concentration with the assumption that the sample's geometric shape does not change. The aim of this study is to determine the dependence of the diffusion coefficient on the moisture concentration taking into account the change in sample thickness during the drying experiment. The experiments were carried out with carrot slices of three different thicknesses: 5 mm, 10 mm and 15 mm thickness on the film infrared dryer at temperature 40 °C. During the experiments, measurements of the weight and thickness of the slices were performed. Using the experimental data the average thickness and diffusion coefficient of slices was calculated depending on the moisture concentration. Obtained results show that thickness depends linearly on the moisture concentration. Using experimental data and obtained average values of samples thickness, the values of diffusion coefficient was calculated. The results indicate that diffusion coefficient value depend linearly on moisture concentration. Their values are close and tend to zero when the concentration decreases if the thickness changes are taken into account during the experiment.

Key words: carrot, diffusion coefficient, drying, thickness.

INTRODUCTION

Vegetables are very important for human nutrition. For example, carrot contains high amount of vitamin, fibre and other valuable nutrients. The importance of carrot is reflected by its global production. Accordingly to the data available on the World Carrot Museum website (2018) and MapsOfWorld (2018) the leading producers of carrot are China, Russia, United States of America, Uzbekistan and Poland. As argue the statistical office of EU Eurostat (2018) in 2016 the total production of vegetables in the EU was 63.9 million tonnes and the vegetable sector was a key sector in EU agriculture, weighting 13.7% of EU agricultural output. The most important producers were Spain (24.1%) and Italy (17.4%). But a year earlier in 2015 the leading supplier of carrots was Israel (56.8%) in EU (Cicco, 2016). Tomatoes, carrots and onions were the most

important vegetables in 2016. There were produced 5.6 million tonnes of carrots, including Latvia with its contribution 14.8 thousand tonnes.

Vegetables contain large quantities of water in proportion to their weight that has important effect on the storage period length (Bastin & Henken, 1997; Rashidi et al., 2010). Drying is one of most popular food preservation methods that causes moisture evaporation in the product. Drying provides safety of product longer time in better quality for consumers and additional economic opportunity for producers.

Many studies have been previously carried out on the food drying (Togrul, 2006; Srikiatden & Roberts, 2008; Aboltins & Upitis, 2011; Aboltins, 2013; Guinē & Barocca, 2013; Aboltins et al., 2017) and the undergoing processes with the main goal to foster optimum utilization of financial, physical and human resources. Without knowledge of drying mechanisms there is no way to predict methods for the improving product quality (Margaris & Ghiaus, 2007). There are various aspects that must be considered when drying small fruits and vegetables, whether for the food or nutraceutical and functional food industries. A system which minimizes exposure to oxidations, light and heat may help conserve quality of product (Ahmed et al., 2013).

Several studies have been carried out using various types of drying. For example, Monghanaki et al. (2013) have compared the advantages of microwave drying with convectional drying methods. While Ahmed with colleagues (2013) has focused upon conventional and new drying technologies and pre-treatment methods based upon drying efficiency, quality preservation, and cost effectiveness. But Daymaz & Kucuk (2017) have investigated drying characteristics of peas in a cabinet dryer at three different temperatures 55, 65 and 75 °C and constant air velocity of 2 m s^{-1}. They have calculated the values of effective diffusivity of the pre-treated and control samples.

Research results focused on drying kinetics investigation have shown that drying rate has strong relationship with drying temperature and drying air velocity (Ndukwu, 2009; Putr & Ajiwiguna, 2017). Drying rate increased with drying temperature and air velocity, but decrease with drying time at the same drying temperature. In addition Putr & Ajiwiguna (2017) have concluded that evaporation process needs more energy when moisture content in the object is low.

The developed mathematical models provide an opportunity to gain an understanding of complex drying process and its dynamics. Most popular are the models of thin layer drying. These models allow many physical variables consider as constants (Aboltins, 2013; Onwude et al., 2016). But they are simplified and based on a number of assumptions, including calculation of diffusion coefficient values. Several studies were focused on the influence of shrinkage investigation on drying kinetics. For example, Dissa et al. (2008) have experimentally established and simulated drying kinetics and bulk shrinkage of the Amelie mangoes.

Researchers group with Botelho (2011) have verified the effective diffusion coefficients at temperatures ranged from 50 °C to 100 °C for carrot slices, but Lamharrar et al. (2017) for Urtica dioica leaves at three temperatures 40, 50, 60 °C. But Aboltins et al. (2017) have shown that the diffusion coefficient is linearly dependent on the moisture concentration with the assumption that the sample's geometric shape does not change. Study results show that the diffusion coefficient increases when the moisture concentration decreases. It was concluded that for approximate calculation of the diffusion coefficient value a simplified formula (Hassini et al., 2004) can be used. But

for highest precision, a series formula with a larger number of terms (at least 15 terms) should be used at small experiment times (Aboltins et al., 2017).

The aim of this study is to determine the dependence of the diffusion coefficient on the moisture concentration taking into account the change in sample thickness during the drying experiment.

MATERIALS AND METHODS

Vegetables materials

The research object of current study is fresh carrots (*Daucus Carota Sativus*) that were purchased from a local market in Jelgava, Latvia. Before the experiment carrots were washed under running water, wiped, prepared with a diameter 25 mm and cut into slices (Fig. 1, a) with three different thicknesses: 5 mm, 10 mm and 15 mm thickness. Carrots were not peeled and blanched. Each sample contains carrot slices that were uniformly placed in a single layer on individual tray with a network base.

Experimental procedure and equipment

In order to obtain experimental data carrot samples were dried in the film infrared dryer (IR) approximately 24 hours at temperature 40 °C (Fig. 1).

The dryer consist of a drying chamber (80 × 50 × 30 cm) with a heat source IR film (South Korea EXCEL) with total area 0.8 m^2 mounted on the top and bottom of the chamber. The drying temperature of dryer is not more than 40–45 °C with IR film power 140 W m^{-2}.

The experiments were performed with the fan with a total maximum capacity of 100 m^3 h^{-1} and power 15 W, which is placed on the top of the side wall of the equipment, the air intake holes located on the bottom of the opposite side wall (Fig. 1).

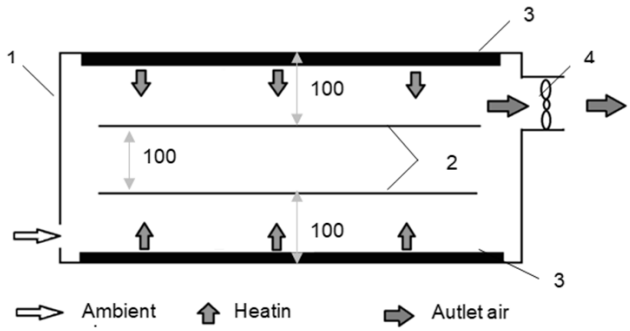

Figure 1. Schematic view of IR dryer: 1 – Body of dryer; 2 – Dryer shelves; 3 – IR drying film; 4 – Fan.

In each experiment, 4 material trays were used for each thickness (Fig. 2), which were placed in the dryer both shelves.

The drying chamber Memmert was used for the determination of dry matter. After experiment carrot samples were dried at temperature 105 °C during the remainder of the experiment until they did not change the weight during the hour in order to obtain dry

matter. During drying process free ventilation was performed. Each tray was weighed before inserting it in dryer.

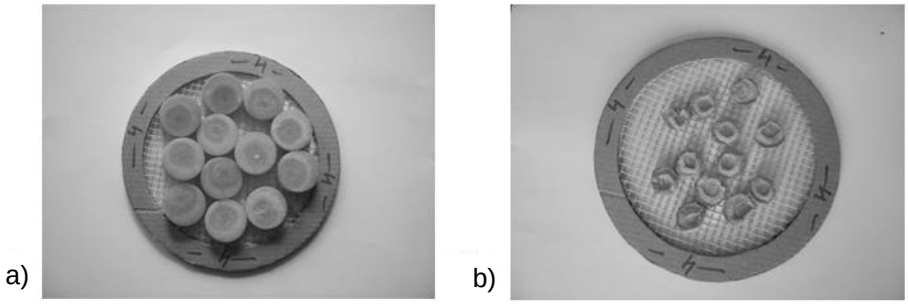

Figure 2. Tray of carrot slices with 15 mm thickness: a) before drying; b) after drying.

During the experiments, measurements of the weight, diameter and thickness of the slices were regularly performed at specified time intervals. Further additional measurements were carried out at the end of the experiment. Values were recorded to determine the mass and geometric shape changes on drying time.

A laboratory balance Kern EW 1500-2M was used for weighing, with measurement accuracy ± 0.01 g. The diameter and thickness were measured using Digital Caliper 1103, with measurement accuracy ± 0.01 mm.

Using the experimental data the average thickness of sample slices and diffusion coefficient were calculated depending on the moisture concentration.

Mathematical modelling

The moisture concentration in % was calculated using the expression (1):

$$C(\%) = \frac{M_i - M_\infty}{M_0} \cdot 100\% \quad (1)$$

where M_i – weight of sample at time moment t_i, g; M_∞ – equilibrium weight of sample, g; M_0 – weight of sample before drying, g.

Drying coefficient value $K(t_i)$ at each time moment t_i was calculated using the formula (2) described by Aboltins & Upitis, 2011:

$$K(t_i) = -\frac{ln\left(\frac{M_i - M_\infty}{M_0 - M_\infty}\right)}{t_i} \quad (2)$$

where t_i – drying time, h.

Diffusion coefficient with constant conditions can be expressed from equation (3) mentioned in Rubina et al. (2016):

$$\frac{M(t)}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} e^{-D\frac{(2n+1)^2\pi^2 t}{L^2}} \quad (3)$$

where L – thickness of carrot slices, mm.

Taking the first member of sum (3) (next will be more that 10 time less) and making simplifications of the obtained expression based on assumption can receive the formula (4). It can be used to calculate diffusion coefficient value $D(t_i)$ at certain moment in time t_i in hours:

$$D(t_i) = \frac{K(t_i)L^2}{\pi^2} \tag{4}$$

where $K(t_i)$ – drying coefficient at time moment t_i, h^{-1}; L – thickness of carrot slices, mm.

The area ration λ was calculated for drying dynamics determination:

$$\lambda = \frac{S_1}{S_2} = \frac{2\pi R^2}{2\pi RH} = \frac{R}{H} \tag{5}$$

where S_1 – sum of top and bottom area of sample, mm^2; S_2 – area of side surface of sample, mm^2; R – half of carrot slices diameter, mm; H – thickness of carrot slices, mm.

Using the experimental data and performing regression analysis the average thickness of sample slices and diffusion coefficient dependence on the moisture concentration were determined, as well as moisture concentration $C(t,\lambda)$ dependence on the drying time and area ratio.

Linear and exponential regression equations and coefficient of determination (R^2) were determined using Microsoft Excel. In order to perform nonlinear multivariable regression analysis and represent analysis results mathematical packages MathCad and Matlab were used.

RESULTS AND DISCUSSION

Three series of measurements concerning the drying of carrot slices were taken: one with 5 mm thick sliced carrots, second with 10 mm thick and third with 15 mm thick sliced carrots with an average diameter of 25 mm ± 0.5 mm.

The evolution of weight for 15 mm thick slices during the drying process is given in Fig. 3. The scattered points show the values of weight of four trays.

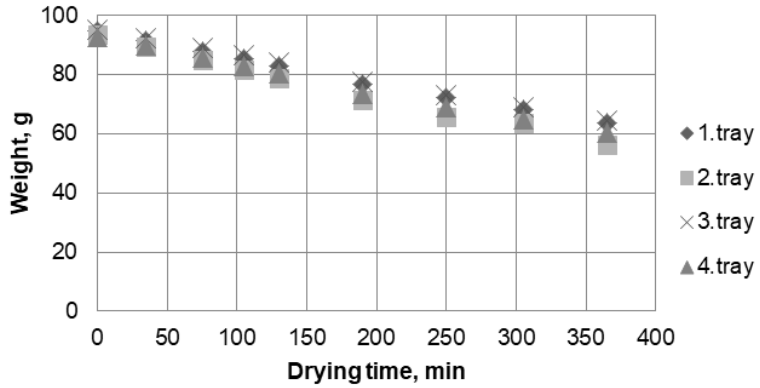

Figure 3. Experimental data of 15 mm thick slices.

Obtained results show that average initial moisture concentration was 94.3% for 5 mm thick carrot slices, 88.3% for 10 mm thick carrot slices and 93.0% for 15 mm thick carrot slices. During the drying experiment after first 4 hours average moisture concentration decreases down to 45.4% ± 9.3, 55.5% ± 5.2 and 67.5% ± 4.6 *(P* = 95%) accordingly. But after a drying period of 20 hours it reached 4.9% ± 0.6, 7.0% ± 2.5 and 28.9% ± 1.9 in slices with the above mentioned thicknesses appropriately.

The evolution of average moisture concentration with confidential interval (*P* = 95%) during the drying process, expressed in (%) is presented in Fig. 4. The red, blue and green scattered points are values of calculated average moisture concentration and the continuous line is the exponential regression.

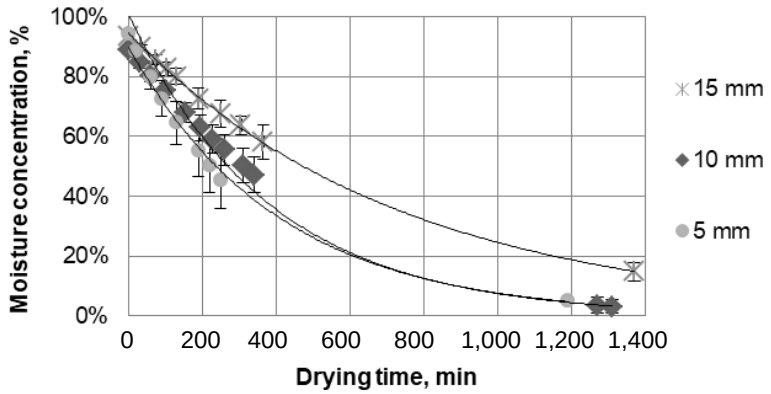

Figure 4. Average moisture concentration dependence on the drying time.

Using experimental data and Excel built in facilities was obtained exponential expression between average moisture concentration *c* and drying time t expressed in minutes for 5 mm (6), 10 mm (7) and 15 mm (8) thick carrot samples:

$$C = 0.8995e^{-0.002t} \quad (6)$$

with determination coefficient R^2 = 0.998;

$$C = 1.0086e^{-0.003t} \quad (7)$$

with determination coefficient R^2 = 0.995;

$$C = 0.9462e^{-0.001t} \quad (8)$$

with determination coefficient R^2 = 0.999.

To achieve the goal set for study during the drying experiments the measurements of slices thickness *L* were recorded. As shown in Fig. 5, the average thickness, expressed in millimetres, depends strongly linearly on the moisture concentration with determination coefficient R^2 = 0.976 for 5 mm thick slices, R^2 = 0.960 for 10 mm and R^2 = 0.999 for 15 mm thick slices. For example, in case of 5 mm thick slices it means that 99.9% of average thickness changes directly depend on moisture concentration changes using linear regression.

Using obtained functional relationship, it became possible to take into account thickness changes in calculation of diffusion coefficient. Evolution of the measured diffusion coefficient including shrinkage changes in fibber direction (thickness) during the drying process, expressed in square millimetres per hour is presented in Fig. 6. The scattered points are values of measured diffusion coefficient and the continuous line is the linear regression of the type:

$$D = k_0 C + k_1 \tag{9}$$

where D – drying coefficient in mm^2h^{-1}; C – moisture concentration in %; k_0, k_1 – regression coefficients.

The results indicate that diffusion coefficient value depends linearly on moisture concentration. In Fig. 5 is shown comparison of diffusion coefficient values obtained from formula (3). Blue scattered points indicate values of diffusion coefficient in case of slices with constant thickness, but red points are values of coefficient which were calculated taking into account thickness changes during drying.

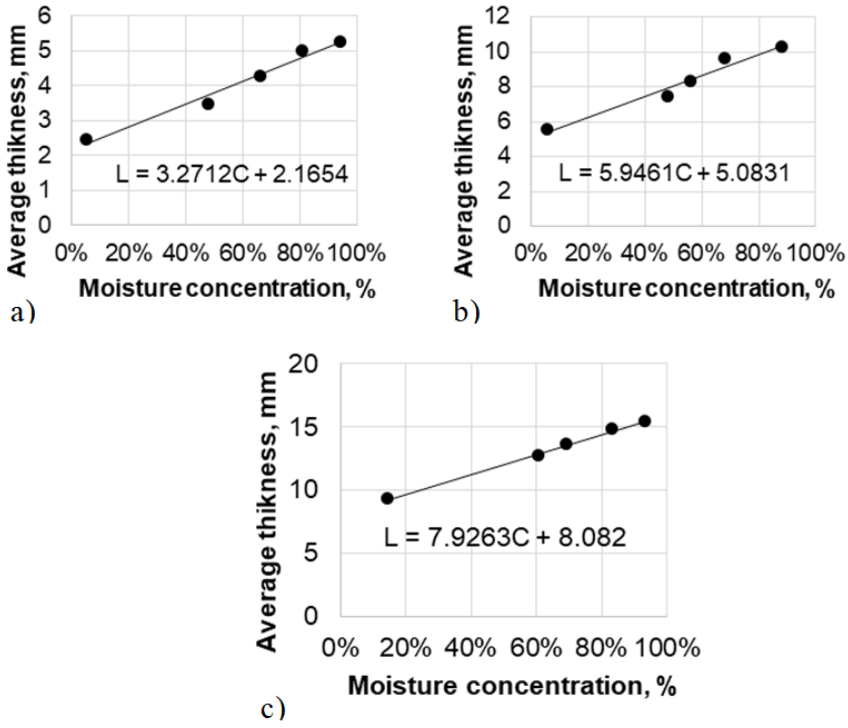

Figure 5. Average thickness dependence on the moisture concentration of slices with: a) 5 mm thickness, b) 10 mm thickness, c) 15 mm thickness.

As shown in Fig. 6 ignoring the thickness change results in values of diffusion coefficient increasing during the drying experiment if the moisture concentration decreases. Totally opposite result is obtained when the thickness variation is taken into account. The results show that diffusion coefficient values tend to zero when the

concentration decreases if the material thickness changes are taken into account during the experiment.

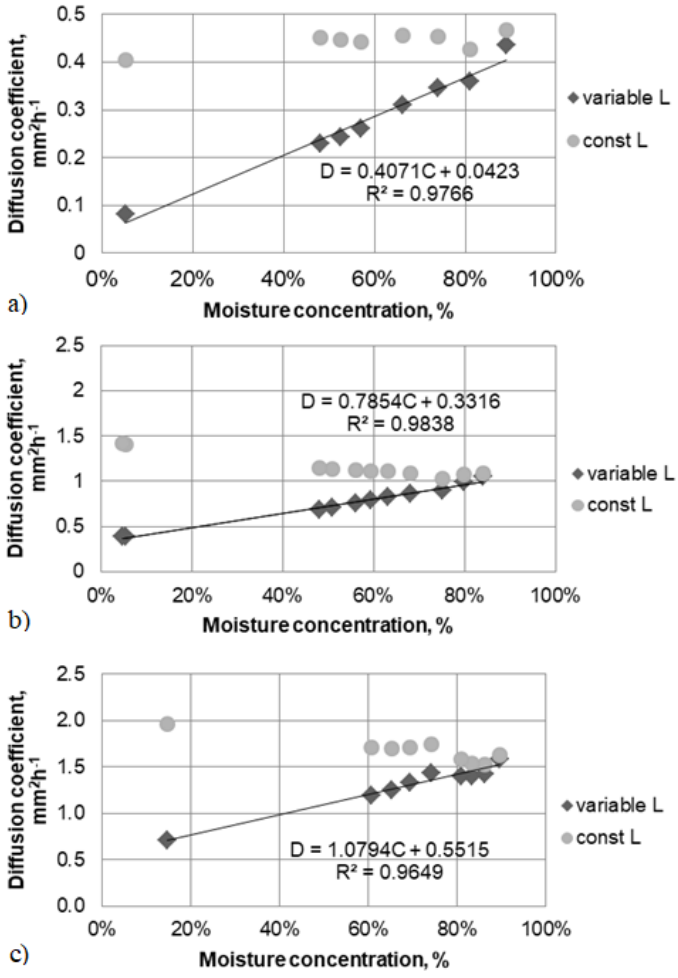

Figure 6. Diffusion coefficient dependence on the moisture concentration of slices taking and not taking into account the change in thickness during drying with: a) 5 mm thickness, b) 10 mm thickness, c) 15 mm thickness.

Taking into account thickness changes it is became possible to describe more precisely diffusion process and its dependence on moisture concentration. Moreover during drying not only shrinkage changes in fibre direction takes place, but also changes in side direction. It means that surface area influences diffusion process. In order to take into consideration the effect of surface area on the moisture concentration changes during drying the area ratio λ coefficient was introduced. Area ratio describes ratio between sum of basic surface area and area of side surface (4).

Using experimental data and mathematical packages MathCad and Matlab capabilities was obtained nonlinear multivariable expression between moisture concentration *C(t,λ)*, drying time *t (hours)* and carrot samples area ratio λ (10) at drying temp erature 40 °C:

$$C(t,\lambda) = 106.3 - 3.43t + 0.014t^2 - 27.7\lambda + 7.41\lambda^2 - 0.5t\lambda \qquad (10)$$

with determination coefficient R^2 = 0.93.

Graphically, this relationship is presented in Fig. 6. It can be seen that at given situation, removal of moisture occurs most rapid if the thickness of the samples is close to the diameter of the sample. It is interesting because the minimum surface of the cylinder at a certain volume is in a situation where its height is equal to the diameter

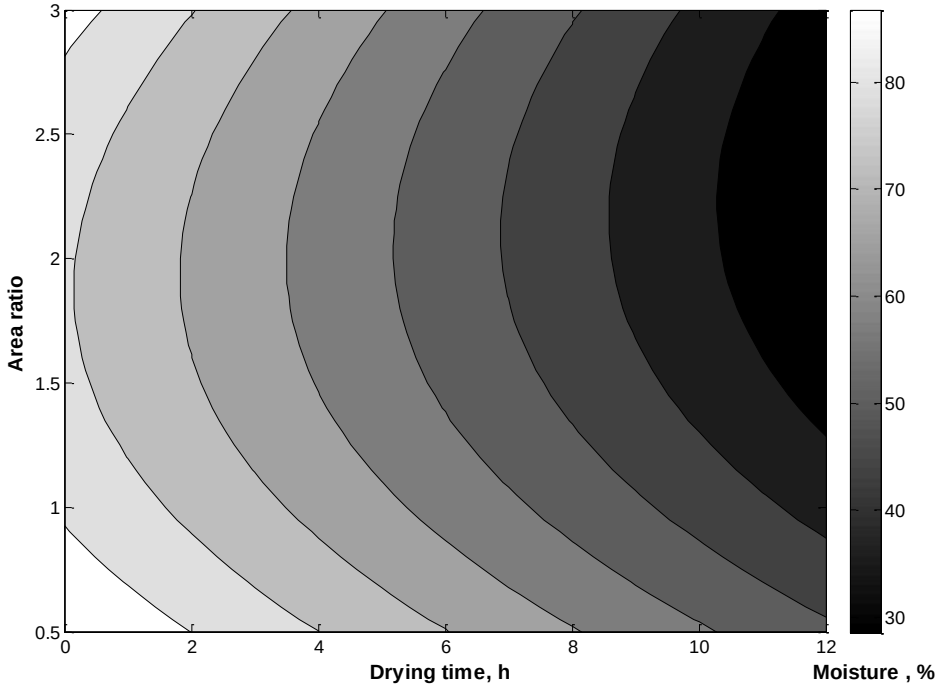

Figure 6. Moisture concentration dependence on the drying time and area ratio.

CONCLUSIONS

Study have shown that, in theoretical description, modelling of the drying process must take into account the dimensional changes in the product itself during the drying process. As the product (carrot cylinder) thickness increases, the effect of its moisture on the thickness changes in the drying process increases: from 3.2 to 5 mm thick to 7.9 to 15 mm thick. Using the obtained results it is possible to determine more precisely the variability of the product's diffusion coefficient, which depends on the moisture content of the product. Since weighing results in a common change in moisture, then the precise study of the diffusion problem requires the effect of the surface of the product on the

drying process, especially if the diffusion in the direction of the fibres (top and bottom) is not the same in the perpendicular direction (side of cylinder).

REFERENCES

Margaris, D. & Ghiaus, A-G. 2008. Fruits and Vegetables dehydration in tray dryers. In: *New food engineering research trends*. New York, pp. 45–101.

Aboltins, A. 2013. Theoretical study of material drying coefficient. *Proceedings of International Scientific Conference Engineering for Rural Development*, Latvia University of Agriculture, Jelgava, Latvia, pp. 153–158.

Aboltins, A., Rubina, T. & Jotautiene, E. 2017. Diffusion coefficient estimation difficulties at the beginning of drying experiment. *Proceedings of International Scientific Conference Engineering for Rural Development*, Latvia University of Agriculture, Jelgava, Latvia, pp. 153–158.

Aboltins, A. & Upitis, A. 2011. The mathematical model of carrot slices drying. Scientific journal: *Agricultural Engineering* **36(**2), 69–75.

Ahmed, N., Singh, J., Chauhan, H., Gupta, P., Anjum, A. & Kour, H. 2013. Different drying methods: their applications and recent advances. *International Journal of food nutrition and safety* **4**(1), 34–42.

Agricultural production – crops. 2017. *http://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agricultural_production_-_crops&oldid =366 258*. Accessed 27.01.2018.

Bastin, S. & Henken, K. 1997. Water content of fruits and vegetables. University of Kentucky: Colleague of Agriculture, 1 p.

Botelho, F., Correa, P., Goneli, A., Martins, M., Magalhaes, F. & Camoos, S. 2011. Periods of constant and falling-rate for infrared drying of carrot slices. *Rev. bras. agric. Ambient.* **15**(8), 845–852.

Cicco, A. 2016. The fruit and vegetable sector in the EU - a statistical overview. *http://ec.europa.eu/eurostat/statistics-explained/index.php/The_fruit_and_vegetable_ sector_in_the_EU_-_a_statistical_overview*. Accessed 27.01.2018.

Dissa, A., Desmorieux, H., Bathiebo, J. & Koulidiati, J. 2008. Convective drying characteristics of Amelie mango (Mangifera Indica L. cv. 'Amelie') with correction for shrinkage. *Journal of Food Engineering* **88**, 429–437.

Daymaz, I. & Kucuk, I. 2017. Pretreatments and temperature effects on the drying kinetics of peas. *Bulgarian Chemical Communications* **49**(1), 90–97.

Guinē, R. & Barocca, M. 2013. Estimation of the Diffusivities and Mass transfer Coefficients for the Drying of D.Joaguina Pears. *Proceedings of the World Congress on Engineering*, London, U.K., July 3–5, **2**, 1–4.

Hassini, L., Azzouz, S. & Belghith, A. 2004. Estimation of the moisture diffusion coefficient of potato during hot-air drying. *Proceedings of the 14th International Drying Symposium* (IDS 2004), São Paulo, Brazil, Vol. B, 22–25 August, pp. 1488–1495.

Lamharrar, A., Idlimam, A., Alouani, A. & Kouhila, M. 2017. Modelling of thin layer solar drying kinetics and effective diffusivity of Urtica dioica leaves. *Journal of Engineering Science and Technology* **12**(8), 2141–2153.

Moghanaki, S., Khoshandam, B. & Mirhal, M. 2013. Calculation of moisture content and drying rate during microwave drying. *Applied Mechanics and Materials* **423–426**, 746–749.

Ndukwu, M. 2009. Effect of drying temperature and drying air velocity on the drying rate and drying constant of cocoa bean. *Agricultural Engineering International: the CIGR Ejournal* **11**, 1–7.

Onwude, D., Norhashila, H., Rimfiel, J., Nawi, N. & Khalina, A. 2016. Modeling the thin-layer drying of fruits and vegetables: A review, *Comprehensive reviews in food science and food safety* **15**, 1327–1332.

Putr, R. & Ajiwiguna, T. 2017. Influence of air temperature and velocity for drying process. *Procedia Engineering* **170**, 516–519.

Rashidi, M., Ranjbar, I., Gholami, M. & Abbassi, S. 2010. Prediction of Carrot Firmness Based on Carrot Water Content. *American-Eurasian J. Agric. & Environ. Sci.* **7**(4), 402–405.

Rubina, T., Aboltins, A., Palabinskis, J. & Jasinskas, A. 2016. Potatoes drying dynamics research. *Proceedings of International Scientific Conference Engineering for Rural Development*, Latvia University of Agriculture, Jelgava, Latvia, pp. 187–192.

Srikiatden, J. & Roberts, J.S. 2008. Predicting moisture profiles in potato and carrot during convective hot air drying using isothermally measured effective diffusivity. *Journal of Food Engineering* **84**, 516–525.

Togrul, H. 2006. Suitable drying model for infrared drying of carrot. *Journal of Food Engineering* **77**(93), 610–619.

Top 10 Carrot Producing Countries. *https://www.mapsofworld.com/world-top-ten/carrot-producing-countries.html*. Accessed 31.01.2018.

World carrot production statistics on the World Carrot Museum website. 2013. *http://www.carrotmuseum.co.uk/worldcarrots.html*. Accessed 30.01.2018.

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Production and investigations of antioxidant rich beverage: utilizing *Monascus purpureus* IHEM LY2014-0696 and various malts

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Abstract. Antioxidant evokes numerous health benefits to the consumer as well as stabilisation of the beverages flavours. Therefore, this paper provides detailed information on the application of *Monascus purpureus* IHEM LY2014-0696 in combination with various malts in brewing antioxidant rich beverage (ARB). Starter culture Angkak was prepared by solid state bioprocessing (SSB). Single infusion method of mashing was used. Physicochemical parameters, volatile compounds, DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity and fermentability of the wort were evaluated. Distillation procedure based on pycnometry technique was use to quantify the alcohol content (%ABV). Fermentability of the wort was found to be $97.6 \pm 0.46\%$ whilst %ABV was equal to 5.42 ± 0.03. It was observed that ARB showed a strong DPPH radical scavenging activity of 1.00×10^{-4} mol × equ ($R^2 = 0.91$) whereas 3.43×10^{-5} mol × equ ($R^2 = 0.81$) for wort. The strong antioxidant activity (AOA) is thought to be caused by pigments produced by *M. purpureus* IHEM LY2014-0696 and other compounds originated from the malts and hops utilised in brewing ARB. A total of 4 volatile compounds were identified in the present study. Incidence of microbial load ranged from $2.14 \pm 0.04 \times 107$ and $0.8 \pm 0.1 \times 10^5$ for *M. purpureus* IHEM LY2014-0696 and bacterial respectively was observed in the ARB. This study contradicts some previous ones, as the ARB brewed did not take the red pigment produced by the *M. purpureus*. Panellists generally expressed their acceptance for the ARB as they assessed it as a new product, moreover, taking account its health benefits.

Key words: Volatile compounds, Fermentability, Angkak, Solid state bioprocessing, Pigments

INTRODUCTION

The genus *Monascus*, subdivided into four species: *M. pilosus*, *M. purpureus*, *M. ruber* and *M. floridanus*, belongs to the family *Monascaceae*, the order *Eurotiales*, the class Ascomycetes, the phylum Ascomycota, and the kingdom Fungi (Hawksworth & Pitt, 1983; Barnard & Cannon, 1987; Lin et al., 2005), can be cultured on a substrate containing starch. These starches are broken into different metabolites. The pigments from which any product fermented with *Monascus* species derives its distinctive red

colour are among them. For centuries, product like rice (Red yeast rice (RYR)) cultivated on red mould was stapled dietary and food additives in Asia continent (Erdoğrul & Azirak, 2004). Moreover, it is used as preservatives in meat and fish, to add colour and flavour to food. RYR is not known by Asians alone but other continents as well. As such, different names are used in identifying the same product, i.e. the Japanese, Chinese, Europeans, and Americans called it Beni-Koji or Red-Koji, *angkak*, *Rotschimmelreis*, Red Mould respectively, (Bakosova et al., 2001). According to Palo et al. (1960), RYR originated from China, however was kept in secrecy probably due to the fear of revenue loss from this product, if other Asian countries get to know this technology. For a decade, the Chinese and the Philippines utilize RYR as starters in brewing *Anchu* and *somsu* alcoholic beverages respectively.

In brewing, the starter culture transform wort into beverage, moreover, that helps to develop volatiles compounds (esters) which give any beverage its distinctive flavour with contributions from other ingredients (hops, species, etc). Alcoholic beverages brewed with *M. purpureus* has higher antioxidant activities than beer brewed with conventional yeasts (Takeshita et al., 2016) and these beverages (food) tend to play a substantial role which evokes health benefits i.e. Cholesterol Lowering, Anti-Diabetic Activity, Effects on Osteoporosis, Anti-Inflammatory inhibitions (Arunachalam & Narmadhapriya, 2011). Moreover, *M. purpureus* can withstand stresses (higher temperature, low pH) than other microorganisms, which makes it more suitable in brewing (Huang, 2000).

Antioxidant plays a role in flavour stability, one of the important characteristics in beverages (Zhao et al., 2010). Malt specifications are vital to brewers all over the globe. Different types of beverage are produced from the variety of malts since malts have different properties and each contribution is crucial in developing sorts of products. However, the geographical location, variety of barley, malting technology could not be ignored as crucial factors that contribute to these properties variation.

To the best of our knowledge, no research has been conducted to investigate the kind of beverage produced from the combined malts (Pilsner, Vienna, Biscuit) and *M. purpureus* (*angkak*: see Fig. 2, b). Therefore, the first objective of this study was to brew antioxidant rich beverage (ARB) from different malts using *angkak* as starter. The second objective was to analyse the antioxidant activity, the microbial load and sensorial properties of the beverage.

MATERIALS AND METHODS

Malts (Pilsner, 181 g, Vienna, 230 g, Biscuit, 200 g), hops (Perle hops, 3 g) and rice (Miracle seeds, Mistral trading, Moscow, Russia, 100 g) used in this study were purchased from Beerfan brewery company and supermarket respectively, whereas, Monascus purpureus IHEM LY2014-0696 was taken from the Belgium Co-ordinated collection of microorganisms (BCCM-IHEM) of the Scientific Institute of Public Health-Section Mycology and Aerobiology, Belgium and maintained on Potato Sucrose Agar (PSA) (100 g of potato, 10 g of sucrose, 10 g of agar, 350 mL of distilled water, pH 5.6) plates.

Starter culture was prepared according to the method described by Takeshita et al., (2016) with some modifications. Solid state bioprocessing (SSB) was performed in preparing the starter called Angkak (Fig. 2, b): 50 g of rice was weighed and soaked in

200 mL distilled water in a 300 mL Erlenmeyer flask for 1 hour. It was then, drained, and autoclaved at 121 °C for 15 min. Autoclaved rice was cooled to ambient temperature and transferred into a plastic Petri dish (Fig. 2, a). M. purpureus IHEM LY2014-0696 (0.2 g) colonies on the PSA plate was cut off with the aid of using a sterile knife and inoculate onto the autoclave rice, and incubated at 30 °C for 6 days to prepare the angkak seed. Again, 50 g of rice was weighed and soaked in 200 mL distilled water for 1 hour then drained, and autoclaved at 121 °C for 15 min. Autoclave rice was once again cooled to an ambient temperature and transferred into plastic Petri dish. 10 g of angkak seed was uniformly mixed with the steam rice with the aid of sterile spatula under lamina flow hood. The mixture was then incubated at 32 °C with 90% humidity for 5 days. The resulting red rice (angkak) was then soaked in 100 mL distilled water for 3 days at room temperature. Water was then drained and angkak ready for pitching. Flowchart of the entire production process is shown in Fig. 1.

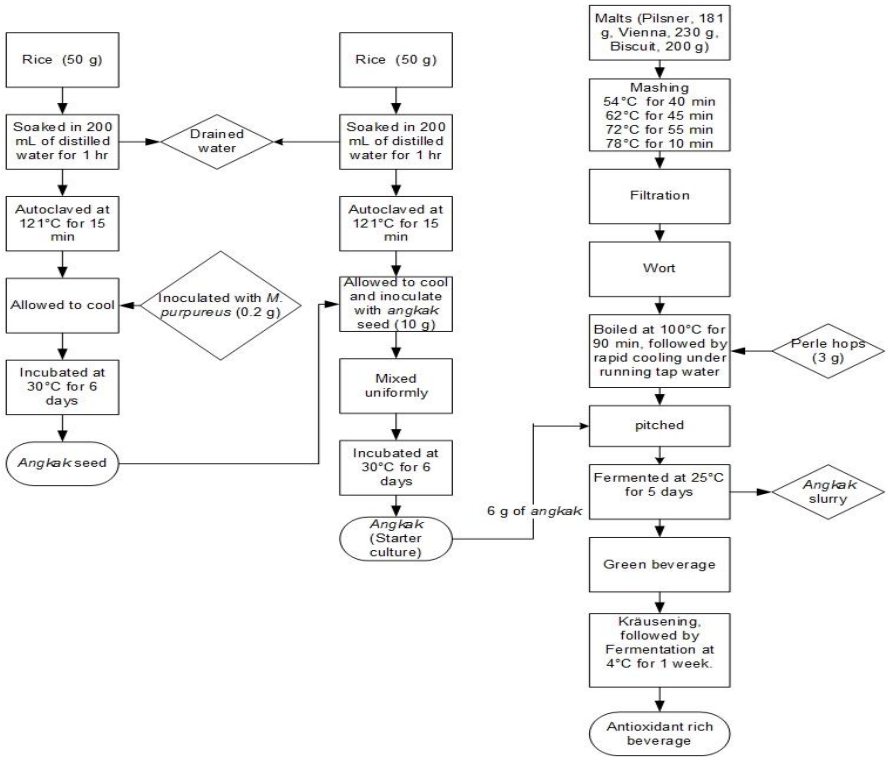

Figure 1. Flowchart of *angkak* and antioxidant rich beverage production.

Single infusion method of mashing was used, malts was mixed with 1.5 L distilled water in mash turn and heated up to 45 °C for 30 minutes. The temperature was increased upto 54 °C and 62 °C with a step times of 40 and 45 min respectively. After these step times, the temperature was once again increased upto 72 °C for 55 mins. Finally, the temperature was increased to 78 °C for 10 minutes. Filtration was carried out whilst 1 L distilled water was used for the sparging process. Wort was then boiled at 100 °C for 90 min, Perle hops (3 g) were added 80 min before the end of the boiling time. Wort was allowed to cool to a desired temperature by placing the boiled wort under

running cold water. Wort was then transferred into 5 L sterilized improvised fermenter (Fig. 3) equipped with airlock bubbler. The entire processes of ARB production is shown in Fig. 1 as well.

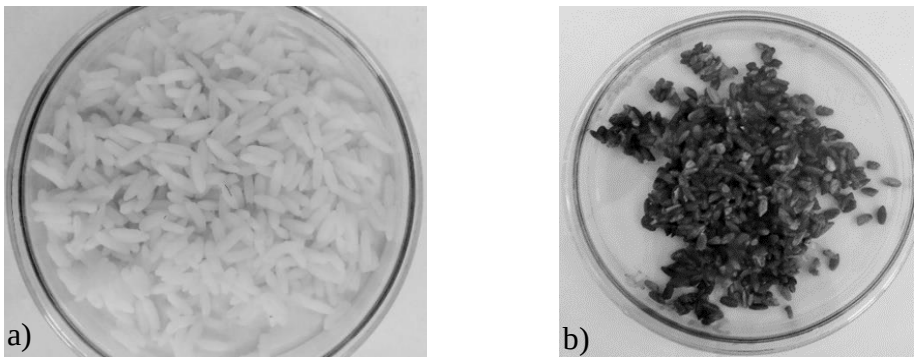

Figure 2. Autoclaved rice (a) and rice cultured with M. purpureus IHEM LY2014-0696 (angkak) (b).

Angkak (6 g) was measured under lamina flow hood, pitched and mixed thoroughly with the wort. Fermentation was carried out at a temperature of 25 °C for 5 days. Filtration was performed to separate green beverage from the fermented angkak. Kräusening was practice to carbonate the green beverage, where 2 mL of sterile wort was added to green beverage and ferment at 4 °C for 1 week. The matured beverage was termed antioxidant rich beverage (ARB).

Figure 3. Hand-made fermenter for fermentation of wort pitched with *angkak*.

Physicochemical parameters (oBrix, Titratable acidity (TA), colour), volatile compounds composition and DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity were evaluated according to the method described by Adadi et al. (2017b). Alcohol content (%ABV) was quantified by distillation technique using pycnometry method (GOST, 2011). Fermentability of the wort was determined according to formula stated by (Briggs et al., 2004).

Serial dilution was performed mixing 1 mL of ARB with 9 mL of sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, and 1,000 mL distilled water, pH = 7.0) and homogenized with the help of pipette. Decimal dilutions were plated. Aerobic mesophiles were enumerated by pour plate on nutrient agar (15 g peptone, 3 g yeast extract, 6 g NaCl, 1 g dextrose and 12 g agar, pH = 7.5) and brain heart infusion agar (HiMedia, Vadhani, India) incubated (Memmert GmbH, model 30-1060, Germany) at 30 °C for 3 days. *M. purpureus* IHEM LY2014-0696 was enumerated by spread plate on PSA and incubated at 25 °C for 5 days. Plates with countable colonies (30–300 colony

forming units (cfu)) were removed and counted according to the method describe by Adadi & Obeng (2017). Sensorial analysis was performed according to previous method use in Adadi et al. (2017a) and Adadi et al. (2017b).

The data were statistically analyzed by Origin statistical software (version 8.1). Values were presented as mean ± standard deviation (S.D.). One-way ANOVA and Fisher test were conducted to test the means results for sensory analysis at 5% significance.

RESULTS AND DISCUSSION

Changes in physiochemical parameters (pH, titratable acidity, ^{o}Brix, colour, fermentability and ABV) of the wort and final beverage are shown in Table 1. There was a gradual decrease in average pH value from 5.53 ± 0.06 to 4.57 ± 0.06 for wort and final beverage respectively. TA increased from 0.83 ± 0.01 to 1.88 ± 0.02% for wort and beverage. Substance in wort and beer can affect their buffering abilities, notable the residue of proteins like aspartate and glutamate (Bamforth, 2001). According to Adadi et al. (2017b), utilisation of substrate in wort and accumulation of metabolites is responsible for fluctuations in TA.

Table 1. Physicochemical characteristics of wort and antioxidant rich beverage

Parameters	Wort	beverage
pH	5.53 ± 0.06	4.57 ± 0.06
Titratable acidity (%)	0.83 ± 0.01	1.88 ± 0.02
^{o}Brix	13.8 ± 0.17	0.33 ± 0.58
Colour (EBC units)	47.7 ± 0.13	33.5 ± 0.24
Alcohol (% ABV)	N.A	5.42 ± 0.03
Fermentability (%)	97.6 ± 0.46	N.A

N.A-not applicable, (n = 3).

The enzyme GOX in *M. purpureus* IHEM LY2014-0696 could produce gluconic acid, which might affect the pH and subsequently TA. Dimerumic and γ-aminobutyric acids found in *M. purpureus* fermented products could contribute to the fluctuation of both the pH and TA (Aniya et al., 2000; Su et al., 2003).

According to Briggs et al. (2004) starter culture (*angkak*) transforms simple sugars in wort to alcohol. ^{o}Brix measures amount of sugar in wort and since starters utilize these sugars a decrease was observed from 13.8 ± 0.17 to 0.33 ± 0.58 for wort and final beer respectively.

It was observed during the fermentation that; the fermenting wort was hazy due to the activity of *M. purpureus* IHEM LY2014-0696. Furthermore, a thick-light layer was seen covering the surface of the fermenting wort after 24 hours of fermentation time, which probably might be the mycelium of the *M. purpureus* IHEM LY2014-0696. In our previous works (Adadi et al., 2017a; Adadi et al., 2017b), we did not observed any layer, when we were working with conventional starters (*S. cerevisiae* and Kölsch Yeast). The mycelium could alter the colour of the fermenting wort. There was a change in colour from 47.7 ± 0.13 to 33.5 ± 0.24 EBC units for wort and beverage respectively which differs averagely ($P < 0.05$). After the end of primary fermentation, it was observed that, *angkak* settled at the bottom of the fermenter.

The alcohol content (% ABV) 5.42 ± 0.03 was measured in the present study. Takeshita and his colleagues, also measured alcohol (% v v^{-1}) 8, 7.9 and 8.6 when they brewed their alcoholic beverages using polished rice, wild rice and black rice respectively (Takeshita et al., 2016). It was noted that, the kind of starting raw material, affected the ethanol content of the final beer. Variety of starters (yeast, bacteria or fungi) utilizes nutrients in wort to support growth, generate energy (Boulton & Quain, 2007) in other to transform wort to beverage.

Fermentability of the wort was found to be $97.6 \pm 0.46\%$ in this study (Table 1). This percentage indicates that not all the sugar in the wort was consumed by the *M. purpureus* IHEM LY2014-0696. Inability of conventional yeasts to ferment wort 100% was report by MacWilliam (1968) when various wort from different countries were used in his work. The procedure and material used to produce wort make it inexorable that is complex was not well characterised. Sugar spectra in these wort differs (Hoekstra, 1974)**,** likewise their fermentability. Fraction of unfermentable sugars (dextrins) accounts roughly 25% in wort. However, it involves both mono (arabinose, xylose and ribose) and trisaccharides (panose and isopanose and β-glucans). Unfermented substances are inevitable in brewing process as majority of α-(1 -6) linkages of malt amylopectin survive wort production intact (Enevoldsen & Schmidt, 1974).

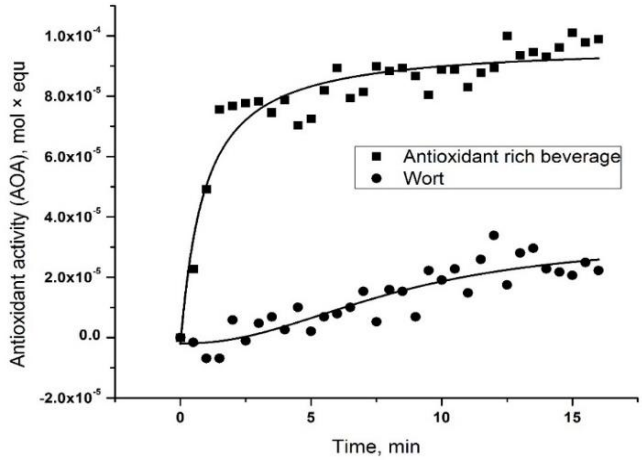

Figure 4. DPPH radical scavenging activity of samples vs. time.

The antioxidant activity (AOA) of the wort and beverage brewed with *angkak* was determined. The DPPH radical scavenging activity of the beverage and wort is shown in Fig. 4. It was observed that beverage showed strong DPPH radical scavenging activity of 1.01×10^{-4} mol × equ ($R^2 = 0.91$) whereas 3.43×10^{-5} mol × equ ($R^2 = 0.81$) for wort. Alcoholic beverage brewed with *beni koji* (*angkak*) showed stronger AOA (3,400 μM Trolox equ.) than the beverage brewed with *ki koji* (1,700 μM Trolox equ.) (Takeshita et al., 2016). The beverage brewed in the current study exhibited stronger AOA than Baltica beer we examined in our previous work (Adadi et al., 2017a). The AOA is thought to be caused by the pigments produced by *M. purpureus* IHEM LY2014-0696 and other compounds originated from the malts and hops utilised in brewing the beer. According to (Sato et al., 1992; Juzlova et al., 1996; Watanabe et al., 1997), the genus

Monascus produced six pigments (rubropunctatin, monascorubrin, rubropunctamine, monascorubramine, monascin and ankaflavin) of polyketide origin which are grouped into 3 major categories.

Monacolin K, γ-aminobutyric acid (GABA), and dimerumic acid (antioxidant) were found in food fermented by *M. purpureus* (Aniya et al., 2000; Su et al., 2003) and are thought to have AOA. Antioxidants are related with the prevention of cardiovascular, neurological diseases, cancer and oxidative stress dysfunction (Bolck, 1992; Diplock, 1995). AOA of beverage decreases as the storage time increases and this phenomenon was observed by Ditrych et al. (2015).

A total of 4 volatile compounds were identified in the presented study (Table 2). Among the volatiles identified 3 were alcohols and 1 ester. These compounds are thought to contribute to flavour of beverage and their availability depends on raw material, mashing style, and fermentation conditions under which the beverage is produced (Adadi et al., 2017a; Adadi et al., 2017b).

Table 2. Volatile compounds identified from antioxidant rich beverage

No	Volatile compounds	Retention time (min)	Formular
1	Ethanol	1.51	C_2H_6O
2	2-Methyl-1-propanol	2.27	$C_4H_{10}O$
3	Ethyl Acetate	2.31	$C_4H_8O_2$
4	3-methyl-1-Butanol	3.27	$C_5H_{12}O$

According to Briggs et al. (2004) yeast metabolites, which contribute to beverage flavour, are diversed chemically and consisted of organic acids, medium chain-length aliphatic alcohols ('fusel alcohols'), aromatic alcohols, esters, carbonyls and various sulphur-containing compounds. It could be obvious that the metabolites produced by *M. purpureus* IHEM LY2014-0696 during fermentation turned to have an inhibitory effect on the formation of these volatiles. Moreover, we practiced Kräusening during the carbonation, this can be another factors why we identified 4 volatiles in this study. Bottles lacks the ability to hold all the gases and volatiles formed during the secondary fermentation as keg, hence the volatiles formed ended up escaping from the bottle (due to pressure built up by the gases formed). In our previous works, 32 volatile were identified in Kölch fruit beer (KFB), (Adadi et al., 2017b) whereas 11 in low alcoholic beer and, 22 sorghum beer (Adadi et al., 2017a) which were all carbonated using kegs.

The incidence of microbial load ranged from $2.14 \pm 0.04 \times 10^7$ and $0.8 \pm 0.1 \times 10^5$ CFU mL^{-1} for *M. purpureus* IHEM LY2014-0696 and bacterial respectively (Table 3) in the ARB. The incidence of *M. purpureus* IHEM LY2014-0696 was shown to be higher than the bacterial load but theren't significantly difference ($P > 0.05$). *M. purpureus* IHEM LY2014-0696 was the sole starter culture use in this study, and after the secondary fermentation there were still viable and capable of fermenting another sterilized wort hence the higher load on the ARB. However, conventional yeast are usually weakened by the alcohol content after secondary fermentation unlike *M. purpureus* which are able to withstand these higher ethanol content and other stressful factors (Huang, 2000). According to Hill (2009) and Suzuki (2011) beer has been recognized as a microbiologically stable beverage due to it range of antimicrobial hindrance that, under most circumstances, prevents the growth of pathogenic microorganisms. Other factors like presence of alcohol, bitter compounds in hops, low pH, played a vital role in keeping

beer safe from microbial contamination. Nevertheless, microbiological contamination sporadically occurs in beverages. Pasteurization was not performed in this study and this might be the reason for the incidence of growth we observed on the plated Petri dishes. We noticed, in our previous work that, the observation strict hygienic protocol could curb the incidence of microbial contaminations (Adadi et al., 2017b).

Table 3. Frequency of microbial load on ARB (Mean ± S.D × CFU mL^{-1})

Microorganisms	Mean	SD	CFU mL^{-1}
Bacteria	0.8	0.1	10^5
M. purpureus	2.14	0.044	10^7

Incidences of bacterial contamination were also observed by Elio (2013), in all beer brewed (young beer; centrifuged beer; beer with priming; bottle beer; bottle conditioned beer) excepting the wort. The presence of bacteria in our study assumed that, the contamination could have happened during the carbonation stage after the primary fermentation as we practiced Kräusening. Hygienic management in brewery and disinfection using hydrogen peroxide, peroxacetic acid and quaternary ammonium compounds (Praeckel, 2009) could help in dealing with the menace of contaminations.

Food acceptance by consumers goes in hand with the impressions of the five human senses. Beverage acceptance by consumers has a role to play by the impression of the human senses i.e sight, hearing etc. Raw materials, technological procedures and storage could positively or negatively influenced the sensorial of beverage. The variables accessed were different statistically ($P < 0.05$) and are tabulated in Table 4. Transparency was not good as the score 1.57 ± 0.53 tells it all. Poor filtration of wort and green beverage might be the reason for the poor feedback from the assessors. Colour was somehow satisfactory with mean scores of 2.14 ± 1.07. Colour and transparency go together as transparent beverage could definitely receive positive responses from panellists. The colour was like conventional beer (brewed from *S. cerevisiae*), did not take the colour (red) of the pigments produced by the *M. purpureus*. Flavour of the ARB was good (2.57 ± 0.98) and this could be attributed to contribution from the hops, malt, and some metabolites produced by *M. purpureus*.

Table 4. Scores of sensory properties of antioxidant rich beverage

Parameters	Mean ± S.D
Transparency	1.57 ± 0.53
Colour	2.14 ± 1.07
Flavour	2.57 ± 0.98
Taste	3.14 ± 1.35
Hops bitterness	4.29 ± 0.49
Foaminess	4.45 ± 0.98
Overall acceptance	3.86 ± 0.89

Figure 5. Sample of antioxidant rich beverage indicating foam formation.

Bitterness from hops balanced the sweet taste of wort when it was fermented with the starter and consumers could reject the sweet beer as they perceived it as juice.

Bittering compounds from hops is thought to play a vital role in stability of the beverage from microbial staling. Kräusening method of carbonation was excellent in this study, judging from the average scored of the beverage foaminess (4.45 ± 0.98). The image of the glass after pouring ARB with massive foam formation is shown in Fig. 5. Foaminess plays a vital role in acceptability of beverage and consumers might reject beverage with poor foaminess. Panellists generally expressed their acceptance for the beverage as they considered it a new product taking into account the health benefit (AOA potential). Flavour of the product also played a role in the acceptability of the beverage studied.

CONCLUSION

Antioxidant rich beverage was brewed from various malts and traditional Asian fungus *M. purpureus* IHEM LY2014-0696. However, the beverage did not take the colour (red) of the *angkak* (*Monascus* pigments) and this contradicts previous work (Takeshita et al., 2016). A thick-light layer was observed covering the surface of the fermenting wort during the 24 hours of fermentation time. It was experimentally established that beer brewed showed a strong DPPH radical scavenging activity when compared with the wort. Incidence of microbial load was recorded in this study and this could be attributed to many factors. The beverage was accepted taking into account the health benefits and flavour.

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REFERENCES

Adadi, P. & Obeng, A.B. 2017. Assessment of bacterial quality of honey produced in Tamale metropolis (Ghana). *J. Food. Drug. Anal*. **25**, 369–373.

Adadi, P., Kovaleva, E.G., Glukhareva, T.V. & Shatunova, S.A. 2017a. Biotechnological production of non-traditional beer. In: Valeeva A.A & Volkovich, V.A. (ed.) Physics, Technologies and Innovation (PTI-2017): *IV International Young Researchers Conference*, AIP Conference Proceedings, Melville, USA, pp. 1–13.

Adadi, P., Kovaleva, E.G., Glukhareva, T.V., Shatunova, S.A. & Petrov, A.S. 2017b. Production and analysis of non-traditional beer supplemented with sea buckthorn. *Agronomy Research* **15**(5), 1831–1845.

Aniya, Y., Ohtani, I.I., Higa, T., Miyagi, C., Gibo, H., Shimabukuro, M., Nakanishi, H. & Taira, J. 2000. Dimerumic acid as an antioxidant of the mold, *Monascus anka*. *Free. Radic. Biol. Med*. **28**, 999–1004.

Arunachalam, C. & Narmadhapriya, D. 2011. Monascus fermented rice and its beneficial aspects: a new review. Asian. *J. Pharm. Clin*. Res. **4**, 29–31.

Bakosova, A., Mate, D., Laciakova, A. & Pipova, M. 2001.Utilization of Monascus purpureus in the production of foods of animal origin. *Bull. Vet. Inst. Pulawy*. **45**, 111–116.

Bamforth, C.W. 2001. pH in brewing: an overview. *Tech Q Master Brew. Assoc. Am*. **38**, 1–9.

Barnard, E.L. & Cannon, P.F. 1987. A new species of *Monascus* from pine tissues in Florida. *Mycologia*. **79**, 479–484.

Bolck, G. 1992. The data support a role of antioxidants in reducing cancer risks. *Nutr. Rev*. **50**, 207–213.

Boulton, C. & Quain, D. 2007. *Brewing yeast and fermentation*. Blackwell Science, New Jessy, p. 660.

Briggs, D.E., Boulton, C.A., Brookes, P.A. & Stevens, R. 2004. *Metabolism of Wort by Yeast: Brewing Science and Practice*. CRC Press, New York.

Diplock, A. 1995. Antioxidant nutrients-efficacy in disease prevention and safety. *Biochem*. **17**, 16–18.

Ditrych, M., Kordialik-Bogacka, E. & Czyżowska, A. 2015 Antiradical and Reducing Potential of Commercial Beers. *Czech J. Food Sci*. **33**, 261–266.

Elio, M. 2013. Development of guidelines for microbiological control in microbrewer. PhD Thesis, University of Perugia.

Enevoldsen, B.S. & Schmidt, F. 1974. Dextrins in brewing. *J. Inst. Brew*. **80**, 520–533.

Erdoğrul, O. & Azirak, S. 2004. Review of the studies on the red yeast rice (Monascus purpureus). *Turkish. Electro. J. Biotechnol*. **2**, 37–49.

GOST 12787-81. *Beer: method of measuring beer actual extract and calculation of dry matter in the initial wort*. (Moscow standard inform, Moscow, 2011), p. 11 [in Russian].

Hawksworth, D.L. & Pitt, J.I. 1983. A new taxonomy for *Monascus* species based on cultural and microscopical characters. *Aust J Bot.* **31**, 51–51.

Hill, A.E. 2009. *Microbiological stability of beer*. In C. Bamforth (ed.), Beer a quality perspective. Academic Press, Cambridge, p.163–184.

Hoekstra, S.F. 1974. Fermentable sugars in wort, brewed with adjuncts and fermented with bottom yeast. Proceedings of the European Brewery Convention Symposium Monograph 1, Ziest. p. 189–97.

Huang, H.T. 2000. *Biology and Biological Technology, Part V: Fermentations and Food Science, Science and Civilisation in China* (Needham, J., ed.). Cambridge University Press, Cambridge, 741 pp.

Jůzlová, P., Martínková, L. & Křen, V. 1996. Secondary metabolites of the fungus Monascus: A review. *J. Ind. Microbiol*. **16**, 163–170.

Lin, C.C., Li, T.C. & Lai, M.M. 2005. Efficacy and safety of *Monascus purpureus* Went rice in subjects with hyperlipidemia. *Eur. J. Endocrinol*. **153**, 679–686.

MacWilliam, I.C. 1968. Wort composition - a review. *J. Inst. Brew*.**74**, 38–54.

Palo, M.A., Vidal-Adeva, L. & Maceda, L.M. 1960. A study on *ang-kak* and its production. *Philippine. J. Sci*. **89**, 1–19.

Praeckel, U. 2009. Cleaning and Disinfecting. In: H. M. Eßlinger (ed.) Handbook of Brewing: Process, Technology, Markets. Wiley-VCH Verlag GmbH, Weinheim, p. 477–490.

Sato, K., Iwakami, S., Goda, Y., Okuyama, E., Yoshihara, K., Ichi, T., Odake, Y., Noguchi, H. & Sankawa, U. 1992. Novel natural colorants from *Monascus anka* U-1**.** *Heterocycles*. **34**, 2057–2060.

Su, Y.C., Wang, J.J., Lin, T.T. & Pan, T.M. 2003. Production of the secondary metabolites γ-aminobutyric acid and monacolin K by *Monascus*. *J. Ind. Microbiol. Biotechnol*. **30**, 40–46.

Suzuki, K. 2011. 125th Anniversary Review: Microbiological Instability of Beer caused by Spoilage Bacteria. *J. Inst. Brew*. **117**, 131–155.

Takeshita, R., Saigusa, N. & Teramoto, Y. 2016. Production and antioxidant activity of alcoholic beverages made from various cereal grains using *Monascus purpureus* NBRC 5965. *J. Inst. Brew*. **122**, 350–354.

Watanabe, T., Yamamoto, A., Nagai, S. & Terabe, S. 1997. Separation and determination of *Monascus* yellow pigments for food by micellar electrokinetic chromatography. *Anal. Sci*. **13**, 571–575.

Zhao, H., Chen, W., Lu, J. & Zhao, M. 2010. Phenolic profiles and antioxidant activities of commercial beers. *Food. Chem*. **119**, 1150–1158.

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Bioactive compounds in herbal infusions

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Abstract. Herbal teas are very popular and known as important source of biologically active compounds. Some of popular Latvian herbal teas: Calendula (*Calendula officinalis* L.), Matricaria chamomilla (*Matricaria chamomilla*), Lady's-mantle (*Alchemilla vulgaris* L.), Jasmine (*Jasminum officinale* L.), Yarrow (*Achillea millefolium* L.) and Linden flowers (*Tilia spp*.) were selected for analysis. This study was carried out with the aim to investigate the effect of extraction time (10, 15, 20 min) on the content of total flavonoids and total phenols as well as antioxidant activity of herbal tea extracts. The infusions were prepared by usual domestic preparation technique using ground air-dried plant materials and boiling deionized water (0.055 μS cm^{-1}) for extraction. Content of total flavonoids, total phenols and antioxidant activity was determined spectrophotometrically. Dry matter content was determined in lyophilized herbal infusions. The obtained results indicated that extraction time did not affected the content of biologically active compounds in the herbal infusions significantly ($P > 0.05$). The highest level of flavonoids was found in Jasmine and Lady's-mantle infusions (average 104.98 ± 9.21 mg quercetin equivalent 100 g^{-1} and 115.28 ± 5.25 QE mg 100 g^{-1} respectivelly), while the lowest was determined in Matricaria chamomilla extract – (average 70.10 ± 4.68 QE mg 100 g^{-1}). Lady's-mantle tea contained the largest amount of total phenols (average 4126.62 ± 26.24 mg gallic acid equivalents 100g^{-1}), the lowest – Calendula tea 1828.04 ± 10.37 mg GAE 100 g^{-1}). Data analysis showed a close linear positive correlation between the content of total flavonoids and total phenols in herbal infusions ($R^2 = 0.872$; $r = 0.934$) with the probability of 99%. In general, all samples tested in this study, demonstrated high level of antioxidant activity (from 75.04 to 91.54 mmol Trolox equivalents 100 g^{-1}). Results of the present experiments demonstrated that content of dry matter in analysed herbal teas was significantly different ($P < 0.05$).

Key words: herbal infusion, phenols, flavonoids, antioxidant activity.

INTRODUCTION

Herbal teas are known as beverages throughout the world. Herbs are mainly used in form of infusion of dried herbs in hot water. Leafy herbal teas are widely known to contain a variety of active phytochemicals with biological properties that promote human health and help reduce the risk of chronic diseases such as allergies, insomnia, headaches, anxiety, intestinal disorders, depression, and high blood pressure (Craig, 1999). Their beneficial effects could be partly attributed to polyphenolic compounds, which are known to possess antioxidant and antimicrobial properties (Miguel, 2010;

Cushnie & Lamb, 2011). There are known many herbal teas in Latvia. Most common are chamomilla, calendula, lady's-mantle, yarrow and linden flowers. Chamomilla contains several classes of biologically active compounds including essential oils, coumarins and several polyphenols, primarily the flavonoids (Avula et al., 2014).

Calendula accumulates large amounts of carotenoids in its flowers. Carotenoids are known as biologically active compounds with multiple applications in therapy. It is important to humans as precursors of vitamin A and retinoids. The plant of lady's-mantle is rich in tannins, flavonoids, salicylic acid, essential oil, bitter substances and phytosterols. It also contains vitamin C and numerous minerals. The most medicinally active part of the yarrow is the flowering tops. Yarrow contains flavonoids (apigenin, luteolin, quercitin) that increase saliva and stomach acid, helping to improve digestion. Yarrow may also relax smooth muscle in the intestine and uterus, which can relieve stomach and menstrual cramps (Lakshmi et al., 2011). Linden flowers contain sugar, tannin, mucilaginous matter, fatty substance, wax, yellow coloring matter, and a volatile oil, to which their fragrant odor is due. The main constituents of linden flower tea are flavonoids (quercetin glycosides, kaempferol glycosides, tiliroside), phenolic acids, essential oils, phytosterols, organic acids, tannins, mucilage, minerals, niacin, and vitamin C. These antioxidants help to prevent and repair DNA damage. Its pharmacological activity is in feverish colds, infectious diseases, and bronchitis, but it also has sedative and diuretic actions (Sroka & Bełz, 2009). Jasmine flower tea is very popular in Asia countries. Phytochemical analysis revealed that the jasmine flower contains antioxidants, coumarins, cardiac glycosides, essential oils, flavonoids, phenolics, saponins, and steroids (Kunhachan et al., 2012).

Antioxidants are molecules that neutralize harmful reactive oxygen species (ROS) by inhibiting oxidative chain reaction, preventing lipid peroxidation, reducing free radical concentration and chelating metal ions (Zhou & Yu, 2004). Reactive oxygen species, produced during aerobic metabolism, are essential mediators of important functions. Studies have demonstrated the involvement of ROS in a number of disorders including Alzheimer, atherosclerosis, diabetes, inflammation, and neurodegenerative and cardiovascular diseases. ROS also plays a key role in certain types of cancers and the ageing process (Salganik, 2001). Free radicals are reactive oxygen species produced in the body as by-products of cellular aerobic respiration and lead to oxidative stress (Yanai et al., 2008). Antioxidant activity is defined as an inhibition of the oxidation of lipids, proteins, DNA or other molecules that occurs by blocking thepropagation step in oxidative chain reactions (Huang et al., 2005).

Primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton's reaction. (Chan et al., 2010).

The antioxidant ability of phenolic components occurs mainly through a redox mechanism and allows the components to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice Evans et al., 1997).

Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8,000 individual compounds known. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellants, and for light screening. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, antiinflammatory, and vasodilating actions. However,

most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals (Pietta, 2000).

Polyphenols are a group of compounds synthesized exclusively by plants, especially for the protection against UV-radiation and activity of pathogens. About 8,000 plant polyphenol compounds have been identified so far whereas only some hundred occur in edible plants. They are present in fruits, flowers, leafs, roots, and woody parts of plants, whereas external tissues include bigger amounts of these components (Manach et al., 2004).

Therefore the aim of current research was to evaluate the effect of extraction time (10, 15, 20 min) on the content of total flavonoids and total phenols as well as antioxidant activity of herbal tea extracts.

MATERIALS AND METHODS

Investigations were carried out at the Latvia University of Life Sciences and Technologies, Department of Chemistry.

Chemicals and spectral measurements

All the reagents used were with the analytical grade from Sigma Aldrich, Germany. JENWAY 630 Spectrophotometer was used for the absorbance measurements.

Plant materials

Plants of Calendula (*Calendula officinalis* L.), Matricaria chamomilla (*Matricaria chamomilla),* Lady's-mantle (*Alchemilla vulgaris* L.), Jasmine (*Jasminum officinale* L.), Yarrow (*Achillea millefolium* L.) and Linden flowers (*Tilia spp.)* were grown in Latvia, Jelgava region (the GPS-coordinates: 56° 39' 3.992" N 23° 43' 16.874" E), collected during the flowering period in May till July 2017 and were dried at room temperature in a dark place.

Preparation of herbal infusions

Infusions were prepared in triplicate from the selected plants by usual domestic preparation technique. For this purpose 0.5 ± 0.0001 g of finely ground air-dried plant material were extracted in 50 mL of boiling distilled water (0.055 μS cm^{-1}) and stirred gently on a magnetic stirrer at room temperature for 10, 15 or 20 min. Each extract was then filtered through paper filter (11 μm, Whatman Inc., Clifton, NJ, USA). The supernatant was used for all determinations.

Analytical methods

Dry matter

The tea infusions were lyophilized (lyophilizator ALPHA 1-2 Ld plus CHRIST, Germany, 72 hours, -56 °C, 0.027 mBar vacuum) to determine the yield of extracted compounds (dried weight) in each of them. The results were expressed as g freeze-dried infusion l00 g^{-1} tea sample.

Total phenolic compound content

The total phenolics of the tea infusions were analyzed spectrometrically according to the Folin-Ciocalteu (Singleton et al., 1999, Dewanto et al., 2002). Briefly, 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times with distilled water) was added to 0.5 mL of

infusion. The mixture was then incubated for 3 min, after which 2 mL of sodium carbonate (Na_2CO_3) (7.5 g 100 g^{-1}) was added and well mixed. The control sample contained all the reaction reagents except the extract. After standing for 30 min at 20 ± 1 °C in dark place for color development, absorbance was measured at 760 nm using JENWAY 630 Spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} dry-matter of herbal tea and analysis were carried out in triplicate for each herbal infusion.

Total flavonoid content

The content was quantified using aluminium chloride method (Xu & Chang, 2007) with some modifications. To 500 µL of tea infusion 2 mL of distilled H_2O was added, then mixed with 150 µL of 5% sodium nitrite $NaNO_2$. After 5 min, 150 µL of 10% aluminium chloride $AlCl_3$ solution was added. The mixture was allowed to stand for another 5 min, and then 1 mL of the 1M sodium hydroxide NaOH was added. The reaction solution was mixed well and incubated at 20 ± 1 °C in dark place for 15 min. The control sample contained all the reaction reagents except the extract. The absorbance was measured at 415 nm using JENWAY 630 Spectrophotometer. Results were expressed as milligram quercetin equivalent 100 g^{-1} in dry weight (mg QE 100 g^{-1} DW).

Antiradical activity

The antiradical activity of tea extracts was determined according to Afify (2012) with some modifications. Method is based on the radical scavenging ability in reacting with stable 2.2-diphenil-1-picrylhydrazyl (DPPH) free radical. 3.5 mL of DPPH solution (4 mg of DPPH reagent dissolved in 100 mL pure ethanol) was added to 0.5 mL sample extract. Solution was well mixed and stand in dark place at 20 ± 1 °C for 30 min. Absorbance was measured at 517 nm using JENWAY 630 Spectrophotometer. The antiradical activity was expressed as TROLOX (6-hydroxy-2.5.7.8-tertamethylchroman-2-carboxylic acid) equivalent antiradical activity (mmoL TE 100 g^{-1} DW).

Mathematical data processing

The results are the means and standard deviation for three replicates. Means were compared by analysis of variance (ANOVA) and correlation analysis. Significance was defined at $P < 0.05$. Statistical analysis was carried out by Microsoft Excel 2010 version software.

RESULTS AND DISCUSSION

Generally, the chemical properties of herbal tea depend on the applied temperature and time, as well as the extraction technique (Ong, 2004).

Based on the absorbance values after reaction with Folin–Ciocalteu reagent, results of the spectrophotometric analysis are given in Fig. 1. The total phenolics content (TPC) in the investigated herbal tea infusions ranged from 1,824.1 ± 9.08 to 4,350.3 ± 13.01 mg GAE 100 g^{-1} (Fig. 1). Lady's-mantle infusions were the most abundant in the total phenols (15 min extraction 4,350.3 ± 13.01 GAE 100 g^{-1} and 20 min extraction 4,226.4 ± 10.32 mg GAE 100 g^{-1}), while the lowest amounts were determined in Calendula tea infusions (in average 1,829.9 ± 9.65 mg GAE 100 g^{-1}). The

obtained results indicated that extraction time did not significantly affected the content of total phenols in the herbal tea infusions ($P > 0.05$).

Previous study Kaya et al. (2012) from Turkey proved the Lady's-mantle are well known as rich sources of polyphenolic compounds, flavonoids and phenolic acids.

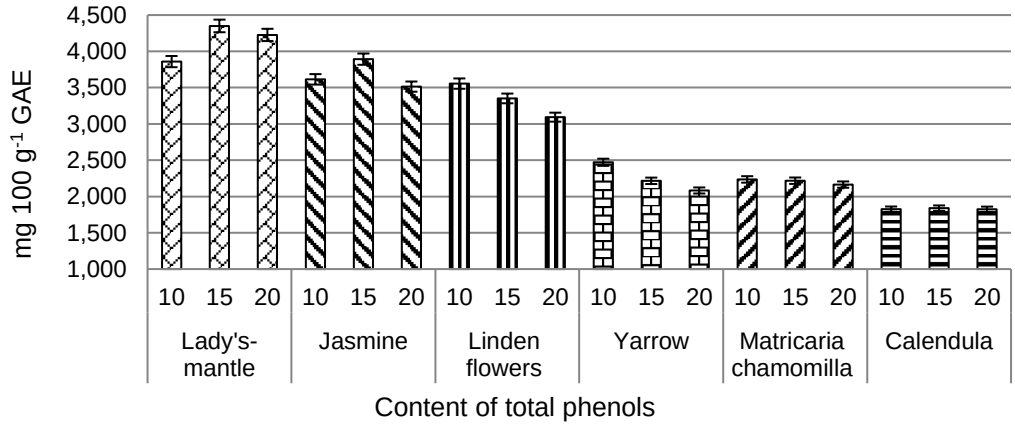

Figure 1. Content of total phenols in herbal tea infusions.

In addition, Rigane et al. (2013) from Tunisia reported that total phenolics content of Calendula herbal tea infusions was 109.27 mg GAE g^{-1} dry weight extracts. Scientist Toda (2011) from Japan reported that total polyphenol content in Arabian jasmine tea-water extract was 101.2 ± 17.8 mg GAE g^{-1}. In a study by Jungmin Oh (2013), it showed that the content of total phenols in green herb tea was 82.21 mg GAE g^{-1}, black herb tea was 82.86 mg GAE g^{-1}, and peppermint tea – 75.31 mg GAE g^{-1}. Accordingly to Formisano et al. (2015) total phenols content in Chamomile herbal tea was $2{,}689.2 \pm 15$ mg GAE 100 g^{-1} DW. Analyzing obtained experimental results we can conclude that our results confirm the results mentioned in scientific literature and that total phenols could be one of the main components responsible for antioxidant activities of these herbal teas.

Flavonoids are a large subgroup of secondary metabolites categorized as phenolic compounds, widely distributed throughout plants. Flavonoids protect plants against various biotic and abiotic stresses and exhibit adiverse spectrum of biological functions and play an important role in the interaction between the plant and their environment. Flavonoids absorbed the harmful UV radiation induced cellular damage (Amalesh et al., 2011). In herbal tea infusions the presence of flavonoids can explain the strong antioxidant activity of them. The elucidation of their structures is complex, as they can range from simple aromatic molecules to highly polymerized compounds and may involve synergism between compounds (Boroski et al., 2011).

The results of this study (Fig. 2.) point out that the highest level of flavonoids was found in Lady's-mantle and Jasmine infusions (average 115.28 ± 5.25 mg quercetin equivalent (QE) 100 g^{-1} and 104.98 ± 9.21 QE mg 100 g^{-1} respectivelly), while the lowest was determined in Matricaria chamomilla extract – (average 70.10 ± 4.68 QE mg 100 g^{-1}). The obtained results indicated that extraction time 10, 15 and 20 min did not significantly affected the content of flavonoids compounds in the herbal tea infusions ($P > 0.05$).

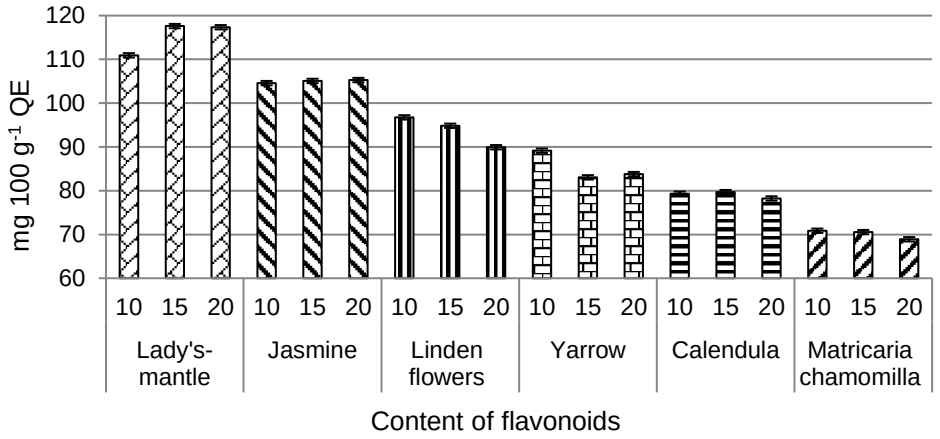

Figure 2. Content of flavonoids in herbal tea infusions.

In a study by Jungmin Oh from Korea (2013), it showed that total flavonoid levels in herb teas can ranged from 2.51 to 48.33 mg QE g^{-1}. In addition, Al-osaj (2016) from Iraq Nahrain University Baghdad, reported that total flavonoids in Lady's-mantle tea infusions was 1,831 quantity mg 100 g^{-1}. Scientist from Iraq reported that the Lady's-mantle is the richest source of flavonoids, containing catechin (250 mg 100 g^{-1}), epicatechin (524 mg 100 g^{-1}) and a significant amount of rutin (1,057 mg 100 g^{-1}) (Al-osaj, 2016). In addition, Rigane et al. (2013) from Tunisia reported that total flavonoids in Calendula herbal tea infusions ranged between 44.91 and 76.44 mg QE g^{-1} dry weight in leaf and flower extracts, respectively. Total flavonoids content in chamomile herbal tea was 710.7 ± 9 mg QE 100 g^{-1} DW to 530.9 ± 20 mg QE 100 g^{-1} DW (Formisano et al., 2015).

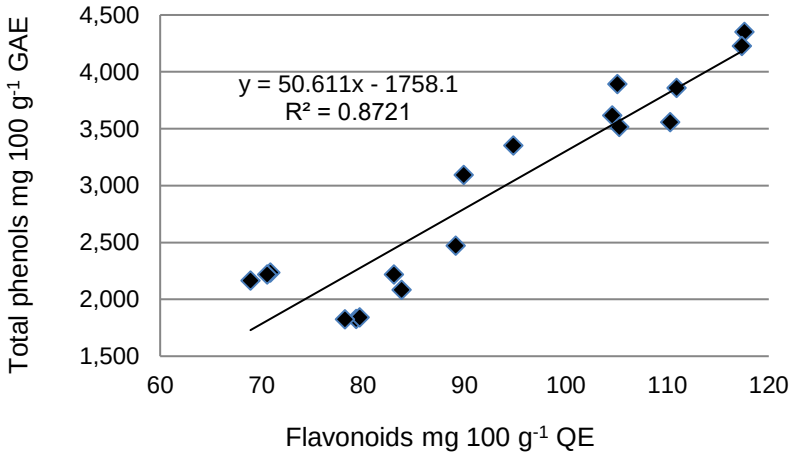

Figure 3. The correlation between total phenols and flavonoids content.

The correlation between total phenols content and flavonoids of the 6 herbal tea infusions is shown in Fig. 3. The result showed a positive linear correlation between total phenols and flavonoids content (R^2 = 0.8721).

The DPPH method is an efficient procedure commonly used to determine antioxidant activity (Scherer et al., 2009). The antioxidant capacity of the herbal tea infusions were studied through the evaluation of their free radical scavenging effect on the 1.1-diphenyl-2-picrylhydrazyl (DPPH) radical. The results were expressed as μM Trolox ekv 100 g^{-1}.

The content of antioxidant activity, mmol Trolox eq. 100 g^{-1} is shown in Table 1. For the herbal tea infusions, the antioxidant activity varied from 75.04 ± 1.15 mmol Trolox eq. 100 g^{-1} to 91.54 ± 2.62 mmol Trolox eq. 100 g^{-1}. The highest antioxidant activity was observed for Linden flowers infusion: 91.19 ± 2.54 mmol Trolox eq. 100 g^{-1} with extraction time 10 min and 91.54 ± 2.62 mmol Trolox eq. 100 g^{-1} (extraction time 15 min), followed by Matricaria chamomilla 90.46 ± 2.66 mmol Trolox eq. 100 g^{-1} (extraction time 15 min).

Calendula had the lowest antioxidant activity – in average 80.08 ± 1.52 mmol Trolox eq. 100 g^{-1}. Extraction time did not have statistically significant influence ($P > 0.05$) on antioxidant activity.

Table 1. Antioxidant activity in herbal tea infusions

Herbal tea infusions	Antioxidant activity, mmol Trolox equivalents 100 g^{-1}		
	Extraction time		
	10 min	15 min	20 min
Calendula	75.04 ± 1.15	83.58 ± 2.02	81.60 ± 1.41
Matricaria chamomilla	89.03 ± 2.62	90.46 ± 2.66	89.13 ± 2.27
Jasmine	86.87 ± 1.64	87.54 ± 1.84	87.69 ± 2.06
Lady's-mantle	82.68 ± 1.35	87.05 ± 1.32	85.32 ± 1.33
Linden flowers	91.19 ± 2.54	91.54 ± 2.62	89.77 ± 2.36
Yarrow	89.50 ± 2.11	90.83 ± 2.21	86.30 ± 1.74

Antioxidant activity, mmol Trolox eq. 100 g^{-1} of the Herbal tea infusions value was expressed as the mean ± standard error (SD).

In addition, Rigane et al. (2013) from Tunisia reported that antioxidant activities of Calendula officinalis extracts was 0.35 ± 0.02 DPPH (IC_{50} mg mL^{-1}) or 28.37 ± 0.12 FRAP (mmol eq. of Trolox). In turn scientist Toda (2011) from Japan proved that antioxidant activity of Arabian Jasmin herb tea water extract was 144.0 ± 1.4 mmol L^{-1} PAO, but Chamomile herbal tea antioxidant activity was 238.2 ± 4 to 811.4 ± 1 mol TE 100 g^{-1} DW (Formisano et al., 2015).

The antioxidant capacities of samples may be influenced by lots of factors, such as cultivation, production, storage conditions and test systems, and cannot be fully described by one single method (Yashin et al., 2011). Most natural antioxidants are multifunctional. A reliable antioxidant evaluation protocol requires different antioxidant activity assessments to be performed to take into account various mechanisms of antioxidant action (Wong et al., 2006).

The obtained results indicated that extraction time did not significantly affected the content of dry matter in the herbal infusions ($P > 0.05$), but it depends on plants type. The highest content of dry matter was determined in Jasmine herbal tea extract (53.37 ± 0.27 g 100 g^{-1}), but the lowest in in Linden flowers and Yarrow herbal tea extracts, respectively 20.64 ± 0.10 g 100 g^{-1} and 20.57 ± 0.14 g 100 g^{-1}.

CONCLUSIONS

In summary, the present paper investigates the possibilities for obtaining herbal tea infusions with high values of bioactive compounds, using in Latvia well known and popular herbs. The obtained results indicated that extraction time (10, 15 or 20 min) did not affect the content of biologically active compounds, as well as antioxidant activity in the herbal infusions significantly, but content of tea`s dry matter was significantly different. The highest level of flavonoids was found in Jasmine and Lady's-mantle infusions, while the lowest (about two times) was determined in Matricaria chamomilla extract. Lady's-mantle tea also contained the highest amount of total phenols – in average 4126.62 ± 26.24 mg GAE $100g^{-1}$. Calendula and Matricaria chamomilla tea infusions are the poorest regarding these compounds. Data analysis showed a close linear positive correlation between the content of total flavonoids and total phenols in herbal infusions ($R^2 = 0.872$; $r = 0.934$). Due to higher content of bioactive compounds in tea infusions, all samples demonstrated high level of antioxidant activity (from 75.04 to 91.54 mmol Trolox equivalents 100 g^{-1}).

REFERENCES

Afify, A.El-M.MR., El-Beltagi, H.S., Abd El-Salam, S.M. & Omran, A.A. 2012. Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. *Asian Pacific Journal of Tropical Biomedicine*, 203–209.

Al-osaj, S.L. 2016. *Cytotoxic Evaluation of Alchemilla vulgaris Flavonoids in Normal Blood Lymphocytes.* A thesis. Al-Nahrain University as a partial fulfillment of the requirement for the Degree, 73 pp.

Amalesh, S, Gouranga, D. & Sanjoy, K.D. 2011. Roles of flavonoids in plants. International Journal of Pharmacy and Pharmaceutical Sciences **6**(1), 12–35.

Avula, B., Wang, Y.-H., Wang, M., Avonto, C., Zhao, J., Smillie, T.J., Rua, D. & Khan, I.A. 2014. Quantitative determination of phenolic compounds by UHPLC-UV–MS and use of partial least-square discriminant analysis to differentiate chemo-types of chamomile/chrysanthemum flower heads. *Journal of Pharmaceutical and Biomedical Analysis* **88**, 278–288.

Boroski, M., Aguiar, A.C., Boeing, J.S., Rotta, E.M., Wibby, C.L., Bonafé, E.G., Souza, N.E. & Visentainer, J.V. 2011. Enhancement of pasta antioxidant activity with oregano and carrot leaf. *Food Chemistry* **125**, 696–700.

Chan, E.W.C., Lim, Y.Y., Chong, K.L., Tan, J.B.L. & Wong, S.K. 2010. Antioxidant properties of tropical and temperate herbal teas. *Journal of Food Composition and Analysis* **23**(2), 185–189.

Craig, W.J. 1999. Health-promoting properties of common herbs. *American Journal of Clinical Nutrition* **70**(3), 491–499.

Cushnie, T.P.T. & Lamb, A.J. 2011. Recent advances in understanding theantibacterial properties of flavonoids. *International Journal of Antimicrobial Agents* **38**, 99–107.

Dewanto, V., Wu, X., Adom, K.K. & Liu, R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* **50**(10), 3010–3014.

Formisanoa, C., Delfineb, S., Olivieroa, F., Tenorea, G.C., Riganoa, D. & Senatorea, F. 2015. Correlation among environmental factors, chemical composition andantioxidative properties of essential oil and extracts of chamomile (Matricaria chamomilla L.) collected in Molise (South-central Italy). *Industrial Crops and Products* **63**, 256–263.

Huang, D., Ou, B. & Prior, R.L. 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry* **53**(6), 1841–1856.

Jungmin, O., Heonjoo, J., Ah Reum, C., Sung-Jin, K. & Jaejoon, H. 2013. Antioxidant and antimicrobial activities of various leafy herbal teas. *Food Control* **31**(2), 403–409.

Kaya, B., Menemen, Y. & Saltan, F.Z. 2012. Flavonoids in the endemic species of Alchemilla L., (Section Alchemilla L. Subsection Calycanthum rothm. ser. Elatae rothm.) from north-east black sea region in Turkey. *Pakistan Journal of Botany* **44**(2), 595–597.

Kunhachan, P., Banchonglikitkul, C., Kajsongkram, T., Khayungarnnawee, A. & Leelamanit, W. 2012. Chemical Composition, Toxicity and Vasodilatation Effect of the Flowers Extract of Jasminum sambac (L.) Ait. 'G. Duke of Tuscany'. *Evidence-Based Complementary and Alternative Medicine*, Article ID 471312, 7 pp.

Lakshmi, T., Geetha, R.V., Roy, A. & Kumar, S.A. 2011. Yarrow (Achillea Millefolium Linn.) a herbal medicinal plant with broad therapeutic use – a review. *International Journal of Pharmaceutical Sciences Review and Research* **9**(2), 22–26.

Manach, C., Scalbert, A., Morand, Ch., Remesy, Ch. & Jimenez, L. 2004. Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition* **5**, 727–747.

Miguel, M.G. 2010. Antioxidant activity of medicinal and aromatic plants. Areview. *Flavour and Fragrance Journal* **25**, 291–312.

Ong, E.S. 2004, Extraction methods and chemical standardization of botanicals and herbal preparations. *Journal of Chromatography B* **812**(1), 23–33.

Pietta, P.-G. 2000. Flavanoids as antioxidants. *Journal of Natural Products* **63**(7), 1035–1042.

Rice Evans, C.A., Miller, N.J. & Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends in Plant Science* **2**(4), 304–309.

Rigane, G., Younes, B., Ghazghazi, S.H. & Salem, B.R 2013. Investigation into the biological activities and chemical composition of Calendula officinalis L. growing in Tunisia. *International Food Research Journal* **20**(6), 3001–3007.

Salganik, R.I. 2001. The benefits and hazards of antioxidants: controlling apoptosis and other protective mechanisms in cancer patients and the human population. *Journal of the American College of Nutrition* **20**, 464–472.

Scherer, R. & Godoy, H.T. 2009. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. *Food Chemistry* **112**(3), 654–658.

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* **29**, 152–178.

Sroka, Z. & Belz, J. 2009. Antioxidant activity of hydrolyzed and non-hydrolyzed extracts of the inflorescence of linden (Tiliae inflorescentia). *Advances in Clinical and Experimental Medicine* **18**(4), 329–335.

Toda, S. 2011. Polyphenol Content and Antioxidant Effects in Herb Teas. *Chinese Medicine* **2**, 29–31.

Wong, S.P., Leong, L.P. & Koh, J.H.W. 2006, Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry* **99**, 775–783.

Xu, B.J. & Chang, S.K.C. 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science* **72**(2), 159–166.

Yanai, N., Shiotani, S., Hagiwara, S., Nabetani, H. & Nakajima, M. 2008. Antioxidant combination inhibits reactive oxygen species mediated damage. *Bioscience, Biotechnology, and Biochemistry* **72**(12), 3100–3106.

Yashin, A., Yashin, Y. & Nemzer, B. 2011. Determination of Antioxidant Activity in Tea Extracts, and Their Total Antioxidant Content. *American Journal of Biomedical Sciences* **3**(4), 322–335.

Zhou, K. & Yu, L. 2004. Effects of extraction solvent on wheat bran antioxidant activity estimation. *LWT — Food Science and Technology* **37**, 717–721.

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Effect of germination and extrusion on the phenolic content and antioxidant activity of raw buckwheat (*Fagopyrum esculentum* Moench)

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Abstract. The aim of the research was to determine the total phenolic content, antioxidant activity and phenolic compounds in raw and germinated common buckwheat grain and their extruded products in order to estimate the effect of germination and extrusion on the total phenolic content, antioxidant activity and phenolic compounds in buckwheat. A total of 10 buckwheat samples were analysed, where the raw buckwheat grain was the control sample, four samples were germinated and then extruded. The total phenolic content was determined using the Folin–Ciocalteu assay. The antioxidant activity was determined using a micro plate assay and phenolic compounds with Liquid Chromatography – Time of Flight Mass Spectrometer method.
The results showed that germination of buckwheat significantly affected the total phenolic content, antioxidant activity, radical scavenging activity and content of phenolic compounds. The decrease of the total phenolic content, antioxidant activity, radical scavenging activity and the content of some phenolic compounds depended on germination time – 48 h of germination provided greater concentrations at the end of a 24 h germination period, whereas the content of some phenolic compounds like rutin, quercetin and vitexin increased substantially after germination, besides, the increase of phenolic compounds depended on the germination time. In total 26 different phenolic compounds were detected in raw and germinated buckwheat samples and only one compound with an *m/z* value 385.1282 was unidentified. The data of extrusion revealed a significant decrease of total phenolic content, antioxidant activity, radical scavenging activity and phenolic compounds content in buckwheat samples ($P < 0.05$). In the extruded buckwheat samples novel phenolic compounds like 4–hydroxybenzoic acid, homovanillic acid, catechin, ferulic acid, and hyperoside were detected.

Key words: buckwheat, germination, extrusion, phenolics, antioxidant activity.

INTRODUCTION

Buckwheat grain is a pseudo–cereal which is characterized as functional food due to its components with biological activity and healing properties like flavonoids, phenolic acids, phytosterols, etc. (Christa & Soral–Smietana, 2008; Filipcev et al., 2011; Torbica et al., 2012; Zhang et al., 2012). Rutin and quercetin compose the major part of bioactive compounds in buckwheat with antioxidant, antimicrobial and anti–

inflammatory activities (Halosava et al., 2002; Cai et al., 2004; Lin et al., 2009; Torbica et al., 2012; Wronkowska et al., 2015). The quantified phenolic compounds in buckwheat are caffeic acid, rutin, vitexin, quercitrin, quercetin and kaempferol (Peng et al., 2017). Zhang et al. (2015) identified 11 phenolic compounds in buckwheat: orientin, isoorientin, vitexin, isovitexin, rutin, kamperol–3–rutinoside, quercitrin, myricetin, luteolin, quercetin and kampferol. It is known that phenolic compounds like rutin, quercetin, etc. cannot be produced in human body, therefore it is important to take them with food. Wang et al. (2009) observed the reduction of chronic diseases risk by using buckwheat bran extract *in vivo*. Kawa et al. (2003) reported the reduction of serum glucose level by feeding buckwheat to rats. Lu et al. (2002) concluded that regular intake of buckwheat can reduce the blood glucose concentration and the risk of diabetes mellitus in humans.

Germination of seeds and grain is characterized as a process with added value because it allows one to improve the nutritional and biological value of product. Kim et al. (2004) observed an increase of phenolic compounds like rutin and quercitrin in buckwheat during germination, whereas Yiming et al. (2015) concluded that germination could be a good way of accumulating rutin in buckwheat grain. Some studies reported that an increase of phenolic compounds concentrations in buckwheat depended on germination time: the longer the time, the faster the increase (Yiming et al., 2015; Zhang et al., 2015).

Total phenolic content, content of phenolic compounds and antioxidant activity could be affected by the processing of buckwheat. A reduction of content of phenolic compounds was observed in buckwheat during heating at a temperature of 150 °C, the loss increased by the prolongation of time (Dietrych–Szostak & Oleszek, 1999). Sensoy et al. (2006) reported that the optimization of temperature and time during processing (roasting or extrusion) could provide the maintenance of phenolic compounds in buckwheat. Jozinovic et al. (2012) concluded that the extrusion of corn meal with buckwheat flour caused the decrease of total phenolic content and antioxidant activity. Zielinska et al. (2007) reported similar observation about the reduction of antioxidant capacity in buckwheat after roasting. In summary, the germination of buckwheat grain provided an improvement of biological value, but there is no information about the extrusion effect on phenolic content and antioxidant activity in germinated buckwheat grain. Therefore, the aim of the current research was to determine the total phenolic content, antioxidant activity and phenolic compounds in raw and germinated buckwheat grain and their extruded products in order to estimate the effect of germination and extrusion on the total phenolic content, antioxidant activity and phenolic compounds in buckwheat. The germination of buckwheat grain was carried out to increase the biological value, to improve the digestibility and the advanced grain will be used for production of flour or new functional products. The extrusion of raw and germinated buckwheat grains was used for production of new products like snacks, which could provide the consumption of buckwheat among the inhabitants of Latvia.

MATERIALS AND METHODS

Materials

Buckwheat (*Fagopyrum esculentum* Moench) grain was purchased from the organic farm 'Bebri' (Saldus area, Latvia).

Germination of buckwheat grain was performed in two time lengths (24 h or 48 h) in the climate control chamber (Memmert, ICH110, Germany). Subsequent drying took place at two different temperatures (40 °C or 60 °C) in the universal oven (Memmert, UF160, Germany).(Fig. 1).

Drying after germination was performed to provide the storage of buckwheat grain and to produce germinated buckwheat flour, which was used further for production of extruded germinated buckwheat products. The selected time of drying was 4 hours till the moisture content in germinated buckwheat grain reached 13–14%.

Extrusion of raw and germinated buckwheat grain was performed with the food extruder (PCE Extrusiometer L–Serie, Göttfert, Germany; Fig. 2). The extruded raw and germinated buckwheat grain were dried in the convective–rotary oven (SVEBA DAHLAN, Sweden).

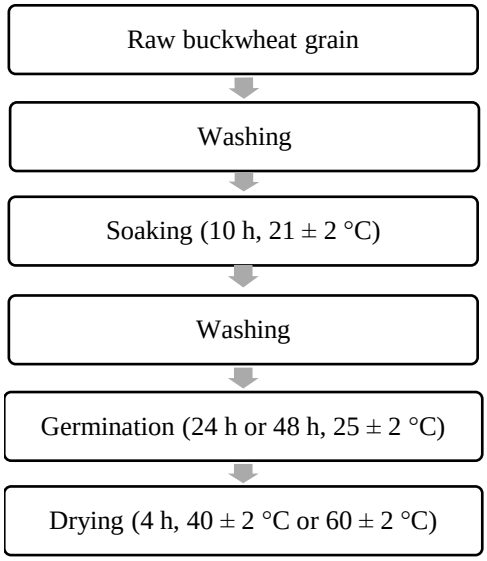

Figure 1. The process of obtaining germinated buckwheat grain.

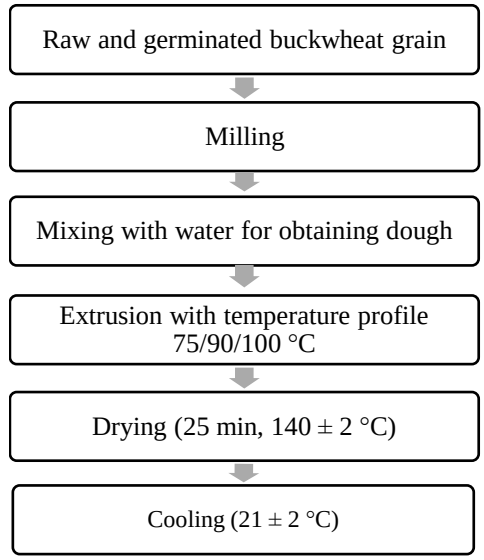

Figure 2. Technological process of obtaining extruded raw and germinated buckwheat products.

Methods

The total phenolic content (TPC) was determined by the Folin–Ciocalteu assay using the high–throughput 96–well plate method as described by Herald et al. (2012) with slight modifications. The measurement was conducted by mixing Folin–Ciocalteu solution (1:1 with water), sodium bicarbonate and ethanolic extract. The absorbance was measured after 90 min of incubation at 765 nm, along with the blank. TPC was expressed

as gallic acid equivalents (GAE mg 100 g^{-1} DW), based on the gallic acid (GA) calibration curve (range 0.025–0.20 mg mL^{-1}, R^2 = 0.9997). Analyses were performed with the Infinite M200 PRO (Tecan Group Ltd., Männedorf, Switzerland) instrument in triplicate. The bandwidth was 9 mm and temperature 24 °C.

Characterisation of antioxidant activity using micro plate assay. The DPPH method is based on the ability to stabilize the free radical 2.2–diphenyl–picrylhydrasyl (DPPH) to react with hydrogen donors. Antiradical activity (ARA) was determined using the DPPH assay using the high–throughput 96–well plate method as described by Herald et al. (2012) with slight modifications. The measurements of buckwheat extracts were done by mixing a 150 μM DPPH solution in ethanol with an extract or standard samples. The absorbance was measured at 517 nm, along with the blank. ARA was expressed as ascorbic acid equivalents (AAE mg 100 g^{-1} DW), based on the calibration curve (0.03–0.09 mg mL^{-1}, R^2 = 0.998). The analyses were performed on the Infinite M200 PRO (Tecan Group Ltd., Männedorf, Switzerland) instrument in triplicate. The bandwidth was 9 mm, temperature 26.4 °C. The percentage of radical scavenging activity of all extracts was calculated using the following formula (1):

$$\%scavenging[DPPH] = [(A0 - A1/A0)] \cdot 100 \quad (1)$$

where $A0$ – the absorbance of the blank; $A1$ – the absorbance in the presence of buckwheat extract.

The content of phenolic compounds was determined with the Liquid Chromatography –Time of Flight Mass Spectrometer (LC–TOF–MS) according to the description offered by Klavina et al. (2015).

During LC–MS analysis, each sample produces its own base peak chromatogram (BPC). In the positive ionization mode each compound can add a proton and can produce its own $[M+H]^+$ mass spectra or stay in a positively charged state as M^+ molecule. Each compound has its own chemical formula and molar mass. This chemical formula using *Mass Hunter Qualitative Analyses B.07.00* software can be used for the calculation of $[M+H]^+/[M]^+$ which is used for the extraction of compounds from the base peak chromatogram. Both values can be compared and the difference (Δ) between them should not be higher than 0.0030. HRMS experiments ensure accurate mass measurements resulting in the removal of background signals of complex matrix interferences. It is useful for non–targeted or retrospective post–targeted identification of unknown compounds by the processing of raw data obtained in different scan modes, including full scan in defined *m/z* windows. The experimental data were handled using the MassHunter version B07.00 software (Agilent Technologies).

All analyses were performed in triplicate.

Preparation of buckwheat extracts was carried as previously described (Carvalho et al. 2015).

Abbreviation of buckwheat samples

In total, ten buckwheat sample types were prepared and analysed, and they were encrypted with specific abbreviations (Table 1).

Table 1. Abbreviation of buckwheat samples

Abbreviations	Sample description
RB	Raw buckwheat
GB–24/40	Germinated buckwheat (germination time 24 h, drying temperature 40 °C)
GB–24/60	Germinated buckwheat (germination time 24 h, drying temperature 60 °C)
GB–48/40	Germinated buckwheat (germination time 48 h, drying temperature 40 °C)
GB–48/60	Germinated buckwheat (germination time 48 h, drying temperature 60 °C)
E–RB	Extruded raw buckwheat
E–GB–24/40	Extruded germinated buckwheat (germination time 24 h, drying temperature 40 °C)
E–GB–24/60	Extruded germinated buckwheat (germination time 24 h, drying temperature 60 °C)
E–GB–48/40	Extruded germinated buckwheat (germination time 48 h, drying temperature 40 °C)
E–GB–48/60	Extruded germinated buckwheat (germination time 48 h, drying temperature 60 °C)

Data processing

The data processing was performed using mathematical and statistical methods with statistical software Microsoft Office Excel 14.0. The results of the research were expressed as a mean ± standard deviation and analysed using the analyses of variance (ANOVA). *T–test* was applied to compare the mean values and *P–value at 0.05* was used to determine the significant differences.

RESULTS AND DISCUSSION

In literature the given total phenolic content (TPC) differed depending on the buckwheat varieties and extractions method used (Inglett et al., 2010). In research of Vollmannova et al. (2013) TPC of five buckwheat varieties varied between 138.10–286.99 GAE mg 100 g^{-1} DW, whereas Mikulajova et al. (2016) evaluated 22 cultivars of buckwheat, and TPC ranged from 89.7 to 145.6 GAE mg 100 g^{-1} DW. Unal et al. (2017) reported significant differences between commercial buckwheat and Güneş variety (207.12 ± 2.67 GAE mg 100 g^{-1} DW and 329.83 ± 3.88 GAE mg 100 g^{-1} DW, respectively). Comparing the literature data with the results of this study about raw buckwheat (Table 2) similar or lower total phenolic content was determined where the differences could be explained by various determination methods used. Analysing the influence of germination on TPC, a significant decrease in TPC of buckwheat samples ($P < 0.05$) was observed. Furthermore, the decrease of TPC depended on the germination time (48 h of germination provided a greater concentration of TPC) and drying temperature after germination (a lower temperature provided a lower decrease of TPC). The greatest concentration of TPC among the germinated buckwheat samples was observed in GB–48/40. The rapid decrease of TPC during germination of first 24 h could be explained by a high demand of energy at the beginning of germination (Jia et al., 2015), whereas a greater TPC after 48 h of germination could be associated with single phenolic compounds increase during germination according to literature (Yiming et al., 2015; Zhang et al., 2015).

Table 2. Total phenolic content, antiradical activity and radical scavenging activity of raw and germinated buckwheat and their extruded products

Samples	TPC GAE mg 100 g^{-1} DW	ARA AAE mg 100g^{-1} DW	DPPH %
RB	132.85 ± 1.03^{a}	90.56 ± 0.51^{a}	94.76 ± 0.48^{a}
GB–24/40	74.13 ± 2.05^{c}	48.39 ± 0.06^{c}	55.03 ± 0.06^{c}
GB–24/60	66.44 ± 1.41^{c}	39.49 ± 0.54^{d}	44.43 ± 0.48^{d}
GB–48/40	96.92 ± 1.18^{b}	64.88 ± 0.59^{b}	71.81 ± 0.56^{b}
GB–48/60	90.81 ± 1.80^{b}	49.73 ± 1.31^{c}	54.79 ± 1.19^{c}
E–RB	34.14 ± 0.70^{d}	12.48 ± 0.09^{e}	21.20 ± 0.08^{e}
E–GB–24/40	28.13 ± 0.11^{d}	10.90 ± 0.60^{e}	19.85 ± 0.55^{e}
E–GB–24/60	30.90 ± 0.84^{d}	9.62 ± 0.05^{e}	18.66 ± 0.05^{e}
E–GB–48/40	31.62 ± 1.08^{d}	13.89 ± 0.01^{e}	22.70 ± 0.01^{e}
E–GB–48/60	28.99 ± 0.65^{d}	9.76 ± 0.09^{e}	18.66 ± 0.08^{e}

Different letters indicate statistically significant difference between buckwheat samples *($P < 0.05$)*.

After extrusion of raw and germinated buckwheat samples, a significant decrease of TPC ($P < 0.05$) was observed which was in concordance with literature (Jozinovic et al., 2012). Similar conclusions were reported by Hes et al. (2014) stating that TPC was affected by the boiling of buckwheat groats where the temperature was close to the maximum temperature of extrusion in this study and by Wronkowska et al. (2015) that after roasting buckwheat TPC was two times lower. In this research the losses of total phenolic content after extrusion composed 74.3% in E–RB and 53.49–68.08% in E–GB samples.

Similar trends were observed for antiradical activity (ARA) and radical scavenging activity (DPPH) of raw and germinated buckwheat grain and their extruded products. The highest ARA and DPPH were determined for raw buckwheat grain ($P < 0.05$). Germination and extrusion processes significantly affected the decrease of ARA and DPPH in buckwheat samples ($P < 0.05$). The decrease of ARA in buckwheat was 1.89 or 2.29 times by 24 h germination and 1.40 or 1.82 times by 48 h germination, whereas the extrusion decreased ARA 7.26 times in raw buckwheat and 4.10–5.09 times in germinated buckwheat samples. The decrease of DPPH was close to the results of ARA in germinated buckwheat samples but not so critical as the results of ARA in extruded buckwheat products. In literature there are reports about an increase of radical scavenging activity in buckwheat during germination, and there are indications that germination for 72 h showed the highest DPPH (Zhang et al., 2015). These conclusions have not been confirmed in the current research. The differences could be explained by different germination methods: in this research germinated buckwheat was dried, while in the research of Zhang et al. (2015) it was lyophilized.

In total, 26 different phenolic compounds were separated from raw and germinated buckwheat samples: catechin hydrate, chlorogenic acid, vanillic acid, caffeic acid, epicatechin, syringic acid, vanillin, p–coumaric acid, sinapic acid, 2–OHcinnamic acid, rutin, quercetin, 2–hydroxy–3–O–b–D-glucopyranosyl–benzoic acid, caffeic acid hexose, swertiamacroside, flavonol–glycosides, catechin–glucosides, epicatechin gallate, (epi)afzelchin–(epi) catechin isomer, vitexin, epiafzelchin–epicatechin–O–methylgallate, (–)epicatechin–O–3.4-dimethyl–gallate, epiafzelchin–epicatechin–O–dimethylgallate, procyanidin B2–dimethylgallate, and epiafzelchin–epiafzelchin–epicatechin. Only one compound with *m/z* value 385.1282 was unidentified.

The main phenolic compounds of buckwheat samples are given in Table 3. The research data confirmed the conclusions found in literature that buckwheat grain contains high level of flavonoids, especially rutin.

Table 3. Phenolic compounds of raw and germinated buckwheat grain and their extruded products, mg 100 g^{-1} DW

Samples	Epicatechin C15H14O6	Rutin C27H30O16	Quercetin C15H10O7	Catechin–glucosides C21H24O11	Vitexin C21H20O10	Epiafzelchin–epicatechin–O–dimethylgallate C39H34O15
RB	39.13^{a}	16.38^{b}	3.84^{b}	165.93^{a}	1.44^{cd}	59.89^{a}
GB–24/40	19.89^{b}	19.59^{b}	4.96^{b}	95.88^{b}	2.25^{c}	20.64^{b}
GB–24/60	14.14^{b}	18.91^{b}	4.79^{b}	91.18^{b}	2.28^{c}	14.44^{c}
GB–48/40	38.34^{a}	32.44^{a}	9.71^{a}	81.84^{c}	63.26^{a}	18.74^{b}
GB–48/60	41.18^{a}	31.21^{a}	8.95^{a}	92.39^{b}	63.3^{4a}	15.50^{c}
E–RB	7.36^{c}	5.51^{c}	1.48^{c}	n.d.	2.44^{c}	5.50^{d}
E–GB–24/40	4.79^{c}	3.98^{c}	0.95^{c}	n.d.	1.13^{cd}	3.25^{e}
E–GB–24/60	4.38^{c}	3.59^{c}	0.96^{c}	n.d.	0.95^{d}	2.55^{e}
E–GB–48/40	4.37^{c}	3.48^{c}	0.82^{c}	n.d.	6.36^{b}	2.29^{e}
E–GB–48/60	4.35^{c}	3.91^{c}	1.04^{c}	n.d.	8.09^{b}	2.05e

n.d. – not detected;
Different letters indicate statistically significant difference between buckwheat samples *($P < 0.05$)*.

Comparing the rutin content of raw buckwheat grain in this research with the data from literature, similar or lower levels of rutin were established. For example, Qin et al. (2010) reported that rutin content in 18 cultivars of common buckwheat ranged between 15.0 and 16.8 mg 100 g^{-1} DW. In the study of Kiprovski et al. (2015) it was indicated that rutin content in buckwheat seeds was from 3.29 to 151.45 mg 100 g^{-1} DW. Vollmannova et al. (2013) revealed that rutin content of 5 cultivars of buckwheat was between 30.99 and 50.77 mg 100 g^{-1} DW. It confirmed the conclusions of literature that rutin content depends on the cultivars, species – common or tartary, and growth conditions (Qin et al., 2010; Mikulajova et al., 2016).

High amounts of epicatechin, catechin-glucosides and epiafzelchin–epicatechin–O–dimethylgallate were determined in raw buckwheat grain. Quercetin content in raw buckwheat grain was lower compared to other phenolic compounds, except vitexin, but high enough compared to literature data where quercetin content in raw common buckwheat groats was reported to be 0.167 mg 100 g^{-1} DW (Hes et al., 2014). Furthermore, in the study of Qin et al. (2010) quercetin was determined only in two cultivars among 18 common buckwheat samples.

After the germination of raw buckwheat grain a significant increase of rutin, quercetin and vitexin was observed. It confirmed the conclusions arrived at in literature that during germination the content of rutin and quercitrin increased gradually (Kim et al., 2004) or that the content of orientin, isoorientin, vitexin, isovitexin, rutin, kamperol–3–rutinoside, quercitrin and kaempferol increased during germination but quercetin was detected only in 24 h of germination (Zhang et al., 2015). Yiming et al. (2015) did not observe regular changes of rutin and quercetin content during germination, though the

total flavonoid content was greater in buckwheat after germination. Similar observation was with regard to epicatechin content of germinated buckwheat grain in this research. The content of catechin-glucosides and epiafzelchin–epicatechin–O–dimethylgallate in raw buckwheat grain decreased after germination. The increase or decrease of phenolic compounds was affected by the germination time – 48 h of germination provided a substantial increase of rutin (approximately 1.9 times), quercetin (approximately 2.5 times) and vitexin (approximately 44.0 times). The other phenolic compounds the content of which increased after 48 h of germination were catechin hydrate, vanillic acid, vanillin, p-coumaric acid, sinapic acid, caffeic acid hexose, and flavonol–glycosides. Similar results regarding the effect of germination time on the content of rutin, quercetin and vitexin had been obtained by Zhang et al. (2015).

The data of extrusion revealed a significant decrease of phenolic compounds in buckwheat samples ($P < 0.05$) which could be a consequence of high temperatures and heating time. The decrease of rutin concentration in E–RB was 66.36% and in E–GB samples – 79.68–89.27% comparing to RB and GB samples, respectively. The losses of quercetin were 2.59 times higher in E–RB than in RB samples and 4.99–11.84 times higher in E–GB samples compared to GB samples. Wronkowska et al. (2015) also reported a decrease of rutin in roasted buckwheat groats, but it was 23% and the losses of phenolic compounds were twofold, whereas Choy et al. (2013) reported about a substantial drop of rutin in instant noodles with buckwheat flour during the cooking process.

In addition, in certain extruded buckwheat samples novel phenolic compounds were detected, such as 4–hydroxybenzoic acid (1.51 mg 100 g^{-1} DW in E–RB, 0.725–0.785 mg 100 g^{-1} DW in E–GB samples), homovanillic acid (0.13 mg 100 g^{-1} DW in E–RB, 0.277–0.962 mg 100 g^{-1} DW in E–GB samples), catechin (3.97 mg 100 g^{-1} DW in E–RB, 2.599–3.190 mg 100 g^{-1} DW in E–GB samples), ferulic acid (7.42 mg 100 g^{-1} DW in E–RB, 1.253–3.595 mg 100 g^{-1} DW in E–GB samples), and hyperoside (0.900 mg 100 g^{-1} DW in E–RB, 0.521–0.694 mg 100 g^{-1} DW in E–GB samples), but vanillic acid, swertiamacroside, and catechin–glucosides were not detected. Consequently, they were not included in Table 3, because only some extruded products contained them.

CONCLUSIONS

By selecting the optimum germination time of raw buckwheat it is possible to increase the content of rutin, quercetin and vitexin. In further studies the germination time of buckwheat should be prolonged to 72 h in order to retain a higher content of phenolic compounds after extrusion. Extrusion had a negative effect on the content of phenolic compounds and their antioxidant activity. There is a need for more profound research to be carried out regarding the possibilities of decreasing the negative effect of extrusion on phenolic compounds in buckwheat by changing the temperature profile during extrusion.

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REFERENCES

Cai, Y.Z., Corke, H. & Li, W.D. 2004. Buckwheat. In Wrigley, C.W., Corke, H. & Walker, C.E. (eds.): *The Encyclopedia of Grain Science*. Vol. 1, Elsiever Academic Press, Oxford UK, pp.120–128.

Carvalho, D.O., Curto, A.F. & Guido, L.F. 2015. Determination of phenolic content in different barley varieties and corresponding malts by liquid chromatography-diode array detection – electrospray ionization tandem mass spectrometry. *Antioxidants* **4**, 563–576.

Choy, A.L., Morrison, P.D., Hughes, J.G., Marriott, P.J. & Small, D.M. 2013. Quality and antioxidant properties of instant noodles enhanced with common buckwheat flour. *Journal of Cereal Science* **57**, 281–287.

Christa, K. & Soral-Smietana, M. 2008. Buckwheat grains and buckwheat products – nutritional and prophylactic value of their components – a Review. *Czech Journal of Food Sciences* **26**, 153–162.

Dietrych-Szostak, D. & Oleszek, W. 1999. Effect of processing on the flavonoid content in buckwheat (*Fagopyrum esculentum* Moench) grain. *Journal of Agricultural and Food Chemistry* **47**, 4384–4387.

Filipcev, B., Simurina, O., Bodroza-Solarov, M. & Vujakovic, M. 2011. Evaluation of physical, textural and microstructural properties of dough and honey biscuits enriched with buckwheat and rye. *Chemical Industry & Chemical Engineering Quarterly* **17**, 291–298.

Halosava, M., Fiedlerova, V., Smrcinova, H., Orsak, M., Lachman, L. & Vavreinova, S. 2002. Buckwheat – the source of antioxidant activity in functional foods. *Food Research International* **35**, 207–211.

Herald, T.J., Gadgil, P. & Tilley, M. 2012. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agriculture* **92**, 2326–2331.

Hes, M., Dziedzic, K., Gorecka, D., Drozdzynska, A. & Gujska, E. 2014. Effect of boiling in water of barley and buckwheat groats on the antioxidant properties and dietary fiber composition. *Plant Foods for Human Nutrition* **69**, 276–282.

Inglett, G.E., Rose, D.J., Chen, D., Stevenson, D.G. & Biswas, A. 2010. Phenolic content and antioxidant activity of extracts from whole buckwheat (*Fagopyrum esculentum* Moench) with or without microwave irradiation. *Food Chemistry* **119**, 1216–1219.

Jia, C.F., Hu, W.H., Chang, Z. & Gao, H.L. 2015. Acid α-galactosidase is involved in D–chiro–inositol accumulation during tartary buckwheat germination. *Acta Societatis Botanicorum Poloniae* **84**, 53–58.

Jozinovic, A., Subaric, D., Ackar, D., Babic, J., Klaric, I., Kopjar, M. & Lendic, K.V. 2012. Influence of buckwheat and chestnut flour addition on properties of corn extrudates. *Croatian Journal of Food Science and Technology* **4**, 26–33.

Kawa, J.M., Taylor, C.G. & Przybylski, R. 2003. Buckwheat concentrate reduces serum glucose in streptozotocin–diabetic rats. *Journal of Agricultural and Food Chemistry* **51**, 7287–7291.

Kim, S.L., Kim, S.K. & Park, C.H. 2004. Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Research International* **37**, 319–327.

Kiprovski, B., Mikulic–Petkovsek, M., Slatnar, A., Veberic, R., Stampar, F., Malencic, D. & Latkovic, D. 2015. Comparison of phenolic profiles and antioxidant properties of European *Fagopyrum esculentum* cultivars. *Food Chemistry* **185**, 41–47.

Klavina, L., Springe, G., Nikolajeva, V., Martsinkevich, I., Nakurte, I., Dzabijeva, D., Steinberga, I. 2015. Chemical composition of analysis, antimicrobial activity and cytotoxicity screening of moss extracts (Moss phytochemistry). *Molecules* **20(9)**, 17221–17243.

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* **112**, 987–991.

Lu, M., Zhang, H., Zhang, Y., Tong, W., Zhao, Y., Shan, S. & Liu, H. 2002. An epidemiological study of relationship between buckwheat in diet and the prevalence of diabetes mellitus as blood glucose concentration. *Modern Preventive Medicine* **29**, 326–327.

Mikulajova, A., Sediva, D., Hybenova, E. & Mosovska, S. 2016. Buckwheat cultivars – phenolic compounds profiles and antioxidant properties. *Acta Chimica Slovaca* **9**, 124–129.

Peng, L.X., Zou, L., Tan, M.L., Deng, Y.Y., Yan, J., Yan, Z.Y. & Zhao, G. 2017. Free amino acids, phenolic compounds in tartary buckwheat of different hull colour. *Czech Journal of Food Sciences* **35**, 214–222.

Qin, P., Wang, Q., Shan, F., Hou, Z. & Ren, G. 2010. Nutritional composition and flavonoids content of flour from different buckwheat cultivars. *International Journal of Food Science and Technology* **45**, 951–958.

Sensoy, I., Rosen, R.T., Ho, C.T. & Karwe, M.V. 2006. Effect of processing on buckwheat phenolics and antioxidant activity. *Food Chemistry* **99**, 388–393.

Torbica, A., Hadnadev, M. & Hadnadev, T.D. 2012. Rice and buckwheat flour characterisation and its relation to cookie quality. *Food Research International* **48**, 277–283.

Unal, H., Izli, G., Izli, N. & Asik, B.B. 2017. Comparison of some physical and chemical characteristics of buckwheat (*Fagopurym esculentum* Moench) grains. *CYTA – Journal of Food* **15**, 257–265.

Vollmannova, A., Margitanova, E., Toth, T., Timoracka, M., Urminska, D., Bojnanska, T. & Cicova, I. 2013. Cultivar influence on total polyphenol and rutin contents and total antioxidant capacity in buckwheat, amaranth, and quinoa seeds. *Czech Journal of Food Sciences* **31**, 589–595.

Wang, M., Liu, J.R., Gao, J.M., Parry, J.W. & Wei, Y.M. 2009. Antioxidant activity of tartary buckwheat bran extracts and its effect on the lipid profile of hyperlipidemic rats. *Journal of Agricultural and Food Chemistry* **57**, 5106–5112.

Wronkowska, M., Honke, J. & Piskula, M.K. 2015. Effect of solid-state fermentation with *Rhizopus oligosporus* on bioactive compounds and antioxidant capacity of raw and roasted buckwheat groats. *Italian Journal of Food Science* **27**, 424–431.

Yiming, Z., Hong, W., Linlin, C., Xiaoli, Z., Wen, T. & Xinli, S. 2015. Evolution of nutrient ingredients in tartary buckwheat seeds during germination. *Food Chemistry* **186**, 244–248.

Zhang, G., Xu, Z., Gao, Y., Huag, X., Zou, Y. & Yang, T. 2015. Effects of germination on the nutritional properties, phenolic profiles, and antioxidant activities of buckwheat. *Journal of Food Science* **80**, H1111–H1119.

Zhang, Z.L., Zhou, M.L., Tang, Y., Li, F.L., Tang, Y.X., Shao, J.R., Xue, W.T. & Wu, Y.M. 2012. Bioactive compounds in functional buckwheat food. *Food Research International* **49**, 389–395.

Zielinska, D., Szawara-Nowak, D. & Michalska, A. 2007. Antioxidant capacity of thermally–treated buckwheat. *Polish Journal of Food Nutrition Sciences* **57**, 465–470.

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The measurement of energy consumption during milling different cereals using the sieve analyses

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Abstract. This paper deals with the measurement of energy consumption required for disintegration of different cereals depending on the desired fineness of obtained grist. The energy consumption necessary for milling was compared with the results of a sieve analysis before and after the disintegration process. The obtained results were compared with energy expended during the disintegration of cereals and were analysed to determine the coefficients of the ratio of fineness of milling/energy consumption. They was found to have good conformity. Special attention was paid to the RRSB distribution for determination of statistic average particle size and specific area of malt grist. Specific area of grist particles from different cereals was determined by calculation of the limited area and x axis in diagrams, this effort is necessary for optimisation of the disintegration process with impact on the quality of final food.

Key words: cereals, two roller mill, hammer mill, sieve analysis, electric energy consumption.

INTRODUCTION

Milling in food processing is the mechanical process of grain disintegration of different cereals in order to increase the surface area of cereal kernels for easier access of enzymes, water or other liquids, and biochemical processes, for example the transfer of starch into simple sugar or heat and mass transfer and so on (Chládek, 1976; Hardwick, 1995; Kunze, 2010). Milling can be either dry or wet. In the agricultural and food industry, two-, four-, five- and six-roller mills, hammer mills, and different disintegrators (dispersion milling) are frequently used for dry milling. For wet milling, only two-roller mills are suitable. The experimental activities involving those these types of equipment are meant to determine electric energy consumption during disintegration (Chládek, 2007; Basařová et al., 2010; Vaculík et al., 2013). In prehistoric times, the barley and husked wheat (*Triticum dicoccum*) used for human food were dehusked by pounding the grain in mortars. The invention of rotary grain mills, for grinding ordinary bread wheats (*T. aestivum*), is attributed to the Romans in the second century B.C. Thereafter, until the development of the rollermill in the mid-nineteenth century, wheat was ground by stone-milling. Today, even in industrial countries, some stone milling is carried out to meet special demands. A stone mill consists of two discs of hard, abrasive stone, approximately 1.20 m in diameter, arranged on a vertical axis. The types of stone used include French burr from La Ferté-sous-Jouarre, Seine-et-Marte, millstone

grit from Derbyshire, German lava, Baltic flint from Denmark, and an artificial stone containing emery obtained from the island of Paxos in Greece. The opposing surfaces of the two stones, which are in close contact, are patterned with a series of grooves leading from the centre to the periphery. During operation, one stone is stationary while the other rotates. Either the upper stone ('upper stone'), or the lower stone ('under runner') may rotate, but it is usually the former. Grain fed into the centre ('eye') of the upper stone is fragmented between the two stones, and the ground products are issued at the periphery ('skirt').

The rollers used in wheat flour milling are usually 250 mm in diameter and either 800 mm, 1,000 mm or 1,500 mm long. The feed is distributed evenly over the length of the rolls by a pair of feed rollers which also control the loading. The succession of grinding stages is grouped into three systems: the *Break* system removes the endosperm from the bran in fairly large pieces, producing as little bran powder as possible. The *Scratch* system removes any small pieces of bran and embryo sticking to the endosperm. A sizing system may be used instead of the scratch system. The *Reduction* system grinds the endosperm into flour, at the same time flattening the remaining bran and embryo particles, and enabling them to be separated (Kent & Evers, 1994).

For the assessment of machines used for production of hard feed and food, quality of final product is a very important parameter. For malt milling used for brewing of traditional beer of Czech type (Pilsner beer Czech origin), it is important to measure dispersity (granulometry) of obtained malt grist. The variations in design and principle of disintegration equipment (roll, hammer and dispersion mills) variations in design and principle an impact on size particles distribution and, of course, on quality of final product. For the optimisation of the milling process (e.g. reduction of electric energy), it is the assessment of average particle size which is very important.

MATERIAL AND METHODS

For the measurement, the following cereals were used:

- barley (variety Bojos, harvest 2017, humidity 10 to 12%),
- malt (variety Sladek, harvest 2017, humidity 3 to 5%),
- wheat (variety Bohemia, harvest 2017, humidity 10 to 12%),
- rye (variety Selgo, harvest 2017, humidity 10 to 12%),
- oat (variety Korok, harvest 2017, humidity 10 to 12%),
- triticale (variety Kolor, harvest 2017, humidity 10 to 12%),
- all cereals were selected on sieve $\geq$ 2.5 mm.

Preparation of grist

Cereal samples (weight 1 kg) were crushed using following equipment:

- two roller mill VKM 130/150 (Czech Republic) with gap adjustments of this mill in the range 0.3, 0.4, 0.5 mm. The capacity of the mill is 200 kg h^{-1} (Figs 1 and 2),
- the hammer mill type Taurus (Fig. 3), supplied by the company TAURUS LTD Chrudim (Czech Republic) was utilized for the milling of cereals.

Material was milled by means of eight hammers as well as by friction between a sieve (size 3.0, 4.0 and 5.0 mm) and milled materials. The capacity of the mill is 200 kg h^{-1} (Fig. 3). Every experiment was repeated 10 times. The average value and standard deviation was calculated and the results are listed in Tables 1 and 2.

Figure 1. Two roller mill VKM 130/150.

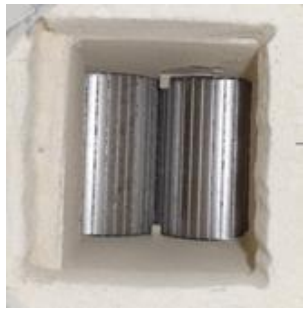

Figure 2. Two rollers (view from below).

Figure 3. Hammer mill Taurus.

Sieve analysis of grist

For the determination of crushed malt dispersity, a test sieve shaker (sieve analyzer) HAVER EML 200 digital plus T (Germany) (Fig. 4). The experiment used a test sieve shaker 2.50, 2.00, 1.60, 1.40, 1.25, 1.00, 0.80, 0.63, 0.50, 0.40, 0.315, 0.25, 0.20, 0.16, 0.125, 0.09 and 0.063 mm (17 pcs) including the bottom. For the measurement of oat grist, 19 sieves were used (because of extremely big oat particles sieves of 3.15 mm and 4.0 mm were added). For the weighing of malt and other cereals samples (1,000 g) and fractions from test sieves, a digital laboratory scale KERN PEJ 2200 – 2M (Germany) (Fig. 5), weighing range 0 to 2,200 g, weighing accuracy +/- 0.01 g was used.

Figure 4. The test sieve shaker (sieve analyzation).

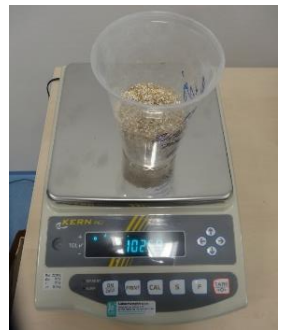

Figure 5. Digital laboratory scale KERN PEJ HAVER EML 200 digital plus T 2 200 – 2M.

The method of evaluation of the test sieving data

This method is described by Maloun (2001). The data obtained from the sieve analysis is relatively difficult to evaluate in a reproducible way. Therefore, a graphical interpretation of the data is often used as it helps in more easily imagining the analytical form of the function (which describes the granulometric composition of the sample).

From the analytical form of the function, it is possible to obtain the essential characteristics of the bulk materials. There are important characteristics such as, 'the co-efficient of polydispersity' and the mean statistical size of the particle $\bar{x}$ which determines the precision of milling.

This non-symmetry does not allow for the standard distribution. RRSB distribution – an exponential relation by Rosin, Rammler and Sperling – was invented for the fine grained materials. According to Bennett modification of this relation, it is possible to express the proportional evaluation of the relative residue on the sieve as (Maloun, 2001):

$$R = 100 \exp\left[-\left(\frac{x}{\bar{x}}\right)^n\right] [\%] \tag{1}$$

where R – the relative residue on the sieve (%); x – the dimension of separate particle (mm); $\bar{x}$ – is the main statistical size of the particle (mm); n – the material constant.

Because the shape of the curve is not suitable for graphical expression, the distribution function can be linearized:

$$\frac{100}{R} = \exp\left[\left(\frac{x}{\bar{x}}\right)^n\right] [\%] \tag{2}$$

and yields the relation:

$$\log\left[\log\frac{100}{R}\right] = n \cdot \log x + C \tag{3}$$

where $C = \log(\log e) - n \cdot \log \bar{x}$

which in turn gives Y and X values:

$$Y = \log\left[\log\frac{100}{R}\right];\ X = \log x \tag{4}$$

The $\bar{x}$ value is defined by the inflexion point of the distribution function curve, which is given by the particular value of the cumulative relative residue. This is possible to obtain from the equation:

$$f''(R) = 0$$

that means from:

$$\left\{y = \log\left[\log\frac{100}{R}\right]\right\}'' = 0 \tag{5}$$

If the calculated second derivative is set equal to zero, the value of the relative residue corresponding to the point of inflexion is obtained:

$$R = \frac{100}{e} = 36.79\ [\%] \tag{6}$$

For the calculated value there is given $x = \bar{x}$. It is possible to locate the $\bar{x}$ value from the diagram (Maloun, 2001; Chládek et al., 2013; Smejtková et al., 2016).

On the base of results obtained from different cereals, diagrams were constructed in which the axis x represents the sizes of sieve holes and the axis y is the mass of the fractions curves on the sieves (log log (100/R)).

The resulting area between the measured points and the x axis was measured and compared to the electricity input (log x); the obtained results are listed in tables 1 and 2 and discussed in the 'Results and discussion' section.

Determination of electric energy consumption during milling of different cereals

For exact determination of electric energy consumption during milling of different cereals, the three-phase power quality analyzer C.A 8332 device (Fig. 6), supplied by the French company Chauvin, was used. This compact device, shock resistant, with graphic representation, enables an instant image of network´s principal characteristics to be obtained and of their variation over a period of time to be monitored.

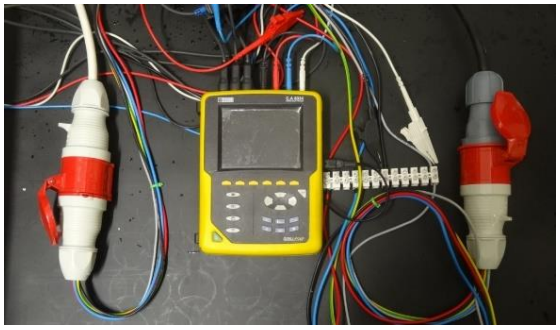

Figure 6. Three-phase power quality analyzer C.A 8332 for electric energy consumption measurement.

The multi-task measurement system simultaneously handles all the measurement functions of various magnitudes, detection, and continuous recording without any constraints. The device is designed for the following activities: measurements of AC rms voltages up to 480 V (phase to neutral) or 830 V (phase to phase); measurements of AC up to 3,000 A. Measurements of the frequency 50 and 60 Hz and other parameters.

Using the above mentioned RRSB theory, the middle particle size and area between the curve and axle x both parameters were compared, and good agreement was observed.

RESULTS AND DISCUSSION

Results of sieve analysis of different cereal grist were evaluated and expressed in following ways:

- as a size of surface area limited by the distribution curve and axle x (Fig. 7),
- as a statistical average size of the particle and standard deviation.

Those figures, depending on gap /sieve) size, type of cereals and measured electricity inputs are listed in Tables 1 and 2.

The size of this area corresponds to the average size of particle. For evaluation, this parameter was divided by initial size of particle 2.5 mm. At the start of measurement, the electricity consumption of roller and hammer mills during idling was determined and the measured value was calculated.

For cereals (the weight of every sample was 1.0 kg, size $\geq$ 2.5 mm, measured 10 times), durations of crushing of every sample were in the range 16 to 18 seconds.

Durations of sieve analysis of every sample were in the range 120 to 200 minutes. The calculated average size of grist particle and standard deviation were obtained from these results. All experimental data are shown in Tables 1 and 2.

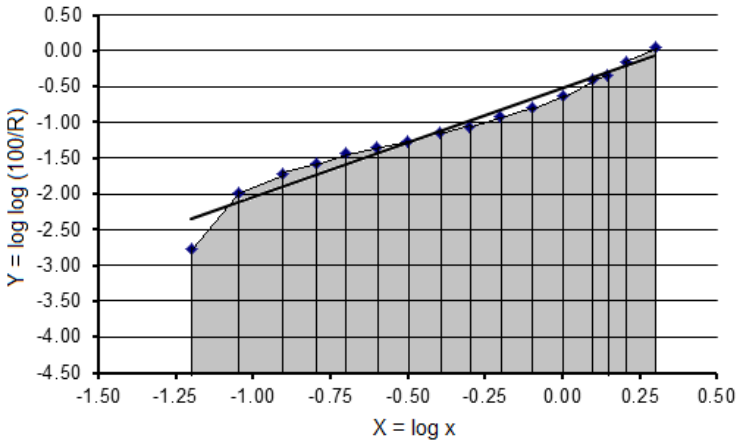

Figure 7. The determination example of the area size limited by the distribution curve and x-axis for malt grist.

Table 1. Two-roller mill - the dependence of power on the middle size of grist particles and different gap size

No	Cereals	Gap	Area beneath curve	Average size of particle	Standard deviation	Initial dimension/ standard deviation	Input	Input/ middle size
		mm	mm^2	mm	mm	mm	kW	kW mm^{-1}
Two-roller mill – gap 0.3 mm								
1.0	Idling	0.3	-	-	-	-	0.52	-
1.1	Barley grist	0.3	9,965	1.09	0.18	2.29	1.26	1.15
1.2	Malt grist	0.3	9,442	0.89	0.25	2.81	0.81	0.91
1.3	Wheat grist	0.3	8,362	1.56	0.27	1.60	1.36	0.87
1.4	Rye grist	0.3	10,220	1.47	0.14	1.70	1.72	1.17
1.5	Oat grist	0.3	6,002	1.96	0.21	1.28	0.92	0.46
1.6	Triticale grist	0.3	8,149	1.53	0.19	1.63	1.21	0.79
Two-roller mill – gap 0.4 mm								
2.0	Idling	0.4	-	-	-	-	0.52	-
2.1	Barley grist	0.4	9,572	1.29	0.15	1.94	1.20	0.93
2.2	Malt grist	0.4	9,085	1.01	0.19	2.48	0.74	0.73
2.3	Wheat grist	0.4	8,029	1.78	0.28	1.40	1.25	0.70
2.4	Rye grist	0.4	9,794	1.68	0.26	1.49	1.60	0.95
2.5	Oat grist	0.4	5,720	2.20	0.11	1.14	0.82	0.37
2.6	Triticale grist	0.4	7,947	1.62	0.21	1,54	0.97	0.59
Two-roller mill – gap 0.5 mm								
3.0	Idling	0.5	-	-	-	-	0.52	-
3.1	Barley grist	0.5	8,529	2.21	0.08	1.13	1.06	0.47
3.2	Malt grist	0.5	8,237	1.20	0.15	2.08	0.71	0.59
3.3	Wheat grist	0.5	7,005	2.12	0.11	1.18	1.16	0.54
3.4	Rye grist	0.5	8,739	2.08	0.30	1.20	1.47	0.70
3.5	Oat grist	0.5	5,198	2.15	0.19	0.86	0.71	0.26
3.6	Triticale grist	0.5	7,361	1.79	0.24	1.57	0.87	0.48

Table 2. Hammer mill - the dependence of power on the middle size of grist particles and different size of sieve

No	Cereals	Sieve	Area beneath curve	Average size of particle	Standard deviation	Initial dimension/ standard deviation	Input	Input/ middle size
		mm	mm^2	mm	mm	mm	kW	kW mm^{-1}
Hammer mill – sieve 3.0 mm								
4.0	Idling	3.0	-	-	-	-	0.76	-
4.1	Barley grist	3.0	9,178	0.93	0.16	2.69	2.90	3.12
4.2	Malt grist	3.0	11,421	0.59	0.11	4.24	1.80	3.05
4.3	Wheat grist	3.0	10,514	0.84	0.14	2.98	3.20	3.80
4.4	Rye grist	3.0	9,261	1.04	0.19	2.40	4.30	4.13
4.5	Oat grist	3.0	9,612	0.89	0.22	2.81	4.90	5.50
4.6	Triticale grist	3.0	9,815	0.88	0.25	2.85	3.70	4.20
Hammer mill – sieve 4.0 mm								
5.0	Idling	4.0	-	-	-	-	0.76	-
5.1	Barley grist	4.0	8,437	0.96	0.14	2.60	2.69	2.80
5.2	Malt grist	4.0	10,426	0.63	0.19	3.97	1.71	2.71
5.3	Wheat grist	4.0	9,763	0.93	0.24	2.69	2.98	3.20
5.4	Rye grist	4.0	8,259	1.07	0.24	2.34	4.06	3.74
5.5	Oat grist	4.0	8,487	1.05	0.28	2.38	4.58	4.36
5.6	Triticale grist	4.0	9,294	0.89	0.19	2.81	3.49	3.92
Hammer mill – sieve 5.0 mm								
6.0	Idling	5.0	-	-	-	-	0.76	-
6.1	Barley grist	5.0	8,155	1.02	0.11	2.45	2.55	2.50
6.2	Malt grist	5.0	10,095	0.68	0.21	3.68	1.68	2.47
6.3	Wheat grist	5.0	9,258	1.07	0.27	2.34	2.45	2.29
6.4	Rye grist	5.0	8,014	1.11	0.15	2.25	3.98	3.59
6.5	Oat grist	5.0	8,298	1.13	0.29	2.21	4.49	3.97
6.6	Triticale grist	5.0	8,928	0.91	0.16	2.75	3.38	3.71

The following figures (Figs 8, 9 and 10) illustrate the area, limited by curve and x-axis, average size of particle and electricity input depending on cereals, type of milling equipment, and gap and sieve size.

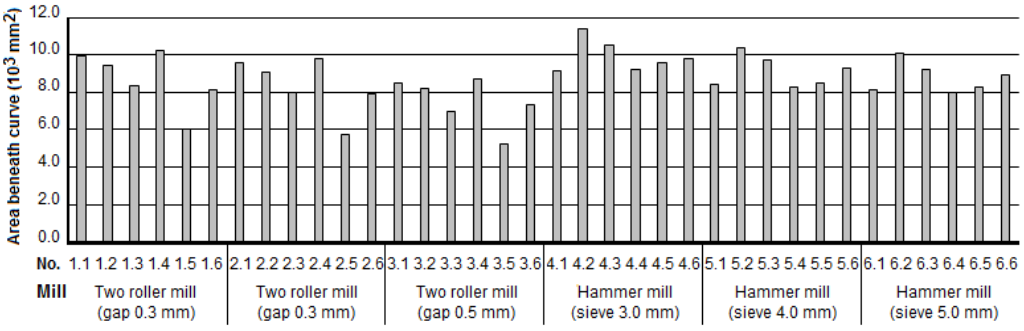

Figure 8. The results - the area limited by the distribution curve and x-axis.

From the analysis of achieved results, it is obvious that the optimal equipment with the lowest electric energy consumption was shown by the two-roller mill using a 0.3 mm gap by milling malt (0.81 kW) and the highest consumption by malt milling using hammer mill was 1.8 kW (sieve 3.0 mm). The highest electric energy consumption was determined to be in the case of oat milling using a hammer mill. (On the other hand should be considered the price of roll mill (appr. 3x higher as the hammer mill) were measured.

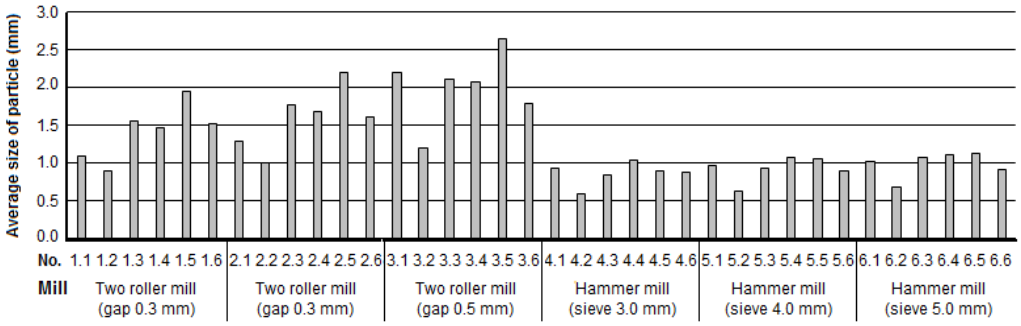

Figure 9. The results – the average size of particle for different cereals and gap (sieve) size.

Malt milling consumed the least energy, due to the very low water content (only 3 to 5% water) in malt kernels and the grain's very fragile structure. The manufacturers of malt mills supply their equipment with unnecessarily energy-consuming electric motors, when smaller motors could do the job well and more economically. The hammer mill has very low efficiency, roughly 3% to 5%, with high electricity consumption, causing financial losses.

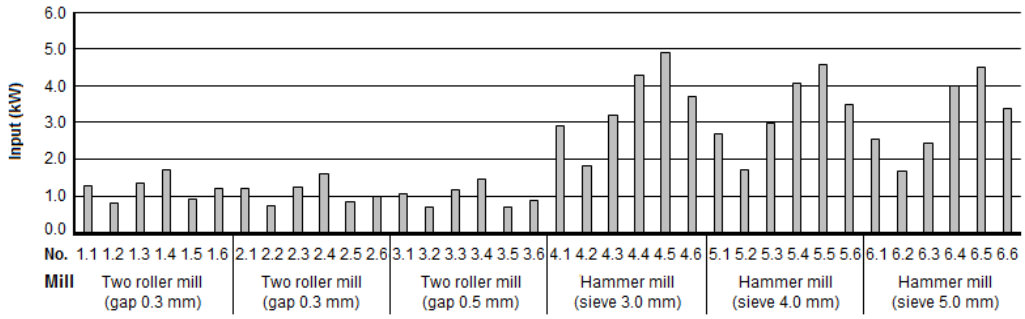

Figure 10. The results – the electrical input for different cereals and gap (sieve) size.

On figures 11 and 12 is shown the comparison of average particle size of malt grist, area beneath the RRSB (Rosin, Rammler, Sperling and Bennett, Eq. 1) distribution curve and x axis on measured input for malt and barley grist. The obtained result confirmed good coincidence with theory. Very similar results were obtained from evaluation of wheat, rye, oak and triticale measurement.

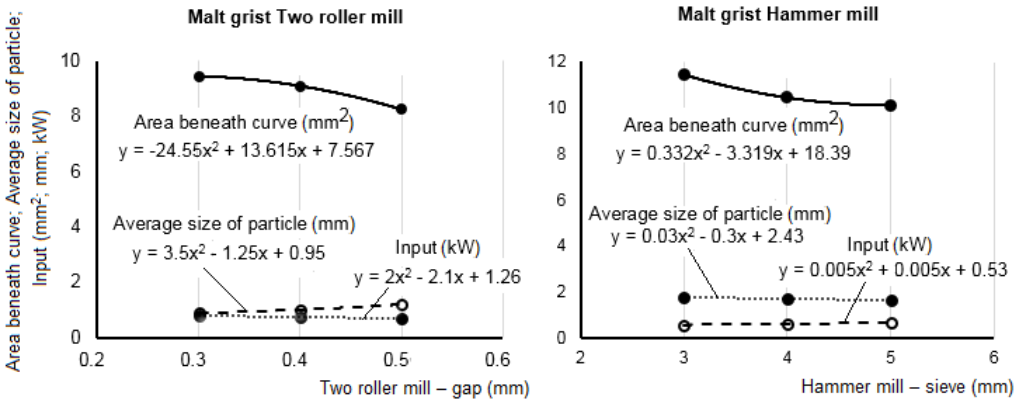

Figure 11. The comparison of impact of average size of grist and area beneath the curve on input for malt grist.

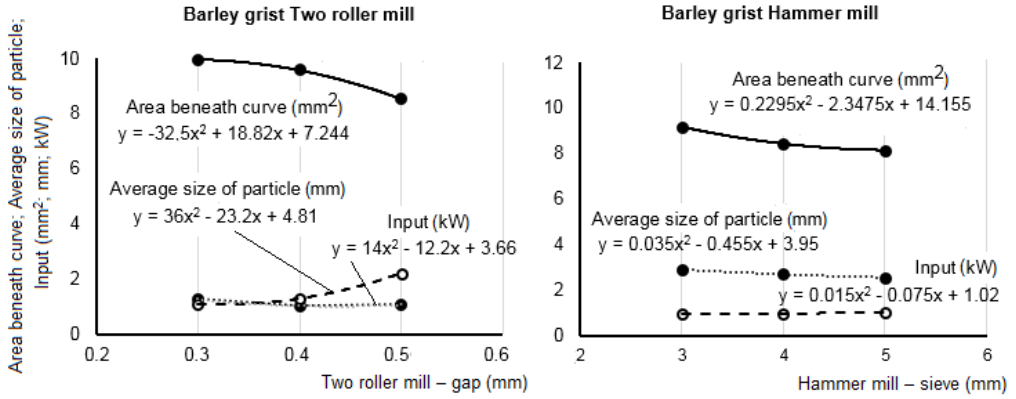

Figure 12. The comparison of impact of average size of grist and area limited by the curve and x-axis on electric input for barley grist.

CONCLUSIONS

All results obtained from sieve analysis and measurement of electricity input are in close agreement and can be used for optimization of electricity consumption during disintegration processes used for feed and food industry.

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REFERENCES

Hardwick, W.A. 1995. *Handbook of Brewing*. Marcel Dekker, Inc., New York. 714 pp.

Basařová, G., Šavel, J., Basař, P. & Lejsek, T. 2010. *Pivovarství: teorie a praxe výroby piva*. Vydavatelství VŠCHT, Prague, 863 pp. (in Czech).

Chládek, L. 1976. *Studium parametrů vyslazovacího procesu (The study of Lautering proces parameters)* (Doctor Theses). Czech Technical University in Prague, Prague, 176 pp. (in Czech).

Chládek, L. 2007. *Pivovarnictví (Breewing industry).* Grada, Prague, 207 pp. (in Czech).

Chládek, L., Vaculík, P., Přikryl, M., Vaculík, M. & Holomková, M. 2013. Impact of malt granulometry on lauter proces. *5th International Conference on Trends in Agricultural Engineering 2013, TAE 2013 03.09.2013, Prague*. Czech University of Life Sciences Prague, Prague, 244–248.

Kent, N.L. & Evers, A.D. 1994. *Kent´s technology of cereals: an introduction for students of food science and agriculture.* Pergamon, Elsevier Science Ltd., 334 pp.

Kunze, W. 2010. *Technology Brewing and Malting.* Versuchs- und Lehranstalt für Brauerei in Berlin (VLB), Berlin, 1047 pp.

Maloun, J. 2001. *Technologická zařízení a hlavní procesy při výrobě krmiv (Technological equipment and main processes in cattle feed production).* Czech University of Life Sciences Prague, Prague, 201 pp. (in Czech).

Smejtková, A., Vaculík, P., Přikryl, M. & Pastorek, Z. 2016. Rating of malt grist fineness with respect to the used grinding equipment. *Research in Agricultural Engineering (Zemědělská technika)* **62**(3), 141–146.

Vaculík, P., Maloun, J., Chládek, L. & Přikryl, M. 2013. Disintegration process in disc crushers. *Research in Agricultural Engineering (Zemědělská technika)* **59**(3), 98–104.

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https://doi.org/10.15159/AR.18.118

Study of potential PCR inhibitors in drinking water for *Escherichia coli* identification

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Abstract. In the last few decades, the polymerase chain reaction (PCR) has become one of the most powerful molecular biological tools. However, the PCR is an enzymatic reaction and therefore sensitive to inhibitors which may occur in drinking water samples. In this work, the possible inhibition effect of chlorine, humic acids, and iron for real-time PCR (qPCR) efficiency was studied and the environmental sample from drinking water treatment system before iron removal was selected and analysed. The results demonstrated that the highest concentrations of humic acids (5 mg L^{-1} and 1 mg L^{-1}) and iron (4 mg L^{-1}) inhibited the PCR reaction while no effect of chlorine was observed. The analysis of the environmental sample with spiked *Escherichia coli* cells demonstrated reduction efficiency of the average threshold cycle (C_t) values compared with control dilution series determining the possible inhibition for qPCR assay.

Key words: Polymerase chain reaction, inhibitors, chlorine, humic acids, iron, *Escherichia coli.*

INTRODUCTION

While most microorganisms play an important role in nature, certain potentially harmful bacteria can contaminate food and water, and cause infectious diseases in both humans and animals (Leonard et al., 2003). Depending on the type of microorganism, conventional identification can take anything from a day to several weeks (Rossen et al., 1992). Therefore, the limitations of culture-based assays (e.g., inability to detect unculturable cells, time consumption) in analysis of microorganisms from oligotrophic environments, such as drinking water, have facilitated the introduction of molecular methods in routine tests for more relevant, rapid and 'real-time' identification (Kim et al., 2013; Fatemeh et al., 2014).

Quantitative real-time PCR (qPCR) has been recommended as a powerful alternative diagnostic tool due to its high sensitivity and specificity (Schriewer et al., 2011). Nevertheless, environmental samples may contain a high concentration of organic and inorganic substances like phenolic compounds, heavy metals and humic acids which may inhibit enzymatic reactions leading to the production of biased results and impact the sensitivity of both conventional and quantitative PCR (Green & Field, 2012; Schrader, 2012; Sidstedt et al., 2015). For instance, PCR inhibition for *E. coli* identification from water samples has been linked with the presence of inhibiotory substances (Walker et al., 2017).

Typical concentrations of dissolved iron in groundwater that is the primary source of drinking water in Nordic countries range from 0.5 to 10 mg L^{-1} (Klove et al., 2016) while other sources, mainly surface water, can be rich in humic substances (Juhna & Klavins, 2001). A major component of naturally occurring organic carbon in water is humic acid, as it can represent up to 90% of dissolved organic carbon in water (Sounthararajah et al., 2015). For instance, Tihomirova et al. (2010) have demonstrated that often the amount of organics directly after water treatment can exceed 5 mg L^{-1}. Finally, the disinfection of drinking water often involves the use of reactive chemical agents such as chlorine. For effective disinfection, the average concentration of free chlorine in the final treatment step might vary from 0.2 to 1 mg L^{-1} (World Health Organization, 2011). Thus, the application of molecular tests, e.g., PCR, in environmental sample, e.g., source waters, and drinking water analyses must be performed with caution to exclude the possibility of inhibition.

Traditionally, to determine the inhibitory effect of the PCR reaction, it has been suggested to carry out the control reactions (Schrader et al., 2012). For instance, serial dilution with variable concentration of possible inhibitors in analysed control samples might provide an excellent mean to determine inhibiting effects when a gene target is present in high copy numbers (Kontanis & Reed, 2006; Schriewer et al., 2011). Therefore, inhibition of qPCR can be measured as the increase in threshold cycle (C_t) relative to an uninhibited control (Huggett et al., 2008). Moreover, the analysis of the PCR product is possible through a measurement of the melt characteristics of the amplicons where the change in the melt curve demonstrates the modification of the PCR product (Opel et al., 2009).

The aim of this study was to estimate if qPCR could be applied for the analysis of drinking water, which is rich in organic and inorganic compounds. The possible inhibition of chlorine, iron and humic acids was examined and evaluated in artificial and water samples. *Escherichia coli* was selected as a test organism because it is the most widely used faecal indicatororganism in microbiology analyses. The research included tests with real-time PCR assay and the results of the threshold cycle (C_t) values were compared in-between the standard curves constructed for *E. coli.*

MATERIALS AND METHODS

Bacterial strain and DNA extraction

Escherichia coli strain (ATCC®25922™) was grown aerobically at 37 °C for 24 h in Tryptone soya broth (Oxoid, UK). The commercial kit GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania) was used according to manufacturer's instructions for DNA extraction and preparation of control dilution series and water sample analyses.

Sample preparation

Inhibitor stock solutions were prepared and serially diluted to obtain final concentrations of 4, 0.8, 0.08 mg L^{-1} of chlorine (from NaClO, Sigma-Aldrich, Germany); 5, 1, 0.3 mg L^{-1} of humic acid (Sigma-Aldrich, Germany), and 4, 0.2, 0.1 mg L^{-1} of iron ($FeCO_3$, Sigma-Aldrich, Germany). All subsequent dilutions were prepared in water.

The water sample of 1 L was collected from a drinking water treatment plant (Dobele, Latvia) during iron removal process (iron concentration > 0.5 mg L^{-1}) in sterile glass bottle, stored at +4 °C and transported to the testing laboratory for further use. For the investigation of possible PCR inhibition from the water sample, a positive control (dilution series of *E. coli*), and negative control (water sample without *E. coli*) was prepared and compared in-between the water sample with spiked *E. coli* with the same DNA concentration as control dilution series. The water sample was analysed with real-time PCR (see below) and tested in triplicates.

Real-time PCR analysis of inhibitors

The amplification was performed in 7300 Real Time PCR System (Applied Biosystems, USA). PCR reaction mixture of 25 µl contained 5 µl template DNA, 12.5 µl SYBR Green/ROX qPCR Master Mix (2x) (Thermo Scientific, Lithuania), 0.5 µl of primer pair EC_5 (5'-AAAGCCGTGGCACAGGCAAGCGT-3') and $EC8c_2$ (5'-TCAATTTGTTATCGCTATCCAGTTGG-3') (Spierings, 1993) and the required amount of PCR-nuclease free water (Thermo Scientific, Lithuania). To enhance the effect of the chlorine, humic acids and iron, the required concentration of inhibitor was added at the end to reach a final reaction volume of 25 µl. Control (without inhibitors) dilution series was performed using the same protocol, with an equivalent volume of PCR water used in place of the inhibitor.

In order to evaluate and compare the success and effectiveness of PCR inhibition, MS Excel 2013 was used for C_t average value and *t*-test statistical calculations. Each sample was tested in triplicates.

RESULTS AND DISCUSSION

Real-time PCR is becoming the method of choice for the detection of pathogenic microorganisms and other target organisms in the environmental samples (Schriewer et al., 2011). Although qPCR can provide more specific and accurate quantification than other molecular techniques, it does have limitations that must be considered when applying it in practice (Kim et al., 2013). Previous research has already demonstrated that water sample analyses with real-time PCR might be inhibited by organic compounds like polyphenol, fulmic and humic acids, metal ions and chlorine (Schrader et al., 2012). However, besides the substance class, there has not been much research on the relationship between the inhibitor concentration and effect caused to the qPCR efficiency. Therefore, within this study, the inhibition of qPCR was investigated and compared with uninhibited control as the increase in the threshold cycle (C_t), which represents the number of cycles required for the fluorescent signal to cross the threshold of background level.

Chlorine effect on qPCR

The treatment of drinking water typically involves a final disinfection process to prevent the discharge of pathogens. For instance, in Latvia, the drinking water treatment process is based on chlorination and the average concentration of free chlorine in the final treatment step might vary from 0.5 to 3 mg L^{-1} (Nescerecka et al., 2014). Based on these observations, three different concentrations (4, 0.8 and 0.08 mg L^{-1}) were investigated in this study.

The results showed that there was no significant difference ($P > 0.05$) in the average crossing threshold (C_t) values of the inhibitors when compared to the control dilution series (Table 1).

Table 1. The comparison of different chlorine concentrations based on the average crossing threshold (C_t) values

Dilution series	C_t			
	Control	4 mg L^{-1}	0.8 mg L^{-1}	0.08 mg L^{-1}
10^1	12.61 (± 0.13)	12.39 (± 0.03)	12.50 (± 0.22)	12.32 (± 0.20)
10^2	16.11 (± 0.07)	16.05 (± 0.12)	15.83 (± 0.10)	15.89 (± 0.06)
10^3	19.57 (± 0.06)	19.53 (± 0.22)	19.46 (± 0.11)	19.59 (± 0.16)
10^4	23.19 (± 0.06)	23.82 (± 1.35)	23.03 (± 0.05)	23.05 (± 0.04)
10^5	nd	26.69 (± 0.14)	nd	nd

nd – C_t was not detected; (±) standard deviation of the average values from 3 replicates.

Previous research (Aken & Lin, 2011) showed that high concentrations of the disinfecting agent, e.g., chlorine, UV, and silver, significantly inhibited the amplification of DNA for *E. coli* bacteria. Furthermore, Delgado-Viscogliosi et al. (2009) demonstrated that the doses of tested chlorine concentration above 0.5 mg L^{-1} are able to bind to DNA molecules, resulting in the inhibition of qPCR. However, here the chlorine did not demonstrate any effect on PCR efficiency. This could be linked to the fact that the incubation time was too short to destruct the DNA molecule and amplification process. Nevertheless, the sensitivity of the qPCR method can depend on the volume of analysed water affecting the final concentration of free chlorine. For instance, the cell number of the target microorganism in drinking water may be small and DNA could be detected by PCR amplification only after concentration of large volumes of drinking water. Thus, the internal controls could be used in order to avoid false-negative results due to chlorine inhibitory effect.

Humic acid effect on real-time PCR

Humic substances are the most commonly reported group of PCR inhibitors in the environmental samples (Filion, 2012). Tihomirova et al. (2010) have indicated that even after drinking water treatment the concentration of humic substances can exceed 5 mg L^{-1}, therefore, great suspicion on possible inhibition of PCR reaction arise. According to this, three different concentrations, 5, 1 and 0.3 mg L^{-1}, of humic acid were evaluated.

The inhibition by humic acids was observed at two concentrations – 5 mg L^{-1} and 1 mg L^{-1} ($P < 0.05$). The efficiency of the average crossing threshold (C_t) values for each dilution series decreased when compared to control, 65% and 16%, respectively. At the same time, no significant change was observed at 0.3 mg L^{-1} humic acid ($P > 0.05$) (Table 2).

Table 2. The comparison of different humic acid concentrations based on the average crossing threshold (C_t) values

Dilution series	C_t			
	Control	5 mg L^{-1}	1 mg L^{-1}	0.3 mg L^{-1}
10^1	12.22 (± 0.03)	19.66 (± 0.07)	14.29 (± 0.43)	12.36 (± 0.14)
10^2	15.68 (± 0.02)	23.16 (± 0.43)	17.45 (± 0.05)	15.90 (± 0.11)
10^3	19.68 (± 0.14)	27.60 (± 0.38)	21.41 (± 0.05)	19.90 (± 0.19)
10^4	23.44 (± 0.02)	31.68 (± 0.28)	25.32 (± 0.10)	23.60 (± 0.02)
10^5	27.24 (± 0.09)	36.33 (± 0.57)	29.41 (± 0.24)	27.53 (± 0.06)

nd – C_t was not detected; (±) standard deviation of the average values from 3 replicates.

Additionally, the same results of PCR inhibition were presented at amplification plot curves with 5 and 1 mg L^{-1} concentrations of humic acids. All inhibitor dilution series did not coincide with non-ihibitory amplification plots (Fig. 1) and demonstrated the influence of humic acids on the PCR efficacy.

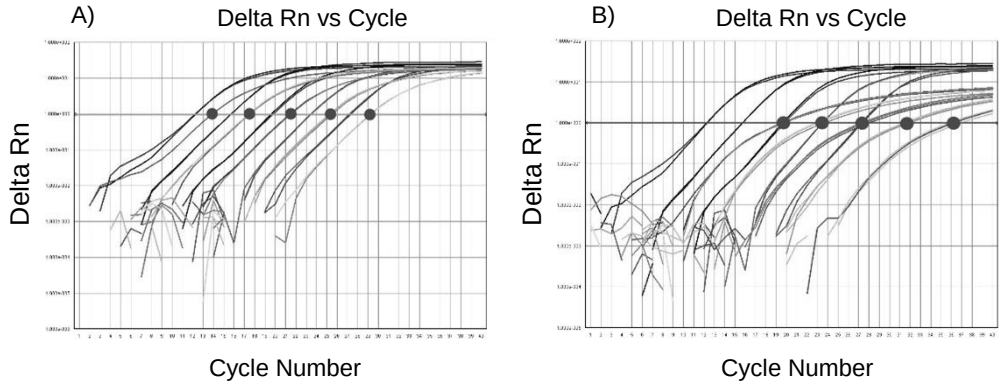

Figure 1. Investigation of humic acid inhibitory properties in real-time PCR amplification with *E. coli* standard curves. **A)** Amplification plot curves from 1 mg L^{-1} chlorine dilution series (red marks at threshold (C_t) point) when compared with control dilution series; **B)** Amplification plot curves from 5 mg L^{-1} chlorine dilution series (marked as **x** at threshold (C_t) point) when compared with control dilution series.

Sidstedt et al. (2015) demonstrated that humic acids from environmental samples are very potent inhibitors that can quench the fluorescence signal of double-stranded DNA binding dyes, including SYBR Green. Furthermore, humic acids have been found to directly disturb the DNA polymerase and form colloids in water and complexes with iron ions, meaning that they could affect the ion content in PCR, for example, by chelating magnesium ions (Sidstedt et al., 2015). As humic substances are amorphous, dark-colored organic compounds, which are relatively resistant to chemical and biological degradation, it is difficult to remove using standard DNA purification procedures (Watson & Blackwell, 2000). Therefore, the most common method used to overcome PCR inhibition by humic substances is to dilute the extract. By diluting the extract the concentration of the inhibitory compounds is reduced to a level where inhibition no longer occurs (Matheson et al., 2010). The results in this paper also indicated that by decreasing the concentration of humic acids from 5 mg L^{-1} to

0.3 mg L^{-1}, increased the efficiency of qPCR. However, dilution of the sample also reduced the concentration of DNA, which can decrease the efficiency of PCR amplification (Filion, 2012). Therefore, Schriewer et al. (2011) have demonstrated absorbent DAX-8 technology to remove humic acids permanently from nucleic acid extracts. This method has a potential to significantly increase the reliability of reported non-detects and measured results obtained by qPCR in environmental monitoring.

Iron effect on real-time PCR

Another group of inhibitors include heavy metals. Generally, the mechanisms of PCR inhibition by heavy metals are still not very well understood. However, one possible explanation could be the variable resistance of DNA polymerases to heavy metal ions (Filion, 2012). The inhibition is attributed to various metals, like mercury, zinc, lead, and also iron (Filion, 2012; Chowdhury et al., 2016) that is often found in water sources. Groundwater can contain > 3 mg L^{-1} iron and not always it is removed sufficiently during the water treatment process. According to the World Health Organization, the concentrations of iron in drinking water is normally below 0.3 mg L^{-1} but may be higher in places where various iron salts are used as coagulating agents in drinking water treatment plants and where iron pipes are used for water distribution (World Health Organization, 2008). The results of the C_t data from 4 mg L^{-1} samples showed a 100% reduction of qPCR efficiency when compared to the control indicating no DNA amplification. At the same time no significant change was observed for 0.2 and 0.1 mg L^{-1} samples ($P > 0.05$) (Table 3).

Table 3. Comparison of different iron concentrations based on the average crossing threshold (C_t) values

Dilution series	C_t			
	Control	4 mg L^{-1}	0.2 mg L^{-1}	0.1 mg L^{-1}
10^1	12.07 (± 0.07)	nd	12.09 (± 0.19)	11.97 (± 0.09)
10^3	19.97 (± 0.14)	nd	20.10 (± 0.01)	20.06 (± 0.03)
10^5	28.17 (± 0.12)	nd	28.30 (± 0.12)	28.34 (± 0.38)

nd – C_t was not detected; (±) standard deviation of the average values from 3 replicates.

The results of this study apparently demonstrated that elevated concentration of iron above 4 mg L^{-1} inhibited the PCR reaction, therefore when analysing the groundwater samples directly, first the inhibition should be tested.

The water sample from drinking water treatment plant was taken in the middle of iron treatment step with iron concentration > 5 mg L^{-1} and analysed for possible PCR inhibition. The results demonstrated no detectable *E. coli*. Meanwhile, the significant difference in the average crossing threshold values in-between *E. coli* control dilution series and the water sample with the same concentration of spiked *E. coli* DNA was observed (Table 4). The results of the water sample with *E. coli* DNA indicated on higher C_t values (14%) when compared to control determining the possible inhibition of uncovered environmental compounds, e.g., iron from DNA extraction.

Table 4. The comparison of control *E. coli* dilution series with the water sample with/without *E. coli* based on the average crossing threshold (C_t) values

		C_t	
Dilution series	Control	Environmental sample with *E. coli*	Environmental sample without *E. coli*
10^7	12.97 (± 0.09)	14.64 (± 0.06)	nd
10^6	16.57 (± 0.12)	18.41 (± 0.03)	nd
10^5	20.35 (± 0.08)	22.24 (± 0.14)	nd

nd – C_t was not detected; (±) standard deviation of the average values from 3 replicates.

According to these investigations, real-time PCR assays must be carefully designed, conducted, and validated with an understanding of the methods limitations and possible inhibitors. Several approaches have attempted to reduce the inhibitory impact of sample quantification with qPCR. For instance, nucleic acid extraction methods that increase DNA yield while removing most qPCR inhibitors have been developed specifically for troublesome water samples (Green & Field, 2012). However, it cannot be guaranteed that the preparations will extract DNA free of PCR inhibitors. Therefore, further analysis of environmental samples should include a pretreatment step before/after the DNA extraction to remove possible inhibitory compounds, e.g., iron, and all reactions should be analysed for the presence of inhibitory effects.

CONCLUSIONS

Three possible inhibitors were examined for the efficiency of drinking water sample analyses with qPCR. The results indicated that humic acids at concentration of 5 and 1 mg L^{-1} reduced the PCR reaction efficiency for 65% and 16%, respectively, while concentration of 4 mg L^{-1} from iron fully inhibited amplification of DNA. The analysis of the environmental sample with spiked *E. coli* cells demonstrated the reduction efficiency of the average C_t values for 14% compared with the control dilution series determining the possible inhibition for qPCR assay. The findings of the research demonstrate that prior PCR analyses, the evaluation of potential inhibitors must be performed.

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REFERENCES

Aken, B.V. & Lin, L.S. 2011. Effect of the disinfection agents chlorine, UV irradiation, silver ions, and TiO2 nanoparticles/near-UV on DNA molecules. *Water Science & Technology* **64**, 1226–1230.

Chowdhury, S., Mazumder, J.A.M., Al-Attas, O., Husain, T. 2016. Heavy metals in drinking water: Occurences, implications, and future needs in developing countries. *Science in the Total Environments* **569**, 476–488.

Delgado-Viscogliosi, P., Solignac, L. & Delattre, J.M. 2009. Viability PCR, a culture – independent method of rapid and selective quantification of viable Legionella pneumophila cells in environmental water samples. *Applied and Environmental Microbiology* **75**, 3502–3512.

Fatemeh, D., Reza, Z.M., Mohammad, A., Solemeh, K., Reza, A.G., Hossein, S., Maryam, S., Azam, A., Mana, S., Negin, N., Reza, K.A. & Saeed, F. 2014. Rapid detection of coliforms in drinking water of Arak city using multiplex PCR method in comparison with the standard method of culture (Most probably number). *Asian Pacific Journal of Tropical Biomedicine* **4**, 404–409.

Filion, M. 2012. Quantitative real-time PCR in applied microbiology. *Caister Academic Press*, 93–94.

Green, H.C. & Field, K.G. 2012. Sensitive detection of sample interference in environmental qPCR. *Water Research* **46**, 3251–3260.

Huggett, J.F., Novak, T., Garson, J.A., Green, C., Morris-Jones, S.D., Miller, R.F. & Zumla, A. 2008. Differential susceptibility of PCR reactions to inhibitors: an important and unrecognised phenomenon. *BMC Research Notes* **1**, 1–9.

Kim, J., Lim, J. & Lee, C. 2013. Quantitative real-time PCR approaches for microbial community studies in wastewater treatment systems: Applications and considerations. *Biotechnology Advances* **31**, 1358–1373.

Juhna, T. & Klavins, M. 2001. Water-quality changes in Latvia and Riga 1980-2000: Possibilities and problems. *Royal Swedish Academy of Science* **30**, 306–314.

Klove, B., Kvitsand, H.M.L., Pitkanen, T., Gunnarsdottir, M.J., Gaut, S., Gardarsson, M.J., Rossi, P.M. & Miettinen, I. 2016. Overview of groundwater sources and water – supply systems, and associated microbial pollution, in Finnland, Norway and Iceland. *Hydrogeology and Human Health* **25**, 1033–1044.

Kontanis, E.J. & Reed, F.A. 2006. Evaluation of real-time PCR amplification efficiencies to detect PCR inhibitors. *American Academy of Forensic Science* **51**, 795–804.

Leonard, P., Hearty, S., Brennan, J., Dunne, L., Quinn, J., Chakraborty, T. & O'Kennedy, R. 2003. Advances in biosensors for detection of pathogens in food and water. *Enzyme and Microbial Technology* **32**, 3–13.

Matheson, C.D., Gurney, C., Esau, N. & Lehto, R. 2010. Assessing PCR inhibition from humic substances. *The Open Enzyme Inhibition Journal* **3**, 38–45.

Nescerecka, A., Rubulis, J., Vital, M., Juhna, T. & Hammes, F. 2014. Biological instability in chlorinated drinking water distribution network. *Plus One* **9**, 1–11.

Opel, K.L., Chung, D. & McCord, B.M. 2009. A Study of PCR inhibition mechanisms using realtime PCR. *Journal of Forensic Science* **10**, 1–8.

Rossen, L., Norskov, P., Holmstrom, K. & Rasmussen, O.F. 1992. Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. *International Journal of Food Microbiology* **17**, 37–45.

Schrader, C., Schielke, A., Ellerbroek, L. & Johne, R. 2012. PCR inhibitors – occurrence, properties and removal. *Journal of Applied Microbiology* **113**, 1014–1026.

Schriewer, A., Wehlmann, A. & Wuertz, S. 2011. Improving qPCR efficiency in environmental samples by selective removal of humic acids with DAX-8. *Journal of Microbiological Methods* **85**, 16–21.

Sidstedt, M., Jansson, L., Nilsson, E., Noppa, L., Forsman, M., Radstrom, P. & Hedman, J. 2015. Humic substances cause fluorescence inhibition in real-time polymerase chain reaction. *Analytical Biochemistry* **487**, 30–37.

Sounthararajah, D.P., Loganathan, P., Kandasamy, J. & Vigneswaran, S. 2015. Effects of humic acid and suspended solids on the removal of heavy metals from water by adsorption onto granular activated carbon. *International Journal of Environmental Research and Public health* **12**, 10475–10489.

Spierings, G., Ockhuijsen, C., Hofstra, H. & Tommassen, J. 1993. Polymerase chain reaction for the specific detection of Escherichia coli/Shigella. *Res. Microbiology* **144**, 557–564.

Tihomirova, K., Rubulis, J. & Juhna, T. 2010. Changes of NOM fractions during conventional drinking water treatment process in Riga, Latvia. *Water Science and Technology: Water Supply* **10**, 157–163.

Walker, D.J., McQuillan, J., Taiwo, M., Parks, R., Stenton, C., Morgan, H., Mowlem, M.C. & Lees, N.D. 2017. A highly specific Escherichia coli qPCR and it comparison with existing methods for environmental waters. *Water Research* **126**, 101–110.

Watson, R.J. & Blackwell, B. 2000. Purification and characterization of a common soil component which inhibits the polymerase chain reaction. *Can. J. Microbiology* **46**, 633–642.

World Health Organization 2008. *Iron in drinking water*. Guidelines for drinking-water quality. WHO/SDE/WSH/03.04.08, pp. 1–2 (in English).

World Health Organization 2011. *Guidelines for drinking-water quality* – 4th edition 141, pp. 333–334 (in English).

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The development of gluten-free sourdough bread technology with rowan powder

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Abstract. A new form of technology was developed which focussed on gluten-free bread with gluten-free sourdough and rowan powder (from the botanical species *Sorbus aucuparia*). This new form of technology allows organoleptic characteristics to be improved, along with structure, texture, microbial spoilage resistance, and the shelf life of gluten-free bread. The gluten-free dry microbial composition with lactic acid bacteria was developed as a starter for sourdough. The lactic acid bacteria, *L. brevis E38*, was experimentally selected for dry microbial composition on the basis of its antagonistic activity against ropy bread disease pathogens (*B. subtilis* and *B. licheniformis*). The dependence was revealed of the accumulation of acetic acid and lactic acid in the sourdough on the microbial composition during fermentation. A gluten-free sourdough technology was developed which involved a new starter, rice, and soy flour at a ratio of 0.2:2:1. It was shown that the use of soy protein slows down the fermentation process in the sourdough. An increase – in acidity levels of between 7.5–9.5 times higher in the dough with sourdough and rowan powder when compared to dough without sourdough. Sourdough usage allowed compressibility of the crumb to be increased by between 1.8–2 times, with a specific volume of 19.0% and a porosity of 9.8% and 11.5%, and for the sensory characteristics to be improved as perceived by consumers. It was proved that microbial composition with a lactic acid bacteria, *L. brevis E38*, inhibits ropy disease and mould development in bread. The results of the present study showed that the addition of sourdough and rowan powder can be used to improve the quality of gluten-free bread.

Key words: gluten-free bread, lactic acid bacteria, dry microbial composition, rowan powder, quality, mould, ropy disease.

INTRODUCTION

The increased demand for gluten-free products proves that celiac disease is one of those food intolerances which is clearly prevalent across the world. However, the absence of gluten, which is necessary for the construction of the protein structure and crumb texture, makes it difficult to produce gluten-free bread. In addition, gluten-free

bread is easily subjected to microbial spoilage. Therefore, obtaining high-quality gluten-free bread is an important technological task.

Recent scientific studies are increasingly focusing on improving the formulation and technology of gluten-free bread. Many researchers have tried to improve the gluten-free quality of bread using gluten-free flour mixtures and starch (Clerici et al., 2009; Torbica et al., 2010; Sakac et al., 2011; Milde et al., 2012; Ozol et al., 2012; Ziobro et al., 2012; Korus et al., 2015), or additives such as hydrocolloids, gums, fibre, emulsifiers, or enzymes (Renzetti et al., 2008; Sciarini et al., 2010; Phimolsiripol et al., 2012; Tsatsaragkou et al., 2014; Różyło et al., 2017).

In traditional technology, the use of sourdough improves the traditional texture of bread, along with its flavour and shelf life. The use of sourdough in technology relating to gluten-free bread is of great interest. The amount of research being carried out on the use of gluten-free sourdough in bakery production has increased in recent years.

Sourdough was developed using buckwheat, oat, quinoa, sorghum, teff, and wheat flour along with the heterofermentative lactic acid bacteria, *Lactobacillus plantarum FST 1.7* (Wolter et al., 2014). But the studies did not provide any satisfactory results. Shelf life for sourdough breads was not prolonged by the addition of sourdough. The inferior aroma of bread that had been prepared using gluten-free flour was also not improved by the addition of sourdough. It can be assumed that the absence of a positive effect on the taste of gluten-free bread is associated with the use of a facultatively heterofermentative lactic acid bacteria strain which generally only produces lactic acid under normal conditions. Therefore, in this study we propose the use not only of facultatively heterofermentative strains but also of obligatory heterofermentative strains and their mixture with a homofermentative strain.

Recent studies also have confirmed the positive effects of adopting sourdough technology in gluten-free breadmaking (Mariotti et al., 2017). The sourdough was created using a stable association between *Lactobacillus sanfranciscensis* (an obligatory heterofermenatative strain) and *Candida humilis*.

Bender et al. (2017) have evaluated the performances of different *Lactobacillus spp*. strains which were applied in the fermentation of millet and buckwheat sourdoughs. They have established that the combination of four strains could be used as potential starter cultures for millet or buckwheat sourdough bread. They have shown that *Lactobacillus pentosus* and *Lactobacillus hammesii* positively influenced the crumb firmness of buckwheat and millet bread respectively, while *Lactobacillus paralimentarius* enhanced this property in both bread types. But in this research only one of the two *Lactobacillus sanfranciscencis* strains was able to improve all functional properties in both gluten-free breads (Bender et al., 2017).

The aim of this study was to investigate different strains of lactic acid bacteria, to create a new starter composition, and to develop gluten-free sourdough bread technology using rowan powder which would improve the quality and microbial stability of bread.

MATERIALS AND METHODS

A characterisation of the ingredients

In this study, the lactic acid bacteria strains *Lactobacillus paracasei/Lactobacillus casei*, *Lactobacillus paracasei E3*, *Lactobacillus plantarum E4*, *Lactobacillus plantarum E5*, *Lactobacillus plantarum E36*, *Lactobacillus parabuchneri E7*,

Lactobacillus fermentum E28, and *Lactobacillus brevis E38* were used. The strains were taken from the collection at the St. Petersburg Branch of the State Research Institute of the Baking Industry.

The dry microbial composition was made by drying a mixture of gluten-free raw materials and pure cultures of lactic acid bacteria strains. A dry microbial composition was used as a starter for sourdough.

The powder from the fruit of the ordinary rowan (the botanical species *Sorbus aucuparia)* was used as an enriching additive.

The pathogen strains of *Bacillus subtilis*, *Bacillus licheniformis*, and *Penicillium chrysogenum* were taken from the collections of the St. Petersburg Branch of the State Research Institute of the Baking Industry, and were used to infect bread when testing microbiological stability.

The study of lactic acid bacteria strains

Strains were cultured in standard liquid medium MRS (BioMerieux, France). Lactic acid bacteria from the St Petersburg collection was inoculated into a liquid medium at the ratio of 1:100, and kept at 37 °C for forty-eight hours. An assessment of microbiological parameters was carried out by examining the following properties: the number of cells in the culture was determined by counting the colonies that had been grown on the MRS with agar, acidity levels were determined using a 0.1 n. solution of NaOH (Afanasjeva, 2003).

Antagonistic activity. lactic acid bacteria's antagonistic activity against ropy bread disease pathogens *B. subtilis* and *B. licheniformis* was investigated. A concentration of 0.1 cm^3 of the pathogen suspension with a cell content of 10^6 cells mL^{-1} was applied to the meat-peptone agar surface in Petri dishes and was distributed over the surface with a spatula, before being covered and left to undergo diffusion for thirty minutes. Then holes with a diameter of 7 mm were cut on the surface of inoculated meat-peptone agar by means of a sterile metal cylinder. A lactic acid bacteria culture of 0.1 cm^3 was inoculated into the holes with the contents of cells at 10^9 cells mL^{-1}. The Petri dishes were kept at 37 °C for twenty-four hours. After that the diameters of the pathogen were measured and no growth zones around the holes were found (Savkina et al., 2015).

Dried microbial composition preparation

A mixture of rice flour and soy protein isolate at a ratio of 2:1 was used to prepare a dry microbial composition. Lactic acid bacteria was grown as follows: a liquid medium of MRS was inoculated by means of lactic acid bacteria from the St Petersburg collection at a ratio of 100:1, and was kept at 37 °C for forty-eight hours. A culture fluid of strains *L. paracasei/L. casei, L. paracasei, L. plantarum* (starter 1), and *L. brevis E38* (starter 2) were mixed with the rice flour and soy protein isolate at a ratio of 1:1. The resulting mixture was granulated through a sieve with a mesh size of 1.5mm and dried in an IR drier (LOIP L3-120/300-VG1, Russia) at a temperature of no higher than 50 °C for between 60–90 minutes to a moisture content of not more than 14.0%.

Sourdough preparation

To prepare the sourdough, the rice flour and soy protein isolate or rice and soy flour at a ratio of 2:1 for both were mixed in a kneading machine, the Kitchen Aid KSM45 (USA), at a speed of 120 revolutions per minute for three minutes with water at a

humidity of 65 ± 0.5%. A dry microbial composition was added to an amount of 2.0% by weight of the mixture. The mixture was mixed and was held at a temperature of between 29–31 °C for between 18–20 hours.

Bread-making procedure

The gluten-free bread formulations used in this study are presented in Table 1. Percentages of ingredients were based on 100 g of gluten-free mixture amount, which include flour, salt, and sugar.

Table 1. Formulations used to prepare different gluten-free bread types

Ingredients, g	Control	Sample 1	Sample 2
Corn starch	57.8	47.5	51.5
Extrusion corn starch	10.0	10.0	-
Soy protein isolate, kg	9.7	9.7	9.7
Rice flour	19.7	-	-
Salt	0.8	0.8	0.8
Sugar	2.0	2.0	2.0
Rowan powder	-	-	6.0
Vegetable oil	3.8	3.8	3.8
Yeast	2.5	2.5	2.5
Sourdough	-	73.0	73.0
Water	until dough humidity achieved of 52% or 49%		

All of the components were mixed in a kneading machine, the Kitchen Aid KSM45 (USA), at a speed of 120 revolutions a minute for seven minutes. After mixing, the dough samples were shaped into 250 g loaves, placed in baking forms, and leavened at 30 °C until the volume was twice that of the initial volume. The leavened dough samples were cooked in an oven, a SvebaDahlen (Sweden), at a temperature of 210 °C for eighteen minutes.

The dry microbial composition and sourdough assessment

The mass proportion of moisture in the dry microbial composition and in the sourdough was determined by drying it at a temperature of 130 °C for forty minutes in a drier, the SHS-1M (Russia). Acidity levels were determined by means of titration, using an 0.1 n. solution of NaOH (State Standard of the Russian Federation, 1996). The lactic acid bacterial cell count was determined by growth on MRS with agar.

The content of acetic and lactic acid in the sourdough were studied using liquid chromatography on a chromatograph Shimadzu LC-10, Japan (State Standard of the Russian Federation, 2014).

An assessment of baked bread

An assessment of quality. An assessment was carried out on bread quality levels in relation to the following properties: organoleptic – appearance (shape, surface, crumb colour), condition of crumb (porosity and texture), and taste and smell; physical and physico-chemical - the mass proportion of moisture was determined by drying out at a temperature of 130 °C for 45 minutes in a drier (the SHS-1M, Russia), and acidity levels were determined by means of titration, using a 0.1 n. solution of NaOH (State Standard of the Russian Federation, 1996); porosity – this being determined as the ratio of pore

volume to the total volume of products; pore volume - the difference between the volume of the product and the volume of the non-porous mass; specific volume - as the ratio of product volume to 100 g of bread, with compressibility being determined on the automatic penetrometer, Labor (Hungary).

The content of gluten in bread was evaluated by means of an enzyme-linked immunosorbent assay.

Ropy disease assessment

In order to determine the effects of the starter on microbial resistance, the bread was infected with the bacteria, *B. subtilis* and *B. licheniformis*. To contaminate the bread, bread crumbs with spores were prepared in the following way: spore-forming bacteria on meat-peptone medium was added to the surface of the sliced gluten-free bread and cultured at a temperature of 37 °C for 96 hours or until signs of disease became apparent. Diseased bread was dried in an oven at a temperature of 50 ± 2 °C and milled to obtain crumbs. A total of 1% of infected crumbs were added while kneading the dough for gluten-free bread. The ready bread was stored at 37 °C prior to any appearance of symptoms of ropy disease (Afanasjeva, 2003).

Moulds spoilage assessment

The impact was investigated in terms of the sourdough and rowan powder and its relation to mould disease in bread. The model experiments with the contamination of sterile bread slices from a pure culture of the mould *Penicillium chrysogenum* were carried out. *Penicillium chrysogenum* was used because the *Penicillium* species are by far the most common for bread. This strain was previously isolated from diseased gluten-free bread and was identified with the species according to cultural and biochemical characteristics. In addition it was contained in the St. Petersburg collection.

Immediately after baking in the oven opening, the loaves were packed into sterile paper, placed in a sterile room, and cooled to a temperature of between 25–28 °C. After cooling the bread was cut in a sterile environment, with slices being taken at a size of 3.5×6.5 cm and at a thickness of 0.3–0.4 cm. The slices were placed in sterile Petri dishes.

An aqueous suspension of a pure culture of the mould, *Penicillium chrysogenum*, was prepared for the infection of slices of bread. The biomaterial of *Penicillium chrysogenum* was transferred from a tube containing a pure culture of mould grown on malt agar to 1ml of sterile water using 'Tween-80' and was thoroughly suspended. The suspension was inoculated into each slice of bread in three shots using a microbiological needle. Petri dishes with infected slices were incubated at a temperature of 25 ± 1 °C until the first signs appeared of a growth of mould colonies.

Statistical analysis of the data

All of the experiments were carried out a total of five times. The accuracy of the experimental data was evaluated by using mathematical statistical methods in Microsoft Excel (2010 version) at a theoretical frequency of 0.95. Results were given as mean ± standard deviation.

RESULTS AND DISCUSSION

Gluten-free bread is easily exposed to microbial damage (ropy disease and mould growth). The use of 6–10% rowan powder in the formulation for making gluten-free bread allows mould spoilage to be slowed down (Dubrovskaya et al., 2017). To inhibit ropy bread disease, it was suggested that a sourdough starter with lactic acid bacteria be developed, which has an increased level of antagonistic activity against ropy disease's causative agent.

The antagonistic activity of lactobacilli against *Bacillus subtilis* and *Bacillus licheniformis* was studied (Fig. 1). This showed that *L. brevis E38* suppressed the *Bacillus* growth more than other strains. The strains *L. paracasei/L. casei*, *L. paracasei E3*, and *L. plantarum E4* showed slightly worse results, but also had rather high levels of antagonistic activity.

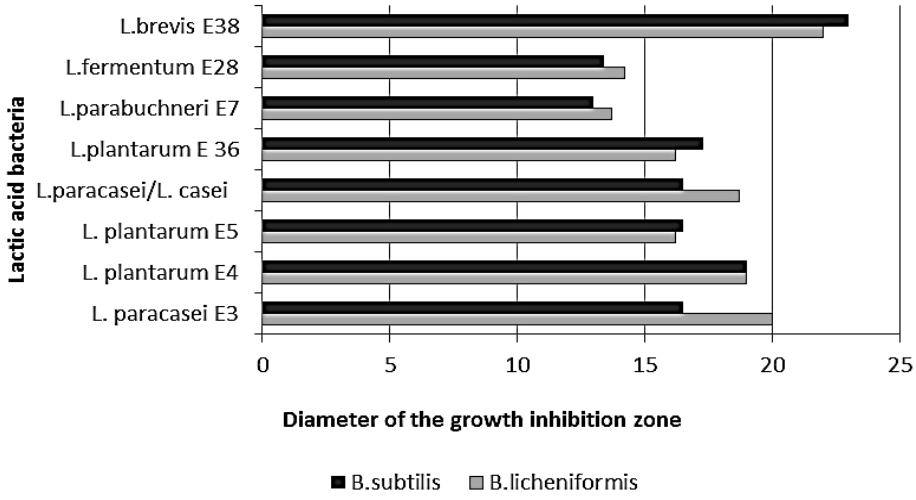

Figure 1. Antagonistic activity by lactic acid bacteria against *B. licheniformis* and *B. subtilis*.

All of the strains are obligatory heterofermentatives or facultatively heterofermentatives, so that they are able to produce organic acid (whether lactic or acetic), thereby having an antimicrobial effect. But investigated lactic acid bacteria had different levels of antagonistic activity. It was found that the species *L. brevis*, *L. paracasei/L. species casei*, *L. paracasei*, and *L. plantarum* had antagonistic activity which were between 30–46% higher than the *L. fermentum* and *L. parabuchneri* species. Thanks to this, the production of organic acids is not the only one mechanism of providing antagonism. Antimicrobial and antibiotic-like compounds – bacteriocins (lactocins) – can also play an important role (Reis et al., 2012).

The antagonistic activity of the mixture of strains (*L. paracasei/L. casei*, *L. paracasei E3*, and *L. plantarum E4)* was higher, being comparable to *L. brevis E38* (data not shown). The increasing in antagonistic activity may be due to synergic action. Therefore, this symbiosis was used to develop the dry microbial composition.

Two types of dry microbial composition were created. *L. paracasei/L. casei*, *L. paracasei E*, and *L. plantarum E4* (starter 1), along with *L. brevis E38* (starter 2) were mixed with the rice flour and soy protein isolate at a ratio of 1:1 and these were subsequently dried.

Biotechnological indicators for two types of dry microbial composition are presented in Table 2. It was found that the number of cells of lactic acid bacteria in starter 2 were 4.85 times higher than in starter 1, although they had practically the same acidity levels. This may be due to competition for food between *L. paracasei/L. casei*, *L. paracasei*, and *L. plantarum.*

Table 2. Biotechnological indicators for two types of dry microbial composition

Biotechnological indicators	Starter 1	Starter 2
Mass proportion of moisture, %	14.0 ± 0.2	13.8 ± 0.2
Acidity, degrees N	9.0 ± 0.4	9.4 ± 0.4
Number of lactobacilli cells, CUFF g^{-1}	$(0.26 \pm 0.01) \times 10^9$	$(1.26 \pm 0.01) \times 10^9$

The number of cells in the starter is of great importance because this reflects an ability to develop in sourdough. That is why starter 1 was selected for further research.

During the sourdough development process, acid accumulation was studied within 36 hours at a temperature of 29–31 °C in two 65% moisture mixtures. This moisture content was chosen based on previous studies which are not listed here. One sourdough sample consisted of rice flour and soy protein isolate, and the other consisted of rice and soy flour at a ratio of 2:1. Rice and soy flour and soy protein isolate were taken from the basic recipe (Dubrovskaya et al., 2017). Starch was not used in the sourdough technology because it is less of a nutrient medium for lactic acid bacteria growth than the flour and the protein.

Besides this, *L. brevis E38* is an obligatory heterofermentative micro-organism, which ferments hexose into lactic acid, acetic acid (ethanol), and CO_2. Pentoses are fermented by them into lactic acid and acetic acid through the pentose-phosphate pathway. Therefore, not only were the acidity levels studied in the sourdoughs, but so was the content of lactic acid and acetic acid.

It was found that acidity accumulation was higher by between 25–48% in sourdough with soy flour than in sourdough with soy protein isolate (Fig. 2). This shows that soy flour is more nutritious for the development and life activity of lactic acid bacteria than soy protein.

As for the metabolite concentration found in the sourdoughs (Table 3), a higher lactic acid amount was produced in sourdough with soy protein isolate. The content of acetic acid was 2.7 times higher in sourdough with soy flour when compared to sourdough with soy protein isolate (Table 3). Higher acidity levels and a higher content of acetic acid can positively influence the taste of bread and increase its microbiological stability during storage.

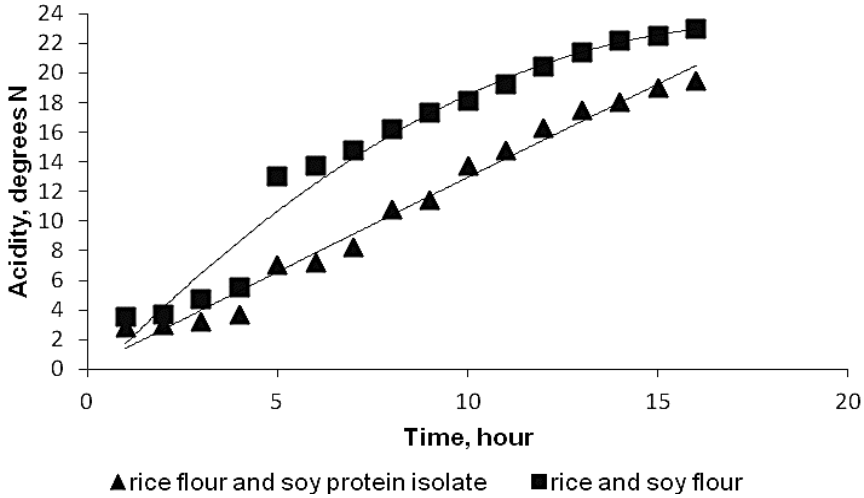

Figure 2. Acidity in two types of sourdough.

Therefore the use of a dry microbial composition with *L. brevis E38*, rice, and soy flour, at a ratio of 2:1 and with water to a moisture content of 65% may be recommended when it comes to preparing gluten-free sourdough.

Table 3 . Biotechnological indicators for two types of sourdough

Indicators	Sourdough with rice flour and soy protein isolate	Sourdough with rice and soy flour
Mass proportion of moisture, %	65.0 ± 1	65.0 ± 1
Acidity, degrees N	13.5 ± 0.5	14.5 ± 0.5
Temperature, °C	30 ± 1	30 ± 1
Duration of fermentation, hour	19 ± 1	19 ± 1
Lactic acid, g kg^{-1}	13.5 ± 2.7	9.7 ± 1.9
Acetic acid, g kg^{-1}	0.70 ± 0.14	1.90 ± 0.38

An investigation was carried out in terms of the influence exhibited by the sourdough on dough and bread quality. It was found that a sourdough and dough combination increases dilution and reduces viscosity (Fig. 3). The diluting effect of the sourdough may be related to the effect of lactic acid bacteria enzymes on the dough biopolymers. This permitted dough humidity to be reduced by 3% (from 52.0% to 49%). When the bread moisture content is reduced, gluten-free bread resistance to microbiological spoilage (mould) may be increased.

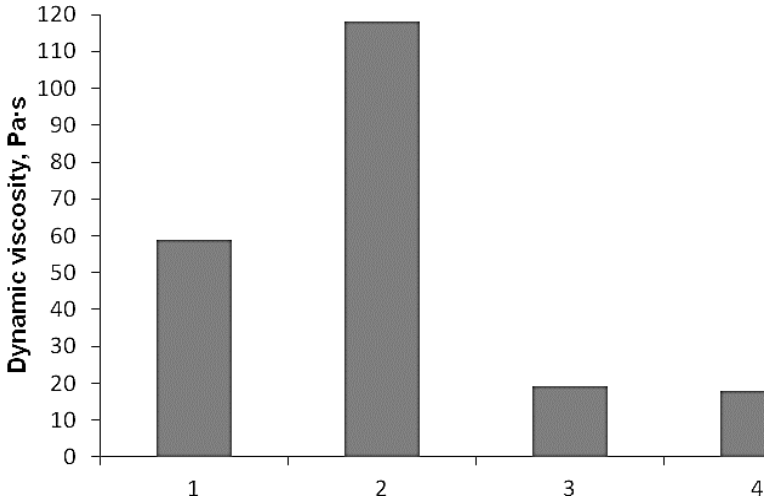

Figure 3. Dough dynamic viscosity at a shear rate of 9 s^{-1}. 1 – control, dough humidity 52%; 2 – control, dough humidity 49%; 3 – Sample 2, dough humidity 49%; 4 – Sample 2, dough humidity 49%.

It was found that sourdough and rowan powder usage increased the dough acidity levels (Fig. 4). Dough acidity in Fig. 4 represented a dough humidity of 49%.

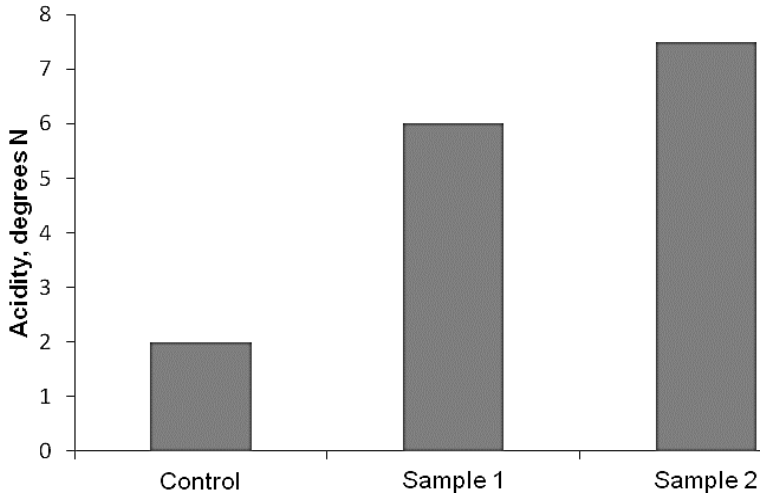

Figure 4. Dough acidity, degrees.

The bread quality indicators are presented in Table 4. It was found that bread with sourdough had an acidity level which was higher than the control by 7.5–9.5 times, while crumb compressibility was higher by between 1.8–2 times, the specific volume was higher by 19.0%, and porosity was higher by 9.8–11.5%. The improvement of gluten-free sourdough bread derives from the metabolic activity of lactic acid bacteria that

provide acidification and the production of exopolysaccharides and antimicrobial substances.

Table 4. Indicators of gluten-free bread quality

Indicators	Control	Sample 1	Sample 2
Mass proportion of moisture, %	51.7 ± 0.5	48.9 ± 0.5	49.1 ± 0.5
Acidity, degrees	0.3 ± 0.1	2.0 ± 0.5	2.8 ± 0.5
Porosity, %	61.0 ± 2	68.0 ± 2	67.0 ± 2
Specific volume, $sm^3 \cdot g^{-1}$	2.1 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
Compressibility, equipment units	19 ± 4	39 ± 4	35 ± 4

Bread organoleptic characteristics are presented in Table 1 (Fig. 5). Sample 1 and Sample 2 had the better crust colour, crumb texture, and porosity than the control. Bread with sourdough and rowan powder (Sample 2) had the best taste and smell. The smell was more intense and pleasant, with fruity notes.

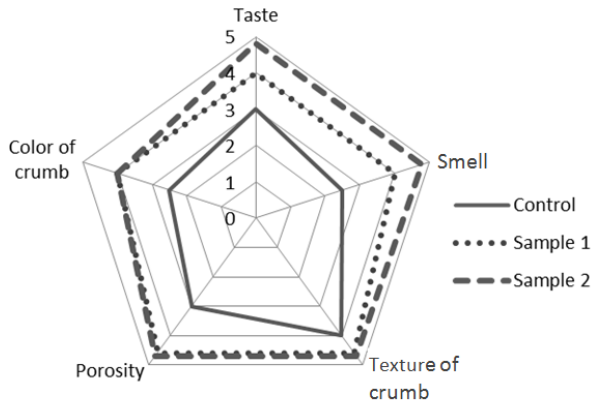

Figure 5. The influence of the sourdough and rowan powder on the organoleptic profiles of gluten-free bread.

The safety of bread with sourdough and rowan powder when it comes to providing nutrition for people who are suffering from coeliac disease was evaluated by means of analysing the gluten content in bread through an enzyme-linked immunosorbent assay. The content of immunoreactive gluten was less than 5 mg for each 1kg of bread, which meets the requirements of diet therapy in celiac disease.

In order to establish the effect of sourdough and rowan powder on ropy bread disease, laboratory baking was carried out with spore-infected breadcrumbs. Bread deposited in precipitating conditions at 37 °C and with a humidity of 70 ± 5%.

It was found that control samples are infected with the disease after a period of seventeen hours, while gluten-free sourdough bread (Sample 1) and bread with sourdough and rowan powder (Sample 2) are not infected at all with ropy disease during the entire storage period (Fig. 6). Therefore, the results of the research have shown that the use of sourdough in gluten-free bread allows the development of the *Bacillus* spore to be completely suppressed.

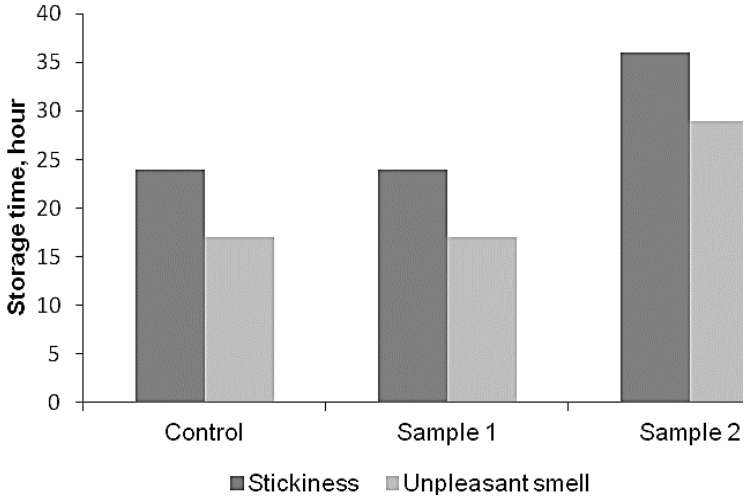

Figure 6. The influence of sourdough and rowan powder on ropy disease in gluten-free bread.

An investigation was carried out into the effect of the sourdough and rowan powder on gluten-free bread's resistance to the moulds. It was found that in the control bread slices were contaminated by *Penicillium chrysogenum*, and a growth of mould colonies was observed between 22–24 hours, while in samples 1 and 2 this was between 36–38 hours. The usage of sourdough with lactic acid bacteria *L. brevis E38* allowed the progression of gluten-free bread mould disease to be slowed down.

Therefore it was proven that the new gluten-free sourdough bread technology using *L. brevis E38* and rowan powder served to increase the microbiological resistance of bread against mould and ropy disease. It also improves the quality, taste, and smell of bread.

CONCLUSIONS

Studied here were the antagonistic activity of a strain of lactic acid bacteria, its biomass, and acidity accumulation during cultivation on an MRS liquid medium. The strain, *L. brevis E38*, was selected to create a dry microbial composition. The dependence was revealed of acetic acid and lactic acid accumulation in the sourdough during fermentation on the microbial composition.

A dry microbial composition was created as a gluten-free sourdough starter. A gluten-free sourdough technology was developed with a new starter, rice, and soy flour at a ratio of 0.2:2:1.

It was found that when using lactic acid bacteria, *L. brevis E38*, the dough liquefies due to the action of the lactobacilli enzymes on dough biopolymers.

It was also shown that sourdough and rowan powder which contained various organic acids and flavouring substances improves the sensory characteristics of gluten-free bread as perceived by consumers. The use of sourdough with a dry microbial composition and rowan powder allowed the compressibility of the crumb to be increased

by between 1.8–2 times, the specific volume by 19.0%, and the porosity by 9.8% and 11.5%.

It was found that sourdough which contained a large volume of lactic acid bacteria ($3.6 \cdot 10^9$), lactic acid (9.7 g kg^{-1}), and acetic acid (1.9 g kg^{-1}), helped to slow down the onset of spore-forming bacteria and mould growth in bread during storage.

REFERENCES

Afanasjeva, O. 2003. Microbiology of bakery production.Beresta,Saint-Peterburg, pp. 217 (in Russian).

Bender, D., Fraberger, V., Szepasvári, P., D'Amico, S., Tömösközi, S., Cavazzi, G., Jäger, H., Domig, K.J. & Schoenlechner, R. 2017. Effects of selected lactobacilli on the functional properties and stability of gluten-free sourdough bread. *https://doi.org/10.1007/s00217-017-3020-1*. Accessed 15.03.2018.

Clerici, M.T.P.S., Airoldi, C. & El-Dash, A. 2009. Production of acidic extruded rice flour and its in fluence on the qualities of gluten-free bread. *Food Science and Technology* **42**, 618–623.

Dubrovskaya, N., Savkina, O., Kuznetsova, L. & Parakhina, O. 2017. Development of gluten-free bread with unconventional raw ingredients of high nutritional value and antimicrobial activity. *Agronomy Research* **15**(2), 1276–1286.

Korus, J., Witczak, M., Ziobro, R. & Juszczak, L. 2015. The influence of acorn flour on rheological properties of gluten-free dough and physical characteristics of the bread. *European Food Research and Technology* **240**(6), 1135–1143.

Mariotti, M., Cappa, C., Picozzi, C., Tedesco, B., Fongaro, L. & Lucisano, M. 2017. Compressed Yeast and Type I Gluten-Free Sourdough in Gluten-Free Breadmaking. *Food and Bioprocess Technology* **10**(5), 62–97.

Milde, L.B., Ramallo, L.A. & Puppo, M.C. 2012. Gluten-free bread based on tapioca starch: texture and sensory studies. *Food and Bioprocess Technology* **5**(3), 888–896.

Ozola, L., Straumite, E., Galoburda, R. & Klava, D. 2012. Application of extruded maize flour in gluten-free bread formulations. *World Academy of Science, Engineering and Technology* **64**, 883–888.

Phimolsiripol, Y., Mukprasirt, A. & Schoenlechner, R. 2012. Quality improvement of rice-based gluten-free bread using different dietary fibre fractions of rice bran. *Journal of Cereal Science* **56**, 389–395.

Reis, J.A., Paula, A.T., Casarotti, S.N. & Penna, A.L.B. 2012. Lactic Acid Bacteria Antimicrobial Compounds: Characteristics and Applications. *Food Engineering Reviews* **4**(2), 124–140.

Renzetti, S., Dal Bello, F. & Arendt, E.K. 2008. Microstructure, fundamental rheology and baking characteristics of batters and bread from different gluten-free flours treated with a microbial transglutaminase. *Journal of Cereal Science* **48**, 33–45.

Różyło, R., Dziki, D., Gawlik-Dziki, U., Biernacka, B., Wójcik, M. & Ziemichód, A. 2017. Physical and antioxidant properties of gluten-free bread enriched with carob fibre. *International Agrophysics* **31**, 411–418.

Sakac, M., Torbica, A., Sedej, I. & Hadnadev, M. 2011. Influence of breadmaking on antioxidant capacity of gluten free breads based on rice and buckwheat flours. *Food Research International* **44**(9), 2806–2813.

Savkina, O., Kuznetsova, L., Krasnikova, L., Dubrovskaya, N., Parakhina, O. 2015. Influence of microflora gluten free raw materials and Rowan powder on the development of ropy bread disease. *Bakery of Russia* **6**, pp. 31–33 (in Russian).

Sciarini, L.S., Ribotta, P.D., Leon, A.E. & Perez, G.T. 2010. Effect of hydrocolloids on gluten-free batter properties and bread quality. *International Journal of Food Science and Technology* **45**, 2306–2312.

State Standard of the Russian Federation 2014. Fodder plants silage. General specifications. GOST R 55986-2014, 12 pp. (in Russian).

State Standard of the Russian Federation. 1996. Bread, rolls and buns. Methods for determination of acidity. GOST 5670-96, 5 pp. (in Russian).

Torbica, A., Hadnadev, M. & Dapčevic, T. 2010. Rheological, textural and sensory properties of gluten-free bread formulations based on rice and buckwheat flour. *Food Hydrocolloids* **24**, 626–632.

Tsatsaragkou, K., Gounaropoulos, G. & Mandala, I. 2014. Development of gluten free bread containing carob flour and resistant starch. *LWT – Food Science and Technology* **58**, 124–129.

Wolter, A., Hager, A.-S., Zannini, E. & Elke K., Arendt. 2014. Impact of sourdough fermented with Lactobacillus plantarum FST 1.7 on baking and sensory properties of gluten-free breads. *European Food Research and Technology* **239**(1), 1–12.

Ziobro, R., Korus, J., Witczak, M. & Juszczak, L. 2012. Influence of modified starches on properties of gluten-free dough and bread. Part II: Quality and staling of gluten-free bread. *Food Hydrocolloids* **29**, 68–74.

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Contents of tocols in different types of dry shell fruits

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Abstract. The aim of this study was to identify all forms of vitamin E in different kinds of dry shell fruits (generally called 'nuts') obtained from different sources and to perform their mutual comparison. All tocols were determined by reversed phase HPLC using isocratic elution with fluorescence detection. Almonds were evaluated as the most important source of α-tocopherol (average value 1,132 mg kg^{-1}), β-tocopherol was present in all samples as a minor component, its maximum content was found in hazelnuts (9.6 mg kg^{-1}). γ-Tocopherol was found in all kinds of nuts and the highest content was found in pistachios (584 mg kg^{-1}), very high amount of δ-tocopherol was contained in Brazil nuts (2,298 mg kg^{-1}). Tocotrienols were found in a smaller number of nut kinds than tocopherols. α-Tocotrienol was found only in three kinds of nuts - Brazil nuts, which contained the highest amount (399 mg kg^{-1}), pine nuts and hazelnuts grown in the Czech Republic, which were analyzed soon after harvest. Similarly, γ-tocotrienol was determined only in four kinds of nuts (pistachios, macadamias, cashew and walnuts grown in the Czech Republic), which were analyzed soon after harvest; the most significant amount was found in pistachios (34.8 mg kg^{-1}), β- and γ-tocotrienols were below the detection limit. In the Czech Republic, walnuts and hazelnuts are the most widely cultivated and consumed nuts. While in walnuts the most important form is γ-tocopherol (459 ± 40 mg kg^{-1}), in hazelnuts it is α-tocopherol (863 ± 10 mg kg^{-1}).

Key words: nuts, tocopherols, tocotrienols, storage, HPLC.

INTRODUCTION

Dry shell fruits (generally called 'nuts') together with the other kinds of fruits and vegetable play an important role in human nutrition, in particular as a source of vitamins, minerals and fiber (Kader et al., 2001; Brufau et al., 2006; Tošić et al., 2015). The ideal recommended daily intake of dry nuts to protect human health is 30 g per day (Kornsteiner et al., 2006).

Vegetable fat is included among the most important macronutrients, its content in nuts is 46–76%. The fats represent about 20 to 30 kJ g^{-1}, depending on the species. Nuts belong to the most important sources of vegetable fats due to its composition, in which the unsaturated fatty acids are predominant. The unsaturated fatty acids have a positive influence on a variety of lifestyle diseases (Ros & Mataix, 2006). The low ratio of n-6/n-3 fatty acids in macadamia nuts, walnuts, chestnuts and almonds is also important (Freitas & Naves, 2010). High content of vegetable fat in dry nuts is associated with a significant content of vitamin E (Wagner et al., 2004); therefore dry nuts are one of the

most important sources of vitamin E (tocopherols and tocotrienols) from all foods that form a usual part of the human diet. The tocopherols, which have antioxidant properties, provide protection against oxidation of unsaturated fatty acids at the same time (Amaral et al., 2005). Antioxidant activity of vitamin E consists mainly in the prevention of free radicals formation, leading to disabling of lipid peroxidation and thereby reducing the content of LDL cholesterol (Brigelius-Flohe & Traber, 1999). Similarly as in the case of fats, it was found that different types of nuts are fundamentally different both in content and in the composition of the various forms of vitamin E (Kornsteiner et al., 2006). Mainly almonds are a rich source of α-tocopherol, which is the most active form of vitamin E, while walnuts contain a significant amount of its isomeric form that is γ-tocopherol (Ros, 2015).

Kornsteiner et al. (2006) dealt with the content of tocopherols (α-, β-, γ-, and δ) in oils pressed from 10 different kinds of nuts. The highest content of α-tocopherol showed almond (31.4 mg in 100 g of extracted oil) and hazelnuts (24.2 mg in 100 g of extracted oil). The relatively high amount of β- and γ-tocopherols, determined together, contained pistachios, Brazil nuts, cashew nuts, peanuts, pecans, pine nuts and walnuts. Average values in 100 g of extracted oil moved in the range from 5.1 mg (cashew nuts) to 29.3 mg (pistachio). Traces of δ-tocopherol (< 4 mg in 100 g of extracted oil) were determined in the cashew nuts, hazel nuts, peanuts, pecan nuts, pine nuts, walnuts, pistachios, and also in the walnuts.

A significant advantage of nuts compared with the other fruits is their good preservation and stability which was appreciated especially in the past (Fraser et al., 1992). The nuts are quite predisposed to mildew, above all in areas with high rainfall and humidity. The main risk factors are aflatoxins that are produced by the fungus *Aspergillus flavus* (Macrae et al., 1993). The risk associated with contamination by mycotoxins may be reduced by correct pre-harvest procedures, proper drying in an adequate time after the harvest and safe storage at the right temperature and humidity (Macrae et al., 1993; Ozilgen & Ozdemir, 2001).

Changes in the contents of tocopherols in peanuts during storage were studied by Silva et al. (2010). According to them the contents of all forms of tocopherols decreased during storage. In contrast, the indicators of lipid oxidation, such as peroxide number and intensity of the oxidized and cardboard taste increased during storage. At the same time a strong negative correlations were evaluated between the content of tocopherols and peroxide number. Chun et al. (2005) came to the conclusion that tocopherols decrease exponentially during storage with the current increase of peroxide number describes. According to the cited authors the contents of tocopherols could be used as an indicator of oxidative state in peanut products. According to Tsantili et al. (2011) the stability and total antioxidant capacity of nuts, which is made up by vitamin E as well, can have been influenced in addition to the external conditions also by selected cultivar.

The goal of this study was to determine the contents of various forms of vitamin E (tocols and tocotrienols) in different kinds of dry nuts purchased at different locations in the Czech Republic or grown and harvested in the Czech Republic and their mutual comparison.

MATERIALS AND METHODS

Analyzed nuts

Various types of nuts, namely walnuts (*Juglans regia* L.), hazelnuts (*Corylus avellana* L.), almonds (*Prunus dulcis* (Mill.) D. A. Webb), cashew nuts (*Anacardium occidentale* L.), pistachios (*Pistacia vera* L.), Brazil nuts (*Bertholletia excelsa* Humb. & Bonpl.), macadamia nuts (*Macadamia integrifolia* Maiden & Betche), pecans (*Carya illinoinensis* (Wangenh.) K. Koch), pine nuts (*Pinus pinea* L.) and peanuts (*Arachis hypogaea* L.) of unknown origin were purchased at two different locations in the Czech Republic market network. In addition, three samples of walnuts and one sample of hazelnuts grown and harvested in different locations of the Czech Republic were collected. All samples of nuts were purchased or harvested in October 2015.

Chemicals

For the preparation of analytical samples the following standards and chemicals were used: DL-α-tocopherol, 98.2% (CALBIOCHEM, Canada), tocopherol set (CALBIOCHEM, Canada), propan-2-ol (Lachner, Czech Republic), methanol super gradient, content min. 99.9% (Lachner, Czech Republic), and treated distilled water (Milipore, France).

Sample preparation

The nuts were shelled and afterwards homogenized in a laboratory grinder. Approximatwly 0.3 g of homogenized sample was weighed into a plastic test tube with a plastic stopper (Falcon type). 10 mL of propan-2-ol was added. The tube was slightly shaken and then placed for 10 minutes into an ultrasound bath. Subsequently, the sample was centrifuged for 5 minutes (Eppendorf 5810R, 20 °C at 11.5 rpm) and the supernatant was decanted. The extraction was 3 times repeated. After that the supernatants were collected and refilled up to 50 mL. Afterwards the tubes were placed for 2 hours into a freezer box (-20 °C) to separate fat layer. Final supernatant was transferred through a syringe filter (nylon, 0.22 μm) into a dark vial for HPLC analysis. All steps were carried out at low light intensity (the windows were darkened with blinds with no direct lighting into the lab). The efficiency of extraction of all vitamers was checked by analyses of all the following extracts of the identical extracted samples. The content of all individual vitamers was close to the limit of detection in the fourth extract, and therefore three consecutive extractions have been evaluated as sufficient approaching to 100% efficiency. Extractions of all analyzed samples were performed in 3 replicates.

Tocopherol and tocotrienol chromatographic determination

HPLC-FD analyses were performed under the following conditions: analytical column Develosil® 5 μ RP-Aqueous (250 × 4.5 mm), (Phenomenex, Torrance, CA, USA), mobile phase H_2O : methanol (3 : 97, v/v), flow 1.0 mL min^{-1}, column temperature 30 °C, injection volume 10.0 μL, time of analysis 30 min. Conditions of detection: fluorescence detector, excitation wavelength $\lambda = 292$ nm, emission wavelength $\lambda = 330$ nm. The contents of analytes in the samples were evaluated by an external standard method. Calibration curve of all tocols was linear in the range from 0.05 to 10 μg mL^{-1}. Limits of detection (LODs), expressed as a ratio of three times the value of the signal-to-noise ratio, for individual tocols δ-T3, γ-T3, β-T3, α-T3, δ-T, γ-T, β-T and α-T were 0.056,

0.111, 0.111, 0.167, 0.056, 0.111, 0.111, and 0.167 μg g^{-1}, respectively. The results were processed with chromatography software Chromeleon and MS Excel. Examples of chromatograms of are given in the Figs 1–3.

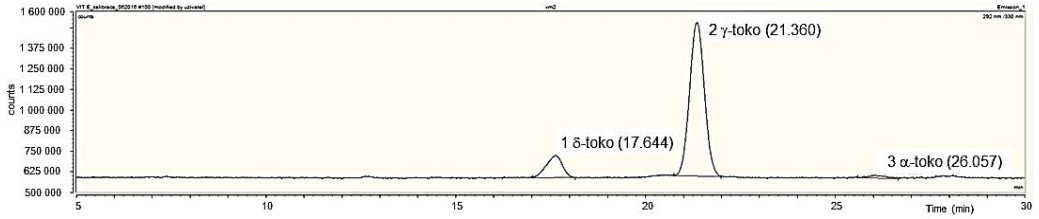

Figure 1. Chromatogram of tocols in walnuts.

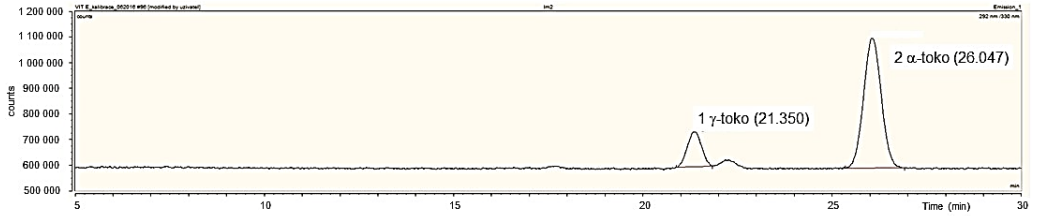

Figure 2. Chromatogram of tocols in hazelnuts.

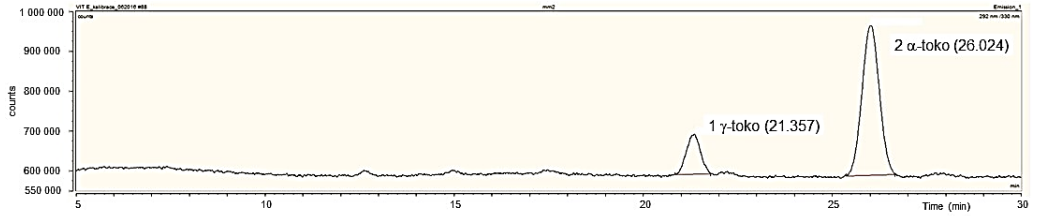

Figure 3. Chromatogram of tocols in almonds.

Statistical evaluation

Statistical analysis was done in *Statistica* Version 12.0 (Statsoft). The measured values were processed by the analysis of one-way variance method (*ANOVA*), using *post-hoc Scheffé's* test for more detailed evaluation, considering $\alpha = 0.05$ and the level of significance $P < 0.05$ as significant.

RESULTS AND DISCUSSION

Contents of tocols in different type of nuts

The determination of individual forms of vitamin E was done in 10 selected types of nuts purchased at 2 different markets (Table 1). Only 6 different forms of vitamin E were determined, β-tocotrienol and δ-tocotrienol in all analyzed types of nuts were below the limit of detection (LOD = 3.33 mg kg^{-1} and 1.67 mg kg^{-1}, resp.) but the composition and contents of the other particular forms of vitamin E in analyzed types of nuts were statistically very different.

Table 1. The contents of individual tocols (mg kg^{-1}) in different types of nuts

Nuts	α-TcP	β-TcP	γ-TcP	δ-TcP	α-TcT	γ-TcT
Walnuts 1	32.7 ± 4.6	< 3.33	432 ± 6.5	44.8 ± 2.3	< 5.00	< 3.33
Walnuts 2	51.8 ± 4.1	< 3.33	453 ± 6.8	59.9 ± 1.3	< 5.00	< 3.33
Mean	**42.2 ± 13.5**	**< 3.33**	**442 ± 14.7**	**52.4 ± 10.7**	**< 5.00**	**< 3.33**
Hazelnuts 1	838 ± 6	7.22 ± 0.1	31.7 ± 0.8	3.12 ± 1.3	< 5.00	< 3.33
Hazelnuts 2	769 ± 26	9.56 ± 0.8	31.3 ± 2.4	< 1.7	< 5.00	< 3.33
Mean	**803 ± 34**	**8.39 ± 1.16**	**31.5 ± 1.4**	**2.39 ± 1.1**	**< 5.00**	**< 3.33**
Almonds 1	1,125 ± 52	4.74±0.2	12.2 ± 0.7	< 1.67	< 5.00	< 3.33
Almonds 2	1,139 ± 7.4	< 3.33	30.6 ± 2	< 1.67	< 5.00	< 3.33
Mean	**1,132 ± 10**	**4.04 ± 1**	**21.4 ± 13**	**< 1.67**	**< 5.00**	**< 3.33**
Cashew 1	< 5.00	< 3.33	116 ± 1.9	6.32 ± 0.5	< 5.00	3.72 ± 0.6
Cashew 2	< 5.00	< 3.33	75.3 ± 5.6	6.60 ± 0.3	< 5.00	< 3.33
Mean	**< 5.00**	**< 3.33**	**95.5 ± 28.7**	**6.46 ± 0.2**	**< 5.00**	**3.52 ± 0.3**
Pistachios 1	42.2 ± 6.4	< 3.33	590 ± 4.8	11.1 ± 1.4	< 5.00	36.3 ± 0.9
Pistachios 2	< 5.00	< 3.33	579 ± 8.4	10.0 ± 1.1	< 5.00	33.3 ± 1.7
Mean	23.6 ± 26	< 3.33	585 ± 8.1	10.6 ± 0.8	< 5.00	34.7 ± 2.2
Brazil nuts 1	175 ± 26	< 3.33	472 ± 11.3	2,932 ± 167	391 ± 39.3	< 3.33
Brazil nuts 2	149 ± 3.7	< 3.33	381 ± 12.8	1,664 ± 154	< 5.00	< 3.33
Mean	**162 ± 18**	**< 3.33**	**427 ± 64.1**	**2,298 ± 897**	**202 ± 279**	**< 3.33**
Macadamia 1	464 ± 22	< 3.33	187 ± 8.46	6.96 ± 0.61	< 5.00	14.0 ± 1.13
Macadamia 2	406 ± 13	< 3.33	< 3.33	< 1.67	< 5.00	< 3.33
Mean	435 ± 41	< 3.33	95.1 ± 129	4.31 ± 3.74	< 5.00	8.67 ± 7.54
Pecans 1	32.5 ± 0.76	< 3.33	586 ± 9.96	39.9 ± 0.71	< 5.00	< 3.33
Pecans 2	97.3 ± 24.3	< 3.33	188 ± 1.28	16.1 ± 0.36	< 5.00	< 3.33
Mean	**64.9 ± 46**	**< 3.33**	**387 ± 282**	**28.0 ± 16.8**	**< 5.00**	**< 3.33**
Pine nuts 1	< 5.00	< 3.33	< 3.33	< 1.67	78.9 ± 7.21	< 3.33
Pine nuts 2	26.7 ± 1.0	< 3.33	67.5 ± 1.27	< 1.67	< 5.00	< 3.33
Mean	**15.8 ± 15**	**< 3.33**	**35.4 ± 45.4**	**< 1.67**	**41.9 ± 52.2**	**< 3.33**
Peanuts 1	267 ± 2.9	< 3.33	124 ± 1.32	5.83 ± 0.52	< 5.00	< 3.33
Peanuts 2	281 ± 6.8	< 3.33	99.8 ± 1.61	6.72 ± 0.53	< 5.00	< 3.33
Mean	**274 ± 10**	**< 3.33**	**112 ± 17.4**	**6.27 ± 0.63**	**< 5.00**	**< 3.33**

TcP – tocopherol, TcT – tocotrienol.

The variability in the content of individual tocols between 2 groups of samples of the same type (purchased in different locations of the Czech Republic) was strongly divergent and ranged from 0.08% (almonds, α-tocopherol) to 138% (Brazil nuts, α-tocotrienol). In general, the highest differences were determined in the content of α-tocotrienol, which was found only in Brazil nuts and pine nuts. In comparison, the average variability of parallel determination of the same sample was 5.10% and only exceptionally (in two cases) was close to 20% (Table 2). According to Tsantili et al. (2011) external conditions during growing play an important role in the content of individual substances. The origin of purchased samples is not known and may be different.

The most contained forms of vitamin E, which are represented in most of the analysed species of dry nuts were α-tocopherol and γ-tocopherol (Table 1). The content of α-tocopherol ranged from 26.7 to 1,139 mg kg^{-1} depending on the type of dry nuts. The highest average amount of α-tocopherol was found in almonds (1,132 ± 10) mg kg^{1} and hazelnuts (863 ± 109) mg kg^{-1}; these two kinds of dry nuts were the most important

source of α-tocopherol also in studies by Maguire et al (2004) and Kornsteiner et al. (2006).

In contrast, the lowest content of α-tocopherol was found in cashew nuts and in one sample of pine nuts, where the presence of α-tocopherol was below the limit of detection (5 μg g^{-1}). These findings are in agreement with the analysis of Kornsteiner et al. (2006) again.

The presence of γ-tocopherol was found in all of the analyzed species of dry nuts (Table 1). Contents of γ-tocopherol ranged between 2.23–590.4 mg kg^{-1} depending on the kind of nuts. The highest amount of γ-tocopherol was found in pistachio (584.7 ± 8.1 mg kg^{-1}) and next to in the samples of walnuts, Brazil nuts and pecan nuts, which is in good accordance with the study by Kornsteiner et al. (2006), where the amount of γ-tocopherol was found in the same descending order in different samples of nuts.

Miraliakbari & Shahidi (2008) reported that the highest amount of γ-tocopherol contained in pecan nuts was 440.2–472.9 mg kg^{-1}. In this study various values of γ-tocopherol in pecan nuts were established in two analyzed samples (586 ± 9.96) mg kg^{-1} and (188 ± 1.28) mg kg^{-1}, resp. It means that other nuts, especially pistachios, have been found as more significant resources of γ-tocopherol.

β-Tocopherol above the limit of detection has been found in only one sample of almonds (4,744 mg kg^{-1}), and in one sample of hazelnuts (9,556 mg kg^{-1}). It can therefore be stated that β-tocopherol is a minor form of vitamin E. This statement is in agreement with the work by Miraliakbari & Shahidi (2008), where β-tocopherol has been found only in samples of almonds and pine nuts. The founded content was in the range 10.9–22.6 mg kg^{-1}.

The highest levels of δ-tocopherol were contained in Brazil nuts (2297 ± 89) mg kg^{-1}. The differences in the content of this vitamer among the various type of nuts were the biggest, because the content of the δ-tocopherol in some next analyzed samples was very low and sometimes even below the limit of detection (< 1.67 μg g^{-1}). In contrary only small amount of δ-tocopherol in 10 different kinds of nuts were detected by Kornsteiner et al. (2006) that found the biggest content 3.8 mg in 100 g extracted oil from walnuts.

A significant higher amount of this form of vitamin E contained also walnuts (52.4 ± 10.7) mg kg^{-1}, which is more than twice higher value than determined Miraliakbari & Shahidi (2008) in their study. According to this authors the content of δ-tocopherol in walnuts is ranging from 19.8–23.4 mg kg^{-1}. This difference could probably be in consequence of different origin and varieties of nuts, or different period of storage before analysis.

In general, the contents of tocotrienols in comparison with tocopherols were found in significantly lower quantities in all dry nut types. Brazil nuts were evaluated as the most important source of α-tocotrienol; in one sample of these nuts the content (399.1 ± 37) mg kg^{-1} was found. α-Tocotrienol was also determined in only one sample of pinenuts (78.9 ± 6.3) mg kg^{-1}. The content of α-tocotrienol in all next analyzed samples was below the limit of detection (< 5.00 μg g^{-1}).

The other vitamer of vitamin E from the tocotrienols group, γ-tocotrienol, was found only in three types of purchased nuts namely cashew, pistachios and macadamia

nuts. The highest amount of γ-tocotrienol has been found in samples of pistachios (34.7 ± 2) mg kg^{-1}.

The contents of tocotrienols could not be compared to the literature data, because the cited studies probably due to minor representation of tocotrienols deal only with tocopherols. Recently, however, more and more information has been available about tocotrienols that are very powerful antioxidants and their antioxidant activity could be even more significant than the antioxidant activity of tocopherols (particularly α-tocopherol), even though their contents in food are usually smaller than the contents of tocopherols (Theriault et al., 1999; Choi & Lee, 2009; Comitato et al., 2017).

Contents of tocols in walnuts and hazelnuts of different origin

Walnuts and hazelnuts are the most cultivated and consumed nuts in the Czech Republic. The contents of tocopherols and tocotrienols were evaluated in five samples of walnuts and three samples of hazelnuts that were either purchased in the market, or grown in different places of the Czech Republic (Table 2).

The average content of all tocols was (563.7 ± 53.1) mg kg^{-1} in walnuts and (914.2 ± 124.9) mg kg^{-1} in hazelnuts. The major tocopherol in walnuts was γ-tocopherol, whose average content was (459.6 ± 40.0) mg kg^{-1}, which is in agreement with data given by Savage et al. (1999), Li et al. (2007) and Oliviera et al. (2002), who established the content of γ-tocopherol in different samples of walnuts in the range of 205–525 mg kg^{-1}.

Table 2. The contents of individual tocols (mg kg^{-1}) in walnuts and hazelnuts of different origin

Nuts	α-TcP	β-TcP	γ-TcP	δ-TcP	α-TcT	γ-TcT
Waln - A	51.4 ± 1.7	< 3.33	419 ± 10	50.3 ± 2.7	< 5.00	14.6 ± 0.61
Waln - B	42.1 ± 8.4	< 3.33	473 ± 9.8	50.5 ± 0.95	< 5.00	16.1 ± 0.28
Waln - C	53.0 ± 3.52	< 3.33	521 ± 8.5	67.6 ± 2.4	< 5.00	15.2 ± 1.3
Waln - D	32.7 ± 4.6	< 3.33	432 ± 6.5	44.8 ± 2.3	< 5.00	< 3.33
Waln - E	51.8 ± 4.1	< 3.33	453 ± 6.8	59.9 ± 1.3	< 5.00	< 3.33
Hazel - A	983 ± 20	< 3.33	36.6 ± 1.2	4.1 ± 0.87	29.0 ± 5.3	< 3.33
Hazel - D	769 ± 26	9.56 ± 0.77	31.3 ± 2.4	< 1.67	< 5.00	< 3.33
Hazel - E	838 ± 6.0	7.22 ± 0.1	31.7 ± 0.8	3.12 ± 1.3	< 5.00	< 3.33

TcP – tocopherol; TcT – tocotrienol; Waln – walnuts; Hazel – hazelnuts; A – harvested in CR (Poteč, location A); B – harvested in CR (Poteč, location B); C – harvested in CR (Valašské Klobouky); D – purchased on the market (Prague–Suchdol); E – purchased on the market (Kolín).

In hazelnuts unlike walnuts the major form of tocols was α-tocopherol, whose average content was (863.3 ± 109.2) mg kg^{-1}. It is more than was reported by Miraliakbari & Shahidi (2008) who have determined the content of α-tocopherol in hazelnuts in the range of 365.0–372.4 mg kg^{-1}. With respect to the total content of vitamin E and to the proportion of α-tocopherol as well, which is considered to be the most active biological form of vitamin E (Jiang et al., 2001; Ros et al., 2004; Wagner et al., 2004; Ros et al., 2015), hazelnuts are even better food source of this substance than the walnuts. β-Tocopherol was found only in purchased samples. According to Lavedrine et al. (1999) and Amaral et al. (2005), there is a link between the content of the major vitamers of vitamin E and the variety of walnuts. Probably there is also a link

between the content of other vitamers of vitamin E and the variety of other types of dry nuts. Unfortunately, nothing is known about the varieties of analyzed hazelnuts.

In samples of walnuts and hazelnuts grown and harvested in the different localities of the Czech Republic have been also determined tocotrienols but in significantly smaller quantities than tocopherols. In sample of hazelnut the most important and the only one detected form of tocotrienols was α-tocotrienol (29.0 ± 5.3) mg kg^{-1}. On the contrary, in all samples of walnuts the most important and the only one detected form of tocotrienols was γ-tocotrienol whose average content was 5.32 ± 0.75 mg kg^{-1}. In the purchased samples these vitamers were below the limit of detection. β-and γ-tocotrienols were below the limit of detection even in all samples of both walnuts and hazelnuts.

It seems likely that the duration and way of storage of nuts has a significant influence on the content of various forms of vitamin E, because the nuts grown in the Czech Republic were analyzed immediately after harvest. Nothing is known about the time of harvest and the way of storage of the purchased nuts. Silva et al. (2010) and Chun et al. (2005) came to the same conclusion that the contents of various forms of vitamin E depend on the duration of nuts storage.

On the basis of statistical analysis it was shown that in five tested samples of walnuts and three tested samples of hazelnuts differing in the place of origin, a statistically significant difference was found between the various forms of vitamin E, both between the purchased samples on the market and samples originating from the home production, as well as among the samples inside each group.

While statistically the highest content of majority form α-tocopherol was set in hazelnut nuts grown in the Czech Republic, in samples of walnuts both statistically the highest and the lowest content of majority form γ-tocopherol were always established in samples of nuts grown in the Czech Republic. According to Lavedrine et al. (1999) and Amaral et al. (2005), there is a link between the content of the major vitamers of vitamin E and the variety of walnuts. In addition to the different geographic origin of nuts this fact could also be the cause of the differentiation in the content of tocols in different samples of walnut and hazelnut.

CONCLUSIONS

Significant differences in the content and composition of the various forms of vitamin E among the 10 kinds of dry nuts were detected. The most important and the most represented vitamers of this lipophilic vitamin have been found α-tocopherol (almonds) and γ-tocopherol (pistachios). High amount of δ-tocopherol contained Brazil nuts. Tocotrienols (α and γ) were found only in a few selected species of dry nuts and γ- and δ-tocotrienols have been in all the samples below the limit of detection.

In the Czech Republic are the most cultivated and consumed walnuts and hazelnuts. Statistically significant differences were found in the content of the various forms of vitamin E among samples of walnut and hazelnut, which came from different geographical areas or were purchased in different location in the Czech Republic. α-and γ-tocotrienols have been also represented in walnuts and hazelnuts in minority quantity, but only in samples of nuts grown and harvested in the Czech Republic and analysed soon after harvest.

In conclusion it can be stated that dry nuts are an excellent source of vitamin E in human diet, but the content and mutual ratio of individual vitamers varies considerably according to the type of nut.

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REFERENCES

Amaral, J.S., Alves, M.R., Seabra, R.M. & Oliveira, B.P.P. 2005. Vitamin E composition of walnut (*Juglans regia* L.): A 3-year comparative study of different cultivars. *Journal of Agricultural and Food Chemistry* **53**, 5467–5472.

Brigelius-Flohe, R. & Traber, M.G. 1999. Vitamin E: function and metabolism. *Faseb Journal* **13**, 1145–1155.

Brufau, G., Boatella, J. & Rafecas, M. 2006. Nuts: source of energy and macronutrients. *British Journal of Nutrition* **96**, 24–28.

Choi, Y. & Lee, J. 2009. Antioxidant and antiproliferative properties of α-tocotrienol - rich fraction. *Food Chemistry* **114**, 1386–1390.

Comitato, R., Ambra, R. & Virgili, F. 2017. Tocotrienols: A Family of Molecules with Specific Biological Activities. *Antioxidants* **6**, Article Number 93.

Chun, J., Lee, J. & Eitenmiller, R.R. 2005. Vitamin E and oxidative stability during storage of raw and dry roasted peanuts packaged under air and vacuum. *Journal of Food Science* **70**, 292–297.

Fraser, G.E., Sabate, J., Beesen, W.L. & Strahan, T.M. 1992. A possible protective effect of nut consumption on risk of coronary heart-disease - the adventist health study. *Archives of Internal Medicine* **152**, 1416–1424.

Freitas, J.B. & Naves, M.M.V. 2010. Chemical composition of nuts and edible seeds and their relation to nutrition and health. *Revista De Nutricao-Brazilian Journal of Nutrition* **23**, 269–279.

Jiang, Q., Christen, S., Shigenaga, M.K. & Ames, B.N. 2001. Gamma-Tocopherol, the major form of vitamin E in the US diet, deserves more attention. *American Journal of Clinical Nutrition* **74**, 714–722.

Kader, A. 2001. Importance of Fruits, Nuts, and Vegetables in Human Nutrition and Health. *Perishables Handling Quarterly* **106**, 4–6.

Kornsteiner, M., Wagner, K.H. & Elmadfa, I. 2006. Tocopherols and total phenolics in 10 different nut types. *Food Chemistry* **98**, 381–387.

Lavedrine, F., Zmirou, D., Ravel, A., Balducci, F. & Blood, A.J. 1999. Cholesterol and walnut consumption: a cross-sectional survey in France. *Preventive Medicine* **28**, 333–339.

Li, L., Tsao, R., Yang, R., Kramer, J.K.G. & Hernandez, M. 2007. Fatty acid profiles, tocopherol contents, and antioxidant activities of heartnut (Juglans ailanthifolia var. Cordiformis) and persian walnut (*Juglans regia* L.). *Journal of Agricultural and Food Chemistry* **55**, 1164–1169.

Macrae, R., Robinson, R.K. & Sadler, M.J. 1993. *Encyclopaedia of Food Science, Food Technology and Nutrition*. 1st ed. Academic Press, London, Volume 1, 5365 pp.

Maguire, L.S., O'sullivan, S.M., Galvin, K., O'connor, T.P., O'brien, N.M., Fallico, B., Ballistreri, G., Arena, E. & Tokuşoğlu, Ö. 2004. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. *International Journal of Food Sciences and Nutrition* **55**, 185–212.

Miraliakbari, H. & Shahidi, F. 2008. Lipid class compositions, tocopherols and sterols of tree nut oils extracted with different solvents. *Journal of Food Lipids* **15**, 81–96.

Oliveira, R., Rodrigues, M. & Bernardo-Gil, M. 2002. Characterization and supercritical carbon dioxide extraction of walnut oil. *Journal of the American Oil Chemists' Society* **79**, 225–230.

Ozilgen, M. & Ozdemir, M. 2001. A review on grain and nut deterioration and design of the dryers for safe storage with special reference to Turkish hazelnuts. *Critical Reviews in Food Science and Nutrition* **41**, 95–132.

Ros, E., Nunez, I., Perez-Heras, A., Serra, M., Gilabert, R., Casals, E. & Deulofeu, R. 2004. A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. *Circulation* **109**, 1609–1614.

Ros, E. & Mataix, J. 2006. Fatty acid composition of nuts - implications for cardiovascular health. *British Journal of Nutrition* **96**, S29–S35.

Ros, E. 2015. Nuts and CVD. *British Journal of Nutrition* **113**, S111-S120.

Savage, G. P., Dutta, P.C. & McNeil, D.L. 1999. Fatty acid and tocopherol contents and oxidative stability of walnut oils. *Journal of the American Oil Chemists Society* **76**, 1059–1063.

Silva, M.P., Martinez, M.J., Casini, C. & Grosso, N.R. 2010. Tocopherol content, peroxide value and sensory attributes in roasted peanuts during storage. *International Journal of Food Science and Technology* **45**, 1499–1504.

Theriault, A., Chao, J.T., Wang, Q., Gapor, A. & Adeli, K. 1999. Tocotrienol: A review of its therapeutic potential. *Clinical Biochemistry* **32**, 309–319.

Tošić, S.B., Mitić, S.S., Velimirović, D.S., Stojanović, G.S., Pavlović, A.N. & Pecev-Marinković, E.T. 2015. Elemental composition of edible nuts: fast optimization and validation procedure of an ICP - OES method. *Journal of the Science of Food and Agriculture* **95**, 2271–2278.

Tsantili, E., Konstantinidis, K., Christopoulos, M.V. & Roussos, P.A. 2011. Total phenolics and flavonoids and total antioxidant capacity in pistachio (*Pistachia vera L.*) nuts in relation to cultivars and storage conditions. *Scientia Horticulturae* **129**, 694–701.

Wagner, K.H., Kamal-Eldin, A. & Elmadfa, I.U. 2004. Gamma-tocopherol - An underestimated vitamin? *Annals of Nutrition and Metabolism* **48**, 169–188.

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3D image analysis of the shapes and dimensions of several tropical fruits

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Abstract. Three dimension virtual models of Avocado (*Persea americana*), Salak (*Salacca zalacca*), Dragon fruit (*Hylocereus undatus*), Mango (*Mangifera indica*), Coconut (*Cocos nucifera*) using 3D scanner Intel RealSense were determined. Calculated models based on arithmetic and geometric diameter were also determined. From statistically analysis implies that virtual models on significance level 0.05 are significantly different with calculated values based on arithmetic or geometric diameter.

Key words: virtual, model, scanner, physical properties.

INTRODUCTION

Accurately understanding to the shapes and dimensions of fruits is one of the key factors which is important for creating mathematical models of their mechanical behaviour (Petrů et al., 2012; Petrů et al., 2014; Herák, 2016). Nowadays with the advent of new progressive technologies the construction process of food processing technologies increasingly utilize 3D virtual models as tools for describing mechanical behaviour of individual fruits as well as bulk fruits (Lizhang et al., 2013; Zhan et al., 2013). These 3D models are usually used in modern simulation processes such are for instance systems based on finite element method (Petrů et al., 2012; Petrů et al., 2014).

Tropical fruits such are Avocado (*Persea americana*), Salak (*Salacca zalacca*), Dragon fruit (*Hylocereus undatus*), Mango (*Mangifera indica*), Coconut (*Cocos nucifera*) are one of the main agricultural products of Southest Asia and they are of great interest to food processors using virtual models to appropriately design the entire food processing chain with respect to high level quality of final products. In previous studies, they were published several 3D models of apple, banana, orange and pear (Barnea et al., 2016). However, the 3D virtual models of tropical fruits which are examined in this study were not described yet in professional literature.

Thus the aim of this research is to determine the three dimension virtual models of Avocado, Salak, Dragon fruit, Mango, Coconut and these virtual models to verify and to compare with real fruits.

MATERIALS AND METHODS

Sample

Avocado (*Persea americana*), Salak (*Salacca zalacca*), Dragon fruit (*Hylocereus undatus*), Mango (*Mangifera indica*), Coconut (*Cocos nucifera*) (Fig. 1) obtained from Medan, Indonesia, were used for the experiment.

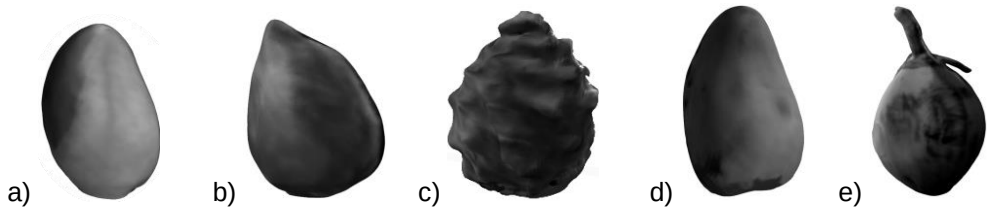

Figure 1. 3D virtual models of Avocado (a); Salak (b); Dragon fruit (c); Mango (d); Coconut (e).

Fruits dimensions

Ten pieces of each fruit were used for determination of dimensions. Dimensions of each fruit, length L (mm), width W (mm), thickness T (mm) were determined by digital calliper (Kmitex 6000.20, Kimtex, Stehelcevs, Czech Republic).

Geometric mean diameter D_g (mm) and arithmetic mean diameter D_a (mm) were calculated using the following equations (Eq. 1; Eq. 2), (Mohsenin, 1970)

$$D_g = \sqrt[3]{W \cdot T \cdot L} \tag{1}$$

$$D_a = \frac{W + T + L}{3} \tag{2}$$

Cross section area based on arithmetic diameter S_a (mm^2) and cross section area based on geometric diameter S_g (mm^2) were calculated by Eq. 3 and Eq. 4.

$$S_a = \frac{1}{4} \cdot \pi \cdot D_a^2 \tag{3}$$

$$S_g = \frac{1}{4} \cdot \pi \cdot D_g^2 \tag{4}$$

Volume based on arithmetic diameter V_a (mm^3) and volume based on geometric diameter V_g (mm^2) were calculated by Eq. 5 and Eq. 6.

$$V_a = \frac{1}{6} \cdot \pi \cdot D_a^3 \tag{5}$$

$$V_g = \frac{1}{6} \cdot \pi \cdot D_g^3 \tag{6}$$

Virtual model

Three dimensional virtual models were created with aid of 3D scanner Intel RealSense. Virtual volumes V_v (mm^3) were determined by Meshmixer (Autodesk Meshmixer, Autodesk Inc., San Rafael, USA). The virtual model is shown in Fig. 2.

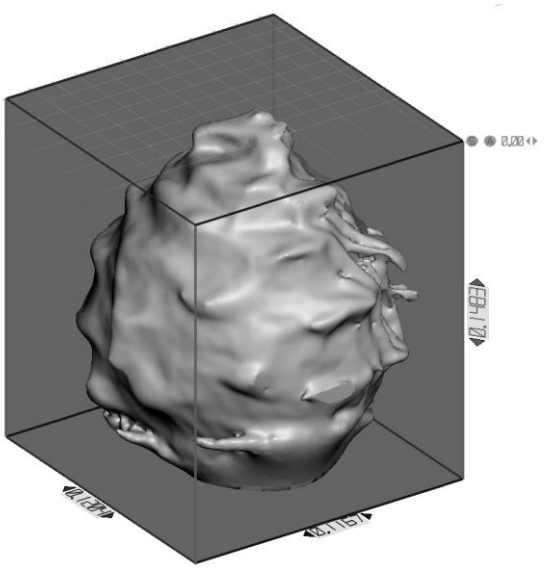

Figure 2. View a virtual model in Meshmixer software.

RESULTS AND DISCUSSION

Measured and calculated dimensions for individual fruits are presented in Table 1. Calculated diameters, volumes and areas are shown in Table 2. From statistical analysis (Table 3) it is evident that determined volumes of virtual models are significantly different on the level of significance 0.05 than calculated models based on arithmetic or geometric diameter. This statement is also clear from the ratio between calculated volumes and virtual volumes such is presented in Table 2.

Table 1. Measured dimensions and calculated diameters, volumes and areas

Fruit	L, mm	W, mm	T, mm	V_v, mm^3
Avocado (SD)	98 (2)	111 (3)	99 (4)	508,049 (16,171)
Salak (SD)	116 (8)	134 (29)	115 (3)	794,008 (174,283)
Dragon fruit (SD)	54 (2)	66 (5)	51 (4)	81,576 (16,750)
Mango (SD)	88 (1)	127 (1)	86 (7)	483,310 (19,745)
Coconut (SD)	160 (4)	242 (43)	164 (10)	2,475,240 (130,480)

Based on measured data (Table 1 and 2), it is evident that 3D models can provide more accurate information about the true geometric properties of the fruit. From these models (Fig. 1) imply that the shapes of the virtual model are similar to shapes of the real fruits and that they can accurately describe surface structures. From determined amounts (Table 1 and 2) it follows that volume standard deviations of analysed fruits except Salak are around fifteen percent which is usual for agricultural products (Mohsenin, 1970). However volume standard deviation of Salak is higher than standard deviation of other analysed fruits that is given by varied and complicated shape which is also evident from Fig. 1c and Fig. 2 (Ismail & Bakar, 2018).

Table 2. Calculated diameters, volumes and areas

Fruit	D_a, mm	D_g, mm	S_a, mm^2	S_g, mm^2	V_a, mm^3	V_g, mm^3	V_a/V	V_g/V
Avocado	103	102	8,263	8,236	565,245	562,418	1.11	1.11
(SD)	(1)	(1)	(178)	(205)	(18,234)	(21,047)	(0.07)	(0.08)
Salak	122	121	11,658	11,581	951,446	941,526	1.20	1.19
(SD)	(13)	(13)	(2,554)	(2,411)	(311,044)	(292,756)	(0.65)	(0.63)
Dragon fruit	57	57	2,555	2,520	97,303	95,308	1.19	1.17
(SD)	(4)	(4)	(319)	(312)	(18,178)	(17,677)	(0.47)	(0.46)
Mango	100	98	7,871	7,596	525,481	498,131	1.09	1.03
(SD)	(1)	(1)	(179)	(175)	(17,946)	(17202)	(0.08)	(0.08)
Coconut	189	185	28,005	26,891	3,529,675	3,319,168	1.43	1.34
(SD)	(10)	(6)	(2,836)	(1,620)	(535,592)	(299,898)	(0.29)	(0.19)

From already published studies as well as from this conducted study follows that these determined 3D virtual models can be used as tools in modern simulation processes such are for instance systems based on finite element method (Petrů et al., 2012; Lizhang et al., 2013; Zhan et al., 2013; Petrů et al., 2014). The benefit in using these models is mainly for accurate simulation of processing, storage and transportation of fruits (Barnea et al., 2016).

Table 3. Statistical analysis measured values with models' values

Compared amount		V_a/V_v	V_g/V_v
T test value	Avocado	9.740	7.803
	Salak	2.296	2.233
	Dragon fruit	2.860	2.539
	Mango	10.752	6.121
	Coconut	4.259	6.630
T test critical value		1.833	1.833

CONCLUSIONS

The three dimension virtual models of Avocado, Salak, Dragon fruit, Mango, Coconut were determined and compared with real fruits. The measured values using standard methods were compared with the values from the models. From statistically analysis implies that this model on significance level 0.05 are significantly different with calculated values based on arithmetic or geometric diameter. Based on measured data, it is evident that 3D models can provide more accurate information about the true geometric properties of the fruit. They can be used, for example, in the construction of machines and equipment for harvesting fruit.

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REFERENCES

Barnea, E., Mairon, R. & Ben-Shahar, O. 2016. Colour-agnostic shape-based 3D fruit detection for crop harvesting robots. *Biosystems Engineering* **146**, 57–70.

Herák, D. 2016. Image analysis of the shapes and dimensions of Teff seeds (Eragrostis tef). *Agronomy Research* **14**, 1299–1305.

Ismail, N. A. & Bakar, M.F.A. 2018. Salak-Salacca zalacca. In *Exotic Fruits*. pp. 383–390.

Mohsenin, N. 1970. *Physical properties of plant and animial materials. Vol. 1. Structure, physical characterisitics and mechanical properties. Physical properties of plant and animial materials.* New York, Gordon and Breach Science Publishers, 742 pp.

Petrů, M., Novák, O., Herák, D. & Simanjuntak, S. 2012. Finite element method model of the mechanical behaviour of Jatropha curcas L. seed under compression loading. *Biosystems Engineering* **111**, 412–421.

Petrů, M., Novák, O., Herák, D., Mašín, I., Lepšík, P. & Hrabě, P. 2014. Finite element method model of the mechanical behaviour of Jatropha curcas L. bulk seeds under compression loading: Study and 2D modelling of the damage to seeds. *Biosystems Engineering* **127**, 50–66.

Lizhang, X., Yaoming, L., Zheng, M., Zhan, Z. & Chenghong, W. 2013. Theoretical analysis and finite element simulation of a rice kernel obliquely impacted by a threshing tooth. *Biosystems Engineering* **114**, 146–156.

Zhan, Z., Yaoming, L., Zhenwei, L. & Zhiqiang, G. 2013. DEM simulation and physical testing of rice seed impact against a grain loss sensor. *Biosystems Engineering* **116**, 410–419.

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Effects of processing conditions on physical parameters of triticale flakes

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Abstract. Consumer interest in breakfast cereal flakes has increased during the last few years. Various technologies, used to produce flakes, significantly influence their quality parameters and shelf-life stability. The main purpose of the present research was to investigate how different processing methods affect the physical parameters of triticale flakes. For obtaining the flakes, cleaned whole triticale grains were treated using the following technologies: dry processing (hot air), steam processing and soaking with subsequent steaming. For preparing the flakes different kilning methods and traditional flaking rolls were used. Traditionally made rolled and dried whole grain triticale flakes were analysed as a control sample. Using standard methods, the flakes' moisture content, water activity, microstructure, swelling capacity and colour changes were analysed. The gap settings of flake rollers do not influence significantly ($P < 0.05$) changes of starch during processing. However, the starch granules were fully transformed into sugars in the flake samples with greater thickness. Non-significant ($P < 0.05$) steaming and hot air drying (toasting) conditions' effects were observed on the changes of the starch granules during processing. Strong correlation was determined during the analysis of water activity and moisture content. The moisture content of the ready- to-eat flakes varied from 2.54% to 10.66%, and the water activity value was from 0.108 to 0.494. Compared with traditionally processed flakes (control sample) the colour of the flakes prepared using other technologies changed significantly, the ΔE values varied from 9.587 to 18.554. The colour of the soaked-steamed-rolled-hot air dried samples was similar but those significantly differed from the colour of soaked-dried-rolled-hot air dried flake samples. The soaked-dried-rolled-hot air dried flakes were darker compared with other analysed flake samples.

Key words: triticale flakes, technology, quality.

INTRODUCTION

Nowadays, breakfast cereals are one of the basic elements in the human diet (Mathebula et al., 2017). In general, breakfast cereals are processed grains for human consumption, typically ready-to-eat. However, ready-to-eat cereals are produced by a variation of several technological operations such as cooking, shape forming, finish drying, flavouring, sweetening, enrichment with vitamins and minerals (Caldwell et al., 2016). Breakfast cereal products historically have been used as milled

grains of wheat and oats, that required further cooking at home prior to consumption. Nowadays, due to the efforts to reduce in-home preparation time, breakfast cereal technology has evolved from a simple procedure to the manufacturing of ready-to-eat products that are convenient and quickly prepared (Mbaeyi-Nwaoha, 2016). Most ready-to-eat cereals may be grouped into several general categories regarding their manufacturing processes: flaked cereals (corn flakes, wheat flakes, and rice flakes), including extruded flakes, gun-puffed whole grains, extruded gun-puffed cereals, shredded whole grains, extruded and other shredded cereals, oven-puffed cereals, granola cereals, extruded expanded cereals, baked cereals, compressed flake biscuits, muesli-type products, and filled bite-size shredded wheat (Fast, 2000). The basic flake making process includes the following main processing stages: starch gelatinisation, browning reaction, interrupting the enzymatic process, sugars dextrinization, caramelisation and moisture reduction (Matz, 1991).

Traditionally, in the world flaked, puffed, shredded, and cooked ready-to-eat cereals are made from whole grains or parts of grains of corn, wheat, rice, or oat (Caldwell et al., 2016). Nowadays, the manufacturers are paying more and more attention to use untraditional cereals in flake production like triticale. For example, triticale flakes should be used as one of the compounds in flake mixtures obtaining a ready-to-consume product with elevated nutritive value, good sensory properties and appropriate shelf-life (Kince et al., 2017).

In general, triticale (× *Triticosecale* Wittmack) was developed by crossing rye and wheat (McGoverin et al., 2011). The results presented in a review by Zhu (2018) conclude, that world production of triticale has been increased during the last few years because triticale has higher starch, arabinoxylans, β-glucans, fructans, lignins, proteins (including α-amylase), alkylresorcinols, vitamins, and polyphenols contents. However, Fraś et al. (2016) indicate, that triticale varieties with a favourable chemical composition from a nutritional and technological perspective can be a good material for flour and bread production. Arendt & Zannini (2013) indicate, that triticale offers a better amino acid balance, mainly due to the high lysine content, resulting a greater biological value than wheat protein.

The main purpose of the present research was to investigate how different processing methods affect the physical parameters of triticale flakes.

MATERIALS AND METHODS

Flake sample preparation

In the present experiment three different technologies for flake production were used: commercially processed flakes (control sample) - purchased ready-made triticale whole grain flakes (mixture of several cultivars) from JSC Dobeles dzirnavnieks (Latvia). The processing technology includes cleaned triticale grain steaming, rolling and drying.

Samples with the first code 1 (cooking technology 1) - cooked triticale flakes were obtained by 1 min steaming, and applying pressure 0.5 ± 0.1 bar for 10 min and at different flaking roll gap settings (respectively, sample 1/3/1 - 0.06 ± 0.01 mm and sample 1/4/1 - 0.04 ± 0.01 mm). Processed triticale flakes were transferred for drying into Mitchell Bach Dryer (GmbH Baker Perkins, UK) for 35 ± 5 min at 80 ± 1 °C. Samples with the first code 2 (cooking technology 2) - triticale grains were transferred

into Rotary Cereal Cooker (GmbH Baker Perkins, UK) with adding of extra water 20 ± 3% from grains total amount, after that samples were 5 min steamed and cooked 30 ± 5 min at pressure 1.2 bar. Samples 2/1/2/1, 2/1/3/1, 2/1/4/1 differed by flaking roll gap settings, respectively, 0.10 ± 0.01, 0.06 ± 0.01 and 0.04 ± 0.01 mm gap. Processed triticale flakes were transferred for drying into Mitchell Bach Dryer (GmbH Baker Perkins, UK) at 80 ± 1 °C for 30 ± 5 min.

Toasting procedure was performed in decreasing temperatures under 200 ± 10 °C for less than one minute in the toaster New Thermoglide Toaster Rig (GmbH Baker Perkins, UK).

Moisture content

Moisture content of triticale flakes was determined using air-oven method (AACC Method 44-15.02) by drying of 5.00 ± 0.05 g sample in the oven Memmert (GmbH Memmert, Germany) - for 1 hour at 110 ± 5 °C temperature. The analyses of moisture content were done in triplicate.

Water activity (aw)

Water activity (aw) was determined using LabSwift-aw (AG Novasina, Switzerland) equipment. Ground flakes were used for the determination of water activity. The analyses of water activity were done in triplicate.

Colour

The colour of triticale flakes was determined using ColorTec-PCM/PSM (Accuracy Inc., USA) in the colour system CIEL*a*b* (L*0 = black, 100 = white, a* + value = red, a* - value = green, b* + value = yellow, b* - value = blue). Ground flakes were used for the determination of colour. Ten readings for each sample determined the colour, and the total colour difference (ΔE) was calculated using Minolta equations (Chakrborty et al., 2011).

Starch microstructure

Starch microstructure was determined using the method described by Rakcejeva et al. (2016) using triocular microscope 'Leica DM 2500 LED HD' (Leica Microsystems, Germany); via 10 × 20 magnification of the microscope.

Statistical analysis

Microsoft Excel 2013 software was used for mathematical data processing. The hypothesis of the thesis was verified with *P*-value method, and factors were evaluated as significant if $P < \alpha_{0.05}$. For the interpretation of the results it was accepted, that $\alpha = 0.05$ with 95% confidence. Tukey's test was used to determine differences among the samples.

RESULTS AND DISCUSSION

Moisture content and water activity. Moisture content influences not only the shelf-life of foodstuffs but also their taste, texture, weight and appearance. Usually, a slight deviation from a defined necessary value has significant impact on the physical properties of a food product. On the other hand, water activity (a_w) of the food product

represents the energy status of water molecules, which influences various biochemical reaction and microbial growth in food products (Labuza, 1975).

Moisture content 10–12% is sufficient to provide appropriate quality for dry cereal product during their storage. In the present research we found a significant technologies' impact ($P < 0.05$) on the moisture content of flakes and water activity changes in triticale flakes. The moisture content of control flake samples was relatively high 10.665% compared with other flake samples (Table 1), that can mainly indicate their comparatively lower quality stability during storage. However, a strong correlation (R = 0.98604) was established between moisture content and water activity changes of the analysed whole-grain triticale flake samples. Significant impact of the different technologies on moisture content and a_w was also observed (Table 1). Compared with control flake sample, it is possible to reduce significantly ($P < 0.05$) the moisture content in the product by using different technological ways maximally by 4.2 times (sample 1/4/1). On the other hand, higher moisture content was obtained in samples 2/1/2/1 (6.23%), 2/1/3/1 (4.68%) and 2/1/4/1 (4.42%) that could be explained with extra water addition during flake processing (cooking technology 2). At the same time, the moisture content of ready-to-consume products should be lower, if the density of flakes is lower.

Table 1. Changes of flake moisture and water activity (a_w)

Flake samples	Moisture content, %	a_w
Control	10.67 ± 0.16^a	0.494 ± 0.040^a
1/3/1	3.62 ± 0.01^d	0.155 ± 0.010^d
1/4/1	2.55 ± 0.01^d	0.108 ± 0.006^d
2/1/2/1	6.23 ± 0.05^b	0.339 ± 0.004^b
2/1/3/1	4.68 ± 0.05^c	0.231 ± 0.001^c
2/1/4/1	4.42 ± 0.02^c	0.225 ± 0.001^c

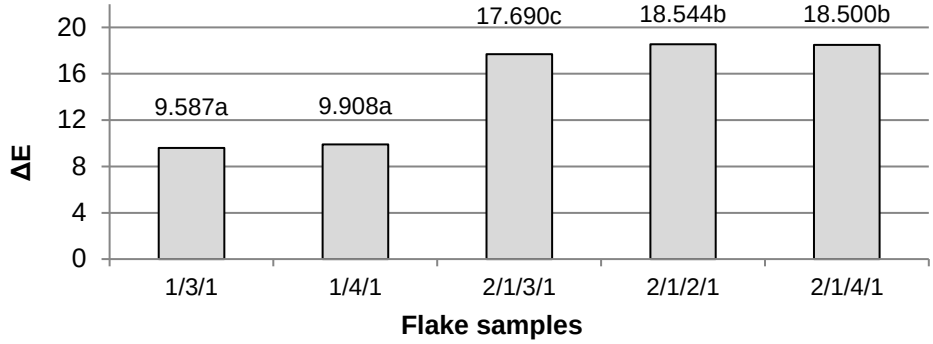
Different letters represent significant differences between values ($P < 0.05$).

Katz & Labuza (1981) indicated in their study, that critical water activities, where the crispy products became sensory unacceptable, generally are in the 0.35–0.50 a_w range. However, Waichungo et al. (2000) indicated, that the critical water activity, the water activity at which the textural characteristics of the extruded puffed corn starch samples significantly changed, was determined to be between 0.36–0.58, which mainly confirms the results obtained from this particular study.

Colour changes. Traditionally, consumers accept food because of their visual aspects before tasting and purchase. Therefore, colour is the most decisive element for food choice (Lee et al., 2013). In this case, ΔE colour value can help evince colour differences of the analysed products, through calculation of values, which correlates with the human visual attitude to differences between two presented colours (McGrath et al., 2017).

In the present research a significantly different ($P < 0.05$) colour was obtained for all flakes with different processing method compared to control flake samples (Fig. 1). After processing, the flakes become two times darker colour than the control sample, especially samples 2/1/2/1 and 2/1/4/1 (ΔE = 18.500–18.544). The darker colour of the obtained samples mainly could be explained with the additional drying during flake production and higher (1.2 bar) cooking pressure (cooking technology 2). However, the

samples produced using technology 1 were slightly lighter in colour, but they were darker compared with the control flake sample (ΔE = 9.908–9.587). Ilo & Berghofer (1999) in their research concluded that the kinetic rate constants for the colour for the extruded products depend on the product temperature and feed moisture content. Therefore, lower processing temperatures, time and moisture content of raw material could provide lighter colour of triticale flakes in the future.



Different letters represent significant differences between values ($P < 0.05$)

Figure 1. Colour differences of analysed flake samples.

Flake swelling. The main changes with starch granules occur in the product during its thermal processing. Food porosity and relative density, as well as granule diameter, granule size distribution play an important role in mastication, bolus formation, and transportation of the bolus in the human gastrointestinal tract and in starch hydrolysis (Alam et al., 2017). For example, rolled oats usually are partially cooked during processing and softened because of the steam treatment. Porridge made from rolled oats requires only a brief domestic cooking time to complete the process of starch gelatinisation (Kent & Evers, 1994). The retrogradation of starch also occurs during the thermal processing of breakfast cereals, resulting textural changes such as increased hardness and reduced stickiness (Kong & Singh, 2011).

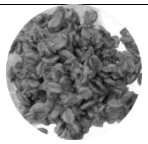
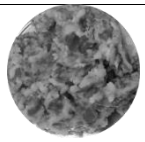
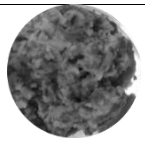
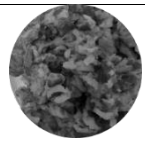
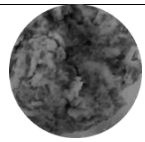
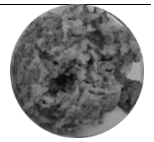
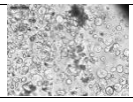
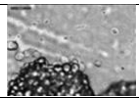
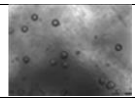
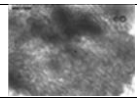
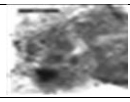
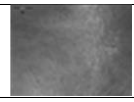
In the present experiments a significant technology influence ($P < 0.05$) was observed in the starch granules of the analysed flake samples. Mainly, starch granules were distinctly visible in the control flake samples (Table 2). However, after making flakes by using different technological parameters, the size of starch granules changed significantly ($P < 0.05$). Use cooking technology 1, the main influence was observed on triticale flake thickness, in the thin samples starch was practically gelatinised (Table 2). Similar results were obtained when using cooking technology 2. However, during flake manufacturing process using both technologies, the size of starch granules changed significantly (approximately by 18%) compared with control flake samples. Starch gelatinisation processes occur more quickly in thin samples processed under pressure and for longer time.

Gelatinisation and retrogradation are the key functional properties of starch that indicate the quality and nutritional value of starchy foods such as breakfast cereals. However, starch retrogradation is advantageous in some technologies, such as in the production of breakfast cereals, due to modification of the structural, mechanical, and

sensory properties of ready-to-eat products (Wang et al., 2015). For example, beans that had been pressure cooked have significantly increased rapidly digestible starch and slowly digestible starch, and decreased resistant starch. But the increased digestibility of rice starch is associated with lower amylose content (Toutounji, 2015).

In the present research for visual appearance of cereal flakes the water absorption capacity of breakfast cereals was determined using method described by Kince et al. (2017) with modifications. Starch granule degradation observed with microscope (Table 2) had strong visual correlation with unacceptable appearance of flakes, soaked in water. The samples with malted starch granules were similar to porridge. However, flake samples with visible starch granules (18.19 μm and 18.32 μm) had better consistency and apperance (samples 1/3/1 and 2/1/2/1). However, it should be noted, that the control flakes practically had not changed their appearance after soaking, which mainly could indicate their inconvenient mastication during their future use in human diet.

Table 2. Microstructure and appearance of cereal flakes

Flake sample	Control	1/3/1	1/4/1	2/1/2/1	2/1/3/1	2/1/4/1
Appearance of cereal flakes						
Overview of starch granules						
Equivalent diameter of starch granules, μm	22.04 ± 8.03	18.19 ± 4.38	gelatinised starch*	18.32 ± 2.65	gelatinised starch	gelatinised starch

*gelatinised starch – starch granules were completely dissolved.

The changes in pressure and temperature during flake processing increase the content of damaged starch up to 80% and induce deep structural modifications of the starch granules which become no longer identifiable - starch is gelatinised. These physical changes may affect starch digestibility too, because the damaged starch is more exposed to amylolytic enzymes than native starch (Cattaneo et al., 2015).

CONCLUSIONS

1. The results of the present research demonstrate significant influence of technology ($P < 0.05$) on the physical properties (moisture content, water activity, colour and swelling capacity) of ready-to-eat flakes.

2. As a result, it is possible to achieve better ready-to-eat product quality by variation of flake thickness and extra water addition during processing - the physical parameters of thicker (0.10 ± 0.01 mm gap) sample changed less than those of the thinner ones.

3. It is possible to significantly influence the starch gelatinisation process resulting increased digestibility.

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REFERENCES

Alam, S.A., Pentikäinen, S., Närväinen, J., Holopainen-Mantila, U., Poutanen, K. & Sozer, N. 2017. Effects of structural and textural properties of brittle cereal foams on mechanisms of oral breakdown and in vitro starch digestibility. *Food Research International* **96**, 1–11.

Arendt, E.K. & Zannini, E. 2013. Triticale. In: *Cereal grains for the food and beverage industries.* Woodhead Publishing Series in Food Science, Technology and Nutrition. pp. 201–219.

Caldwell, E.F., McKeehen, J.D. & Kadan, R.S. 2016. Cereals: Breakfast Cereals. In: *Encyclopedia of Food Grains*. Second Edition, pp. 262–267.

Cattaneo, S., Hidalgo, A., Masotti, F., Stuknytė, M., Brandolini, A. & De Noni, I. 2015. Heat damage and in vitro starch digestibility of puffed wheat kernels. *Food Chemistry* **188**(1), 286–293.

Chakrborty, R., Bera, M., Mukhopadhyay, P. & Bhattacharya, P. 2011. Prediction of optimal conditions of infrared assisted freeze-drying of aloe vera (*Aloe barbadensis*) using response surface methodology. *Separation and Purification Technology* **80**(2), 375–384.

Fast, R.B. 2000. Chapter 2: Manufacturing Technology of Ready-to-Eat Cereals. In*: Breakfast Cereals and How They Are Made.* Second Edition. pp. 17–54.

Fraś, A., Gołębiewska, K., Gołębiewski, D., Mańkowski, D.R., Boros, D. & Szecówka, P. 2016. Variability in the chemical composition of triticale grain, flour and bread. *Journal of Cereal Science* **71**, 66–72.

Ilo, S. & Berghofer, E. 1999. Kinetics of colour changes during extrusion cooking of maize grits. *Journal of Food Engineering* **39**(1), 73–80.

Katz, E.E. & Labuza, T.P. 1981. Effect of water activity on the sensory crispness and mechanical deformation of snack food products. *Journal of Food Science* **46**(2), 403–409.

Kent, N.L. & Evers, A.D. 1994. *Technology of cereals*. Elsevier Science Ltd. pp. 244–245.

Kince, T., Galoburda, R., Klava, D., Tomsone, L., Senhofa, S., Straumite, E., Kerch, G., Kronberga, A., Sturite, I., Kunkulberga, D. & Blija, A. 2017. Breakfast cereals with germinated cereal flakes: changes in selected physical, microbiological, and sensory characteristics during storage. *European Food Research and Technology* **243**(9), 1497–1506.

Kong, F. & Singh, R.P. 2011. Chemical deterioration and physical instability of foods and beverages. In. *Food and Beverage Stability and Shelf Life*. pp. 29–62.

Labuza, T.P. 1975. Sorption phenomena in foods: Theoretical and practical aspects. In: *Theory, determination and control of physical properties of food materials*. Springer Netherlands, Dordrecht. pp. 197–219.

Lee, S.-M., Lee, K.-T., Lee, S.-H. & Song, J.-K. 2013. Origin of human colour preference for food. *Journal of Food Engineering* **119**(3), 508–515.

Mathebula, M.W., Mandiwana, K. & Panichev, N. 2017. Speciation of chromium in bread and breakfast cereals. *Food Chemistry* **217**(15), 655–659.

Matz, S.A. 1991. Processing ready-t-eat breakfast cereals. In: *The chemistry and technology of cereals as food and feed*. Second edition, pp. 654–655.

Mbaeyi-Nwaoha, I.E. & Uchendu, N.O. 2016. Production and evaluation of breakfast cereals from blends of acha and fermented soybean paste (okara). *Journal of Food Science and Technology* **53**(1), 50–70.

McGoverin, C.M., Snyders, F., Muller, N., Botes, W., Fox, G. & Manley, M. 2011. A review of triticale uses and the effect of growth environment on grain quality. *Journal of the Science of Food and Agriculture* **9**(7), 1155–1165.

McGrath, J.R., Matthew, M.B. & Jr, E.H. 2017. Replicating Red: Analysis of ceramic slip colour with CIELAB colour data. *Journal of Archaeological Science* **14**, 432–438.

Rakcejeva, T., Klava, D., Cinkmanis, I., Galoburda, R., Straumite, E. & Kronberga, A. 2016. Starch breakdown and formation of sugars during triticale grains germination. In: *Proceedings of 8th International Congress FLOUR-BREAD '15 & 10th Croatian Congress of Cereal Technologists*. pp. 188–200.

Toutounji, M.R. 2015. *Molecular characterisation of breakfast cereals and rice to understand their digestibility*. *A thesis submitted in fulfilment of the requirement of Master of Science (Honours)*. University of Western Sydney, Australia, 220 pp.

Waichungo, W.W., Heymann, H. & Heldman, D.R. 2000. Using descriptive analysis to characterize the effects of moisture sorption on the texture of low moisture foods. *Journal of Sensory Studies* **15**(1), 35–46.

Wang, S., Li, C., Copeland, L., Niu, Q. & Wang, S. 2015. Starch retrogradation: A comprehensive review. *Food Science and Food Safety* **14**, 568–585.

Zhu, F. 2018. Triticale: Nutritional composition and food uses. *Food Chemistry* **241**(15). 468–479.

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https://doi.org/10.15159/AR.18.101

Sonochemical effects on food emulsions

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Abstract. Acoustic cavitation of food emulsions is widely applied as the main processing method to improve the quality of a finished product and its organoleptic characteristics, as well as to increase production performance. To identify the optimal modes of ultrasonic emulsification, we propose a model of emulsion droplet breakup in an acoustic cavitation field, which allows us to determine the dependence of emulsion droplets' diameter on exposure time and intensity of action. The developed models enabled us to pioneer complex research of the dependence of emulsion droplets' diameter on time given the maximum radius of cavitation bubbles and physical properties of liquid phases in the emulsion composition. We carried out the first complex theoretical and practical research of how shapes and positions of absolutely fixed boundaries influence the propagation of oscillations in a activating liquid medium (food emulsion). To verify the adequacy of the obtained theoretical models, we studied the dependence of emulsion droplets' breakup rate (by the example of a model water/oil emulsion) on the exposure time and the intensity of ultrasonic action. The calculation results revealed that the results of a series of experiments and the results obtained with the use of the developed mathematical model are consistent. Based on the theoretical data obtained, we designed an industrial flow- type acoustic cavitation device aimed at acting on food emulsions; it differs from analogous devices in that it has within it a cylindrical wave acting through solid walls of the tunnel for transmitting processed liquid.

Key words: ultrasound, cavitation, model, food emulsion, sonochemistry.

INTRODUCTION

Emulsification processes are one of the most important foundations of the modern food industry (Taylor, 1963; Khmelev, 2012). Acoustic cavitation is one of the most promising means of forming emulsions, since it has a low energy intensity, the possibility of obtaining fine emulsions (up to fractions of 3 μm and less), and high productivity (Álvarez et al., 2007; Khmelev et al., 2014; Krasuly, et al., 2016).

To form a cavitation zone with maximum possible energy effect in liquid media of different properties by means of secondary effects (shock and capillary waves), it is necessary to set certain modes according to the intensity of introduced ultrasonic oscillations, and to ensure certain conditions the volume of a processed medium, the form of a technological volume, the exposure time, etc. (Khmelev, 2007).

The industrial application of ultrasonic devices in modern food production necessitates searching for and providing the most effective (optimal) modes of action on food media (Brotchie et al., 2009; Krasulya, 2013). Setting and maintenance of optimal ultrasonic mode is possible only by knowing the implementation mechanisms of ultrasonic processes and identifying and continuously monitoring the processing media parameters which characterize the change in their properties (Chandrapala, et al., 2012).

Having generalized theoretical and practical results on the research topic, we established that most of the existing theories which describe the process of emulsification under the action of cavitation are aimed at study the behavior of a single cavitation bubble and its interaction with emulsion droplets (Kentish et al., 2008; Patista & Bates, 2008; Krasulya et al., 2014; Shanmugam & Ashokkumar, 2015). However, in real processes involving ultrasonic cavitation of liquid food media, their cavitation zone is generated, as it is practically impossible to obtain a single cavity. Therefore, it is obvious that the efficiency of ultrasonic will be determined by the macroscopic characteristics of the activating medium as a whole (wave impedance, volume content of bubbles or cavitation index, etc.) (Truhaut, 1991).

The purpose of this work is to identify the optimal modes of exposure and the conditions for the propagation of oscillations to create a uniform ultrasonic field throughout a mixture of two mutually insoluble fluids and to obtain emulsions with given dispersion characteristics.

MATERIALS AND METHODS

To study the effects of acoustic cavitation emulsification, we developed a laboratory reactor where the source of ultrasonic treatment is an ultrasonic device, consisting of an electronic generator and a radiator with an immiscible titanium waveguide, which has a mushroom-shaped running end. The device allows for vertical shifting of the working body in order to carry out different modes of exposure.

Since, according to the generalization of the results of theoretical and practical studies, the maximum efficiency of cavitation is ensured at a frequency of 20–25 kHz, we focused special attention on detecting the intensity of ultrasonic oscillations necessary to obtain emulsion droplets of the required diameter in a specific frequency range.

We used 'vegetable oil/water' food emulsions as objects for the research. Sunflower-seed oil (deodorized, refined with a 99.9% fat content) was used as vegetable oil. The vegetable oil used had the following chemical and physical characteristics of crude vegetable oil: Relative density (at 20 °C) – 0.9 ± 0.02 C/water, Refractive index – 1.464 ± 0.002 (ND 40 °C), Saponification Value – 191 ± 0.5 mg KOH g^{-1} oil, Iodine value – 119 ± 0.5. The tap water used had the following quantitative characteristics: hydrogen index 6.5, total hardness – 6.8 mg L^{-1}, phenol index – 0.20 mg L^{-1}. To conduct the experiment, we used food emulsions with a different ratio of ingredients:

- the first option – 30% vegetable oil + 70% water;
- the second option – 50% vegetable oil + 50% water;
- the third option – 5% vegetable oil + 95% water;

– the fourth option – 30% vegetable oil + 70% water.

For the preparation of reference samples, we used technological device an RKU-type with a piezoceramic radiator (Russia). Ultrasound details: frequency mechanical oscillations – 22 ± 1.5 kHz, the maximum power consumption – 1,000 W. The time of ultrasound exposure varied from 20 to 40 minutes, and the power of sonication varied from 500 W to 1,000 W:

– mode №1 – 20 min, 700 W;
– mode №2 – 20 min, 1,000 W;
– mode №3 – 20 min, 500 W;
– mode №4 – 40 min, 500 W.

The temperature of the food emulsion before its exposure to ultrasound was 15 °C.

The distribution of fat globules in food emulsion according to its concentration and processing time was photographed with the help of an optical microscope.

The software package Altami Studio was used to process the obtained fat globule images.

Mathematical measurement processing was carried out by conventional statistics methods and expressed as the arithmetic mean (M) and it's standard error (m). We used the Mann – Whitney test (U) to determine the statistically significant differences between the test and reference groups. Differences were considered significant at $P < 0.05$. Statistical interrelations were studied using nonparametric correlation analysis by calculating the Spearman correlation coefficients (Rs). Results were obtained using generally accepted methods of statistical analysis and expressed as an arithmetical average and its standard deviation. Differences were deemed significant where $P < 0.05$.

RESULTS AND DISCUSSION

To identify the optimal ultrasonic emulsification modes, we developed a model of emulsion droplet breakup in an acoustic cavitation field. The model is based on droplet deformation Eq. (1) proposed by foreign researchers:

$$m\ddot{x} = F - kx - d\dot{x} \quad (1)$$

where m is the mass of the droplet, kg; F is the external force acting on the droplet from the direction of fluid flow, N; k is the coefficient of elasticity of the droplet, N m^{-1}; and d is the damping ratio of the droplet, kg s^{-1} (Taylor, 1963).

According to this equation, the droplet wall is regarded as a load of mass m on a spring (equivalent to the surface tension forces) with a damper (equivalent to the viscosity of a disperse phase).

The coefficient of elasticity of a droplet is determined by formula 2:

$$\frac{d}{m} = C_d \frac{\sigma}{\rho_d R^3} \quad (2)$$

where C_d is the coefficient of proportionality, which depends on the droplet deformation mode; σ is the surface tension in the interface between the carrier phase and the dispersed phase, N m^{-1}; and ρ_d is the density of the dispersed phase, kg m^{-3}.

The damping ratio of a droplet is determined by formula 3:

$$\frac{d}{m} = C_d \frac{\mu_d}{\rho_d R^2} \quad (3)$$

where C_d is the coefficient of proportionality, which depends on the mode of droplet deformation and μ_d is the viscosity of the dispersed phase, Pa·s.

The external force F under acoustic cavitation is proportional to the amplitude of the shock wave pressure in its front when a cavitation bubble collapses.

The solution of differential Eq. (1) enables us to find the maximum value of droplet deformation and determine the possibility of its breakup. According to Taylor (1963), if the maximum droplet deformation is greater than a quarter of its diameter d, the droplet breaks up into 2 identical droplets of the diameter $\frac{d}{\sqrt[3]{2}}$.

Therefore, the dependence of the droplet diameter on time is defined by differential Eq. 4:

$$\frac{\partial d}{\partial t} = dt_{bu}(d) \ln \frac{1}{\sqrt[3]{2}} \quad (4)$$

where $t_{bu}(d)$ is the dependence of the breakup time of a single droplet on its diameter.

The dependence of the breakup time of a single droplet on its diameter is determined as follows. According to Eq. (1), the maximum value of droplet deformation is proportional to the external force acting on the droplet from the direction of fluid flow. This force is proportional to the amplitude of the shock wave pressure as it reaches the droplet walls. Since a droplet breaks up if and only if its maximum deformation exceeds half the radius, accordingly, the breakup will occur when the amplitude of the shock wave pressure near the droplet walls exceeds a certain threshold value.

This means that a droplet will break up by force of the action of cavitation bubbles formed in its neighborhood due to shock wave scattering.

Based on this information, the droplet breakup time is determined by the interval when at least one cavitation bubble forms in the zone V_b.

The time interval for the formation of bubbles leading to the droplet breakup is determined on the basis of the probabilistic approach in accordance with obtained expression 5:

$$t \approx \frac{T}{nV_b} \quad (5)$$

where n is the concentration of cavitation bubbles determined according to (Khmelev et al., 2014), m^{-3}; T is the period of bubble collapse, s; and V_b is the volume of the zone of bubble collapse leading to the droplet breakup, m^3.

We obtained the dependences of the droplet breakup time, represented by a graph and shown in Fig. 1.

As follows from the dependences obtained (Fig. 1), the greatest breakup time is required for droplets of small diameter (the breakup time of 20 μm droplets three times exceeds the breakup time of 100 μm droplets). The reason for this is the small volume of the neighborhood of a droplet, where collapsing bubbles lead to droplet breakup. Therefore, in order to effectively carry out the process of obtaining an emulsion with dispersed phase particles of a small diameter, it is necessary to increase the radius of cavitation bubbles, and, consequently, to increase the intensity of ultrasound.

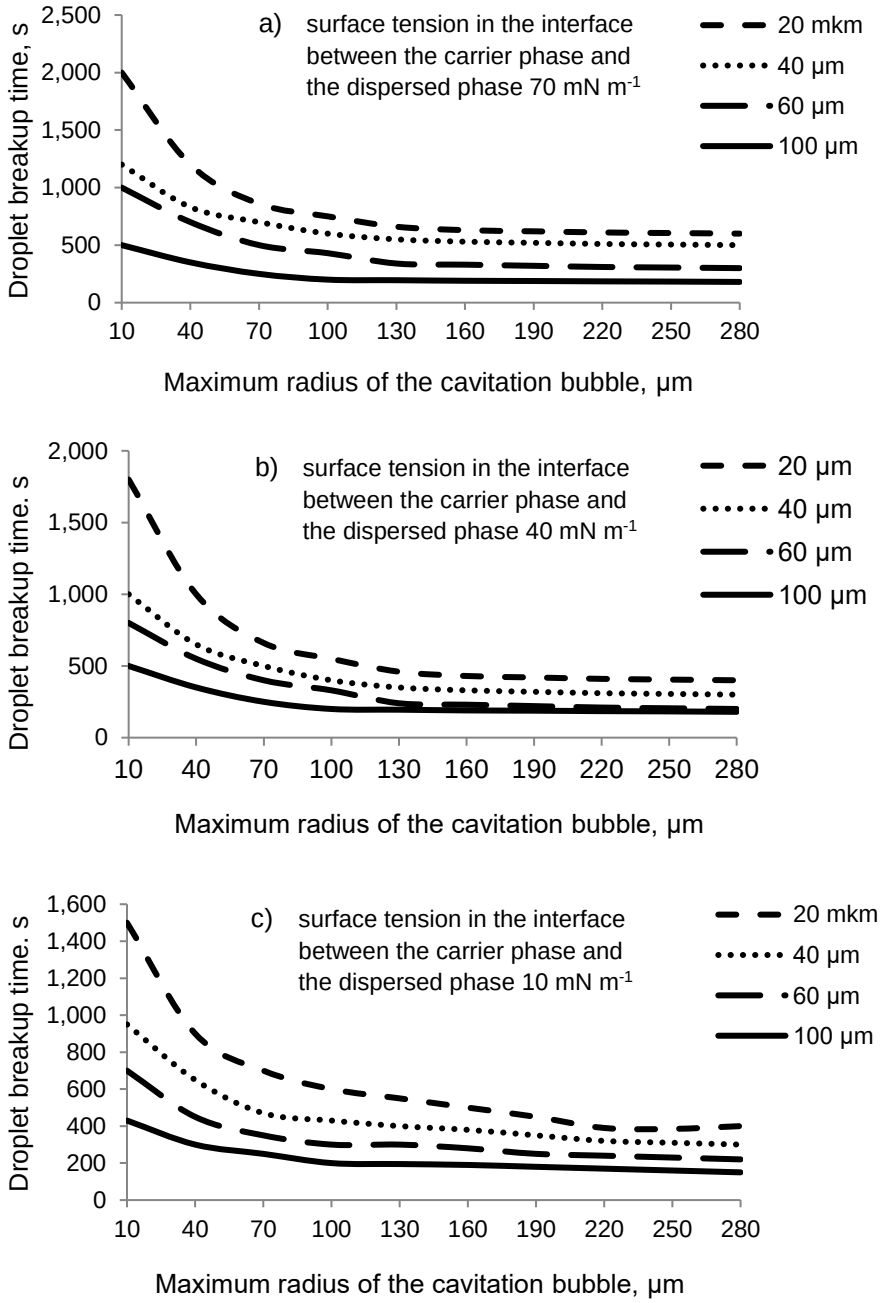

Figure 1. Dependences of the droplet breakup time on the maximum radius of the cavitation bubble depending on its various diameters and surface tensions in the interface between the carrier phase and the dispersed phase.

There is a certain threshold radius for each droplet size, from which decrease in the breakup time of the droplet ceases. For example, the minimum breakup time of 20 μm droplets is achieved when the radius of cavitation bubbles is 250 μm. And for the breakup

of 100 μm droplets, the bubble radius should be 130 μm. Exceeding the threshold radius of cavitation bubbles will not lead to an increase in the efficiency of emulsification; moreover, if the bubble radius exceeds 300 μm, according to (Khmelev, 2012), a degenerate cavitation will form, and the emulsification process might stop.

The obtained droplet breakup time allows us to find the dependence of the droplet diameter on time (Fig. 2) when emulsifying sunflower oil in water.

As follows from the presented dependence, under the action with the intensity of 1.5 W cm^{-2}, even after a 20-minute exposure, the droplet diameter still exceeds 20 μm, which is insufficient for a number of technological processes.

Action with the intensity of 3 W cm^{-2} allows for emulsions with a droplet diameter of 15 μm within 20 minutes, while action with the intensity of 9 W cm^{-2} and more allows obtaining emulsion droplets with a diameter of less than 7–10 μm within just 20 minutes of exposure time.

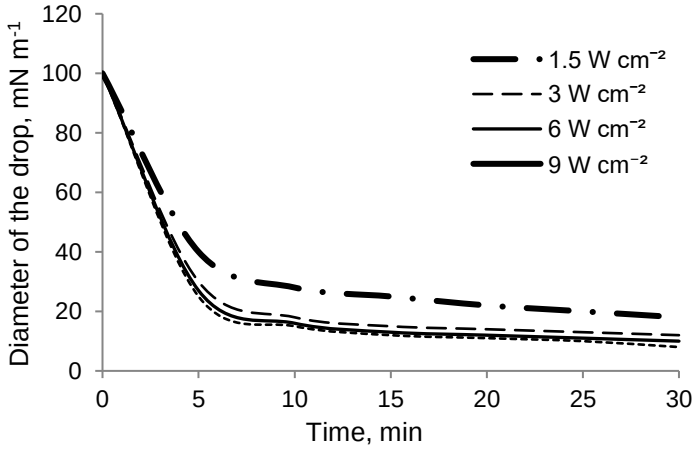

Figure 2. Dependence of emulsion droplets' diameter on time under the action of different intensity.

To verify the adequacy of the obtained theoretical models, we studied the dependences of the breakup rate of emulsion droplets (by the example of a model emulsion of water/oil type) on time and intensity of ultrasound (Fig. 3).

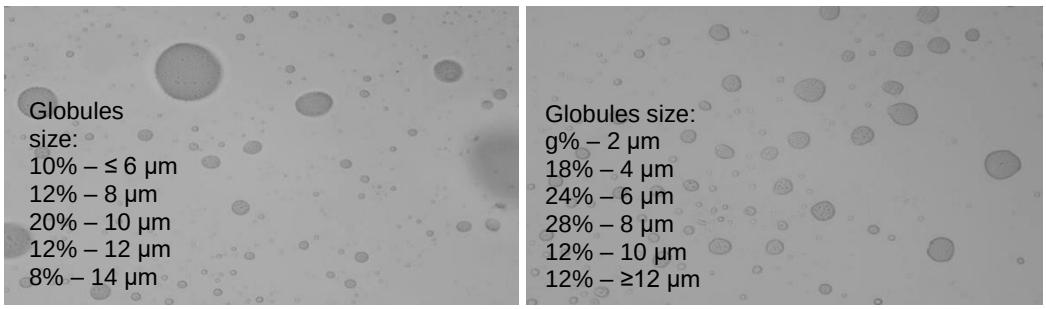

Figure 3. Photos of the model emulsion (magnification × 1,600): left – before the processing, right – after the processing (US 700 W, 20 min).

The resulting images were processed with the use of software, and the hexograms of the distribution of fat globule sizes at different modes of sonochemical action were plotted based on the obtained results (Fig. 4).

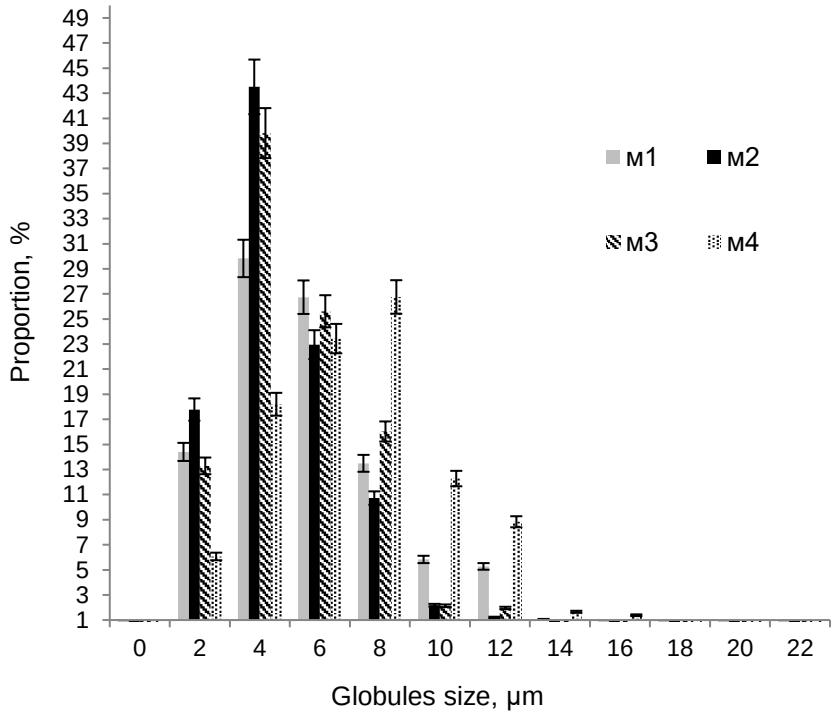

Figure 4. Hexagram of the distribution of fat globules at the different processing modes: M1: 30% vegetable oil + 70% water; US 20 min, 700 W; M2: 50% vegetable oil + 50% water; US 20 min, 1,000 W; M3: 5% vegetable oil + 95% water; US 20 min, 500 W; M4: 30% vegetable oil + 70% water; US 40 min, 500 W.

Based on the results of droplet size calculations, we found that the results of a series of experiments and the results, obtained with the help of the mathematical model, are consistent. We established that the optimal concentration of emulsion is 30% – vegetable oil and 70% – water, and the optimal processing time is 40 minutes with an ultrasonic power of 500 W, which corresponds to a processing intensity of 9 W cm^{-2} (mode M4, Fig. 4). These modes ensure the maximum uniform distribution of fat globules of an average diameter within the range of 6–8 μm.

We undertook the first complex theoretical and practical studies of how the shapes and positions of absolutely fixed boundaries influence

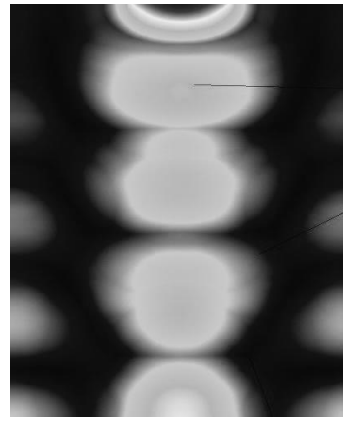

Figure 5. Distribution of oscillation amplitudes of medium pressure.

the propagation of oscillations in a activating liquid medium (food emulsion). We carried out a finite element simulation for the obtained wave equation of oscillation propagation in order to find the distribution of acoustic pressure and the degree of cavitation development in technological volumes. We obtained a visual image (Fig. 5) of the distribution of the amplitudes of pressure oscillation in the medium. It allows for the design of flow working chambers of technological volumes with optimal geometrical dimensions which avoids zones in which the process medium remains unprocessed and thus leads to a decrease in the performance of the device as a whole.

With the help of a specially developed measuring bench, we determined the optimum shapes and sizes of technological volumes for ultrasonic cavitation processing of food emulsions.

To this purpose, longitudinal shifting of the end reflecting boundary (placed opposite the radiator) was constructively generated in order to realize the modes of both a travelling and a stationary wave. The optimal values for the size of process chambers were determined: the diameter should be at least 130 mm; the length of a process chamber should be at least 230 mm.

On the basis of the obtained theoretical data, we developed a design of an acoustic cavitation industrial device for acting on food emulsions, which differs from its analogues in that it has a setup cylindrical wave acting through solid walls of the tunnel for transmitting processed liquid.

CONCLUSIONS

The research resulted in the development of a model of ultrasonic emulsification, which enables the determination of the dependence of the diameter of emulsion droplets on the exposure time and intensity of the action. The analysis of the model allowed us to establish that the optimal time for ultrasonic emulsification is 40 minutes.

In order to obtain an emulsion droplet diameter of less than 20 μm in a carrier phase having a viscosity like that of water (1 mPa·s), the action intensity should be at least 5 W cm^{-2}, while for a liquid with the viscosity of 40 mPa·s, a droplet diameter of less than 20 μm is achieved under the action intensity of 8 W cm^{-2}.

The obtained results may be used to select the modes of operation of ultrasonic technological equipment and to design a process chamber, which would provide the required residence time of an emulsion in the device.

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REFERENCES

Álvarez, D., Castillo, M., Payne, F.A., Garrido, M.D., Bañón, S. & Xiong, Y.L. 2007. Prediction of meat emulsion stability using reflection photometry. *Journal of Food Engineering* **82**, 310–315.

Brotchie, A., Grieser, F. & Ashokkumar, M. 2009. Effect of power and frequency on bubble-size distributions in acoustic cavitation. *Physical Review Letters* **102**(8), art. no. 084302.

Chandrapala, J., Oliver, C., Kentish, S. & Ashokkumar, M. 2012. Ultrasonics in food processing. *Ultrasonics Sonochemistry* **19**(5), 975–983.

Khmelev, V.N. 2007. *Ultrasonic multifunctional and specialized devices for the intensification of technological processes in industry, agriculture and household.* Barnaul, 400 pp. (in Russian).

Khmelev, V.N., Golykh, R.N. & Shalunov, A.V. 2012. Optimization of these modes and conditions of ultrasonic influence on various technological mediums by mathematical modeling International Conference and Seminar on Micro/Nanotechnologies and Electron Devices. In: *Conference Proceedings* NSTU, Novosibirsk, pp. 124–134 (in Russian).

Khmelev, V.N., Golykh, R.N., Shalunov, A.V., Khmelev, S.S. & Karzakova, K.A. 2014. Determination of Ultrasonic Effect Mode Providing Formation of Cavitation Area in High-Viscous and Non-Newtonian Liquids. In: *15th International Conference of Young Specialist on Micro/Nanotechnologies and Electron Devices EDM 2014*. NSTU, Novosibirsk, pp. 203–207 (in Russian).

Kentish, S., Wooster, T.J., Ashokkumar, M., Balachandran, S., Mawson, R. & Simons, L. 2008. The use of ultrasonics for nanoemulsion preparation. *Innovative Food Science and Emerging Technologies* **9**(2), 170–175.

Krasulya, O., Bogush, V., Khmelev, S.S., Potoroko, Yu.I., Tsirulnichenko, L.A., Kanina, K.A., Yuzhina, E.A. & Anandan, S. 2017. Sonochemical Effects on Food Emulsions. *Bulletin of the South Ural State University, series Food and Biotechnology* **5**(2), 38–46.

Krasulya, O.N, Bogush, V.I, Trishina, V., Potoroko, I., Khmelev, S., Sivashanmugam, P. & Anandan, S. 2016. Impact of acoustic cavitation on food emulsions. *Ultrasonics Sonochemistry* **30**, 98–102.

Krasulya, O.N., Bogush, V.I., Dolgova, O.A. & Misharina, T.A. 2013. Use of sonochemistry at production of cooked sausage products. *J. Meat Ind.* **7**, 20–24.

Krasulya, O., Shestakov, S., Bogush, V., Potoroko, I., Cherepanov, P. & Krasulya, B. 2014. Applications of sonochemistry in Russian food processing industry. *Ultrasonics Sonochemistry* **21**(6), 2112–2116.

Patista, A. & Bates, D. 2008. Ultrasonic innovations in the food industry: From the laboratory to commercial production. *Innovative Food Science and Emerging Technologies* **9**(2), 147–154.

Shanmugam, A. & Ashokkumar, M. 2015. Characterization of Ultrasonically Prepared Flaxseed oil Enriched Beverage/Carrot Juice Emulsions and Process-Induced Changes to the Functional Properties of Carrot Juice. *Food and Bioprocess Technology* **8**(6), 1258–1266.

Taylor, G.I. 1963. The Shape and Acceleration of a Drop in a High Speed Air Stream. in: *Batchelor GK, The Scientific Papers of GI Taylor*. University Press, Cambridge 3, pp. 457–464.

Truhaut, R. 1991. Toxicology, social science and the humanitarian. *Bulletin de l'Academie Nationale de Medecine* **175**(9), 1385–1402.

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Changes in the nutritional value of breakfast cereals containing germinated spring grain flakes during storage

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Abstract. The aim of current research was to assess the nutritional value of breakfast cereals containing germinated spring grain flakes and its changes after 6 month storage. Three types of breakfast cereals were prepared and packaged in two types of Standup pouches – Pap50g/Alu7/Pe60 (AL), Pap40g/PELD20/PE40 (PE). For the accelerated shelf life test the samples were stored at 35 ± 2 °C and dietary fibre, protein, fat, B-group vitamins, sugars, total phenol content and DPPH, ABTS+ radical scavenging activity were determined. Developed breakfast cereals have high nutritional value and all are high in fibre and thiamine. Additionally, sample S2 is source of protein, riboflavin, niacin, and S3 – is source of riboflavin and high in niacin. Comparing total phenolic content and antioxidant capacity of tested samples S3 showed the highest values. Storage and selected packaging influenced stability of nutrients, and for S1 and S2 AL showed better results whereas for S3 – PE.

Key words: breakfast cereals, nutritional value, germination, spring cereals, packaging, storage.

INTRODUCTION

Breakfast cereals are popular food product and different approaches are developed to improve their nutrition quality by use of whole grain cereals, cereals not widely used in similar products like germinated cereals, triticale (further – non-traditional cereals), specific ingredients or processes with beneficial effect to final product. Whole grain products are increasingly being used in food, due to their beneficial composition – increased content of fibre, vitamins, minerals and phytochemicals, including phenolics, carotenoids, vitamin E, lignans, β-glucan, inulin, resistant starch, sterols, and phytates (Lafiandra et al., 2014; Oliveira et al., 2015; Sumczynski et al., 2015; Bucsella et al., 2016). Non-traditional types of cereals contain significant amounts of biologically active compounds and can be assumed that breakfast flakes and muesli products made from non-traditional cereals will have higher nutritional properties (Sumczynski et al., 2015).

Cereal consumption as part of a healthy lifestyle may play a role in maintaining adequate nutrient intake all day long and the study about selection of breakfast cereals in diet, showed that the main parameters determining the beneficial effects in the body is increased amount of fibre, protein, calcium and reduced fat content (Barton et al., 2005; Albertson et al., 2008). Dietary fibre is important for a healthy diet and can lower

risk factors for cardiovascular disease and type 2 diabetes mellitus (Lafiandra et al., 2014). Also, cereals contain various essential nutrients including B-group vitamins (Capozzi et al., 2012). Human body does not accumulate B-group vitamins and that is why, in order to comply with daily recommended intake, products enriched in B-group vitamins should be included in every meal (Lebiedzińska & Szefer, 2006).

For development of breakfast cereals it is important to combine different raw materials to obtain products with the target composition. Barley is a desirable food ingredient, with health benefits provided by a β-glucan fibre fraction (Škrbić et al., 2009). Hull-less barley flakes have better nutritional value than hulled ones and they contain more proteins, lipids and soluble dietary fibre (Soares et al., 2007). Oat flakes are the main commercial oat products around the world (Hu et al., 2014). β-glucan is the major form of soluble dietary fibre in oat, and it is known for its effectiveness in lowering blood cholesterol (Kapica, 2001). Rye grain contributes significant quantities of energy, protein, selected micronutrients and non-nutrients to a human diet (Edge et al., 2005) Wheat is one of the oldest food crops, which has achieved a central role as a staple food and contains different classes of bioactive compounds as phenolic acids, carotenoids, tocopherols, alkylresorcinols, etc. (Luthria et al., 2015). Triticale is a hybrid crop developed by crossing wheat (Triticum) and rye (Secale cereale). Comparing to wheat triticale has similar content of protein (Fraś et al., 2016), with a slightly higher amount of lysine (0.33–0.71%) and also similar content of fibre, but with a higher amount of soluble fraction, especially water-extractable arabinoxylans (Rakha et al., 2011).

Germination is a complex process causing physical, chemical, and structural changes in grains, and has been identified as an inexpensive and effective technology for improving cereal quality (Wu et al., 2013). During the germination process a significant changes in the biochemical, nutritional, and sensory characteristics of cereals occur due to degradation of reserve materials used for respiration and synthesis of new cell constituents for developing embryo in the seed. As compared to un-germinated seed, germinated seeds contain high protein, vitamin, low unsaturated fatty acids, low carbohydrate content (Sharma et al., 2016).

For preserving quality of food products an optimum package design should balance the packaging material properties, product protection requirements, environmental and transport conditions, and cost (Macedo et al., 2013). Breakfast cereals are traditionally packed in different paper pouches, and for packaging of breakfast cereal products a paper or polymer package, with particular emphasis on the moisture permeability must be selected.

Development of breakfast cereals should result in high fibres and B group vitamins, formulation made using hull-less grains and germinated samples from barley, oat, rye and wheat to prepare nutritionally enriched breakfast cereals.

The aim of current research was to assess the nutritional value of breakfast cereals containing germinated spring grain flakes and its changes after 6 month storage.

MATERIALS AND METHODS

Raw materials

The grains of hull-less barley (cv ′Irbe′), hull-less oat (cv ′Lizete′), rye (cv ′Kaupo′), and wheat (cv ′Elvis′) conventionally grown at Institute of Agricultural Resources and Economics (Latvia) in 2015, and triticale (cv ′Tulus′) cultivated at Norwegian Institute for Agricultural and Environmental Research (Norway) in 2015 were used in the study. Varieties for breakfast cereals were selected based on their nutritional composition. The following experiments were carried out in the scientific laboratories of the Faculty of Food Technology, Latvia University of Agriculture. Cereal germination, flaking and drying was done according to methodology described by Kince et al. (2017).

Preparation of breakfast cereals

Three types of breakfast cereals with increased nutritional value were developed selecting raw materials (germinated and ungerminated cereals) based on their chemical composition (Fig. 1). Sample S1 was developed from the cereals with higher content of fibre (triticale, germinated triticale, rye, germinated hull-less oats, and germinated hull-less barley (germinated cereals – 40%)). Sample S2 was developed from the cereals with increased content of proteins (triticale, oats, germinated wheat, germinated triticale, and germinated hull-less barley (germinated cereals – 30%)). Sample S3 was developed from the cereals with high B group vitamin content (rye, triticale, germinated rye, germinated hull-less barley, germinated hull-less oats, and wheat (germinated cereals – 50%)).

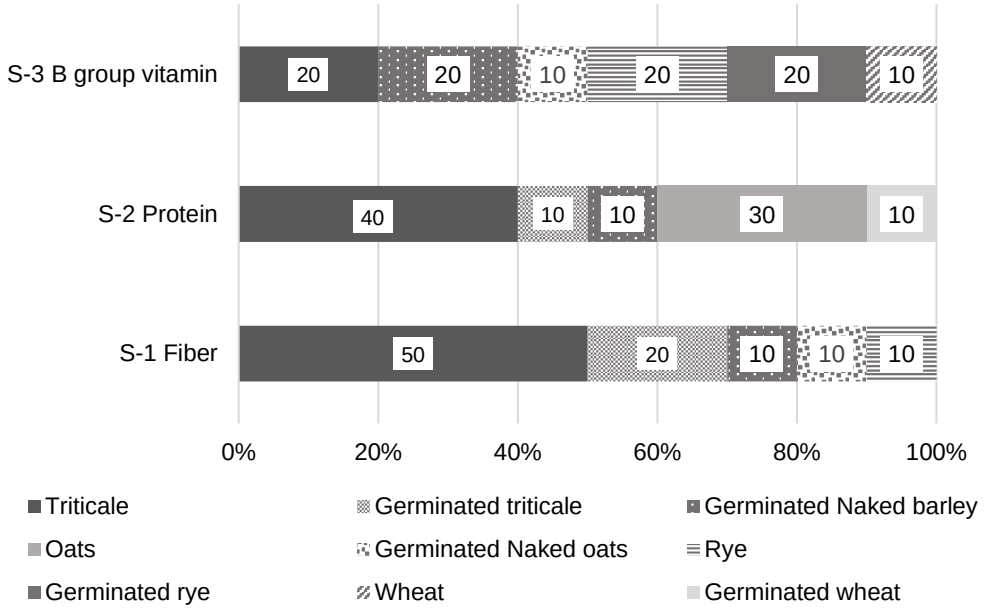

Figure 1. Recipes of developed breakfast cereals.

Packaging and storage of breakfast cereals

Breakfast cereals samples were packaged in *Doypack* (stand up pouches) made from Pap50g/Alu7/Pe60 (Pap/Alu/PE) and stand up pouches Fibrecote® HB MG 40/60 (PE/EvOH/Pap). The size of Doypack was 110 × 65 × 185 mm, volume – 250 mL, the amount of breakfast cereals in each package was 200 ± 5 g. Samples were stored for 6 months at temperature t = 35 ± 2 °C and relative air humidity φ = 55 ± 3%. Abbreviations of the samples stored for 6 month in *Doypack* made from Pap/Alu/PE are as follows: S1-Pap/Alu/PE, S2- Pap/Alu/PE, S3- Pap/Alu/PE, and for samples stored in PE/EvOH/Pap: S1- PE/EvOH/Pap, S2- PE/EvOH/Pap, S3- PE/EvOH/Pap.

Analytical methods

Total protein content was determined by Kjeldahl; standard method AACC 46-20. Total fat content was determined by the standard method ISO 6492. Dietary fibre was determined according to the standard method AOAC No 985.29 using Fibertec E System (Foss, Sweden). Sugars were determined by HPLC as described by Senhofa et al. (2016). *B-group vitamins* were determined by the following methods: vitamin B_1 (thiamine) – AOAC Official Method 986.27, vitamin B_2 (riboflavin) – AOAC Official Method 970.65, vitamin B_3 (niacin) – AOAC Official Method 961.14., vitamin B_5 (pantothenic acid) – AOAC Official Method 992.07, vitamin B_6 – AOAC Official Method 985.32.

Total phenol content and antioxidant activity

Extraction for determination of total phenol content and antioxidant activity of breakfast cereals were performed as described in previous experiments about germinated grains (Kruma et al., 2016). The total phenolic content (TPC) of the grain extracts was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999). Total phenols were expressed as the gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of grain material. Antioxidant activity of the breakfast cereal extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl (DPPH) radical as outlined by (Yu et al., 2003). The absorbance was measured at 517 nm. The cation scavenging activity of extract was measured by 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid ($ABTS^+$) radical cation assay (Re et al., 1999). The radical scavenging activity was expressed as Trolox equivalents (TE) 100 g^{-1} DW of plant material.

Statistical analysis

Experimental results are presented as means of three parallel measurements and were analysed by Microsoft Excel 2010 and SPSS 17.00. Analysis of variance (ANOVA) and Tukey test were used to determine differences among samples. A linear correlation analysis was performed in order to determine relationship between TPC, antioxidant activity such as $DPPH^{\cdot}$, ABTS+. Differences were considered as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Nutritional composition of breakfast cereals

Data on the nutritional composition and energy value of three developed breakfast cereal types is shown in Table 1. The highest content of fats was in the sample S2.

Fat content of various cereals differs significantly and the highest content is in oats (Koletta et al., 2014). The sample S2 contained the highest proportion of oats (30%), comparing to S1 (10%) and S3 (10%), thus it had the highest fat content.

The parameters presented in Table 1 indicates that the main compounds providing beneficial nutritional value of breakfast cereal are proteins and fibre. In regulation **(**Regulation (EC) No 1924/2006) it is determined that product can be labelled as source of protein if at least 12% of energy value is provided by proteins. Comparing the analysed samples, only in S2 it exceeds the required value, reaching 12.23%.

Breakfast cereals were made from whole grains. Wholegrain wheat flour has lower protein content comparing to bread wheat flour (Bucsella et al., 2016). In non-traditional wheat flakes crude protein content ranged between 11.9% and 16.1% (Sumczynski et al., 2015). Analysed breakfast cereal samples contained raw materials that differed in protein content. Rye whole grain flours has significantly lower protein content comparing to whole grain wheat flour (Bucsella et al., 2016). Comparing different grains the lowest protein content was observed for oat flakes (13.58%) whereas the highest was for rye wholegrain flour (Koletta et al., 2014). In whole grain oat flours protein content is 13 g per 100 g whereas proteins in whole grain triticale flours were 11.5% (Fraś et al., 2016). As part of raw materials are germinated also it is necessary to take into account transformations of proteins during germination process. Comparing raw materials wheat and oats contain more proteins, and it could explain higher content of proteins in S2 because these cereals contained the highest (40%) ratio of these cereals, comparing to S1 (10%) and S3 (20%).

Table 1. Nutritional composition and energy value of breakfast cereals

Parameters per 100 g	Samples [A]		
	S1	S2	S3
Fats, g	$1.87 \pm 0.06^{a*}$	2.72 ± 0.03^{b}	1.87 ± 0.08^{a}
Carbohydrates, g	61.44 ± 1.62^{b}	57.84 ± 0.94^{a}	57.52 ± 1.9^{a}
Protein, g	8.82 ± 0.11^{a}	10.05 ± 0.22^{c}**	9.04 ± 0.11^{b}
Fibre, g	15.12 ± 0.16^{a}*	16.27 ± 0.02^{b}*	18.08 ± 0.26^{c}*
Ash, g	1.21 ± 0.03^{a}	1.50 ± 0.07^{b}	1.77 ± 0.05^{c}
Energy value, kcal	328^{b}	329^{b}	319^{a}
Energy value, kJ	$1{,}385^{b}$	$1{,}385^{b}$	$1{,}345^{a}$
Energy value provided by proteins, %	10.75^{a}	12.23^{c}**	11.33^{b}

[A] Results are expressed as mean values (n = 3) ± standard deviation. Mean values followed by the same letter within the row are not significantly different ($P < 0.05$).

* According to regulation (EC) No 1924/2006 product can bear nutrition claim High Fibre.

Breakfast cereals were developed using whole grain flakes, as a result all three samples had high fibre content – 15.12 g 100 g^{-1}, 16.27 g 100 g^{-1}, 18.08 g 100 g^{-1} in samples S1, S2, S3, respectively. Based on the Regulation all three samples can be labelled with nutritional claim high fibre. A claim that a food is high in fibre, may be made only where the product contains at least 6 g of fibre per 100 g of product. Cereal

dietary fibre contributes to the health benefits associated with the consumption of whole grain cereal products, including reduced risk of obesity, type 2 diabetes, cardiovascular disease and colorectal cancer (Lafiandra et al., 2014).

Energy value of the sample S3 was lower, comparing to S1 and S2 due to the lower content of carbohydrates and higher content of fibre.

The analysed breakfast cereals were characterised by a variable content of B-group vitamins and the Table 2 demonstrates percentages of significant values provided by 100 grams of breakfast cereals relative to nutrient reference value (NRV). The highest content of thiamine was determined in the sample S3 and sample S2, which contains 61% and 53% of NRV per 100 grams of product, respectively. Riboflavin content was similar in sample S2 (27% of NRV) and sample S3 (26% of NRV). The highest niacin content was determined in sample S3 (6.39 mg 100 g^{-1}, providing 40% of NRV). Panthotenic acid and vitamin B_6 content was relatively low in all the analysed products. Cereals contain B-group vitamins, but during processing as milling, cooking etc. their content is reduced (Capozzi et al., 2012) whilst in the current technology minimal thermal treatment of cereals was applied and it resulted in relatively high content of B-group vitamins in new breakfast cereals. Also whole grain products and seeds, are better sources of the B-group vitamins than technologically processed products, and therefore more nutritionally efficacious (Lebiedzińska & Szefer, 2006).

Table 2. Content of B-vitamins in breakfast cereals (mg 100 g^{-1}) and nutrient reference values

Vitamin	Samples [A]			Source of	High
	S1	S2	S3	vitamin	vitamin
Thiamine (B_1)	0.35 ± 0.01^{a}*	0.58 ± 0.02^{b}**	0.67 ± 0.01^{c}**	0.165	0.33
Riboflavin (B_2)	0.18 ± 0.00^{a}	0.38 ± 0.02^{b}*	0.36 ± 0.01^{b}*	0.21	0.42
Niacin (B_3)	3.85 ± 0.10^{a}*	4.5 ± 0.16^{b}*	6.39 ± 0.17^{c}**	2.4	4.8
Pantothenic acid (B_5)	0.35 ± 0.02^{b}	0.26 ± 0.01^{a}	0.25 ± 0.02^{a}	0.9	1.8
Vitamin B_6	0.07 ± 0.01^{b}	0.06 ± 0.00^{a}	0.08 ± 0.01^{b}	0.21	0.42

[A] Results are expressed as mean values (n = 3) ± standard deviation. Mean values followed by the same letter within the row are not significantly different ($P < 0.05$).

* According to regulation (EC) No 1924/2006 and Regulation (EU) No 1169/2011 product can bear nutrition claim Source of Vitamin (Name) if it provides more than 15% of NRV, and NRV for riboflavin is 1.4 mg 100 g^{-1}, niacin 16 mg 100 g^{-1}, pantothenic acid 6 mg 100 g^{-1}, vitamin B_6 1.4 mg 100 g^{-1}.

** According to regulation (EC) No 1924/2006 product can bear nutrition claim High Vitamin (Name) if it contains at least double value specified for source of vitamin.

In general addition of germinated flakes for nutritive value improvement is beneficial because germination increases protein and carbohydrate digestibility, increases vitamin bioavailability, and antioxidants activity (Donkor et al., 2012).

Changes of nutritional value after 6 month storage

During storage significant decrease in content of fats was observed but tendencies was influenced by both sample and packaging material (Table 3). Reduction of fat content most probably is the result of oxidation. Breakfast cereals were prepared by avoiding thermal treatment to maintain biologically active substances, but in unheated samples due to lipase activity in oat, hydrolysis of lipids to free fatty acids starts, which

furthermore may promote lipid oxidation during storage (Lampi et al., 2015). No significant changes in total protein content during storage were observed.

Table 3. Content of total fat and protein content of breakfast cereals

Samples	Fats, g 100 g^{-1} DW [A]	Proteins, g 100 g^{-1} DW [A]
S1	2.11 ± 0.07^{c}	9.99 ± 0.12^{a}
S1-Pap/Alu/PE	1.68 ± 0.01^{b}	10.23 ± 0.15^{a}
S1-PE/EvOH/Pap	1.23 ± 0.05^{a}	10.05 ± 0.13^{a}
S2	3.08 ± 0.03^{b}	11.37 ± 0.25^{a}
S2-Pap/Alu/PE	3.04 ± 0.08^{b}	11.35 ± 0.03^{a}
S2-PE/EvOH/Pap	2.42 ± 0.06^{a}	11.38 ± 0.08^{a}
S3	2.12 ± 0.09^{b}	10.24 ± 0.12^{a}
S3-Pap/Alu/PE	0.88 ± 0.04^{a}	10.26 ± 0.06^{a}
S3-PE/EvOH/Pap	2.05 ± 0.07^{b}	10.21 ± 0.08^{a}

[A] Results are expressed as mean values (n = 3) ± standard deviation. Mean values followed by the same letter within the column for the each sample group separately are not significantly different ($P < 0.05$).

Changes of fibre content during storage was significant and decrease of insoluble and soluble fibre was observed. Insoluble fibre content decreased less, reaching 13% in S3-PE/EvOH/Pap, whereas soluble fibre was more unstable and the highest decrease reached 65% in S2-PE/EvOH/Pap (Table 4).

Table 4. Fibre in breakfast cereals (g 100 g^{-1} DW) [A]

Samples	Insoluble fibre	Soluble fibre	Ratio soluble: insoluble	Total fibre
S1	13.92 ± 0.33^{a}	3.18 ± 0.13^{b}	1:4	17.09 ± 0.18^{b}
S1-Pap/Alu/PE	13.74 ± 0.62^{a}	2.46 ± 0.10^{a}	1:6	16.19 ± 0.05^{a}
S1-PE/EvOH/Pap	15.21 ± 0.17^{a}	2.69 ± 0.18^{a}	1:5	17.91 ± 0.15^{c}
S2	16.28 ± 0.35^{b}	2.13 ± 0.16^{c}	1:8	18.41 ± 0.03^{c}
S2-Pap/Alu/PE	14.51 ± 0.53^{a}	1.42 ± 0.14^{b}	1:10	15.93 ± 0.02^{a}
S2-PE/EvOH/Pap	16.28 ± 0.26^{b}	0.73 ± 0.08^{a}	1:22	17.01 ± 0.03^{b}
S3	17.19 ± 0.78^{c}	4.43 ± 0.33^{b}	1:4	20.48 ± 0.30^{c}
S3-Pap/Alu/PE	16.22 ± 0.09^{b}	2.02 ± 0.26^{a}	1:8	18.24 ± 0.03^{a}
S3-PE/EvOH/Pap	14.91 ± 0.14^{a}	4.12 ± 0.14^{b}	1:4	19.03 ± 0.06^{b}

[A] Results are expressed as mean values ($n = 3$) ± standard deviation. Mean values followed by the same letter within the column for the each sample group separately are not significantly different ($P < 0.05$).

For tested samples the lowest proportion of soluble fibre was found in S2 and during storage it decreased significantly, reaching ratio 1:22 in S2-PE/EvOH/Pap. In this sample was the highest proportion of oats (30%), and oat grain is rich in dietary fibre (10–12%) with the soluble component accounting for about 55% of the total fibre (Singh et al., 2013).

Sugars during storage also changed significantly and for monosaccharides as fructose and glucose significant increase was observed, whereas content of disaccharide – sucrose decreased significantly, and maltose was not detected in any of the samples after storage (Table 5).

Table 5. Sugars in breakfast cereals (g 100 g^{-1} DW) [A]

Sample	Fructose	Glycose	Sucrose	Maltose	Sum of identified sugars
S1	0.08 ± 0.00^{a}	0.15 ± 0.00^{a}	0.55 ± 0.02^{b}	0.75 ± 0.03	1.53
S1-Pap/Alu/PE	0.55 ± 0.02^{b}	0.92 ± 0.02^{b}	0.24 ± 0.01^{a}	n.d.	1.71
S1-PE/EvOH/Pap	0.64 ± 0.02^{c}	0.87 ± 0.02^{b}	0.22 ± 0.01^{a}	n.d.	1.73
S2	0.28 ± 0.02^{a}	0.15 ± 0.01^{a}	0.73 ± 0.02^{c}	0.54 ± 0.04	1.7
S2-Pap/Alu/PE	0.68 ± 0.02^{b}	0.80 ± 0.02^{b}	0.24 ± 0.01^{b}	n.d.	1.72
S2-PE/EvOH/Pap	0.89 ± 0.02^{c}	0.84 ± 0.02^{b}	0.16 ± 0.01^{a}	n.d.	1.89
S3	0.10 ± 0.00^{a}	0.19 ± 0.01^{a}	0.77 ± 0.04^{c}	1.18 ± 0.02	2.24
S3-Pap/Alu/PE	0.36 ± 0.02^{b}	1.07 ± 0.03^{b}	0.07 ± 0.01^{a}	n.d.	2.2
S3- PE/EvOH/Pap	0.70 ± 0.02^{c}	1.20 ± 0.04^{b}	0.26 ± 0.02^{b}	n.d.	2.16

[A] Results are expressed as mean values ($n = 3$) ± standard deviation. Mean values followed by the same letter within the column for the each sample group separately are not significantly different ($P < 0.05$).

One of the reasons for increase of simple sugars could be breakage of starch by enzymes that are activated during germination. During the germination, the most significant changes occur in starch, which is broken to simpler sugars by amylases. Starch degradation changes during the sprouting process directly affects grain germination temperature, germination time, relative humidity, moisture content, culture media, steeping time and light (López-Amorós et al., 2006; Kruma et al., 2016).

TPC in breakfast cereals differed significantly and the highest content was observed in sample S3 (Table 6).

Table 6. Phenolic compounds and antioxidant activity

Samples	TPC, mg 100 g^{-1} DW [A]	DPPH, mM 100 g^{-1} DW [A]	ABTS, mM 100 g^{-1} DW [A]
S1	47.79 ± 1.79^{c}	2.63 ± 0.05^{a}	4.40 ± 0.15^{a}
S1-Pap/Alu/PE	43.01 ± 0.81^{a}	3.76 ± 0.07^{b}	5.57 ± 0.27^{b}
S1-PE/EvOH/Pap	45.11 ± 0.54^{b}	3.6 ± 0.17^{b}	5.69 ± 0.18^{b}
S2	47.02 ± 0.48^{b}	2.74 ± 0.03^{a}	5.98 ± 0.13^{a}
S2-Pap/Alu/PE	35.38 ± 0.56^{a}	3.28 ± 0.09^{b}	6.27 ± 0.14^{b}
S2-PE/EvOH/Pap	35.63 ± 2.12^{a}	3.38 ± 0.05^{b}	6.62 ± 0.23^{b}
S3	57.01 ± 2.17^{c}	3.42 ± 0.13^{a}	6.23 ± 0.18^{b}
S3-Pap/Alu/PE	48.40 ± 0.54^{b}	3.96 ± 0.16^{b}	7.27 ± 0.43^{c}
S3-PE/EvOH/Pap	41.29 ± 1.88^{a}	3.79 ± 0.16^{b}	5.28 ± 0.08^{a}

[A] Results are expressed as mean values ($n = 3$) ± standard deviation. Mean values followed by the same letter within the column for the each sample group separately are not significantly different ($P < 0.05$).

This sample contained the highest proportion of hull-less barley, followed by rye and wheat and previous results showed that these cereals has higher TPC (Kruma et al., 2016). During storage TPC significantly decreased in all tested samples. The highest decrease was detected in sample S2, and it was similar for both S2- Pap/Alu/PE and S2- PE/EvOH/Pap, 24.75%, 24.22%, respectively. Packaging material influenced TPC in sample S3.

DPPH and ABTS scavenging activity during storage significantly increased, except sample S3- PE/EvOH/Pap. The highest increase of 29.32% was observed in S1- PE/EvOH/Pap. For S3 sample contrary results were found, in S3- Pap/Alu/PE increase for 16.7% whereas for S3-PE/EvOH/Pap 15.2% decrease was observed. Radical scavenging activity, analysed by both assays in S1 and S2 was significantly higher. Contrary to phenolic compounds, scavenging activity during storage increased in all three tested samples.

To understand the role of phenolic compounds in antioxidant activity of flakes, correlation analyses were performed. No correlation between TPC and DPPH scavenging activity (r = 0.08) and TPC and ABTS scavenging activity (r = 0.05) was observed. Only between both tested antioxidant assays moderate correlation (r = 0.47) was determined. The cereals with high TPC are not efficient for DPPH inhibition and it could be explained by ferulic acid, the main phenolic acid in cereal grains, which showed a weak antiradical effect in experiments with the DPPH radical, which may explain the discrepancies (Đorđević et al., 2010). Also it could be explained by the contribution of other compounds to antioxidant activity.

Phenolic compounds have been investigated due to their diverse health benefits as antioxidants both in food and in human body. Thereby for selection of packaging material it is also important to select material for the best presevation of phenolic compounds. In current experiment accelerated storage test was performed, and it could also influence changes of phenolic compounds and antioxidant acitivity. But results about processing of sorghum with dry heat did not affect the total phenolic compounds and antioxidant activity, whereas the wet heat decreased total phenolic compounds and antioxidant activity (Cardoso et al., 2014). Also in the sorghum TPC reduced after storage for 180 days at a temperature of 40°C, the retentions of these compounds were high, 87.6% in flour and 93% in grains (Oliveira et al., 2017).

The permeability of packaging materials to water vapour and oxygen as well as light transmittance are critical factors determining the quality and bio-chemical stability of dried products during storage. Aluminum polyethylene pouches, with a greater protective barrier, better preserved TPC and the antioxidant compared to polyamide/polyethylene pouches (Udomkun et al., 2016). Our results showed that breakfast cereal composition also is critical factor and in the current research the highest decrease of TPC in S3 was detected and it could be explained by the highest proportion of germinated grains. These samples were microwave vacuum dried at low temperature and still some enzymatic activity may remain resulting in oxidation of phenolic compounds.

As different whole grains have different composition and health benefits, technologies should be developed to allow the use of versatile grain raw materials, also multigrain products and new product concepts. More varied use of raw materials and ingredients would also diversify the taste of cereal foods' (Poutanen et al., 2014). Developed breakfast cereals are multigrain products, with additional value of germinated cereals, but it also can cause problems in stability of product during its storage.

CONCLUSIONS

Breakfast cereals containing germinated grains has high nutritional value. All developed samples are high in fibre and thiamine (vitamin B1). Additionally S2 is a source of protein, riboflavin (vitamin B2) and niacin (vitamin B3); S3 – is a source of riboflavin (vitamin B2) and high in niacin (vitamin B3). Comparing TPC and antioxidant capacity of tested samples S3 showed the highest values. Storage and selected packaging material influenced stability of nutrients, except proteins. During storage fat content and TPC reduced, whereas antioxidant activity increased. For better preserving of samples nutrition value for S1 and S2 Pap50g/Alu7/Pe60 (AL) but for S3 Pap40g/PELD20/PE40 (PE) should be selected.

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REFERENCES

Albertson, A.M., Thompson, D., Franko, D.L., Kleinman, R.E., Barton, B.A. & Crockett, S.J. 2008. Consumption of breakfast cereal is associated with positive health outcomes: evidence from the National Heart, Lung, and Blood Institute Growth and Health Study. *Nutrition Research* **28**,744–752.

Barton, B.A., Eldridge, A.L., Thompson, D., Affenito, S.G., Striegel-Moore, R.H., Franko, D.L., Albertson, A.M. & Crockett, S.J. 2005. The relationship of breakfast and cereal consumption to nutrient intake and body mass index. *Journal of the American Dietetic Association* **105**, 1383–1389.

Bucsella, B., Molnár, D., Harasztos, A.H. & Tömösközi, S. 2016. Comparison of the rheological and end-product properties of an industrial aleurone-rich wheat flour, whole grain wheat and rye flour. *Journal of Cereal Science* **69**, 40–48.

Capozzi, V., Russo, P., Dueñas, M.T., López, P. & Spano, G. 2012. Lactic acid bacteria producing B-group vitamins: A great potential for functional cereals products. *Applied Microbiology and Biotechnology* **96**, 1383–1394.

Cardoso, L. de M., Montini, T.A., Pinheiro, S.S., Pinheiro-Sant'Ana, H.M., Martino, H.S.D. & Moreira, A.V.B. 2014. Effects of processing with dry heat and wet heat on the antioxidant profile of sorghum. *Food Chemistry* **152**, 210–217.

Donkor, O.N., Stojanovska, L., Ginn, P., Ashton, J. & Vasiljevic, T. 2012. Germinated grains – Sources of bioactive compounds. *Food Chemistry* **135**, 950–959.

Đorđević, T.M., Šiler-Marinković, S.S. & Dimitrijević-Branković, S.I. (2010). Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. *Food Chemistry* **119**, 957–963.

Edge, M.S., Jones, J.M. & Marquart, L. 2005. A New Life for Whole Grains. *Journal of the American Dietetic Association* **105**, 1856–1860.

Fraś, A., Gołębiewska, K., Gołębiewski, D., Mańkowski, D.R., Boros, D. & Szecówka, P. (2016). Variability in the chemical composition of triticale grain, flour and bread. *Journal of Cereal Science* **71**, 66–72.

Hu, X.-Z., Zheng, J.-M., Li, X., Xu, C. & Zhao, Q. 2014. Chemical composition and sensory characteristics of oat flakes: A comparative study of naked oat flakes from China and hulled oat flakes from western countries. *Journal of Cereal Science* **60**, 297–301.

Kapica, C. 2001. Oats-Nature's Functional Food. *Nutrition Today* **36**, 56–60.

Kince, T., Galoburda, R., Klava, D., Tomsone, L., Senhofa, S., Straumite, E., Kerch, G., Kronberga, A., Sturite, I., Kunkulberga, D. & Blija, A. 2017. Breakfast cereals with germinated cereal flakes: changes in selected physical, microbiological, and sensory characteristics during storage. *European Journal of Food Research and Technology* **243**, 1497–1506.

Koletta, P., Irakli, M., Papageorgiou, M. & Skendi, A. 2014. Physicochemical and technological properties of highly enriched wheat breads with wholegrain non wheat flours. *Journal of Cereal Science* **60**, 561–568.

Kruma, Z., Tomsone, L., Ķince, T., Galoburda, R. & Senhofa, S. 2016. Effects of germination on total phenolic compounds and radical scavenging activity in hull-less spring cereals and triticale. *Agronomy Research* **14**, 1372–1383.

Lafiandra, D., Riccardi, G. & Shewry, P.R. 2014. Improving cereal grain carbohydrates for diet and health. *Journal of Cereal Science* **59**, 312–326.

Lampi, A.-M., Damerau, A., Li, J., Moisio, T., Partanen, R., Forssell, P. & Piironen, V. 2015. Changes in lipids and volatile compounds of oat flours and extrudates during processing and storage. *Journal of Cereal Science* **62**, 102–109.

Lebiedzińska, A. & Szefer, P. 2006. Vitamins B in grain and cereal-grain food, soy-products and seeds. *Food Chemistry* **95**, 116–122.

López-Amorós, M.L., Hernández, T. & Estrella, I. 2006. Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis* **19**, 277–283.

Luthria, D.L., Lu, Y. & John, K.M.M. 2015. Bioactive phytochemicals in wheat: Extraction, analysis, processing, and functional properties. *Journal of Functional Foods* **18**, 910–925.

Macedo, I.S.M., Sousa-Gallagher, M.J., Oliveira, J.C. & Byrne, E.P. 2013. Quality by design for packaging of granola breakfast product. *Food Control* **29**, 438–443.

Oliveira, L.C., Rosell, C.M. & Steel, C.J. 2015. Effect of the addition of whole-grain wheat flour and of extrusion process parameters on dietary fibre content, starch transformation and mechanical properties of a ready-to-eat breakfast cereal. *International Journal of Food Science and Technology* **50**, 1504–1514.

Oliveira, K.G. de, Queiroz, V.A.V., Carlos, L. de A., Cardoso, L.D.M., Pinheiro-Sant'Ana, H.M., Anunciação, P.C., Menezes, C.B., Silva, E.C.D. & Barros, F. 2017. Effect of the storage time and temperature on phenolic compounds of sorghum grain and flour. *Food Chemistry* **216**, 390–398.

Poutanen, K., Sozer, N. & Della Valle, G. 2014. How can technology help to deliver more of grain in cereal foods for a healthy diet? *Journal of Cereal Science* **59**, 327–336.

Rakha, A., Åman, P. & Andersson, R. 2011. Dietary fiber in triticale grain: Variation in content, composition, and molecular weight distribution of extractable components. *Journal of Cereal Science* **54**, 324–331.

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* **26**, 1231–1237.

Senhofa, S., Ķince, T., Galoburda, R., Cinkmanis, I., Sabovics, M. & Sturite, I. 2016. Effects of germination on chemical composition of hull - less spring cereals. In: P*rocedings of Research for Rural Development*. p. 91–97.

Sharma, S., Saxena, D.C. & Riar, C.S. 2016. Analysing the effect of germination on phenolics, dietary fibres, minerals and γ-amino butyric acid contents of barnyard millet (Echinochloa frumentaceae). *Food Bioscience* **13**, 60–68.

Singh, R., De, S. & Belkheir, A. 2013. Avena sativa (Oat), a potential neutraceutical and therapeutic agent: an overview. *Critical Reviews in Food Science and Nutrition* **53**, 126–144.

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* **119**, 152–178.

Škrbić, B., Milovac, S., Dodig, D. & Filipčev, B. 2009. Effects of hull-less barley flour and flakes on bread nutritional composition and sensory properties. *Food Chemistry* **115**, 982–988.

Soares, R.M.D., De Francisco, A., Rayas-Duarte, P. & Soldi, V. 2007. Brazilian hull-less and malting barley genotypes: I. chemical composition and partial characterization. *Journal of Food Quality* **30**, 357–371.

Sumczynski, D., Bubelova, Z., Sneyd, J., Erb-Weber, S. & Mlcek, J. 2015. Total phenolics, flavonoids, antioxidant activity, crude fibre and digestibility in non-traditional wheat flakes and muesli. *Food Chemistry* **174**, 319–325.

Udomkun, P., Nagle, M., Argyropoulos, D., Mahayothee, B., Latif, S. & Müller, J. 2016. Compositional and functional dynamics of dried papaya as affected by storage time and packaging material. *Food Chemistry* **196**, 712–719.

Wu, F., Yang, N., Touré, A., Jin, Z. & Xu, X. 2013. Germinated Brown Rice and Its Role in Human Health. *Critical Reviews in Food Science and Nutrition* **53**, 451–453.

Yu, L., Perret, J., Harris, M., Wilson, J. & Haley, S. 2003. Antioxidant properties of bran extracts from 'Akron' wheat grown at different locations. *Journal of Agricultural and Food Chemistry* **51**, 1566–1570.

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Influence of technological parameters on chemical composition of triticale flakes

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Abstract. Triticale is hybrid crop developed by crossing wheat (*Triticum*) and rye (*Secale*) and in last years it become more popular for food applications, including flake production. Different approaches are developed to improve flakes technology by applying different cooking, rolling, toasting parameters resulting in high quality products. All these technologies influence also nutrition quality of product due to the different stability of these compounds during mechanical and thermal treatment. The aim of current experiment was to investigate the influence of technological parameters on chemical composition of triticale flakes. In current experiment triticale grains and triticale flakes obtained by different technologies was tested. For evaluation of the influence of technological parameters, different flaking and rolling parameters were tested. For all samples were determined composition of basic nutrients (fats, proteins, fibres, sugars, ash), minerals (Ca, Mg, K, Zn, P), vitamins, total phenolics and antioxidant activity. Triticale has high nutritional quality, containing significant amounts of protein, fibres, vitamins and minerals. Technological processes significantly influence cereals composition, but it depends on parameters tested. Control sample showed lower results and hierarchical cluster analyses showed that samples 1/3/1, 2/1/2/1, 2/1/3/1, 2/1/4/1 are similar in composition of bioactive compounds. Results showed that for selection of the best method for flaking physical and/or sensory properties should be taken in account.

Key words: triticale, flakes, technology, chemical composition.

INTRODUCTION

The health benefits of cereal products and consumer's acceptance should be prioritised (Fardet, 2014). Cereal consumption is significant factor influencing healthful lifestyle that helps to maintain healthful body mass index (Barton et al., 2005). Different trends become popular in last years to improve the nutrient composition of cereals, often in response to public health concerns and consumer demands, for example decrease of sugar and sodium, increase level of fiber level, use of whole grain, fruit powders (Oliveira et al., 2018), legumes etc. (Thomas et al., 2013). Part of consumers prefer use of traditional flakes for not only making of porridge but also consuming with milk or yogurt. Cereal flakes traditionaly are produced by steaming to certain moisture content followed by flaking, but for consumption without hot water treatment harder structure

remains. Different approaches is developed to improve flakes production technology by using reduced or high pressure treatment, selected rolling and toasting parameters resulting in high quality products. One of the property that could be influenced by these techniques is bulk density and improved sensory properties of products. Volume of flakes is very important because consumer researchs shows influence of volume to selection of portion size and results about breakfast cereals showed that by reducing flake size, panelists took smaller volume of sample, but still they took more energy value (Rolls et al., 2014). Technology used in current experiemnts could increase bulk density and could result in lower consumption of food.

Triticale is hybrid crop developed by crossing wheat (Triticum) and rye (Secale cereale) and in last years it become more popular for food applications, including muesli production (Senhofa et al., 2015). Comparing to wheat triticale has similar content of protein, with a slightly higher amount of lysine (0.33–0.71%) (Fraś et al., 2016) and also similar content of fibre, but with a higher amount of soluble fraction, especially water-extractable arabinoxylans (Rakha et al., 2011). Technologies applied influence also nutrition quality of product due to the different stability of these compounds during mechanical and thermal treatment (Fraś et al., 2016).

The aim of current experiment was to investigate the influence of technological parameters on chemical composition of triticale flakes.

MATERIALS AND METHODS

Triticale flakes samples preparation

In the present experiment three different technologies for flakes production were used: traditionally commercially processed flakes (control sample) – purchased ready-made triticale whole grain flakes from JSC Dobeles dzirnavnieks (Latvia). Processing technology includes purified triticale grains steaming, rolling and drying.

Samples numbered with first code 1 (cooking method 1)– cooked triticale flakes obtained by 1 min steaming and 0.5 ± 0.1 bar pressure 10 min and different flaking rolls gap settings (respectively, sample 1/3/1 – 0.06 ± 0.01 mm and sample 1/4/1 – 0.04 ± 0.01 mm). Processed triticale flakes were transferred for drying in the Mitchell Bach Dryer (GmbH Baker Perkins, UK) for 35 ± 5 min at 80 ± 1 °C. Samples numbered with first code 2 (cooking method 2) – triticale grains were transferred in the Rotary Cereal Cooker (GmbH Baker Perkins, UK) with adding of extra water 20 ± 3% from grains total amount, after that samples were 5 min steamed and cooked 30 ± 5 min at 1.2 bar pressure. Samples 2/1/2/1, 2/1/3/1, 2/1/4/1 differing by flaking rolls gap settings, respectively, 0.10 ± 0.01, 0.06 ± 0.01 and 0.04 ± 0.01 mm gap. Processed triticale flakes were transferred for drying in the Mitchell Bach Dryer (GmbH Baker Perkins, UK) at 80 ± 1 °C for 30 ± 5 min.

Toasting procedure was performed in falling temperatures under 200 ± 10 °C for less than one minute in the toaster New Thermoglide Toaster Rig (GmbH Baker Perkins, UK).

Methods

Basic nutrients were determined by following methods: fat – § 64 LFGB L 20.01/02-5, fatty acids – § 64 LFGB L 13.00-26/-27/20, carbohydrates (calculated by difference), sugars (fructose, glucose, sucrose, maltose, lactose) – § 64 LFGB L 00.00-

143, salt (from sodium) – calculated, fibres – § 64 LFGB L 00.00-18, protein – § 64 LFGB L 17.00-15, energy value – calculated in kcal and kJ.

Vitamins were determined by following methods: vitamin B1 (thiamine) – § 64 LFGB L 00.00-83, vitamin B2 (riboflavin) – § 64 LFGB L 00.00-84, vitamin B6 – § 64 LFGB L 00.00-97, niacin – SLMB 62/12.2.1.

Mineral substances were determined by following methods: calcium, iron, potassium, magnesium, phosphorus – DIN EN ISO 11885, ICP-OES, zinc – DIN EN 15763, sodium – DIN EN ISO 11885, ICP-OES.

Extraction process and determination of total phenolic compounds, $DPPH^{\cdot}$ radical scavenging activity and $ABTS^{+}$ radical scavenging activity was determined according methodology used for grains (Kruma et al., 2016).

Data Treatment

Experimental results presented are means of three parallel measurements and were analysed by Microsoft Excel 2013, XLSTAT 2018 and SPSS 23. Analysis of variance (ANOVA) and Tukey test was used to determine differences among samples. Hierarchical cluster analysis was used to identify relatively homogeneous groups of cases based on tested parameters, using an algorithm that starts with each case (or variable) in a separate cluster and combines clusters until only one is left. Standardizing transformations were performed and agglomeration schedule, cluster membership for a range of solutions were tested for selection of best solution.

RESULTS AND DISCUSSION

Carbohydrates are the major nutrients in triticale grain and flakes (Table 1) and no significant differences ($p > 0.05$) in content of carbohydrates were observed in flakes produced by different technological parameters. Starch content was the lowest in control flakes, but also no significant influence of technological parameters was observed. The lowest content of fibres were in control sample, and only in sample 2/1/2/1 it remained in the same level as in triticale grains.

Table 1. Content of carbohydrtaes, starch, sugars, fibers in triticale grains and flakes

Samples	Carbohydrates, g 100 g^{-1}	Starch, g 100 g^{-1}	Sugars, g 100 g^{-1}	Fibres, g 100 g^{-1}	Protein, g 100 g^{-1}
Triticale grains	68.49 ± 1.78a*	48.12±1.05a	1.07 ± 1.11c	15.64 ± 0.7c	11.79 ± 0.14a
Control Flakes	69.41 ± 2.72a	50.4 ± 2.05a	1.06 ± 0.05c	12.79 ± 0.18a	13.13 ± 0.58b
1/3/1	70.56 ± 3.04a	52.65±2.00ab	0.58 ± 0.01a	14.41 ± 0.62b	11.27 ± 0.44a
1/4/1	71.46 ± 1.12a	54.73±1.75b	0.59 ± 0.02a	14.06 ± 0.23b	10.86 ± 0.45a
2/1/2/1	69.28 ± 1.27a	51.02±1.35a	0.72 ± 0.01b	15.36 ± 0.39c	11.49 ± 0.26a
2/1/3/1	69.78 ± 2.06a	52.41±1.42ab	0.70 ± 0.03b	14.95 ± 0.16b	11.35 ± 0.29a
2/1/4/1	70.48 ± 1.17a	51.46±1.22a	0.68 ± 0.01b	14.6 ± 0.66b	11.22 ± 0.50a

* Results are expressed as mean values (n = 3) ± standard deviation. Mean values followed by the same letter within the column for the each sample group separately are not significantly different ($P < 0.05$).

Protein content of triticale grains was 11.79 g 100 g^{-1} and it was similar comparing to data found in different triticale varieties grown in Poland ranging from 11.8 until

15.2. g 100 g^{-1} (Fraś et al., 2016). Proteins in triticale flakes ranged between 10.86 to 13.13 mg 100 g^{-1}. In control sample content of proteins was higher comparing to grains, but in flakes made by Cooking method 1 and Cooking method 2 no significant differences were obtained. Cereals are not rich in fats and analysed triticale grains contained only 2.1 g 100 g^{-1} (Table 2) mainly consisting of polyunsaturated acids (61%). Comparing flakes, the highest content of fats and its fractions were observed in control sample followed by sample 2/1/2/1 treated in increased pressure and cooked for 35 minutes. Cereal consumption was related to increased intake of fiber and decreased intake of fat and cholesterol and predictive of lower body mass index (Barton et al., 2005).

Table 2. Content of fats in triticale grains and flakes

Samples	Fat, g 100 g^{-1}	Fatty acid, saturated, g 100 g^{-1}	Fatty acid, monounsaturated, g 100 g^{-1}	Fatty acid, polyunsaturated, g 100 g^{-1}
Triticale grains	2.1 ± 0.06c*	0.47 ± 0.02b	0.35 ± 0.01a	1.28 ± 0.05b
Control Flakes	3.11 ± 0.1d	0.56 ± 0.02c	0.67 ± 0.02b	1.89 ± 0.02c
1/3/1	1.88 ± 0.04a	0.42 ± 0.02b	0.31 ± 0.01a	1.15 ± 0.02a
1/4/1	1.76 ± 0.04a	0.31 ± 0.01a	0.31 ± 0.02a	1.14 ± 0.03a
2/1/2/1	2.04 ± 0.07bc	0.43 ± 0.01b	0.32 ± 0.01a	1.29 ± 0.03b
2/1/3/1	1.91 ± 0.09b	0.42 ± 0.02b	0.32 ± 0.01a	1.17 ± 0.01a
2/1/4/1	1.9 ± 0.07b	0.32 ± 0.01 a	0.32 ± 0.01a	1.16 ± 0.04a

* Results are expressed as mean values (n = 3) ± standard deviation. Mean values followed by the same letter within the column for the each sample group separately are not significantly different ($P < 0.05$).

Cereal consumption was related to increased intake of calcium, iron, folic acid, vitamin C, and zinc (Barton et al., 2005). Grain products are source of B group vitamins. Triticale grains contained 0.350 mg 100g^{-1} vitamin B1, and during processing it decreased significantly, showing the lowest results in control flake sample (0.073 mg 100 g^{-1}) and the highest in sample 1/3/1 treated at lower pressure (cooking method 2) (0.167 mg 100 g^{-1}) (Table 3). Content of thiamine in triticale was reported 0.378 mg 100 g^{-1} (Zhu, 2018). Sumczynski et al (2018) reported thiamine content in wheat flakes ranging from 0.15–1.05 mg 100 g^{-1} and it is mainly higher comparing to results of current study. Content of B2 vitamin decreased during thermal tretment, except sample 2/1/2/1 (no significant differences comparing to triticale grains). The lowest level in control flakes were observed. Content of B2 vitamin in wheat flakes were in similar range 0.032–0. 350 mg 100 g^{-1} (Sumczynski et al.,2018). In tested triticale grains content of vitamin B6 was 0.257 mg 100 g^{-1} , whereas in experiments reported in literature twice higher content was detected – 0.403 mg 100 g (Zhu, 2018). Vitamin B6 level the lowest was in control sample and for all samples made with cooking method 2 content was the highest. Niacin was only one vitamin from tested that increased during thermal treatment up to two times. Similar results were obtained for the bread and in this case nicotinic acid and nicotinamide increased during toasting steps (Nurit et al., 2016).

Table 3. Content of vitamins in triticale grains and flakes

Samples	Vitamin B1, mg 100 g^{-1}	Vitamin B2, mg 100 g^{-1}	Vitamin B6, mg 100 g^{-1}	Niacin, mg 100 g^{-1}
Triticale grains	0.350 ± 0.011f*	0.096 ± 0.003c	0.257 ± 0.004d	0.548 ± 0.019a
Control flakes	0.073 ± 0.001a	0.040 ± 0.002a	0.110 ± 0.005a	0.667 ± 0.027b
1/3/1	0.167 ± 0.008e	0.085 ± 0.002b	0.219 ± 0.008b	0.877 ± 0.026c
1/4/1	0.134 ± 0.004d	0.082 ± 0.001b	0.217 ± 0.007b	0.869 ± 0.011c
2/1/2/1	0.107 ± 0.004c	0.093 ± 0.003c	0.236 ± 0.010c	1.182 ± 0.040d
2/1/3/1	0.098 ± 0.005c	0.085 ± 0.001b	0.223 ± 0.009bc	1.273 ± 0.019d
2/1/4/1	0.087 ± 0.001b	0.088 ± 0.002bc	0.222 ± 0.008bc	1.270 ± 0.051d

* Results are expressed as mean values (n = 3) ± standard deviation. Mean values followed by the same letter within the column for the each sample group separately are not significantly different ($P < 0.05$).

Content of total phenolic compounds in triticale grain was 34.22 mg 100 g^{-1} (Table 4) and comparing to literature it is similar – 295.02 μg g^{-1} (29.5 mg 100 g^{-1}) range (Irakli et al., 2012). In samples made by cooking method 2 and also 1/3/1/ significant TPC increase were determined. DPPH antioxidant activity in flakes was significantly higher compared to grains, and similar trend for ABTS antioxidant activity was observed. Exception was sample 2/1/4/1 were significant lower activity was observed. For both antioxidant assays higher results for Cooking method 2 was observed. Processing of sorghum with dry heat did not affect the total phenolic compounds and antioxidant activity, whereas the wet heat decreased total phenolic compounds and antioxidant activity (Cardoso et al., 2014). In our experiment wet treatment was used but decrease of antioxidant activity for two samples was not observed (1/4/1 and 2/1/3/1).

Table 4. Content of total phenolics and antioxidant activity of triticale grain and flakes

Samples	TPC, mg 100 g^{-1}	DPPH, mM TE 100g^{-1}	ABTS, mM TE 100g^{-1}
Triticale grains	34.22 ± 0.97c*	1.15 ± 0.44a	7.05 ± 0.22b
Control Flakes	26.96 ± 1.36a	1.79 ± 0.22b	8.45 ± 0.29c
1/3/1	36.15 ± 1.42c	2.31 ± 0.03c	6.97 ± 0.21b
1/4/1	29.8 ± 0.86b	2.35 ± 0.05c	9.21 ± 0.33d
2/1/2/1	38.15 ± 0.59d	2.83 ± 0.02d	10.11 ± 0.27e
2/1/3/1	42.89 ± 0.47e	2.78 ± 0.04d	12.35 ± 0.11f
2/1/4/1	42.74 ± 1.35e	2.86 ± 0.08d	6.3 ± 0.26a

* Results are expressed as mean values (n = 3) ± standard deviation. Mean values followed by the same letter within the column for the each sample group separately are not significantly different ($P < 0.05$).

For antioxidant activity even increase was observed and mainly it could be explained by transformation of some bound antioxidants to free form with higher activity. Oat-based breakfast cereals, irrespective of brand, variety or cost, are a significant source of polyphenols and antioxidant compounds (Ryan et al., 2011) and use of triticle could also be perspective source of phenolics.

The main mineral substances were determined in triticale grain and flakes. The highest content of zinc, phosphorus, magnesium and potasiums were in triticale grains, whereas iron was in higher concentration in flakes made by cooking method 1 and cooking method 2 (Table 5 and 6). It is possible to conclude that tendencies of changes

of mineral substances differ and mainly flakes production resulted in decrease in mineral content. In quinoa's 100% of phosphorus remained for steamed preparation. In steamed buckwheat retention of minerals ranged from 87% for zinc to 98% to calcium and 100% for iron (Mota et al., 2016).

Calcium was the only one mineral in the highest concentration in control sample, but other substances as Fe, K, Mg and P were in lowest concentration in control flakes.

Table 5. Content of calcium, iron, potassium and magnesium in triticale grains and flakes

Samples	Calcium, mg 100 g^{-1}	Iron, mg 100 g^{-1}	Potassium, mg 100 g^{-1}	Magnesium, mg 100 g^{-1}
Triticale grains	45.04 ± 1.96d*	3.77 ± 0.16a	582.61 ± 6.71c	161.03 ± 3.52d
Control flakes	55.06 ± 2.7e	3.49 ± 0.08a	486.1 ± 12.99a	127.03 ± 2.52a
1/3/1	34.76 ± 1.33 a	4.31 ± 0.22b	501.88 ± 18.71a	137.89 ± 5.63b
1/4/1	40.85 ± 1.13bc	4.50 ± 0.20b	552.22 ± 13.58b	149.33 ± 7.30c
2/1/2/1	38.45 ± 1.35 b	4.19 ± 0.18b	510.53 ± 10.04a	143.72 ± 7.16c
2/1/3/1	40.93 ± 0.62bc	4.31 ± 0.17b	516.65 ± 11.9a	150.69 ± 6.34c
2/1/4/1	42.01 ± 1.68c	4.28 ± 0.18b	518.94 ± 16.77a	151.64 ± 6.86c

* Results are expressed as mean values (n = 3) ± standard deviation. Mean values followed by the same letter within the column for the each sample group separately are not significantly different ($P < 0.05$).

No significant differences in the content of minerals between cooking methods was observed, because loses of minerals were mainly influenced by rolling thickness. The major losses occur probably due to the solubilisation of minerals by water during the steaming process, with the exception of manganese, iron and calcium (Mota et al., 2016). Comparing to literature (Zhu, 2018) content of mineral substances in tested triticale grains were higher, except phosphorus level was lower.

Table 6. Content of phosphorus, zinc and total ash in triticale grains and flakes

Samples	Phosphorus, total, mg 100 g^{-1}	Zinc, mg 100 g^{-1}	Ash, g 100 g^{-1}
Triticale grains	436.64 ± 9.08d*	3.12 ± 0.07c	1.98 ± 0.05b
Control flakes	346.61 ± 5.93a	2.84 ± 0.06b	1.56 ± 0.07a
1/3/1	368.37 ± 5.01b	2.49 ± 0.07a	1.88 ± 0.07b
1/4/1	408.69 ± 6.39c	2.65 ± 0.04a	1.86 ± 0.03b
2/1/2/1	382.49 ± 13.25b	2.63 ± 0.13a	1.93 ± 0.05b
2/1/3/1	402.55 ± 15.96c	2.68 ± 0.13a	1.91 ± 0.02b
2/1/4/1	394.29 ± 17.44bc	2.57 ± 0.35a	1.90 ± 0.06b

* Results are expressed as mean values (n = 3) ± standard deviation. Mean values followed by the same letter within the column for the each sample group separately are not significantly different ($P < 0.05$).

In current research triticale grains and flakes were classified based on the vitamin, mineral, total phenol content and antioxidant properties, using the hierarchical cluster analysis (Fig. 1).

According to agglomeration schedule coefficients samples can be divided in four clusters and three of them contains only one sample:

A – triticale grains; C – 1/4/1/;

B – control flakes; D – 1/3/1, 2/1/2/1, 2/1/3/1, 2/1/4/1.

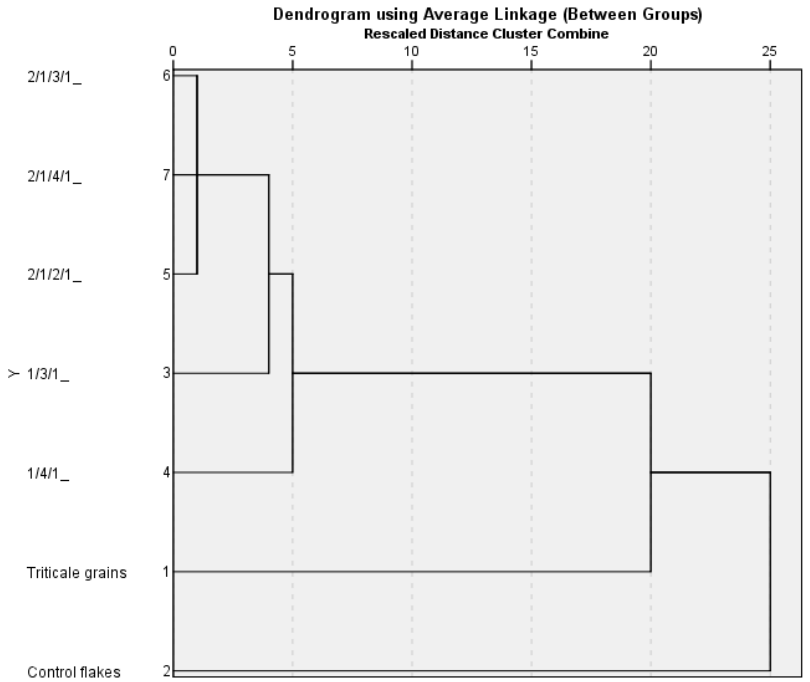

Figure 1. Dendrogram obtained by hierarchical cluster analysis using means of vitamins, minerals, total phenolics and antioxidant activity.

Triticale grains, control flakes and sample 1/3/1 did not show similarities with other samples, whereas the fourth cluster classify together samples prepared by cooking method 2 and also 1/3/1, showing similarities of these samples.

CONCLUSIONS

Triticale has high nutritional quality, containing significant amounts of protein and fibres, vitamins and minerals. Technological processes significantly influence cereals composition, but it depends on parameters tested. Control sample showed lower results and hierarchical cluster analyses showed that samples 1/3/1, 2/1/2/1, 2/1/3/1, 2/1/4/1 are similar in composition of bioactive compounds. Generally better results showed samples prepared by cooking method 2 with adding of extra water and after that samples were steamed and cooked 30 ±5 min at 1.2 bar pressure. Results showed that for selection of the best method for flaking physical and/or sensory properties should be taken in account.

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REFERENCES

Barton, B.A., Eldridge, A.L., Thompson, D., Affenito, S.G. & Striegel-Moore, R.H., Franko, D.L., Albertson, A.M. & Crockett, S.J. 2005. The relationship of breakfast and cereal consumption to nutrient intake and body mass index. *Journal of the American Dietetic Association* **105**(9), 1383–1389.

Cardoso, L. de M., Montini, T.A., Pinheiro, S.S., Pinheiro-Sant'Ana, H.M., Martino, H.S.D. & Moreira, A.V.B. 2014. Effects of processing with dry heat and wet heat on the antioxidant profile of sorghum. *Food Chemistry* **152**, 210–217.

Fardet, A. 2014. How can both the health potential and sustainability of cereal products be improved? A French perspective. *Journal of Cereal Science* **60**(3), 540–548.

Fraś, A., Gołębiewska, K., Gołębiewski, D., Mańkowski, D.R., Boros, D. & Szecówka, P. 2016. Variability in the chemical composition of triticale grain, flour and bread. *Journal of Cereal Science* **71**, 66–72.

Irakli, M.N., Samanidou, V.F., Biliaderis, C.G. & Papadoyannis, I.N. 2012. Development and validation of an HPLC-method for determination of free and bound phenolic acids in cereals after solid-phase extraction. *Food Chemistry* **134**(3), 1624–1632.

Kruma, Z., Tomsone, L., Kince, T., Galoburda, R., Senhofa, S., Sabovics, M. & Sturite, I. 2016. Effects of germination on total phenolic compounds and radical scavenging activity in hull-less spring cereals and triticale. *Agronomy Research* **14**(S2), 1372–1383.

Mota, C., Cláudia, A., Santos, M., Delgado, I., Coelho, I., Rego, A. & Castanheira, I. 2016. The effect of cooking methods on the mineral content of quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus* sp.) and buckwheat (*Fagopyrum esculentum*), *Journal of Food Composition and Analysis* **49**, 57–64.

Nurit, E., Lyan, B. & Pujos-Guillot, E. 2016. Change in B and E vitamin and lutein, β -sitosterol contents in industrial milling fractions and during toasted bread production, *Journal of Cereal Science* **69**, 290–296.

Oliveira, L.C., Alencar, N.M.M. & Steel, C.J. 2018. Improvement of sensorial and technological characteristics of extruded breakfast cereals enriched with whole grain wheat fl our and jabuticaba (*Myrciaria cauli flora*) peel. *LWT - Food Science and Technology* **90**, 207–214.

Rakha, A., Åman, P. & Andersson, R. 2011. Dietary fiber in triticale grain: Variation in content, composition, and molecular weight distribution of extractable components. *Journal of Cereal Science* **54**(3), 324–331.

Ryan, L., Thondre, P.S. & Henry, C.J.K. 2011. Oat-based breakfast cereals are a rich source of polyphenols and high in antioxidant potential. *Journal of Food Composition and Analysis* **24**(7), 929–934.

Rolls, B.J., Meengs, J.S. & Roe, L.S. 2014. Variations in cereal volume affect the amount selected and eaten for breakfast. *Journal of the Academy of Nutrition and Dietetics* **114**(9), 1411–1416.

Senhofa, S., Straumite, E., Sabovics, M., Klava, D., Galoburda, R. & Rakcejeva, T. 2015. The effect of packaging type on quality of cereal muesli during storage. *Agronomy Research* **13**(4), 1064–1073.

Sumczynski, D., Koubová, E., Sneyd, J., Erb-weber, S. & Orsavová, J. 2018. Preparation of non-traditional Dickkopf and Richard wheat flakes: Phenolic and vitamin profiles and antioxidant activity. *LWT - Food Science and Technology* **90**, 31–37.

Thomas, R.G., Pehrsson, P.R., Ahuja, J.K.C., Smieja, E. & Miller, K.B. 2013. Recent trends in rReady-to-eat breakfast cereals in the U.S. *Procedia Food Science* **2**, 20–26.

Zhu, F. 2018. Triticale : Nutritional composition and food uses, *Food Chemistry* **241**, 468–479.

Agronomy Research **16**(S2), 1425–1434, 2018
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Flavonoids and total phenolic content in extruded buckwheat products with sweet and salty taste

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Abstract. The aim of research was to evaluate the effect of added ingredients used for taste improvement on flavonoids and total phenolic content in extruded buckwheat products. The added ingredients were – sugar, vanilla sugar, stevia, agave syrup, cinnamon, caraway, garlic powder, sweet pepper powder and salt. Six extruded buckwheat products were analysed, where an extruded buckwheat product without added ingredients was a control sample, two extruded buckwheat products were with sweet taste and three products with salty taste. Total phenolic content was determined using the modified Folin–Ciocalteu method but flavonoid content according to LC-TOF-MS method.

The highest total phenolic content ($p < 0.05$) was determined in extruded buckwheat product with vanilla sugar + stevia (91.17 ± 0.67 GAE mg $100g^{-1}$DW). It was almost three times higher than in the control sample. The significant differences were observed in extruded buckwheat products with caraway + salt + agave syrup (41.5 ± 0.12 GAE mg $100g^{-1}$DW) and sweet pepper powder + salt + agave syrup (42.39 ± 0.80 GAE mg $100g^{-1}$DW) comparing to other extruded products ($p < 0.05$). The highest content of rutin and quercetin ($p < 0.05$) was established in extruded buckwheat product with garlic powder + salt + agave syrup, whereas the highest content of catechin and epicatechin – in extruded buckwheat product with vanilla sugar + stevia. The extruded buckwheat product with caraway + salt + agave syrup in addition contained luteolin, kaempferol and isoquercitrin. The results of research showed that some added ingredients used for taste improvement can significantly influence the total phenolic content and flavonoid content.

Key words: buckwheat, extrusion, flavonoids, total phenolic content.

INTRODUCTION

Buckwheat is an alternative crop with high consumption in countries like China, Japan and Taiwan due to its functional food properties and biological value – great concentration of bio–active compounds such as phenols and flavonoids (Lin et al., 2009; Inglett et al., 2010; Qin et al., 2010; Zhang et al., 2012; Vollmannova et al., 2013). The diverse total phenolic content of buckwheat is reported in literature, the differences of results establish buckwheat species: common (*Fagopyrum esculentum* Moench) or tartary (*Fagopyrum tataricum* Gaertn); cultivars, growth conditions and technological production process. Mikulajeva et al. (2016) analysed 22 common buckwheat cultivars,

which total phenolic content ranged between 0.897 and 4.226 mg GAE g^{-1} DW. Unal et al. (2017) reported about significant differences of total phenolic content between commercial buckwheat (produced in Kazakhstan) and Günes variety (produced in Turkey) – 207.12 ± 2.67 and 329.83 ± 3.88 mg GAE 100 g^{-1}, respectively. The diverse results about flavonoids content like rutin can be found in literature, too. Mikulajova et al. (2016) established rutin content in common buckwheat cultivars between 59.93 ± 2.16 and 304.45 ± 5.45 μg g^{-1} DW, whereas Vollmannova et al. (2013) – between 309.97 ± 10.84 and 507.78 ± 17.11 mg kg^{-1} DW.

The increase of buckwheat consumption in Latvia could be possible by production of new buckwheat products. In Asian countries and Italy there are buckwheat noodles, in Latvia there could be some buckwheat snacks using extrusion. There is not a single view about the effect of processing on phenolic and flavonoid content in buckwheat. Zielinska et al. (2007) and Wronkowska et al. (2015) concluded significant differences of phenolic compounds in raw and roasted buckwheat groats, whereas Hes et al. (2014) did not establish any negative effect on the nutritional properties in buckwheat groats after 30 min boiling in water. Sensoy et al. (2006) determined insignificant decrease of total phenolic content in dark buckwheat flour after roasting (200 °C, 10 min), whereas antioxidant activity was significantly lower in roasted dark buckwheat flour comparing to raw dark buckwheat flour. Our previous research (Beitane et al., 2018) showed significant decrease of total phenolic content, antiradical activity, radical scavenging activity and phenolic compounds concentration in buckwheat after extrusion.

In production of new products their taste has got important role, which could be improved with several ingredients. Furthermore the added ingredients used for taste improvement can affect the nutritional and biological value of the final product. In this research the added ingredients for taste improvement of extruded buckwheat products were sugar, vanilla sugar, stevia, agave syrup, cinnamon,-caraway, garlic powder, sweet pepper powder and salt. The brief description of used ingredients and their importance in food product production is given below.

Vanillin is the most used flavour in the food industry with antimicrobial and antioxidant properties (Zabkova et al., 2006).

Stevia rebaudiana or steviol glycosides are thermally stable and natural sweeteners with high sweetening potential, which are successfully used in cocoa and chocolate products, in chewing gums, flour or starch–based snacks etc. to reduce sugar consumption in the industrialized countries (Olsson et al., 2016; Shannon et al., 2016).

Agave syrup is a natural sweet product with functional properties due to biological active compounds, prebiotic molecules and antioxidant content, which is used as sugar replacement due to low glycaemic index (Foster–Powell et al., 2002; Phillips et al., 2009; Mellado–Mojica & Lopez, 2015, Muniz–Marquez et al., 2015).

Cinnamon is a culinary spice with anti–oxidant properties and free radical scavenging activity, which is used in traditional Chinese medicine and which has beneficial effects on serum glucose, triglyceride and total cholesterol level in diabetic patients (Parkash et al., 2007; Crawford, 2009; Otto, 2010; Adisakwattana et al., 2011).

Caraway is the most cultivated spice in Europe with wide use in folk medicine (Ghoneem et al., 2016; Kluz et al., 2016). Particular attention in scientific researches is paid to caraway essential oil with the main components – carvone and limonene (Ghoneem et al., 2016).

Garlic is a vegetable, which is known worldwide, with anti–bacterial and anti–fungal properties, high flavonoids content, especially quercetin, and antioxidant capacity (Griffiths et al., 2002; Beato et al., 2011; Onyeoziri et al., 2016).

Sweet pepper is a vegetable with high content of vitamins, carotenoids and phenols, which could be included in daily diet or used as additive in food production or in traditional medicine (Marti et al., 2011; Hernandez–Ortega et al., 2012; Sharma et al., 2016; Raybaudi–Massilia et al., 2017)

The aim of research was to evaluate the effect of added ingredients used for taste improvement on flavonoids and total phenolic content in extruded buckwheat products.

MATERIALS AND METHODS

Materials

Raw common buckwheat (Fagopyrum esculentum Moench) grown in Latvia in 2017, was produced by the organic farm 'Bebri'.

The added ingredients for taste improvement of extruded buckwheat products were purchased in grocery: sugar (Dan Sukker, Denmark), vanilla sugar produced from sugar and flavouring material vanillin (Mood, Lithuania), stevia (Canderel, The Czech Republic), agave syrup (Super Garden, Mexico), cinnamon (Santa Maria, Estonia), caraway (Santa Maria, Estonia), garlic powder (Kotanyi, Austria), sweet pepper powder (Santa Maria, Estonia) and salt (Artiomosol, Ukraine).

Dough preparation for extrusion

Raw buckwheat grain was milled using Mühle 2 (Hawo's, Germany) to obtain whole grain flour. Buckwheat flour with added ingredients and water was mixed with Sirman Planetary Mixers, model Pluton 10 (Minneapolis, Italy). The added ingredients for taste improvement of extruded buckwheat products were sugar, vanilla sugar, stevia, agave syrup, cinnamon, caraway, garlic powder, sweet pepper powder and salt. Six different doughs were prepared (Table 1), where buckwheat dough without added ingredients was prepared as control, two doughs were with sweet taste (vanilla sugar + stevia; sugar + cinnamon) and three – with salty taste (caraway + salt + agave syrup; garlic powder + salt + agave syrup; sweet pepper powder + salt + agave syrup).

Table 1. The formation of dough and abbreviations in buckwheat products

	Control	B+VS	B+SC	B+CSA	B+GSA	B+PSA
Buckwheat flour, g	100	100	100	100	100	100
Water, mL	75	75	75	75	75	75
Sugar, g	–	–	5	–	–	–
Vanilla sugar, g	–	5	–	–	–	–
Stevia, g	–	4	–	–	–	–
Agave syrup, g	–	–	–	5	5	5
Cinnamon, g	–	–	3	–	–	–
Caraway, g (milled)	–	–	–	5	–	–
Garlic powder, g	–	–	–	–	1.5	–
Sweet pepper powder, g	–	–	–	–	–	5
Salt, g	–	–	–	1.5	1.5	1.5

Extrusion

The prepared buckwheat doughs with different tastes were extruded with the food extruder PCE Extrusiometer L–Serie (Göttfert, Germany) by temperature profile 75/90/100 °C. After extrusion the products were cut in squares of 1 cm, dried in the convective–rotary oven (SVEBA DAHLAN, Sweden) at the temperature 140 ± 2 °C for 25 min and cooled to room temperature. Afterwards, six acquired extruded buckwheat products were used for further analysis.

Methods

Total phenolic content (TPC) of added ingredients for taste improvement and extruded buckwheat products was determined using the modified Folin–Ciocalteu method as described by Herald et al. (2012). The measurement was conducted by mixing Folin–Ciocalteu solution (1:1 with water), sodium bicarbonate and ethanolic extract. The absorbance was measured after 90 min of incubation at 765 nm againts a blank. TPC was expressed as gallic acid equivalents (GAE mg 100 g^{-1} DW), based on the gallic acid (GA) calibration curve (range 0.025–0.20 mg mL^{-1}, $R^2 = 0.9997$). Analyses were performed with the Infinite M200 PRO (Tecan Group Ltd., Männedorf, Switzerland) instrument in triplicate. The bandwidth was 9 mm and temperature 24 °C.

Determination of flavonoid content in extruded buckwheat products was made with the Liquid Chromatography – Time of Flight Mass Spectrometer (LC–TOF–MS) as described by Klavina et al. (2015). During LC–MS analysis, each sample produces its own base peak chromatogram (BPC). In the positive ionization mode each compound can add a proton and can produce its own $[M+H]^+$ mass spectra or stay in a positively charged state as M^+ molecule. Each compound has its own chemical formula and molar mass. This chemical formula using *Mass Hunter Qualitative Analyses B.07.00* software can be used for the calculation of $[M+H]^+/[M]^+$ which is used for the extraction of compounds from the base peak chromatogram. Both values can be compared and the difference (Δ) between them should not be higher than 0.0030. HRMS experiments ensure accurate mass measurements resulting in the removal of background signals of complex matrix interferences. It is useful for non–targeted or retrospective post–targeted identification of unknown compounds by the processing of raw data obtained in different scan modes, including full scan in defined *m/z* windows. The experimental data were handled using the MassHunter version B07.00 software (Agilent Technologies).

The extruded buckwheat products were analysed in three replications.

Data processing

The data of research were analysed using mathematical and statistical methods of Microsoft Office Excel 14.0. To determine the significant differences between the mean values were used the analyses of variance (ANOVA), *T–test* and *P–value* at 0.05.

RESULTS AND DISCUSSION

The total phenolic content (TPC) of ingredients used for taste improvement in extruded buckwheat products is given in Table 2.

The obtained data allows better to understand and explain the results of current research.

Table 2. Total phenolic content in extruded buckwheat products with different taste

Ingredients for taste improvement	TPC, GAE mg 100 g^{-1}
Sugar	n.d.
Vanilla sugar	125.86 ± 6.09
Stevia	n.d.
Agave syrup	26.68 ± 2.17
Cinnamon, g	1,534.07 ± 34.18
Caraway	202.62 ± 7.39
Garlic powder	51.60 ± 1.78
Sweet pepper powder	285.47 ± 8.01
Salt	n.d.

n.d. – not detected.

The extruded buckwheat products with sweet and salty taste displayed higher TPC as control sample (Fig. 1). It means that in generally the added ingredients for taste improvement of extruded buckwheat products had favourable effect on TPC. However statistically significant differences were observed between control and extruded buckwheat products with vanilla sugar + stevia; caraway + salt + agave syrup and sweet pepper powder + salt + agave syrup.

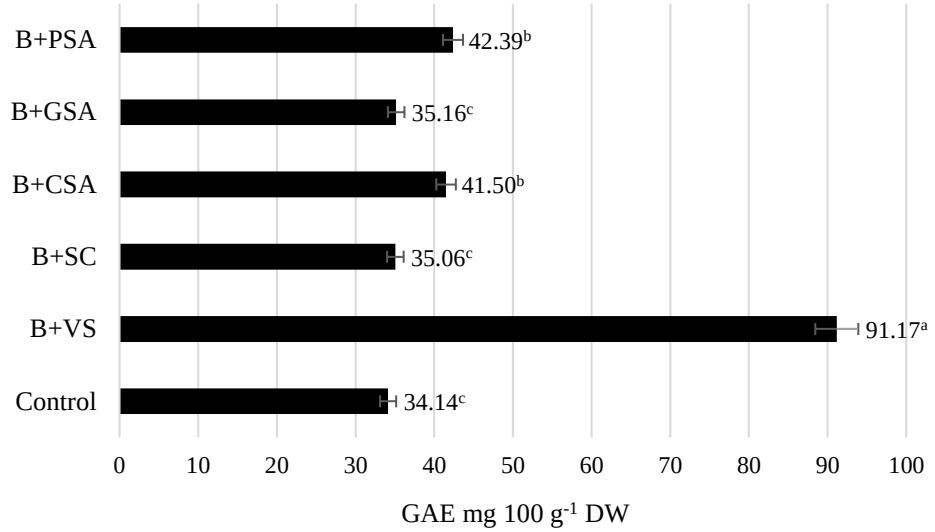

Figure 1. Total phenolic content in extruded buckwheat products with different taste.
Used letters (a, b, c) point to statistically significant difference between buckwheat products *($P < 0.05$)*.

The highest TPC (*$P < 0.05$*) was determined in extruded buckwheat product with vanilla sugar + stevia (91.17 ± 0.67 GAE mg 100 g^{-1} DW). The impressive increase of TPC in extruded buckwheat product with vanilla sugar + stevia could be explained with added vanilla sugar. The results of current research (Table 2) showed, that vanilla sugar had a high total phenolic content (125.86 ± 6.09 GAE mg 100 g^{-1}), whereas in stevia it was not detected at all. There are not researches in literature about content of possible phenolic compounds in vanilla sugar. Only Burri et al. (1989) concluded that vanillin

acts as an antioxidant in products of cereals. There are necessary profound researches about vanilla sugar, but it could be concluded, that the addition of vanilla sugar to extruded buckwheat product provided significant increase of TPC.

The great TPC ($P < 0.05$) in comparison to control was determined in extruded buckwheat products with caraway + salt + agave syrup and sweet pepper powder + salt + agave syrup, too. The increase of TPC in extruded buckwheat products could be based on addition of caraway and sweet pepper powder, because both presented great TPC (202.62 and 285.47 GAE mg 100 g^{-1}, respectively). Sharma et al. (2016) reported about high total phenolic content in red sweet pepper, which varied between 488.81 ± 8.18 and 977.63 ± 25.40 μg GAE g^{-1} FW (fresh weight). It is known that production process of sweet pepper powder can markedly affect the total phenolic and flavonoid content, however it is rich source of phenols. Shotorban et al. (2012) indicated that different processing techniques can significantly affect the content of bioactive compounds like polyphenols. Therefore the increase of TPC in extruded buckwheat product with sweet pepper powder + salt + agave syrup was not so marked. The other reason for increase of TPC in extruded buckwheat products with salty taste could be related with addition of agave syrup. Agave syrup is produced from agave plant and it rightly pointed to the presence of TPC in agave syrup (26.68 GAE mg 100 g^{-1}). Unfortunately, the few researches in literature about agave syrup chemical composition are focused on sugar composition.

The extruded buckwheat products with added sugar + cinnamon and garlic powder + salt + agave syrup did not provide expected increase of TPC, though cinnamon had the greatest total phenolic content among the added ingredients for taste improvement. It could be explained with the small amount of added cinnamon to dough, and extrusion, which had adverse effects on total phenolic content according to previous research (Beitane et al., 2018).

Extruded buckwheat product with garlic powder + salt + agave syrup had significantly greater concentration of rutin and quercetin ($P<0.05$) comparing to other products (Table 3).

Table 3. Flavonoids content in extruded buckwheat products with different taste, mg 100 g^{-1} DW

	Rutin	Quercetin	Catechin	Epicatechin
Control	5.51 ± 0.72^{b}	1.48 ± 0.13^{b}	3.97 ± 0.23^{a}	7.36 ± 0.62^{a}
B+VS	6.75 ± 0.81^{b}	1.68 ± 0.12^{b}	4.45 ± 0.26^{a}	8.02 ± 0.69^{a}
B+SC	6.72 ± 0.77^{b}	1.59 ± 0.19^{b}	4.01 ± 0.20^{a}	6.24 ± 0.48^{b}
B+CSA	5.35 ± 0.90^{b}	1.28 ± 0.21^{b}	2.20 ± 0.18^{b}	4.20 ± 0.33^{c}
B+GSA	8.99 ± 0.69^{a}	2.27 ± 0.17^{a}	2.47 ± 0.17^{b}	7.26 ± 0.52^{a}
B+PSA	5.90 ± 0.72^{b}	1.52 ± 0.11^{b}	2.65 ± 0.22^{b}	5.07 ± 0.66^{c}

Mean values with different letters (a, b, c) within a column point to statistically significant difference between buckwheat products *($P < 0.05$).*

The increase of quercetin concentration in extruded buckwheat product with garlic powder + salt + agave syrup could be based on literature, where Griffiths et al. (2002) reported about high content of flavonoids, especially, quercetin and its conjugates in garlic; Miean & Mohamed (2001) determined relatively high content of quercetin – 47.0 mg kg^{-1} DW, myricetin – 693.0 mg kg^{-1} DW and apigenin – 217.0 mg kg^{-1} DW in garlic. The extruded buckwheat products with garlic powder + salt + agave syrup

contained great concentration of rutin in the present study. It could be due to the presence of added garlic powder and agave syrup, because both presented the content of phenols. Scientific reports about flavonoids composition in garlic did not provide any information about rutin concentration. However the conclusions of researches about flavonoids content in garlic are contrary, for example Beato et al. (2011) did not detect myricetin, quercetin, kaempferol and apigenin in garlic. Therefore it cannot exclude the possibility that the addition of garlic powder provided the increase of rutin content in extruded buckwheat product with garlic powder + salt + agave syrup. The aim of current research was not to evaluate the flavonoid content of added ingredients, therefore it could be investigate in the following research. It was positive that all products except extruded buckwheat product with caraway + salt + agave syrup had rutin and quercetin concentration higher than control sample.

The greatest concentration of catechin and epicatechin was observed in extruded buckwheat product with vanilla sugar + stevia. Product which had the highest TPC, too. Catechin concentration among extruded buckwheat product with vanilla sugar + stevia, control sample and extruded buckwheat product with sugar + cinnamon was insignificant ($P > 0.05$) as well as epicatechin concentration was insignificant among extruded buckwheat product with vanilla sugar + stevia, control sample and extruded buckwheat product with garlic powder + salt + agave syrup ($P > 0.05$).

The extruded buckwheat product with caraway + salt + agave syrup in addition contained luteolin – 0.24 mg 100 g^{-1} DW, kaempferol – 0.46 mg 100 g^{-1} DW and isoquercitrin – 1.55 mg 100 g^{-1} DW. Isoquercitrin was detected in extruded buckwheat products with garlic powder + salt + agave syrup and sweet pepper powder + salt + agave syrup (0.22 and 0.35 mg 100 g^{-1} DW, respectively).

The extruded buckwheat product with sugar + cinnamon showed insignificant results about TPC, rutin, quercetin and catechin content comparing to control sample, except epicatechin content, which was significantly lower. The addition of cinnamon did not give the expected result, though Adisakwattana et al. (2011) reported that in cinnamon extract the TPC varied between 0.17–0.21 g GAE g^{-1} and flavonoids content – between 48.85–65.52 mg QE g^{-1}.

CONCLUSIONS

The aim to produce new buckwheat product with increased nutritional value was achieved, because some added ingredients used for taste improvement can significantly influence the total phenolic content and flavonoid content. However there is a need for further researches to understand and explain how and why the added ingredients affected/not affected the total phenolic and flavonoid content in extruded buckwheat products.

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REFERENCES

Adisakwattana, S., Lerdsuwankij, O., Poputtachai, U., Minipun, A. & Suparpprom, C. 2011. Inhibitory activity of cinnamon bark species and their combination effect with acarbose against intestinal *α*–glucosidase and pancreatic *α*–amylase. *Plant Foods for Human Nutrition* **66**, 143–148.

Beato, V.M., Orgaz, F., Mansilla, F. & Montano, A. 2011. Changes in phenolic compounds in garlic (*Allium sativum* L.) owing to the cultivar and location of growth. *Plant Foods for Human Nutrition* **66**, 218–223.

Beitane, I., Krumina–Zemture, G & Sabovics, M. 2018. Effect of germination and extrusion on the phenolic content and antioxidant activity of raw buckwheat (*Fagopyrum esculentum* M.). *Journal Agronomy Research* **16**. (Accepted for publication)

Burri, J., Graf, M., Lambelet, P. & Loliger, J. 1989. Vanillin – more than a flavouring agent – a potent antioxidant. *Journal of the Science of Food and Agriculture* **48**, 49–56.

Crawford, P. 2009. Effectiveness of cinnamon for lowering haemoglobin A1C in patients with type 2 diabetes: A randomized, controlled trial. *The Journal of the American Board of Family Medicine* **22**, 507–512.

Foster–Powell, K., Holt, S.H.A. & Brand–Miller, J.C. 2002. International table of glycemic index and glycemic load values: 2002. *The American Journal of Clinical Nutrition* **76**, 5–56.

Ghoneem, K.M., Saber, W.I.A., El–Awady, A.A., Rashad, Y.M. & Al–Askar, A.A. 2016. Alternative preservation method against *Sclerotium* tuber rot of Jerusalem artichoke using natural essential oils. *Phytoparasitica* **44**, 341–352.

Griffiths, G.F., Trueman, C.T., Thomas, B.M. & Smith, B.M. 2002. Onions, a global benefits to health. *Phytotherapy Research* **16**, 603–615.

Herald, T.J., Gadgil, P. & Tilley, M. 2012. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agriculture* **92**, 2326–2331.

Hernandez–Ortega, M., Ortiz–Moreno, A., Hernandez–Navarro, M.D., Chamorro–Cevallos, G., Dorantes–Alvarez, L. & Necoechea–Mondragon, H. 2012. Antioxidant, antinociceptive, and anti–inflammatory effects of carotenoids extracted from dried pepper (*Capsicum annuum* L.). *Journal of Biomedicine and Biotechnology*, 1–10. DOI:10.1155/2012/524019

Hes, M., Dziedzic, K., Gorecka, D., Drozdzynska, A. & Gujska, E. 2014. Effect of boiling in water of barley and buckwheat groats on the antioxidant properties and dietary fiber composition. *Plant Foods for Human Nutrition* **69**, 276–282.

Inglett, G.E., Rose, D.J., Chen, D., Stevenson, D.G. & Biswas, A. 2010. Phenolic content and antioxidant activity of extracts from whole buckwheat (*Fagopyrum esculentum* Moench) with or without microwave irradiation. *Food Chemistry* **119**, 1216–1219.

Klavina, L., Springe, G., Nikolajeva, V., Martsinkevich, I., Nakurte, I., Dzabijeva, D., Steinberga, I. 2015. Chemical composition of analysis, antimicrobial activity and cytotoxicity screening of moss extracts (Moss phytochemistry). *Molecules* **20**(9), 17221–17243.

Kluz, M., Terentjeva, M., Puchalski, C., Hutkova, J., Kantor, A., Petrova, J., Mellen, M., Cubon, J., Hascik, P., Kordiaka, R., Kunova, S. & Kacaniova, M. 2016. The extension of shelf–life of chicken meat after application of caraway and anise essential oils and vacuum packaging. *Potravinarstvo* **10**(1), 132–138.

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* **112**, 987–991.

Marti, M.C., Camejo, D., Vallejo, F., Romojaro, F., Bacarizo, S., Palma, J.M., Sevilla, F. & Jimenez, A. 2011. Influence of fruit ripening stage and harvest period on the antioxidant content of sweet pepper cultivars. *Plant Foods for Human Nutrition* **66**, 416–423.

Mellado–Mojica, E. & Lopez, M. 2015. Identification, classification, and discrimination of agave syrups from natural sweeteners by infrared spectroscopy and HPAEC–PAD. *Food Chemistry* **167**, 349–357.

Miean, K.H. & Mohamed, S. 2001. Flovonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry* **49**, 3106–3112.

Mikulajova, A., Sediva, D., Hybenova, E. & Mosovska, S. 2016. Buckwheat cultivars – phenolic compounds profiles and antioxidant properties. *Acta Chimica Slovaca* **9**, 124–129.

Muniz–Marquez, D.B., Contreras, J.C., Rodriguez, R., Mussatto, S.I., Wong–Paz, J.E., Teixeira, J.A. & Aguilar, C.N. 2015. Influence of thermal effect on sugars composition of Mexican Agave syrup. *Journal of Food* **13**, 607–612.

Olsson, K., Carlsen, S., Semmler, A., Simon, E., Mikkelsen, M.D. & Moller, B.L. 2016. Microbial production of next-generation stevia sweeteners. *Microbial Cell Factories* **15**(207), 1–14. DOI 10.1186/s12934–016-0609–1

Onyeoziri, U.P., Romanus, E.N. & Onyekachukwu, U.I. 2016. Assessment of antioxidant capacities and phenolic contents of Nigerian cultivars of onions (*Allium cepa* L.) and garlic (*Allium sativum* L.). *Pakistan Journal of Pharmaceutical Sciences* **29**, 1183–1188.

Otto, A.D. 2010. Cinnamon as a supplemental treatment for impaired glucose tolerance and type 2 diabetes. *Current Diabetes Reports* **10**, 170–172.

Parkash, D., Suri, S., Upadhyay, G. & Singh, B.N. 2007. Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. *International Journal of Food Sciences and Nutrition* **58**, 18–28.

Phillips, K.M., Carlsen, M.H. & Blomhoff, R. 2009. Total antioxidant content of alternatives to refined sugar. *Journal of the American Dieteric Association* **109**, 64–71.

Qin, P., Wang, Q., Shan, F., Hou, Z. & Ren, G. 2010. Nutritional composition and flavonoids content of flour from different buckwheat cultivars. *International Journal of Food Science and Technology* **45**, 951–958.

Raybaudi–Massilia, R., Suarez, A.I., Arvelo, F., Zambrano, A., Sojo, F., Calderon–Gabaldon, M.I. & Mosqueda–Melgar, J. 2017. Cytotoxic, antioxidant and antimicrobial properties of red sweet pepper (*Capsicum Annuum* L. var. Llaneron) Extracts: *In vitro* study. *International Journal of Food Studies* **6**, 222–231.

Sensoy, I., Rosen, R.T., Ho, C.T. & Karwe, M.V. 2006. Effect of processing on buckwheat phenolics and antioxidant activity. *Food Chemistry* **99**, 388–393.

Shannon, M., Rehfeld, A., Frizzell, C., Livingstone, C., McGonagle, C., Skakkebaek, N.E., Wielogorska, E. & Connolly, L. 2016. *In vitro* bioassay investigations of the endocrine disrupting potential of steviol glycosides and their metabolite steviol, components of the natural sweetener *Stevia*. *Molecular and Cellular Endocrinology* **427**, 65–72.

Sharma, V.K., Chandel, C., Kumar, R., Meena, R.D. & Mawliya, M. 2016. Influence of planting time and fruit maturity stage on antioxidant activity, phenols and anthocyanin contents in sweet pepper. *Research on Crops* **17**, 360–368.

Shotorban, N.I.Y., Jamei, R. & Heidari, R. 2012. Antioxidant activities of two sweet pepper Capsicum annuum L. varieties phenolic extracts and the effects of thermal treatment. *Avicenna Journal of Phytomedicine* **3**, 25–34.

Unal, H., Izli, G., Izli, N. & Asik, B.B. 2017. Comparison of some physical and chemical characteristics of buckwheat (*Fagopurym esculentum* Moench) grains. *CYTA – Journal of Food* **15**, 257–265.

Vollmannova, A., Margitanova, E., Toth, T., Timoracka, M., Urminska, D., Bojnanska, T. & Cicova, I. 2013. Cultivar influence on total polyphenol and rutin contents and total antioxidant capacity in buckwheat, amaranth, and quinoa seeds. *Czech Journal of Food Sciences* **31**, 589–595.

Wronkowska, M., Honke, J. & Piskula, M.K. 2015. Effect of solid-state fermentation with *Rhizopus oligosporus* on bioactive compounds and antioxidant capacity of raw and roasted buckwheat groats. *Italian Journal of Food Science* **27**, 424–431.

Zabkova, M., Otero, M., Minceva, M., Zabka, M. & Rodrigues A.E. 2006. Separation of synthetic vanillin at different pH onto polymeric adsorbent Sephabeads SP206. *Chemical Engineering and Processing* **45**, 598–607.

Zhang, Z.L., Zhou, M.L., Tang, Y., Li, F.L., Tang, Y.X., Shao, J.R., Xue, W.T. & Wu, Y.M. 2012. Bioactive compounds in functional buckwheat food. *Food Research International* **49**, 389–395.

Zielinska, D., Szawara–Nowak, D. & Zielinski, H. 2007. Comparison of spectrophotometric and electrochemical methods for the evaluation of the antioxidant capacity of buckwheat products after hydrothermal treatment. *Journal of Agricultural and Food Chemistry* **55**, 6124–6131.

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Development of the composition and technology of the frozen dessert without sugar, using sesame flour

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Abstract. The article presents the results of the analysis of the entry dose of stevioside, topinambur syrup, sesame flour and the type of stabilizer added to the milk ice cream formula, theoretically calculated, using solids according to GOST 31457-2012 for development of a functionally oriented dessert with low content of fat and without sugar. It has been established that the entry dose of the stevioside and the topinambur syrup in an amount of 0.03 and 5% respectively to the mixture mass of the frozen dessert allows us to get the product with pleasant flavor and scent. The use of the PGX-1 stabilizer (Germany) with an entry dose of 0.4 to the mixture mass allows us to get the product with the best indicators for whipping, thawing resistance, density and uniformity of consistency. It has been determined that the entry dose of sesame flour added to the mixture mass varies from 1.5 to 2%. It is expedient to put it into the normalize mixture after the process of homogenization before milling, pre-brewing with water in the ratio of 1:10 for 15–20 minutes to humidity (80 ± 1) %. It has been found that the expiration date of the frozen dessert without sugar with the use of sesame flour is 6 months at a temperature of – 18 °C.

Key words: frozen dessert, topinambur syrup, stevioside, stabilizer, sesame flour, prophylactic and functional products.

INTRODUCTION

A hot topic in the food industry is the creation of dietary, therapeutic food products of functional orientation with low content of fat, without sugar, with innovative useful additives to prevent the diseases connected with malnutrition of children and adults and to protect and promote the health of the population. The problem of diabetes is alarmingly increasing (Saad et al., 2013). According to preliminary data, there were 4,418,305 patients with diabetes in the Russian Federation in 2015 and every year the number increases. For this reason, the development of the technology of the frozen low-fat desserts without sugar, without sugar, with sesame flour for diabetics and people with obesity and calcium deficiency is a relevant and timely aim.

Stevioside is a glycoside from an extract of Stevia plants. In comparison with sucrose, stevioside is 250–300 times more sweet. As a result of a patent and information search, stevioside and topinambur syrup were chosen as a sugar substitute (Erashova &

Pavlova, 1997; Arseneva & Yakovleva, 2012; Ozdemir et al., 2015). Stevia extract contains over 70 chemical elements, providing its unique therapeutic and prophylactic properties, flavonoids, soluble chlorophyll and xanthophyll, oxycinnamic acids (coffee, chlorogenic, etc.), neutral water-soluble oligosaccharides, free sugar, 17 amino acids (including 8, which are indispensable), fiber, tannins, essential oil (containing 53 components), mineral compounds, vitamins A, B, C, D, E, K, P, PP, trace elements: iron, zinc, phosphorus, potassium, magnesium, calcium, selenium, sodium, iodine, emulsified fat, saponins and other components, including silver (0.0006 mg mL^{-1}), which ensures the antimicrobial properties of the Stevia extract (Arseneva, 2011a).

Stevioside is a harmless natural sugar substitute of low energy value without mutagenic and carcinogenic features, non-toxic, heat-resistant, stable in acid and alkalis. It does not require a large dosage and is harmless with a long-term use. Stevioside is used for the prevention and treatment of type 1 and type 2 diabetes.

Topinambur syrup has a sweet taste due to the fructans of rare polymers, which act on humans differently than glucose and fructose than usual glucose. Fructans are contained in a very small amount of plants. Tubers of topinambur are the absolute leaders in their concentration (Arseneva & Yakovleva, 2011). Polysaccharide inulin, which is contained in topinambur syrup, improves metabolism, maintains normal intestine microflora, reduces cholesterol; pectin and fiber clear toxins and wastes from the body; vitamins C, B1, B2 and PP and trace elements, especially biogenic silicon, iron, magnesium, potassium, etc. strengthen joints, bones, heart, contribute to the maintenance of immunity and health in general. Topinambur syrup glycemic index is only 13–16 gl, which is the lowest indicator among all known sweeteners. The natural topinambur syrup, obtained from the topinambur tubers, contains about 40% of the plant fiber, where the sweet polymer is concentrated. This fiber gives a lasting sense of saturation, because it does not decompose in stomach. The process of glucose releasing, so necessary for the full brain and other body organs function, begins only in intestine (Arsenyeva, 2002; Arsenyeva, 2011b).

In dietotherapy topinambur is recommended for diabetics, as it has a sugar-reducing property, a beneficial effect on the pancreas. It also reduces blood pressure and increases hemoglobin (Vozhdaeva & Sorochkina, 2000; Suhas et al., 2015). Topinambur syrup contains prebiotics, specific substances necessary for nutrition and healthy activity of intestinal cultures (probiotics). Biologically active topinambur substances also stimulate heart, reduce blood pressure and level of ‘bad’ cholesterol in blood (Yakovleva, 2012).

The benefit of sesame flour is primarily due to the vitamin-mineral product structure, which contains a wide amount of calcium and other useful compounds of natural origin. Percent of consumption (based on 100 g of sesame per day): Food fiber – 28%; Vitamin B1 (thiamin) – 80%; Vitamin B2 – 20%; Vitamin E – 15%; Vitamin PP – 55%; Potassium – 20%; Calcium – 140%; Magnesium – 135%; Phosphorus – 90%; Iron – 90%. Sesame helps to strengthen the entire bone system. It contributes to the blood vessels purification (including brain), relieves spasms, cramps and muscle pain. It also strengthens immunity, removes toxins and gently cleanses intestine. Sesame is recommended in case of high cholesterol level (Zhukovsky, 1997).

MATERIALS AND METHODS

In this research the entry dose of stevioside, topinambur syrup, sesame flour and the type of stabilizer added to the milk ice cream formula, theoretically calculated using solids according to GOST 31457-2012 was determined in order to obtain a sugar-free ice cream formula using sesame flour(Onopriyko et al., 2004; Arseneva, 2009).

The experimental samples of the frozen dessert mixture were prepared in a laboratory in the following way: pre-mixed dry components were added to water heated to a temperature of 35−40 °C, and were mixed until their almost complete dissolution. The mixture was subjected to a thermal treatment at a temperature of 85 °C with 60 second extract time. Homogenization was carried out at the same temperature, the homogenization pressure was about 10–12 MPa (Olenev et al., 2004).

Sesame flour was added to the homogenized mixture, cooled to a temperature of 4−6 °C, and left in a freezer for ripening the mixture for 4−12 hours. A batch freezer was used without forced air supplying for freezing. Upon the completion of this process, the temperature of the frozen dessert was about -4−-6 °C.

Standard techniques were used in this research to determine physico-chemical parameters, and a score system of the organoleptic parameters presented in Tables 1, 2 and 3 was developed.

The characteristics of the sweetness and scent of the frozen dessert are presented in Table 1 in points.

Table 2 shows the characteristics of organoleptic indicators (sweetness and color) of the frozen dessert samples in points.

Table 1. The characteristics of the sweetness and scent of the frozen dessert

Sweetnes and scent characteristics	Number of points
Unsweetened, without stevia scent	1
Lack of sweetness, without stevia scent	2
Not enough sweetened, stevia scent	3
Excessively sweetened, stevia scent	4
Sweetened, stevia scent	5

Table 2. The characteristics of organoleptic indicators (sweetness and color) of the frozen dessert samples in points

Flavor and color characteristics	Number of points
Excessively sweetened, coffee color	1
Highly sweetened, light coffee color	2
Sugary, beige color	3
Not sweetened enough, milk color	4
Pleasantly sweetened, milk color	5

The characteristic of the quality indicators was developed according to the 5-point scale to make an organoleptic assessment of the frozen dessert samples (Table 3).

Table 3. The characteristic of the quality indicators was developed according to the 5-point scale to make an organoleptic assessment of the frozen dessert samples

Indicator	Product organoleptic characteristics	Points
Consistency	Homogeneous, moderately dense	5
	Homogeneous, not dense	4
	Heterogeneous, slightly liquid	3
	Heterogeneous, excessively dense	2
	Heterogeneous throughout the mass, excessively liquid	1

Table 3 (continued)

Color	Uniform, white, barely visible particles of sesame flour are allowed	5
	Uniform, white, with visible particles of sesame flour	4
	Uneven, white-gray, with visible particles of sesame flour	3
	Uneven, white-gray, with significant particles of sesame flour	2
	Uneven, taupe, with large particles of sesame flour	1
Sweetness	Pure, with pronounced sweetness, without foreign flavor	5
	Moderately sweet, insufficiently pronounced	4
	Unpronounced flavor	3
	Unpronounced, with foreign flavor	2
	Pronounced foreign flavor	1
Scent	Pure, with pronounced aroma, without foreign odor	5
	Pure, without foreign odor	4
	Unpronounced	3
	Unpronounced odor of foreign filler	2
	With pronounced unpleasant foreign odor	1

RESULTS AND DISCUSSION

At the first stage of the research the concentration of stevioside was selected. It varied from 0.03 to 0.07% to the mixture mass within 0.005%.

Organoleptic features of the frozen dessert samples were assessed on a five-point scale. It is noted that the concentration of stevioside did not influence the consistency of the final product, the color remained unchanged, therefore all the samples received 5 points for consistency and color.

Fig. 1 presents the profilograms of the organoleptic assessment of the frozen dessert samples with a sweetener dose, % to the mixture mass: from 0.030 to 0.050 (a) and from 0.055 to 0.070 (b).

As seen from the profilograms (Fig. 1), the highest score for the organoleptic indicators was obtained by an experimental sample with the entry dose of stevioside of 0.05%. However, a frozen dessert with this concentration had a pronounced unpleasant specific flavor of stevioside. At a concentration of 0.03% there was no flavor, but it was not sweetened enough. Therefore, further research was devoted to the selection of the topinambur syrup concentration, which varied from 1 to 9% to the mixture mass within 1%. Products consistency did not change even at various topinambur syrup concentration and scored five points.

As seen from the profilogram (Fig. 2), the highest score for the organoleptic indicators was obtained by the experimental sample with the entry dose of stevioside of 5% to the mixture mass.

It is established that the frozen dessert sample had the highest score (5 points according to flavor characteristics) when adding 5% topinambur syrup and 0.03% stevioside to the mixture mass.

The selection of stabilizer entry dose and type was done at the following stage (Vzor & Nikitkov, 1998; Arseneva, 2011b). PGX-1 (Germany), Kremodan SE 334 VEG (USA), Denyse 805 R (Russia) at a concentration of 0.4% recommended by the producer were used as stabilizers. The quality indicators of the frozen dessert experimental samples with the researched stabilizers are presented in Table 4.

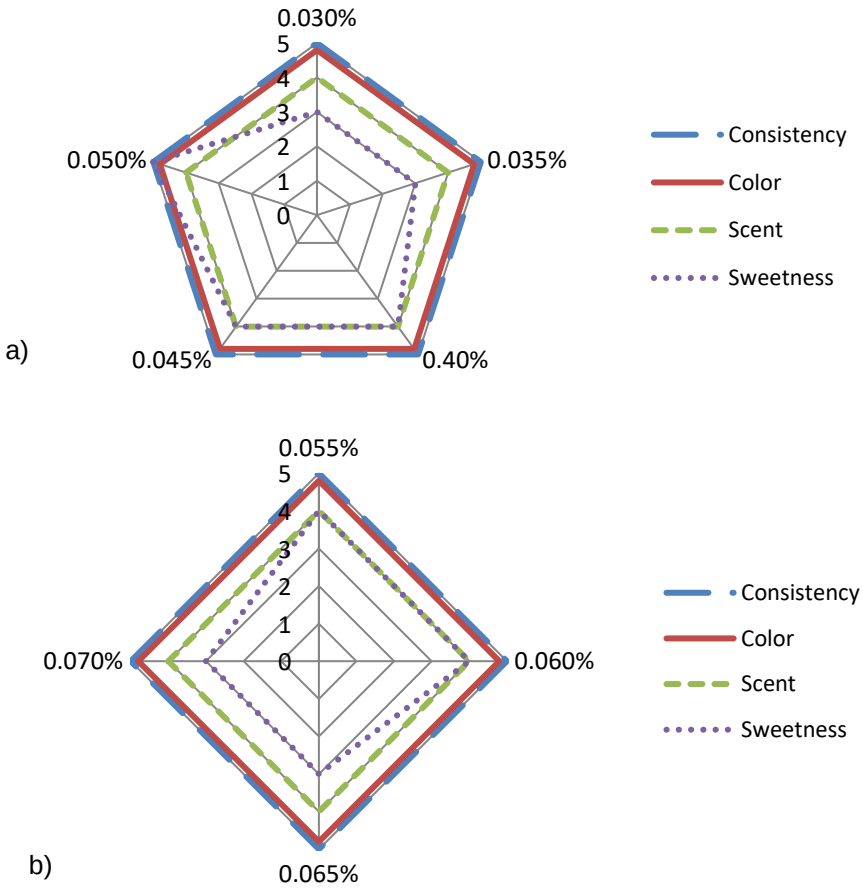

Figure 1. profilograms of the organoleptic assessment of the frozen dessert samples with a sweetener dose, % to the mixture mass: from 0.030 to 0.050 (a) and from 0.055 to 0.070 (b).

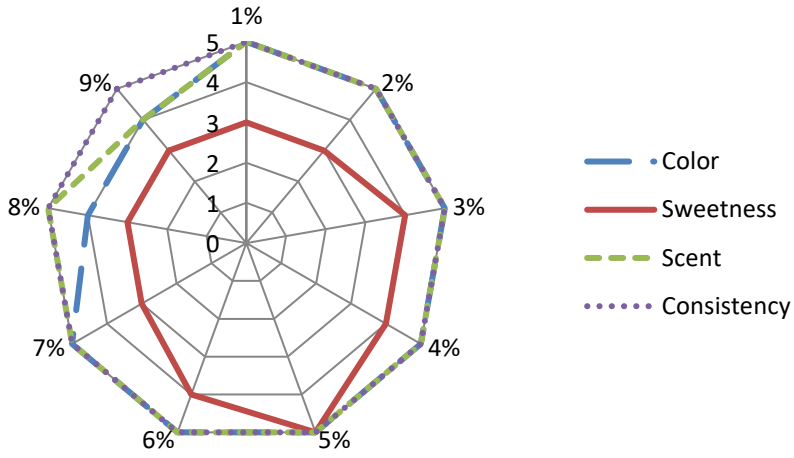

Figure 2. The profilogram of organoleptic assessment of the frozen dessert samples with various concentration of topinambur syrup in points.

As seen from the data presented in Table 4, the experimental samples, regardless of the stabilizer entry, had a creamy, sweet flavor, sufficiently dense, homogeneous consistency without any visible ice crystals, according to the organoleptic characteristics. When using the PGX-1 stabilizer, the experimental samples had higher whipping and thawing resistance indicators, therefore the PGX-1 stabilizer was used in further research.

Table 4. The quality indicators of the frozen dessert experimental samples with the researched stabilizers

Indicators	Stabilizer entry type		
	PGX-1 (Germany)	Kremodan SE 334 VEG (USA)	Denyse 805 R (Russia)
Organoleptic:	Creamy	Creamy	Creamy
Sweetness	Sweet	Sweet	Sweet
Scent	Gentle and moderately dense consistency, homogeneous without any visible ice crystals	Sufficiently dense consistency, homogeneous without any visible ice crystals	Moderately dense consistency, homogeneous without any visible ice crystals
Consistency			
Whipping, %	36 ± 2	34 ± 2	32 ± 2
Thawing resistance, min	43 ± 2	40 ± 1	40 ± 1
Temperature after freezing, °C	-4…-6	-4…-6	-4…-6

It is generally accepted that it is recommended to use no more than 7 g sesame flour per day. A portion of the final product weighing 100 g corresponds to 7% of frozen dessert mixture mass. In this proportion, the consistency became excessively thick when adding sesame flour to the mixture. Therefore, the amount of flour varied from 1 to 5% to the mixture mass within 0.5%.

For an organoleptic assessment which results are presented in Table 5, 9 frozen dessert samples with various dosage of sesame flour, % to the mixture mass (sample 1–1; 2–1.5; 3–2; 4–2.5; 5–3; 6–3.5; 7–4; 8–4.5; 9–5), were offered to a group of 8 people aged 23–26 (graduate students in the 1st and 2nd years of studies).

Table 5. Results of organoleptic assessment of 9 frozen dessert samples with various dosage of sesame flour, % to the mixture mass (sample 1–1; 2–1.5; 3–2; 4–2.5; 5–3; 6–3.5; 7–4; 8–4.5; 9–5)

Sample number	Assessment (points)				Points in total
	Color	Scent	Consistency	Flavor	
№ 1	4.9	4.8	4.8	4.9	19.4
№ 2	4.9	5.0	4.9	4.9	19.7
№ 3	4.9	4.9	4.9	5	19.7
№ 4	4.8	4.8	4.5	4.8	18.9
№ 5	4.8	4.8	4.3	4.8	18.7
№ 6	4.8	4.0	4.3	4.8	17.9
№ 7	4.6	4.0	3.5	4.3	16.4
№ 8	4.6	4.0	3.4	4.3	16.3
№ 9	4.5	4.0	3.4	4.3	16.2

As seen from the data presented, samples 2 and 3 with 1.5 and 2% sesame flour addition respectively received the highest assessment.

Since sesame flour does not dissolve during pasteurization, it is not possible to add it into the mixture before pasteurization as the homogenization comes immediately after pasteurization (Olenev, 2001). Sesame flour was brewed with water in the ratio 1:10 for 15–20 minutes to a humidity of (80 ± 1)% and was added directly to the normalized mixture after the homogenization process before maturing the mixture. Organoleptic and physico-chemical indicators of the frozen dessert sample without sugar with the use of sesame flour are presented in Table 6.

Table 6. Organoleptic and physico-chemical indicators of the sample

Indicator	Characterictics and description
Flavor and scent	Pure, creamy with a pleasant sesame flavor
Consistency	Moderately dense, with barely visible sesame particles
Structure	Homogeneous, without visible fat globules, protein, lactose, with barely visible sesame particles
Acidity, 0 T	22 ± 0.5
Fat mass fraction, %	5 ± 0.1
Whipping, %	48 ± 2
Thawing resistance, min	45 ± 2
Average diameter of air bubbles, μm	62 ± 0.1
Average diameter of fat globules, μm	1.8 ± 0.1

Research on the establishment of expiration dates of the frozen dessert were conducted in the laboratory of the Department of Applied Biotechnology. The frozen dessert sample was stored at -18 °C and all the changes of quality indicators were examined monthly within 8 months (results are presented in Table 7).

Table 7. The changes of quality indicators frozen dessert samples examined monthly within 8 months

Indicator	Storage time, months								
	0	1	2	3	4	5	6	7	8
Flavor and scent	Pure, creamy with a pleasant sesame flavor								
Consistency	Moderately dense, with barely visible sesame particles								
Structure	Homogeneous, without visible fat globules, protein, lactose.								
Average diameter of air bubbles, μm	63 ± 1	63 ± 1	63 ± 1	62 ± 1	62 ± 1	62 ± 1	62 ± 1	62 ± 1	
Average diameter of lactose crystals, μm	No more than 10								
Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (QMAFAnM), CFU g^{-1}	No more than $1*10^5$								
Amount of yeast, CFU g^{-1}	No more than 50								
Amount of mold, CFU g^{-1}	No more than 50								
Coliform bacteria per $0.01cm^3$	No coliform bacteria								

The results of the research show that during the storage period there were no changes in quality indicators in the sample of frozen dessert with sesame flour. However, according to the technique (Gapparov et al., 1999), an expiration date for the products is 6 months.

CONCLUSIONS

1. The entry dose of the stevioside and the topinambur syrup in an amount of 0.03 and 5% respectively added to the mixture mass of the frozen dessert allows us to get the product with pleasant flavor and scent.

2. The frozen dessert samples were got with the best indicators for whipping, thawing resistance, density and uniformity of consistency when using the PGX-1 stabilizer with an entry dose of 0.4 to the mixture mass.

3. The entry dose of sesame flour that is needed to be pre-brewed with water in the ratio 1:10 for 15–20 minutes to a humidity of (80 ± 1)%, varies from 1.5% to 2% to the mixture mass.

4. It is expedient to add brewed sesame flour before freezing.

5. An expiration date of the frozen dessert without sugar with the use of sesame flour is 6 months at a temperature of – 18 °C.

REFERENCES

Arsenyeva, T.P. 2002. *Handbook of the technologist of dairy production. Technology and recipes*, GIORD, SPb., pp. 180 (in Russian).

Arseneva, T.P. 2011a. Selection of components of plant origin for diabetic ice cream. *The science* **176**, 50–52 (in Russian).

Arsenyeva, T.P. 2011b. Development of ice cream with plant components for diabetic nutrition. *Low-Temperature and Food Technologies in the 21st Century: Sat. scientific works of the V international. scientific-practical. Conf.* **425,** 304–305 (in Russian).

Arseneva, T.P. 2009 Development of theoretical bases and elaboration of technologies low-lactose dairy products and adjustable fatty acid composition, *Pishchevaya tekhnologiya* **192**, 22–31 (in Russian).

Arseneva, T.P. & Yakovleva, Y.A. 2012. Formulation of ice cream with plant ingredients. *Pishchevaya tekhnologiya* **215**, 75–78 (in Russian).

Arseneva, T.P. & Yakovleva, Y.A. 2011. Jerusalem artichoke as a means of treating the prevention of various diseases. *Processes and devices of food production* **86**, 1–6 (in Russian).

Erashova, L.D. & Pavlova, G.N. 1997. Source of natural sweetener food. *Food industry* **98**, 9 (in Russian).

Gapparov, M.M., Panchenko, S.N. & Ugreninov, V.G. 1999. Natural products - food of the 21st century. *Food industry* **85**, pp. 12 (in Russian).

Olenev, Y.A., Tvorogova, A.A., Kazakova, N.V & Soloveva, L.N. 2004. *Reference book on production of ice cream*. DeLiprint, Moscow, 798 pp. (in Russian).

Olenev, Y.A. 2001 *Technology and equipment for production of ice cream*, DeLi, Moscow, 323 pp. (in Russian).

Onopriyko, A.V., Khramtsov, A.G. & Onopriyko, V.A. 2004. *Production of dairy products*. March, Rostov-on-Don, 411 pp. (in Russian).

Ozdemir, C., Arslaner, A., Ozdemir, S. & Allahuari, M. 2015. The production of ice cream using stevia as a sweetener. *J. Food Sci. Technol.* **842**, 246–264.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J. & Bressollier, P. 2013. An overview of the last advances in probiotic and prebiotic field. *LWT – Food Science and Technology* **50**, 1–16.

Suhas, M.G., Preeti, M. & Rahul, R.G. 2015. Analytical hierarchy process to recommend an ice cream to a diabetic patient based on sugar content in it, *Procedia Computer Science* **264**, 64–72.

Vozhdaeva, L.I. & Sorochkina, I.G. 2000. About the functional properties of Jerusalem artichoke. *Problems and prospects of healthy food* **197**, p. 43 (in Russian).

Vzor, A.L. & Nikitkov, V.A. 1998. The use of stabilizers and emulsifiers in modern ice cream production. *Food industry* **118**, 40–43 (in Russian).

Yakovleva, Y.A. 2012. Development of the composition and technology of low-lactose cream ice cream for people suffering from diabetes mellitus *Collected works of young scientists. Part I: Proceedings of the Conference (65th\\Student Scientific and Practical Conference)* **222**, 22–24 (in Russian).

Zhukovsky, P.M, 1997. *Cultivated plants and their relatives*. VNIIMSa, Uglich, 751 pp. (in Russian).

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The possibility of using powdered sea-buckthorn in the development of bakery products with antioxidant properties

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Abstract. The article examines ways of increasing the antioxidant capacity of bakery products (referred to here as BP) by adding powdered peel, powdered seeds, and powdered marc produced from sea-buckthorn berries. Three different versions of BP were developed with the maximum addition of the following powders: from the peel (3%), from the marc (5%) with a sugar content of 14.5%; and from seeds (5%) with a sugar content of 5%, and with a potato flake content of 5%. BPs with the addition of sea-buckthorn powders were baked at two temperature regimes: 200°C and 220 °C. The content of phenolic compounds, flavonoids, and ascorbic acid were determined for the sea-buckthorn powder, for the crust of BP, and in BP crumbs. The antioxidant activity of BP was determined by the use of two methods: by chemiluminescence, and by their reaction to the DPPH-radical. Cyclic amides (lactams) were determined in BP crusts and crumbs with the use of the IR spectroscopy method. The AOA of powdered sea buckthorn depended upon the volume of phenolic compounds and ascorbic acid in them: peel > marc > seeds. The antioxidant properties of BPs decreased in the following order and were aligned the following way: BP with marc > BP with peel > BP with seeds. They displayed higher AOA levels than was calculated in theoretical tests, depending upon the volume of powders in the recipe. An increase of the baking temperature led to a loss of phenolic compounds and vitamin C, as well as to the formation of poly lactams. BP baked at the temperature regime of 200 °C displayed the greatest AOA levels.

Key words: sea buckthorn, peel, seed, marc, bakery products, antioxidant activity, lactams.

INTRODUCTION

Most bread and bakery products (BP) are made of low-yield wheat flour, or what is generally known as refined flour. Such flour has good baking properties, but contains almost no vitamins, minerals, or dietary fibres (Pashchenko & Zharkova, 2006; Akhtar et al., 2011; Nechaev, 2013). In human nutrition, bread and BP which is made of low-yield wheat flour serve only as a source of energy. Besides this, melanoidins which are formed during the baking process may cause a pro-oxidative effect in the absence of natural antioxidants, thereby damaging human health (Putilina et al., 2006; Nilova & Pilipenko, 2016).

The development of alimentary products, including bread and BP which has been enriched with natural antioxidants (AO) is a popular direction when it comes to

functional nutrition. Natural raw materials containing water-soluble AO – fruit and vegetable powders, juices, and extracts (Belyavskaya & Rodicheva, 2013; Dziki et al., 2014; Jaisanthi & Banu, 2014; Karrar, 2014; Nilova et al., 2015) – or lipid-soluble AO such as vegetable oils (Caponio et al., 2013; Nilova et al., 2017) are used as the source of natural AO. A valuable source of water-soluble and lipid-soluble AO is sea-buckthorn (*Hippóphae rhamnoídes L.*).

The composition of biologically active substances (BAS) in sea-buckthorn – its berries, seeds, and leaves – has been studied by a good many researchers (Bal et al., 2011; Chaman et al., 2011; Kant et al., 2012; Saikia & Handique, 2013; Fatima et al., 2015). Berries and seeds from sea-buckthorn contain phenolic compounds, ascorbic acid, tocochromanols, carotenoids, and phytosterols. They effectively combat free radicals (Zeb, 2006; Chaman et al., 2011; Kant et al., 2012; Ursache et al., 2017). It is impossible to precisely affirm which parts of sea-buckthorn contain more AO as the quantitative composition can be influenced by the botanical breed of sea-buckthorn, the area of its cultivation, or the method of research being used to study it (Jalakas et al., 2003; Bal et al., 2011). The peel and the pulp contain a lot of ascorbic acid, carotenoids, and phytosterols, while the seeds are rich in cochromanol and lignans (Li et al., 2007; Christaki, 2012; Smeds et al., 2012). Phenolic compounds can dominate in the pulp and the peel of berries or seeds (Saikia & Handique, 2013), while flavonoids (with a predominance of rutin and quercetin) are concentrated mostly in the leaves of sea-buckthorn (Fatima et al., 2015). The amount of lipid-soluble AO in processed sea-buckthorn products depends upon the amount of fat that remains in them after the extraction of sea-buckthorn oil has taken place.

Due to their valuable biochemical composition, sea-buckthorn berries are recommended for use in nutrition in their natural form or as part of food products (Bal et al., 2011). Sea-buckthorn is used mostly for making products such as oil, squash, and juices (Zeb, 2004; Cenkowski et al., 2006; Lipowski et al., 2009). The remaining marc could be used as an enriching supplement for BP, but the high percentage of ascorbic acid in the marc strengthens gluten and can reduce the quality of the BP.

This work is aimed at the development of BP which is made from wheat flour with AO properties due to the use of sea-buckthorn powders in the recipe.

MATERIALS AND METHODS

Preparing sea-buckthorn powders

Sea-buckthorn berries were harvested in the Leningrad region of Russia. The marc left over after squeezing out the juice was dried at 50–55 °C for six hours. Three types of powder were obtained: from the marc, from the seeds, and from the peel. The powder was ground down immediately before any research or BP baking took place. The ground-down powder was then sifted in order to obtain powder with a particle size less than 150 mm. The larger fractions of the powder were milled repeatedly.

The bakery product recipe and baking process

The following ingredients were used in the BP recipe: wheat flour (gluten 28.9%, ash content 0.55) produced by St Petersburg Mill Plant OJSC; refined and deodorised sunflower oil produced by EFCO FOODS PLC, Russia; sugar (99.8% sucrose) by JSC ‘Lebedyansky Sugar Plant’, Russia; pressed bakery yeast by JSC ‘Food Factory’, Russia;

and salt and potato flakes (carbohydrates 83%, proteins 7%, fats 1%) by JSC Hercules Plus, Russia.

The BP were produced by using a straight dough method according to three recipes presented in Table 1.

Table 1. Basic and experimental recipes for bakery products

Ingredient, g	Basic recipes			Experimental recipes		
	Recipe 1 (R1)	Recipe 2 (R2)	Recipe 3 (R3)	with seed powder (based on R2)	with marc powder (based on R3)	with peel powder (based on R3)
Wheat flour	1,000	1,000	1,000	900	950	970
Seed powder	-	-	-	50	-	-
Marc powder	-	-	-	-	50	-
Peel powder	-	-	-	-	-	30
Potato flakes	-	-	-	50	-	-
Sugar	-	50	145	50	145	145
Vegetable oil	-	40	145	40	145	145
Yeast	20	20	20	20	20	20
Salt	15	15	15	15	15	15
Water, mL	620	570	490	570	490	490

The amount of water in the dough was determined through calculations and depended upon the moisture and gluten content of the flour and its additional ingredients (Pashchenko & Zharkova, 2006).

To optimise the amount of sea-buckthorn powder in BP recipes, part of the wheat flour was replaced by sea-buckthorn powders: the first one made from marc; another one made from seeds; and the final one made from peel, to the amount of 1–7%.

The entire dough mass was prepared according to the recipe, from flour and water, being first kneaded for seven minutes and then left to ferment for 150 minutes at 30 °C; the dough was punched down after ninety minutes from the beginning of the fermentation period. Baking the BP which weighed 100g was done at two temperature regimes: at 200 °C for 25 minutes and at 220 °C for twenty minutes. The crust and the crumb were separated after cooling the BP and were dried separately at 40 °C, to reach a constant mass.

Methods for researching the quality of BP

The assessment of BP quality was carried out according to the following parameters:

– the sensory parameters – appearance (shape, surface, and colour of the crust), crumb condition (porosity and texture), and taste and flavour;

– the physico-chemical and physical parameters – the mass proportion of moisture was determined by drying at a temperature of 130 °C for forty minutes; the acidity by use of the titration method 0.1 with a normal NaOH solution; the porosity by determining the ratio of the volume of pores to the total product volume; the pore volume as the difference between the product volume and the volume of the non-porous mass; the specific volume by determining the ratio of product volume to that of 100 g of flour. The quality of experimental BP samples which were produced with the addition of sea-buckthorn powders was compared with control BP samples which had been produced

using the same recipe but without having any of the supplements added (Shevchenko et al., 2009).

Methods of researching individual antioxidants

The goal was to determine individual AO levels in sea-buckthorn powders, as well as in the crust and the crumb of the developed BP.

A determination of ascorbic acid was carried out by use of the titrimetric method, using a 2.6-dichlorophenolindophenolate sodium solution. The extraction of ascorbic acid from the raw materials was carried out with the use of 2% hydrochloric acid. A total of 5 g of the powder was mixed with 5 mL of 2% hydrochloric acid solution, 20 g of crust or crumb were mixed with 20 mL of a 2% solution of hydrochloric acid, infused for ten minutes and then filtered. The ascorbic acid was determined in extracts without any delay (Shevchenko et al., 2009).

Total phenol assay by Folin-Ciocalteau reagent. Ethanol extracts of sea-buckthorn powder, as well as the crust and the crumb powders from CBS, were kept in the dark with Folin-Ciocalteau reagent for thirty minutes at room temperature (1 g of powder in 50 mL of 80% ethanol) with periodic shaking. After the incubation period all samples were centrifuged at 3,500 rpm for ten minutes. The optical density was measured using a SHIMADZU 1240 spectrophotometer (SHIMADZU, Japan) at a wavelength of 735 nm. The results obtained were expressed in mg of gallic acid (Rogozhin & Rogozhina, 2015).

The total content of flavonoids was determined spectrophotometrically by reaction with aluminium chloride. The extraction of flavonoids was carried out with 60% ethyl alcohol. The optical density was measured after thirty minutes on a SHIMADZU 1240 spectrophotometer (SHIMADZU, Japan) at a wavelength of 420 nm. The results obtained were expressed in mg of routine (Rogozhin & Rogozhina, 2015).

Methods for assessing overall antioxidant activity

The antioxidant activity (AOA) of aqueous extracts from sea-buckthorn powders, along with the crust and crumb of BP, was determined by use of the chemiluminescent method using the BCL-06M biochemiluminometer (Nizhny Novgorod, Russia) in a model system which contained riboflavin, hydrogen peroxide, and ferrous iron (Putilina et al., 2006). The extraction was carried out using distilled water, and was centrifuged for ten minutes at 3,500 rpm. Measurements were taken for luminescence in volts (I_{50}) at room temperature for fifty seconds. The information obtained from I_{50} of water extracts was used in making up the charts. The charts were used to determine the concentration of the substance, which reduced the intensity of chemiluminescence by 50%. Trolox 97% (Acros organics, USA) was used as the standard.

A determination of AOA of BP was carried out by use of the Glavind method (Rogozhin & Rogozhina, 2015). The hydro-alcoholic extracts of sea-buckthorn, and the crumb and the crust powder samples, were prepared in the same manner as described above, but extraction was carried out with the use of a 50% ethanol solution. The DPPH solution was prepared by dissolving a 5 mg test sample in 5 mL of 16.4M (96%) ethanol which was heated on a bain-marie until dissolved. A total of 0.2 mL of the extract was added to 2 mL of DPPH solution. The resulting solution was incubated in the dark for thirty minutes, after which the absorbance levels of the resulting solution were measured at a wavelength of 517 nm using a SHIMADZU 1240 spectrophotometer (SHIMADZU,

Japan). The results were calculated using the standard calibration curve for trolox (trolox 97%, Acros Organics, USA).

A determination of cyclic amides (lactams) in BP

Cyclic amides (lactams) were studied with the use of IR-Fourier spectroscopy in the area of 1,680–1,800 cm^{-1} (Silverstein, 2011). The infrared spectra were determined using the IR-Fourier 'ФСМ 1202' spectrometer produced by Monitoring LLC, Russia, with the automatic counting of peaks as compared to the baseline. Spectral registration parameters were as follows: spectral range 400–4,000 cm^{-1}; number of scans, twenty; resolution, 4 cm^{-1}; mode, interferogram. The absolute error in calibration for the wave number scale did not exceed ± 0.1cm^{-1}. Deviation of the 100% transmission line from the nominal value (1,950–2,050 cm^{-1}, resolution 4 cm^{-1}, twenty scans) did not exceed ± 0.5%. The mean square deviation of the 100% transmission line (1,950–2,050 cm^{-1}, resolution 4 cm^{-1}, twenty scans) did not exceed 0.025%. The interferograms obtained were transformed into transmission spectra. The samples for testing were prepared by pressing the BP crust or crumb with potassium bromide. For the preparation of tablets, an exact quantity of potassium bromide was ground down using an agate mortar with 2 g of BP. A measure of 100 mg of the mixture was then pressed in press moulds for fifteen minutes on each side. The identification of lactams was carried out on the basis of the peaks area at an interval of 1,800–1,680 cm^{-1} (Bellami, 1971; Silverstein, 2011): monocyclic γ-lactams were identified at an interval of 1,700 cm^{-1}; polycyclic at 1,700–1,750 cm^{-1}; monocyclic ß-lactams at 1,760–1,730 cm^{-1}; and polycyclic, condensed with other cycles, at an interval of 1,770–1,800 cm^{-1}.

Statistic analysis

The research was conducted in triplicate. The reliability of the experimental data was evaluated by methods involving mathematic statistics with the use of the Microsoft Excel 2007 application for Windows. The data confidence level is 0.95.

RESULTS AND DISCUSSION

The powders had the characteristic aroma of sea-buckthorn berries of varying levels of intensity, and also by the sour taste. The main distinction of the powders lay in their colour. The peel-based powder was of a rich orange colour, the one made with seeds was grey with brown inclusions, while the marc-based powder had a pale orange colour. All of the powders contained ascorbic acid, phenolic compounds, and flavonoids in varying amounts (Table 2).

Phenolic compounds prevailed in all of the powders, followed by flavonoids. The ratio of phenolic compounds and flavonoids in different sea-buckthorn powders was different. According to their content, the powders were aligned in the following order: skin-based powders > marc-based powders > seed-based powders. The share of flavonoids in the peel-based powders came to 89.5% of all phenolic compounds, and in marc-based powders it was 79.9%. The amount of flavonoids in the seed-based powders was less: 39.6% of the total amount of phenolic compounds. The content of ascorbic acid was also higher in the peel and marc-based powders than in those made from the seeds.

The studied sea-buckthorn skin and marc-based powders contained ten times more vitamin C, five times more phenolic compounds, and four times more flavonoids when

compared to those powders that were obtained after oil extraction (Zolotareva et al., 2005; Nikulina & Ivanova 2006), but less vitamin C than in fruit juice powder (Hussain et al., 2010; Selvamuthukumaran & Khanum, 2014). The content of phenolic compounds is almost the same as in powder that was based on sea-buckthorn berries (Roidaki et al., 2015). Differences in the composition of biologically active substances are associated both with the method of obtaining the powders (Guan et al., 2005), and with the botanical variety of sea-buckthorn types and their region of growth. The content of vitamin C in various sea buckthorn types can differ from two to fifteen times, while the content of phenolic compounds and flavonoids can differ between eight to ten times (Ercisli et al., 2007; Ershova, 2009; Wani et al., 2016). The higher amount of biologically active substances increases the AOA of the berries and other sea buckthorn products (Kant et al., 2012; Zeb & Hussain, 2014; Fatima et al., 2015).

The content of individual AO had an impact on the results of studies of AOA in sea-buckthorn powders. The more AO the powder contained, the higher was its AOA: sea-buckthorn peel > sea-buckthorn marc > sea-buckthorn seeds. The AOA of water extracts was lower than that of hydro-alcoholic extracts in the peel powders. In seed powders, on the other hand, the AOA of the aqueous extract was higher than that of the hydro-alcoholic extract, probably because of the inclusion of phenolic compounds.

To assess the possibility of being able to use sea-buckthorn powders in the production of BP from wheat flour, three standard recipes were used, where a certain proportion of the flour was replaced by sea-buckthorn powder. Recipe 1 did not provide for the use of a sugar and fat component. With addition of 1% of any sea-buckthorn powder, the BP acquired a sour taste, while the fragrance of sea-buckthorn was not felt. The specific volume of BP increased by 4% when applying the marc powder, and by 3% after adding powder which was based on the peel or seeds. The further increase in concentration of powders in Recipe 1 engendered a pronounced sour taste and a decrease in the specific volume of BP. The sea-buckthorn aroma became apparent only with addition of sea-buckthorn powder to the amount of 3%. Recipe 1 cannot be used for BP which is enriched with sea-buckthorn powders.

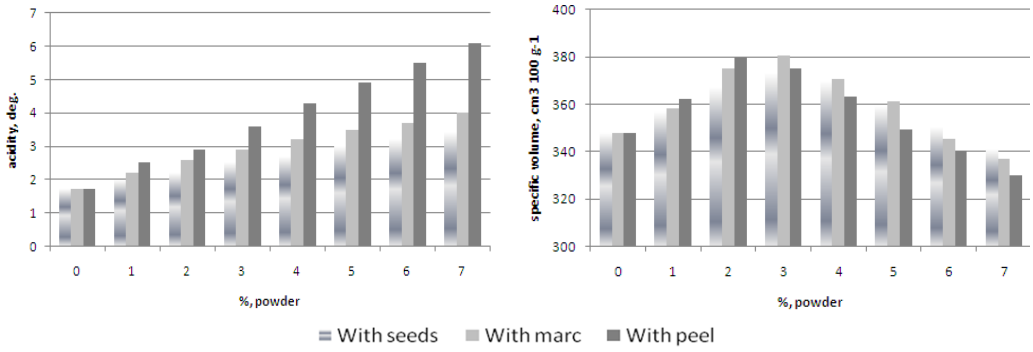
Table 2. The content of antioxidants and the total antioxidant activity of sea-buckthorn powders, ± standard deviation

Powdered form	The content of antioxidants, mg per g DM			AOA, μg Trolox per g DM	
	Ascorbic acid	Total phenolic content	Total flavonoids content	DPPH-radical assay	Chemi-lumnescence
Marc	2.52 ± 0.08	8.61 ± 0.20	6.88 ± 0.28	45.10 ± 1.50	40.20 ± 1.30
Seeds	0.65 ± 0.02	6.95 ± 0.22	2.75 ± 0.16	12.32 ± 0.50	15.40 ± 0.60
Peel	3.21 ± 0.05	9.16 ± 0.20	8.20 ± 0.22	58.62 ± 1.51	51.80 ± 1.80

Recipe 2 provided for the use of 4% vegetable oil and 5% sugar. The gradual increase in the amount of powder in the BP content led to the appearance of a sour taste, confirmed by the values of titratable acidity of BP (Fig. 1). The sour taste was noticed when applying 2% of the peel and the marc powders, and 3% of the seed powder. At the same time, the sea-buckthorn smell was practically indistinguishable.

The main factor which served to inhibit the increase in the number of sea-buckthorn powders in BP recipes was the high content of ascorbic acid in the powders. The ascorbic acid tightens gluten, thereby restraining the increase in the specific volume of BP

(Pashchenko & Zharkova, 2006). As a result, the maximum specific volume of BP was achieved only when using 2% of the peel-based powder and 3% of powder which used the marc or seeds (Fig. 1). Increasing the extent of powders in Recipe 2 resulted in a decrease in the BP-specific volume.



a) Change in acidity, deg. b) Change in specific volume, cm^3 $100g^{-1}$

Figure 1. The effect of sea-buckthorn-based powders on the acidity (a) and specific volume (b) of BP (Recipe 2).

BP with seed-based powder acquired a sea-buckthorn aroma only with the addition of 5% powder. The crumb colour was grey. Therefore the use of powder from the seeds in Recipe 3 is not appropriate**.** In order to increase the BP-specific volume in Recipe 2, it was necessary to use ingredients which prevent gluten from thickening. Potato flakes were chosen as such an ingredient. Potato flakes to the amount of 3%, 5%, and 7% were added to Recipe 2 with the further addition of 5% of the seed-based powder. BP quality was controlled according to specific volume (Fig. 2).

The introduction of 3% of potato flakes increased the specific volume of BP by 1.2% with the addition of 5% of seed-based powder; while the introduction of 5% of potato flakes increased it by 2.7%, which almost reached the specific volume of BP with the addition of 3% of the seed-based powder. The values were only 1% lower. However, a further increase of the proportion of potato flakes resulted in an excessive relaxation of gluten and a decrease of the specific volume. Therefore, powder which was obtained from seeds can be used to the amount of 5% in Recipe 2 for BP, but only in combination with potato flakes to the amount of 5% (Table 1).

In order to relax gluten, one can use a significant amount of sugar in BP recipes (Pashchenko & Zharkova, 2006). For this purpose, Recipe 3 was used to increase the proportion of peel-based and marc-based powders (Fig. 3). The high content of ascorbic acid in the peel-based powder (Table 1) did not allow us to increase its quantity in BP above 3%, but the BP acquired a specific sea-buckthorn aroma, a nice orange tinged crumb colour, and a sweet-and-sour taste. Due to the reduced amount of ascorbic acid in the marc powder, its optimal amount in Recipe 3 for BP was 5%. The further increase in the amount of the peel and marc-based powders in Recipe 3 for BP led to a reduction of the specific volume of BP (Fig. 3), the appearance of a pronounced acidic taste, and also an excessive colour.

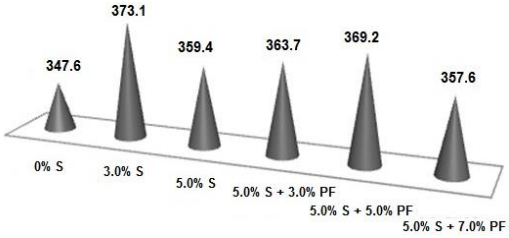


Figure 2. Changes in specific volume, cm^3 100 g^{-1}, BP with seed-based powder (S), with the addition of potato flakes (PF).

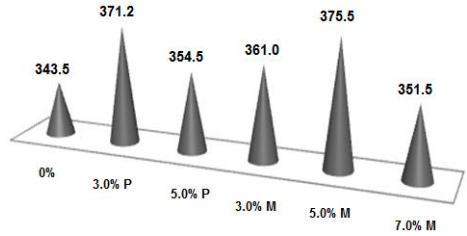


Figure 3. Changes in specific volume, cm^3 $100g^{-1}$, BP with peel-based powder (P) and marc-based powder (M), Recipe 3 (R3).

The most optimal recipes for BP with sea-buckthorn powders are presented in Table 1. The characteristics of quality indicators for the developed BP with sea-buckthorn powders are presented in Table 3.

Table 3. Physico-chemical characteristics of enriched bakery products

Physico-chemical characteristics	Bakery products in powdered form				
	Recipe 2 (R2)		Recipe 3 (R3)		
	Control R2	Seeds	Control R3	Marc	Peel
Moisture, %	39.5 ± 1.0	39.1 ± 1.0	35.3 ± 1.0	35.2 ± 1.0	35.0 ± 1.0
Acidity, deg,	1.7 ± 0.1	3.2 ± 0.1	1.7 ± 0.1	3.4 ± 0.1	3.6 ± 0.1
Porosity, %	71.7 ± 1.3	74.5 ± 1.7	69.3 ± 2.1	72.9 ± 1.8	72.8 ± 1.8
Specific volume, cm^3 $100g^{-1}$	347.5 ± 8.8	369.2 ± 8.8	343.5 ± 9.3	375.5 ± 8.6	371.2 ± 8.9

The sea-buckthorn powders have enriched BP with phenolic compounds that were found both in the crust and the crumb (Table 4). When compared with the control samples, the amount of phenolic compounds in BP crumb which had been produced with the use of seed-based powder increased by 26.7%, those made with marc-based powder by 42.3%, and those made with peel-based powder by 13.5%. The amount of flavonoids in BP was below the figure for phenolic compounds. In the crust, they were found only in BP with the addition of marc and peel-based powder. In the crumb, the amount of flavonoids was less than phenolic compounds by a factor of six in BP with seed-based powders and a factor of three in BP with peel and marc-based powders.

Lowering the temperature of baking increased the amount of phenolic compounds and flavonoids in the crust and crumb of all BP samples which had been produced with the addition of sea-buckthorn powders. The maximum amount of phenolic compounds and flavonoids was contained in the crumb of BP with the marc-based powder, having been baked at a temperature of 200 °C.

The actual content of phenolic compounds and flavonoids in BP with sea-buckthorn powders was below the theoretically possible extent, given their amount in the sea-buckthorn powders and the BP control samples. The loss of phenolic compounds in the crumb of BP which had been produced with the addition of sea-buckthorn powders was between 23.8–26.5%, when baked at 220 °C. Lowering the baking temperature to 200 °C reduced the loss of phenolic compounds by between 7–13%. The content of phenolic compounds in the crust decreased by between 2.2–3.0, depending upon the baking

temperature and the BP recipe. The loss of flavonoids during baking was more significant.

Table 4. The content of ascorbic acid, phenolic compounds, and flavonoids in the crust and crumb of bakery products, per g DM (mean ± standard deviation)

Bakery products	Ascorbic acid		Total phenolic content		Total flavonoids content	
	Crust	Crumb	Crust	Crumb	Crust	Crumb
Baking at 220 °C						
Control R2	nd	nd	0.05 ± 0.01	0.45 ± 0.03	nd	nd
With seeds	nd	nd	0.08 ± 0.01	0.57 ± 0.02	nd	0.09 ± 0.01
Control R3	nd	nd	0.08 ± 0.01	0.52 ± 0.01	nd	nd
With marc	nd	0.021 ± 0.001	0.18 ± 0.02	0.74 ± 0.01	0.05 ± 0.01	0.24 ± 0.01
With peel	nd	0.015 ± 0.001	0.15 ± 0.01	0.59 ± 0.01	0.06 ± 0.00	0.18 ± 0.01
Baking at 200 °C						
Control R2	nd	nd	0.10 ± 0.01	0.52 ± 0.02	nd	nd
With seeds	nd	nd	0.18 ± 0.01	0.72 ± 0.01	nd	0.11 ± 0.01
Control R3	nd	nd	0.12 ± 0.02	0.66 ± 0.01	nd	nd
With marc	nd	0.032 ± 0.001	0.25 ± 0.02	0.95 ± 0.02	0.12 ± 0.01	0.28 ± 0.01
With peel	nd	0.025 ± 0.002	0.20 ± 0.01	0.76 ± 0.02	0.10 ± 0.01	0.20 ± 0.01

nd – not detected.

The high baking temperatures completely destroyed the ascorbic acid in all BP crusts. Ascorbic acid was found only in the crumb of BP which had been produced with the addition of sea-buckthorn marc and peel-based powders. Its quantity was up to 30% of the theoretically possible extent, depending upon the recipe used to produce the BP. Baking at the lower temperature of 200 °C made it possible to preserve the ascorbic acid by 50% more, when compared with baking at a temperature of 220 °C.

The presence of individual AO in BP led to an increase in their AOA (Table 5). The AOA of BP with sea-buckthorn powders depended upon the amount and type of the powder: BP with sea-buckthorn marc-based powder > BP with sea-buckthorn peel-based powder > BP with sea-buckthorn seed-based powder.

Table 5. The antioxidant activity of bakery products, μg Trolox per g DM (mean ± standard deviation)

Bakery products	DPPH-radical assay		Chemiluminescence	
	Crust	Crumb	Crust	Crumb
Baking at 220 °C				
Control R2	3.19 ± 0.06	4.54 ± 0.08	7.10 ± 0.08	10.50 ± 0.10
With seeds	4.10 ± 0.15	6.10 ± 0.02	8.80 ± 0.18	12.90 ± 0.20
Control R3	4.48 ± 0.10	6.35 ± 0.12	9.10 ± 0.08	12.88 ± 0.14
With marc	7.50 ± 0.12	10.48 ± 0.15	14.70 ± 0.10	20.02 ± 0.22
With peel	6.25 ± 0.12	8.95 ± 0.16	11.00 ± 0.16	15.28 ± 0.02
Baking at 200 °C				
Control R2	3.45 ± 0.06	5.10 ± 0.09	7.75 ± 0.10	11.10 ± 0.18
With seeds	4.45 ± 0.10	6.55 ± 0.15	9.50 ± 0.22	12.96 ± 0.12
Control R3	4.80 ± 0.04	6.82 ± 0.10	9.62 ± 0.20	13.26 ± 0.00
With marc	8.00 ± 0.10	11.45 ± 0.20	16.10 ± 0.05	22.40 ± 0.20
With peel	6.81 ± 0.10	9.60 ± 0.05	11.70 ± 0.10	17.00 ± 0.25

The experimentally determined AOA of BP was higher than the one that had been calculated theoretically. Therefore, the crumb of BP with a marc-based powder theoretically should have the AOA of 8.55 μg g^{-1} DM and 8.36 μg g^{-1} DM, as determined by DPPH and chemiluminescence methods respectively. But the experimental values of AOA of BP crumb using a marc-based powder (determined according to the DPPH and chemiluminescence methods) were higher by 22.6% and 20.02% respectively. An even greater difference in AOA was recorded for BP which had been produced with the addition of seed-based powder: 24.5% and 28.3% respectively. This tendency is traced not only in the crumb, but also in the crust, irrespective of the baking temperature. But in case of baking at a lower temperature (200 °C), the AOA figures were higher, both for the crumb and the crust. BP crumb had an AOA level that was higher than that of the crust when using two temperature profiles during baking.

Biologically active compounds such as tocopherols and carotenoids which are found in sea-buckthorn powders, something that was not considered in the present study, can also contribute to the AOA of BP, as can melanoidins that are formed during baking (Martins et al., 2001; Nilova et al., 2015).

Melanoidins include mono- and polycyclic lactams – δ-(six-membered ring), γ-(five-membered ring) and ß-(four-membered ring). Their AO properties are determined by the system of conjugated double bonds in heterocyclic and quinoid chains (Selemenev et al., 2008). The IR-spectroscopy method makes it possible to identify lactams by intensity of absorption bands of the carbonyl group in the area of 1,680–1,800 cm^{-1}, which is characteristic of mono- and polycyclic γ- and β-lactams. The decrease in the number of members in the ring leads to a shift of bands towards higher frequencies. For a quantitative interpretation of the data obtained, peak areas were used which characterised oscillations in the selected space. The results are shown in Table 6.

Table 6. The characteristics of mono- and polycyclic lactams (β- and γ-forms) of the crust and the crumb of bakery products, relative standard units, ± standard deviation

Bakery products	Lactams			
	Mono β- & γ-lactams		Poly β- & γ-lactams	
	Crust	Crumb	Crust	Crumb
Baking at 220 °C				
Control R2	49.32 ± 0.36	73.09 ± 0.44	77.37 ± 0.60	110.72 ± 0.68
With seeds	27.49 ± 0.40	40.98 ± 0.40	42.88 ± 0.72	61.52 ± 0.70
Control R3	54.18 ± 0.70	81.20 ± 0.35	86.82 ± 0.80	120.55 ± 0.70
With marc	2.35 ± 0.45	38.25 ± 0.60	42.72 ± 0.82	61.46 ± 0.65
With peel	28.19 ± 0.50	41.50 ± 0.40	46.01 ± 0.45	63.72 ± 0.49
Baking at 200 °C				
Control R2	50.08 ± 0.30	75.10 ± 0.55	75.85 ± 0.58	105.40 ± 0.60
With seeds	28.81 ± 0.55	41.96 ± 0.35	41.20 ± 0.65	57.94 ± 0.45
Control R3	55.90 ± 0.38	81.91 ± 0.30	83.62 ± 0.72	116.89 ± 0.70
With marc	28.75 ± 0.42	42.47 ± 0.65	39.45 ± 0.75	57.23 ± 0.50
With peel	29.80 ± 0.50	44.00 ± 0.56	43.70 ± 0.60	61.06 ± 0.62

The explored areas of IR spectra for the crust and the crumb of BP had a similar number of bands with different levels of intensity. The intensity of the bands depended upon the recipe and added ingredients, as well as the baking temperature. The different intensity of the bands resulted in a change in the aggregate area of peaks for mono- and

polycyclic lactams. A common pattern was noted for all infrared spectra of BP samples: the total number of lactams was greater in the crumb than in the crust by 1.5 times on average; the predominating elements in the crust and the crumb were polycyclic lactams (60–62%); the share of polycyclic lactams in the crumb was less than that in the crust by between 1–2%. The use of sea-buckthorn powders inhibited formation of cyclic lactams in BP by between 1.8–2 times. The decrease in baking temperature led to the increased intensity of infrared spectra bandwidths of BP, which were characteristic of monocyclic lactams, as concerns the crust and the crumb.

CONCLUSIONS

Powders made from various parts of sea-buckthorn berries (from the marc, seeds, or peel) display AOA that depends upon the content of particular AO. Regardless of the part that is used for preparing such powders, the AOA increased in the following order: seeds < marc < peel.

When using sea-buckthorn powders in the production of BP, it is necessary to consider the high levels of acidity in the powders, something that has an impact on the taste of the products; as well as the high content of ascorbic acid that has a tightening effect on gluten (and a decrease in the specific volume of BP). The maximum usage of powders from sea-buckthorn are best as follows: peel-based powder, 3%; marc-based powder, 5%, with a sugar content of 14.5%; seed-based powder, 5%, with a sugar content of 5%; and potato flakes with a content of 5%.

The use of sea-buckthorn powders in the production of BP increases the amount of AO in them – in other words those phenolic compounds and flavonoids which number less than the theoretically possible extent by between 23.8–26.5%. Ascorbic acid disintegrates under the influence of the high baking temperature; therefore it is absent in the crust of BP, but it is found in the crumb of BP where these have been produced with the addition of peel-based and marc-based powders, to a volume of up to 30% of the theoretical value.

It was established that BP with sea-buckthorn powders have a greater AOA than the control samples. The AOA of BP which use sea-buckthorn powders increases in the following order: BP with seed-based powder < BP with peel-based powder < BP with marc-based powder. The formation of AOA depends not only on the type and amount of sea-buckthorn powders in the BP recipe, but also on the baking temperature. A decrease in the baking temperature results in a reduction of losses specific to biologically active substances, as well as in formation of monocyclic lactams that have an impact on the AOA of BP.

REFERENCES

Akhtar, S., Anjum, F.M. & Anjum, M.A.A. 2011. Micronutrient fortification of wheat flour: recent development and strategies. *Food Research International* **44**, 652–659.

Bal, L.M., Meda, V., Naik, S.N. & Satya, S. 2011. Sea buckthorn berries: A potential source of valuable nutrients for nutraceuticals and cosmoceuticals. *Food Research International* **44**(7), 1718–1727.

Bellami, L. 1971. New data on IR spectrums of difficult molecules. *Word, Moscow*, 318 pp., (in Russian, transl. from English).

Belyavskaya, I.G. & Rodicheva, N.V. 2013. Determination of antioxidant capacity of bakery products vegetables processing. *Khleboproducty* **11**, 52–53, (in Russian, English abstr.)

Caponio, F., Giarnetti, C., Summo, C., Paradiso, V.M., Cosmai, L. & Gomes, T. 2013. A comparative study on oxidative and hydrolytic stability of monovarietal extra virgin olive oil in bakery products. *Food Research International* **54**(2), 1995–2000.

Chaman, S., Syed, N.H., Danish, Z. & Khan, F.Z. 2011. Phytochemical analysis, antioxidant and antibacterial effects of sea buckthorn berries. *Pak. J. Pharm. Sci.* **24**(3), 345–351.

Cenkowski, S., Yakimishen, R., Przybylski, R. & Muir, W.E. 2006. Quality of extracted sea buckthorn seed and pulp oil. *Canadian biosystems engineering* **48**(3), 9–16.

Christaki, E. 2012. Hippophae Rhamnoides L. (Sea Buckthorn): a Potential Source of Nutraceuticals. *Food and Public Health* **2(**3), 69–72.

Dziki, D., Różyło, R., Gawlik-Dziki, U. & Świeca, M. 2014. Current trends in the enhancement of antioxidant activity of wheat bread by the addition of plant materials rich in phenolic compounds. *Trends in Food Science & Technology* **40**(1), 48–61.

Ercisli, S., Orhan, E. & Ozdemir, O. 2007. The genotypic effects on the chemical composition and antioxidant activity of sea buckthorn (*Hippophae rhamnoides L.*) berries grown in Turkey. *Scientia Horticulturae* **115**(1), 27–33.

Ershova, I.V. 2009. Estimation of Altai varieties and sea buckthorn hybrids according to the biochemical composition of fruits. *Achievements of Science and Technology of AICis.* **7**, 11–12.

Fatima, T., Kesari, V., Watt, Ia., Wishart, D., Todd, J.F., Schroeder, W.R., Paliyath, G. & Krishna, P. 2015. Metabolite profiling and expression analysis of flavonoid, vitamin C and tocopherol biosynthesis genes in the antioxidant-rich sea buckthorn (*Hippophae rhamnoides L.*). *Phytochemistry* **118**, 181–191.

Guan, T.T.Y., Cenkowski, S. & Hydamaka, A. 2005. Effect of drying on the nutraceutical quality of sea buckthorn (*Hippophae rhamnoides L. ssp. sinensis*) Leaves. *Journal of Food Science* **70**(9), 514–518.

Hussain, A., Abid, H., Ali, G. & Ullah, Sh. 2010. Studies on the preparation of Sea buckthorn (*Hippophae rhamnoides L.*) powder drink and its nutritional evaluation. *J. Chem. Soc. Pak.* **32**(4), 519–524.

Jaisanthi, J. & Banu, T.A. 2014. Phytonutrient composition, antioxidant activity and acceptability of baked product incorporated with grape seed extract. *Journal of Human Nutrition & Food Science* **2**(6), 1049–1054.

Jalakas, M., Kelt, K. & Karp, K. 2003. The yield and fruit quality of sea buckthorn (*Hippóphae rhamnoídes* L.) after rejuvenation cutting. *Agronomy Research* **1**, 31–36.

Kant, V., Mehta, M. & Varshneya, C. 2012. Antioxidant potential and total phenolic contents of seabuckthorn (*Hippophae rhamnoides*) pomace. *Free Radicals and Antioxidants* **2**(4), 79–86.

Karrar, E.M. 2014. A Review on: Antioxidant and its impact during the bread making process. *International Journal of Nutrition and Food Sciences* **3**(6), 592–596.

Li, T.S.C., Beveridge, T.H.J. & Drover, J.C.G. 2007. Phytosterol content of sea buckthorn (*Hippophae rhamnoides* L.) seed oil: Extraction and identification. *Food Chemistry* **101**, 1633–1639.

Lipowski, J., Marszałek, K. & Skąpska, S. 2009. Sea Buckthorn – an innovative raw material for the fruit and vegetable processing industry. *Journal of Fruit and Ornamental Plant Research* **17**(2), 121–126.

Martins, S., Jongen, W. & Martinus, A. 2001. A review of Maillard reaction in food and implications to kinetic modeling. *Food science and technology* **11**, 364–373.

Nechaev, A.P. 2013. Food ingredients in production of bakery products. *Deli plus*, Moscow, 527 pp. (in Russian).

Nikulina, O. & Ivanova, G. 2006. Sea-buckthorn meal for bakery and macaroni products. *Khleboproducty* **5**, 40–46, (in Russian, English abstr.).

Nilova, L., Orlova, O. & Nasonova, J. 2015. The role cyclic amides in the formation of antioxidant capacity of bakery products. *Agronomy Research* **13**(4), 1020–1030.

Nilova, L.P. & Pilipenko, T.V. 2016. Evaluation antioxidant properties enriched bakery products in experiment on laboratory animals. *Problems of Nutrition* **85**(6), 39–47, (in Russian, English abstract).

Nilova, L., Pilipenko, T. & Malyutenkova, S. 2017. An investigation into the effects of bioactive substances from vegetable oils on the antioxidant properties of bakery products. *Agronomy Research* **15**(S2), 1399–1410.

Pashchenko, L.P. & Zharkova, I.M. 2006. Technology of bakery products. *Publishing house «Colossus»,* Moscow, 389 pp. (in Russian).

Putilina, Ph.E., Galkina, O.V., Eschenko, I.D., Digi, G.P., Krasovskaya, I.E. & Prokopenko, V.M. 2006. Workshop on free radical oxidation. *St.Peterburg state University*, St. Peterburg, 82–83 (in Russian).

Roidaki, A., Zoumpoulakis, P.G. & Proestos, C. 2015. Comparison of Extraction Methods for the Determination of Antioxidant Activity in Extracts of *Hippophae Rhamnoides L*. and *Lippia Citriodora*. The Effect of Seasonal Collection. *Austin J. Nutri Food Sci*. **3**(1), 1057–1065.

Rogozhin, V.V. & Rogozhina, T.V. 2015. Workshop on biochemistry of agricultural production. *GIORD.* St. Petersburg, 480 pp. (in Russian).

Saikia, M. & Handique, P.J. 2013. Antioxidant and antibacterial activity of leaf, bark, pulp and seed extracts of seabuckthorn (*Hippophae salicifolia D. Don*) of Sikkim Himalayas. *Journal of Medicinal Plants Research* **7**(19), 1330–1338.

Selvamuthukumaran, M. & Khanum, F. 2014. Effect of modified atmosphere packaging on physicochemical, sensory and microbiological properties of spray-dried Sea Buckthorn fruit juice powder. *Journal of Food Quality* **37**, 149–156.

Shevchenko, V.V., Vytovtov, A.A., Nilova, L.P. & Karaseva, E.N. 2009. Measuring methods for monitoring the quality and safety of food. Vegetable products. *Publishing House «Troitsky Bridge»*, St. Petersburg, 304 pp. (in Russian).

Selemenev, V.F., Rudakov, O.B., Slavinskaya, G.I. & Drozdova, N.V. 2008. Pigments of food productions. *DeLi print*, Moscow, 246 pp. (in Russian)

Silverstein, P. 2011. Spectrophotometric identification of organic compounds. *Binom. Laboratoriya znaniy*, Moscow. 557 pp., (in Russian, transl. from English).

Smeds, A.I., Eklund, P.C. & Willfor, S.M. 2012. Content, composition, and stereochemical characterisation of lignans in berries and seeds. *Food Chemistry* **134**, 1991–1998.

Ursache, F.M., Ghinea, I.O., Turturică, M., Aprodu, I., Râpeanu, G. & Stănciuc, N. 2017. Phytochemicals content and antioxidant properties of sea buckthorn (*Hippophae rhamnoides L.*) as affected by heat treatment – Quantitative spectroscopic and kinetic approaches. *Food Chemistry* **233**, 442–449.

Wani, T.A., Wani, S.M., Ahmad, M., Ahmad, M., Gani, A. & Masoodi, F.A. 2016. Bioactive profile, health benefits and safety evaluation of sea buckthorn (*Hippophae rhamnoides L.*): A review. *Cogent Food and Agriculture* **2**(1). http://www.tandfonline.com/doi/full/10.1080/23311932.2015.1128519

Zeb, A. 2006. Anticarcinogenic Potential of Lipids from Hippophae – Evidence from the Recent Literature. *Asian Pacific Journal of Cancer Prevention* **7**, 32–35.

Zeb, A. 2004. Chemical and Nutritional Constituents of Sea Buckthorn Juice. *Pakistan Journal of Nutrition* **3**(2), 99–106.

Zeb, A. & Hussain, Sh. 2014. Sea Buckthorn Seed Powder Provides Protection in the Oxidative Stress Produced by Thermally Oxidized Sunflower Oil in Rabbits. *Food Biochemistry* **38**(5), 498–508.

Zolotareva, A.M., Gabanova, G.V. & Chirkina, T.F. 2005. Seeds of sea-buckthorn as a food source of biologically active substances. *Storage and processing of farm products* **1**, 30–31, (in Russian, English abstract).

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The effect of sapropel extracts on microflora and physicochemical parameters of Dried Distillers' Grain

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Abstract. This article is devoted to the effect of ultradispersed humate sapropel extracts, obtained from air-dry samples of sapropel, from Seryodka deposit (Pskov region, Russia) by alkaline extraction under the action of ultrasonic radiation, on microbial and physicochemical parameters of Distillers' grain (DG) during storage. At the end of the distillation, wet DG was divided and treated with sapropel extract. The untreated sample served as a control. Both (treated and untreated) were then dried to 10% moisture content. Microbial and physicochemical parameters (moisture content, pH, titrable acidity (TA), acid value (AV) of fats) were assessed before storage of dried DG. A similar experiment was repeated after one week of storage but on microbial load. The microbial counts ranged from 2.3×10^4 CFU g^{-1} (untreated) to 1.77×10^4 CFU g^{-1} (treated) before storage while 2.5×10^4 CFU $g^{-1}$to 2.18×10^4 CFU g^{-1} accounted for after a week of storage. The pH had increased from 4.5 ± 0.1 to 6.1 ± 0.1 before and after treatment respectively. TA likewise decreased from 3.2 ± 0.4 to 2.03 ± 0.06%. The results showed that sapropel extracts had effects on microflora and physicochemical parameters of DDG.

Keywords: Grains, pH, Acid Value, microflora, humic acid, fungicidal properties.

INTRODUCTION

Distillers' grains (DG) are primary fermentation by-products obtained after the fermentation of cereal grains by yeast into alcohol (Clark et al., 1987). It is valuable secondary by-product after ethanol production but due to its high moisture content pose a challenge in storing it. Wet DG approximately contains 77–81% of moisture (Aliyu & Bala, 2011). High moisture, the residue of fermentable sugars and other nutrients make DG a niche for microorganisms resulting in rapid degradation (Russ et al., 2005).

To curtail this menace (microbial instability), multi-stage separation and drying of DG were adapted. Drying is an alternative for DG preservation with the additional merit of reduced DG volume, hence decreased transport and storage costs (Santos et al., 2003).

According to GOST 31809-2012, the shelf life of the dried DG is 6 months. However, improper storage of DG can lead to the development of foreign microflora, which results in excretion of mycotoxins. These mycotoxins can interfere with the immune system of livestock's predisposing them to diseases (Emtsev, 2006).

DG has been designated as high-protein-energy substitutes for feeding livestock. Series of research has been carried out concerning the feeding (nutritional) value of DG. With respect to energy, DG possesses equal to slightly greater feeding value in comparison to corn. Nevertheless, vital research has suggested that DG could be employed as a protein source, which is equivalent to soybean and other protein base feeds utilized in feeding cattle (Coupe et al., 2008). The sort of grain utilized, technology and drying procedure can significantly impact the end product of DG, thus varies in chemical composition (pH, proteins, reducing sugar, amino acids, total acidity, an acid value of fat, etc.) and physical parameters (moisture content, odour, colour, and lightness) (Cromwell et al., 1993).

According to Emeis & Weissert (2009), sapropel is the term used to describe 'organic-rich fine-grained sediments deposited in lake and oceans' water. Also, Avdeyeva et al. (2009) defined sapropel as dark-colour sediments that are rich in organic matter, formed under anaerobic conditions from a dead organic matter of anhydrobiotic microflora and microfauna.

It has been noted that three vital constituents of sapropel interact with each other: biologically active, organic and mineral (Kireycheva & Khokhlova, 1998). Our previous work has shown the fungicidal potentials of ultradispersed humic sapropel suspensions. Sapropel extracts have proven to have significant fungicidal properties depending on volume applied, even in small doses (Barakova et al., 2017).

The important role of sapropel on the stored Dried DG (DDG) cannot be ignored since it alters the chemical composition and physical parameters due to the humic acid contained in it. The biological effects of humic substances are based on the diversity of their reaction groups, which enable them to participate in a variety of biochemical transformations (Perminova, 2008; Savchenko, 2015). The presence of carboxyl groups allows participation in ion exchange, hydroxyl reactions, carbonyl in oxidation-reduction reactions, etc. (Perminova, 2008).

Sapropel extracts treatment of animal feed has reported improving livestock productivity (Kireycheva & Khokhlova, 2000). Sapropel is a unique organic feed, which is promising to use in various sectors of industry, agriculture, livestock farming, medicine and balneology (Plaksin & Krivonos, 2007).

The aim of the present study is to show the effect of sapropel extract on microflora, chemical composition and physical parameters of DDG obtained after production of ethanol from barley grains.

MATERIALS AND METHOD

The objects of the study were ultradispersed humic sapropel suspensions obtained in RAS Limnology Institute with alkaline extraction and ultrasound treatment of air-dry sapropel from Seryodka deposit (Pskov region, Russia). Sapropel extracts used was obtained from hot method extraction at 40 °C.

Analysis and optimization of sapropel extracts

A rotary evaporator EV 130 (LabTech) was used to increase the concentration of sapropel extracts. The sapropel obtained from hot method extraction had 3.5% of dry matter and the evaporation was carried out to increase the concentration to 20%.

Automatic Titrator 848 Titrino Plus (Metrohm) was used for determination of sapropel extracts pH. The device operates in two modes: titration with automatic endpoint determination and pH measurement. The latter was used in the experiments. Time of assay was no less than 5 minutes, and sometimes more in case of decimals other than the last digit being unstable.

Refractometer PTR 46 (Index Instruments) was used for measurement of the concentration of dry matter in sapropel extracts.

Determination of moisture and starch content of barley

Moisture analyser MOC-120H (Shimadzu) was used for assessment of all barley grains and flour moisture content according to ISO/TC 34 (ISO/TC 34, 2009).

The barley used in this experiment was harvested in Russia within the 2016 season.

The starch content of the barley was determined by using Polarimeter (PolAA FF55). The determination of the starch content of barley was conducted according to ISO/TC 93- Ewers polarimetric method (ISO/TC 93, 1997).

Production of ethanol

Barley grains (10 kg) were weighted and milled in a milling machine (SINBO SCM-2929). Mashing, fermentation and distillation were carried out in Dr Guber factory.

The milled barley was then mixed with 35 L of warm water (50 °C). An amount of alpha amylase (13.6 mL) and xylanase (13.7 mL) was then pipetted into the solution, for a rest period of 30 minutes. The temperature was then increased to 70 °C, for a rest period of 4 hours.

A sample of wort was taken at 30 min intervals and the concentration of dry matter (°Brix) was measured using a refractometer. The final wort was allowed to cool down to a temperature of 30 °C. *Saccharomyces cerevisiæ* (8.4 g) was reactivated, 10 min before pitching. Glucoamylase (5.6 mL) was then added to the pitched wort. All enzymes used are from Erbslöh (Germany).

Fermentation was then carried out at a temperature of 30 °C for 72 hrs. After the 72 hrs of fermentation, a distillation of the fermented wash was then performed.

Distiller's grain collection and treatment

After distillation, Wet distillers' grains was then collected and centrifuged at 4,600 rpm for 10 minutes and stored in the freezer before their analysis (Fig. 1).

An amount of 20 mL of sapropel extracts (20% of dry matter and pH 7) (Fig. 2) was sprinkled on 100 g of distillers' grain followed by uniform mixing. The mixture was allowed for a 30 minutes rest period (undisturbed). A control sample of the same mass (untreated) was also observed (Fig. 3). Samples (treated and untreated distillers' grains) were then dried in cabinet dryer (ES-4610) at a temperature of 100 °C.

Figure 1. Wet Distiller's grain in plastic packet before drying and analysis.

Figure 2. Sapropel extracts (20% of dry matter and pH 7) before sprinkling on wet distillers' grain.

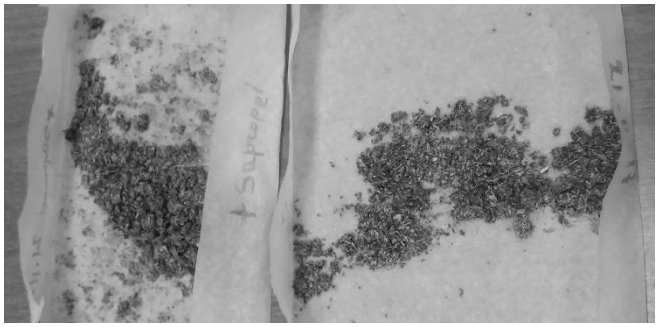

Figure 3. Dried DG 10% of moisture content, treated sample (left) and untreated (right).

Microbiological analysis of DDG

For microbiological analysis, 10 g of each sample (treated and untreated) of DDG, was taken and mixed with 100 mL sterilized distilled water in the different conical flask and the latter was shaken for 10 minutes. Quantification of the microbial load was then carried out according to the method described by Adadi and Obeng (2017). The

remaining DDG was stored in a thermostat TS-1/80 SPU at 37 °C for further microbial investigation after one week.

Physicochemical parameters determination

After microbiological analysis, physicochemical parameters (Moisture content, pH, Titratable acid (TA) and Acid value (AV) of fat) were then determined.

The moisture content of Dried Distiller's grains was measured according to ISO/TC 34 (ISO/TC 34, 2009).

The titratable or total acidity (TA) of DDG was determined according to GOST 13496.12-98 (GOST 13496.12, 1998).

Fat Acid Value (AV) was determined according to GOST 13496.18-85. In our research, the potentiometric titration method written in GOST 13496.18-85 was used to determine AV. The essence of this method consists in potentiometric titration of fatty acids extracted from the product by extraction with a mixture of chloroform and ethyl alcohol (GOST 13496.18, 1986).

Data generated were subjected to analysis of variance (ANOVA) using Origin statistical software (version 8.1) at 5% significance. All measurements were made at least in triplicate. Results were reported as means ± standard deviations.

RESULTS AND DISCUSSION

The moisture and starch content of the raw barley grains determination

The moisture and starch content of the raw barley grains were determined in the present study and results are reported in Table 1.

The determination of the amount of moisture is one of the most fundamental and important analytical procedures that can be performed on a food product (Aurand et al., 1987). In brewing and whiskey ventures, starch content of the raw material is very crucial. This has a significant impact on the concentration of ethanol and composition of dried DG.

Table 1. Starch and moisture content of barley

Parameters	Concentration (%)
Moisture content	8.53 ± 0.07
Starch content	52.05 ± 0.05

Variation of the concentration of dry matter during mashing process

The concentration of dry matter was measured and results were recorded in Table 2.

Wort samples were taken at 30 min interval until the mashing was over (270 min) which allowed for the concentration of solids (°Brix) to be plotted against time (Fig. 4).

Table 2. Variation of the concentration of dry matter (°Brix) during mashing process

Time (min)	Concentration of dry matter (°Brix)
30	3.7 ± 0.3
60	12.3 ± 0.4
90	14.1 ± 0.5
120	16.2 ± 0.1
150	16.9 ± 0.1
180	17.6 ± 0.2
210	17.7 ± 0.1
240	17.9 ± 0.1
270	18.2 ± 0.1

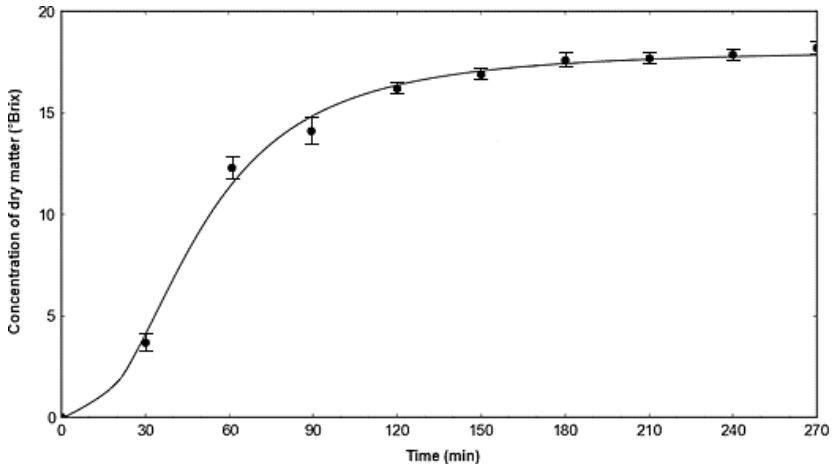

Figure 4. Variation of the concentration of dry matter (°Brix) during mashing process.

Amylase enzymes involved in the transformation of starch into fermentable sugars is temperature dependent, so as the mashing proceeds heat also increase aiding the activations of the enzymes. The increase in concentration of dry matter (°Brix) cannot remain persistent due to the deactivation of the enzymes at high temperature. Adadi et al. (2017) studied the concentration of dry matter (°Brix) kinetic during fermentation of beer supplemented with *Hippophae rhamnoides.*

The effect of sapropel on DDG's microflora

Microbiological analysis of DDG was carried out and after incubation, colonies were counted manually and the results were reported in Table 3.

Table 3. Microbiological results of un- and treated dried distillers' grain before and after one week of storage at 37 °C

Treatment	Before storage (CFU g^{-1})	After storage (CFU g^{-1})
Untreated DDG	2.3×10^4	2.5×10^4
Treated DDG	1.77×10^4	2.18×10^4

CFU - colony forming unit; g- gram of dried distillers 'grain.

In the present study, sapropel inhibited the growth of microflora on treated DDG. However, there was an inhibition of the untreated DDG, which could be a result of environmental effects (temperature, pressure, pH etc.). According to Adadi & Obeng (2017), pH is very important and has a major influence on microbial growth. Moreover, the antibacterial and antifungal properties of sapropel have been reported. Barakova et al. (2017) showed the fungicidal potency of sapropel extracts. However, these properties depend on the quantity utilized. Bactericidal properties of sapropel have also been elucidated by Buzlama & Chernov (2010), which are due to humates and humic substances (humic acids in humic acids group). These compounds could inhibit the proliferation of some groups of bacteria. Russell & Diez-Gonzalez (1998) explained how coupling mechanism is altered by acids transported across the cell membrane. This blocks the production of ATP thereby arresting the ability of anabolism.

The effect of sapropel extracts on physicochemical parameters of DDG

Physicochemical parameters of DDG were determined during this experiment and recorded in Table 4.

Chemical parameters of DDG were undoubtedly changed due to the application of sapropel extract. The chemical and physical characteristics of DDG were determined routinely as part of quality management. According to GOST 31809-2012, the moisture content of DG feed should be not less than 5 and not more than 10. DDG (treated and untreated) in the present study were in range: $5 < 9.96 < 10$ (Table 4).

Table 4. Physicochemical parameters of un- and treated dried distillers grain

Physicochemical parameters	Untreated DDG	Treated DDG
Moisture content (%)	9.96 ± 0.06	9.96 ± 0.06
pH	4.5 ± 0.1	6.1 ± 0.1
Titratable acidity (TA) (%)	3.2 ± 0.4	2.03 ± 0.06
Acid Value (AV) (mg g^{-1})	2.89 ± 0.04	1.26 ± 0.01

The pH had increased from 4.5 ± 0.1 to 6.1 ± 0.1 before and after treatment respectively. TA likewise decreased from 3.2 ± 0.4 to 2.03 ± 0.06%. Prior to the treatment, DDG was more acidic than after treatment. pH is important to assess the ability of a microorganism to grow in a specific environment. According to Adadi et al. (2017a) and Adadi (2017b), the lower a pH value, the higher TA.

The role in acidulation is influenced by inorganic acids like carbonic acid and phosphoric acid from carbon and phosphorus elements respectively whereas organic acids affect flavour, colour, microbial stability and keeping the quality of feed (Tyl & Sadler, 2017). Sapropel is not rich in inorganic acids but it could affect the TA of DDG due to its organic-rich sediments. A decrease (from 2.89 ± 0.04 to 1.26 ± 0.01 mg g^{-1}) in acid value (AV) was also observed. AV was expressed in milligrams of potassium hydroxide per gram of fat (Table 4).

CONCLUSION

In this study, sapropel extracts have shown its effect on microflora and physical parameters and chemical composition on DG. Sapropel extracts could be of great help to the food industries and other grain-related fields due to its unique organic acids. The potency of sapropel extracts depends on the amount used, its pH and concentration of dry matter hence these parameters should always be monitored for optimal results. Sapropel could aid to reduce the quantity of microorganisms in DG after treatment. On the other hand, sapropel can increase the growth rate of microorganisms during storage. Further research is needed in order to get better understanding of these relations.

REFERENCES

Adadi, P. & Obeng, A.K. 2017. Assessment of bacterial quality of honey produced in Tamale metropolis (Ghana). *J. Food. Drug.Anal*. **25**, 369–373.

Adadi, P., Kovaleva, E.G., Glukhareva, T.V. & Shatunova, S.A. 2017a. Biotechnological production of non-traditional beer. In: Valeeva A.A & Volkovich, V.A. (ed.) Physics, Technologies and Innovation (PTI-2017): *IV International Young Researchers Conference*, AIP Conference Proceedings, Melville, USA, pp. 1–13.

Adadi, P., Kovaleva, E.G., Glukhareva, T.V., Shatunova, S.A. & Petrov, A.S. 2017b. Production and analysis of non-traditional beer supplemented with sea buckthorn. *Agronomy Research* **15**, 1831–1845.

Aliyu, S. & Bala, M. 2011. Brewer's spent grain: a review of its potentials and applications *Afr. J. Biotechnol*. **10**, 324–331.

Aurand, L.W. & Woods, A.E. & Wells, M.R. 1987. *Food composition and analysis*. Van Nostrand Reinhold, New York, 19–34, 178–230 pp.

Avdeyeva, L.N., Kovalenko, T.A., Krivonos, O.I., Plaksin, G.V. & Strunina, N.N. 2009. Determination of sapropel chemical composition. *Chemistry and Chem. Eng.* **52**, 121–123 (in Russian).

Barakova, N.V., Sharova, N.Y., Juskauskajte, A.R., Mityukov, A.S., Romanov, V.A. & Nsengumuremyi, D. 2017. Fungicidal activity of ultradispersed humic sapropel suspensions. *Agronomy Research* **15**, 639–648.

Buzlama, A.V & Chernov, Yu.N. 2010. Analysis of pharmacological properties, mechanisms of action and prospects for the use of humic substances in medicine. *Experimental and clinical pharmacology* **73**, 43–48.

Clark, P., Givens, D.I. & Brunnen, J.M. 1987. The chemical composition, digestibility, and energy value of fodder-beet roots. *Anim. Feed Sci. Technol*. **18**, 225–231.

Coupe, L.R., Lardy, G.P., Meyer, A.M., Neville, B.W., Leupp, J.L. & Canton, J.S. 2008. Effects of corn condensed distillers solubles supplementation on performance and dry matter intake of beef cows consuming forage – based diets. *J. Anim Sci*. **59**, 346–349.

Cromwell, G.L., Herkelmad, K.L. & Stah, T.S. 1993. Physical, Chemical, and Nutritional Characteristics of Distillers Dried Grains with Solubles for Chicks and Pigs. *J. him. Sci*. **71**, 679–686.

Emeis, K.C. & Weissert, H. 2009.Tethyan-Mediterranean organic carbon-rich sediments from Mesozoic black shales to sapropels. *Sedimentology* **56**, 247–266.

Emtsev, V.T. & Mishustin, E.N. 2006. *Microbiology: a textbook for universities- 6thed*. Drofa, Moscow, 444 pp. (in Russian).

GOST 13496.12-98 (Interstate Standard), 1998. Mixed fodders, mixed fodder raw materials: Method for determination of total acidity. *http://internet-law.ru/gosts/gost/8879* updated: 04.04.2015 (in Russian).

GOST 13496.18-85 (Interstate Standard). 1986. Mixed fodder and fodder raw stuff. Methods for determination of fat acid value. *http://internet-law.ru/gosts/gost/20120* updated: 21.12.2017 (in Russian).

ISO/TC 34.(International Organization for Standardization Technical Committee 34). 2009. ISO 712: 2009 Cereals and cereal products – Determination of moisture content. *https://www.iso.org/obp/ui/#!iso:std:44807:en*. Reviewed in 2014.

ISO/TC 93 (International Organization for Standardization Technical Committee 93), 1997.ISO10520:1997 Native starch – Determination of starch content – Ewers polarimetric method. *https://www.iso.org/obp/ui/#iso:std:iso:10520:ed-1:v1:en.* Accessed: 03.31.2017.

Kireycheva, L.V. & Khokhlova, O.B. 2000. Elemental composition of humic substances in sapropel deposits. *RAAS Newsletter* **4**, 59–62 (in Russian).

Kireycheva, L.V., Khokhlova, O.B. 1998. Sapropels: composition, properties, application. *M. Science*. 124 pp. (in Russian).

Perminova, I.V. 2008. Humic substances – a challenge to chemists of the XXI century. *Chem. Life* **1**, 50–55 (in Russian).

Plaksin, G.V. & Krivonos, O.I. 2007. Thermochemical processing of lake sapropels: composition and properties of products. *Russian Chemical Journal* **4**, 140–147 (in Russian).

Russ, W., Mortel, H. & Meyer-Pittroff, R. 2005.Application of spent grains to increase porosity in bricks. *Adv. Chem. Eng. Sci*. **4**, 117–126.

Russell, J.B. & Diez-Gonzalez, F. 1998.The effects of fermentation acids on bacterial growth. *AdvMicrob Physiol*. **39**, 205–234.

Santos, J.E.P., Villaseñor, M., Robinson, P.H., DePeters, E.J. & Holmberg, C.A. 2003. Type of cottonseed and level of gossypol in diets of lactating dairy cows: plasma gossypol, health, and reproductive performance. *J. Dairy Sci.* **86**(3), 892–905.

Savchenko, I.A. 2015. *Chemical and pharmaceutical research of humic substances of sapropel of Lake Gorchakov*. Dissertation of the candidate of Pharmaceutical Sciences, Ulan-Ude, 22 pp. (in Russian).

Tyl, C. & Sadler, G.D. 2017. *pH and Titratable Acidity*. Food Analysis, 5th Ed. Food Science Text Series. Springer, Cham. pp. 389–406.

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Effect of impact-activating-disintegration treatment on grain protein fraction of autumn rye

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Abstract. This paper studies the distribution of rye protein fractions according to their mass and amino acid composition while milling by machines with different work tools. The research was conducted on the autumn rye of harvest year 2017 with kernel moisture content of 8%. Cereals were milled in the machine of an impact-activating-disintegrating type DEZI-15 with three-row and five-row rotors which rotate at 120 s^{-1}, and in the laboratory mill with a knife-rotor work tool. The milling grain size was determined by a diffraction-type grain analyser Malvern Mastersizer 2000. The average grain size obtained from three-row disintegrating rotor was 167 μm, from the five-row rotor 158.1 μm, and from the laboratory knife-rotor mill 384 μm, respectively. The free amino acids composition in flour samples was investigated using the size exclusion chromatography method (SEC-method). The polypeptide composition of total grain protein has been determined by One-dimensional SDS-acrylamide gel electrophoresis. According to the electropherogram results obtained from all the the content of high-protein fraction of 200 kDa. The glutelin fraction with molecular weight of 116.25 kDa is definitely observed in the sample obtained from the three-row disintegrating rotor. Whereby the lowest glutelin content has been detected in the flour sample obtained from the five-row disintegrating rotor. Fractions with molecular weight of 60–75 kDa – globulin fractions – come up frequently in the sample obtained from the three-row disintegrating rotor. Prolamine fractions of 45–47 kDa are clearly observed in the flour sample obtained from the laboratory knife-rotor mill. The albumin fraction with molecular weight of 17–28 kDa are mostly observed in the samples obtained by three-row and five-row disintegrating rotors. Few LMW fractions (from 6.5 to 15 kDa) are found in samples obtained using the impact-activating-disintegrating technique, mostly in the sample milled in the laboratory knife-rotor mill. Based on the data of free amino acid content in sample investigated it can be concluded that the impact-activating-disintegrating techniques does not cause reduction in protein biological value. The albumin rich flour milled in the disintegrator can be used for production of functional food. Due to the low content of glutelin protein fraction the flour obtained from the five-row disintegrating rotor offers the greatest promise for production of gluten-free foods.

Key words: impact-activating-disintegrating technique, autumn rye, protein fractions, amino acid composition, biological value foods.

INTRODUCTION

Cereal products constitute an important part of everyday diet. They provide 21% of calories, 38% of carbohydrates and 20% of protein in human nutrition in protein being the second only to meat products (Bulgakov, 1976).

The promising raw material for food production is rye. Having enhanced root system this crop is one of the most cold-resistant with no demand of soil fertility. Moreover, rye protein is better balanced in composition of essential amino acids (8–16%) compared to wheat and barley protein because of high content of lysine (up to 0.619 g 100 g^{-1}), valine, threonine and methionine. Rye contains antioxidants, especially phenolic acid and polyphenols that protect body tissues from oxidative stress and cell death and prevent chronic diseases such as cardiovascular, nervous degenerative disorders and carcinomata. The merits of rye are rich vitamin composition, especially B group, and the content of macro- and microelements such as potassium, phosphorus, magnesium, manganese, iron, copper, zinc, selenium and others (Bakhitov et al. 2008).

The rye protein consists of the following fractions. Concentration of water-soluble proteins – albumins – is usually 5–15% of the total amount, but can be up to 30%. Salt-soluble proteins – globulins in the rye grain are about 15–25%, alcohol-soluble proteins – prolamines – account for 15–25%, alkali-soluble proteins – glutelins are about 30–40%.

The rye grain contains also other nitrogen compounds in small amounts: free amino acids and their amides, peptides, basic nitrogen and nucleotides, nucleic acids, etc. They account for 5–10% of the total amount of grain nitrogenous matter. Mostly they are found in the kernel and aleurone layer.

The rye protein fractions vary in amino acid composition as well as in the content of essential amino acids which determine the biological value of proteins. Albumins are of the greatest biological value, they contain the best ratio of all the essential amino acids, with some deficit in the content of methionine. Globulins are also characterized by a well balanced amino acid composition, although their content of some essential amino acids is lower compared to albumin (methionine, tryptophan, leucine).

Rye glutelins are characterized by a rather strong deficit of lysine, tryptophan and methionine. Prolamins have the lowest biological value. They are distinguished by a very low content of essential amino acids such as lysine, tryptophan, methionine, and a high concentration of glutamic acid and proline which accounts for up to 40–55% of the mass of these proteins (Rybakova & Glebova, 2012).

In food production technology the presence of different protein fractions of raw material plays an essential role. In the zymurgy the greater presence of free amino acids and LMW protein fractions intensify the output of ethyl alcohol due to consumption of α- amine nitrogen by yeasts (Rimareva et al.; 2008, Rimareva, 2010). In starch and grain syrup production and brewing reduction in content of HMW proteins facilitates the processes of filtration and clarification of wort, as well stabilizes the fobbing (Tretiak, 2009; Donkova & Donkov, 2014; Danina & Ivanchenko, 2015; Sergeeva, 2016). Thus, the main task of most food production technologies based on grain raw materials is to destruct the protein matrix into LMW components that increase their solubility (Maltseva, 1999; Sereda et al., 2010; Shakir et al., 2017). Additionally, the degradation of protein frame may lead to greater availability of other valuable components of grain

raw materials such as starch (Stepanov et al., 2007; Alekseeva et al., 2011; Amelyakina et al., 2011).

Currently, the following machines can be employed for fine grain milling: mills – ball mills, vibration mills, ball electromagnetic mills, disintegrators and dismembrators, roll-oscillating mills, jet mills, ultrasonic mills, devices with velocity layer of ferromagnetic particle etc. (Kalinina et al., 2002: Sotnikov et al., 2002; Smirnova & Krechetnikova, 2005; Likhtenberg et al., 2007; Barakova & Ustinova, 2010; Oshkordin et al., 2011; O. Lomovsky & I. Lomovsky, 2011; Romanyuk et al., 2013).

The latest methods of grain handling also include the extrusion technology. The application of extrusion devices allows to produce new products from the vegetable feedstock as well as to enhance the current processes such as production of wort with high content of dry solids weight ratio up to 36 percent, usage of raw materials with high content of no starch polysaccharides, etc. (Nachetova & Barakova, 2013).

The deep grain destruction can be conducted by infrared grain handling (infrared radiation). Since the fluence rate of infrared radiation is high enough, the moisture concentrates in the grain which is heated up to 110–115 °C causing rapid increase of the vapour pressure and decrease of grain strength properties (Krikunova et al.; 2004, Andrienko et al., 2007).

However, we have the evidence that the employment of rough grain processing methods, such as extrusion and micronization (IR-treatment), reduces the biological value of processed grain. As a result the main amino acids of the grain milled are limited with the total amount of α-amine nitrogen reduction (Martirosyan & Malkina, 2010; Bikchantaev et al., 2016).

Another way of deep grain processing techniques of special interest is the impact devices such as disintegrators. One of the most important features of disintegrators is that the material processed is subjected to mechanical activation. The activation of particles applying high mechanical energy is the new advanced type of improvement of technological processes in various industries (Boldyrev, 2006; Sabirov et al., 2017a).

Disintegrators are the devices which function is based on the free impact principle (that's why the device name includes the term impact). In the 1980s, the specialists of the design and technology bureau 'isintegrator' (Tallinn, Estonia) developed the advanced design of disintegrators allowing to obtain fine milled mixtures. The material being milled is continuously fed into the mixing chamber in the middle between two high-speed counterrotating rotors. Impact tools are located on each rotor along the concentric circles. Rotors run into each other in such a way that the concentric circles with pins of one rotor come into the concentric circles with pins of another one.

The advantage of such disintegrators compared to other milling units is the rapidity of milling processes when the material processed therein within 10 s^{-1} receives 2 to 7 high-intensity impacts (Alimova et al., 2014; Sabirov et al., 2017b).

Research in the field provides the evidence of deep mechanochemical destruction of grain components on the example of winter wheat grains subjected to the impact-activating-disintegrating technique. As the electron microscopy method shows in the case of the impact-activating-disintegrating treatment the grain endosperm is deeply destructed. The starch grain of the endosperm takes an oval-round and lenticular shape, its size ranges from 25 to 40 μm. The protein matrices after the impact-activating-disintegrating treatment have more developed surface, the interspace protein can be separated much easier releasing more starch grains and partially leaving the starch grains

in the protein matrix provided that the interspace protein of glassy endosperm is destructed during milling together with the firmly linked starch kernel.

Using the gas chromatography method it was found that during the impact-activating-disintegrating treatment of grain we observe the following: glucose (percent) – 1.41 (three-row rotor), 1.48 (five-row rotor), as well as maltose (percent) – 2.05 (three-row rotor), 1.84 (five-row rotor) while in the case of the rotor milling (close to treatment by rolls) the glucose content in the sample is 1.21 percent. In this case we observe the total absence of maltose. This fact indicates that during the impact-activating-disintegrating treatment of grain starch is destructed without an enzymatic process (Barakova & Tishin, 2010).

It is worth mentioning that today scientific literature in the field provides no information on size distribution of grain proteins processed by a disintegrator. Thus, the paper studies the change in the size distribution of proteins as well as free amino acids of winter rye after the impact-activating-disintegrating treatment.

MATERIALS AND METHODS

This paper studies rye flour samples milled by machines with different work tools. The research was conducted on the first class rye of harvest year 2017. The moisture and starch content of test kernels were found to be 8 and 65% respectively. The rye grain moisture content was detected with Shimadzu MOC-120H. The amount of highly soluble carbohydrates was determined with the polarimeter AA-55 by Optical Activity.

For the experimental purposes cereals were milled in the machine of an impact-activating-disintegrating type DEZI-15 with three-row and five-row rotors (Central research institute for engineering materials 'Prometheus', Saint Petersburg). The reference sample was the grain milled in a laboratory mill with a knife-rotor work tool. The milling grain size was determined by diffraction-type grain analyzer MalvernMastersizer 2000. The average grain size obtained from three-row disintegrating rotor was 167 μm, from the five-row rotor – 158.1 μm, and from the laboratory knife-rotor mill – 384 μm, respectively.

The free amino acids composition in flour samples was investigated using the size exclusion chromatography method (SEC-method). In order to determine the quantitative composition of free amino acids in flour the sample was put into 1n hydrochloric acid, then trichloracetic acid was added for sedimentation of non-hydrolized proteins. The sample was kept during 30 minutes, then centrifuged. The supernatant (filter cake) was used for the analysis of free amino acids applying the SEC-method. The analytical tolerance is 0.5% or less.

The polypeptide composition of total grain protein was determined by One-dimensional SDS-acrylamide gel electrophoresis of acrylamide concentration gradient 10–20% in a separating gel (pH = 8.8) and 6% acrylamide in a stacking gel (pH 6.8). The running buffer was *tris*-glycine buffer (pH = 8.3) containing 0.1% SDS. Electrophoresis was conducted at constant current (25–30 mA) within 6–7 h at 4–60 °C.

RESULTS AND DISCUSSION

Analysis of protein fractions of rye flour samples studied is shown in Fig. 1. According to the electropherogram obtained all samples showed the content of high-

protein fraction of 200 kDa. 116.25 kDa fraction is definitely observed in the sample obtained from the three-row disintegrating rotor – this is a glutelin fraction. The lowest content of glutelins can be found in the flour samples obtained from the five-row disintegrating rotor.

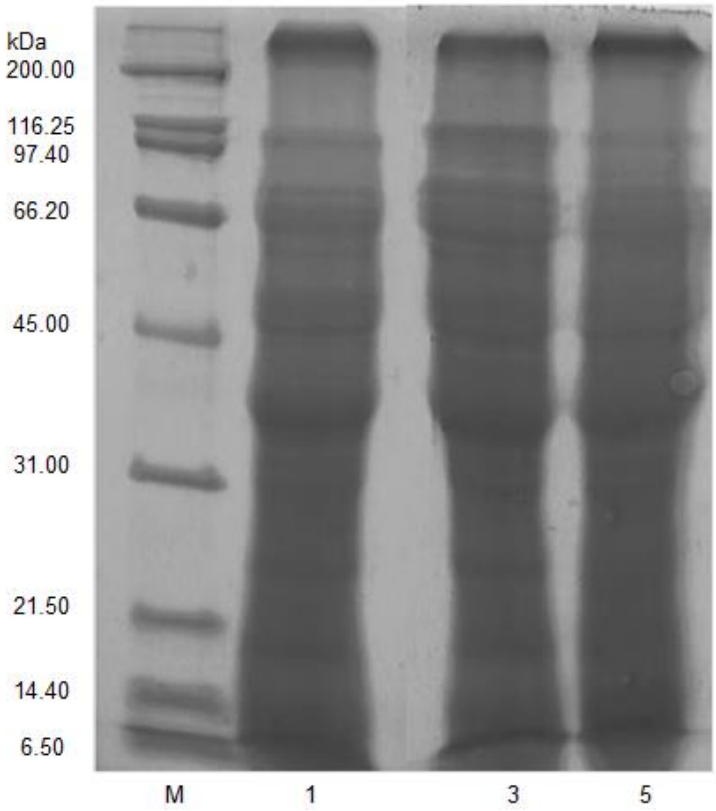

Figure 1. Electropherograms of protein fractions of rye flour samples. M – marker; 1 – electropherogram of the rye flour sample obtained in the laboratory mill with a knife-rotor work tool; 3 – electropherogram of the rye flour sample obtained from the three-row disintegrating rotor; 5 – electropherogram of the rye flour sample obtained from the five-row disintegrating rotor.

Fractions with molecular weight of 60–75 kDa – globulin fractions – are frequently found in the sample obtained from the three-row disintegrating rotor. Prolamine fractions of 45–47 kDa are clearly observed in the flour sample obtained from the laboratory knife-rotor mill. The albumin fraction with molecular weight of 17–28 kDa are mostly observed in the samples obtained by the three-row and five-row disintegrating rotors.

Few LMW fractions (from 6.5 to 15 kDa) are found in samples obtained using the impact-activating-disintegrating technique, mostly – in the sample milled in the laboratory knife-rotor mill.

Thus, the flour milled by the three-row disintegrating rotor is richer in the glutelin protein fraction and hence is more suitable for bakery product production. The glutelin fraction present in flour comprises of two proteins gliadine and glutenin plays role for

the dough making process and its qualitative properties. The flour obtained from the five-row disintegrating rotor contains the highest amount of the albumin fraction which is mostly rich in essential amino acids and is suitable for production of functional foods. The low content of glutelin fraction can make such foods with low glutelin content fit for a gluten-free diet (Kolpakova et al., 2001).

In order to evaluate the influence of grain handling by a disintegrator on other nitrogen compounds we have measured the content of free amino acids in the flour samples. The results obtained are shown in Table 1.

Table 1. Free amino acids of rye flour samples investigated

Amino acid	Content of free amino acids, mg g^{-1}		
	Flour obtained by the knife-rotor mill	Flour obtained by three-row disintegrating rotor	Flour obtained by five-row disintegrating rotor
Aspartic acid	0.2752	0.3426	0.3720
Serine	0.0379	0.0414	0.0469
Threonine	0.6093	0.5083	0.6019
Glutamic acid	0.2529	0.2865	0.2934
Proline	0.0676	0.1639	0.2487
Glycine	0.0223	0.0397	0.0328
Alanine	0.1177	0.1847	0.2132
Cysteine	0.0	0.0	0.0
Valine	0.0414	0.0449	0.0467
Methionine	0.0	0.0	0.0
Isoleucine	0.0164	0.0285	0.0285
Leucine	0.0207	0.0224	0.0276
Tyrosine	0.0	0.0216	0.0250
Phenylalanine	0.0129	0.0181	0.0319
Histidine	0.3918	0.3529	0.3789
Lysine	0.0440	0.0397	0.0449
Tryptophane	0.5320	0.5057	0.5782
Arginine	0.1562	0.1812	0.1648
Total amount	2.5993	2.7822	3.1354
Total content of essential amino acids	1.2767	1.1676	1.3594

* The error in the content of amino acids in the test samples is not more than 0.0001%; $p = 0.95$.

As seen from Table 1 the total content of free amino acids increases according to the intensification of the grain milling process. Thus, the content of free amino acids in the flour milled by the three-row disintegrating rotor is higher by 6.5 percent compared to the flour obtained from the knife-rotor mill. And the flour milled by the five-row disintegrating rotor shows the content of free amino acids higher by 17 percent than the sample obtained from the knife-rotor mill and by 11.3 percent compared to the flour milled by the three-row disintegrating rotor.

Concerning the total content of essential free amino acids we have obtained mixed result. We observe the highest content of these amino acids in the sample obtained from the five-row disintegrating rotor. The sample shows the content of essential amino acids higher by 6 percent than the sample obtained from the knife-rotor mill and by 14.1 percent than the sample from the three-row disintegrating rotor. This proves that the

three-row disintegrating rotor has less mechanochemical effect on the raw material milled. Amino acids such as arginine and histidine, which are essential for a child's body, are totally the same in all three flour samples investigated (Soboleva et al., 2015).

Based on the data proven we can assume that grain milling by the disintegrator shall changes the mechanochemical structure of the raw materials handled causing the increase of the diffusion properties of LMW components. Moreover, the impact-activating-disintegrating technique does not reduce the biological value of proteins compared to such methods as the extrusion or micronization.

CONCLUSIONS

The paper shows that the impact-activating-disintegrating technique for rye milling allows to obtain a fine milled grain with the high biological value compared to the techniques and devices employed today because:

- the total content of water-soluble protein particles is higher in flour samples obtained by disintegrator;
- the total content of free amino acids is also higher in flour samples obtained from rye treated by the impact-activating-disintegrating technique.

The albumin rich flour milled in the disintegrator can be used for the production of functional food. Due to the low content of glutelin protein fraction the flour obtained from the five-row disintegrating rotor is likely to be the most promising for the production of gluten-free foods.

REFERENCES

Alekseeva, N., Faradzheva, E. & Chusova, A. 2011. Development of comprehensive grain to alcohol processing technology with partial separation of starch. *Production of alcohol and liqueurs and spirits* **1**, 7–8 (in Russian).

Alimova, D., Barakova, N., Nachetova, M. & Samodelkin, E. 2014. Water-heat treatment of rye mashes with a reduced hydromodule and preservation of activity of native alpha-amylase. *Storage and processing of agri supplie* **11**, 18–21 (in Russian).

Amelyakina, M., Sharikov, A. & Stepanov, V. 2011. Innovation production technology of ethanol and protein concentrate based on the integrated processes of grain single stage. In: *Scientific papers of the 5th Conference of young scientists and specialists of the Department for storage and processing of agricultural products of the Russian Agricultural Academy*, pp. 28–30 (in Russian).

Andrienko, T., Polyakov, V. & Krikunova, L. 2007. Production of saccharified wort from IR-processed rye grain. *Storage and processing of agree supplies* **7**, 59–62 (in Russian).

Bakhitov, T., Chelnokova, E., Zaitseva, L. & Kolpakova, V. 2008. Resource efficient rye grain processing techniques. *News of Higher Educational Institutions. Food technology* **5**(6), 49–51 (in Russian).

Barakova, N. & Tishin, V. 2010. Investigation of the influence of enzyme preparations on the viscosity of high-concentrated barley mashes in the production of ethyl alcohol. *Production of alcohol and liqueurs and spirits* **4**, 24–26 (in Russian).

Barakova, N. & Ustinova, A. 2010. Study of the influence of grain dispersion degree on technological parameters in the production of alcohol from wheat. *Technologies and equipment of chemical, biotechnological and food industries: collection of research papers*. Biysk, pp. 220–224 (in Russian).

Bikchantaev, I., Shakirov, S., Yagund, E. & Yakhin, R. 2016. Analysis of changes in the IR spectra of winter rye seeds subjected to different types of moisture-thermal treatment. *Chemistry of plant products* **1**, 135–141 (in Russian).

Boldyrev, V. 2006. Mechanical chemistry and mechanochemical activation of solids. *Advances in Chemistry* **75**, 203–212 (in Russian).

Bulgakov, N. 1976. Biochemistry of malt and beer, Moscow, *Food industry*, 278 pp. (in Russian).

Danina, M. & Ivanchenko, O. 2015. Usage of extruded wheat in the breeding. *Bulletin of the International Academy of refrigeration* **2**, 18–22 (in Russian).

Donkova, N. & Donkov, S. 2014. Biotechnology of sugar production from grain raw materials. *Bulletin of the Krasnoyarsk state Agrarian University* **6**, 211–213 (in Russian).

Kalinina, O., Ledenev, V. & Krikunova, L. 2002. Development of high-performance, low-waste technology of ethanol from rye grain based on the mechanical and cavitational processing, 1st stage of mash production. *Storage and processing of agri supplies* **6**, 35–40 (in Russian).

Kolpakova, V., Krikunova, L. & Kononenko, V. 2001. Research of the possibility of obtaining protein preparations from differentiated fractions of grain of rye and barley. *News of higher educational institutions. Food technology* **5**(6), 35–39 (in Russian).

Krikunova, L., Omisova, O. & Zhurba, O. 2004. IR processing of raw materials in alcohol production. *News of Higher Educational Institutions* **4**, 46–49 (in Russian).

Likhtenberg, L., Plyanitsa, A., Alekseeva, L., Fedoseev, V. & Parfennikova, N. 2007. Implementation of wort production lines with hydrodynamic machining of grain mash at alcohol plants. *Production of alcohol and liqueurs and spirits* **4**, 13–18 (in Russian).

Lomovsky, O. & Lomovsky, I. 2011. *Mechanochemically Assisted Extraction. Enhancing Extraction Processes in the Food Industry*. NY – London, CRC Press, pp. 361–398 (in Russian).

Maltseva, B. 1999. Resources-saving production technologies of lysine protein feed \supplements based on the bioconversion of plant wastes from processing agrarian business. *Ecological Compliance in the agrarian business*. *Abstract journal 3*, 724 pp. (in Russian).

Martirosyan, V. & Malkina, V. 2010. Change in the amino acid composition of cereal crops after extrusion treatment. *Basics of the state policy in the area of production of healthy foods: technological aspects*, pp. 51–53 (in Russian).

Nachetova, M. & Barakova, N. 2013. Choice and verification of the temperature of water-heat treatment of mash from extruded wheat. *Production of alcohol and liqueurs and spirits* **3**, 29–32 (in Russian).

Oshkordin, A., Lavrov, L. & Usov, G. 2011. Kinetics and dynamics of milling of plant raw materials for food production. *Polzunovsky Bulletin* **2**, 202–206 (in Russian).

Rimareva, L. 2010. *Theoretic and realistic basis of yeast biotechnology*. DeLi Print, 252 pp. (in Russian).

Rimareva, L., Overchenko, M., Ignatova, N. & Abramova, L. 2008. Theoretic and realistic basis of enzyme catalysis of polymers of grain raw materials in the alcohol production. *Production of alcohol and liqueurs and spirits* **3**, 5–9 (in Russian).

Romanyuk, T., Chusova, A. & Agafonov, G. 2013. Production of clarified wort from rye grain and its fermentation to ethanol. *Production of alcohol and liqueurs and spirits* **4**, 13–16.

Rybakova, T. & Glebova, S. 2012. Changes in protein content and amino acid composition of rye and barley in flaking. *Bulletin of Novosibirsk State Agrarian University* **1**, 93–97 (in Russian).

Sabirov, A., Barakova, N. & Samodelkin, E. 2017a. Influence of the impact-activating-disintegrating technique on the technological parameters of hydrolyzates from rye. *Academic journal of ITMO University. Series: Food production processes and plants* **3**, 43–52 (in Russian).

Sabirov, A., Barakova, N. & Samodelkin, E. 2017b. Reasons to employ the impact-activating-disintegrating technique in the sirup production technologies from starch-containing products. *Bulletin of the South Ural State University. Series: Food and biotechnologies* **2**, 60–66 (in Russian).

Sereda, A., Kostyleva, E. & Smirnova, I. 2010. Application of enzyme preparations of serine proteases at hydrolysis of toasted and extruded soybean meal. *Storage and processing of agri supplies* **10**, 5–8 (in Russian).

Sergeeva, I. 2016. Classification of turbidity components of plant-based beverages. *Food processing and techniques* **42(**3), 70–76 (in Russian).

Shakir, I., Grosheva, V., Karetkin, B., Baurin, D. & Panfilov, D. 2017. Comprehensive processing of renewable vegetable feedstock obtaining the high-protein and probiotic feed stuff. *Butlerov's reports* **50(**5), 73–80 (in Russian).

Smirnova, I. & Krechetnikova, A. 2005. Influence of ultrasound processing on components of wheat chemical composition in the alcohol production. *Production of alcohol and liqueurs and spirits* **3**, 27–29 (in Russian).

Soboleva, O., Kondratenko, E. & Vityaz, S. 2015. Influence of the electromagnetic field on the amino acid composition and biological value of the grain of a new winter crop. *Bulletin of the Altai State Agrarian University* **11**, 58–64 (in Russian).

Sotnikov, V., Fedorov, A., Gamayurova, V., Kotelnikova, N. & Kotelnikov, M. 2002. Low-temperature cooking method of starch-containing raw materials for alcohol production. *Production of alcohol and liqueurs and spirits* **1**, 13–15 (in Russian).

Stepanov, V., Rimareva, L., Ivanov, V. & Sharikov, A. 2007. Processing methods of starch-containing raw materials at production of concentrated grain wort. *Production of ethyl alcohol and liqueurs and spirits* **3**, 16–17 (in Russian).

Tretiak, L. 2009. Transformation of malt proteins in the brewing technology. *Industry of beverages* **4**, 16–18 (in Russian).

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Encapsulation of Gallic acid in solid lipid core surrounded with maltodextrin shell

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Abstract. Multiple phase capsules had been prepared in a single spray drying process. The main goal of the present study was to investigate whether the conversion of a portion of the modified starch (wall material used in spray drying) to resistant starch (RS) would offer added protection of encapsulated material. To achieve this, dry gallic acid (GA; a model water soluble phenol compound used in the present study) was initially dispersed in palm oil and stabilized with Polyglycerol Polyricinoleate (PGPR 4175) as an emulsifier. This dispersion was homogenized with a modified starch (MS, dextrose equivalent of 15) solution, that was previously treated with high pressure and increased temperature to achieve starch retrogradation, and then spray dried. It was possible to produce only small amounts of RS from modified starch, varying from 0.1 to 0.2% of total carbohydrate content. GA content in the lipid phase of the capsule was determined by lipid droplet size in the O/W emulsion (the feeding solution), as smaller droplets results in the significantly bigger surface area, and more intensive GA diffusion from O to W phase. Maltodextrin shell wall was able to prevent leaking of the melted palm oil form the capsule core to the surface during seating tests, preventing agglomeration of capsules. This could be very important for the storage/transportation of capsules in the uncontrolled temperature conditions.

Key words: gallic acid, spray drying, encapsulation, resistant starch.

INTRODUCTION

Protection of sensitive bioactive compounds is an important aspect of modern food industry in order to provide high nutritional value added food products. Alternatively, there are many compounds that cannot be incorporated into food systems in their regular form due to specific taste, biochemical activity, and physical properties. In this situation, encapsulation steps in as a possible solution. It is a process of entrapping a specific component (‘active compound’ or core material) within a protective matrix (‘encapsulant’, wall or matrix material) (Garti & McClements, 2012).

Present work is focused on encapsulation of a water soluble phenol compound - gallic acid (GA). Phenolics are an extremely heterogeneous class of secondary plant metabolites. They protect plant against biotic (caused by herbivores, insects, and pathogens) and abiotic (caused by free radical) stresses (Schieber & Saldana, 2009). In human diet, phenolics are the most common antioxidants (Friedman, 1997; Lima et al.,

2014). Their preservation and application in food production can offer added nutritional value for human nutrition, and provide antioxidative protection for the food product itself.

In food industry, an efficient encapsulation system for protection of biologically active compound must be formulated using only food grade compounds, which were manufactured with solvent-free production methods (McClements et al., 2007; Acosta, 2009). This system should have minimum interaction with the encapsulated compound, provide high physicochemical stability (McClements et al., 2007; Donsi et al., 2011), and shield active compound from interaction with another food ingredients and environment factors such as temperature, pH, oxygen, and light (McClements et al., 2007). Additionally, this system should maximize the uptake of encapsulated compounds upon consumption (Acosta, 2009; Dordevic et al., 2015) and ensure controlled release in response to a specific stimulus in the environment (McClements et al., 2007). From technical point of view, it should be scalable to industrial production (Desai & Park, 2005; Donsi et al., 2010a; Donsi et al., 2010b).

There are many different encapsulation techniques, and spray drying is a most often used in food industry. It is cost-effective, flexible (drying parameters are easily controllable), and can be operated continuously (Desai & Park, 2005; Fang & Bhandari, 2010; Anandharamakrishnan & Padma Ishwarya, 2015). In short, core and wall materials are homogenised, fed into a spray dryer, and atomized with a nozzle or spinning wheel. Water is evaporated by the hot air stream that contacts the atomized material. During the drying process, droplets shrink and form round capsules, which are than collected (Gibbs et al., 1999). Most common wall materials for spray drying encapsulation are gum arabic, maltodextrin and modified starch (MS) (Munin & Edwards-Levy, 2011). For encapsulation of phenolic compounds, maltodextrins are frequently chosen as wall material. They can wary in dextrose equivalents (DE, a measurement of the amount of reducing sugars in the product), depending on the final product application (Ersus & Yurdagel, 2007; Krishnaiah et al., 2012; Kaderides et al., 2015; Pasrija et al., 2015; Saikia et al., 2015). Most phenolics possess bad sensory properties and maltodextrins had been successfully applied in spray drying processes to stabilize phenolics and mask their unpleasant bitter taste (Sansone et al., 2011). In addition, maltodextrin is able to provide good thermal protection, and was able to preserve the integrity of the anthocyanins during their encapsulation (Ersus & Yurdagel, 2007; Robert et al., 2010). Unfortunately, maltodextrin doesn't possess good enough emulsifying properties for lipophilic material encapsulation. As a result, it should be modified by adjusting a hydrophobic part to its molecule, forming a material called modified starches (MS). MS is a good choice for hydrophilic material encapsulation in the hydrophilic matric, and it will be used in present study for oil-in-water emulsion formation.

Despite good overall encapsulation properties, capsules and shells made from maltodextrin and MS are not stable against enzymatic digestion. It was shown that stability to enzymatic digestion of coatings (prepared form high amylose corn starch) can be improved by addition of resistant starch (RS) (Dimantov et al., 2004b). RS is a physiologically important indigestible starch fraction in human diet. It is usually present in relatively low amounts in food products. RS is enzyme resistant and is not digestible in the small intestine, but can be fermented by microorganisms in the large intestine (Eerlingen et al., 1993). Incorporation of 20% RS in the wall material, made of high amylose corn starch, is suitable for achieving targeted release of encapsulated active

compound in the colon (Dimantov et al., 2004a). Increased concentrations of RS considerably improve the stability of the coatings to enzymatic digestion, although resulting in the crack on the capsule surface (Dimantov et al., 2004b).

Alternatively, to ensure capsule stability in the hydrophilic media and against non-lipid digestible enzymes, active compounds can be encapsulated in the solid hydrogenated lipids (Mukherjee, 2009; Eltayeb et al., 2013; Wolfe et al., 2015). Resulted capsule particles possess a great water barrier, but their thermal stability is highly dependent on the melting point of the chosen lipid phase. For additional protection, hydrophilic (modified starch) and hydrophobic (solid lipids) systems can be combined to produce multiple layered capsules. In this case, solid lipid core usually is surrounded by MS shell by using fluidized bed technology (Arshadey, 1999). In this process, solid particles are moved in the air in fluidized bed reactor and simultaneously sprayed with a liquid coating material, which is solidifying due to the temperature and humidity control system. It is a two-step process consistent of preparation of solid core and coating it with shell material.

In present study, multiple phase capsules (consistent of solid lipid core and modified starch shell) will be prepared in a single spray drying process for water soluble phenolic compound encapsulation. The aim of the present study is to investigate whether the conversion of a portion of the modified starch (wall material, used in spray drying) to resistant starch (RS) would offer added protection of encapsulated material (water soluble phenolics).

MATERIALS AND METHODS

Chemicals and Reagents

Modified starch 'Capsul®' was provided by Ingredion, Inc. (Westchester, USA); Calcium Chloride dihydrate, Gallic acid, Glacial Acetic acid, Potassium Hydroxide, Sodium Azide, Sodium Carbonate, Maleic acid, and Sodium Hydroxide were purchased from Sigma-Aldrich (St. Louis, USA); Ethanol (200 proof, anhydrous 99.5%) was purchased from Pharmco-Aaper (Brookfield ,USA); Folin-Ciocalteu phenol reagent purchased from VWR International (Radnor, USA); Palm Oil kernels were purchased form Bulk Apothecary (Aurora, USA); Polyglycerol Polyricinoleate (PGPR 4175) was provided by Palsgaard, Inc. (Morris Plains, USA).

Preparation of the wall material

To produce resistant starch (RS), Eerlingen et al. (1993) method was used. MS was dispersed in distilled water in ratio 1:2, and autoclaved at 120 °C for 1 h. in AMSCO, American Sterilizer (Erie, USA). When temperature and pressure dropped to the safe limit, samples were immediately transferred to baths at 22 ± 2, 68 ± 2 and 100 ± 2 °C for 24 h. Control sample, without autoclave treatment, was incubated under magnetic stirring at 80 °C for 30 min to ensure full MS gelatinization. Prepared solutions were used for further oil-in-water (O/W) emulsion preparation, forming W phase (shell wall material).

Resistant starch content determination

RS content was determined by using Megazyme RS analyse kit K-RSTAR 10/15, AOAC method 2002.02, AACC method 32-40.01 (Mccleary et al., 2002; AACC 32-40.01).

Sample preparation

Capsule core had been made as a suspension of GA in the melted lipids, forming a O phase for the further O/W emulsion preparation. To achieve this, palm oil kernels were melted (with melting point at ≈50 °C) at 50 °C and GA was added in proportion 4:1, and 0.1% of PGPR (w/w) was added as stabiliser. PGPR is broadly used in chocolate production water-in-oil emulsifier, and had been chosen for the present research as it is food grade (E 476), not carcinogenic, 98% digestible in rats, and is utilized in the body as a source of energy superior to starch (Wilsona et al., 1998). Mixture was homogenized by magnetic stirring at 600 rpm for 5 min. Obtained dispersion was added to the prepared wall material solution at a proportion 1:2, and homogenized by high shear mixer for 30 sec, obtaining O/W emulsion. Mixture was immediately fed to Buchi Mini Spray Dryer B-290 (New Castle, USA). Drying parameters were set as followed: inlet and outlet temperatures – 170 and 75 °C, respectively; sample feed rate – 15 mL min^{-1}; spray gas flow – 667 L h^{-1}. Feed solution had been kept on the hot plate at 60 °C under constant intensive stirring to prevent oil solidification. Collected samples were stored in double plastic bags at -20 °C. Whole experiment was repeated three times.

Gallic acid extraction from different capsule parts

All samples were divided in three parts. First part was used to determine total GA content (TGA): 0.5 g of sample was mixed with 5 ml of 70% ethanol and magnetically stirred for 30 min at 50 °C, and filtered through a paper filter Nr.3. Second part was used for the surface GA content determination (SGA): 0.5 g of sample material was washed with 5 mL ethanol and filtered through the paper filter. Third part was used for GA content determination in the wall material: 0.5 g of sample was mixed with 5 mL of 70% ethanol and magnetically stirred for 30 min at room temperature to dilute wall material, and filtered through the paper filter.

All filtrates were collected and used for the GA content determination in different parts of produced capsules.

GA content determination

For the GA content determination in the filtrates, Ragazzi & Veronese (1973) Folic-Ciocalteu method with slight modifications. Briefly, 200 μL of the sample were mixed with 1 mL of distilled water and 100 μL of Folin-Ciocalteu phenol reagent (preciously diluted with distilled water 1:1, v/v). After 5 min, 2 mL of the 10% sodium carbonate solution was added. Acquired mixture was allowed to stand at room temperature for 60 min. The absorbance was measured at 725 nm by laboratory spectrophotometer UV-1800 (Shimadzu, Japan).

Encapsulation efficiency

Encapsulation efficiency (EE) was calculated by the following equation:

$$EE = 100 \times \left(1 - \frac{SGA}{TGA}\right) \quad (1)$$

Heat stability and optical microscopy

Heat stability was analysed visually. 0.5 g of each sample were placed in the oven on the filter paper for 30 min at 100 °C. Sample optical microscopy was prepared by using BX40 (Olympus, United States) microscope.

Particle size distribution

Particle size distribution was analysed using laser diffraction particle size analyser LA 960 (HORIBA, Japan).

Moisture content determination

Moisture content was determined using HTF 2000 Moisture analyser (DSC, United States). Data was used for GA content recalculations on dry weight.

Statistical analysis

One-way ANOVA ($P \leq 0.05$) and Tukey's test were applied for the statistical analysis of the distribution of GA in the different capsule fractions.

RESULTS AND DISCUSSION

In present study, three phase encapsulation system (solid lipid core, containing water soluble phenolic compound, surrounded with modified starch shell wall) had been developed in a single spray drying process.

Based on the fact that RS fractions can be obtained by hydrothermal treatment and retrogradation of starches (Herman & Remon, 1989; Sievert & Pomeranz, 1989; Eerlingen et al., 1993), wall material previously was autoclaved at 121 °C in excess water with further incubation at 22 °C, 68 °C, and 100 °C (Eerlingen et al., 1993). Unfortunately, it was possible to produce only small amounts of RS in the chosen MS material: 0.12% in samples incubated at 22 °C and 68 °C, and 0.19% in samples incubated at 100 °C, against 0.10% for control sample. Achieved concentrations are not enough to ensure enzymatic resistance of the shell, as RS is not a chemically reactive substance in present conditions and will not influence physical or chemical properties of the capsule shell at present concentrations. It can be seen that increase in the incubation temperature directly influence RS formation, and incubation at 100 °C results almost in double amounts of RS in comparison to incubation at 22 and 68 °C. This fact matches previously reported data by Eerlingen et al. (1993). No correlation had been detected between RS concentration in the wall material and GA content in the lipid core of produced capsules (Fig. 1). It was expected that (at the sufficient concentrations) higher increase in RS content could influence GA diffusion from the lipid core into the coating material, or from the coating to the surface (as both GA and MS are water soluble, GA is evenly distributed inside the coating matrix, and GA content on the surface is dependent on the volume-to-surface ratios of the coating itself). At the same time, too high RS concentrations can cause cracking in the MS coating due to reduced intermolecular interactions, caused by the nature of RS (Dimantov et al., 2004b). This cracking results in lower integrity of the shell and could be a reason for further additional GA losses, due to the more intensive water evaporation during the spray drying process. Alternatively, high RS content could result in bigger amount of free water in the W phase

of the O/W solution that could increase GA diffusion from the lipid core phase or capsule surface into the wall material phase.

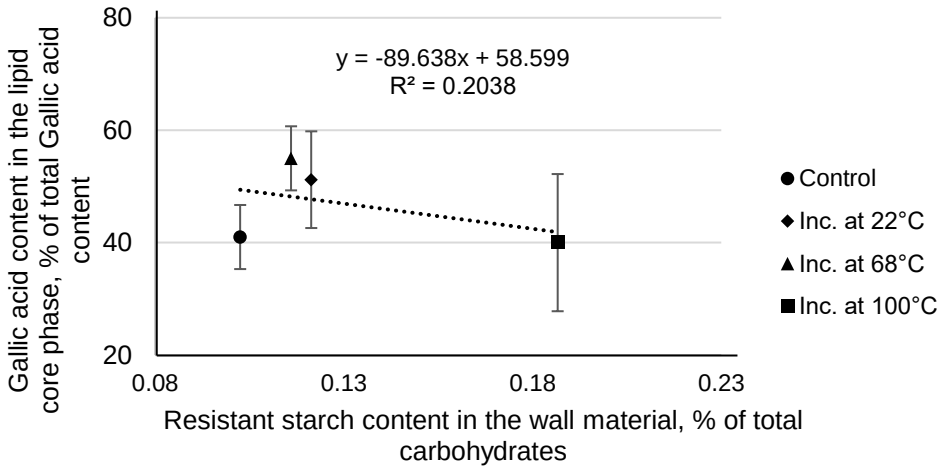

Figure 1. Gallic acid content in the produced capsules depending on the resistant starch concentration. Where, Inc. – incubation.

In the produced capsules, GA was approximately evenly distributed between core and shell fractions, with little remains on the capsule surface (Fig. 2). It is a result of GA diffusion during O/W emulsion preparation, when O phase was mixed with W right before mixture feeding into the spray dryer. It is possible that increase in PGPR concentration or addition of another emulsifier could cause better system stabilization and decrease diffusion and increase GA content in the lipid phase. In this case, bigger portion of lipid phase would be concentrated on the O/W phase surface, blocking GA migration by stronger hydrophilic polarity.

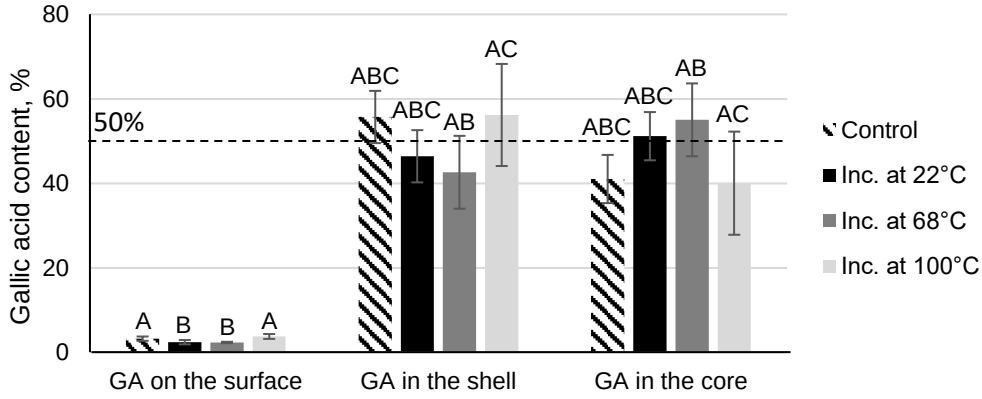

Figure 2. Distribution of gallic acid in different capsule fractions. The data is presented as a mean (n = 9); similar uppercase letters indicate no significant difference among samples of the same fraction ($P \leq 0.05$). Where, GA – gallic acid; Inc. – incubation.

Holser (2013) showed that ferulic acid, another phenolic compound with antioxidative properties, encapsulated in lipid matrix capsules was stable during 3 months storage at ambient conditions. In another study, lipid nanoparticles placed in water were able to stabilize (-)-epigallocatechin-3-gallate during 4 weeks period while (-)-epigallocatechin-3-gallate solubilized in water exhibited 100% degradation within 4 hours period (Barras et al., 2009). Solid lipid fraction, used in present study, had a melting point approximately of ≈50 °C. This allows to conclude that GA in produced capsules is well protected from environmental factors. It is not only encapsulated in the hydrophobic core, but also surrounded by additional MS shell that should protect melted lipids from leakage and capsule agglomeration, in case of capsule storage at temperature that exceeds 50 °C.

Fig. 2 shows that big part of total encapsulated GA had been diffused into the water phase during sample preparation period and was fixed in the MS matrix. In present study used commercial MS is known for its great encapsulation properties and ability to mask undesirable odours and flavours of encapsulated substance, including phenolics (Spinelli et al., 2016). This means that GA in the shell can be considered as shielded, till capsule will come in contact with aqueous phase that will dilute shell wall material or cause GA diffusion from MS phase into the surrounding aqueous phase.

Based on discussed factors, it can be assumed that produced capsules could be added to different types of food products (ground meat or fish, or dough) in order to release phenolics from the shell wall to the surrounding media. Released phenolics could serve as antioxidants for the food product itself. If there will be no temperature rises combined with intensive mixing that would break integrity of lipid core (as in sausage production), lipid phase phenolics will remain fixed in the lipid phase and protected till will come into the human digestive system. Wada & Fang (1992) had shown that phenolics are able to inhibit polyunsaturated fatty acid oxidation in frozen-crushed bonito meat. In whole and ground meat products, phenolics were effective in retarding rancid odour and flavour, and colour changes (Shah et al., 2014). Better retention of colour and protection of lipids against peroxidation due to the addition of phenols had been observed also in irradiated meats (Kanatt et al., 2005). Similar results were acquired for minced horse mackerel (Sabeena Farvin et al., 2012). In case of human nutrition, phenolics are known to have multiple positive effects on the health by alleviating oxidative stress (Singh & Rajini, 2004; Singh & Rajini, 2008).

Only small percentage of total GA had been exposed on the capsule surface: 2.4 ± 0.5% for samples incubated at 22 °C, 2.3 ± 0.2% for samples incubated at 68 °C, 3.8 ± 0.6% for samples incubated at 100 °C, against 3.3 ± 0.5% for control sample. This amounts can be considered as permanent loses, bringing the total encapsulation efficiency to 97.1 ± 0.4%. In present study, particles with mean size form 23.1 ± 0.5 to 30.7 ± 2.1 μm were produced (Table 1), resulting in fine white powder that should not be visible and sensible in case of adding it to the ground meat/fish products or dough.

Table 1. Particle size and distribution of microcapsules incubated at different temperatures

Parameter	Control	22 °C	68 °C	100 °C
Median, μm	18.7 ± 1.4	21.4 ± 0.8	22.3 ± 1.0	20.2 ± 1.6
Mean, μm	23.1 ± 0.5	27.7 ± 1.9	30.7 ± 2.1	26.5 ± 3.3
Variance, μm^2	306.9 ± 168.8	615.51 ± 184.6	947.9 ± 105.6	597.5 ± 250.9
Mode, μm	18.6 ± 2.5	21.2 ± 0.0	21.3 ± 0.1	19.5 ± 1.6

Fig. 2 shows GA distributions between different capsule fractions (core, wall, and surface). Statistical analysis showed that GA amounts in the lipid core phases are dependent on the particle mean size (Fig. 3, Table 1). This can be explained by the fact that in present experiment produced particle size is dependent on the lipid droplet size in the O/W emulsion. Bigger lipid droplet can accommodate more GA than small ones. With increase of lipid core size, core-to-shell ration also increases. As a result, the smaller is the shell in proportion to the core, the less GA in proportion to the core GA it can contain.

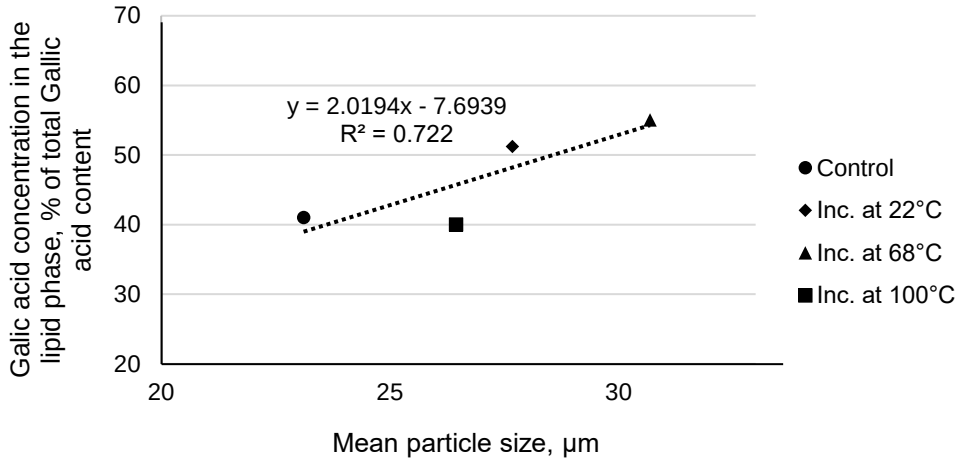

Figure 3. Gallic acid content in the lipid phase of produced capsules depending on the mean particle size (n = 9). Where, Inc. – incubation.

Capsules had been heated at 100 °C for 30 min to test integrity of the shell, and visually no melted lipid leakage or capsule agglomeration had been detected. In combination with previously discussed aspects, it can be concluded that produced capsules can be considered as stable and can be stored at ambient conditions. As an example, in case of uncontrolled temperature increase, capsule shell will maintain its integrity and particles will not stick together lowering powdered product quality, as it can happen during capsule transportation in the summer time without specialized cooling equipment. As starch is not a reactive material, capsules does not require specific storage conditions and package material, except protection from the excess moisture to prevent dilution of the shell and GA diffusion/dissolution. This minimises storage expenses and overall potential produced capsule price.

CONCLUSIONS

In present study, three phase encapsulation system (solid lipid core, containing water soluble phenolic compound, surrounded with modified starch shell wall) had been developed in a single spray drying process for GA encapsulation. Produced RS amounts in the maltodextrin were too small to impact encapsulation process. Present capsule structure can be considered as stable for the long term storage at ambient conditions. Real storage study should be conducted to determine optimal storage conditions and time

limits. Due to diffusion processes during capsule formation, GA was evenly distributed between core and shell wall materials. Depending on the capsule application, this fact can be considered as negative (if GA content in the shell wall is considered as loses) or as positive (assuming that shell wall GA can be released in the surrounding media in order to provide antioxidative and antiradical protection for the mentioned media). Produced capsules have a great potential for application in food production, as GA can be replaced with any another water or oil soluble biologically active compound to produce stable encapsulation system for protection of the active compound and masking its possible undesirable sensory properties. In case of hydrophilic compound encapsulation, it is assumed that chosen compound will be evenly distributed between capsule phases and, after adding capsules to the moisture rich food system, will partially participate in the antioxidative protection of the chosen food system. Remaining active compound could remain protected till it will reach digestive system. Present work makes a basis for future studies on natural plant phenolic compound encapsulation and application in food industry.

REFERENCES

Acosta, E. 2009. Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Current Opinion in Colloid & Interface Science* **14**, 3–15.

Anandharamakrishnan, C. & Padma Ishwarya, S. 2015. *Spray drying techniques for food ingredient encapsulation*. John Wiley & Sons, Chichester, 294 pp.

Arshadey, R. 1999. *Microspheres, microcapsules and liposomes*. United Kingdom, London: Citus Books (v.1), 550 pp.

Barras, A., Mezzetti, A., Richard, A., Lazzaroni, S., Roux, S., Melnyk, P., Betbeder, D. & Monfilliette-Dupont, N. 2009. Formulation and characterization of polyphenol-loaded lipid nanocapsules. *International Journal of Pharmaceutics* **379**, 270–277.

Desai, K.G.H. & Park, H.J. 2005. Recent developments in microencapsulation of food ingredients. *Drying Technology* **23**, 1361–1394.

Dimantov, A., Kesselman, E. & Shimoni, E. 2004a. Surface characterization and dissolution properties of high amylose corn starch-pectin coatings. *Food Hydrocolloids* **18**, 29–37.

Dimantov, A., Greenberg, M., Kesselman, E. & Shimoni, E. 2004b. Study of high amylose corn starch as food grade enteric coating in a microcapsule model system. *Innovative Food Science and Emerging Technologies* **5**, 93–100.

Donsi, F., Annunziata, M., Sessa, M. & Ferrari, G. 2011. Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *LWT- Food Science and Technology* **44**, 1908–1914.

Donsi, F., Senatore, B., Huang, Q. & Ferrari, G. 2010a. Development of novel pea protein-based nanoemulsions for delivery of nutraceuticals. *Journal of Agricultural and Food Chemistry* **58**, 10653–10660.

Donsi, F., Sessa, M. & Ferrari, G. 2010b. Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *Journal of Biotechnology* **150**, S67–S67.

Dordevic, V., Balanc, B., Belscak-Cvitanovic, A., Levic, S., Trifkovic, K., Kalusevic, A., Kostic, I., Komes, D., Bugarski, B. & Nedovic, V. 2015. Trends in encapsulation technologies for delivery of food bioactive compounds. *Food Engineering Reviews* **7**, 452–490.

Eerlingen, R.C., Crombez, M. & Delcour, J.A. 1993. Enzyme-Resistant Starch. I. Quantitative and qualitative influence of incubation time and temperature of autoclaved starch on resistant starch formation. *Cereal Chemistry* **70**, 339–344.

Eltayeb, M., Bakhshi, P.K., Stride, E. & Edirisinghe, M. 2013. Preparation of solid lipid nanoparticles containing active compound by electrohydrodynamic spraying. *Food Research International* **53**, 88–95.

Ersus, S. & Yurdagel, U. 2007. Microencapsulation of anthocyanin pigments of black carrot (Daucus carota L.) by spray drier. *Journal of Food Engineering* **80**, 805–812.

Fang, Z. & Bhandari, B. 2010. Encapsulation of polyphenols – a review. *Trends in Food Science & Technology* **21**, 510–523.

Sabeena Farvin, K.H., Grehsen, H.D. & Jacobsen, C. 2012. Potato peel extract as a natural antioxidant in chilled storage of minced horse mackerel (*Trachurus trachurus*): Effect on lipid and protein oxidation. *Food Chemistry* **131**, 843–851.

Friedman, M. 1997. Chemistry, biochemistry, and dietary role of potato polyphenols. A review. *Journal of Agriculture and Food Chemistry* **45**, 1523–1540.

Gibbs, B.F., Kermasha, S., Alli, I. & Mulligan, C.N. 1999 Encapsulation in the food industry: a review. *International Journal of Food Science and Nutrition* **50**, 213–224.

Garti, N. & McClements, J. 2012. *Encapsulation technologies and delivery systems for food ingredients and nutraceuticals*. Woodhead Publishing, Oxford, 612 pp.

Herman, J. & Remon, J.P. 1989. Modified starches as hydrophilic matrices for controlled oral delivery. II. In-vitro drug-release evaluation of thermally modified starches. *International Journal of Pharmaceutics* **56**, 65–70.

Holser, R. 2013. Stability of lipid encapsulated phenolic acid particles. *Recent Research Developments in Lipids* **9**, 47–52.

Kaderides, K., Goula, A.M. & Adamopoulos, K.G. 2015. A process for turning pomegranate peels into a valuable food ingredient using ultrasound-assisted extraction and encapsulation. *Innovative Food Science and Emerging Technologies* **31**, 204–215.

Kanatt, S.R., Chander, R., Radhakrishna, P. & Sharma, A. 2005. Potato peel extract – a natural antioxidant for retarding lipid peroxidation in radiation processed lamb meat. *Journal of Agriculture and Food Chemistry* **53**, 1499–1504.

Krishnaiah, D., Bono, A., Sarbatly, R., Nithyanandam, R. & Anisuzzaman, S.M. 2012. Optimisation of spray drying operating conditions of *Morinda citrifolia* L. fruit extract using response surface methodology. *Journal of King Saud University – Engineering Sciences* **27**, 26–36.

Lima, G.P.P., Vianello, F., Correa, C.R., da Silva Campos, R.A. & Borguini, M.G. 2014. Polyphenols in fruits and vegetables and its effect on human health. *Food and Nutrition Science* **5**, 1065–1082.

Mccleary, B.V., Mcnally, M., Monaghan, D. & Mugford, D.C. 2002. Measurement of a-Amylase Activity in White Wheat Flour, Milled Malt, and Microbial Enzyme Preparations, Using the Ceralpha Assay: Collaborative Study. *Journal of AOAC International* **85**, 1096–1102.

McClements, D.J., Decker, E.A. & Weiss, J. 2007. Emulsion-based delivery systems for lipophilic bioactive components. *Journal of Food Science* **72**, 109–124.

Mukherjee, S., Ray, S. & Thakur, R.S. 2009. Solid lipid nanoparticles: a modern formulation approach in drug delivery system. *Indian Journal of Pharmaceutical Science* **71**, 349–358.

Munin, A. & Edwards-Levy, F. 2011. Encapsulation of natural polsyphenolic compounds; a review. *Pharmaceuticals* **3**, 793–829.

Pasrija, D., Ezhilarasi, P.N., Indrani, D. & Anandharamakrishnan, C. 2015. Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. *LWT - Food Science and Technology* **64**, 289–296.

Ragazzi, E. & Veronese, G. 1973. Quantitative analysis of phenolic compounds after thin layer chromatography. *Journal of Chromatography* **77**, 369–375.

Resistant starch in starch samples and plant materials: AACC method 32-40.01. (2002) Available at: http://methods.aaccnet.org/summaries/32-40-01.aspx, 22 March 2018.

Robert, P., Gorena, T., Romero, N., Sepulveda, E., Chavez, J. & Saenz, C. 2010. Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying. *International Journal of Food Science and Technology* **45**, 1386–1384.

Saikia, S., Mahnot, N.K. & Mahanta, C.L. 2015. Optimisation of phenolic extraction from Averrhoa carambola pomace by response surface methodology and its microencapsulation by spray and freeze drying. *Food Chemistry* **171**, 144–152.

Sansone, F., Mencherini, T., Picerno, P., D'Amore, M., Aquino, R.P. & Lauro, M.R. 2011. Maltodextrin/pectin microparticles by spray drying as carrier for nutraceutical extracts. *Journal of Food Engineering* **105**, 468–476.

Shah, M., Bosco, S. & Mir, S. 2014. Plant extracts as natural antioxidants in meat and meat products. *Meat science* **98**, 21–33.

Shieber, A. & Saldana, M.D.A. 2009. Potato peels: a source of nutritionally and pharmacologically interesting compounds – a review. *Food* **S3**, 23–29.

Sievert, D. & Pomeranz, Y. 1989. Enzyme-resistant starch. I. Characterization and evaluation by enzymic, thermosanalytical, and microscopic methods. *Cereal Chemistry* **66**, 342–347.

Singh, N. & Rajini, P.S. 2004. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry* **85**, 611–616.

Singh, N. & Rajini, P.S. 2008. Antioxidant-mediated protective effect of potato peel extract in erythrocytes against oxidative damage. Chemico-Biological Interactions **173**, 97–104.

Spinelli, S., Conte, A. & Del Nobile, M.A. 2016. Microencapsulation of extracted bioactive compounds from brewer's spent grain to enrich fish-burgers. *Food and Bioproducts Processing* **100**, 450–456.

Wada, S. & Fang, X. 1992. The synergistic antioxidant effect of rosemary extract and α-tocopherol in sardine oil model system and frozen-crushed fish meat. *Journal of Food Processing and Preservation* **16**, 263–274.

Wilsona, R., van Schieb, B.J. & Howesa, D. 1998. Overview of the preparation, use and biological studies on polyglycerol polyricinoleate (PGPR). *Food and Chemical Toxicology* **36**(9–10), 711–718.

Wolfe, L.A., Roberts, R.F. & Coupland, J.N. 2015. Encapsulation of aqueous components in solid fat beads: studies of a model dye and a probiotic culture. *Journal of the American Oil Chemists' Society* **92**, 87–94.

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Comparison of power consumption of a two-roll mill and a disc mill

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Abstract. Grinding or milling is often used process, for example in the production of feed, grinding of malt in beer production, grinding of cereals on flour, etc. In order to optimize the energy intensity of the whole production process, it is necessary to know the energy consumption of individual processes. The grinding of malt influences the mashing process, the drawing-off and the boiling yield. Correct grain grinding makes the endosperm available for enzyme and physicochemical reactions during wort production. Husks affect the drawing-off process. Two-, four- or six-roll mills, in some cases a hammer mill or disc mill, are most commonly used for grinding of malt. Power consumption was monitored when light, Munich, caramel and coloring malt were grinding. A two-roll mill and a disc mill with engine speeds of 1,500 rpm and 2,800 rpm were used for grinding. The gap between the mill rollers and the mill disks was set to 0.4 mm. The fineness of the grinding was evaluated for all types of malt on all used equipment. The energy intensity of the grinding was correlated on 1.0 kg of malt and then compared.

Key words: malt, grinder, dispersant, electrical power, mechanical power.

INTRODUCTION

At present, the process of production or processing also tracks the energy intensity of processes, as it is one of the ways to reduce production costs (Kunze, 2010; Chládek et al., 2013).

Traditional procedures may not always be the least energy consuming, and it may sometimes be appropriate to adjust the production process with respect to energy consumption while maintaining the other requirements.

Beer production is demanding energy consumption. Throughout the manufacturing process, there are a number of energy-intensive technological processes - malting, malting, boiling, cooling and more. The energy intensity of breweries depends on their size, equipment level and the introduction of austerity measures. As has been said, one of the processes that consume energy is malting.

Malt grinding is a mechanical and seemingly simple process that fundamentally influences the process of mashing, drawing-off and the brewing yield. Mechanical breakdown of malt grain is necessary to make extractive substances available and accelerate their dissolution.

Malt grain consists of husks and endosperm. The endosperm is composed mainly of starch, glycids and proteins. Correct grain grinding makes the endosperm available for enzymatic and physical-chemical reactions in the wort production. Husks affect the process of drawing-off. The requirements on fineness of grinding vary depending on the process of drawing-off used, which is influenced by the type of beer produced.

For filtration vat, the grist should have the most thoroughly grinded, at least damaged husks, a low proportion of coarse semolina and a high proportion of fine semolina. These requirements are most often met by malt mills (roller mills) which contain two, four or six milling rollers (Kosař & Procházka 2000; Basařová, 2010).

The grist for mash filter should have, on the contrary, well-milled husks. For grinding for mash filter is usually preferred hammer mill or a disc mill (Chládek, 2007; Vaculík et al., 2013).

In this work, the energy intensity of grinding on a two-roll mill and disc mill is monitored. It builds on the previous work, where a sieve analysis of the malt grist was carried out for these grinding devices.

MATERIAL AND METHODS

The energy consumption of grinding of different types of malt when using different devices was measured. It was used two-roller mill KVM 130/150 (Fig. 1), made by KVM Uničov, Czech Republic, with maximum performance 250.0 kg hour^{-1} and two electric motors, each with input 2.05 kW. Grinding gap width was set to 0.4 mm. Roll speed was 250 rpm.

The second device was disc mill Skiold SK 2500 (Fig. 2), made by Skiold Sæby, Denmark**.** To this disc mill is connected a drive dynamometer type DS 546-4/V made by Mezservis Vsetín, Czech Republic, with output 26.0 kW. Gap width between the grinding discs was set to 0.4 mm. Disc mill was used with 1,500 rpm and 2,800 rpm.

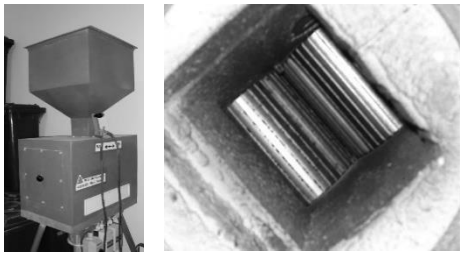

Figure 1. Two-roll mill KVM 130/150.

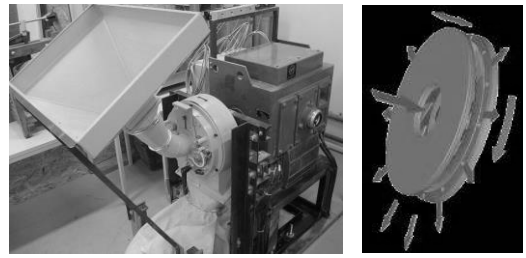

Figure 2. Disc mill Skiold SK 2500.

Both grinding devices, on which the measurement was made, are part of he laboratories that fall under the Department of Technological Equipment of Buildings of Faculty of Engineering at the Czech University of Life Sciences Prague.

Dry grinding of malt was made on both devices and four kinds of malt were processed (Table 1).

Table 1. Basic properties of malt

Malt type	Producer	Malt colour (EBC)	Humidity (%)
Light barley malt (pilsner type)	Soufflet, Hodonice, Czech Republic	3–4	5.1
Barley malt called Munich	Heinz Weyermann, Bamberg, Germany	15	4.8
Barley malt called colouring	Heinz Weyermann, Bamberg, Germany	1,100–1,300	4.1
Barley malt called caramel	Heinz Weyermann, Bamberg, Germany	150–200	4.5

Two-roll mill

Samples of malt weighing 3.0 kg were used for individual measurements. The Nanovip Plus network analyzer was used to measure the values.

The first measurement took place idling speed and then was open a flap blocking the supply of malt between the cylinders. Values were recorded at two second intervals. From the measured values of current, voltage and power factor, the electrical power is calculated using the following formula (Pokorný, 2003; Feynman et al., 2011).

$$P_e = U \cdot I \cdot \cos\ \varphi \tag{1}$$

where P_e – electrical power (W); U – voltage (V); I – current (A); $cos\ \varphi$ – power factor (-).

Disc mill

Samples of 3.0 kg malt were used for individual measurements. A computer with original software from MEZ Vsetín was used to measure the values. Due to the fact that the device allows changing the engine speed, two values were chosen for the given measurements, namely 1,500 rpm and 2,800 rpm.

The first measurement took place idling speed and then was open a flap blocking the supply of malt between the discs.

From the measured speed and torque values, the mechanical power is calculated using the following formula (Čadil, 1976; Feynman et al., 2011).

$$P_m = M \cdot 2\pi \cdot \frac{n}{60} \tag{2}$$

where P_m – mechanical power (W); M – torque (N.m); n – speed (rpm).

RESULTS AND DISCUSSION

All obtained samples of malt grist were categorized on Pfungstadt sifter. Using a roller mill, the obtained grist was the coarsest for all types of malt, the finest grist was obtained using a dispersant at 2,800 rpm (Vaculík et al., 2010; Smejtková et al., 2016).

The course of power in dependence on time for individual devices and malt samples is shown in the Figs 3–6.

Fig. 3 illustrates the energy consumption over time when grinding a light malt using a two-roll mill, disc mill 1.500 rpm and disc mill 2.800 rpm. The highest values of energy consumption were achieved for the disc mill 2.800 rpm, but grinding took off the shortest time.

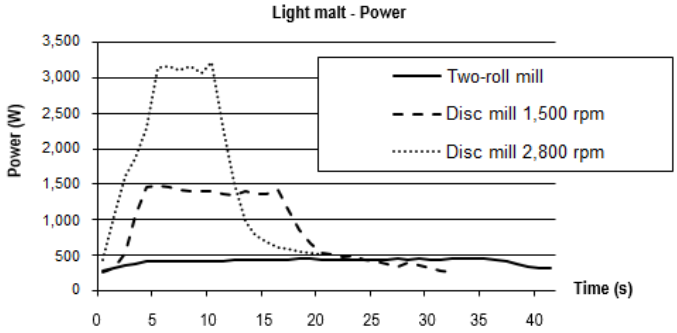

Figure 3. The course of power when grinding light malt.

Fig. 4 illustrates the energy consumption over time when grinding Munich malt. Compared to the light malt grinding, the disc mill 2.800 rpm has a lower power consumption, but the highest between used equipment.

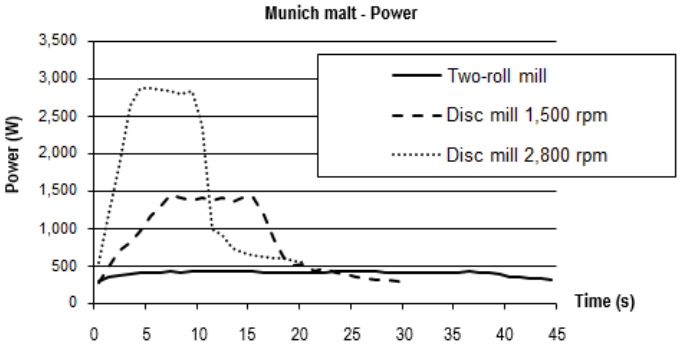

Figure 4. The course of power when grinding Munich malt.

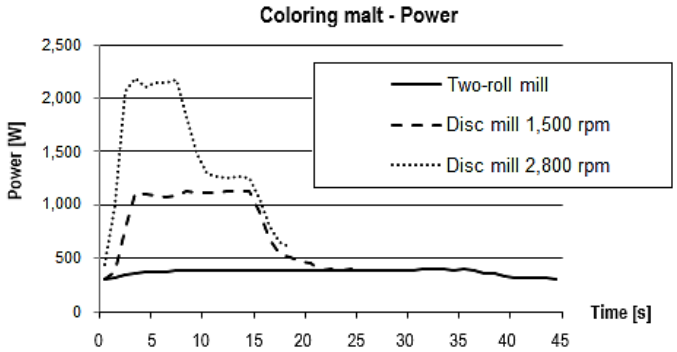

Figure 5. The course of power when grinding colouring malt.

Fig. 5 illustrates the energy consumption over time when grinding coloring malt. Energy consumption is the lowest when grinding coloring malt, especially when using a disc mill 2,800 rpm. This is because the coloring malt is very fragile.

Fig. 6 illustrates the energy consumption over time when grinding caramel malt. Compared to the previous measurement, the energy consumption increased while using the disc mill 1,500 rpm.

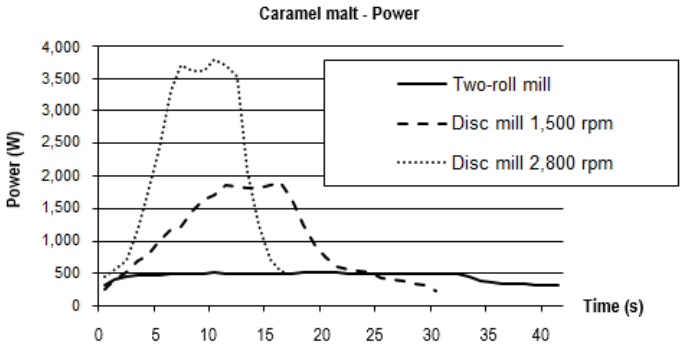

Figure 6. The course of power when grinding caramel malt.

The results obtained correspond to those of other authors (Boehm et al., 2015; Salonitis, 2015). Also, when using similar grinding equipment and grinding other materials, the results are comparable (Chohan et al., 2009; Mohd Rozalli et al., 2015).

In the Fig. 7 is shown energy consumption per 1.0 kg of malt for all used devices. From average power needed to grinding, power idling speed was subtracted. This value was multiplied by the time needed to grinding of each sample and related to 1.0 kg of sample.

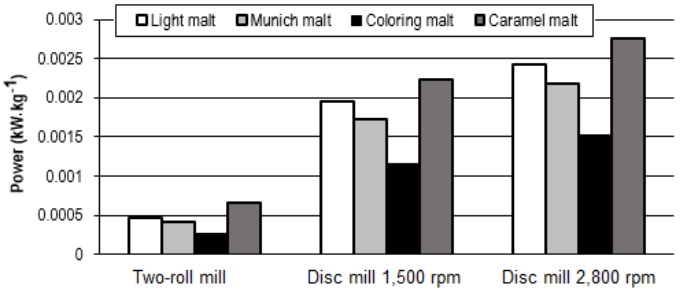

Figure 7. Energy consumption per 1 kg of malt.

Idling speed, for the two-roll grinder is electrical power 321 W, for the disc mill 1,500 rpm is mechanical power 220 W and for the disc mill 2,800 rpm is mechanical power 425 W.

The most energy consumption of the compared devices has the disc mill with 2,800 rpm for all types of malt. The least demanding is the two-roll grinder. Of the used malt, the highest demand on energy consumption of grinding has caramel malt, followed by light malt, Munich malt and the least demanding coloring malt.

Used grinding devices also differed in the speed of grinding. Fig. 8 shows a comparison of the time needed to grinding of 1.0 kg of malt. For the disc mill, the time required for grinding is decreasing as the number of revolutions increases.

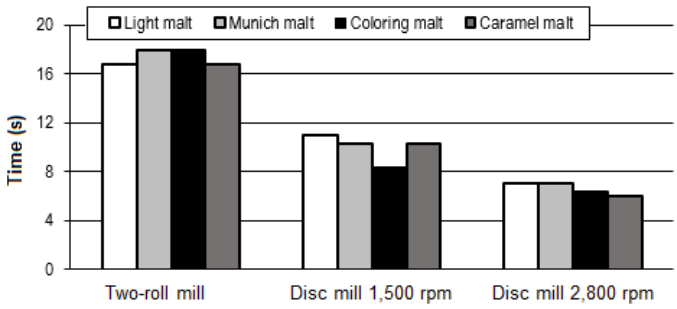

Figure 8. Grinding time of 1 kg of malt.

From these values, we can approximately determine the capacity of individual grinding devices for each type of malt (Table 2).

Table 2. Capacity of the grinding device

	Efficiency		
Malt	Two-roll mill (kg hour^{-1})	Disc mill (1,500 rpm) (kg hour^{-1})	Disc mill (2,800 rpm) (kg hour^{-1})
Light malt	214	327	514
Munich malt	200	349	514
Coloring malt	200	434	571
Caramel malt	214	349	600

CONCLUSION

The paper presents the results of the measurement of the required power of a two-roll mill and a disc mill 1,500 rpm and 2,800 rpm for the grinding of light, Munich, caramel and coloring malt.

The rotational speed of the disc mill influences the fineness of grinding, energy consumption and grinding time. The higher the speed, the shorter the grinding time and the finer the grist, but the higher the energy consumption. Regardless of the speed used, the disc mill has a higher energy consumption than a two-roll mill, but the grinding time is shorter and the malt finer.

Regardless of the use of the grinding device, the highest energy consumption is used to grind caramel malt, followed by light malt, Munich malt, and the least demanding is coloring malt.

Due to the short period of grinding of small malt samples, the performance values expressed in kg h^{-1} should be considered as indicative only. A small inaccuracy in the measurement is a larger deviation when referring to time 1.0 hour.

For a two-roll mill, the performance in grinding different types of malt is not much different and is significantly lower compared to the disc mill. For the disc mill 1,500 rpm, the highest performance was achieved when the coloring malt was grinded.

Disc mill 2,800 rpm has the highest performance of the monitored devices, the performance of grinding of caramel malt is higher than that of other malt.

The subject of the next measurement will be the monitoring of the energy consumption of the four-roll and six-roll grinders and the comparison of the obtained results with the results of this work. Based on the evaluation of all measured quantities, it will be possible to select a suitable type of grinder, taking into account its energy consumption.

REFERENCES

Boehm, A., Meissner, P. & Plochberger, T. 2015. An energy based comparison of vertical roller mills and tumbling mills. *International Journal of Mineral Processing* **136**, 37–41.

Basařová, G. 2010. Pivovarství: teorie a praxe výroby piva. 1st edition. Publisher: Vydavatelství VŠCHT. Prague. 863 pp. (in Czech).

Čadil, F. 1976. Elektrické pohony: učebnice pro elektrotechn. fak. 1st edition. Publisher: SNTL (Mír 1). Prague. 558 pp. (in Czech).

Chládek, L. 2007. Pivovarnictví. 1st edition. Publisher: Grada. Prague. 207 pp. (in Czech).

Chládek, L., Vaculík, P., Přikryl, M., Vaculík, M. & Holomková, M. 2013. Impact of malt granulometry on lauter proces. In *5th International Conference on Trends in Agricultural Engineering 2013, TAE 2013 03.09.2013*, Prague. Prague: Czech University of Life Sciences Prague, pp. 244–248.

Chohan, P. & Garson, C.A. 2009. Performance of a tandem of Bundaberg two-roll mills. *International Sugar Journal* **111**(1330), 650–655.

Feynman, R.P., Leighton, B.R. & Sands, M. 2011. *The Feynman Lectures on Physics, boxed set: The New Millennium Edition.* 1st edition. Publisher: Basic Books. 1552 pp.

Kosař, K. & Procházka, S. 2000. Technologie výroby sladu a piva. 1st edition. Publisher: Výzkumný ústav pivovarský a sladařský, Prague. 398 pp. (in Czech).

Kunze, W. 2010. *Technology Brewing and Malting*. 4th updated English Edition. Berlin: Versuchs- und Lehranstalt für Brauerei in Berlin (VLB), 1047 pp. (in German).

Pokorný, K. 2003. Elektrotechnika I. 1st edition. Publisher: Česká zemědělská univerzita v Praze, Technická fakulta, NAROMA. Prague. 172 pp. (in Czech).

Mohd Rozalli, N.H., Chin, N.L. & Yusof, Y.A. 2015. Grinding characteristics of Asian originated peanuts (Arachishypogaea L.) and specific energy consumption during ultra-high speed grinding for natural peanut butter production. *Journal of Food Engineering* **152**. pp 1–7.

Salonitis, K. 2015. Energy efficiency assessment of grinding strategy. *International Journal of Energy Sector Management* **9**(1), pp. 20–37.

Smejtková, A., Vaculík, P., Přikryl, M. & Pastorek, Z. 2016. Rating of malt grist fineness with respect to the used grinding equipment. *Research in Agricultural Engineering (Zemědělská technika)* **62**(3), 141–146.

Vaculík, P., Malaťák, J. & Chládek, L. 2010. Recent trends in the processing of construction and demolition waste. In *4th International Conference on Trends in Agricultural Engineering 2010, TAE 07.09.2010*, Prague. Prague: Czech University of Life Sciences Prague, pp. 619–622.

Vaculík, P., Maloun, J., Chládek, L. & Přikryl, M. 2013. Disintegration process in disc crushers. *Research in Agricultural Engineering (Zemědělská technika)* **59**(3), 98–104.

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Single cell protein production from waste biomass: review of various agricultural by-products

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Abstract. Agricultural waste constitutes for most of the manmade waste streams. Processing of biodegradable waste materials ensures the treatment of harmful substances and allows to reduce environmental pollution. In addition, conversion of these waste materials in value-added products makes these recycling methods more economically viable. Single-cell protein is one of the value-added products that can be produced by microbial fermentation of waste materials. In this review various biodegradable agricultural by-products as substrates for production of SCP are categorized and compared.

Key words: single cell protein, waste biomass, agricultural by-products, resource availability, aquaculture, fish feed, fish meal.

INTRODUCTION

The recycling of biodegradable waste into environmentally harmless compounds is one of the bases for waste management. However, simply treating biodegradable waste is no longer considered efficient waste management practice. Depending on the type of waste used, nowadays it is possible to produce a range of value added products that make waste management not only an environmentally friendly endeavour, but also a promising business activity. However, a large proportion of biodegradable waste is currently being processed into products with a relatively low added value, such as biogas (Kost et al., 2013), bioenergy and biofuels (Lipinsky, 1981; Browne et al., 2011). Thanks to the development of various waste recycling technologies, an increasing number of technological solutions are emerging today that provide the opportunity to extract high value-added products such as enzymes (El-Bakry et al., 2015), single-cell oil (Finco et al., 2016), building block chemicals (Werpy & Petersen, 2004; FitzPatrick et al., 2010) and others. One such high-value added product is single-cell protein (SCP).

SCPs are known as dietary single-cell microorganisms whose biomass or protein extracts are derived from pure or mixed microscopic algae, yeasts, mushrooms or bacterial cultures (Anupama & Ravindra, 2000). These microorganisms can be used as protein-rich foods or food ingredients or dietary supplements (Ritala et al., 2017), but they are mainly used as food for human and animal consumption (Ugalde & Castrillo, 2002). SCPs are a good alternative to replacing protein of agricultural origin, since SCP production is not characterized by high water consumption (Mekonnen & Howkstra,

2014), it does not cover large areas of land, does not endanger environmental diversity (Tilman, 1999), does not contribute to climate change and does not produce high greenhouse gas emissions (Vermeulen et al., 2012), as it is the case with agriculture.

To reduce the cost of production of SCP, it is essential to use biodegradable agroindustrial by-products and waste as a source of nutrients for the cultivation of microorganisms. In this context, currently known agricultural waste products suitable for the cultivation of microorganisms synthesizing SCP will be reviewed and compared. Industrial wastes applicable for SCP production will be compared in another review.

In other reviews (Anupama & Ravindra, 2000; Nasseri et al., 2011; Ritala et al., 2017), which summarize the reported findings on the waste products suitable for the cultivation of SCP producing microorganisms, information mainly focuses on the used microorganisms (fungi, bacteria, algae, etc.) and not so much on the properties of the waste products themselves. However, nowadays, for both research and industrial applications, access to various strains of microorganisms is relatively simple, but availability of waste products is very specific for every local economy. Consequently, in order to further facilitate the finding of the most suitable waste products, this review seeks to categorize and describe waste products suitable for the production of SCP.

WASTE TYPES

Finco et al. (2016) described the most suitable waste products for the production of single-cell oils and divided them into four groups: mono and disaccharide-rich waste products; starch rich waste products; glycerol-rich waste products; lignocellulose-rich waste products. This distribution was used as bases for the division of four groups of agricultural wastes. The proposed agricultural waste groups for SCP production are: Mono and disaccharide rich sources; Starch rich sources; Structural polysaccharides rich sources; Protein or lipid rich sources (Fig. 1).

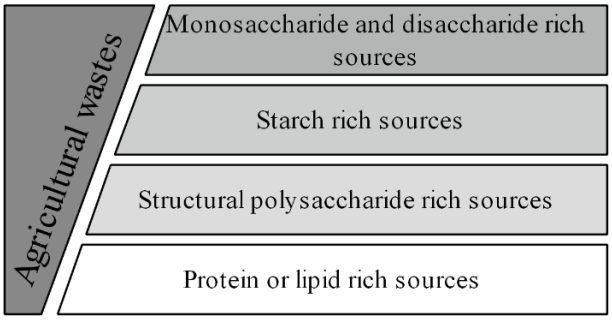

Figure 1. Categorization of agricultural wastes applicable for cultivation of SCP producing microorganisms.

COMPARISION OF WASTE RESOURCES

Monosaccharides and disaccharides rich sources

Monosaccharides and disaccharides rich sources such as molasses, dairy (lactose rich) and fruit processing wastes can be directly processed by microorganisms with good

SCP yields. Consequently, this waste group requires much less pre-treatment prior to its use in the cultivation of microorganisms, which significantly reduces the overall SCP production costs.

Molasses

Molasses is a rich source of carbohydrates suitable for the cultivation of various microorganisms without the need for pre-treatment of the waste material (Kopsahelis et al., 2007; Aggelopoulos et al., 2014). Molasses are mainly a by-product of the production of sugar from sugar cane and sugar beet or by-product from soy protein concentrate production. From all potential sources soy molasses, a by-product of soy protein concentrate (SPC) production, has become more accessible in recent years due to rapid increase in the production of SPC (Deak & Johnson, 2006; Gao et al., 2012). Depending on the extraction method, plant species and plant age, molasses usually contain about 45–60% fermentable carbohydrates, 10% nitrogen compounds, 20% fat and 10% minerals (Kinney, 2003; Gao et al., 2012). The use of molasses in SCP production is determined by its availability, price, composition, and whether it contains impurities of fermentation inhibitors and toxic compounds that could be transferred from culture medium to the final SCP product (Bekatorou et al., 2006; Aggelopoulos et al., 2014).

Dairy waste

Whey and other dairy wastes have high levels of chemical oxygen demand (COD) and biological oxygen demand (BOD), as well as high concentrations of oils and fats and nitrogen and phosphorus compounds (Braio & Taveres, 2007; Singh et al., 2011), which can lead to ecological problems if such wastes enter local environments without prior treatment. From cheese production alone, the global dairy industry generates around 139 million tonnes of whey every year (Ghaly et al., 2007; Yadav et al., 2013; Yadav et al., 2014), of which about 50% are simply dumped in sewage systems or in local water bodies (Ghaly et al., 2007; Yadav et al., 2014). Consequently, the dairy industry needs to develop effective solutions for the processing of whey and other waste products, in order to remove organic and nitrogen compounds from the wastes. Depending on the technology used in milk processing, dairy waste can have high levels of either lactose or protein (Kim & Lebeault, 1981; Singh et al., 2011; Aggelopoulos et al., 2014), therefore whey can be categorized as monosaccharides and disaccharides rich source or protein or lipid rich source (see Tables 1, 4). If dairy waste is high in lactose, these by-products can be used as suitable substrate for SCP production using microorganisms capable of fermenting lactose (Singh et al., 2011).

Fruit waste (simple sugar rich)

The content of fruit processing waste is highly dependent on the type of fruit and the part of the fruit that forms the main mass of the waste. If the waste is mainly whole fruit, then a large amount of monosaccharides and disaccharides will be available in the waste, as it is in the case with banana wastes, where 5 to 30% of harvested bananas are discarded as waste due to export regulations (Baldensperger et al., 1985). A similar situation exists for figs where, due to incorrect transport, storage and market changes, large quantities of figs are not realized, and they need to be disposed of when they begin to deteriorate (Hashem et al., 2014).

Table 1. Monosaccharides and disaccharides rich sources. Recent reports of protein content (% of biomass after fermentation) from mono and disaccharide rich wastes

Mono and disaccharides rich sources	Microorganisms	Protein content (%)	References
Molasses	*Kluyveromyces marxianus*	50.5	Anderson et al., 1988
Soy molasses	*Candida tropicalis*	56.4	Gao et al., 2012
Sugarcane juice	*Phaffia rhodozyma*	-	Fontana et al., 1996
Whey (lactose rich)	*Penicillium cycloplum*	54	Kim & Lebeault, 1981
Cheese whey	*Kluyveromyces marxianus, Candida krusei*	43.4	Yadav et al., 2014
	kefir microorganisms	53.9	Paraskevopoulou et al., 2003
Cheese whey filtrate	*Trichoderma harzianum*	34.2	Sisman et al., 2013
Banana wastes	*Aspergillus niger*	18	Baldensperger et al., 1985
Spoiled date palm fruits	*Hanseniaspora uvarum, Zygosaccharomyces rouxii*	48.9	Hashem et al., 2014

Table 2. Starch rich sources. Recent reports of protein content (% of biomass after fermentation) from starch rich wastes

Starch rich sources	Microorganisms	Protein content (%)	References
Sorghum hydrolysate	*Candida krusei*	47.5	Konlani et al., 1996
Wheat bran	*Rhodopseudomonas gelatinosa*	66.7	Shipman et al., 1975
Rice bran	*Aspergillus flavus*	11.5	Valentino et al. 2016
Starch hydrolysate	*Fusarium graminearum*	-	Anupama & Ravindra, 2000
Starch	*Schwanniomyces alluvius*	52.8	Calleja et al., 1986
	Schwanniomyces occidentali	-	Deibel et al., 1988
Leaf juice	*Saccharomyces cerevisiae*	45.6	Chanda & Chakrabarti, 1996
	Torula utilis	54.3	
	Candida lipolytica	50.5	

If fruit processing waste mainly consists of outer and inner shells, peels and seeds, as it is the case with juice producing wastes (Bhalla & Joshi, 1994; Scerra et al., 1999; De Gregorio et al., 2002), then there will be more fibres and hence the waste can be categorized as structural polysaccharides rich sources (see subsection 3.3.4. and Table 3).

Starch rich sources

Starch rich waste products, such as grains and tuber residues, account for a large proportion of agricultural waste. Starch rich substrates need to be hydrolysed to monosaccharides prior to use them in cultivation, which may increase the cost of production of SCP, if microorganisms that are capable of dissolving and metabolising starch are not used in fermentation. However, expenses can be reduced if associative fermentation is ensured, where amylolytic microorganisms with SCP synthesizing microorganisms are used in tandem (Calleja et al., 1986; Deibel et al., 1988). In this way amylolytic microorganisms digest starch and provide SCP producing microorganisms with the simple carbohydrates they require.

Bran

Bran is a by-product of grain processing, which is commonly used in oil extraction, animal feed or as a food additive (Hanmoungjai et al., 2000; Valentino et al., 2016). In general, all types of grain bran have a high content of starch, fibre and protein (Shipman et al., 1975; Konlani et al., 1996) and bran is also a good source for lipids, iron, vitamin B, phenolic acid, phytosterol and antioxidants (Godber & Juliano, 2004; Kahlon, 2009; Valentino et al., 2016). Consequently, bran should be used as a substrate for SCP production only if it is not possible or it is economically unprofitable to use bran in animal or human consumption.

If bran is deoiled, then during this process starch content in the bran is significantly reduced, which leaves only proteins and fibres in the by-product (Revinder et al., 2003); therefore, use of starch fermenting microorganisms in deoiled bran is no longer effective and it is necessary to use cellulolytic microorganisms instead (see Table 3).

Table 3. Structural polysaccharides rich sources (agricultural waste). Recent reports of protein content (% of biomass after fermentation) from structural polysaccharides rich wastes

Structural polysaccharides rich sources	Microorganisms	Protein content (%)	References
Corn cobs	*Aspergillus niger*	30.4	Singh et al., 1991
Maize stalk Cotton stalk	*Aspergillus niger*, *Sporotrichum pulverulentum*	-	Anupama & Ravindra, 2000
Wheat straw	*Pleurotus florida*	62.8	Ahmadi et al., 2010
Rice bran (deoiled)	*Aspergillus oryzae*	57	Revinder et al., 2003
Soy bean hull	*Bacillus subtilis*	12.3	Wongputtisin et al., 2014
Potato starch processing waste (cellulose rich)	*Bacillus licheniformis*	38.2	Liu et al., 2014
	Bacillus pumilus, Candida utilis, Aspergillus niger	46.1	Liu et al., 2013
Sugarcane bagasse	*Pleurotus eryngii*	-	Zadrazil & Puniya, 1995
	Candida tropicalis	31.3	Pessoa et al., 1996
Beet-pulp	*Trichoderma reesei, Kluyveromyces marxianus*	54	Ghanem, 1992
Apple pomace	*Aspergillus niger*	20	Bhalla & Joshi, 1994
Citrus pulp (pectin rich)	*Trichoderma viride*	31.9	De Gregorio et al., 2002
Citrus fruit peel	*Penicillium roquefort*	5.7	Scerra et al., 1999
Poltry manure (fibre rich)	*Pseudomonas fluorescens*	59.1	Shuler et al., 1979
Poultry litter (fibre rich)	*Candida utilis*	29	Jalasutram et al., 2013
Brewery's spent grains hemicellulosic hydrolysate	*Debaryomyces hansenii*	31.8	Duarte et al., 2008
Pawn shell waste	*Candida species*	70.4	Rhishipal & Philip, 1998
	Pichia kudriavzevii	40	Revah-Moiseev & Carroad, 1981

Leaf juice

Deproteinized leaf juice (DIJ) is a vegetable protein production by-product from leaf juice. This waste product has high COD and BOD and low pH levels. Consequently, DIJ discharges without prior treatment can cause damage to the environment (Chanda et al. 1984; Pirie 1987; Chanda & Chakrabarti, 1996). Thus, microbiological pre-treatment of DIJ is desirable and in combination with SCP production, this treatment may also

serve as a good business perspective, since SCP yields reported so far on using DIJ as substrate are high (see Table 2). The amount of starch and other substances in DIJ are affected mostly by used plant species and applied extraction methods for production of vegetable protein. The starch content in leaves of legumes, peas and beans (*Papilionoideae* family) is high (Pirie, 1987), but in general, other carbohydrates (both monosaccharides and polysaccharides), amino acids, fats, vitamins and minerals are found in the DIJ as well (Pirie, 1987; Chanda & Chakrabarti, 1996; Choi & Chung, 2003).

Structural polysaccharides rich sources (agricultural waste)

Lignocellulosic agricultural waste is the most widely available waste in the world. According to the International Grains Council, in 2015, 737 million tonnes of wheat, 984 million tonnes of maize and 474 million tonnes of rice (IGC, 2018) were harvested globally. Rice, wheat and corn straw-to-grain ratio varies from 0.7 to 1.7 (Skidmore, 1988; Nelson, 2002; NLA, 2013), which means that straw and stover volumes are usually equal or greater than actual produced grain volumes. This alone indicates that cereal straws are available in enormous quantities in virtually all regions of the world where crops are grown. Consequently, the use of these residues in the production of SCP is highly desirable, both economically and ecologically. However, the use of lignocellulosic agricultural waste is much more complicated than using starch or simple sugar-rich waste because lignocellulose is more difficult to process, and lignocellulosic substrates need to be subjected to extensive mechanical and chemical or biochemical pre-treatments, which greatly increase the total cost of production.

Lignocellulose essentially consists of 30–56% cellulose, 3–30% lignin, 10–24% hemicellulose and 3–7.2% protein, but its applicability, for example in animal feed, is limited due to low digestibility and low protein content (Ahmadi et al., 2010).

In order to reduce the costs associated with the use of straw, researchers have explored the possibilities of using cellulolytic microorganisms that are able to delignify and ferment lignocellulose-rich waste materials (Singh et al., 1991; Valmaseda et al., 1991; Vares et al., 1995; Agosin et al., 1999; Ahmadi et al., 2010; Brijwani & Vadlani, 2011).

Soybean hull

Soybean hull (SBH) is an agricultural by-product from the extraction of soybean oil and production of soybean meal (Zervas et al., 1998). SBH accounts for about 8–10% of the total soybean mass (Wongputtisin et al., 2014), and given the fact that around 350 million tonnes of soybeans (IGC, 2018) are harvested annually globally, then the amount of available SBH is significant. In small amounts, SBH is used to regulate the protein content of soy bean meal used as animal feed (Wongputtisin et al., 2014). SBH is suitable and also used as a source of fibre for ruminants, but unprocessed SBH cannot be used as feed for monogastric animals as they cannot digest cellulose and hemicellulose compounds (Zervas et al., 1998). In general, SBH contains about 36% fibres, about 12% protein and 3% fat (Wongputtisin et al., 2014). So far, SBH fermentation studies have succeeded in increasing simple sugar and protein content and reducing fibre concentrations, but the overall increase in protein concentration has been relatively small (Wongputtisin et al., 2014).

Starch and sugar processing waste

Similarly, as for other previously described by-products, also liquid wastes from starch and sugar production have high COD and BOD values, therefore these wastewaters have potential of being harmful to environment if released into natural water bodies (Lettinga et al., 1980; Frostell, 1983; Rajeshwari et al., 2000). Environmentally friendly starch and sugar refineries are too expensive; therefore, they need to be combined with the production of other products (Cibis et al., 2006; Krzywonos et al., 2009; Lasik et al., 2010; Lui et al., 2014). Multiple studies have been carried out on the use of potato starch processing residues using microbial fermentation in the production of biologically active compounds and animal feeds (Suzuki et al 2010; Wang et al 2010; Lui et al., 2013; Lui et al., 2014). Studies have also been carried out on how to obtain high-quality SCP from sugarcane bagasse and beet pulp (Ghanem, 1992; Zadrazil & Puniya, 1995; Pessoa et al., 1996). Additionally, sugarcane bagasse and beet and potato pulp can be used in limited amounts as feed additive for ruminants (Zadrazil & Puniya, 1995; Wang et al., 2010). Despite being a by-product of starch or sugar processing, potato starch processing waste, sugarcane bagasse and sugar beet pulp itself are low in starch and sugar and other non-structural carbohydrates, but high in energy and fibre. These residues, similarly as cereal straws, contain large amounts of cellulose and hemicellulose, which is the reason why potato, sugarcane and sugar beet wastes need to be pre-treated before SCP producing fermentation stage. Fermentation process can be divided in two stages, where in the first stage cellulolytic microorganisms are used for pre-treatment of the substrate and SCP synthesizing microorganisms are used in the second stage (Ghanem, 1992; Lui et al., 2013; Lui et al., 2014). By doing so it is possible to ensure a good SCP yields, while at the same time limiting the pollution created by sugar and starch industries.

Fruit waste (fibre rich)

Similarly, as for sugar and starch production waste, also fruit processing residues can be used as feed for cattle, however, this is often not possible, or the transportation of the residues is too expensive (Bhalla & Joshi, 1994; Scerra et al., 1999; De Gregorio et al., 2002). Consequently, in order to reduce the production costs, usually the waste from fruit processing is simply discarded (Upadhyay & Sohi 1988, De Gregorio et al., 2002). Mainly dietary fibre rich fruit processing wastes come from juice and essential oil production factories (De Gregorio et al., 2002). Pomace and juice pulps make up to about 25 to 65% of the total fruit volume used for juicing (Walter & Sherman, 1976; Bhalla & Joshi, 1994; Scerra et al., 1999). Considering that about 25% of the harvested fruits are used in industrial processing (Bhalla & Joshi, 1994), the global annual amount of pomace and juice pulp produced from apples and citrus fruits is estimated at around 15 million tonnes (FAO, 2018a). From a nutritional point of view, fruit remnants are not suitable for monogastric animal feeds because of their low digestibility and low protein content (Bhalla & Joshi, 1994). Due to the large amount of generated waste and the limited use of it, researchers have looked at ways to improve the nutritional value of fibrous fruit wastes by using them as substrates for the production of SCP (Bhalla & Joshi, 1994; Scerra et al., 1999; De Gregorio et al., 2002; Aggelopoulos et al., 2014).

Poultry waste

Disposal of poultry waste has always been problematic because of the price of this waste is too low to be used as a cost-effective fertilizer, and its direct use without destroying pathogens is potentially dangerous for public health (Shuler et al., 1979; Jalasutram et al., 2013). Consequently, SCP production is a good alternative to the processing of poultry wastes because of the relatively high concentrations of micro and macroelements and the high composition of organic compounds compared with other animal wastes (Mitchell & Tub, 2003; Stanely et al., 2004; Kargi et al., 2005; Jalasutram et al., 2013). In general, poultry waste has a high content of nitrogen compounds (5–7% of which 60–70% is uric acid nitrogen, 10–15% is protein nitrogen, 10% is ammonia nitrogen) (Shuler et al., 1979) and fibre, thus appropriate pre-treatment needs to be performed, which hydrolyses polysaccharides and eliminates potential pathogens (Jalasutram et al., 2013). Microorganisms such as *Candida*, *Saccharomyces* and *Rhodotorula spp*. are suitable for the conversion of nitrogen-rich materials into SCP (El-Deek et al., 2009; Jalasutram et al., 2013).

Spent grains

The composition of brewery's spent grains (BSG) can be very different from one beverage plant to the other, and the composition can also vary within a single production unit, depending on which type of beverage is being brewed at that moment (Duarte et al., 2008). Consequently, before using the BSG in the fermentation of microorganisms, it is necessary to find out the concentrations of simple sugars, polysaccharides and proteins in order to choose the most suitable pre-treatments and microorganisms. Usually BSG has high concentration of hemicellulose, lignin and proteins (Duarte et al., 2008), and BSG can be rich in various minerals and vitamins (Mussatto et al., 2006), which makes BSG a potentially complete culture for the cultivation of microorganisms. By hydrolysing BSG, it is possible to significantly increase the concentrations of simple sugars such as xylose and glucose (Duarte et al., 2008).

Pawn shell waste

According to FAO, in 2015, crustacean production from aquaculture and wild capture was 13.9 million tonnes (FAO, 2018b), of which 70–80% constitutes as processing waste (Mauldin & Szabo, 1975; Anderson et al., 1978; Revah-Moiseev & Carroad, 1981). Consequently, the amount of crustacean processing waste is huge. Those wastes that cannot be fed to aquaculture or farm animals are simply dumped into the ocean, burned or landfilled (Revah-Moiseev & Carroad, 1981). These are generally environmentally unfriendly solutions, that considerably increase overall production expenses due to transportation of the waste materials (Kreag & Smith, 1975). In crustacean processing waste chitin content ranges from 13–27% of the dry mass (Ashford et al., 1977; Revah-Moiseev & Carroad, 1981). Chitin is a structural polysaccharide that is a glucose derivate. The high concentration of chitin in crustacean waste limits its options for recycling if economically sound and environmentally friendly operation principles are considered (Revah-Moiseev & Carroad, 1981). Hydrolysis of chitin results in the production of carbohydrates available to SCP synthesizing microorganisms, therefore it is possible to utilize the already developed enzymatic lignocellulose hydrolysis technologies for pre-treating of crustacean processing wastes (Revah-Moiseev & Carroad, 1981). By using hydrolysed crustacean waste as source of

nutrients for SCP production, it is possible to obtain very high protein concentrations in microbial biomass (Rhishipal & Philip, 1998).

Protein or lipid rich sources

By using protein-rich waste products in the production of SCP, it is possible to obtain very high protein concentrations in the final biomass (Table 4). In order to break down the fibrous protein compounds in the waste, they need to be hydrolysed using enzymes from proteolytic microorganisms. The hydrolysis of proteins usually complicates and raises the cost of the SCP production. If it is possible to hydrolyse the waste products by providing associative fermentations (Atalo & Gashe, 1993), then protein-rich waste products can become one of the most suitable waste products for the production of SCP due to their high protein yields.

Wastewaters (protein rich)

Protein rich wastewaters usually have high levels of COD and BOD, therefore expensive waste treatment is required to ensure that it will not cause serious pollution (Kam et al., 2012).

Stickwater

Stickwater is a liquid by-product from fish feed production (Kam et al., 2012). The volume of fish feed production has been relatively constant since the 1980s (6–7 million tonnes a year) (Stickney & McVey, 2002). In comparison, soybean protein production, which produces soy whey wastewater as by-product, has increased in production volumes more than 4-fold in the same time period (Wang et al., 2013; FAO, 2018a). Therefore, there has been no ecological or economical pressure to look for solutions to stickwater treatment and the use of stickwater in the production of SCP was first described relatively recently (Kam et al., 2012). In general, stickwater, as a substrate, can provide high protein concentrations in microbial biomass (Kam et al., 2012). Stickwater has high concentrations of protein, phosphorus and calcium (Kam et al., 2012).

Waste liquor from glutamic acid factory

Approximately 4 tonnes of wastewater are produced from each tonne of L-glutamate (Chiou et al., 2001). Around 1.5 million tons of L-glutamate per year are produced globally (Perosa & Zecchini, 2007), therefore L-glutamate industry generates a huge amount of wastewaters. Solid particles of the waste liquor are rich in protein (around 20%) (Chiou et al., 2001), and therefore this by-product can be used in pig and ruminant nutrition (Yang & Lee, 1982; Chen et al., 1983). However, so far studies on the use of L-glutamate production wastes in animal nutrition have shown that the use of these wastes should be limited in order to avoid metabolic disorders (Yang & Lee, 1982; Chen et al., 1983). Therefore, use of glutamic acid rich wastewaters in SCP production is a promising alternative that can improve the nutritional quality of the waste products and treat created wastewaters at the same time.

Waste capsicum powder

Paprika oleoresin is a natural pigment that is usually obtained from plants of *Capsicum* genus (Uquiche et al., 2004). This pigment is widely used in the food industry and annually around 1,400 tonnes of paprika oleoresin are produced globally (Buckenhusks, 2001; Topuz & Ozdemir, 2003). Waste capsicum powder is a by-product of pigment extraction (Zhao et al., 2010). Approximately 98.6% of the used peppers turn

into wastes during pigment extraction (Zhao et al., 2010). Waste capsicum powder contains capsaicin (Perva-Uzunalic et al., 2004), which is irritant for mammals that produces a sensation of burning, therefore, while waste capsicum powder has high protein content, it is not usable as additive in animal feeds and is usually simply dumped in landfills (Zhao et al., 2010). In recent studies, waste capsicum powder has shown good protein yields after fermentation (Zhao et al., 2010).

Slaughterhouse waste

Horns, feathers, nails and hair make up large part of the waste products from slaughterhouses (Lehninger, 1975; Baden & Kubilus., 1983; Dalev, 1990). These fibrous protein rich wastes are suitable for bioconversion using proteolytic microorganisms, which can produce protein or amino acid concentrates (Atalo & Gashe, 1993). For example, ram horns are rich in cysteine and other amino acids (Kurbanoglu & Algur 2002), and by using ram horn hydrolysate as a medium for SCP production it is possible to obtain very high protein concentrations in microbial biomass (Kurbanoglu & Algur 2002) (see Table 4). This suggests that slaughterhouse waste products are a very promising raw material for the production of SCP.

Table 4. Protein or lipid rich sources. Recent reports of protein content (% of biomass after fermentation) from protein or lipid rich wastes

Protein or lipid rich sources	Microorganisms	Protein content (%)	References
Stickwater	*Aspergillus niger*	48.7	Kam et al., 2012
	Lactobacillus acidophilus	68.4	
Glutamic acid waste liquor	*Aspergillus niger*	50.2	Chiou et al., 2001
Waste capsicum powder	*Candida utilis*	48.2	Zhao et al., 2010
Combined agricultural waste (mostly protein rich)	*Saccharomyces cerevisiae*	38.5	Aggelopoulos et al., 2014
	Kluyveromyces marxianus	33.7	
	Kefir microorganisms	23.6	
Ram horn	*Escherichia coli*	66	Kurbanoglu & Algur, 2002
	Bacillus cereus	68	
	Bacillus subtilis	71	
Soy bean meal	*Bacillus subtilis*	-	Wongputtisin et al., 2012

Soybean meal

Soybean meal (SBM) is one of the most widely used sources of protein for farm animals. SBM is a by-product of soybean oil extraction and contains about 50% proteins, 35% carbohydrates and 2% fat, as well as various minerals (Wongputtisin et al., 2007). SBM can be with and without shell, and as we previously discussed, soy bean hull is polysaccharide rich soy processing by-products that can be used with SBM to regulate its protein concentration (Wongputtisin et al., 2012). SBM fermentation greatly improves its nutritional value, increases feed conversion ratios and neutralize compounds that are not desirable in animal feeds (Feng et al., 2007; Wongputtisin et al., 2012).

Combined agricultural wastes

A good approach to waste utilization for SCP production is combination of different wastes. In this way it is possible to ensure that the medium contains all nutrients and

elements necessary for the cultivation of microorganisms. Aggelopoulos et al. (2014) managed to produce good SCP yields by using a combination of agricultural waste feedstocks containing simple sugars-rich molasses, fibre-rich orange and potato pulps, and protein-rich brewer's spent grains, whey and malt spent rootlets.

CONCLUSIONS

In this review, most of the agricultural wastes that can be used in the production of SCP have been categorized and discussed more closely. Each agricultural waste group has its own advantages and disadvantages if used as substrate for SCP production.

Monosaccharides and disaccharides rich sources require minimal pre-treatment, which give these wastes distinct technological and economical advantage over other waste types, since simpler bioreactor designs can be used, and no sophisticated pre-treatment processes are required.

Fermentation of polysaccharides, protein or lipid rich sources improve the overall digestibility of these by-products, which makes them more applicable as animal feeds. More extensive pre-treatment of these wastes can result in higher SCP yields, but cost effectiveness of applied pre-treatments needs to be considered in order to justify the expenses.

Structural polysaccharides rich wastes are available in huge quantities all over the world; therefore, using these wastes have limited competition with other industries which use waste as resource for production of other value-added products. In comparison, competition over monosaccharides, disaccharides and starch rich sources is greater, since those wastes are not so abundant and are more easily used. If other waste types have limited local availability and efficient and economically reasonable pre-treatment process can be used for hydrolysis, structural polysaccharides rich wastes can be used extensively for production of SCP.

In general, the key considerations for choosing the most suitable waste product for SCP production are: (1) local availability of the particular waste product; (2) pre-treatment costs of the waste product before using it in fermentation; (3) the costs of transportation of the waste product; (4) SCP concentrations in the final microbial biomass after fermentation.

In the future, it is also necessary to thoroughly review and compare the different industrial wastes in regard to their use as substrates for SCP production.

REFERENCES

Aggelopoulos, T., Katsieris, K., Bekatorou, A., Pandey, A., Banat, I.M. & Koutinas, A.A. 2014. Solid state fermentation of food waste mixtures for single cell protein, aroma volatiles and fat production. *Food Chemistry* **145**, 710–716.

Agosin, E., Monties, B. & Odier, E. 1999. Structural changes in wheat straw components during decay by lignin-degrading white-rot fungi in relation to improvement of digestibility for ruminants. *J. Sci. Food Agric.* **36**, 925–935.

Ahmadi, A.R., Ghoorchian, H., Hajihosaini, R. & Khanifar, J. 2010. Determination of the Amount of Protein and Amino Acids Extracted from the Microbial Protein (SCP) of Lignocellulosic Wastes. *Pakistan Journal of Biological Sciences* **13**(8), 355–361.

Anderson, C.G., DePablo, N. & Romo, D.R. 1978. Antartic krill (*Euphasia superba*) as a source of chitin and chitosan. Proceedings of the First International Conference on Chitinl Chitosan, R.A.A. Muzzarelli, and E.R. Pariser, Eds., M.I.T. *Sea Grant Report MITSG* 78–7 (M.I.T., Cambridge, MA, 1978), pp, 54–63.

Anderson, P.J., McNeil, K.E. & Watson, K. 1988. Thermotolerant single cell protein production by *Kluyveromyces marxianus* var. *marxianus*. *Journal of Industrial Microbiology* **3**, 9–14.

Anupama & Ravindra, P. 2000. Value-added food: single cell protein. *Biotech. Adv.* **18**, 459–479.

Ashford, N.A., Hattis, D. & Murray, A.E. 1977. Industrial prospects for chitin and protein from shellfish wastes, M.I.T. *Sea Grant Program*, 77–3 (M.I.T., Cambridge, MA, 1977).

Atalo, K. & Gashe, B.A. 1993. Protease production by a thermophilus *Bacillus species* which degrades various kinds of fibrous proteins. *Biotechnol. Lett.* **15**, 1151–1156.

Baden, H. & Kubilus, J. 1983. Fibrous proteins of bovine hoof. *J. Invest. Dermatol.* **81**, 220–224.

Baldensperger, J., Le Mer, J., Hannibal, L. & Quinto, P.J. 1985. Solid State Fermentation of Banana Wastes. *Biotechnology Letters* **7**(10), 743–748.

Bekatorou, A., Psarianos, C. & Koutinas, A.A. 2006. Production of food grade yeasts. *Food Technology and Biotechnology* **44**, 407–415.

Bhalla, T.C. & Joshi, M. 1994. Protein enrichment of apple pomace by co-culture of cellulolytic moulds and yeasts. *World Journal of Microbiology & Biotechnology* **10**, 116–117.

Braio, V.B. & Taveres, C.R. 2007. Effluent generation by the dairy industry: preventive attitude and opportunities. *J. Chem. Eng.* **24**(4), 487–497.

Brijwani, K. & Vadlani, P. 2011. Solid state fermentation of soybean hulls for cellulolytic enzymes production. In Soybean – *Applications and Technology* ed. Ng, T.-B. pp. 305–322.

Browne, J., Nizami, A.S., Thamsiriroj, T. & Murphy, JD. 2011. Assessing the cost of biofuel production with increasing penetration of the transport fuel market: A case study of gaseous biomethane in Ireland. *Renewable and Sustainable Energy Reviews* **15**, 4537–4547.

Buckenhuskes, H.J. 2001. Actual requirements on paprika powder for the food industry. Presented at *the 9th Meeting on Genetics and Breeding of Capsicum and Eggplant*, Antalya, Turkey.

Calleja, G.B., Yaguchi, M., Levy-Rick, S., Segun, J.R.H., Roy, C. & Lusena, C.V. 1986. Single-Cell Protein Production from Potato Starch by the Yeast *Schwanniomyces alluvius*. *J. Ferment. Teehnol*. **64**(1), 71–75.

Chanda, S. & Chakrabarti, S. 1996. Plant origin liquid waste: A resource for single cell protein production by yeast. *Biores. Technol.* **57**, 51–4.

Chanda, S., Chakrabarti, S. & Matai, S. 1984. Whey as the medium for production of microbial metabolites and biomass. *Current Trends in Life Sciences* **9**, 377–389.

Chen, M.C., Shiao, T.F., Lee, C.F., Huang, C.Y., Chen, C.P. & Shien, Y.S. 1983. Studies on the utilization of glutamate mother liquor as a feed for cattle. *Chin. Agric. Soc.* **124**, 98–115.

Chiou, P.W.S., Chiu, S.W. & Chen, C.R. 2001. Value of *Aspergillus niger* fermentation product as a dietary ingredient for broiler chickens. *Animal Feed Science and Technology* **91**, 171–182.

Choi, S. & Chung, M.H. 2003. A Review on the Relationship Between *Aloe vera* Components and Their Biologic Effects. *Seminars in Integrative Medicine* **1**(1), 53–62.

Cibis, E., Krzywonos, M. & Miskiewicz, T. 2006. Aerobic biodegradation of potato slops under moderate thermophilic conditions: effect of pollution load. *Bioresour. Technol.* **97**(4), 679–685.

Dalev, P. 1990. An enzyme-alkaline hydrolysis of feather keratin for obtaining a protein concentrate for fodder. *Biotechnol. Lett.* **12**, 71–72.

De Gregorio, A., Mandalari, G., Arena, N., Nucita F., Tripodo M.M. & Lo Curto, R.B. 2002. SCP and crude pectinase production by slurry-state fermentation of lemon pulps. *Bioresource Technology* **83**, 89–94.

Deak, N.A. & Johnson, L.A. 2006. Functional properties of protein ingredients prepared from high-sucrose/low-stachyose soybeans. *J. Am. Oil Chem. Soc.* **83**, 811–818.

Deibel, M.R.J., Hiebsch, R.R. & Klein, R.D. 1988. Secreted amylolytic enzymes from *Schwanniomyces occidentalis*: purification by fast protein liquid chromatography (FPLC) and preliminary characterization. *Preparative biochemistry* **19**(1), 77–120.

Duarte, L.C., Carvalheiro, F., Lopes, S., Neves, I. & Girio, F.M. 2008. Yeast biomass production in brewery's spent grains hemicellulosic hydrolyzate. *Appl. Biochem. Biotech.* **148**, 119–129.

El-Bakry, M., Abraham, J., Cerda, A., Barrena, R., Ponsa, S. & Gea, T. 2015. From Wastes to High Value Added Products: Novel Aspects of SSF in the Production of Enzymes. *Journal Critical Reviews in Environmental Science and Technology* **45**, 18.

El-Deek, A.A., Ghonem, K.M., Saffa, M.H., Aser, M.A., Fahad, M.A. & Osman, M.M. 2009. Producing single cell protein from poultry manure and evaluation for broiler chickens diets. *Int. J. Poult. Sci.* **8**, 1062–1077.

Feng, J., Liu, X., Liu, Y.Y. & Lu, Y.P. 2007. The effect of Aspergillus oryzae fermented soybean meal on growth performance, digestibility of dietary components and activities of intestinal enzymes in weaned piglets. *Anim. Feed Sci. Technol.* **134**, 295–303.

Finco, A.M.O., Mamani, L.D.G., Carvalho, J.C., Pereira, G.V.M., Soccol, V.T. & Soccol, C.R. 2017. Technological trends and market perspectives for production of microbial oils rich in omega-3. *Critical Reviews in Biotechnology* **37**(5), 656–671.

FitzPatrick, M., Champagne, P., Cunningham, M.F. & Whitney, R.A. 2010. A biorefinery processing perspective: Treatment of lignocellulosic materials for the production of value-added products. *Bioresource Technology* **101**, 8915–8922.

Fontana, J.D., Czeczuga, B., Bonfim, T.M.B., Chociai, M.B., Oliveira, B.H., Guimaraes, M.F. & Baron, M. 1996. Bioproduction of carotenoids: the comparative use of raw sugarcane juice and depolymerized bagasse by *Phaffia rhodozyma*. *Bioresource Technology* **58**, 121–125.

Food and Agricultural Organization of United Nations (FAO). 2018b. Global statistical collections: global capture production and global aquaculture production. *http://www.fao.org/fishery/statistics/*. Accessed 21.2.2018.

Food and Agricultural Organization of United Nations (FAO). FAOSTAT. 2018a. *http://www.fao.org/faostat/en/#data/QC*. Accessed 21.2.2018.

Frostell, B. 1983. Anaerobic–aerobic biological treatment of starch industry waste waters. *Starch-Stärke* **35**, 185–189.

Gao, Y., Li, D. & Liu, Y. 2012. Production of single cell protein from soy molasses using *Candida tropicalis*. *Ann. Microbiol.* **62**, 1165–1172.

Ghaly, A.E., Mahmoud, N.S., Rushton, D.G. & Arab, F. 2007. Potential environmental and health impacts of high land application of cheese whey. *Am. J. Agric. Biol. Sci.* **2**, 106–117.

Ghanem, K.M. 1992. Single cell protein production from beet pulp by mixed culture. *Qatar Univ. Sci. J.* **12**, 85–88.

Godber, J. & Juliano, B. 2004. Rice lipids. In E.T. Champagne (Ed.), Rice: Chemistry and technology. *Minnesota: American Association of Cereal Chemists Inc.*, 163–190.

Hanmoungjai, P., Pyle, D.L. & Niranjan, K. 2000. Extraction of rice bran oil using aqueous media. *J. Chem. Technol. Biotechnol.* **75**, 348–352.

Hashem, M., Hesham, A.E.L., Almari, S.A. & Alrumman, S.A. 2014. Production of single-cell protein from wasted date fruits by *Hanseniaspora uvarum* KKUY-0084 and *Zygosaccharomyces rouxii* KKUY-0157. *Ann. Microbiol.* **64**, 1505–1511.

International Grains Council. 2018. International Grains Council Market Report. *http://www.igc.int/downloads/gmrsummary/gmrsumme.pdf*. Accessed 21.2.2018.

Jalasutram, V., Kataram, S., Gandu, B. & Anupoju, G.R. 2013. Single cell protein production from digested and undigested poultry litter by Candida utilis: optimization of process parameters using response surface methodology. *Clean Techn. Environ. Policy* **15**, 265–273.

Kahlon, T.S. 2009. Rice Bran: Production, Composition, Functionality and Food Applications, Physiological Benefits. In: Choo SS PS, editor. Fiber Ingredients: *Food Applications and Health benefits* 305–321.

Kam, S., Kenari, A.A. & Younesi, H. 2012. Production of Single Cell Protein in Stickwater by *Lactobacillus acidophilus* and *Aspergillus niger*. *J. of Aqua. Food Prod. Tech.* **21**, 403–417.

Kargi, F., Shuler, M.L., Vashon, R., Seeley, H.W., Henry, A. & Austic, R.E. 2000. Continuos aerobic conversion of poultry waste into single cell protein using a single reactor: kinetic analysis and determination of optimal conditions. *Biotechnol. Bioeng.* **22**, 1567–1600.

Kim, J.H. & Lebeault, J.M. 1981. Protein Production from Whey Using *Penicillium cyclopium*; Growth Parameters and Cellular Composition. *European J. Appl. Microbiol. Biotechnol.* **13**, 151–154.

Kinney, A.J. 2003. Engineering soybeans for food and health. *Ag. Bio. Forum* **6**, 18–22.

Konlani, S., Delgenes, J.P., Moletta, R., Traore, A. & Doh, A. 1996. Optimization of cell yield of *Candida krusei* SO1 and *Saccharomyces spp.* LK3G cultured in sorghum hydrolysate. *Bioresource Technology* **57**, 275–281.

Kopsahelis, N., Agouridis, N., Bekatorou, A. & Kanellaki, M. 2007. Comparative study of delignified and non-delignified brewer's spent grains as yeast immobilization supports for alcohol production from molasses. *Bioresource Technology* **98**, 1440–1447.

Kost, C., Mayer, J.N., Thomsen, J., Hartmann, N., Senkpiel, C., Philipps, S., Nold, S., Lude, S., Saad, N. & Schlegl, T. 2013. Levelized cost of electricity renewable energy technologies. Fraunhofer ISE *https://www.ise.fraunhofer.de/content/dam/ise/en/documents/publications/studies/FraunhoferISE_LCOE_Renewable_Energy_technologies.pdf.* Accessed 21.2.2018.

Kreag, R. & Smith, F. 1975. Seafood solid waste in Oregon: disposal or recovery? *Oregon State University Extension Marine Advisory Program Report 395*.

Krzywonos, M., Cibis, E., Lasik, M., Nowak, J. & Miskiewicz, T. 2009. Thermo- and mesophilic aerobic batch biodegradation of high-strength distillery wastewater (potato stillage)-utilisation of main carbon sources. *Bioresour. Technol.* **100**(9), 2507–2514.

Kurbanoglu, E.B. & Algur, O.F. 2002. Single-cell protein production from ram horn hydrolysate by bacteria. *Bioresource Technology* **85**, 125–129.

Lasik, M., Nowak, J., Krzywonos, M. & Cibis, E. 2010. Impact of batch, repeated-batch (with cell recycle and medium replacement) and continuous processes on the course and efficiency of aerobic thermophilic biodegradation of potato processing wastewater. *Bioresour. Technol.* **101**(10), 3444–3451.

Lehninger, A.L. 1975. *Biochemistry*, second ed. Worth Publishers, 444 Park Avenue South, New York, 1104 pp.

Lettinga, G., van Velsen, A.F.M., Hobma, S.W., de Zeeuw, W. & Klapqijk, A. 1980. Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering* **12**, 699–734.

Lipinsky, E.S. 1981. Chemicals from biomass: petrochemical substitution options. *Science* **212**, 1465–1471.

Liu, B., Li, Y., Song, J., Zhang, L., Dong, J. & Yang, Q. 2014. Production of single-cell protein with two-step fermentation for treatment of potato starch processing waste. *Cellulose* **21**, 3637–3645.

Liu, B., Song, J., Li, Y., Niu, J., Wang, Z. & Yang, Q. 2013. Towards Industrially Feasible Treatment of Potato Starch Processing Waste by Mixed Cultures. *Appl. Biochem. Biotechnol.* **171**, 1001–1010.

Mauldin, A.F. & Szabo, A.J. 1975. Shrimp canning waste treatment study," *Environmental Protection Agency Publication* **2**, 74–061.

Mekonnen, M.M. & Howkstra, A.Y. 2014. Water footprint benchmarks for crop production: A first global assessment. *Ecological Indicators* **46**, 214–223.

Mitchell, C.C. & Tub, S. 2003. Long-term evaluation of poultry litter as a source of nitrogen for cotton and corn. *Agron. J.* **97**, 399–407.

Mussatto, S.I., Dragone, G. & Roberto, I.C. 2006. Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science* **43**, 1–14.

Nasseri, A.T., Sasoul-Amini, S., Morowvat, M.H. & Ghasemi, Y. 2011. Single Cell Protein: Production and Process. *American Journal of Food Technology* **6**(2), 103–116.

Nelson, R.G. 2002. Resource assessment and removal analysis for corn stover and wheat straw in the Eastern and Midwestern United States - rainfall and wind-induced soil erosion methodology. *Biomass and Bioenergy* **22**, 349–363.

NL Agency. 2013. Rice straw and Wheat straw. Potential feedstocks for the Biobased Economy. Netherlands Ministry of Economic Affairs. *https://english.rvo.nl/sites/default/files/2013/12/Straw%20report%20AgNL% 20June%202013.pdf*. Accessed 21.2.2018.

Paraskevopoulou, A., Athanasiadis, I., Kanellaki, M., Bekatourou, A., Blekas, G. & Kiosseoglou, V. 2003. Functional properties of sigle cell protein produced by kefir microflora. *Food Res. Int.* **36**, 431–438.

Perosa, A. & Zecchini, F. 2007. *Methods and Reagents for Green Chemistry: An Introduction*. John Wiley & Sons. 25 pp.

Pessoa, A.J., Mancilha, I.M. & Sato, S. 1996. Cultivation of Candida tropicalis in sugar cane hemicellulosic hydrolyzate for microbial protein production. *J. of Biotech.* **51**, 83–88.

Pirie, N.W. 1987. *Leaf Protein: And Its By-products in Human and Animal Nutrition*. Cambridge University Press, 209 pp.

Rajeshwari, K.V., Balakrishnan, M., Kansal, A., Lata, K. & Kishore, V.V.N. 2000. State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. *Renewable and Sustainable Energy Reviews* **4**(2), 135–156.

Revah-Moiseev, S. & Carroad, P.A. 1981. Conversion of the Enzymatic Hydrolysate of Shellfish Waste Chitin to Single-Cell Protein. *Biotechnology and Bioengineering* **23**, 1067–1078.

Rhishipal, R. & Philip, R. 1998. selection of marine yeasts for the generation of single cell protein from prawn-shell waste. *Bioresource Technology* **65**, 255–256.

Ritala, A., Hakkinen, S.T., Toivari, M. & Wiebe, M.G. 2017. Single Cell Protein—State-of-the-Art, Industrial Landscape and Patents 2001–2016. *Front. Microbiol.* **8**, 2009.

Scerra, V., Caridi, A., Foti, F. & Sinatra, M.C. 1999. Influence of dairy *Penicillium spp*. on nutrient content of citrus fruit peel. *Animal Feed Science and Technology* **78**, 169–176.

Shipman, R.H., Kao, I.C. & Fan, L.T. 1975. Single-cell protein production by photosynthetic bacteria cultivation in agricultural by-products. *Biotech. and Bioeng.* **17**, 1561–1570.

Shuler, M.L., Roberts, E.D., Mitchell, D.W., Kargi, F., Austic, R.E., Henry, A., Vashon, R. & Seeley, H.W.J. 1979. Process for the aerobic conversion of poultry manure into high-protein feedstuff. *Biotechnology and Bioengineering* **21**, 19–38.

Singh, A., Abidi, A.B., Agrawal, A.K. & Darmwal, N.S. 1991. Single cell protein production by *aspergillus niger* and its evaluation. *Zentralbl. Mikrobiol.* **146**, 181–184.

Singh, J.K., Meshram, R.L. & Ramteke, D.S. 2011. Production of Single cell protein and removal of 'COD' from dairy waste water. *European Journal of Experimental Biology* **1**(3), 209–215.

Sisman, T., Özlem, G., Dogan, N., Özdal, M., Algur, Ö.F. & Ergon, T. 2013. Single-cell protein as an alternative food for zebrafish, Danio rerio: a toxicological assessment. *Toxicol. Ind. Health* **29**, 792–799.

Skidmore, E.L. Wind erosion. In: Soil erosion research methods. Anakeny, IA: Soil and Water Conservation Society, 1988.

Stanely, V.G., Gray, C., Daley, M., Krueger, W.F. & Sefton, A. 2004. An alternative to antibiotic based drugs in feed for enhancing performance of broilers grown on *Eimeria spp*. infected litter. *Poult. Sci.* **83**, 39–44.

Stickney, R.R. & McVey, J.P. 2002. *Responsible Marine Aquaculture*. CABI, 391 pp.

Suzuki, S., Fukuoka, M., Tada, S., Matsushita-Morita, M., Hattori, R., Kitamoto, N. & Kusumoto, K.I. 2010 Production of polygalacturonase by recombinant Aspergillus oryzae in solid-state fermentation using potato pulp. *Food Sci. Technol. Res.* **16**(5), 517–521.

Zadrazil, F. & Puniya, A.K. 1995. studies on the effet of particle size on solid-state fermentation of sugarcane bagasse into animal feed using white-rot fungi. *Biores. Technol.* **54**, 85–87.

Zervas, G., Fegeros, K., Koytsotolis, K., Goulas, C. & Mantzios, A. 1998. Soy hulls as a replacement for maize in lactating dairy ewe diets with or without dietary fat supplements. *Anim. Feed Sci. Technol.* **76**, 65–75.

Zhao, G., Zhang, W. & Zhang, G. 2010. Production of single cell protein using waste capsicum powder produced during capsanthin extraction. *Letters in Applied Microbiol.* **50**, 187–191.

Tilman, D. 1999. Global environmental impacts of agricultural expansion: the needfor sustainable and efficient practices. *Proc. Natl. Acad. Sci.* **96**(11), 5995–6000.

Topuz, A. & Ozdemir, F. 2003. Influences of γ-Irradiation and Storage on the Carotenoids of Sun-Dried and Dehydrated Paprika. *J. Agric. Food Chem.* **51**, 4972–4977.

Ugalde, U.O. & Castrillo, JI. 2002. Applied mycology and biotechnology. *Agriculture and food production* **2**, 123–149.

Upadhyay, R.C. & Sohi, H.S. 1988. Apple pomace - a good substrate for the cultivation of edible mushrooms. *Current Science* **57**, 1189–1190.

Valentino, M.J.G., Ganado, L.S. & Undan, J.R. 2016. Single cell protein potential of endophytic fungi associated with bamboo using rice bran as substrate. *Adv. in Appl. Sci. Res.* **7**(3), 68–72.

Valmaseda, M., Almendrs, G. & Martinez, A.T. 1991. Chemical transformation of wheat straw constituents after solid state fermentation with selected lignocellulose-degrading fungi. *Biomass Bioenergy* **1**, 261–266.

Walter, R.C. & Sherman, R.M. 1976. Fuel value of grape and apple processing wastes. *Journal of Agricultural and Food Chemistry* **24**, 1244–1245.

Wang, L., Wu, Z., Zhao, B., Liu, W. & Gao, Y. 2013. Enhancing the adsorption of the proteins in the soy whey wastewater using foam separation column fitted with internal baffles. *Journal of Food Engineering* **119**(2), 377–384.

Wang, T.Y., Wu, Y.H., Jiang, C.Y. & Liu, Y. 2010. Solid state fermented potato pulp can be used as poultry feed. *Br. Poult. Sci.* **51**(2), 229–234.

Vares, T., Kalsi, M. & Hatakka, A. 1995. Lignin peroxidases and other ligninolytic enzymes by *Phlebia radiate* during solid-state fermentation of wheat straw. *Environ. Microbiol.* **61**, 3515–3520.

Vermeulen, S.J., Campbell, B.M. & Ingram, J.S.I. 2012. Climate change and food systems. *Annual Review of Environment and Resources* **37**, 195–222.

Werpy, T. & Petersen, G. 2004. Top value added chemicals from biomass. Volume I - results of screening for potential candidates from sugars and synthesis gas, U.S.D. o. Energy. *https://www.nrel.gov/docs/fy04osti/35523.pdf*. Accessed 21.2.2018.

Wongputtisin, P., Khanongnuch, C., Kongbuntad, W., Niamsup, P. & Lumyong, S. 2012. Screening and selection of Bacillus spp. for fermented corticate soybean meal production. *Journal of Applied Microbiology* **113**, 798–806.

Wongputtisin, P., Khanongnuch, C., Kongbuntad, W., Niamsup, P., Lumyong, S. & Sarkar, P.K. 2014. Use of *Bacillus subtilis* isolates from Tea-nao towards nutritional improvement of soya bean hull for monogastric feed application. *Letr. in Appl. Microbiol.* **59**, 328–333.

Wongputtisin, P., Khanongnuch, C., Pongpiachan, P. & Lumyong, S. 2007. Antioxidant activity improvement of soybean meal by microbial fermentation. *Res. J. Microbiol.* **2**, 577–583.

Yadav, J.S.S., Bezawada, J., Ajila, C.M., Yan, S., Tyagi, R.D. & Surampalli, R.Y. 2014. Mixed culture of *Kluyveromyces marxianus* and *Candida krusei* for single-cell protein production and organic load removal from whey. *Bioresource Technology* **164**, 119–127.

Yadav, J.S.S., Bezawada, J., Elharche, S., Yan, S., Tyagi, R.D. & Surampalli, R.Y. 2013. Simultaneous single-cell protein production and COD removal with characterization of residual protein. *Bioprocess Biosyst. Eng.* **37**(6), 1017–1029. http://dx.doi.org/10.1007/s00449-013-1072-6.

Yang, Y.F. & Lee, P.K. 1982. Can molasses and glutamate fermentation end liquor as feed ingredients on growth and feed efficiency on growing fattening pigs. *Taiwan Livest. Res.* **15**(1), 9–16.

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A study of dynamics of bitter acids and xanthohumol in hop pellets during storage

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Abstract. Eight varieties of hop pellets were analyzed for the contents of α-acids, ß-acids and xanthohumol according to the EBC 7.7 analytical method. The pellets were extracted with acidified mixture methanol - diethylether and analyzed using HPLC with a diode-array detector and a Nova-Pak column C_{18}. Four series of analyses were performed: immediately after the unpacking of the pellets and then after five, seven and nine months of storage at 4 °C. According to the first series of analyses, the contents were assayed as following (α-acids, ß-acids, xanthohumol resp., all in weight % in pellets): Galaxy (13.4, 8.0, 0.74), Citra (11.1, 3.0, 0.48), Tradition (8.2, 8.0, 0.58), Cascade (4.5, 5.2, 0.25), Northern Brewer (4.0, 2.9, 0.37), Sládek (3.5, 4.0, 0.48), Saaz (2.0, 3.4, 0.24), and Triskel (1.7, 3.6, 0.18). According to these results, variety Galaxy was found as the richest in all three parameters. After nine months of storage at 4 °C, the weight loss of α-acids ranged from 4.1% (Citra and Triskel) to 66.4% (Galaxy). The losses of β-acids and xanthohumol were less distinctive (from zero to 31.3% and 25.7%, resp.) and indicated good long storage possibilities of these compounds at convenient conditions (darkness, low temperature, elimination of direct influence of oxygen).

Key words: hop pellets, hop varieties, storage, α-acids, ß-acids, xanthohumol, HPLC.

INTRODUCTION

Hop growing at the territory of the present-day Czech Republic has a long tradition; the first news concerning hop growing come already from the 8th and 9th centuries. Thanks to the specific soil and climatic conditions, the best results in the quality of hops have been achieved in Žatec (Saaz) region. Several important hop varieties exported to many foreign countries originate from this region, e.g. Saaz semi-early red-bine hop, Saaz Late or Sládek (Nesvadba, 2013).

From the chemical point of view, the most important criteria of hop quality are the contents of hop resins (especially α-acids and β-acids), hop polyphenols (xanthohumol and related compounds), and the total contents and composition of essential oils (European Brewery Convention, 2013). The main constituents of α-acids are humulone,

cohumulone and adhumulone, the main constituents of β-acids are lupulone, colupulone and adlupulone (Fig. 1).

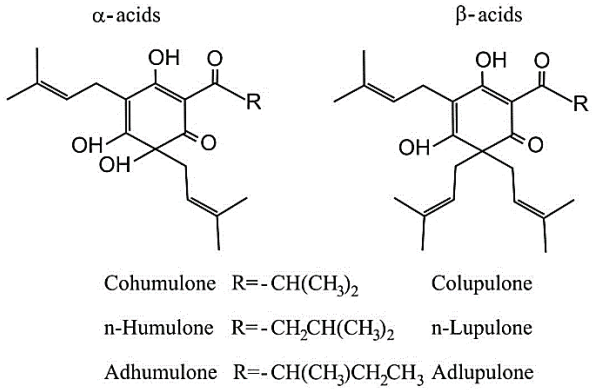

Figure 1. Chemical formulas of the main constituents of α- and β-acids (Saugspier et al., 2012).

During the brewing process, humulones are thermally transformed into iso-humulones (iso-α-acids), which are responsible for the specific bitter taste and the stability of beer foam (Česlová et al., 2009). Due to structural differences, many properties of β-acids are different from the properties of α-acids. The presence of another isoprenyl side chain causes the molecule as a whole to have a more hydrophobic character. Therefore, β-acids are much less soluble in water and cannot isomerize during the brewing process, but their oxidation occurs in the course of processing and storage of hops and to a small extent also during beer production. The unique chemical structure of β-acids is a source of their antimicrobial properties and other physiological effects on the human organism (Krofta & Mikyška, 2014). Detailed analytical studies of α- and β-acids using high-performance liquid chromatography (Hermans-Lokkerbol & Verpoorte, 1994) and HPLC-mass spectrometry (Česlová et al., 2009) were published.

The hops contain many polyphenolic compounds in addition to bitter acids. The important hop flavonoids are xanthohumol and related prenylflavonoids as isoxanthohumol, desmethylxanthohumol, 6-prenylnaringenin and many others. They have a positive effect on the human health due to their antioxidant, anticancer, antimicrobial, and anti-inflammatory properties (Stevens & Page, 2004). Xanthohumol is the most frequently investigated polyphenol in hops and hop products (Fig. 2).

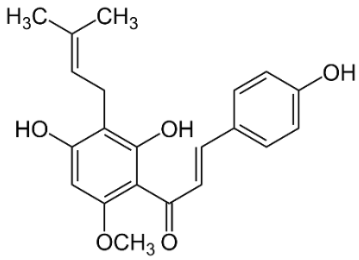

Figure 2. Chemical formula of xanthohumol (https://en.wikipedia.org/wiki/Xanthohumol#/media/File:Xanthohumol.svg).

Hop pellets are a hop product added to the kettle to provide bitterness and a hop character that is not distinguishable from that achieved using raw hops. Pellets provide improved homogeneity, better storage stability and reduced storage/transport costs

compared to raw hops. Their chemical composition and the demands for their quality assessment are similar as for the raw hops. Hop pellets are usually packed in laminated foils with an aluminium layer as a barrier against diffusion of oxygen and they can be shipped for long distances (Barth & Schönberger, 2014). Thus, the using of hop pellets made of miscellaneous hop varieties is typical for contemporary brewery practice including breweries in the Czech Republic, where the use of traditional Czech varieties predominated in past years (Nesvadba, 2013).

Because the quality of input raw materials is very important, we decided to arrange an analytical study including the determination of α-acids, β-acids and xanthohumol in eight varieties of hop pellets being frequently used in the Czech Republic. The main goal of this study was to evaluate the influence of storage on the quality of hop pellets and to compare the properties of analyzed hop varieties with data from literature.

MATERIALS AND METHODS

Hop pellets

Hop pellets manufactured from eight miscellaneous hop varieties were studied (country of origin is given in brackets): Cascade (USA), Citra (USA), Galaxy (Australia), Northern Brewer (Germany), Saaz (Czech Republic), Sládek (Czech Republic), Tradition (Germany), and Triskel (France). All pellets were type 90, cylindrical shape with diameter about 6 mm, packed in laminated aluminium foils per 5 kg, year of harvest 2015 (only Cascade 2014, weight of package 10 kg).

All samples were obtained in cooperation with a medium-size brewery located in East Bohemia (beer production ca. 130 000 hl per year). Most of analyzed hop pellets were supplied by Joh. Barth & Sohn Gmbh & Co. KG (Nuremberg, Germany), only the Saaz variety was supplied by a Czech company Chmel Polepská blata, Ltd., Polepy. All other identification data (batch numbers, delivery sheets etc.) are available at the authors.

Sampling and storage

About 100 g of hop pellets was taken from each batch delivered into the brewery immediately after packing foil opening and placed into a zip reclosable plastic bag. Before closing the bag, the access of air was removed carefully and tightly closed bags were stored in a refrigerator at 4 °C. The first series of analyses was performed up to 48 hours from sampling, the next series of analyses were performed after five, seven, and nine months of storage in the refrigerator at the same conditions.

Extraction of pellets

Extraction of pellets was performed according to the EBC 7.7 analytical method (Krofta, 2008; European Brewery Convention, 2013). 10 g of hop pellets were blended and poured into a 250 mL glass jar with a screw cap. 20 mL of methanol, 100 mL of diethyl ether, and 40 mL of hydrochloric acid ($c = 0.1$ mol L^{-1}) were added. Tightly closed jars were shaken for 40 minutes in a laboratory shaker and then left to stand for 10 minutes at least (to let liquid phases to separate). 5 mL of the clear upper layer were pipetted into a 50 mL volumetric flask, filled up with methanol and homogenized carefully. About 2 mL of this solution were taken into a plastic syringe and transferred through a syringe filter into a glass vial for the HPLC analysis. For each sample of pellets, two parallel extractions were made.

High-performance liquid chromatography

HPLC instrument Waters equipped with a diode-array detector and a Nova-Pak column C_{18} (150 mm x 3.9 mm i.d., particle size 4 μm) was used for the analysis of samples. The composition of the mobile phase was slightly modified to improve the separation (isocratic elution with methanol/water/orthophosphoric acid = 764 : 227 : 9, v/v/v, flow rate 0.8 mL min^{-1}, column temperature 40 °C). The new International Calibration Standard ICE–3 (Labor Veritas, Zürich) was used for the calibration of the HPLC instrument according to the EBC 7.7 method. Two repeated injections (10 μl) of the calibration solution were made before and after the ending of each series of analyses for the quantification of α- and ß-acids at 314 nm. The solutions of a pure analytical standard (Sigma-Aldrich, Prague) in the concentration range 0.012–0.24 mg mLl^{-1} were used for the quantification of xanthohumol at 370 nm. Two repeated injections (10 μl) were made for each extract of hop pellets. Example of a chromatogram at 314 nm (variety Triskel) is given in Fig. 3

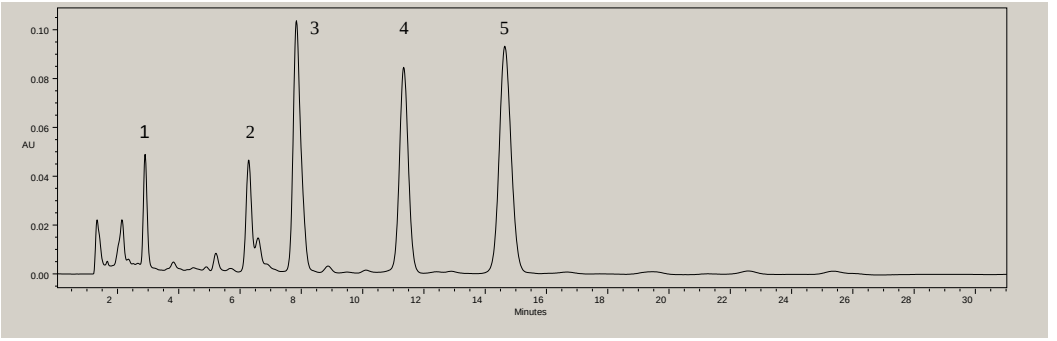

Figure 3. Chromatogram of hop pellets at 314 nm, variety Triskel (1 = xanthohumol, 2 = cohumulone, 3 = humulone + adhumulone, 4 = colupulone, 5 = lupulone + adlupulone).

RESULTS AND DISCUSSION

The contents of α-acids, β-acids, and xanthohumol were determined in pellets manufactured of eight hop varieties. The first series of analyses included pellets which were analyzed immediately after packing foil opening (up to 48 hours), the next series of analyses were done after five, seven and nine months storage of pellets in tightly closed plastic bags at 4 °C. The percentual loss of weight after nine months storage for each analyzed component and the α/β ratio were calculated. All results are given in Table 1 (including data from literature).

According to the contents of α-acids measured in the first series of analyses, the richest varieties were Galaxy (13.4%) and Citra (11.1%), the lowest contents were in Saaz (2.0%) and Triskel (1.7%). In most cases, the measured values of α-acids were almost in the same range (Cascade, Citra, Galaxy) or about 20% lower (Saaz and Sládek) than data from literature (see notices under Table 1). Substantial differences between measured values and literature data were found for three varieties: Tradition (8.2% vs. 4–7% in lit.), Northern Brewer (4.0% vs. 8–10%), and especially Triskel (1.7% vs. 8–9%).

Table 1. Contents of α-acids, β-acids and xanthohumol in hop pellets

Variety (country of origin)	Component	Contents (data from literature)	Measured contents* (after package opening)	Measured contents* (after 5 months)	Measured contents* (after 7 months)	Measured contents* (after 9 months)	Loss of mass
		% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (w/w)	% (rel.)
Cascade	α-acids	4.5–7.0[2)]	4.45 ± 0.15	4.21 ± 0.16	4.20 ± 0.06	4.22 ± 0.06	5.2
(USA)	β-acids	4.8–7.0[2)]	5.24 ± 0.26	5.12 ± 0.25	5.12 ± 0.11	5.19 ± 0.11	1.0
	xanthohumol	0.30–0.32[3)]	0.25 ± 0.01	0.26 ± 0,01	0.25 ± 0,01	0.26 ± 0,01	0.0
	α/β ratio	0.9–1.0[2)]	0.85	0.82	0.82	0.81	
Citra	α-acids	11.0–13.0[2)]	11.10 ± 0.14	10.92 ± 0.10	10.81 ± 0.04	10.65 ± 0.03	4.1
(USA)	β-acids	3.5–4.5[2)]	3.01 ± 0.04	3.01 ± 0.03	2.98 ± 0.01	2.93 ± 0.06	2.7
	xanthohumol	0.44 [2)]	0.48 ± 0.01	0.50 ± 0.00	0.49 ± 0.00	0.49 ± 0.00	0.0
	α/β ratio	2.4–3.7 [2)]	3.69	3.62	3.63	3.63	
Galaxy	α-acids	13.5–14.8[2)]	13.38 ± 0.09	7.74 ± 0.02	5.84 ± 0.03	4.49 ± 0.02	66.4
(Australia)	β-acids	5.0–6.5[2)]	8.03 ± 0.06	6.46 ± 0.0.1	5.86 ± 0.02	5.52 ± 0.02	31.3
	xanthohumol	0.5–0.9[2)]	0.74 ± 0.01	0.65 ± 0.00	0.59 ± 0.00	0.55 ± 0.00	25.7
	α/β ratio	2.1–2.9[2)]	1.67	1.19	0.99	0.81	
Northern Brewer	α-acids	8.0– 0.0[2)]	3.98 ± 0.11	3.90 ± 0.04	3.78 ± 0.05	3.81 ± 0.06	4.3
(Germany)	β-acids	3.0–5.0[2)]	2.87 ± 0.09	2.92 ± 0.03	2.87 ± 0.05	2.96 ± 0.06	0.0
	xanthohumol	0.6[2)]	0.37 ± 0.01	0.38 ± 0.00	0.37 ± 0.01	0.38 ± 0.01	0.0
	α/β ratio	no data found	1.39	1.33	1.32	1.29	
Saaz	α-acids	2.5–4.5[1)]	1.95 ± 0.04	1.81 ± 0.06	1.80 ± 0.05	1.80 ± 0.02	7.7
(Czech R.)	β-acids	4.0–6.0[1)]	3.41 ± 0.07	3.32 ± 0.12	3.27 ± 0.09	3.35 ± 0.04	1.8
	xanthohumol	0.3–0.5[1)]	0.24 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.24 ± 0.00	0.0
	α/β ratio	0.6–1.0[1)]	0.57	0.55	0.55	0.54	
Sládek	α-acids	4.5–8.0[1)]	3.47 ± 0.04	3.08 ± 0.03	2.83 ± 0.08	2.76 ± 0.01	20.5
(Czech R.)	β-acids	4.0–7.0[1)]	3.95 ± 0.06	3.81 ± 0.06	3.50 ± 0.13	3.67 ± 0.02	7.1
	xanthohumol	0.50–0.75[1)]	0.48 ± 0.01	0.48 ± 0.01	0.47 ± 0.01	0.47 ± 0.00	2.1
	α/β ratio	0.7–1.3[1)]	0.88	0.81	0.81	0.75	

Table 1 (continued)

Tradition (Germany)	α-acids	4.0–7.0[2)]	8.24 ± 0.11	6.97 ± 0.24	6.42 ± 0.05	6.05 ± 0.02	26.6
	β-acids	3.0–6.0[2)]	7.98 ± 0.07	7.58 ± 0.21	7.24 ± 0.09	7.28 ± 0.05	8.8
	xanthohumol	0.3–0.5[2)]	0.58 ± 0.00	0.57 ± 0.02	0.55 ± 0.01	0.54 ± 0.01	6.9
	α/β ratio	no data found	1.03	0.92	0.89	0.83	
Triskel (France)	α-acids	8.0–9.0[2)]	1.71 ± 0.01	1.62 ± 0.01	1.66 ± 0.02	1.64 ± 0.03	4.1
	β-acids	4.0–4.7[2)]	3.60 ± 0.05	3.55 ± 0.03	3.61 ± 0.06	3.65 ± 0.08	0.0
	xanthohumol	0.2–0.6[2)]	0.18 ± 0.00	0.18 ± 0.00	0.17 ± 0.00	0.18 ± 0.00	0.0
	α/β ratio	no data found	0.48	0.46	0.46	0.45	

[1)] Nesvadba (2013); [2)] Barth & Schönberger (2014); [3)] Forster & Gahr (2014)

[*]calculated as the arithmetic mean of n = 4 (two repeated injections of two extracts).

The highest contents of β-acids were in Galaxy (8.0%) and Tradition (8.0%), the lowest contents were in Citra (3.0%) and Northern Brewer (2.9%). The greatest differences compared to literature data were found for Galaxy (8.0% vs. 5.0–6.5% in lit.) and Tradition (8.0% vs. 3.0–6.0%). Extremely high value of α/β ratio was found in Citra (3.69), the lowest value in Triskel (0.48). The trend of α/β ratio in analyzed hop varieties was similar as the trend of α-acids contents.

It is generally known that contents and ratios of α- and β-acids can be very variable depending on the year of harvest. For example, in a long-term study of Czech hops (1994–2013) the contents of α-acids in dried hop cones of Saaz variety ranged from 1.85% to 5.41%, the contents of β-acids ranged from 1.56% to 5.36%, and the α/β ratios ranged from 0.60 to 1.40 (Mikyška & Jurková, 2014). These data collected in a very long time period are in a good agreement with reference values given in other literature sources (Nesvadba, 2013) and also with data given in our study.

The differences depending on the growing area were also recorded. For example, the average values of α-acids contents in Saaz hop cones from three different growing areas in the Czech Republic (Žatec, Úštěk and Tršice) harvested in 2013 were resp. 3.5%, 3.2% and 2.9% (Mikyška & Jurková, 2014). The declared contents of β-acids in variety Cascade from Oregon (USA) were 4.8–7.0%, in the same variety from Hallertau (Germany) only 3.4% (Barth & Schönberger, 2014). Further literature data about varieties Northern Brewer and Triskel, which showed the most remarkable differences in our study, were not available according to our best knowledge and analyses of these varieties should be the subject of additional investigations.

After nine months of storage at 4 °C, the contents of α- and β-acids decreased in most of analyzed varieties. The relative decline of α-acids was more distinctive than the decline of β-acids; the α/β ratio decreased in all cases consequently. The loss of α-acids ranged from 4.1% (Citra and Triskel) to 66.4% (Galaxy), the loss of β-acids ranged from zero (Northern Brewer and Triskel) to 31.3% (Galaxy). Generally, the highest losses of both groups of acids occurred in Galaxy, where the value of α/β ratio changed remarkably from 1.67 to 0.81% during storage.

Relatively low losses of β-acids measured in this study are in a certain disagreement with a generally accepted opinion about their low stability. For example, the loss of pure β-acids after their 96 h deposition on an inert carrier (micronized cellulose) under open air was 94% at 4 °C and 25.7% at -18 °C (Krofta & Mikyška, 2014); the same authors also refer to low stability of β-acids in dried hop cones stored under open air. On the base of all these results it is possible to assume that principal factors causing low stability of β-acids are influence of oxygen and storage temperature. On the contrary, relatively simple adjustment of storage conditions can improve the stability of these compounds.

CONCLUSIONS

The measured contents of α- and β-acids in eight analyzed hop varieties were in most cases in a good agreement with literature data excluding varieties Tradition (much higher measured contents), Northern Brewer and Triskel (much lower measured contents). The measured contents of xanthohumol responded very well to literature data with the exception of Northern Brewer. According to the results acquired in this study, variety Galaxy was found as the richest in all three analyzed parameters (α-acids, β-acids, xanthohumol).

After nine months of storage at 4 °C, considerable decline of α-acids was observed, but substantial differences among analyzed varieties were recorded. The losses of β-acids and xanthohumol were less distinctive and indicated good long storage possibilities of these compounds at convenient conditions (low temperature, elimination of direct oxygen influence).

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REFERENCES

Barth, S. & Schönberger, C. (Eds.). 2014. *The Hop Aroma Compendium. A Flavour Guide. Volume 1–3.* Fachverlag Hans Carl, Nuremberg, 270 pp.

Češlová, L., Holčapek, M., Fidler, M., Drštičková, J. & Lísa, M. 2009. Characterization of prenylflavonoids and hop bitter acids in various classes of Czech beers and hop extracts using high performance liquid chromatography-mass spectrometry. *Journal of Chromatography. A* **1216**, 7249–7257.

European Brewery Convention. 2013. *Analytica-EBC (on-line version): Hops and Hop Products.* http://www.analytica-ebc.com

Forster, A. & Gahr, A. 2014. A comparison of the analytical and brewing characteristics of Cascade and Comet hop varieties as grown in Yakima (USA) and Hallertau (Germany). *BrewingScience* **67**, 137–148.

Hermans-Lokkerbol, A.C.J. & Verpoorte, R. 1994. Development and validation of a high-performance liquid chromatography system for the analysis of hop bitter acids. *Journal of Chromatography A* **669**, 65–73.

Krofta, K. 2008. *Evaluation of hops quality.* Hop Research Institute, Co., Ltd., Žatec, Czech Republic, 52 pp. (in Czech).

Krofta, K. & Mikyška, A. 2014. Hop beta-acids: Properties, significance and utilization. *Kvasny Prum.* **60**, 96–105.

Mikyška, A. & Jurková, M. 2014. Evaluation of bitter acids and polyphenols content in Czech hops harvest in 2013 – I: Contents of α- and β-bitter acids. *Kvasny Prum.* **60**, 88–95.

Nesvadba, V. (Ed.). 2013. *Development and Tradition of Czech Hop Varieties.* Hop Research Institute, Co., Ltd., Žatec, Czech Republic, 104 pp.

Saugspier, M., Dorn, C., Czech, B., Gehrig, M., Heilmann, J. & Hellerbrand, C. 2012. Hop bitter acids inhibit tumorigenicity of hepatocellular carcinoma cells in vitro. Oncol. Rep. 28, 1423–1428

Stevens, J.F. & Page, J.E. 2004. Xanthohumol and related prenylflavonoids from hops and beer: to your good health! *Phytochemistry* **65**, 1317–1330.

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Cadmium-induced oxidative damage and protective action of fractioned red beet (*Beta vulgaris*) root juice in chickens

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Abstract. Cadmium (Cd) is one of the most dangerous environmental bioaccumulative pollutants that affects many organs in humans and animals. Present investigation was conducted to evaluate the protective effect of fractioned red beetroot juice on Cd-induced oxidative stress in chickens. The named red beetroot juice fraction (BJF) was received by juice ultrafiltration. Per oral administration of BJF for 10 days followed by dietary Cd exposure (50 mg kg^{-1} of diet) was evaluated in the *in vivo* experiments in chickens. The prominent increase of Cd concentration in blood plasma, liver and kidney provoked the rise of oxidative processes activity in organs. BJF treatment attenuated the Cd-induced oxidative stress. The changes of oxidative stress markers - the reduction of hepatic and kidney malondialdehyde amount, the increase of glutathionperoxidase level in liver and blood catalase activity indicated the possible antioxidative influence of BJF. Chickens exposed to Cd showed no evidence of clinical toxicity, but exhibited some features of adverse action of this heavy metal. The increase of uric acid concentration in blood serum is associated with protein catabolic processes intensified by Cd affect. Suppressive effect of Cd on the immune response in chickens manifested in alteration of cell and humoral immunity parameters. The data of the most investigated oxidative stress markers, biochemical and immunological indices in Cd-exposed chickens were almost back to the values, when received BJF *per os* during 10 days. Administration of fractioned red beetroot juice to Cd-treated chickens prevented the oxidative impact of this heavy metal and provided immunomodulating effect.

Key words: oxidative stress, cadmium, red beetroot juice, antioxidative effect, chickens.

INTRODUCTION

Heavy metal cadmium (Cd) is a naturally occurring element that is present everywhere in the environment – in almost all soils, surface waters, plants and wildlife. In comparison with other heavy metals, Cd possesses high mobility in soil and is taken up by plants in various degrees (Kah et al., 2012; Brzóska et al., 2016). Cd content in the environment is increasing, because this element is continuously released from its natural sources (the Earth's crust), and introduced through various human activities, including industrial processes, it does not undergo biodegradation (Moulis & Thévenod, 2010).

Cd intake by humans mainly occurs via the food chain and this heavy metal is considered one of the most dangerous occupational and environmental poisons. Hazards

from Cd are associated with its high bioaccumulative capacity. Adverse human, animal and plant physiological effects of Cd are numerous (Bernhoft, 2013). At the cellular level, the pro-oxidative Cd action results in oxidative damage to the cellular macromolecules and cellular structures (Curcic et al., 2014; Rahman et al., 2017). Disbalanced antioxidant system and developed oxidative stress produce injury to healthy tissues and immune cells by free radicals, which results in compromised immune functions in chickens (Vasiljeva, Berzina & Remeza, 2011). Cd is among the immunosuppressive agents, it excessive and even low doses in daily food can cause diverse physiological and immunological disturbances in human, animals and poultry (Bokori & Fekete, 1995; El-Boshy et al., 2015).

Since Cd-induced damage in an organism is believed to be irreversible, the question of primary prevention is of great importance. Efficient beneficial results may be achieved by addition of nutrients with antioxidative properties to the diet (Nair et al., 2013). Antioxidants are known to play a vital role in the health due to cell protection from damage induced by oxidative stress (Surai, 2003; Brzóska & Rogalska, 2013).

Cultivated forms of red beet (*Beta vulgaris L.*) have been used for medical purposes since ancient times. In recent years, interest in the biological activity of red beets and its potential utility as a functional nutrition and disease prevention has been growing. Red beetroot juice is also considered to promote therapeutic treatment in a number of clinical pathologies associated with oxidative stress and inflammation (Clifford et al., 2015; Cho et al., 2017).

Red beet contains betalain pigments belonging to the group of cationic antioxidants. Betalains are divided into two groups: red betacyanins (predominantly betanin) and yellow betaxanthins (vulgaxanthine I and vulgaxanthine II). Red betanin is primarily responsible for the antioxidant ability of red beets (Czapski et al., 2009). Betanin is both a free radical scavenger and an inducer of an antioxidant defense mechanism in cells. Its effect is dose dependent (Esatbeyoglu, 2014). Attempts to concentrate the fraction of juice betalains by gel filtration on Sephadex using juice extracts in water or ethanol were unsuccessful (Lee et al., 2005). Despite the failure of mentioned experiment the idea of red beetroot fractionation seems prospective. Recently, using the modern method of membrane separation (diafiltration) (Mereddy et al., 2017), as well as ultrafiltration (Krumina et al., 2016), allowed to obtain more concentrated pigment fractions of beet juice. In these cases, freshly squeezed beet juice was used. Ultrafiltration is more suitable for industrial use in comparison with chromatography. Ultrafiltration as industrial technology has several advantages over chromatography. Ultrafiltration use is cheaper, has higher productivity and provides possibility to prepare natural compounds product with more accurate parameters (i.e. cut off point).

Present investigation was conducted to evaluate the protective effect of red beetroot juice fraction (BJF) in chickens exposed to Cd.

MATERIALS AND METHODS

Ethics statement

All experimental procedures were approved by the Animal Ethics Committee of the Food and Veterinary Service (Riga, Latvia, authorisation reference number 13, December 22, 2008).

Animals and experimental design

35-day-old male Lohmann brown chickens were obtained from SIA BALTICOVO (Iecava, Latvia) and used for the laboratory investigations. Chickens were divided into four groups of 7 heads each. Food and water were provided *ad libitum* for 10 days. Group 1 (Control) received the fool-fed basal diet without any supplements. Group 2 (+Cd) was given the same diet with addition of Cd 50 mg kg^{-1} (as $CdCl_2$, Sigma, EU). Group 3 (+BJF) received the basal diet without any supplements and each chicken was administered by 1 mL of BJF *per os* daily. Group 4 (+Cd+BJF) was fed the same diet supplemented with Cd as group 2 and administered by 1 mL of BJF *per os* daily. The chosen high experimental level of dietary Cd exposure was based on our previous unpublished experimental data and had hazard effect for 35–45-days old chickens. It allows investigate a risk of health damage by this heavy metal. In accord with our previous study the best administered dose of BJF for chicken was 1 mL per one head (Smirnova et al., 2017). Red beet (*Beta vulgaris*) root juice fraction (BJF) was produced using the laboratory equipment**.** The red beetroots were washed, skinned, shredded, and the juice was extracted mechanically by juice press. The juice was deproteinized using heating at 85ºC for 10 min followed by centrifugation. The supernatant was fractionated with ultrafiltration using ultrafilter membrane with cut-off-point 150 KDa. 100 mL of the obtained fraction was contained: 65.80 mg of betanin, 40.10 mg of vulgaxanthine-I, 7.80 g of sucrose, 11.00 mg of ascorbic acid, 1.20 mg of lysozyme and 0.20 mg of iron. Obtained ultrafiltrate BJF was used in the experiment.

At the end of experiment, chickens were weighed and sacrificed by decapitation in accordance with the Recommendation for Experimental Animals of the European Convention (Close et al., 1997). Whole blood, blood plasma, liver, kidneys of chickens were collected and used for analyses.

Biochemical assays

Cd determination of the tissue samples was performed after dry ashing in atomic absorption spectrophotometer Perkin-Elmer (model AAnalyst 700), according to the procedures of the AOAC (1999). The antioxidant status was evaluated by measuring the level of lipid peroxidation product malondialdehyde (MDA) in liver and kidney homogenates by the thiobarbituric acid reaction (Surai et al., 1996). Activity of glutathionperoxidase (GSH-Px) in liver homogenate was measured based on the method described by Pinto and Bartley (Pinto & Bartley, 1969) with some modifications. Catalase (CAT) activity in blood was estimated by the method of Aebi (Aebi, 1984). The determination of uric acid in blood serum was performed enzymatically (Trivedi et al., 1978). Hemoglobin (Hb) level was estimated by cyanohemoglobin method with commercial kit (SIA 'Divi Dent', Latvia). Total protein concentration in blood plasma was determined spectrophotometrically by the biuret method, using a commercial kit (Boehringer, Germany).

Immunological analyses

Immune cell T- and B- populations were estimated as responses to T- and B-markers: sheep erythrocytes for T-cells and zymozan (C3 complexes) for B-cells. Serum lysozyme content was evaluated by nefelometric assay (Shugar, 1952) with some modification, by absorptiometric determination of the decrease in turbidity of a suspension of *Micrococcus lysodeicticus.* Nonspecific circulating immune complexes

(CIC) in serum were estimated spectrophotometrically using precipitation with polyethylenglycol (Riha et al., 1979).

Statistical analysis

All statistics were performed using the software *Statistica* 7. Results of Cd content and biochemical parameters are presented as mean ± SE. Multiple group comparision was done using *one-way* ANOVA and *Post-hoc* Tukey HSD test.

RESULTS AND DISCUSSION

In general, the variance analysis revealed that there was a statistically significant difference between the treatment groups of the experiment for most of analyzed parameters, except MDA level in chicken liver (Table 1).

Table 1. Results of multiple group comparison with one-way ANOVA, analysing Cd content and biochemical parameters in chickens under Cd exposure and administration with red beetroot juice fraction (BJF) (in bold – significant *P* values, $P < 0.05$, n = 28, df = 3)

Parameter	F*, stated	P-value	F, critical
Cd, blood plasma	250.097	**3.12346E-18**	3.009
Cd, liver	1899.165	**1.19352E-28**	3.009
Cd, kidney	703.131	**1.64691E-23**	3.009
MDA, liver	2.174	0.11734499	3.009
MDA, kidney	51.925	**1.20918E-10**	3.009
GSH-px	27.599	**5.95067E-08**	3.009
Catalase	5.994	**0.003371862**	3.009
Hemoglobin	34.762	**6.73753E-09**	3.009
Total protein	45.681	**4.49054E-10**	3.009
Uric acid	29.528	**3.18001E-08**	3.009
T- lymphocytes	64.860	**1.17286E-11**	3.009
B(C3)- lymphocytes	10.276	**0.000153855**	3.009
Lysozyme	22.609	**3.56601E-07**	3.009
CIC	28.842	**3.95871E-08**	3.009

* Fisher's value.

The Cd content in blood plasma, liver and kidney of chickens did not differ between the control group and the 3rd group, which received BJF (Table 2). Additional Cd administration provided a significant increase of this heavy metal concentration in chickens: by 2.3 times in blood plasma, 80 times and 93 times in liver and in kidney, respectively, compared to the control. After additional per oral intake of BJF reduced Cd accumulation in chickens of the 4th group was found: in blood and liver by 1.3 times and by 1.2 times, respectively. On contrary, there was a slight increase in Cd content by 1.1 times in kidney of the group 4 compared to the single Cd treatment (group 2). The observed increase of Cd concentration in kidneys can be associated with the growing intensity of removing of this heavy metal from organism (Zabulyte et al., 2007).

A marked increase of Cd concentration in blood plasma, liver and kidneys of chickens is accompanied by enhance of oxidative processes in organs (Erdogan et al., 2005; Berzina et al., 2007). MDA is one of the final products polyunsaturated fatty acids peroxidation in cells. This is the final product of lipid peroxidation. An increase in free

radicals causes overproduction of MDA followed by cell oxidative injury (Babu et al., 2006; Moitra et al., 2014). Cd triggers adverse effects in organs *via* oxidative stress induction. With Cd dietary overload, peroxidation of membrane lipids causes liver and kidney injury by free radical generation, as evidenced by an increase in MDA production (Nemmiche, 2017). It is known that one of the most sensitive organs to the action of Cd is kidney (Surai, 2003). Addition of Cd in chicken diet resulted in a significant increase of MDA level in kidney by 25.4% (Table 3). Administration of BJF to Cd-treated chickens slightly reduced this effect in kidneys. MDA level decreased by 7.0% in comparison with the group 2. The level of hepatic MDA in chickens of (+ BJF) and (+ Cd + BJF) groups did not differ from the control data.

Table 2. The effect of red beetroot juice fraction (BJF) administration on cadmium (Cd) concentration in chicken's organs after Cd dietary intake

Group	Cd, $\mu g \cdot g^{-1}$		
	Blood plasma	Liver	Kidney
1. Control	$0.044 \pm 0.002^{a*}$	0.12 ± 0.01^{a}	0.20 ± 0.03^{a}
2. + Cd	0.103 ± 0.005^{c}	9.61 ± 0.48^{c}	18.6 ± 1.56^{b}
3. + BJF	0.046 ± 0.007^{a}	0.10 ± 0.05^{a}	0.19 ± 0.08^{a}
4. + Cd + BJF	0.080 ± 0.003^{b}	8.05 ± 0.35^{b}	20.6 ± 1.65^{b}

* Statistically different within column according to Post-hoc Tukey's test ($P < 0.05$).

Cd is known to cause oxidative stress by increasing lipid peroxidation. This heavy metal may damage enzymatic antioxidative defense system and disturb oxidative/ antioxidative status of the cells (Nair et al., 2013). GSH-Px is selenium-dependent enzyme that catalyses the reduction of hydroxyperoxides by glutathione. Its main function is to protect against the damaging effect of endogenously formed hydroxyperoxides (Galazyn-Sidorczuk et al., 2012). It was established, that Cd exposure to rats caused the inhibition of GSH-Px and CAT activities in kidneys and liver (Jihen et al., 2009). The results of our study demonstrated that the level of hepatic GSH-Px decreased in Cd-treated groups (2 and 4) by 14.6% and 11.5%, respectively, compared to the control.

Table 3. The effect of red beetroot juice fraction (BJF) administration on oxidative stress indices in organs of Cd-treated chickens

Group	MDA, mM g^{-1}		GSH-Px, mM GSH min^{-1} g^{-1}	Catalase, k g^{-1} Hb
	Liver	Kidney	Liver	Blood
1. Control	$17.7 \pm 0.76^{a*}$	13.8 ± 0.59^{a}	9.60 ± 0.23^{a}	56.4 ± 2.52^{a}
2. + Cd	18.3 ± 0.68^{a}	17.3 ± 0.56^{b}	8.20 ± 0.28^{b}	50.6 ± 2.25^{b}
3. + BJF	17.6 ± 0.83^{a}	14.1 ± 0.27^{a}	9.51 ± 0.19^{a}	57.0 ± 1.98^{a}
4. + Cd+BJF	17.4 ± 0.69^{a}	16.1 ± 0.60^{b}	8.50 ± 0.36^{b}	56.9 ± 2.00^{a}

* Statistically different or similar within column according to Post-hoc Tukey's test ($P < 0.05$).

CAT is a common antioxidant enzyme, which is produced naturally in almost all living organisms. It is very important enzyme in protecting the cell from oxidative damage by reactive oxygen species. The effects of the exposure to Cd on CAT activities have been studied rather extensively and are depending on the experimental conditions (Wang et al., 2015). Cd stimulates the formation of reactive oxygen species, thus causing

oxidative damage to erythrocytes and various tissues resulting in loss in membrane functions (Sarkar et al., 1998). The activity of CAT in blood of Cd-exposed chickens significantly decreased by 10.3 % compared to the enzyme activity of the control group (Table 3). It characterizes the disturbing action of Cd on enzyme state. BJF administration to chickens of the group 4 showed the increase of CAT activity to the same level as in control data. The restored changes of GSH-Px and CAT activities in chicken tissues of group 4 indicated a protective effect of BJF against toxic impact of Cd. It is notable that MDA level and investigated enzymes activities did not change in chickens administered by BJF alone (group 3) compared to the control.

There was no difference between Cd-treated chickens and control group in blood hemoglobin level and concentration of total protein in blood plasma (Table 4). After intake of BJF an increase of hemoglobin index and total protein level in blood plasma of chickens was found compared to the control. Administration of BJF alone also increased hemoglobin level in chicken blood.

Table 4. The influence of red beetroot juice fraction (BJF) intake on hemoglobin and protein metabolic indices in Cd-treated chickens

Group	Blood	Blood plasma	
	Hemoglobin, g dl^{-1}	Total protein, mg L^{-1}	Uric acid, g dl^{-1}
1. Control	$8.63 \pm 0.12^{a*}$	28.95 ± 0.33^{a}	2.51 ± 0.28^{a}
2. + Cd	8.63 ± 0.06^{a}	28.93 ± 0.80^{a}	3.31 ± 0.12^{b}
3. + BJF	9.15 ± 0.18^{b}	29.01 ± 0.46^{a}	2.60 ± 0.17^{a}
4. + Cd+ BJF	9.21 ± 0.14^{b}	32.74 ± 1.07^{b}	2.82 ± 0.07^{a}

* Statistically different within column according to Post-hoc Tukey's test ($P < 0.05$).

Cd exposure caused the intensification of protein catabolic processes. It is mainly related to a risk of damage in kidneys (Ferraro et al., 2010; Akesson et al., 2014; Wallin et al., 2014). Uric acid in blood plasma, a product of protein catabolism, may be considered as a marker of kidney function in organism (Cohen et al., 2007; Braun & Sweazea, 2008). It is in accord with the data of our study. Significant increase of uric acid concentration in blood plasma was recorded for chickens of the group 2 by 31.9% (Table 4). The elevated accumulation of uric acid in chickens of group 2 suggests Cd-induced disturbance of kidney excretion function associated with a change in purine metabolism intensity. Although uric acid acts as an antioxidant and has a free-radical scavenging effect, when accumulates to a high level in blood it can cause health problems (Mielcarz et al., 2006). The BJF intake provided a decline in Cd adverse effect on protein metabolism in chickens. The BJF administered alone did not significantly influence the protein catabolic processes in chickens.

Suppressive Cd action on chicken immune response was manifested by a significant decrease of cell-mediated parameters in the group 2 (Table 5). Compared with the control values the count of T-lymphocytes decreased by 58.3%, and B (C3)-lymphocyte number – by 33.3%.

The influence of Cd on humoral immunity also was unfavourable. The data of serum lysozyme and CIC illustrated the response of nonspecific immunity on Cd exposure and BJF intake in chickens. Lysozyme concentration in Cd-treated chickens was significantly lower than in control group. The level of nonspecific antigen-antibody circulating complexes in blood serum of Cd-exposed chickens increased by 19.8%

compared to the control. The results of immunological studies showed that intake of BJF eliminated a Cd immunosuppressive impact in chickens, but it was not notably effective when administered alone.

Table 5. Cd-induced immunological changes and the effect of red beetroot juice fraction (BJF) administration in chickens

Group	Blood		Blood serum	
	T-lymphocytes, %	B(C3)-lymphocytes, %	Lysozyme, μg mL^{-1}	Circulating immune complexes (CIC), extinction x 100
1. Control	$36.0 \pm 3.0^{a*}$	15.0 ± 1.8^{a}	8.0 ± 1.23^{a}	3.1 ± 0.11^{a}
2. + Cd	15.0 ± 4.0^{b}	10.0 ± 2.1^{b}	4.7 ± 0.88^{b}	3.7 ± 0.14^{c}
3. + BJF	34.0 ± 2.0^{a}	16.0 ± 3.0^{a}	7.8 ± 1.08^{a}	3.2 ± 0.15^{ab}
4. + Cd+ BJF	31.0 ± 3.2^{a}	12.0 ± 2.0^{b}	5.0 ± 0.76^{b}	3.3 ± 0.12^{b}

* Statistically different within column according to Post-hoc Tukey's test ($P < 0.05$).

The results of biochemical and immunological studies are in accordance with chicken growth parameters. Chickens exposed to Cd showed no evidence of clinical toxicity. After 10 days of Cd treatment, chickens body weight did not differ from the control data. Chicken body weight of the control and Cd-treated groups was 475.4 ± 24.5 g and 474.4 ± 60.2 g, correspondingly. BJF administration had a positive influence on the chicken body weight both of the 3rd (485.3 ± 51.2 g) and the 4th Cd-exposed (484.1 ± 39.9 g) groups.

The observed suppressive action of Cd supplementation in chicken diet during 10 days was expressed by an increase of Cd accumulation in kidneys, liver and blood plasma; development of oxidative stress in organs; disturbance of protein metabolic processes; decrease of cell-mediated and humoral immune response.

Administration of 1 mL BJF *per os* daily alone had no significant effect on the investigated parameters, but prevented the Cd-induced damage in chickens. Experimental betalain rich fraction from red beetroot juice was obtained using ultrafiltration. Antioxidative capacities of BJF provide pigments of betalain group, including betanin. Betanin is both a free radical scavenger and an inducer of antioxidant defense mechanism in cells (Esatbeyoglu et al., 2014). The similar protective effect of plant extract (*Fragaria ananassa*) against Cd adverse impact also was observed in rats, because of its polyphenolic composition, described by Elkhadragy et al. (2018).

CONCLUSION

Administration of red beetroot (*Beta vulgaris*) juice fraction to Cd-treated chickens during 10 days protected against Cd-induced oxidative damage and provided immunomodulating effect.

REFERENCES

Aebi, H. Catalase *in vitro*. 1984. *Methods Enzymology* **105**, 121–126.

AOAC. 9.1.09 Official Method. 1999. *https://www.scribd.com/document/357719355/9-1-09-AOAC-Official-Method-999-11-Determination-of-Lead-Cadmium-pdf.*

Akesson, A., Barregard, L., Bergdahl, I.A., Nordberg, G.F., Nordberg, M. & Skerfving, S. 2014. Non-renal effects and the risk assessment of environmental cadmium exposure. *Environmental Health Perspectives* **122**, 431–438.

Babu, K.R., Rajmohan, H.R. Rajan, B.K. & Kumar, K.M. 2006. Plasma lipid peroxidation and erythrocyte antioxidant enzymes status in workers exposed to cadmium. *Toxicology & Industrial Health* **22**, 329–335.

Bernhoft, R.A. 2013. Cadmium toxicity and treatment. *Scientific World Journal*. Article ID 394652, 7pp. http://dx.doi.org/10.1155/2013/394652.

Berzina, N., Markovs, J., Isajevs, S., Apsite, M. & Smirnova, G. 2007. Cadmium-induced enteropathy: biochemical and histological study. *Basic & Clinical & Pharmacology & Toxicology* **101**(1), 29–34.

Bokori, J. & Fekete, S. 1995. Complex study of the physiological role of cadmium. 1. Cadmium and its physiological role. *Acta Veterinaria Hungarica* **43**(1), 3–43.

Braun, E.J. & Sweazea, K.L. 2008. Glucose regulation in birds. *Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology* **151**, 1–9.

Brzóska, M., Borowska, S. & Tomczyk, M. 2016. Antioxidants as a potential preventive and therapeutic strategy for cadmium. *Current Drug Targets* **17**, 1350–1384.

Brzóska, M. & Rogalska, J. 2013. Protective effect of zinc supplementation against cadmium-induced oxidative stress and the RANK/RANKL/OPG system imbalance in the bone tissue of rats. *Toxicology and Applied Pharmacology* **272**, 208–220.

Cho, J., Bing, S.J., Kim, A., Lee, N.H., Byen, S.H., Kim, G.O. & Jee, Y. 2017. Beetroot (*Beta vulgaris*) rescues mice from gamma-ray irradiation by accelerating hematopoiesis and curtailing immunosuppression. *Pharmaceutical Biology* **55**, 306–316.

Clifford, T., Howatson, G., West, D.J. & Stevenson, E.J. 2015. The potential benefits of red beetroot supplementation in health and disease. *Nutrients* **7**(4), 2801–2822.

Close, B., Banister, K., Baumans, V., Bernoth, E.M., Bromage, N., Bunyan, J., Gregory, N., Hackbarth, H., Morton, D. & Warwick, C. 1997. Recommendation for euthanasia of experimental animals. Part 2. DGXT of the European Commission. *Laboratory Animals* **31**, 1–32.

Cohen, A., Klasing, K. & Ricklefs, R. 2007. Measuring circulating antioxidants in wild birds. *Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology* **147**, 110–121.

Curcic, M., Durgo, K., Kopjar, N., Ancic, M., Vucinic, S. & Antonijevic, B. 2014. Cadmium and decabrominated diphenyl ether mixture: In vitro evaluation of cytotoxic, prooxidative and genotoxic effects. *Environmental Toxicology and Pharmacology* **38**(2), 663–671.

Czapski, J., Mikołajczyk, K. & Kaczmarek, M. 2009. Relationship between antioxidant capacity of red beet juice and contents of its betalain pigments. *Polish Journal of Food and Nutrition Sciences* **59**(2), 119–122.

El-Boshy, M.E., Risha, E.F., Abdelhamid, F.M., Mubarak, M.S. & Hadda, T.B. 2015. Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. *Journal of Trace Elements in Medicine and Biology* **29**, 104–110.

Elkhadragy, M., Al-Olayan, E., Al-Amiery, A. & Moneim, A. 2018. Protective effects of *Fragaria ananassa* extract against cadmium chloride-Induced acute renal toxicity in rats. *Biological Trace Element Research* **181**(2), 378–387.

Erdogan, Z., Erdogan, S., Celik, S. & Unlu, A. 2005. Effects of ascorbic acid on cadmium – induced oxidative stress and performance of broilers. *Biological Trace Element Research* **104**(1), 19–32.

Esatbeyoglu, T., Wagner, A.E., Motafakkerazad, R., Nakajima, Y., Matsugo, S. & Rimbach, G. 2014. Free radical scavenging and antioxidant activity of betanin: electron spin resonance spectroscopy studies and studies in cultured cells. *Food and Chemical Toxicology* **73**, 119–126.

Ferraro, P.M., Costanzi, S., Naticchia, A., Sturniolo, A. & Gambaro, G. 2010. Low level exposure to cadmium increases the risk of chronic kidney disease: analyses of the NHANES 1999-2006. *BMC Public Health* **10**, pp. 304.

Galazyn-Sidorczuk, M., Brzóska, M., Rogalska, J., Roszczenko, A. & Jurczuk, M. 2012. Effect of zinc supplementation on glutathione peroxidase activity and selenium concentration in the serum, liver and kidney of rats chronically exposed to cadmium. *Journal of Trace Elements in Medicine and Biology* **26**, 46–52.

Jihen, E., Imed, M., Fatima, H. & Abdelhamid, K. 2009. Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver of the rat: Effects on the oxidative stress. *Ecotoxicology and Environmental Safety* **72**(5), 1559–1564.

Kah, M., Levy, L. & Brown, C. 2012. Potential for effects of land contamination on human. 1. The case of cadmium. *Journal of Toxicology and Environmental Health Part B* **15**, 348–363.

Krumina, G., Babarykin, D., Smirnova, G., Vasiljeva, S., Basova, N., Krumina, Z., Babarykina, A. & Markov, J. 2016. Red beet (*beta vulgaris*) root juice membrane ultrafiltration use to modify product's functionality. In Jakobsone, I. (ed): *2nd International Conference Nutrition and Health, Conference Program and Book of Abstracts*, Rīga, University of Latvia Press, pp. 66.

Lee, C.H, Wettasinghe, M, Bolling, B.W, Ji, L.L. & Parkin, K.L. 2005. Betalains, phase II enzyme-inducing components from red beetroot (*Beta vulgaris* L.) extracts. *Nutrition and Cancer* **53**(1), 91–103.

Mereddy, R., Chan, A., Fanning, K., Nirmal, N. & Sultanbawa, Y. 2017. Betalain rich functional extract with reduced salts and nitrate content from red beetroot (*Beta vulgaris* L.) using membrane separation technology. *Food Chemistry* **215**, 311–317.

Mielcardz, G., Linke, K., Barinow-Wojewodzki, A. & Morawska-Staszak, K. 2006. Effect of creatine supplementation on selenium, copper and zinc status in patients with chronic liver disease. In Anke, M., Flachovsky, G., Kisters, K., Shafer, U., Schekel, H., Seifert, M. & Stoeppler, M. (eds): *Macro and Trace elements, 23. Workshop, September 27. Friedrich Schiller University Jena*, pp. 632–634.

Moitra, S., Brashier, B. & Sahu, S. 2014. Occupational cadmium exposure associated oxidative stress and erythrocyte fragility among jewelry workers in India. *American Journal of Industrial Medicine* **57**, 1064–1072.

Moulis, J-M. & Thévenod, F. 2010. New perspectives in cadmium toxicity: and introduction. *BioMetals* **23**(5), 763–768.

Nair, A.R., De Gheselle, O., Smeets, K., Van Kerkhove, E. & Cuypers, A. 2013. Cadmium-induced pathologies: where is the oxidative balance lost (or not)? *The International Journal of Molecular Sciences* **14**, 6116–6143.

Nemmiche, S. 2017. Oxidative signaling response to cadmium exposure. *Toxicological Sciences* **156**(1), 4–10.

Pinto, R.E. & Bartley, W. 1969. The effect of age and sex on glutathione reductase and glutathione peroxidase activities and on aerobic glutathione oxidation in rat liver homogenates. *Biochemical Journal* **112**(1), 109–115.

Rahman, M., Ukiana, J., Uson-Lopez, R., Sikder, M.T., Saito, T. & Kurasaki, M. 2017. Cytotoxic effects of cadmium and zinc co-exposure in PC12 cells and the underlying mechanism. *Chemico-Biological Interactions* **269**, 41–49.

Riha, I., Haskova, V., Kaslik, J., Maierova, M. & Stransky, J. 1979. The use of polyethylene glycol for immune complex detection in human sera. *Molecular Immunology* **16**, 489–493.

Sarkar, S., Yadav, P. & Bhatnagar, D. 1998. Lipid peroxidation damage on cadmium exposure and alterations in antioxidant system in rat erythrocytes: a study with relation to time. *BioMetals* **11**(2), 153-157.

Shugar, D. 1952. The measurement of lysozyme activity and the ultra-violet inactivation of lysozyme. *Biochimica et Biophysica Acta* **8**(3), 302–309.

Smirnova, G.Ju., Vasiljeva, S.V., Basova, N.A., Babarykin, D.A. & Krumina, G.G. 2017. Effect of betain – rich fraction of beet root juice under high dietary level of cadmium. *Russian Journal of Gastroenterology, Hepatology, Coloproctology* **27**(5), Suppl. 50, pp. 89 (in Russian).

Surai, P., Noble, R. & Speake, B. 1996. Tissue-specific differences in antioxidant distribution and susceptibility to lipid peroxidation during development of the chick embryo. *Biochimica et Biophysica Acta* **1304**, 1–10.

Surai, P. 2003. *Natural antioxidants in avian nutrition and reproduction*. Nottingham University Press, Nottingham, England. 651 pp.

Trivedi, R.C., Rebar, L., Berta, E. & Stong, L. 1978. New enzymatic method for serum uric acid at 500 nm. *Clinical Chemistry* **24**(11), 1908–1911.

Vasiljeva, S., Berzina, N. & Remeza, I. 2011. Dietary modulation of immune responses by ascorbic acid in healthy and cadmium-induced immunocompromised chickens. *Proceedings of the Latvian Academy of Sciences* **65**(3/4), 110–116.

Wallin, M., Sallsten, G., Lundh, T. & Barregard, L. 2014. Low-level cadmium exposure and effects on kidney function. *Occupational and Environmental Medicine* **71**, 848–854.

Wang, J., Zhang, H. & Zhang, T. 2015. Molecular mechanism on cadmium-induced activity changes of catalase and superoxide dismutase. *International Journal of Biological Macromolecules* **77**, 59–67.

Zabulyte, D., Paltanaviciene, A., Uleckiene, S., Kalibatas, J., Juozulynas, A., Jascaninienė, N., Sąlyga, J. & Stosik, M. 2007. Cadmium accumulation in the kidneys and its excretion with urine, and possible influence of PAH's on these processes in rats. *Bulletin of the Veterinary Institute in Pulawy* **51**(2), 257–260.

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Meat chemical composition of pasture pure lambs and crossbreeds

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Abstract. Increased customer interest of safe, healthy and environmentally friendly food consumption promote development of sheep farming industry in Latvia. Aim of the paper – explain of different pasture-fattened sheep breed and their crosses lamb meat composition traits. A study of pasture fattened lamb meat chemical composition was carried out from year 2013 to 2017. Fattening lambs were kept in breeding rams control station 'Klimpas' (57°50'58.8''N 25°19'39.6''E) pasture array. Lambs were slaughtered in a certified slaughterhouse, but analysis of meat chemical composition were conducted in laboratory of Institute of Food Safety, Animal Health and Environment (BIOR). For the analysis of the meat chemical composition were used up to 1 kg heavy *Quadriceps femoris* muscle samples. In meat were analysed following elements of its chemical composition: dry matter, protein, fat, minerals, pH, cholesterol and unsaturated fatty acids. Data analysis shows that the lambs before slaughter ranged in age from 5 to 8 months. Lamb meat obtained from the study groups had a significantly different total amount of dry matter and fat. The lowest total fat, but the highest ash content was obtained in the lamb meat of the extensive breed group. The lowest total fat and the highest ash content were obtained in the lamb meat from the extensive breed group. In meat obtained a small (in individual samples < 0.10%) cis-10-pentadecenoic acid, cis-11-eicosenoic acid and myristoleic acid content. Of unsaturated fatty acids in lamb meat were represented higher amount of oleic acid, linoleic acid and elaidic acid.

Key words: breeds, lamb, pasture, meat, composition.

INTRODUCTION

Rapidly growing consumers interest of safe and healthy food in their diet are encouraging development of sheep husbandry. In particular, this refers to the products of animal origin derived in environmentally friendly farming conditions.

Feeding ruminants with grass-based feed provides cheap and high quality production.

In suckling period lamb diet main nutrients provides milk, after weaning different feedstuffs, for example, meadow hay and concentrates (Scerra, et al., 2007).

Research studies in different animal species meat composition were repeated by Strazdiņa (2014); less studied was the chemical composition of small ruminants.

Latvia Darkhead and crossbreed lamb fattening results in 2005 showed that 10 month old lambs meat contain 25.7% dry matter and in dry matter were 18% protein, 6.6% fat and 1.3% ash (Kairiša, 2005).

Studies of sheep meat chemical composition and its influencing factors are important for farmers and consumers.

It is common practice to make crossbreed lambs by using local breed sheep and different meat type breeds to improve their adaptation to different environmental conditions.

The main focus of scientists in context of meat composition and quality is concerning their age and sex, various feedstuffs and the influence of lamb fattening technologies on their meet quality (Tejeda et al., 2008; Abdullah et al., 2009; Jandasek, 2013).

The aim of study – explain meat chemical composition of pasture fattened pure breed and crossbreed lambs.

MATERIALS AND METHODS

Study was carried out from 2014 to 2017 in Jeri parish, Rujiena region, Latvia. Lambs were kept in pasture all day in one group with unlimited access to hay, mineral licks and water.

Hay contained 869.5 g kg^{-1} of consumed dry matter with 96.6 g kg^{-1} crude protein and 9.72 MJ kg^{-1} metabolizable energy.

Quality of pasture grass varied depending of month. Dry matter in 1 kg grass were in range from 177 g on May to 205.8 g on September, protein in 1 kg dry matter were from 134.9 g on September to 162.5 g on May. Good quality high yielding grass fodder contained, high quality botanical composition and good chemical composition. Grass productivity depends on various factors suchas climate, soil, botanical composition, country and grazing patterns (Priolo et al., 2002; Beyene & Mlambo, 2011; El-Shesheny et al., 2014).

Lamb meat samples used in of Latvia Darkhead, four meat type breeds and most popular crossbreeds among Latvia sheep breeders (Table 1).

Table 1. Study materials

Trial group	Breed (abbreviation)	Number of meat samples
Local sheep breed	Latvia Darkhead (LT)	5
Meat type sheep breeds	Charollais (SA)	4
	Ile de France (IF)	3
	German Merino Local (VMV)	4
	Oxforddown (OX)	5
Latvia Darkhead × meat type sheep crossbreeds	LT × T (Texel)	4
	LT × S (Suffolk)	6
	LT × VMV	5
	LT × Dorper (DOR)	3

Lambs were slaughtered in certified slaughterhouse when reached 40 kg body weight. Analysis of meat chemical composition tested in science centre BIOR laboratory. *Quadriceps femoris* muscles samples were used for chemical content

analysis. In meat established various nutrients: moisture (%), protein (%), crude fat (%), ash (%), pH, cholesterol (mg g^{-1}) and several unsaturated fatty acids (Table 2).

Table 2. The used methods in meat chemical analysis

Meat chemical composition, %	Methods
Moisture	LVS ISO 1442:1997
Protein	LVS ISO 937:1998
Crude fat	LVS ISO 1443:1973
Ash	ISO 936:1998
Cholesterol	BIOR-T-012-132-2011*
Unsaturated fatty acids, %	
Alpha-linolenic acid [C18:3 n3]	BIOR-T-012-131-2011** (fatty acid composition in fats)
Arachidonic acid [C20:4 n6]	
Linolenic acid [C18:2 n6c]	
Oleic acid [C18:1 n9c]	
Palmitoleic acid [C16:1 n9c]	

* Method of gas chromatography; analysed choresterol composition in sample; ** Accredited method of gas chromatography; analysed fatty acid composition in fats.

Data of study was analysed by using mathematical methods. Data obtained from analysis in laboratory were converted to g kg^{-1}. We calculated trait values – mean, standard error and coefficient of variation. Significantly difference between mean values determined by *t*-test and signed with lowercase Latin letters a, b, c ($P \leq 0.05$), also calculated correlation of results.

RESULTS AND DISCUSSION

Average age of lambs before slaughtering was 191 days or little more than 6 months. In meat samples in average 730.2 g kg^{-1} were water, 195.6 g kg^{-1} protein, 67.3 g kg^{-1} crude fat and 10.5 g kg^{-1} ash (Table 3). The chemical composition of Polish Lowlands breed lamb meat samples (taken from *Musculus adductor)* was similar that the present study, it contained 25.55% dry matter, 19.28% protein, 4.15% fat and 1.05% ash (Niedziółka & Pieniak-Lendzion, 2006). Similar nutrient composition in different region lamb meat was shows a similarity of lamb fattening technologies.

Table 3. Meat chemical composition (g kg^{-1}) and pH value in different sheep breed feeding with pasture grass

Trait	LT	SA	IF	OX	VMV
	mean ± standard error				
Age, days	211 ± 12.0	175 ± 17.5	186 ± 14.5	199 ± 12.1	192 ± 4.5
Water, g kg^{-1}	721.4 ± 7.5	753.5 ± 7.1	740.3 ± 9.3	709.2 ± 23.9	728.3 ± 20.8
Dry matter, g kg^{-1}	278.4 ± 7.8	246.5 ± 7.1	259.7 ± 9.3	292.0 ± 24.3	274.3 ± 20
Protein, g kg^{-1}	194.0 ± 3	200.5 ± 2.5	206.0 ± 1.2	195.0 ± 6.6	193.3 ± 3.4
Crude fat, g kg^{-1}	75.6 ± 9.1^{a}	38.3 ± 9.7^{b}	45.7 ± 11.7^{a}	87.4 ± 30.8^{a}	73.3 ± 23.6^{a}
Ash, g kg^{-1}	10.4 ± 0	11.3 ± 0.2	11.1 ± 0.2	10.1 ± 0.4	10.3 ± 0.3
pH, at 20°C	5.70 ± 0.1	5.67 ± 0.	5.77 ± 0.1	5.75 ± 0.1	5.72 ± 0.1
Cholesterol, mg 100 g^{-1}	88.0 ± 10.4^{a}	63.7 ± 5.4^{b}	61.4 ± 3.8^{b}	77.0 ± 12.5^{ab}	88.8 ± 15.5^{ab}

$^{a, b}$ – different letter represent significantly different between meat traits results ($P \leq 0.05$); LT – Latvia Darkhead; SA – Charollais; IF – Il de France; OX – Oxforddown; VMV – German Merino Local.

Age of lambs before slaughtering was in range from 175 days or 5.5 months for Charollais lambs up to 211 days (7 months) for Latvia Darkhead lambs. In end of fattening were recorded large age difference of SA lambs. Age difference of age between SA and LT group lambs was 36 days which did not different significantly.

The maximal protein (206.0 g kg^{-1} IF, 200.5 g kg^{-1} SA) and minimal crude fat (45.7 g kg^{-1} IF and 38.3 g kg^{-1} SA) content in meat were obtained from France origin sheep breeds (Table 4).

Table 4. Chemical content and pH value of crossbreed lamb meat samples

Traits	LT × T	LT × S	LT × DOR	LT × VMV
	mean ± standard error			
Age, days	192 ± 9.8	196 ± 5.7	170 ± 10.7	201 ± 9.1
Water, g kg^{-1}	736.0 ± 16	738.5 ± 14.9	703.3 ± 30.6	730.80 ± 12.5
Dry matter, g kg^{-1}	276.0 ± 12.3	262.5 ± 14.6	296.7 ± 30.5	272.9 ± 12.5
Protein, g kg^{-1}	188.0 ± 2.7	192.8 ± 3.1	190.0 ± 7.8	199.6 ± 5.5
Crude fat, g kg^{-1}	78.5 ± 10.1	60.2 ± 16.4	97.7 ± 38.2	65.2 ± 16.7
Ash, g kg^{-1}	10.0 ± 0.2	10.2 ± 0.3	10.7 ± 0.4	10.5 ± 0.3
pH, at 20°C	5.82 ± 0	5.76 ± 0	5.69 ± 0	5.94 ± 1.6
Cholesterol, mg 100 g^{-1}	66.0 ± 5.6[a]	75.8 ± 8.2[ac]	53.2 ± 4.3[b]	89.5 ± 7[c]

[a, b, c] – Different letter represent significantly differences between meat traits results ($P \leq 0.05$).

Less protein was from LT and VMV lamb meat samples 193.3–194 g. SA and IF lamb meat contained 63.7 mg g^{-1} and 61.4 mg g^{-1}. In a study where used the father breeds as Oxforddown, Texel, Charollais or Suffolk, but as a mother breed German Merino Local in meat had significantly different pH values, protein amount and meat juiciness post mortem 24 hours and 48 hours ($P < 0.05$). Charollais × German Merino Local crossbreed lamb meat had the best quality, meat contained more protein, intramuscular fats, less juice loss and better juiciness and texture (Jandasek et al., 2013).

The amount of cholesterol in different animal meat compared with other food products was low, because it correlated to amount of muscle fibre and its composition. In comparison hen eggs may containe 380 mg g^{-1} and beef, pork and lamb meat 60–70 mg 100 g^{-1} cholesterol (Chizzolini et al., 1999). The youngest lamb in the present study were LT × DOR crossbreed, lamb meat had increased fat and decreased cholesterol (53.2 g mg^{-1}) amount, meat had pH 5.59 as an average.

The pH value of lamb meat varied from 5.5 to 5.9 in an average, 3–4 month of age. Chilled meat from heaviest lambs (19–24 kg body weight) had bigger pH value compared to smaller lambs ($P < 0.01$). Heaviest carcass meat has a stronger taste and aroma (Teixeira et al., 2005).

Most meat food have similar ratio of saturated to unsaturated fatty acid amount, but ruminant meat contained less polyunsaturated fatty acids. Stearic acid (30% of saturated acid amount) have neutral effect to plasma cholesterol synthesis (Bonanome & Grundy, 1988).

The studied meat samples contained 495 g kg^{-1} to 580 g kg^{-1} of unsaturated fatty acids (Table 5).

Table 5. Unsaturated fatty acid amount in purebreds lamb meat samples, g

Unsaturated fatty acid	LT	SA	IF	OX	VMV
	mean ± standard error				
C18:1 n9c	369.5 ± 11.3	347.0 ± 13.2	397.3 ± 26.1	386.8 ± 14.5	360.0 ± 24.5
C18:2 n6c	33.5 ± 3.5^{a}	57.0 ± 10^{ab}	49.7 ± 3^{b}	36.6 ± 4.9^{ab}	36.5 ± 4.5^{ab}
C18:3 n3	11.9 ± 1.9^{a}	24.8 ± 4.2^{b}	21.0 ± 6.7^{ab}	17.0 ± 2.4^{ab}	15.8 ± 1.2^{ab}
C16:1 n9c	17.7 ± 2.6	13.0 ± 1.2	14.7 ± 2.7	17.4 ± 0.9	15.3 ± 3.4
C20:4 n6	2.9 ± 0.1	8.0 ± 3.8	5.7 ± 1.5	5.0 ± 1.6	5.0 ± 2.1

$^{a,\ b}$ – Different letter represent significantly differences between unsaturated fatty acid traits results ($P \leq 0.05$).

From unsaturated fatty acids profile biggest part of lamb fat took oleic acid [C18:1 n9c] (from 347 g kg^{-1} SA lambs to 397.3 g kg^{-1} IF lambs). Similar results were obtained in Ricardo et al. (2015) and Santos-Silva et al. (2002) studies. In lamb fat linolenic acid [C18:2 n6c] took largest part of omega 6 fatty acids, for example, in SA lamb fat linoleic acid was 57.0 g kg^{-1}, but in LT lamb fats less 33.5 g kg^{-1}.

LT 11.9 g kg^{-1} and SA 24.8 g kg^{-1} breed lamb fat contained significantly different amount of alpha-linolenic acid [C18:3 n3] ($P < 0.05$).

Fatty acid profile between purebreds and crossbreed lamb fat was similar (Table 6).

Table 6. Fatty acids composition (percentage of total fatty acids) of intramuscular fat (m. *Longissimus dorsi*) from lambs fed with grass

Fatty acids	LT × T	LT × S	LT × DOR	LT × VMV
	mean ± standard error			
C18:1 n9c	405.0 ± 4.6^{a}	369.8 ± 15^{b}	377.0 ± 8^{b}	356.8 ± 16.6^{b}
C18:2 n6c	36.3 ± 3.5	40.3 ± 2.9	41.3 ± 3	33.0 ± 2.5
C18:3 n3	16.3 ± 5	17.8 ± 2.5	25.0 ± 3	16.7 ± 2.7
C16:1 n9c	21.3 ± 3.6	16.3 ± 1.4	15.0 ± 0	16.6 ± 2.5
C20:4 n6	2.7 ± 0.9	4.4 ± 0.9	4.0 ± 1.5	5.0 ± 2

$^{a,\ b}$ – Different letter represent significantly differences between unsaturated fatty acid results ($P \leq 0.05$).

The amount of unsaturated acids do not significantly different between breeds, only LT × T crossbreed lamb meat contained more oleic acid 405 g kg^{-1}.

Correlation coefficients among meat chemical composition (Table 7) show that meat moisture was related to protein (r = -0.61), crude fat (r = -0.96) and ash (r = 0.62).

Positive correlation was found between protein, protein to ash (r = 0.71), but negative correlation between protein and crude fat (r = 0.72).

Correlations between cholesterol and unsaturated fatty acids were negative and moderate by related, for example cholesterol to alpha-linoleic acid (r = -0.45), cholesterol to linoleic acid (r = -0.48) and positive related choresterol content were to palmitolenic acid (r = 0.39).

Correlations of unsaturated fatty acids are moderate-close related (Table 8). Ratio alpha-linolenic acid to arachidonic acid had moderately positive correlation (r = 0.47), correlation to linoleic acid (r = 0.77), but alpha-linolenic to palmitoleinic acid had negative correlation (r = -0.48).

Table 7. Correlations of meat chemical composition and unsaturated fatty in sheep meat, feeding in pastures

Trait	Moisture	Protein	Crude fat	Ash	Cholesterol	pH
Protein	0.61*	1.00				
Crude fat	-0.96*	-0.77*	1.00			
Ash	0.62*	0.71*	-0.72*	1.00		
Cholesterol	-0.02	0.02	0.03	-0.15	1.00	
pH	0.01	-0.27	0.07	-0.16	-0.10	1.00
Alpha-linoleic acid [C18:3 n3]	0.05	0.14	-0.14	0.16	-0.45*	-0.05
Arachidonic acid [C20:4 n6]	0.11	-0.01	-0.13	0.06	-0.05	-0.20
Linolenic acid [C18:2 n6c]	0.24	0.10	-0.27	0.21	-0.48*	-0.03
Oleic acid[C18:1 n9c]	-0.21	-0.05	0.19	-0.20	-0.28	-0.01
Palmitoleic acid[C16:1 n9c]	-0.12	-0.15	0.20	-0.27	0.39*	-0.19

* – significant difference ($P < 0.05$).

Table 8. Correlations amount of unsaturated fatty acids

Trait	Alpha-linoleic acid [C18:3 n3]	Arachidonic acid [C20:4 n6]	Linolenic acid [C18:2 n6c]	Oleic acid [C18:1 n9c]
Arachidonic acid [C20:4 n6]	0.47*	1.00		
Linoleic acid [C18:2 n6c]	0.77*	0.80*	1.00	
Oleic acid [C18:1 n9c]	-0.07	-0.07	0.07	1.00
Palmitolenic acid [C16:1 n9c]	-0.48*	-0.18	-0.44*	0.44*

* – significantly difference ($P < 0.05$).

Arachidonic acid obtained a close positive correlation with linoleic acid, but linolenic acid have moderate positive collation with palimitolenic acid (r = -0.44). Although the highest proportion of unsaturated fatty acids is for oleic acid, it had positive correlation to palmitoleinic acid (r = 0.44).

CONCLUSIONS

The results of lamb meat nutrient amount proved that pasture fattened crossbreed lambs have meat with lowest fat and cholesterol amount and highest amount of omega 3 and omega 6 fatty acids.

Omega 3 and omega 6 fatty acids had a positive correlation, but omega 3 to omega 9 fatty acids – negative.

Pasture-based lamb fattening provides good lamb meat production, best precocity was recorded from lambs with France origin - Charollais and Il de France.

Meat type ram crossbreeding with local breed ewes had a positive effect on amount of nutrient and unsaturated fatty acids in crossbreed lamb meat.

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REFERENCES

Abdullah, A. & Qudsieh, R. 2009. Effect of slaughter weight and time on the quality of meat from Awassi ram lambs. *Meat Science* **82**, 309–316.

Beyene, S.T. & Mlambo, V. 2011. Botanical and chemical composition of common grass species around dip–tank areas in semi–arid communal rangelands of swaziland. *Tropical and Subtropical Agroecosystems* **14**, 1–10.

Bonanome, A. & Grundy, S.M. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *New England Journal of Medicine* **318**, 1244–1248.

Chizzolini, R., Zanardi, E. & Derigoni, V. 1999. Calorific value and cholesterol content of normal and low-fat meat and meat products. *Trends in Food Science and Technology* **10**, 119–128.

El-Shesheny, M.A., Hendawy, S.H. & Ahmed, K.M. 2014. Assessment of productivity, botanical composition and nutritive value of some plant communities at Sidi-Barrani in North Western Coast of Egypt. *Annals of Agricultural Science* **59**(2), 155–163.

Jandasek, J., Milersik, M. & Lichovnikova, M. 2013. Effect of sire breed on physio-chemical and sensory characteristics of lamb meat. *Meat Science* **96**, 88–93.

Kairiša, D. 2005. Scientifical purposes of lambs meat production in Latvia*:* summary of Doctoral Thesis of Dr. agr. Scientific degree. Jelgava: LLU, 58 pp. (in Latvian).

Niedziółka, R. & Pieniak-Lendzion, K. 2006. Chemical composition of meat (*M. Adductor*) and fatty acids in intramuscular fat of goat kids and ram lambs. *Slovak Journal of Animal Science* **39** (4), 197–200.

Priolo, A., Micola, D., Agabriela, J., Prachea, S. & Dransfieldb, E. 2002. Effect of grass or concentrate feeding systems on lamb carcass and meat quality. *Meat Science* **62**, 179–185.

Ricardo, H.A., Fernandes, A.R.M., Mendes, L.C.N., Oliveira, M.A.G., Protes, V.M., Scatenac, E.M., Roc, R.O., Athaydef, N.B., Girãog, L.V.C. & Alves, L.G.C. 2015. Carcass traits and meat quality differences between a traditional and an intensive production model of market lambs in Brazil: Preliminary investigation. *Small Ruminant Research* **130**, 141–145.

Santos-Silva, J., Bessa, R.J.B. & Santos-Silva, F. 2002. The effect of genotype, feeding system and slaughter weight on the quality of light lambs 2. Fatty acid composition of meat. *Livestock Production Science* **77**, 187–194.

Scerra, M., Caparra, P., Foti, F., Galofaro, V., Sinatra, M.C. & Scerra, V. 2007. Influence of ewe feeding systems on fatty acid composition of suckling lambs *Meat Science* **76**, 390–394.

Strazdiņa, V. 2014. Biochemical evaluation of game meat in Latvia: summary of Doctoral Thesis for the Academic Degree in Engineering and Food Sciences (Dr.sc.ing) Latvia University of Agriculture, 66 pp. (in Latvian).

Teixeira, A., Batista, S., Delfa, R. & Cadavez, V. 2005. Lamb meat quality of two breeds with protected origin designation. Influence of breed, sex and live weight. *Meat Science* **71**, 530–536.

Tejeda, J., Pena, R. & Andres, A. 2008. Effect of live weight and sex on physio chemical and sensorial characteristics of Merino lamb meat. *Meat science* **80**, 1061–1067.

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Optimisation of biologically active compounds ultrasound assisted extraction from potatoes using response surface methodology

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Abstract. Potato (*Solanum tuberosum* L.) is source of phenolic compounds and from plant matrixes can be extracted by several methods. In recent years ultrasound assisted extraction has become more popular due to its efficiency for recovery of phenolic compounds and antioxidants and response surface methodology is an effective tool for optimisation of extraction procedure by evaluating different variables and their interaction. The aim of the current research was to optimize ultrasound assisted extraction of biologically active compounds from potatoes by response surface methodology. For experiment purple-flesh potato variety 'Blue Congo' was selected. Control sample was extracted by stirring for 1 hour. Box-Behnken design was used for optimization of extraction conditions from fresh potatoes and as variables were selected: ethanol concentration (% v/v), hydrochloric acid concentration (molarity) and time (min). For extracts as responses total phenolic, total flavonoid, total anthocyanin content and antioxidant activity (DPPH, ABTS+ scavenging activity) were determined using a spectrophotometric methods. Significant models were obtained for antocyanins, total phenols and DPPH radical scavenging activity. Optimisation of extraction showed that for maximising all responses optimal HCl concentration is 2.5M, ethanol concentration 79.4% and extraction time 60 minutes, resulting in following responses: 57.41 mg 100 g^{-1} of anthocyanins, 238.52 mg 100 g^{-1} of TPC, 24.58 mM TE 100 g^{-1} of DPPH scavenging activity and 12.99 mM TE 100 g^{-1} of ABTS scavenging activity. Conventional extraction method showed significantly lower results. It could be concluded that ultrasound assisted extraction is effective method for recovery of phenolic compounds and solvents and extraction time is significant parameter influencing efficiency.

Key words: purple-flesh potatoes, phenolics, ultrasound assisted extraction, response surface methodology, Box-Behnken design.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is widely grown and consumed crop and it contains many vitally important elements that can benefit human diet (Leo et al., 2008).

Significant and valuable nutrient group in potatoes are phenolics which are secondary metabolites with health promoting effect (Velioglu et al., 1998; Espin et al., 2000; Manach et al., 2004). Potatoes are so important source of phenolics that they range

as a third consumed crop right after apples and oranges which are good source of phenolics as well (Chun et al., 2005).

Phenolics show antioxidant, anticarcinogenic, antibacterial, anti-inflammatory, antiglycemic, antiviral and vasodilatory qualities (Duthie et al., 2000; Cai et al., 2004; Reyes et al., 2005; Tsao & Deng, 2005; Mattila & Hellstrom, 2006; Leo et al., 2008; Berghe, 2012; Kazeem et al., 2012; Konaté et al., 2012; Lolayekar & Shanbhag, 2012). They also present a positive impact on human longevity, ocular organs, mental health as well as cardiovascular system (Parr & Bolwell, 2000; Manach et al., 2004; Scalbert et al., 2005), and phenolics usage in human diet protects from degenerative diseases (Pourcel et al., 2007; Im et al., 2008). Studies show that in terms of safety as natural antioxidant in form of extract phenolics are not mutagenic (Sotillo et al., 2007).

Analysis of potatoes have shown significant correlation between existence of phenolics and total antioxidant level lighting up the fact that higher phenolics amount bring higher antioxidant levels (Andre et al., 2007).

While all potatoes contain phenolics, the amounts differ in varieties, i.e., red and purple flesh potatoes might contain approximately twice as much total phenolics compared to white flesh potatoes (Ezekiel et al., 2013). It may be explained with high amount of anthocyanins that are pigments in those varieties (Im et al., 2008; Al-Weshahy & Rao, 2009). Preparation process for different coloured potatoes for consumption purposes also affects level of phenolics in different ways (Reyes & Zevallos, 2003; Brown et al., 2005). The phenolics appear in the whole tuber in potatoes, still the skin has highest level of phenolics (Lewis et al., 1999; Nara et al., 2006).

Phenolics extraction optimisation is important to reach most accurate analysis, therefore response surface methodology can be used as an effective tool for this purpose. It is used as an alternative to classical optimization methods, and is more time saving, cheaper and helps in data evaluation process (Myers et al., 2004; Amado et al., 2014). This methodology may help to evaluate the effect of the variables and their interactions (Wettasinghe & Shahidi, 1999; Farris & Piergiovanni, 2009; Asfaram et al., 2015). Methanol and ethanol are one of the most widely used solvents for phenolics extraction from potatoes (Singh & Rajini, 2004; Mohdaly et al., 2010; Amado et al., 2014). Conventional way of phytochemical extraction includes maceration and Soxhlet extraction, and these methods have quite high organic solvent consumption which limits bioactive extract usage range because of solvent toxicity, as well as time required for extraction is long and consumed energy in the process is high (Da Porto et al., 2012). The alternative and modern method, called ultrasound assisted extraction, has recently gained more and more popularity (Bendicho et al., 2012) which shortens the time and energy spent on the process and it also limits final costs. This technology is sustainable as it protects the environment and consumers health as well as saves time and money (Armenta et al., 2015).

The aim of the current study was to optimize ultrasound assisted extraction of biologically active compounds from potatoes by response surface methodology.

MATERIALS AND METHODS

Plant material

Purple-flesh potato variety 'Blue Congo', grown at the test fields of Institute of Agricultural Resources and Economics, was selected for the experiment. Harvested tubers were kept in the storage facility at 4 °C and at 80 ± 5% relative air humidity until analysis. Potatoes were homogenized before extraction experiment.

Extraction of phenolic compounds

The homogenized potato samples (2.0 g) were extracted with 20 mL solvent (according to optimisation model described in experimental design) in an ultrasonic bath YJ5120-1 (Oubo Dental, USA) at 35 kHz for certain time (according to optimisation model described below).

Extraction solvent was acidified ethanol (ethanol: HCl solution 85:15 (v/v)). Ethanol concentration and molarity of HCl were varied in the experiment. The extracts were then centrifuged in a centrifuge CM-6MT (Elmi Ltd., Latvia) at 3,500.00 rpm for 5 min.

For comparison, extraction methodology used in previous studies was tested (Kampuse et al., 2016). Phenolic compounds extraction from potatoes - the homogenized samples were extracted with ethanol (80/20 w/w) in a conical flask with a magnetic stirrer (magnet 4.0 cm × 0.5 cm) at 700 rpm for 1 h at room temperature (20 ± 1 °C). The extracts were then filtered (paper No.89).

Experimental design

A response surface methodology using Box-Behnken design (Design Expert) was used for optimization of extraction conditions of anthocyanins, total phenols and antioxidants from fresh potatoes and variables were selected as follows: ethanol concentration (% v/v), hydrochloric acid concentration (molarity) and time (min) (Table 1).

Table 1. Independent variables, their levels and responses

Run order	A HCl, M	B Ethanol, %	C Time, min	Anthocyanin, mg $100g^{-1}$	TPC, mg $100g^{-1}$	DPPH, mM TE $100g^{-1}$	ABTS, mM TE $100g^{-1}$
1	0.5	50	40	29.55	218.36	13.46	20.96
2	2.5	50	40	44.76	218.99	20.99	14.01
3	0.5	90	40	28.35	189.24	14.64	14.79
4	2.5	90	40	34.95	175.70	24.92	17.76
5	0.5	70	20	42.38	258.84	15.04	20.26
6	2.5	70	20	49.75	243.06	22.16	13.97
7	0.5	70	60	42.73	263.07	14.97	21.01
8	2.5	70	60	58.27	256.88	22.52	13.70
9	1.5	50	20	54.76	243.06	15.31	20.06
10	1.5	90	20	32.78	205.01	20.69	25.02
11	1.5	50	60	31.16	178.84	18.56	18.20
12	1.5	90	60	44.08	204.09	22.15	12.88
13	1.5	70	40	29.77	248.22	18.70	15.89
14	1.5	70	40	29.97	248.68	18.76	15.36
15	1.5	70	40	30.70	248.22	18.95	16.13

Experiment was performed by 15 runs with three replicates of central run. Estimation of error was performed by 3 runs of central points. Optimisation process was based on evaluation of responses of samples designed according to model. Coefficients of response function was calculated, predicting response of fitting model. Statistical significance was examined by analysis of variance (Anova), lack of fit, pure error, adeq.precision was tested to check models adequacy.

Optimisation was made by both numerical and graphic analysis using contour curves and desirability functions. In current experiment different maximum response variable values were obtained.

Analytical methods

The total phenolic content (TPC) of the potato extracts was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999). The absorbance was measured at 765 nm and total phenols were expressed as the gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of plant material. The TFC was measured by a spectrophotometric method (Kim et al., 2003).

The absorbance was measured at 415 nm and total flavonoids were expressed as catehin equivalents (CE) 100 g^{-1} DW of the sample. Antioxidant activity of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl ($DPPH^{\bullet}$) radical as outlined by Yu et al. (2003) and 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid ($ABTS^{\bullet+}$) radical cation assay (Re et al., 1999). Antioxidant activity was expressed as TE 100 g^{-1} DW of plant material. Total anthocyanins were determined by method described by Mane et al. (2015). The pH shift method (pH 0.6 and pH 3.5) for determination of anthocyanins in potatoes extracts were used based on the absorbance at 700 nm and 520 nm. Results were calculated as pigment cyanindin-3-glucoside equivalents and expressed to dry matter.

RESULTS AND DISCUSSION

The response surface methodology using Box-Behnken is widely used for optimisation of extraction (Espada-Bellido et al., 2017). Extraction variables were HCl molarity (A), ethanol concentration (B) and time (C). Results of analysis of variance for the quadratic model of responses are shown in Table 2 for total anthocyanins, in Table 3 for total phenols, in Table 4 for DPPH radical scavenging activity and Table 5 for ABTS scavenging activity. Models for response were significant for anthocyanins (P = 0.0310), total phenols (P = 0.0231), DPPH radical scavenging activity (P = 0.0018) but not for ABTS scavenging activity (P = 0.1274). Coefficient of variation ranged between 5.2 until 8.4.

The second order polynomial equation express relationship between tested factors their interaction and anthocyanins fitted model is:

$$\text{Anthocyanins} = 30.15 + 3.54A - 0.46B - 0.43C + 1.95AB + 2.04AC + 8.72BC + 3.87A^2 - 3.72B^2 + 14.72C^2$$

Current equation could be used for prediction of responses in the range of tested factors.

Table 2. Analysis of variance for the quadratic model of extracted anthocyanins

Source	Sum of squares	Degree of freedom	Mean square	*F* value	*P*-value	Coefficient estimate
Model	1,313.42	9	145.94	6.03	0.0310	30.15
A-HCl	100.14	1	100.14	4.14	0.0975	3.54
B-Ethanol	1.67	1	1.67	0.0691	0.8031	-0.4572
C-Time	1.48	1	1.48	0.0612	0.8144	-0.4301
AB	15.25	1	15.25	0.6308	0.4631	1.95
AC	16.66	1	16.66	0.6891	0.4443	2.04
BC	304.41	1	304.41	12.59	0.0164	8.72
A^2	55.30	1	55.30	2.29	0.1909	3.87
B^2	51.08	1	51.08	2.11	0.2059	-3.72
C^2	751.36	1	751.36	31.07	0.0026	14.27
Residual	120.91	5	24.18			
Lack of Fit	120.43	3	40.14	165.84	0.0060	
Pure Error	0.4841	2	0.2421			
Cor. Total	1,434.33	14				
Adeq. precision	7.24					

Based of coefficient estimate showed at Table 3 the second order polynomial equation express relationship between tested factors their interaction and total phenol fitted model is:

$TPC = 248.37 - 8.08A - 6.93B - 5.89C + 3.90AB + 2.40AC + 15.82BC - 3.76A^2 - 51.48B^2 + 10.85C^2$

Table 3. Analysis of variance for the quadratic model of extracted TPC

Source	Sum of squares	Degree of freedom	Mean square	*F* value	*P*-value	Coefficient estimate
Model	1,2870.42	9	1,430.05	6.94	0.0231	248.37
A-HCl	521.91	1	521.91	2.53	0.1724	-8.08
B-Ethanol	384.48	1	384.48	1.87	0.2302	-6.93
C-Time	277.24	1	277.24	1.35	0.2985	-5.89
AB	60.75	1	60.75	0.2948	0.6105	3.90
AC	23.00	1	23.00	0.1116	0.7519	2.40
BC	1,001.56	1	1,001.56	4.86	0.0786	15.82
A^2	52.25	1	52.25	0.2536	0.6360	-3.76
B^2	9,783.96	1	9,783.96	47.48	0.0010	-51.48
C^2	434.75	1	434.75	2.11	0.2061	10.85
Residual	1,030.35	5	206.07			
Lack of Fit	1,030.20	3	343.40	4,845.27	0.0002	
Pure Error	0.1417	2	0.0709			
Cor. Total	1,3900.76	14				
Adeq. precision	7.6617					

Also for DPPH antioxidant activity the second order polynomial equation expresing relationship between tested factors their interaction fitted model is:

$DPPH = 18.80 + 3.27A + 2.55B + 0.63C + 2.28AB + 0.11AC - 0.44BC - 1.20A^2 - 0.69B^2 + 1.07C^2$

For ABTS antioxidant activity developed model was not significant it was not used for optimisation procedure.

Table 4. Analysis of variance for the quadratic model of extracted DPPH

Source	Sum of squares	Degree of freedom	Mean square	*F* value	*P*-value	Coefficient estimate
Model	174.15	9	19.35	21.34	0.0018	18.80
A-HCl	85.31	1	85.31	94.08	0.0002	3.27
B-Ethanol	52.16	1	52.16	57.52	0.0006	2.55
C-Time	3.13	1	3.13	3.45	0.1224	0.6254
AB	20.70	1	20.70	22.83	0.0050	2.28
AC	0.0467	1	0.0467	0.0515	0.8295	0.1080
BC	0.7916	1	0.7916	0.8730	0.3930	-0.4449
A^2	5.29	1	5.29	5.84	0.0604	-1.20
B^2	1.77	1	1.77	1.96	0.2209	-0.6929
C^2	4.19	1	4.19	4.62	0.0842	1.07
Residual	4.53	5	0.9068			
Lack of Fit	4.50	3	1.50	87.16	0.0114	
Pure Error	0.0344	2	0.0172			
Cor. Total	178.69	14				
Adeq. precision	14.9679					

Table 5. Analysis of variance for the quadratic model of extracted ABTS

Source	Sum of squares	Degree of freedom	Mean square	*F* value	*P*-value	
Model	107.27	6	17.88	2.38	0.1274	No
A-HCl	35.26	1	35.26	4.70	0.0621	significant
B-Ethanol	1.59	1	1.59	0.2116	0.6578	
C-Time	22.87	1	22.87	3.05	0.1191	
AB	20.90	1	20.90	2.78	0.1338	
AC	0.2625	1	0.2625	0.0350	0.8563	
BC	26.40	1	26.40	3.52	0.0976	
Residual	60.07	8	7.51			
Lack of Fit	59.76	6	9.96	65.11	0.0152	
Pure Error	0.3060	2	0.1530			
Cor. Total	167.35	14				
Adeq. precision	5.0943					

Results are visualized in nine response surface graphs which provide visual representation of the relationship between responses and levels of each variable and the type of interactions between two test variables in each case. Circular or elliptical form of the contour plots show significance level of the interactions between the variables (Fig.1).

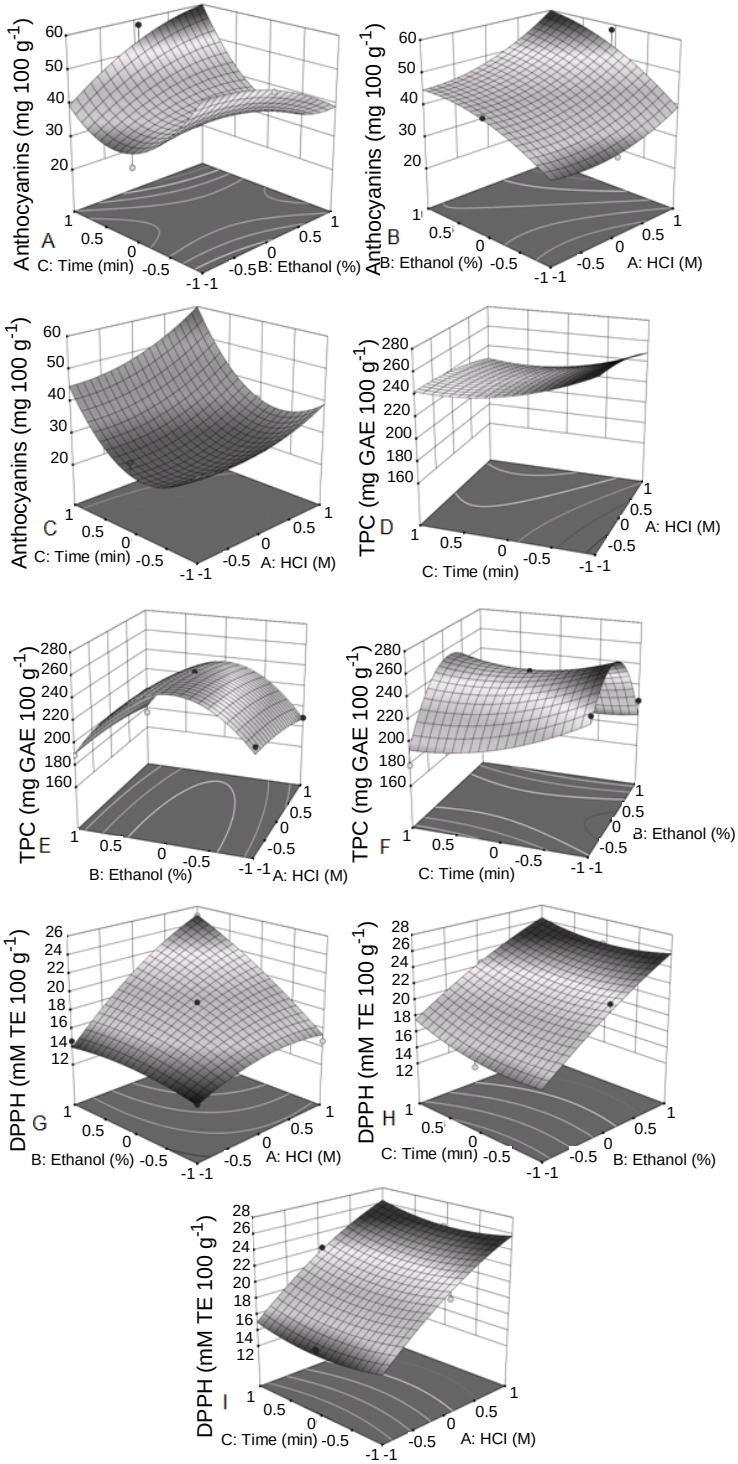

Figure 1. Response surface 3D plots (A (Anthocyanins), F (TPC), H (DPPH)) presenting the effect of extraction time and extraction ethanol concentration; (B (Anthocyanins), E (TPC), G (DPPH)) extraction ethanol and HCI concentration; (C (Anthocyanins), D (TPC), I (DPPH)) extraction time and HCI concentration.

For extraction of anthocyanins the best conditions are with higher concentration of ethanol and longer extraction time (Fig. 1, A), and also with higher concentration of HCl and ethanol (Fig. 1, B). Analysis of purple sweet potatoes showed that significant factors influencing anthocyanins extractability are temperature, ethanol concentration and ultrasound power, but time is not significant that is opposite to results obtained in current experiment (Cai et al., 2016). It is confirmed that anthocyanins are not stable in alkaline and neutral environment, and for improvement of their stability and extractability hydrochloric acid addition is beneficial to reduce pH up to 2 til 2.3 (He et al., 2016). Anthocyanins extraction yield and composition of sweet potatoes are also dependent on extraction method and ultrasound assisted extraction could result in higher impurities of other phenolics (Cai et al., 2016). In current experiment it is not disadvantage because coextraction is tested.

Analysing tendencies for extraction of TPC, higher values wereobtained extracting shorter time, and with lower concentration of HCl as solvent (Fig. 1, D), ethanol concentration and HCl interaction showed that higher values were obtained by medium ethanol concentration and the result was not dependent on HCl concentration (Fig. 1, E). For extraction of phenolics from potatoe peels, use of acidified ethanol resulted in pure and stable extracts avoiding side reactions (Maldonado et al., 2014). Extraction of phenolic compounds from eggplant showed that acidified solvent gave better yield, and it decreased by the increase of pH (Ferarsa et al., 2018). Whereas analysing interaction of time and ethanol, higher results were obtained by medium concentration of ethanol and shorter time (Fig. 1, F). Extraction efficiency of phenolics from eggplants increased by increasing water content in ethanol up to 50% (Ferarsa et al., 2018). It could be explained by polarity of solvents and addition of water to organic solvent enhances extract separation efficiency (Ferarsa et al., 2018). Comparing the two extracts (in water and ethanol), water was less effective than ethanol for the extraction of phenolic compounds. This difference can be explained by the polarity of both solvents. It can also be seen that the yields of aqueous ethanol extracts (50 and 75%) are higher than those of pure ethanol (100%) and pure water (0%). These results indicate that adding water to the organic solvent enhances extraction yield (Ferarsa et al., 2018). For DPPH activity the maximal values analysing ethanol and HCl concentration was obtained using highest concentrations tested (Fig. 1, G), higher activity was observed with higher concentration of HCl (Fig.1.H), and ethanol (Fig. 1, I), and in both cases time was not significant factor. Ultrasound assisted extraction of sweet potatoes resulted in samples with high radical scavenging activity (Cai et al., 2016).

Box Behnken methodology gives possibility to analyse interaction of different factors for obtaining desirable results. In current experiment possibility to make one extraction for testing all responses were tested. If all responses were maximised then optimal parameters for extraction were following: HCl concentration 2.5M, ethanol concentration 79.4% and extraction time 60 minutes, and as a result can be obtained 57.41 mg 100 g^{-1} of anthocyanins, 238.52 mg 100 g^{-1} of TPC, 24.58 mM TE 100 g^{-1} of DPPH scavenging activity and 12.99 mM TE 100 g^{-1} of ABTS scavenging activity. It is also possible to optimise one parameter and if content of anthocyanins is maximised then optimal HCl concentration is 2.42M, ethanol concentration 88.8% and extraction time 59.8 min, as a result can be obtained 58.31 mg 100 g^{-1} of anthocyanins, but results of other parameters are lower – 210.57 mg 100 g^{-1} of TPC, 25.91 mM TE 100 g^{-1} of DPPH scavenging activity and 12.69 mM TE 100 g^{-1} of ABTS scavenging activity. For

extraction of antocyanins from mulberries were 76% MeOH in water at pH 3 (Espada-Bellido et al., 2017) that is according to our results that higher concentration of HCl gives better results. Whereas for extraction of anthocyanins from purple sweet potatoes maximal yield was obtained at 50 °C, 45 minutes using 90% (v/v) ethanol as the solvent (Cai et al., 2016).

For maximal TPC extraction the optimal HCl concentration is 0.82M, ethanol concentration is 68.2% and extraction time is 27.2 min, as a result 37.87 mg 100 g^{-1} of anthocyanins, 263.58 mg 100 g^{-1} of TPC, 15.52 mM TE 100 g^{-1} of DPPH scavenging activity and 20.03 mM TE 100 g^{-1} of ABTS scavenging activity can be obtained. The optimum conditions for extraction of total phenolic compounds from berries were 61% MeOH in water at pH 7 (Espada-Bellido et al., 2017), that is also in accordance with our results showing that lower concentration of acid gives higher TPC.

To maximise DPPH antioxidant activity, the optimal HCl concentration would be 2.46M, ethanol concentration 89.2% and extraction time 48.4 minutes, as a result 41.92 mg 100 g^{-1} of anthocyanins, 192.90 mg 100 g^{-1} of TPC, 25.10 mM TE 100 g^{-1} of DPPH scavenging activity and 15.17 mM TE 100 g^{-1} of ABTS scavenging activity can be obtained.

For comparison of results, conventional extraction by stirring for one hour was tested and results obtained were significantly lower, namely, TPC 200.56 mg $100g^{-1}$, DPPH scavenging activity 6.27 mM TE $100g^{-1}$, ABTS 12.77 mM TE $100g^{-1}$. Obtained results are in accordance with results reported in literature showing efficiency of ultrasound for extraction for anthocyanins (Mane et al., 2015; Espada-Bellido et al., 2017) and phenolic compounds (Espada-Bellido et al., 2017).

CONCLUSIONS

In current experiment coextraction of total phenols, anthocyanins and antioxidants characterised by antiradical activity were tested. Significant models were obtained for antocyanins, total phenols and DPPH radical scavenging activity. Optimisation of extraction showed that for maximising all responses, the optimal HCl concentration is 2.5M, ethanol concentration 79.4% and extraction time 60 minutes, resulting in following responses: 57.41 mg 100 g^{-1} of anthocyanins, 238.52 mg 100 g^{-1} of TPC, 24.58 mM TE 100 g^{-1} of DPPH scavenging activity and 12.99 mM TE 100 g^{-1} of ABTS scavenging activity. Results of conventional extraction showed significantly lower results. If one of the responses are maximised, content of others reduces significantly. For extraction of anthocyanins, the optimal HCl concentration is 2.42M, ethanol concentration is 88.8% and extraction time is 59.8 minutes, whereas for TPC optimal HCl concentration is 0.82M, ethanol concentration is 68.2% and extraction time is 27.2 minutes. For obtaining extracts with higher DPPH activity optimal HCl concentration is 2.46M, ethanol concentration is 89.2% and extraction time is 48.4 minutes. It could be concluded that ultrasound assisted extraction is effective method for recovery of phenolic compounds and solvents and extraction time is significant parameter influencing efficiency.

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REFERENCES

Al-Weshahy, A. & Rao, V.A. 2009. Isolation and characterization of functional components from peel samples of six potatoes varieties growing in Ontario. *Food Research International* **42**(8), 1062–1066.

Amado, I.R, Franco, D, Sánchez, M. & Vázquez, J.A. 2014. Optimisation of antioxidant extraction from Solanum tuberosum potato peel waste by surface response methodology. *Food Chemistry* **165**, 290–299.

Andre, C.M., Ghislain, M., Bertin, P., Oufir, M., Herrera, M.R., Hoffmann, L., Hausman, J.F., Larondelle, Y. & Evers, D. 2007. Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. *Journal of Agricultural and Food Chemistry* **55**(2), 366–378.

Armenta, S., Garrigues, S. & De Guardia, M. 2015. The role of green extraction techniques in Green Analytical Chemistry. *Trends in Analytical Chemistry* **71**, 2–8.

Asfaram, A., Ghaedi, M., Goudarzi, A., Soylak, M. & Langroodi, S.M. 2015. Magnetic nanoparticle based dispersive micro-solid-phase extraction for the determination of malachite green in water samples: optimized experimental design. *New Journal of Chemistry* **39**(12), 9813–9823.

Bendicho, C., De La Calle, I., Pena, F., Costas, M., Cabaleiro, N. & Lavilla, I. 2012. Ultrasound-assisted pretreatment of solid samples in the context of green analytical chemistry. *TrAC Trends in Analytical Chemistry* **31**(8), 50–60.

Berghe, W.V. 2012. Epigenetic Impact of Dietary Polyphenols in Cancer Chemoprevention: Lifelong Remodeling of Our Epigenomes. *Pharmacological Research* **65**(6), 565–576.

Brown, C.R., Wrolstad, R., Durst, R., Yang, C.P. & Clevidence, B. 2005. Breeding studies in potatoes containing high concentrations of anthocyanins. *American Journal of Potato Research* **80**(4), 241–249.

Cai, Y., Luo, Q., Sun, M. & Corke, H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences* **74**(17), 2157–2184.

Cai, Z., Qu, Z., Lan, Y., Zhao, S., Ma, Z., Wan, Q., Jing, P. & Li, P. 2016. Conventional, ultrasound-assisted, and accelerated-solvent extractions of anthocyanins from purple sweet potatoes. *Food Chemistry* **197**(A), 266–272.

Chun, O.K., Kim, D.O., Smith, N., Schroeder, D., Han, J.T. & Lee, C.Y. 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture* **85**(10), 1715–1724.

Da Porto, C., Porretto, E. & Decorti, D. 2012. Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds. *Ultrasonics Sonochemistry* **20**(4), 1076–1080.

Duthie, G., Duthie, S. & Kyle, J. 2000. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutrition Research Reviews* **13**(1), 79–106.

Espada-Bellido, E., Ferreiro-González, M., Carrera, C., Palma, M., Barroso, C.G. & Barbero, G.F. 2017. Optimization of the ultrasound-assisted extraction of anthocyanins and total phenolic compounds in mulberry (*Morus nigra*) pulp. *Food Chemistry* **219**, 23–32.

Espin, J.C, Rivas, C.S., Wichers, H.J. & Viguera, C.G. 2000. Anthocyanin based natural colorants: a new source of antiradical activity for foodstuff. *Journal of Agricultural and Food Chemistry* **48**(5), 1588–1592.

Ezekiel, R., Singh, N., Sharma, S. & Kaur, A. 2013. Beneficial phytochemicals in potato – a review. *Food Research International* **50**(2), 487–496.

Farris, S. & Piergiovanni, L. 2009. Optimization of manufacture of almond paste cookies using response surface methodology. *Journal of Food Process Engineering* **32**(1), 64–87.

Ferarsa, S., Zhang, W., Moulai-Mostefa, N., Ding, L., Jaffrin, M.Y. & Grimi, N. 2018. Recovery of anthocyanins and other phenolic compounds from purple eggplant peels and pulps using ultrasonic-assisted extraction. *Food and Bioproducts Processing* **109**, 19–28.

He, B., Zhang, L., Yue, X., Liang, J., Jiang, J., Gao, X. & Yue, P. 2016. Optimization of Ultrasound-Assisted Extraction of phenolic compounds and anthocyanins from blueberry (*Vaccinium ashei*) wine pomace. *Food Chemistry* **204**, 70–76.

Im, H.W., Suh, B.S., Lee, S.U., Kozukue, N., Ohnisi-Kameyama, M., Levin, C.E. & Friedman, M. 2008. Analysis of phenolic compounds by high-performance liquid chromatography and liquid chromatography/mass spectrometry in potato plant flowers, leaves, stems, and tubers and in home-processed potatoes. *Journal of Agricultural and Food Chemistry* **56**(9), 3341–3349.

Kampuse, S., Tomsone, L., Krūma, Z., Šabovics, M. & Skrabule, I. 2016. Effect of lovage phenolics to formation of acrylamide in French fries. *Agronomy Research* **14**(S2), 1315–1327.

Kazeem, M.I., Akanji, M.A., Hafizur, R.M. & Choudhary, M.I. 2012. Antiglycation, Antioxidant and Toxicological Potential of Polyphenol Extracts of Alligator Pepper, Ginger and Nutmeg from Nigeria. *Asian Pacific Journal of Tropical Biomedicine* **2**(9), 727–732.

Kim, D., Jeong, S.W. & Lee, C.Y. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* **81**(3), 321–326.

Konaté, K., Hilou, A., Mavoungou, J.F., Lepengué, A.N., Souza, A., Barro, N., Datté, J.Y., M'Batchi, B. & Nacoulma, O.G. 2012. Antimicrobial Activity of Polyphenol-Rich Fractions from *Sida alba* L. (Malvaceae) against Cotrimoxazol-Resistant Bacteria Strains. *Annals of Clinical Microbiology and Antimicrobials* **11**(1), 1–6.

Leo, L., Leone, A., Longo, C., Lombardi, D.A., Raimo, F. & Zacheo, G. 2008. Antioxidant compounds and antioxidant activity in early potatoes. *Journal of Agricultural and Food Chemistry* **56**(11), 4154–4163.

Lewis, C.E., Walker, J.R.L. & Lancaster, J.E. 1999. Changes in anthocyanin, flavonoid and phenolic acid concentrations during development and storage of coloured potato (*Solanum tuberosum* L.) tubers. *Journal of the Science of Food and Agriculture* **79**(2), 311–316.

Lolayekar, N. & Shanbhag, C. 2012. Polyphenols and Oral Health. *RSBO* **9**(1), 74–84.

Maldonado, A.F.S., Mudge, E., Gänzle, M.G. & Schieber, A. 2014. Extraction and fractionation of phenolic acids and glycoalkaloids from potato peels using acidified water/ethanol-based solvents. *Food Research International* **65**(A), 27–34.

Manach, C., Scalbert, A., Morand, C., Remesy, C. & Jimenez, L. 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition* **79**(5), 727–747.

Mane, S., Bremner, D.H., Tziboula-Clarke, A. & Lemos, M.A. 2015. Effect of ultrasound on the extraction of total anthocyanins from Purple Majesty potato. *Ultrasonics Sonochemistry* **27**, 509–514.

Mattila, P. & Hellstrom, J. 2006. Phenolic acids in potatoes, vegetables, and some of their products. *Journal of Food Composition and Analysis* **20**(3–4), 152–160.

Mohdaly, A.A.A., Sarhan, M.A., Smetanska, I. & Mahmoud, A. 2010. Antioxidant properties of various solvent extracts of potato peel, sugar beet pulp and sesame cake. *Journal of the Science of Food and Agriculture* **90**(2), 218–226.

Myers, R.H., Montgomery, D.C., Vining, G.G. & Borror, C. 2004. Response surface methodology: a retrospective and literature survey. *Journal of Quality Technology* **36**(1), 53–78.

Nara, K., Miyoshi, T., Honma, T. & Koga, H. 2006. Antioxidative activity of bound form phenolics in potato peel. *Bioscience, Biotechnology, and Biochemistry* **70**(6), 1489–1491.

Parr, A.J. & Bolwell, G.P. 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture* **80**(7), 985–1012.

Pourcel, L., Routaboul, J.M., Cheynier, V., Lepiniec, L. & Debeaujon, I. 2007. Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Trends in Plant Science* **12**(1), 29–36.

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* **26**(9–10), 1231–1237.

Reyes, L.F. & Zevallos, L.C. 2003. Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum* L.). *Journal of Agricultural and Food Chemistry* **51**(18), 5296–5300.

Reyes, L.F., Miller, J.C. & Zevallos, L.C. 2005. Antioxidant capacity, anthocyanins and total phenolics in purple and red fleshed potato (*Solanunm tuberosum* L.) genotypes. *American Journal of Potato Research* **82**(4), 271–277.

Scalbert, A., Manach, C., Morand, C., Remesy, C. & Jimenez, L. 2005. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition* **45**(4), 287–306.

Singh, N. & Rajini, P. 2004. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry* **85**(4), 611–616.

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 Enzymology* **299C**(1), 152–178.

Sotillo, D.R., Hadley, M. & Wolf-Hall, C. 2007. Potato peel extract a nonmutagenic antioxidant with potential antimicrobial activity. *Journal of Food Science* **63**(5), 907–910.

Tsao, R. & Deng, Z. 2005. Separation procedures for naturally occurring antioxidant phytochemicals. *Journal of Chromatography* B **812**(1–2), 85–99.

Velioglu, Y.S, Mazza, G., Gao, L. & Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry* **46**(10), 4113–4117.

Wettasinghe, M. & Shahidi, F. 1999. Evening primrose meal: A source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals. *Journal of Agricultural and Food Chemistry* **47**(5), 1801–1812.

Yu, L., Perret, J., Harris, M., Wilson, J. & Haley, S. 2003. Antioxidant properties of bran extracts from 'Akron' wheat grown at different locations. *Journal of Agricultural and Food Chemistry* **51**(6), 1566–1570.

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Comparison of phenolic compounds and antioxidant activity of fresh and freeze-dried potatoes

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Abstract. Potatoes (*Solanum tuberosum* L.) contain a wide range of compounds with health benefits, and different techniques have been developed for the determination of these compounds. Freeze-drying is a common method for the preservation and preparation of samples for the analyses of bioactive compounds, but it is well known that drying influences the composition of food products. The aim of the current study was to compare phenolic compounds and antioxidant activity of fresh and freeze-dried potatoes. In the experiment 11 cultivar potatoes grown in experimental fields of the Institute of Agricultural Resources and Economics in 2016 were analysed. Potatoes were freeze-dried. Homogenized fresh and freeze-dried samples were extracted with an ethanol/water (80/20 w/w) solution. Total phenolic content (TPC) was determined using the Folin-Ciocalteu method, and the antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH˙) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. The highest TPC and radical scavenging activity were determined in cultivars 'Peru Purple' and 'Blue Congo' potatoes. Freeze-drying influenced all the tested parameters but more significantly the TPC, and for certain cultivars a reduction of more than 30% was observed. A strong correlation between TPC, DPPH and ABTS in fresh and freeze-dried samples was observed, r = 0.81, r=0.93, r = 0.92, respectively. It could be concluded that the effect of freeze-drying on TPC and antioxidant activity is cultivar dependent.

Key words: potatoes, phenolic compounds, antioxidant activity, freeze-drying.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most widely grown and consumed crop in the world that contains many dietary valuable compounds (Leo et al., 2008). Potatoes are a source of carbohydrates, proteins and minerals which makes them a vital part of the human diet (Casanas et al., 2002). They are highly recommended as a part of everyday diet because many of the potatoes' compounds have a beneficial effect on human health (Bravo, 1998; Katan & Roos, 2004). In addition, potatoes also contain secondary metabolites phenolic compounds which are highly beneficial for health (Velioglu et al., 1998; Espin et al., 2000; Manach et al., 2004). They are valuable phytochemicals which present antioxidant, anticarcinogenic, antibacterial, anti-

inflammatory, antiglycemic, antiviral and vasodilatory properties (Duthie, Duthie & Kyle, 2000; Reyes, Miller & Zevallos, 2005; Mattila & Hellstrom, 2006; Leo et al., 2008).

Phenolic compounds are a group that consists of thousands of different compounds and some of which, in addition to the above listed qualities; also have a positive effect on longevity, mental health, cardiovascular system and ocular organs (Parr & Bolwell, 2000; Manach et al., 2004; Scalbert et al., 2005). A high correlation has been found between the presence of phenolic compounds in potatoes and the total antioxidant capacity (Andre et al., 2007). Potatoes as a phenolic compound source are the third consumed crop after apples and oranges (Chun et al., 2005). Total phenolic content in potatoes is significantly higher ranging from approximately 530 to 1,770 mg 1 kg^{-1} fresh weight (Al Saikhan, Howard & Miller, 1995), that is why the studies on the phenolic compound and antioxidant properties in context of potatoes are highly important. Phenolic compounds can be found in potatoes' flesh and skin, although the skin contains the highest concentration (Lewis, Walker & Lancaster, 1999; Nara et al., 2006). All potato cultivars have phenolic compounds, but some of them contain different total amounts, for example, red and purple flesh potato cultivars have twice as much total phenolic content compared to white flesh potatoes (Ezekiel et al., 2013), and also the technological process affects potatoes with different colour flesh differently (Brown et al., 2005; Reyes & Zevallos, 2003). For example, freeze-drying of purple flesh potatoes neither significantly affects total phenolic neither content nor total antioxidant content (Nayak, 2011). Although potatoes are naturally rich in phenolic compounds having high antioxidant activity, this factor can change based on conditions (storage, wounding, technological processes) to which potatoes are exposed.

The aim of this study was to evaluate and compare phenolic compound and antioxidant activity changes of fresh and technologically affected (freeze-drying) potatoes.

MATERIALS AND METHODS

Raw materials

The potatoes were planted in the middle of May and harvested in the last ten days of August or first days of September in 2016. The soil type in the field was sod-podzolic (PVv), sandy loam, pHKCl 5.3, organic matter 1.8%, contained P_2O_5 120 mg kg^{-1}, K_2O 143 mg kg^{-1}. Pre-crop – winter cereals, applied fertilisers before planting NPK 60:55:90 kg ha^{-1} herbicides, insecticides and fungicides were applied according to conventional growing technology. Harvested tubers were kept in the storage facility at an air temperature of 4 °C and at a relative air humidity of 80 ± 5%. In the experiment eleven potato (*Solanum tuberosum* L.) cultivars with white, yellow and purple coloured flesh were evaluated (Table 1).

For the experiment a total of 10 kg (around 50–60 potato tubers) of table potato tubers per cultivar were selected into small piles, from ten different wooden boxes (the size of the box: 90 cm (l) ×50 cm (w) × 40 cm (h)). Five potatoes were selected from several location points of each box.

Table 1. Description of potato cultivars

Cultivar	Breeder / country	Earliness	Tuber shape	Colour of skin	Colour of flesh
Imanta	AREI / Latvia	medium late	long oval	yellow with pink eyes	white
S 01085-21	AREI / Latvia	early	oval	light yellow	light yellow
S 04009-37	AREI / Latvia	medium late	oval	purple	white
Brasla	AREI / Latvia	medium late	round	yellow	yellow
Lady Claire	Meijer Research BV / The Netherlands	medium early	oval	cream	light yellow
Lenora	AREI / Latvia	medium early	round oval	yellow	yellow
Gundega	AREI / Latvia	medium late	round	pink	yellow
2000-49.82	AREI / Latvia	medium late	round	yellow	light yellow
19514.20	AREI / Latvia	medium late	round oval	purple	white
Blue Congo		late	oval	dark purple	purple
Peru Purple		late	oval	dark purple	purple

Cut potato tuber cubes (1 x 1 cm) were placed in a horizontal freezer (Zanussi, Pordenone, Italy) at a temperature of minus 18 °C before freeze-drying. Frozen potatoes were transferred to a freeze drier (CHRIST Alpha 2-4 LD plus, Osterode am Harz, Germany), which operates at the following conditions: temperature in a sample chamber – 83 ± 2 °C, vacuum – 1.0 mbar; freezing time – 34 hours.

Chemical analysis

Extraction of phenolic compounds from potatoes – the homogenized samples were extracted with ethanol (80/20 w/w) in a conical flask with a magnetic stirrer (magnet 4.0 cm × 0.5 cm) at 700 rpm for 1 h at room temperature (20 ± 1 °C). The extracts were then filtered (paper No.89). The extraction process was done in triplicate.

Determination of total phenolic content (TPC) – the TPC of potato extracts was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. The absorbance was measured at 765 nm and total phenolics were expressed as the gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of plant material.

Determination of antioxidant activity – antioxidant activity of potato extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical as outlined by Yu et al. (2003). The absorbance was measured at 517 nm. The radical scavenging activity (RSA) of extracts was also measured by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) radical cation assay (Re et al., 1999). For the assessment of extracts, the $ABTS^{\bullet+}$ solution was diluted with a phosphate buffer solution to obtain the absorbance of 0.800 ± 0.030 at 734 nm. The RSA was expressed as TE 100 g^{-1} DW of plant material. The higher the Trolox equivalent antioxidant capacity (TEAC) of a sample, the stronger is the antioxidant activity.

Statistical analysis

Experimental results are means of three replications and were analyzed by Microsoft Excel 2010 (descriptive statistics) and SPSS 17.00 (ANOVA and correlation

analysis). Analysis of variance (ANOVA) and Tukey's test were used to determine differences among samples. Differences were considered as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Total phenolics

TPC of analysed potatoes ranged from 82–282 mg 100 g^{-1} (Fig. 1) and presence of phenolics in potatoes complies with the previous studies indicating that potatoes contain highly health-beneficial phenolic compounds (Velioglu et al., 1998; Espin et al., 2000; Manach et al., 2004). Comparing TPC between tested potatoes, cultivar 'Blue Congo' showed the highest content. It is purple potato cultivar, and it has been evaluated that purple potatoes are one of the richest phenolics and anthocyanins source (Jansen & Flamme, 2006). Antioxidative activity in purple potatoes is demonstrated by anthocyanins (Reyes, Miller & Zevallos, 2005; Han et al., 2007; Steed & Truong, 2008). Purple potatoes compared to white potatoes have a higher health promoting effect and now are widely used as functional food in Europe, Southeast Asia and North America (Lei et al., 2014). In addition to acylated anthocyanins pigmented cultivars, for example, purple and red contains also greater levels of chlorogenic acid (the main phenolic acid in potatoes) compared to white and yellow-fleshed cultivars. While white and yellow-fleshed cultivars are more commonly used in human diet, this study highlights pigmented potatoes potential in contribution of higher amounts of phenolics and higher retention of valuable compounds during and after commercial processing. The latest studies suggest that that the usage of fresh or processed whole (unpeeled) potatoes provide higher levels of phenolics to human diet (Furrer et al., 2017).

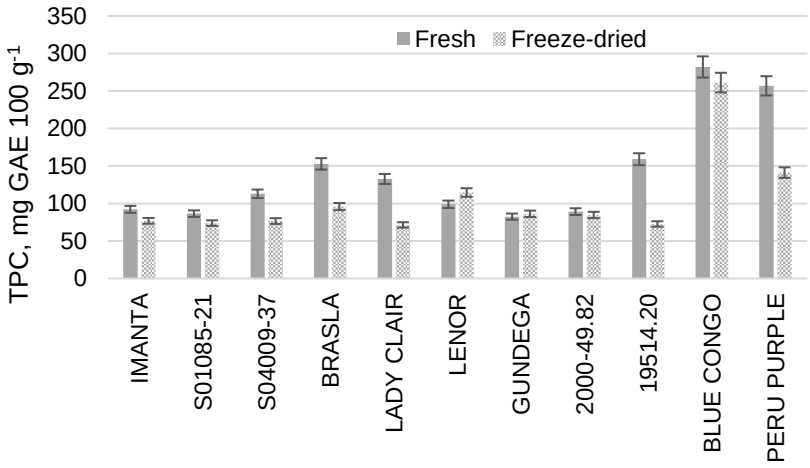

Figure 1. Total phenolic content of fresh and freeze-dried potatoes.

Phenolics and anthocyanins affect the colour of flesh and skin of potatoes forming a specific shade, i.e., red and purple potatoes. Potato flesh and skin can have different levels of pigmentation – entire pigmentation or partial. Potatoes with red colour flesh and skin contain acylated glucosides of pelargonidin, whereas potatoes with purple flesh and skin also contain acylated glucosides of delphinine, petunidin, malvidin, peonidin

(Brown, 2005). The availability of phytonutrients in potatoes nowadays is a highly important factor for consumers due to the increased focus on human health and a healthier diet which works as a developing force for future cultivar. Studies have indicated some potato cultivars that have significantly higher phytonutrients level compared to most widely used potato cultivars, also suggesting that focusing on potato selection with higher phytonutrients amount can raise the level of phytonutrients even more than it is now on average, for example, already identified are white-flesh potatoes with higher phenolics level compared to the average amount of phenolics in this colour potatoes, which proves that selection works well in the case of higher phytonutrients level (Navarre et al., 2011).

Technological processes, for example freeze-drying, affect the chemical content of plants (Angela & Meireles, 2009) so it is important that any kind of technological treatment is done using as low temperature as possible helping to retain as high biologically active compound levels as possible.

In all potato cultivars TPC after treatment was significantly lower compared to fresh samples and reduction was up till 45%.

Antioxidant activity

Antioxidant activity is closely linked to total phenolics and total anthocyanins (Reyes, Miller & Zevallos, 2005; Leo et al., 2008). In the current study 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity ranged from 4.6–12.2 mM $100g^{-1}$ with the highest activity in potatoes 'Blue Congo' and 'Peru Purple' (Fig. 2). It also corresponds to other studies that state that antioxidant activity of fresh purple and red-fleshed potatoes is high (Cevallos-Casals & Cisneros-Zevallos, 2003).

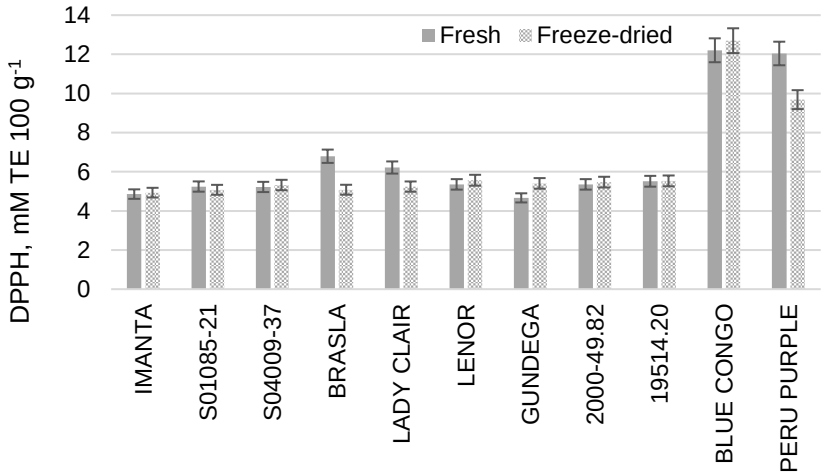

Figure 2. DPPH scavenging activity of fresh and freeze-dried potatoes.

Antioxidant activity is the ability of redox molecules in potatoes to encompass free radicals and taking into account the health promoting influence of all potatoes antioxidants it is important to study health-beneficial compounds (Puchau et al., 2010). Potatoes are considered to be a good source of antioxidants since they contain, for

example polyphenols, ascorbic acid and α-tocopherol (Yanishlieva-Maslarova & Heinonen, 2001).

Antioxidant activity is presented by some classes of biologically active compounds and compounds with such property could be used as an oxidation inhibitor (Jimenez et al., 2017). High costs of freeze-drying does not make it a widely used technological process, but it has some beneficial effects compared to other methods, for example, it helps retain phenolic compounds (Torres et al., 2010) and antioxidant activity (Chan et al., 2009; Zhao et al., 2017).

Potatoes 'Blue Congo' exhibited the highest antioxidant activity after freeze-drying, although generally in almost all freeze-dried samples no significant antioxidant activity reduction has been observed compared to fresh samples. Valadez-Carmona et al. (2017) reported increase in antioxidant activity after freeze drying of cacao, and explained it by extraction efficiency of electrons or hydrogen to stabilize the DPPH$^{\bullet}$ turning them into intermediary stable radicals. The contribution of the sum of the bioactive phenolic compounds and flavonoids suggests that consumers could choose those cultivars with a high content of phenolic compounds and presumably high antioxidative properties. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging activity ranged from 3–47 mM 100 g^{-1} with the highest activity in 'Blue Congo' followed by 'Peru Purple' (Fig. 3). ABTS scavenging for ABTS cation scavenging temperature is a significant factor. The advantage of the ABTS radical is its high reactivity, and thus likely the ability to react with a broader range of antioxidants.

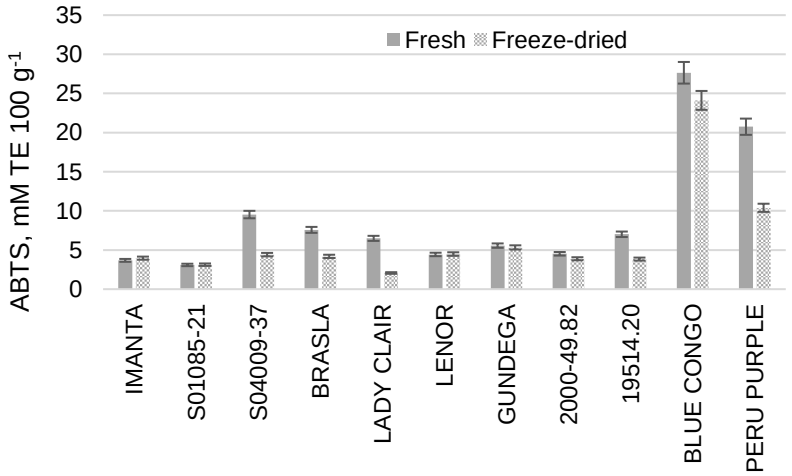

Figure 3. ABTS scavenging activity of fresh and freeze-dried potatoes.

On the other hand, the preparation of the ABTS reagent is more complicated and its stability is lower compared to DPPH (Marecek et al., 2017).

Relationship between phenolic compounds and antioxidant activity

Phenolic compounds have radical scavenging activity. Table 2 shows the Pearson`s correlation coefficients between the phenolic compounds levels and antioxidant activity in fresh and freeze-dried samples.

Table 2. Pearson`s correlation coefficients between antioxidant activity and total phenolic content of fresh and freeze-dried potatoes

Parameters	Fresh	Freeze-dried	Significance level
Total phenolic content / 2,2-diphenyl-1-picrylhydrazyl (DPPH)	0.96	0.95	Substantial
Total phenolic content / 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)	0.95	0.98	Substantial
2,2-diphenyl-1-picrylhydrazyl (DPPH) / 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)	0.95	0.96	Substantial

Potato extracts evaluation presented a highly positive correlation between the content of phenolic compounds and antioxidant activity, thus the increase of one indicator will increase the other indicator. A strong correlation between antiradical activity and phenolic compounds was found in different potato materials.

CONCLUSIONS

This study showed that cultivar and freeze-drying are significant factors affecting TPC, $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ activity. The highest TPC and radical scavenging activity were determined in cultivars 'Peru Purple' and 'Blue Congo' potatoes. Freeze-drying caused the decrease of the content of phenolic compounds and antioxidant activity in potatoes. Freeze-drying influenced all the tested parameters but more significantly the TPC, and for certain cultivars a reduction of more than 30% was observed. A strong correlation between TPC, DPPH and ABTS in fresh and freeze-dried samples was observed, $r = 0.81$, $r = 0.93$, $r = 0.92$, respectively. It could be concluded that the effect of freeze-drying on TPC and antioxidant activity is cultivar dependent.

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REFERENCES

Al Saikhan, M., Howard, L. & Miller, J. 1995. Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum* L.). *Journal of Food Science* **60**(2), 341–343.

Andre, C.M., Ghislain, M., Bertin, P., Oufir, M., Herrera, M.R., Hoffmann, L., Hausman, J.F., Larondelle, Y. & Evers, D. 2007. Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. *Journal of Agricultural and Food Chemistry* **55**(2), 366–378.

Angela, M. & Meireles, A. (Eds.). 2009. Extracting Bioactive Compounds for Food Products Theory and Applications. Taylor & Francis Group, LLC, UK.

Bravo, L. 1998. Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. *Nutrition Reviews* **56**(11), 317–333.

Brown, C.R., Wrolstad, R., Durst, R., Yang, C.P. & Clevidence, B. 2005. Breeding studies in potatoes containing high concentrations of anthocyanins. *American Journal of Potato Research* **80**(4), 241–249.

Casanas, R., Gonzalez, M., Rodriguez, E., Marrero, A. & Diaz, C. 2002. Chemometric Studies of Chemical Compounds in Five Cultivars of Potatoes from Tenerife. *Journal of Agricultural and Food Chemistry* **50**(7), 2076–2082.

Cevallos-Casals, B. & Cisneros-Zevallos, L. 2003. Stoichiometric and kinetic studies of phenolic antioxidants from Andean purple corn and red-fleshed sweet potato. *Journal of Agricultural and Food Chemistry* **51**(11), 3313–3319.

Chan, E.W.C., Lim, Y.Y., Wong, S.K., Lim, K.K. & Yong, M.Y. 2009. Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chemistry* **113**, 166–172.

Chun, O.K., Kim, D.O., Smith, N., Schroeder, D., Han, J.T. & Lee, C.Y. 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture* **85**(10), 1715–1724.

Duthie, G., Duthie, S. & Kyle, J. 2000. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutrition Research Reviews* **13**(1), 79–106.

Espin, J.C, Rivas, C.S., Wichers, H.J. & Viguera, C.G. 2000. Anthocyanin based natural colorants: a new source of antiradical activity for foodstuff. *Journal of Agricultural and Food Chemistry* **48**(5), 1588–1592.

Ezekiel, R., Singh, N., Sharma, S. & Kaur, A. 2013. Beneficial phytochemicals in potato – a review. *Food Research International* **50**(2), 487–496.

Furrer, A., Cladis, D.P., Kurilich, A., Manoharan, R. & Ferruzzi, M.G. 2017. Changes in phenolic content of commercial potato varieties through industrial processing and fresh preparation. *Food Chemistry* **218**, 47–55.

Han, K.H., Matsumoto, A., Shimada, K., Sekikawa, M. & Fukushima, M. 2007. Effects of anthocyanin-rich purple potato flakes on antioxidant status in F344 rats fed a cholesterol-rich diet. *British Journal of Nutrition* **98**(5), 914–921.

Jansen, G. & Flamme, W. 2006. Coloured potatoes (Solanum tuberosum L.) – anthocyanin content and tuber quality. *Genetic Research and Crop Evolution* **53**(7), 1321–1331.

Jiménez, P., García, P., Bustamante, A., Barriga, A. & Robert, P. 2017. Thermal stability of oils added with avocado (*Persea americana cv. Hass*) or olive (*Olea europaea cv. Arbequina*) leaf extracts during the French potatoes frying. *Food Chemistry* **221**, 123–129.

Katan, M.B. & Roos, N.M. 2004. Promises and problems of functional foods. *Critical Reviews in Food Science and Nutrition* **44**(5), 369–377.

Lei, C., Ma, Q., Tang, Q.Y., Ai, X.R., Zhou, Z., Yao, L., Wang, Y., Wang, Q. & Dong, J.Z. 2014. Sodium selenite regulates phenolics accumulation and tuber development of purple potatoes. *Scientia Horticulturae* **165**, 142–147.

Leo, L., Leone, A., Longo, C., Lombardi, D.A., Raimo, F. & Zacheo, G. 2008. Antioxidant compounds and antioxidant activity in early potatoes. *Journal of Agricultural and Food Chemistry* **56**(11), 4154–4163.

Lewis, C.E., Walker, J.R.L. & Lancaster, J.E. 1999. Changes in anthocyanin, flavonoid and phenolic acid concentrations during development and storage of coloured potato (*Solanum tuberosum* L.) tubers. *Journal of the Science of Food and Agriculture* **79**(2), 311–316.

Manach, C., Scalbert, A., Morand, C., Remesy, C. & Jimenez, L. 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition* **79**(5), 727–747.

Marecek, V., Mikyska, A., Hampel, D., Cejka, P., Neuwirthova, J., Malachova, A., Cerkal, A. 2017. ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. *Journal of Cereal Science* **73**, 40–45.

Mattila, P. & Hellstrom, J. 2006. Phenolic acids in potatoes, vegetables, and some of their products. *Journal of Food Composition and Analysis* **20**(3–4), 152–160.

Nara, K., Miyoshi, T., Honma, T. & Koga, H. 2006. Antioxidative activity of bound form phenolics in potato peel. *Bioscience, Biotechnology, and Biochemistry* **70**(6), 1489–1491.

Navarre, D.A., Pillai, S.S., Shakya, R. & Holden, M.J. 2011. HPLC profiling of phenolics in diverse potato genotypes. *Food Chemistry* **127**(1), 34–41.

Nayak, B. 2011. Effect of thermal processing on the phenolic antioxidants of colored potatoes. Doctoral dissertation, Washington State University, Washington, USA.

Parr, A.J. & Bolwell, G.P. 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture* **80**(7), 985–1012.

Puchau, B., Zulet, M.A., Echavarri, A.G., Hermsdorff, H.H.M. & Martínez, J.A. 2010. Dietary total antioxidant capacity is negatively associated with some metabolic syndrome features in healthy young adults. *Nutrition* **26**(5), 534–541.

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* **26**(9–10), 1231–1237.

Reyes, L.F. & Zevallos, L.C. 2003. Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum* L.). *Journal of Agricultural and Food Chemistry* **51**(18), 5296–5300.

Reyes, L.F., Miller, J.C. & Zevallos, L.C. 2005. Antioxidant capacity, anthocyanins and total phenolics in purple and red fleshed potato (*Solanunm tuberosum* L.) genotypes. *American Journal of Potato Research* **82**(4), 271–277.

Scalbert, A., Manach, C., Morand, C., Remesy, C. & Jimenez, L. 2005. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition* **45**(4), 287–306.

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 Enzymology* **119**, 152–178.

Steed, L. & Truong, V.D. 2008. Anthocyanin content, antioxidant activity, and selected physical properties of flowable purple-fleshed sweetpotato purees. *Journal of Food Science* **73**(5), 215–221.

Torres, C., Díaz-Maroto, M.C., Hermosín-Gutiérrez, I. & Pérez-Coello, M.S. 2010. Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin. *Analytica Chimica Acta* **660**(1–2), 177–182.

Valadez-Carmona, L., Plazola-Jacinto, C.P., Hernández-Ortega, M., Hernández-Navarro, M.D., Villarreal, F., Necoechea-Mondragón, H., Ortiz-Moreno, A. & Ceballos-Reyes, G. 2017. Effects of microwaves, hot air and freeze-drying on the phenolic compounds, antioxidant capacity, enzyme activity and microstructure of cacao pod husks (*Theobroma cacao* L.) *Innovative Food Science & Emerging Technologies* **41**, 378–386.

Velioglu, Y.S, Mazza, G., Gao, L. & Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry* **46**(10), 4113–4117.

Yanishlieva-Maslarova, N.V. & Heinonen, I.M. 2001. Sources of natural antioxidants: vegetables, fruits, herbs, spices and teas. **In** J. Pokorny, N. Yanishlieva & M. Gordon (Eds.), *Antioxidants in food: Practical applications,* pp. 210–266. UK: Woodhead Publishing.

Yu, L., Perret, J., Harris, M., Wilson, J. & Haley, S. 2003. Antioxidant properties of bran extracts from 'Akron' wheat grown at different locations. *Journal of Agricultural and Food Chemistry* **51**(6), 1566–1570.

Zhao, G., Zhang, R., Liu, L., Deng, Y., Wei, Z., Zhang, Y., Ma, Y. & Zhang, M. 2017. Different thermal drying methods affect the phenolic profiles, their bioaccessibility and antioxidant activity in Rhodomyrtus tomentosa (Ait.) Hassk berries. *LWT – Food Science and Technology* **79**, 260–266.

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A study of commercial β-galactosidase stability under simulated *in vitro* gastric conditions

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Abstract. β-Galactosidase activity in milk may be affected by several factors, such as temperature, pH, milk composition, and metal ions. It is important to note that digestive proteases and gastrointestinal pH can affect enzyme activity during transit through the gastrointestinal tract. For the investigation of commercial β-galactosidase stability in human and animal gastric tracts, human gastrointestinal tract (GIT) models were employed, enabling prediction of enzyme activity under *in vivo* conditions. The aim of this study was to analyse and compare commercial β-galactosidase stability under simulated *in vitro* gastric conditions. Commercial enzymes (Ha Lactase 5200 produced by *Kluyveromyces lactis* and NOLA™Fit5500 produced by *Bifidobacterium bifidum* expressed in *Bacillus licheniformis*, Chr. Hansen, Hørsholm, Denmark; GODO-YNL2 produced by *Kluyveromyces lactis*, Danisco, Copenhagen, Denmark) were used for this study. Commercial enzymes were added to GIT models at 1 and 5 mL L^{-1}. The enzyme activity was assessed as the percentage of lactose hydrolysis by the enzymes from *Kluyveromyces lactis* and *Bacillus licheniformis* using HPLC after digestion. β-Galactosidase extracted from yeast (*Kluyveromyces lactis*) and bacteria (*Bacillus licheniformis*) was found to be effective as a strategy for improving lactose tolerance.

Key words: β-galactosidase, simulated gastric conditions, lactose hydrolysis.

INTRODUCTION

Lactose is the major disaccharide in milk and can be hydrolysed into glucose and galactose by β-galactosidase (Gambelli, 2017). Lactose is digested *in vivo* by a membrane-bound enzyme of the small intestinal epithelial cells in the human body (O'Connell & Walsh, 2010). Lactose is hydrolysed most slowly out of all of the dietary sugars. The hydrolysis of lactose occurs at only half the rate of sucrose hydrolysis. Generally, carbohydrates increase intestinal calcium absorption, and lactose, which is predominantly present in dairy products, is the most effective calcium absorbing carbohydrate (Pérez et al., 2008). Consumption of lactose-containing dairy products should be minimised for individuals with lactose intolerance (Dutra Rosolen et al., 2015). Certain ethnic and racial groups have increased propensity for β-galactosidase deficiency in the digestive tract. Most Asians (more than 90%), Africans (80–100%), Native Americans (more than 90%) and Southern Europeans (more than 80%) are

reported to be lactose intolerant (Mlichová & Rosenberg, 2006), mainly due to a low level of β-galactosidase in the intestinal walls.

One potential solution to the lack of this enzyme is a strategy of oral consumption of β-galactosidase for deficient individuals whilst consuming products containing lactose (Bosso et al., 2015). The market offers a great variety of caplets, chewable tablets, or soft gel capsules which contain β-galactosidase that improve gastrointestinal digestion of lactose and eliminate the symptoms caused by β-galactosidase deficiency. The ability of β-galactosidase preparations in the gastrointestinal tract depends upon degradation and enzyme activity under physiological conditions (Wang et al., 2009). There are several factors which can affect activity of β-galactosidase, such as temperature, pH, gastric enzymes, and bile acids. Furthermore, depending upon the source of β-galactosidase extraction, the enzyme may display diverse properties in a variety of applications (Bosso et al., 2016). Enzymes which need to be released in the intestine, such as β-galactosidase, necessarily have to pass intact through the stomach and need to be resistant to acidic pH in the range of 1.0 to 3.0 (Bosso et al., 2015). Therefore, most supplemental β-galactosidase preparations are coated to prevent gastric inactivation, and become activated in the intestine (Wang et al., 2009).

Bosso et al. (2015) have shown that *A. oryzae* and *K. lactis* β-galactosidases are inactivated under simulated gastrointestinal digestive conditions. The enzyme from *A. oryzae* was less effective for lactose hydrolysis than the enzyme from *K. lactis.* These authors determined that lactose hydrolysis was over 90% with the highest concentration of the enzyme from *K. lactis* at 40 °C. The highest levels of hydrolysis were at 37 °C and at enzyme concentrations of 3 and 5 mL L^{-1} (Bosso et al., 2015). The principal objective of this study was to analyse and compare commercial β-galactosidase stability under simulated *in vitro* gastric condition at different concentrations.

MATERIALS AND METHODS

B-Galactosidase resistance to inactivation was evaluated under simulated human stomach and small intestine digestive conditions.

Chemicals

All chemicals KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$, $(NH_4)_2CO_3$, NaOH, HCl, $CaCl_2$, bile salt, porcine pepsin (EC 3.4.23.1), porcine trypsin (EC 3.4.21.4), bovine chymotrypsin (EC 3.4.21.1), porcine pancreatic α-amylase (EC 3.2.1.1), porcine pancreatic lipase (EC 3.1.1.3), porcine pancreatic colipase and D-lactose monohydrate, D-(+)-glucose, D-(+)-galactose (≥ 98%, HPLC) were purchased from Sigma-Aldrich (Riga, Latvia).

Commercial enzymes

Three commercial β-galactosidase preparations were used in this study: NOLA™Fit5500 and Ha-Lactase 5200 (Chr. Hansen, Hørsholm, Denmark) and GODO YNL2 (Danisco, Copenhagen, Denmark). NOLA™Fit5500 is a *Bifidobacterium bifidum* ß-galactosidase (lactase) produced by submerged fermentation on a vegetable substrate using a selected strain of *Bacillus licheniformis* kept under contained conditions and not present in the final product (NOLA™Fit5500 Product Information, 2017). The other two enzymes are of *Kluyveromyces lactis* origin, GODO-YNL2 and

Ha-Lactase 5200 are active at neutral condition pH 6.5–8.0 (Ha-Lactase 5200 Product Information, 2014). NOLA™Fit5500 enzyme is active under acidic conditions (optimum pH 5.0–7.0) according to the manufacturers' data. GODO-YNL2 and Ha-Lactase 5200 enzymes have an activity of 5,000 NLU (neutral lactase units) mL^{-1} and 5,200 NLU mL^{-1}, respectively, and NOLA™Fit5500 has an activity of 5,500 BLU (bifido lactase units) mL^{-1}. All enzymes remained fully active throughout the study.

***In vitro* digestion of commercial β-galactosidase**

Three experiments were carried out to determine the effect of simulated gastrointestinal tract conditions on commercial GODO-YNL2 and Ha-Lactase 5200 (*Kluyveromyces lactis*) and NOLA™Fit5500 (*Bacillus licheniformis*). The modified method of Minekus et al. (2014) for gastrointestinal incubation of β-galactosidase was used in order to determine enzyme activity. Concentrations of gastric (SGF) and intestinal (SIF) electrolyte stock solutions, as well as gastrointestinal enzyme activities (U ml^{-1} digesta) were calculated according to the international consensus reported by Minekus et al. (2014).

In all experiments, the enzyme concentrations were 1 and 5 mL L^{-1}, calculated as 5,000 and 25,000 NLU mL^{-1} for GODO-YNL2 enzyme, 5,200 and 26,000 NLU mL^{-1} for Ha-Lactase 5200 enzyme, and 5,500 and 27,500 BLU mL^{-1} for NOLA™Fit5500 enzyme (Fig. 1).

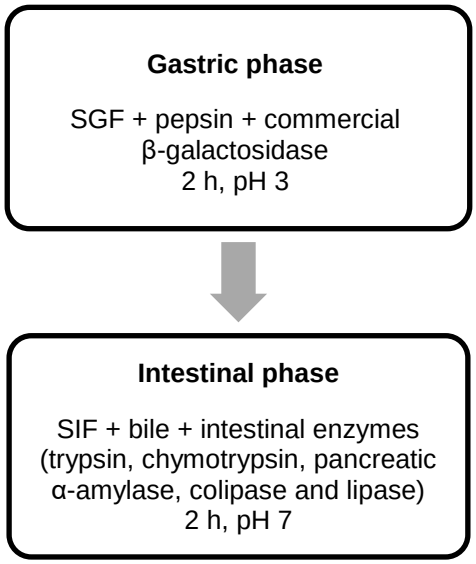

Figure 1. Diagram of a simulated *in vitro* digestion system. The GIT system (static gastrointestinal tract simulator) comprised of a single stirred tank reactor (Labfors 5, INFORS HT, Bottmingen, Switzerland) connected to the computer and controlled by bioprocess monitoring and control software 'Iris 6' (INFORS HT).

Each phase concentration/dosage of stock solution and enzyme activity were prepared according to Minekus et al. (2014).

Gastric phase

Liquid commercial β-galactosidase enzyme samples were mixed with 120 mL of simulated gastric fluid (SGF), which is the medium simulating gastric conditions in the fasted state, water, pepsin and 1 M HCl was added to reduce the pH to 3.0. Solution was incubated at 37 °C for 2 hours.

Intestinal phase

After 2 hours of gastric digestion, 160 mL of simulated intestinal fluid (SIF), intestinal enzymes and bile salt were added. NaOH (1 M) was used to raise the pH to 7.0 and the solution was incubated at 37 °C for another 2 hours. At this stage, 5% lactose solution was added to the flasks.

Sample preparation after digestion

The digested samples were taken after the small intestine digestion at the end of experiment, and β-galactosidase activity was measured. The samples were heated at 90–95 °C to inactivate the enzymes and kept at 4–6 °C until further analysis. The experiments were carried out with three replicates for each test.

Determination of lactose, glucose and galactose

The enzyme activity was determined according to percentage of lactose hydrolysis by the enzymes from *Kluyveromyces lactis* and *Bifidobacterium bifidum* using HPLC (Prominence HPLC system, Shimadzu LC-20, Torrance, CA, USA), refractive index detector RID-10A; Alltech NH_2, 4.6 mm x 250 mm, 5 μm column; temperature of 30 °C; mobile phase isocratic elution: A – acetonitrile 84%, B – deionized water 16%; capacity of the injection sample: 10 μL; total analysis time of up to 25 min; rate of flow: 1.0 mL min^{-1}.

Statistical analysis

Statistical analyses were performed with analysis of variance ANOVA and *Tukey* test at the significance level $P < 0.05$.

RESULTS AND DISCUSSION

All enzymes at a concentration of 1 mL L^{-1} showed higher lactose hydrolysis compared with 5 mL L^{-1} after simulation of enzyme passage through the stomach and small intestines (Table 1).

Table 1. Lactose hydrolysis (%) by *Kluyveromyces lactis* and *Bacillus licheniformis* β-galactosidases at different concentrations after intestinal phase digestion

Commercial enzyme	Hydrolysis, %	
	1 mL L^{-1}	5 mL L^{-1}
GODO-YNL2 *	42.5 ± 2.5 [a]	39.8 ± 4.8 [a]
NOLA™Fit5500 **	57.3 ± 4.3 [b]	49.1 ± 5.7 [b]
Ha-Lactase 5200 *	62.5 ± 3.9 [b]	56.7 ± 4.1 [c]

*Enzyme from *Kluyveromyces lactis;* **Enzyme from *Bacillus licheniformis.*
Results indicated with the same letter within a column do not differ significantly ($P < 0.05$).

Results showed that there is no significant difference between commercial enzyme concentrations ($F = 11.8$, $P > 0.05$), but in turn a significant difference between commercial enzyme preparates within each concentration was noticed ($F = 44.5$, $P < 0.05$).

Lactose hydrolysis was observed for all enzymes, however Ha-Lactase 5200 β-galactosidase showed greater hydrolysis for all concentrations studied. Contrary to yeast neutral lactase, acid lactase continues to hydrolyse lactose to approximately pH 4.5 and remains active in the presence of digestive enzymes and bile acids (Selvarajan & Mohanasrinivasan, 2015). This result can be explained by the findings of Vrese et al. (2001) where the effect was substantiated, largely due to digestion of lactose by the probiotic lactase activity, effectively performing the functions of a defective human enzyme, and also due to a slower transit time of the product.

Kwak (2001) evaluated the enzyme activity of microencapsulated β-galactosidase from *K. lactis* in a simulated human intestinal system (pH 7–8) and found 60.8 to 68.8% hydrolysis after 60 min of testing. The percentages are higher than the results found in the present study, where lactose hydrolysis up to 42.5% was obtained for GODO-YNL2 *K. lactis* β-galactosidase at 37 °C. However, lactose hydrolysis up to 62.5% was obtained for Ha-Lactase 5200 *K. lactis* β-galactosidase indicating a stronger resistance to digestion and higher activity of this enzyme. Lower lactose hydrolysis rates can be explained by the temperature. A temperature of 37 °C used in the GIT is lower than the optimal temperature of GODO-YNL2 enzyme (40 °C), but for NOLA™Fit5500 (35–50 °C) and Ha-Lactase 5200 (35–45 °C), is within the optimal range. Furthermore, Vidya et al. (2014) pointed out that the enzyme activity can be affected by the type of strain, cultivation conditions (temperature, pH, aeration, agitation and incubation time) and the growth media composition (particularly carbon and nitrogen sources). This leads to the conclusion that commercial β-galactosidase preparations produced from the same species, but using a different method which includes cultivation conditions and the growth medium composition, can impact enzyme physical properties.

Kotz et al. (1994) analysed β-galactosidase activity in conventional yoghurt and high lactase (HL) yoghurt during 60 min incubation at 37 °C and pH 3.5. The β-galactosidase in HL yoghurt was much less acid resistant than was the β-galactosidase in conventional yoghurt, likely due to the deactivation of β-galactosidase in the human gastrointestinal system. The authors assumed that β-galactosidase at high concentrations and at low pH is more sensitive to denaturation, and the dose of the enzyme is one of the factors which can impact degree of hydrolysis.

The results show that the highest degree of hydrolysis can be obtained using a β–galactosidase concentration of 1 mL L^{-1} (Fig. 2), rather than 5 mL L^{-1} (Fig. 3). The method used in this study was based on the proportions between the intake of food, digestive enzymes, and stock solution. This indicates that low concentrations of enzymes have a greater chance of maintaining activity by dissolving in a fermentable environment.

Metal ions can affect enzyme activity. For the yeast β-galactosidase which is isolated from *Kluyveromyces lactis* K^+ and Mg^{2+} worked as activators, whereas Ca^{2+} and Na^+ worked as inhibitors; for β-galactosidase from *Bacillus licheniformis* Ca^{2+}, Mn^{2+}, and Mg^{2+} were activators, while Cu^{2+}, Zn^{2+}, and Fe^{2+} were inhibitors (Zolnere, Liepins, & Ciprovica, 2017). For preparation of stock solutions according to Minekus (2014) a variety of salts were added (KCl; KH_2PO_4; $NaHCO_3$; NaCl; $MgCl_2(H_2O)_6$; $(NH_4)_2CO_3$;

$CaCl_2$) in different concentrations. This can also impact the amount of hydrolysed lactose (Adalberto et al., 2010).

Studying kinetic parameters of β-galactosidase from *Kluyveromyces lactis* Mateo et al., 2004 found that galactose was competitive and glucose noncompetitive inhibitor. Juajun et al. 2011 analysed bioconversion of lactose using β-galactosidase from *Bacillus licheniformis*, and the results showed that mainly D-galactose is an inhibitor.

Transgalactosylation is the reaction by which the enzyme β-galactosidase hydrolyzes lactose and transfers galactose to another carbohydrate, forming galactooligosaccharides (Otieno, 2010). Such reactions may explain why glucose and galactose concentrations are not equal upon lactose hydrolysis (Figs 2, 3).

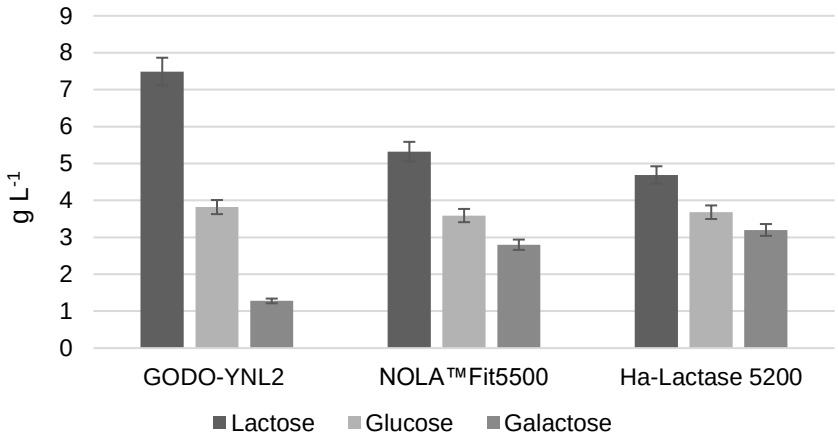

Figure 2. Sugars remaining after hydrolysis of an initial amount of 12.50 g in the GIT by *Kluyveromyces lactis* and *Bacillus licheniformis* commercial β-galactosidases at a concentration of 1 mL L^{-1}.

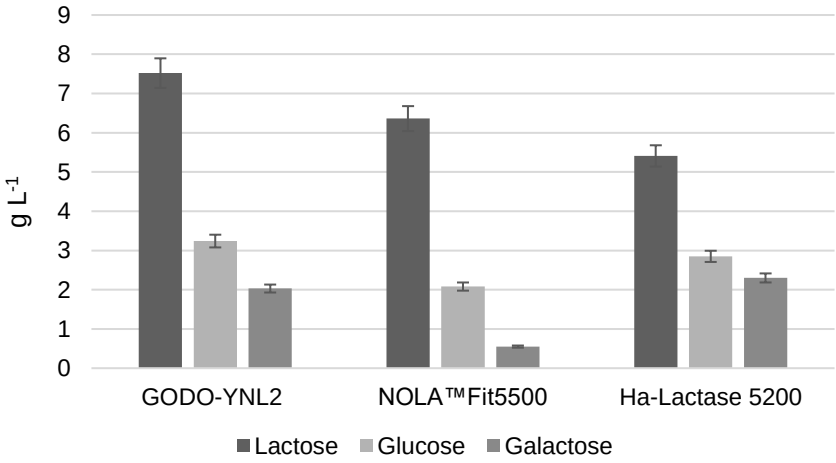

Figure 3. Sugars remaining after hydrolysis of an initial amount of 12.50 g in the GIT by *Kluyveromyces lactis* and *Bacillus licheniformis* commercial β-galactosidases at a concentration of 5 mL L^{-1}.

There were no statistical differences ($P < 0.05$) in lactose hydrolysis by enzymes from *B. licheniformis* and *K. lactis* under simulated intestinal conditions. Relatively high levels of lactose hydrolysis were obtained, especially for enzyme concentrations of 1 mL L^{-1}.

CONCLUSIONS

The present study indicates that β-galactosidases extracted from yeast (*Kluyveromyces lactis*) and bacteria (*Bacillus licheniformis*) were effective under *in vitro* digestive conditions as a strategy for improving lactose tolerance. The dose of β galactosidase enzyme is one of the factors which can impact upon the degree of lactose hydrolysis in the human gastrointestinal system. Enzymes at high concentrations and low pH are more sensitive to denaturation. Under *in vitro* conditions, the highest hydrolysis percentages were for NOLA™Fit5500 and Ha-Lactase 5200 enzymes, thus these were most effective at mitigating against lactose intolerance. In future, an encapsulation method may allow the creation of orally used β-galactosidase preparations to be consumed with food products containing lactose.

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REFERENCES

Adalberto, P.R.R., Massabni, A.C.C., Carmona, E.C.C., Goulart, A.J.J., Marques, D.P.P. & Monti, R. 2010. Effect of divalent metal ions on the activity and stability of β-galactosidase isolated from *Kluyveromyces lactis*. *Journal of Basic and Applied Pharmaceutical Sciences* **31**, 143–150.

Bosso, A., Hoch Batista de Souza, C. & Hiroshi Suguimoto, H. 2015. Enzyme activity of β-galactosidase from *Kluyveromyces lactis* and *Aspergillus oryzae* on simulated conditions of human gastrointestinal system. *Journal of Basic and Applied Pharmaceutical Sciences* **36**, 153–157.

Bosso, A., Morioka, L.R.I., Santos, L.F. & Suguimoto, H.H. 2016. B-galactosidase hydrolysis in processed milk. *Food Science and Technology* **36**, 159–165.

Dutra Rosolen, M., Gennari, A., Volpato, G. & Volken De Souza, C.F. 2015. Lactose hydrolysis in milk and dairy whey using microbial β-galactosidases. *Enzyme Research* **2015**, 1–7.

Gambelli, L. 2017. Milk and its sugar – lactose: a picture of evaluation methodologies. *Beverages* **3**, 35.

Ha-Lactase 5200 Product Information 2014. https://hjemmeriet.com/no/uploads/dokumenter/PI_GLOB_Ha-Lactase5200_450804_EN.pdf. Accessed 28.1.2018

Juajun, O., Nguyen, T.H.H., Maischberger, T., Iqbal, S., Haltrich, D. & Yamabhai, M. 2011. Cloning, purification, and characterization of β-galactosidase from *Bacillus licheniformis* DSM 13. *Applied Microbiology and Biotechnology* **89**, 645–654.

Kotz, C.M., Furne, J.K., Savaiano, D.A. & Levitt, M.D. 1994. Factors affecting the ability of a high β-galactosidase yogurt to enhance lactose absorption. *Journal of Dairy Science* **77**, 3538–3544.

Kwak, H.M. 2001. Microencapsulation of β galactosidase with fatty acid esters. *Journal of Dairy Science* **84**, 1576–1582.

Mateo, C., Monti, R., Pessela, B.C.C., Fuentes, M., Torres, R., Guisan, J.M. & Fernandez-Lafuente, R. 2004. Immobilization of lactase from *Kluyveromyces lactis* greatly reduces the inhibition promoted by glucose. Full Hydrolysis of Lactose in Milk. *Biotechnology Progress* **20**, 1259–1262.

Minekus, M., Alminger, M., Alvito, P, Balance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D.J., Ménard, O., Recio, I., Santos, C.N., Singh, R.P., Vegarud, G.E., Wickham, M.S., Weitschies, W. & Brodkorb, A. 2014. A standardised static *in vitro* digestion method suitable for food – an international consensus. *Food and Function* **5**, 1113–1124.

Mlichová, Z. & Rosenberg, M. 2006. Current trends of β-galactosidase application in food technology. *Journal of Food and Nutrition Research* **45**, 47–54.

NOLA™Fit5500 Product Information 2017. https://hjemmeriet.com/da/ChrHansen/Products/NOLA-Fit/PI_GLOB_NOLA_Fit5500_350502_EN.pdf. Accessed 28.1.2018

O'Connell, S. & Walsh, G. 2010. A novel acid-stable, acid-active β-galactosidase potentially suited to the alleviation of lactose intolerance. *Applied Microbiology and Biotechnology* **86**, 517–524.

Otieno, D.O. 2010. Synthesis of β-Galactooligosaccharides from Lactose Using Microbial β-Galactosidases. *Comprehensive Reviews in Food Science and Food Safety* **9**, 471–482.

Pérez, A.V., Picotto, G., Carpentieri, A.R., Rivoira, M.A., Peralta, L.M.E. & Tolosa de Talamoni, N.G. 2008. Minireview on regulation of intestinal calcium absorption. *Digestion* **77**, 22–34.

Selvarajan, E. & Mohanasrinivasan, V. 2015. Kinetic studies on exploring lactose hydrolysis potential of β galactosidase extracted from *Lactobacillus plantarum* HF571129. *Journal of Food Science and Technology* **52**, 6206–6217.

Vrese, M., Stegelmann, A., Richter, B., Fenselau, S., Laue, C. & Schrezenmeir, J. 2001. Probiotics – compensation for lactase insufficiency. *American Journal of Clinical Nutrition* **73**, 421–429.

Wang, H., Luo, H., Bai, Y., Wang, Y., Yang, P., Shi, P. & Fan, Y. 2009. An acidophilic β-galactosidase from *Bispora* sp. MEY–1 with high lactose hydrolytic activity under simulated gastric conditions. *Journal of Agricultural and Food Chemistry* **57**, 5535–5541.

Vidya, B., Palaniswamy, M. & Gopalakrishnan, V.K. 2014. Screening and optimization of β-galactosidase from fungal strains by using agro residues. *World Journal of Pharmacy and Pharmaceutical Sciences* **3**, 1809–1821.

Zolnere, K., Liepins, J. & Ciprovica, I. 2017. The impact of calcium ions on commercially available β-galactosidase. **In** E. Straumite (Ed.), 1*1th Baltic Conference on Food Science and Technology 'Food science and technology in a changing world*' FOODBALT 2017 Conference, Jelgava, pp. 27–30.

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