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Dear participants,

The FOODBALT 2019 13th Baltic Conference on Food Science and Technology "Food. Nutrition. Well-Being" joined with 5th North and East European Congress on Food "NEEFood 2019" is organized by the Faculty of Food Technology on 2nd to 3rd May, 2019 in the Latvia University of Life Sciences and Technologies (LLU). The conference is a representation of the multidisciplinarity, transdisciplinarity and interdisciplinarity of food science, technology and experiential knowledge. It brought together food scientists, researchers as well as students from different countries, promoting the dissemination of new knowledge and allowing to exchange the latest research results in the area of food science and technology. -

The conference attracted more than 110 delegates from 13 countries. The conference programme contains 4 Key lectures, 37 oral and 18 e-poster presentations in 7 sessions. Additionally, a total of 68 posters are presented. The conference call accepted papers from a range of different topics: Health-relevant food products and issues, Quality and safety of processed foods, Trends in new food product and technology development, Food chemistry, analysis and quality assessment, Food material/packaging interaction, Sustainable processing and packaging, Consumer behaviour and sensory issues, Food chain management (raw materials, logistics, economics, information systems, etc.). The conference Organising Committee received 61 full paper submissions from 7 countries. A peer review process was enforced with the help of 45 food and related sciences experts from 8 different countries. Totally, 3 reviews, 40 original papers and 10 short communications were selected for publishing.

The conference could not be realized without the vital contribution of the participants who present research papers in the sessions or posters. Your participation is invaluable for both us and the conference as a whole. The Organizing Committee hope that you will find the presentations valuable, stimulating and interesting for your scientific activities. Also, we are convinced that your stay in Jelgava will be educational, scientific and socially beneficial.

The chair of the joined FOODBALT 2019 13th Baltic Conference on Food Science and Technology and 5th North and East European Congress on Food "NEEFood 2019

Dr.sc.ing. Martins Sabovics

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REVIEW

INFLUENCE OF THE ROASTING PROCESS ON BIOACTIVE COMPOUNDS AND AROMA PROFILE IN SPECIALTY COFFEE: A REVIEW

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Abstract

The coffee roasting process is one of the most important parts of coffee aroma formation, and also has a varied influence on the biologically active compounds composition in coffee. It is essential to understand the point of the roasting process when the pleasant specialty coffee aroma compounds and health-beneficial bioactive compounds are at the best ratio. The aim of the review was to evaluate the technological parameters in the specialty coffees roasting process to obtain optimal aroma profile and composition of bioactive compounds. The loss of aroma compounds with fruity, floral and sweet acidic notes in roasting process happens slower than the loss of chlorogenic acid. Meanwhile compounds like melanoidins with anti-oxidative properties and chlorogenic acid lactones mostly form in medium roasting level, but due to their bitter sensory characteristics, they have low cupping score. Both, the aroma compounds and bioactive compounds start rapidly decreasing by the medium–dark roast. It is proven, that antioxidant activity stays at the highest point in the light–medium roast level when coffee melanoidins start to form and the chlorogenic acid is still at high concentration and good cup quality remains. By knowing the roasting process influence on chemical properties of coffees aroma and bioactive compound composition it is possible to maintain high coffee cupping score without losing the valuable bioactive compounds.

Keywords: specialty coffee, roasting, aroma, phenolic compounds

Introduction

Moderate coffee consumption is more associated with positive health benefits. The bioactive compounds are responsible for the antioxidant, antibacterial and anti–inflammatory activities. Their wide and powerful variety contributes to regular coffee consumption benefits. Moderate coffee consumption is associated with lower cardiovascular diseases risk, for example, Martínez-Lopez et al. (2018) research suggests that drinking 3 cups of coffee a day positively affects blood pressure, blood glucose and triglyceride levels, which overall contributes to lower metabolic syndrome risk. According to Gutiérrez-Grobe et al. (2012), higher coffee consumption is associated with lower non-alcoholic fatty liver disease risk. To better understand the antioxidant properties of coffee it is important to evaluate the bioactive compounds individually. Chlorogenic acid has shown the highest antioxidant activity in coffee. Chlorogenic acid can decrease breast and pancreatic cancer risk, as also empowering overall cardiovascular health (Keefe et al., 2013). Aside from the strong bitter flavour, phenylindanes are known with their neuroprotective effect in Alzheimer's disease pathologies (Mancini et al., 2018). Caffeine is one of the most powerful bioactive compounds in coffee. By drinking 3–5 cups of coffee a day it can prevent dementia risk in future (Carman et al., 2014). Also, caffeine has proven antidiabetic properties, caffeine has the ability to increase fat metabolism and control blood sugar level (Nuhu, 2014). Both, trigonelline and nicotinic acid show antioxidant properties to pancreatic cancer cells and trigonelline has ability to reduce blood glucose levels (Yoshinari, Igarashi, 2010). Melanoidins have powerful antibacterial activity against Gram–positive (*Staphylococcus aureus*) and Gram–negative (*Escherichia coli, Salmonella typhimurium*) bacteria (Moreira et al., 2012). Also, melanoidins can act as dietary fibre (Carman et al., 2014), this could approve the positive influence of melanoidins on healthy gut microbiota (Lopes et al., 2016; Coelho, 2014). All bioactive compounds from sensory point of view have bitter flavour notes, which can influence the overall coffee taste.

Specialty coffee is coffee which is standardized by the whole coffee process cycle from choosing coffee plantation criteria's till coffee brew serving to client. Specialty coffee flavour is focused on more fruity, floral and acidic notes. The presence of at least five different flavour notes within balance in coffee is valued with higher sensory score (McCoy et al., 2017; Alex et al., 2016). The sensory analysis system for specialty coffee is cup tasting or cupping, which is done by Specialty Coffee Association (SCA) guidelines and cupping protocol (2015).

The roasting process is responsible for cascade chemical reactions, in which from non-volatile compound degradation forms new compounds with potential flavour attributes (Toledo et al., 2016).

Despite the fact that the roasting process is widely analysed it is challenging to compare the roasting levels between studies because the roasting parameters are significantly different (Table 1). Several studies only indicate roasting level without specific time and temperature parameters. In previous studies the roasting temperature for specialty coffee was mostly chosen in range between 160–200 °C and roasting time range between 4–12 minutes (Fassio et al., 2017; Tolessa et al., 2016; Piccino et al., 2014). From specialty coffee point of view, Moon, Shibamoto, (2009) research have the most similar roasting parameters with roasting

standards in Latvia specialty coffee roasteries, these parameters were chosen as roast level measures in this review.

Main chemical reactions during roasting are Maillard reaction, Strecker degradation. Non–volatile compounds involved in Maillard reaction are amino acids and reducing sugars forming end products melanoidins during roasting (Vignoli et al., 2014). Chlorogenic acid degradation is also involved in melanoidin formation and passes the antioxidant activity (Bartel et al., 2015). Strecker degradation is responsible for free amino acid reactions in roasting process, creating beneficial flavour compounds. One of Strecker compounds are 2,3-pentanedione with buttery flavour notes, methanethiol with fresh, airy flavour notes (Kim et al., 2018; Cheong et al., 2013).

Table 1

Coffee roasting parameters

References	Roasting level		
	Light	Medium	Dark
Moon, Shibamoto (2009)	170–230 °C	230–240 °C	240–250 °C
Bhumiratana et al. (2011)	180–205 °C 6 min 30 s	180–238 °C 7 min 30 s	180–238 °C 8 min 30 s
Somporn et al. (2011)	230 °C 12 min	240 °C 14 min	250 °C 17 min
Vignoli et al. (2014)	215 °C 7 min		225 °C 10 min

In the process of roasting, various compounds, that differ in structure and chemical properties, are formed, because of that, roasting parameters are very crucial in creating specific coffee flavours and overall quality (Vignoli et al., 2014). By understanding the roasting process effects on bioactive compound composition and aroma profile development, it could be possible to suggest guideline criteria to achieve high quality coffee with high beneficial bioactive compound content. The aim of the review was to evaluate the technological parameters in the specialty coffees roasting process to obtain optimal aroma profile and composition of bioactive compounds.

Materials and Methods

The study was preceded by using the monographic method and summarizing actual researches of influence of roasting process on individual bioactive compounds and aroma profile. The review summarises and evaluates the bioactive compound and aroma compound changes during roasting process.

Results and Discussion

Bioactive compounds change during the roasting process

Roasting process significantly decreases the concentration and therefore antioxidant ability of such biologically active compound as chlorogenic acid. Mojica et al. (2018) evaluated anticancer activities of coffee samples with different roast levels and in the result at light roast level (225 °C) coffee extracts had the highest total phenolic content and antioxidant capacity. Chlorogenic acid concentration decreased rapidly after roasting process, but gallic and caffeic acids had the highest concentration in light level roasted coffee (225 °C). The light roasted coffee extracts also showed the highest antiproliferative effect on colon cancer cells (Mojica et al., 2018). Other studies show that light roasted coffee can even increase antioxidant activity of melanoidins (Bekedam et al., 2008). But overall total polyphenol concentration is rapidly decreasing during roasting process. Several studies show that chlorogenic acid isomer 5-caffeoylquinic acid has the most significant concentration reduction: Duarte et al. (2005) study confirms that from green coffee to light roasted coffee 5-caffeoylquinic acid concentration decreases three times, but Vignoli et al. (2014) shows that 5-caffeoylquinic acid concentration decreases twice during the time from green to light roasted and three times from light roasted to medium roasted coffee (Jeszka-Skowron et al., 2016). While the 5-caffeoylquinic acid concentration is decreasing, the 4-caffeoylquinic acid and 3-caffeoylquinic acid concentration is increased from light to medium roast and only then the concentration is decreasing. By the degradation of chlorogenic acid chlorogenic acid lactones are formed, most common are 3-caffeoylquinic-1,5-lactone and 4-caffeoylquinic-1,5-lactone forms. The highest chlorogenic acid and chlorogenic acid lactones concentration is found in light roast level (203 °C for 7 min) coffee (Farah et al., 2005). From sensory point of view chlorogenic acid and chlorogenic acid lactones have light coffee–like bitterness. If the roasting process goes further than medium roast level, phenylindanes are formed with harsh bitterness and are one of the main bitter compounds in dark roasted coffees (Frank et al., 2007). While polyphenol concentration decreases in roasting process, the concentration of other bioactive compounds as melanoidins is increasing till medium–dark level roast (Coelho et al., 2014). In melanoidin formation polysaccharides, proteins and also phenolic compound like chlorogenic acid are involved. It is the reason why chlorogenic acid concentration has an opposite correlation between roasting level and melanoidin content (Moreira et al., 2012). It also suggests that higher roasting temperature helps the melanoidin compound formation from chlorogenic acid. Coffees roasted at 220 °C temperature had higher antioxidant concentration than coffees roasted at 217 °C, but at the same time coffees roasted above 228 °C lose their antioxidant activity (Pilipczuk, Kusznierewicz, 2014). Similar conclusions were stated in Bekedam et al. (2008) research, suggesting, that light roast level helps form melanoidins from chlorogenic acid, which also gives the antioxidant activity and characteristics. The given statement is approved by Coelho et al. (2014) and Vignoli et al. (2014) research results, that at the beginning of coffee melanoidin formation at light roast

level, the chlorogenic acid content is still high and gives higher total antioxidant activity to coffee. It also slows down the more bitter phenolic compound formation (Farah et al., 2005). From cup quality point of view, melanoidins have important role in coffee *crema* formation and also bringing bittersweet flavour notes to coffee brew (Kim et al., 2018; Coelho et al., 2014).

During the roasting process, trigonelline converts to nicotinic acid. In Taguchi et al. (1985) research trigonelline concentration decreased rapidly with the roasting time. Meanwhile, nicotinic acid in the roasting process started to form at 180 °C temperature and after 20 min roasting at 220 °C reached the highest concentration. Taguchi et al. (1985) suggests that trigonelline concentration is the highest in light roasted coffees (roasting temperature below 180 °C and roasting time is no longer than 10 minutes). Trigonelline is important precursor of volatile compounds and from sensory point of view higher trigonelline concentration is associated with better sensory scores. Fassio et al. (2017) research shows that trigonelline content in coffee brew has positive correlation with sucrose content and cup quality, while chlorogenic acid has negative correlation.

Kahweol and cafestol concentration decreases significantly after 8 minute roasting time at 230 °C, and dehydro derivatives are formed. Kahweol and cafestol stability in roasting process depends on roasting temperature – if the temperature is below 230 °C reduction of these compounds is slower (Carlos et al., 2014).

For maintaining beneficial bioactive compound content in roasted coffee, the medium roast level is the most appropriate. At this roast level from temperature point of view coffee roasted in the range between 200 to 225 °C could have the highest composed content of trigonelline, chlorogenic acid, kahweol and cafestol content and also presence of melanoidin and chlorogenic acid lactones could be possible.

Aroma and flavour compound changes during roasting process

The roasting process significantly influences the chemical composition of coffee beans. Light or light–medium roasted coffees are more intense with the fruity, acidic and citrusy notes. Although in light roast coffees, there is a higher chance to detected green coffee bean defects (physiological and microbiological defects). Meanwhile, dark roasted coffees have lost the pleasant aroma, flavour compounds and are focused on high concentrations of coffee-like, roasted and burned notes (Parenti et al., 2014; Steen et al., 2017). In Table 2 is shown odour and roast level description of roasted coffee brew compounds. The roast level parameters used in Table 1 are according to Moon, Shibamoto (2009) research.

The presence of a higher content of phenolic compounds, for example, 2-methylphenol, 4-vinylguaiacol, in roasted coffee is associated with immature bean defects (coffee cherries picked before ripeness or affected with rust disease), but by adjusting roasting process it is possible to reduce the concentrations of these compound (Craig, 2018; Steen, 2017). With increasing roasting time 5-feruloylquinic acid hydrolyzes to form ferulic acid, and the ferulic acid decarboxylates to 4-vinylguaiacol (spicy, smoky flavour), but this formation process can happen if the roasting temperature is kept below 170 °C (Dorfner et al., 2003). To avoid the unpleasant flavour notes, at this point it is important to increase roast temperature above 170 °C (Wei et al., 2017).

Table 2

Aroma, flavour compound description

Compound	Odour	Roast level with the highest concentration
*positive effect on cup quality**		
(E)-β-damascenone	cooked apple, sweet [a]	light
3-methylbutanal	malt[b]	medium
2-phenylacetaldehyde	floral, fruit[c]	light
hydroxymethylfurfural	honey[d]	light
2-methylpyrazine	chocolate, nutty[e]	light
2-pentanone	sweet, fruity[f]	light
2,3-butanedione	buttery[f]	medium
methanethiol	fresh[g]	medium
*negative effect on cup quality**		
dimethyl disulphide	sulphury[g]	dark
2- methyl-3-furanthiol	sulphury, vegetable flavour[b]	dark
2-methylpyrazine	overripe, harsh[g]	dark
4-vinylguaiacol	smoky[h]	light (low temperature)

[a]Mayer et. al., 1999; [b]Kim et al., 2018; [d]Moon, Shibamoto, 2009; [e]Wei et al., 2017; [f]Toledo et al., 2016; [g]Poltronieri, Rossi, 2016; [g]Yang et al., 2016; [h]Dorfner et al., 2003

*Cup quality term for sensory evaluation of coffee brew (flavour, aroma, texture) (Cheong et al., 2013; Donnet et al., 2008; Suslick et al., 2010).

Strecker aldehydes like 2-methylpropanal and 3-methylbutanal are responsible for malt flavour notes in coffee (Kim et al., 2018). Also as positive indicators of coffee quality after roasting is 3-metilbutanal and 2,3-pentanedione (Toledo et al., 2016; Ribeiro et al., 2010). Piccino et al. (2014) highlight 2-phenylacetaldehyde and hexanal as compounds with a pleasant specialty coffee like flavours (sweet fruits, citrus or green vegetables). These compounds are in high concentration in green coffee beans and remain only after light roast level (roasting temperature 210 °C for 4–5minutes) (Kim et al., 2018; Piccino et al., 2014).

Ketones like 2,3-butanedione and 2,3-pentanedione are responsible for buttery flavour notes and their highest concentration is at medium roast level. Sweet and fruity

flavour notes in coffee are associated with the presence of compounds such as 2-pentanone (Toledo et al., 2016) and (E)-β-damascenone (pleasant cooked apple, fruity, tea flavour notes), but their concentration rapidly decreases after light roast level (Mayer et al., 1999). Ketones and aldehydes are good cup quality markers, because of the sensibility to changes, especially in roasting process (Poltronieri, Rossi, 2016).

Furans are responsible for coffee–like, caramel flavour notes in light roasted coffees, but with increasing roast level the bitter, spicy furan flavour compounds are developed. Furfural is associated with coffee–like, fresh bread notes and has the highest concentration in light roasted coffee (Cheong et al., 2013; Somporn et al., 2011). Similar to trigonelline, furfural concentration is the highest at light roast level if roasting temperature balance at 200 °C. Hydroxymethylfurfural (honey flavour notes) concentration is the highest in light roasted coffee at roasting temperature 230 °C for 12 minutes (Vignoli et al., 2014; Moon, Shibamoto, 2009). Murkovic, Bornik (2007) research shows, that by increasing roasting temperature to 240 °C hydroxymethylfurfural reaches the highest concentration in 3 minutes, and afterwards the concentration rapidly decreases. Furanones have similar characteristics. For example, furaneol is associated with sweet caramel, but only in light roasted coffees (Bressanello et al., 2017; Cheong et al., 2013).

Pyrazine compounds can bring both negative and positive flavour aroma variations in coffee. Pyrazine like 2-methylpyrazine (chocolate, nutty flavour notes) and 2-ethyl-6-methylpyrazine (roasted hazelnut flavour notes) have positive flavour notes if the roast level is light (Wei et al., 2017), because in dark roasted coffees 2-methylpyrazine and 2-ethyl-6-methylpyrazine associates with "black roast defect" (overripe, harsh, fermented) flavour (Yang et al., 2016). Velásquez et al. (2019) research confirms, that immature coffee beans after roasting at light roast level have a higher concentration of volatile phenols and pyrazines with an earthy odour, meanwhile having lower concentration of carbohydrate degradation products (caramel, sweet flavour notes) and worse sensory score. In this conclusion, specialty coffee eliminates the immature and overripe green coffee bean factor.

Despite the fact that sulphuric compounds in coffee beans is present only in minor quantities, they significantly affect the quality of coffee (Kim et al., 2018). The concentration of these compounds may vary depending on type of roasting. It has been observed that higher temperature promotes an increase in the content of sulphur compounds (Wei et al., 2017). At this point, for the quality, the balance of compounds is more important, than the highest concentration. For example, 2-furfurylthiol is known with pleasant roasted coffee–like aroma in low concentration (in light–medium roast coffees). At dark roast level, there are formed sulphuric compounds like 2-methyl-3-furanthiol (sulphury / vegetable flavour) and dimethyl disulphide (sulphury / cabbage flavour) from methanethiol oxidation (Kim et al., 2018; Mayer et al., 1999). Methanethiol increases with the roasting process till dark roast level, and then by oxidation process, it converts to dimethyl disulphide. The presence of this compound in coffee indicates the quality loss and could be considered as a quality marker (Poltronieri, Rossi, 2016).

By increasing roasting process time and temperature, the pleasant aroma, flavour compounds of pyrazines, aldehydes and ketones are imparting to bitter and harsh notes. Meanwhile, this transformation sets these compound groups as cup quality measurements. Specialty coffee sensory characteristics are associated with floral, fruity compound notes, which are found in the highest concentration in light–medium roast level if the roasting fluctuates in the range of 200–240 °C temperature.

Specialty coffees set higher goals towards sensory attributes, and specialty coffee standards help to exclude the damaged coffee defects before roasting and after the roasting process during storage.

Conclusions

Bioactive compounds like chlorogenic acid, trigonelline and caffeine have the highest value in light roasted coffee, while chlorogenic acid lactones and melanoidins are formed at light–medium roast level. The light–medium roast level using range of 200–225 °C ensures better preservation of phenolic compounds and therefore higher antioxidant activity. Specialty coffee aroma profile includes sweet, fruity flavour notes, which are developed in light–medium, medium roast level when temperature between 200–240 °C is applied. In conclusion, the profile and concentration of biologically active compounds in coffee beans mainly depends on the degree of roasting which in turn affects aroma and taste. Observing all the rules of coffee roasting, it is possible to maintain the quality and nutritional value of coffee.

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References

1. Alex M. de C., Juliana C. de R., Tiago T. R., Andre D. F., Ramiro M. R., Antonio N. G. M., Gladyston R. C. (2016) Relationship between the sensory attributes and the quality of coffee in different environments. *African Journal of Agricultural Research*, Vol. 11(38), p. 3607–3614.
2. Bartel, C., Mesias, M., Morales, F. J. (2015) Investigation on the extractability of melanoidins in portioned espresso coffee. *Food Research International*, Vol. 67, p. 356–365.
3. Bekedam E., Loots M., Schols H., Van Boekel M., Smit G. (2008) Roasting effects on formation mechanisms of coffee brew melanoidins. *Journal of Agricultural and Food Chemistry*, Vol. 56, p. 7138–7145.

4. Bhumiratana N., Adhikari K., Chambers E. (2011) Evolution of sensory aroma attributes from coffee beans to brewed coffee. *LWT-Food Science and Technology*, Vol. 44(10), p. 2185–2192.
5. Bressanello D., Liberto E., Cordero C., Rubiolo P., Pellegrino G., Ruosi M. R., Bicchi C. (2017) Coffee aroma: Chemometric comparison of the chemical information provided by three different samplings combined with GC–MS to describe the sensory properties in cup. *Food Chemistry*, Vol. 214(Supplement C), p. 218–226.
6. Carlos R., Mercadante A., Machado-Faria A., Bragagnolo N. (2014) Roasting process affects the profile of diterpenes in coffee. *European Food Research and Technology*, Vol. 239(6), p. 961–970.
7. Carman A. J., Dacks P. A., Lane R. F., Shineman D. W., Fillit H. M. (2014) Current evidence for the use of coffee and caffeine to prevent age-related cognitive decline and Alzheimer's disease. *Journal of Nutrition, Health and Aging*, Vol. 18(4), p. 383–392.
8. Cheong M. W., Tong K. H., Ong J. J. M., Liu S. Q., Curran P., Yu B. (2013) Volatile composition and antioxidant capacity of Arabica coffee. *Food Research International*, Vol. 51(1), p. 388–396.
9. Coelho C., Ribeiro M., Cruz A. C. S., Domingues M.R.M., Coimbra M. A., Bunzel M., Nunes F. M. (2014) Nature of phenolic compounds in coffee melanoidins. *Journal of Agricultural and Food Chemistry*, 62(31), p. 7843–7853.
10. Donnet M. L., Weatherspoon D. D., Hoehn J. P. (2008) Price determinants in top-quality e-auctioned specialty coffees. *Agricultural Economics*, Vol. 38(3), p. 267–276.
11. Dorfner R., Ferge T., Kettrup A., Zimmermann R., Yeretzian C. (2003) Real-time monitoring of 4-vinylguaiacol, guaiacol, and phenol during coffee Roasting by resonant laser ionization time-of-flight mass spectrometry. *Journal of Agricultural and Food Chemistry*, Vol. 51, p. 5768–5773.
12. Farah A., Paulis T., Trugo L. C., Martin P. R. (2005) Effect of roasting on the formation of chlorogenic acid lactones in coffee. *Journal of Agricultural and Food Chemistry*, Vol. 53, p. 1505–1513.
13. Fassio L. O., Malta M. R., Liska G. R., Alvarenga S. T., Sousa M. M. M., Farias T. R. T., Pereira R. G. F. A. (2017) Sensory profile and chemical composition of specialty coffees from Matas de Minas Gerais, Brazil. *Journal of Agricultural Science*, Vol. 9(9), p. 78.
14. Frank O., Blumberg S., Kunert C., Zehentbauer G., Hofmann T. (2007) Structure determination and sensory analysis of bitter-tasting 4-vinylcatechol oligomers and their identification in roasted coffee by means of LC-MS / MS. *Journal of Agricultural and Food Chemistry*, Vol. 5, p. 1945−1954.
15. Gutiérrez-Grobe Y., Chávez-Tapia N., Sánchez-Valle V., Gavilanes-Espinar J., Ponciano-Rodríguez G., Uribe M., Méndez-Sánchez N. (2012) High coffee intake is associated with lower grade nonalcoholic fatty liver disease : The role of peripheral antioxidant activity. *Annals of Hepatology*, Vol. 11(3), p. 350–355.
16. Jeszka-Skowron M., Sentkowska A., Pyrzyńska K., De Peña M. P. (2016) Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: Influence of green coffee bean preparation. *European Food Research and Technology*, Vol. 242(8), p. 1403–1409.
17. Keefe J. H. O., Bhatti S. K., Patil H. R., Dinicolantonio J. J., Harm P. D., Lucan S. C., Lavie C. J. (2013) Effects of habitual coffee consumption on cardiometabolic disease, cardiovascular health, and all-cause mortality. *Journal of the American College of Cardiology*, Vol. 62(12), p. 1043–1051.
18. Kim S.Y., Ko J.A., Kang B.S., Park H.J. (2018) Prediction of key aroma development in coffees roasted to different degrees by colorimetric sensor array. *Food Chemistry*, Vol. 240, p. 808–816.
19. Lopes G. R., Ferreira A.. S., Pinto M., Passos C. P., Coelho E., Rodrigues C., Figueira C., Rocha S. M, Fernando M. N., Coimbra M. A. (2016) Carbohydrate content, dietary fibre and melanoidins: Composition of espresso from single-dose coffee capsules, *Food Research International*, Vol.89(2016), p. 989–996.
20. Mancini R. S., Wang Y., Weaver D. F. (2018) Phenylindanes in brewed coffee inhibit amyloid-beta and tau aggregation. *Frontiers in Neuroscience*, Vol. 12(October), p. 1–14.
21. Duarte S. M. da S., Abreu C. M. P. de, Henezes H. C. de, Santos M. H. dos, Gouvea, C. M. C. P. (2005) Effect of processing and roasting on the antioxidant activity of coffee brews. *Ciencia y Tecnología de Alimentos*, Vol. 25(2), p. 387–393.
22. Martínez-Lopez S., Sarriá B., Mateos R., Bravo-Clemente L. (2018) Moderate consumption of a soluble green/ roasted coffee rich in caffeoylquinic acids reduces cardiovascular risk markers: results from a randomized, cross-over, controlled trial in healthy and hypercholesterolemic subjects. *European Journal of Nutrition*, Vol. 61, p. 1–14.
23. Mayer F., Czerny M., Grosch W. (1999) Influence of provenance and roast degree on the composition of potent odorants in Arabica coffees. *European Food Research and Technology*, Vol. 209, p. 242–250.
24. McCoy M. J., Hoppe Parr K. A., Anderson K. E., Cornish J., Haapala M., Greivell J. (2017) Diacetyl and 2,3-pentanedione in breathing zone and area air during large-scale commercial coffee roasting, blending and grinding processes. *Toxicology Reports*, Vol. 4, p. 113–122.
25. Mojica B. E., Fong L. E., Biju D., Muharram A., Davis I. M., Vela K. O., Rios D., Osorio-Camacena E., Kaur B., Rojas S.M., Forester S. C. (2018) The impact of the roast levels of coffee extracts on their potential anticancer activities. *Journal of Food Science*, Vol. 00(Nr. 0,2018), p. 1–6.
26. Moon J. K., Shibamoto T. (2009) Role of roasting conditions in the profile of volatile flavor chemicals formed from coffee beans. *Journal of Agricultural and Food Chemistry*, Vol. 57(13), p. 5823–5831.
27. Moreira A. S. P., Nunes F. M., Domingues M. R., Coimbra M. A. (2012) Coffee melanoidins: structures, mechanisms of formation and potential health impacts. *Food & Function*, Vol. 3(9), p. 903.
28. Murkovic M., Bornik M. (2007) Formation of 5-hydroxymethyl-2-furfural (HMF) and 5-hydroxymethyl-2-furoic acid during roasting of coffee. *Molecular Nutrition & Food Research*, Vol. 51(4), p. 390–394.
29. Nuhu A. A. (2014) Bioactive micronutrients in coffee: recent analytical approaches for characterization and quantification. *ISRN Nutrition*, Vol. 2014, p. 1–13.
30. Parenti A., Guerrini L., Masella P., Spinelli S., Calamai L., Spugnoli P. (2014) Comparison of espresso coffee brewing techniques. *Journal of Food Engineering*, Vol. 121(1), p. 112–117.
31. Piccino S., Boulanger R., Descroix F., Sing A. S. C. (2014) Aromatic composition and potent odorants of the "specialty coffee" brew "Bourbon Pointu" correlated to its

three trade classifications. *Food Research International*, Vol. 61, p. 264–271.
32. Pilipczuk T., Kusznierewicz B. (2014) The influence of roasting and additional processing on the content of bioactive components in special purpose coffees. *Journal of Food Science and Technology*, Vol. 52(9), p. 5736–5744.
33. Poltronieri P., Rossi F. (2016) Challenges in Specialty coffee processing and quality assurance. *Challenges*, Vol 7(19), p. 1–22.
34. Ribeiro J. S., Augusto F., Ferreira M. M. C., Salva T. J. G. (2010) Uso de perfis cromatográficos de voláteis de cafés arábicas torrados para a diferenciação das amostras segundo o sabor o aroma e a qualidade global da bebida. *Quimica Nova*, Vol. 33(9), p. 1897–1904.
35. Specialty Coffee Association (2015) *Cupping Specialty Coffee* [accessed on 10.02.2019.] Available: http://www.scaa.org/PDF/resources/cupping-protocols.pdf
36. Somporn C., Kamtuo A., Theerakulpisut P., Siriamornpun S. (2011) Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of Arabica coffee beans (*Coffea arabica* L. cv. Catimor). *International Journal of Food Science and Technology*, Vol. 46(11), p. 2287–2296.
37. Steen I., Waehrens S. S., Petersen M. A., Münchow M., Bredie W. L. P. (2017) Influence of serving temperature on flavour perception and release of Bourbon Caturra coffee. *Food Chemistry*, Vol. 219, p. 61–68.
38. Suslick B. A., Feng L., Suslick K. S. (2010). Discrimination of complex mixtures by a colorimetric sensor array: Coffee aromas. *Analytical Chemistry*, Vol. 82(5), p. 2067–2073.
39. Taguchi H., Sakaguchi M., Shimabayashi Y. (1985) Trigonelline content in coffee beans and the thermal conversion of trigonelline into nicotinic acid during the roasting of coffee beans *Graecum* L. (Fenugreek) from which the was found in coffee beans, amongthe various Assay for trigonelline After meth. *Agricultural and Biological Chemistry*, Vol. 49(12), p. 3467–3471.
40. Toledo P. R. A. B., Pezza L., Pezza H. R., Toci A. T. (2016) Relationship between the different aspects related to coffee quality and their volatile compounds. *Comprehensive Reviews in Food Science and Food Safety*, Vol. 15(4), p. 705–719.
41. Tolessa K., Rademaker M., De Baets B., Boeckx P. (2016) Prediction of specialty coffee cup quality based on near infrared spectra of green coffee beans. *Talanta*, Vol. 150, p. 367–374.
42. Velásquez S., Peña N., Carlos J., Gutierrez N., Sacks G. L. (2019) Volatile and sensory characterization of roast coffees – Effects of cherry maturity. *Food Chemistry*, Vol. 274, p. 137–145.
43. Vignoli J. A., Viegas M. C., Bassoli D. G., Benassi M. de T. (2014) Roasting process affects differently the bioactive compounds and the antioxidant activity of arabica and robusta coffees. *Food Research International*, Vol. 61, p. 279–285.
44. Wei L., Yu G., Wai M., Curran P., Yu B. (2017) Modulation of the volatile and non-volatile profiles of coffee fermented with *Yarrowia lipolytica* : II. Roasted coffee. *LWT-Food Science and Technology*, Vol. 80, p. 32–42.
45. Yang N., Liu C., Liu X., Degn T. K., Munchow M., Fisk I. (2016) Determination of volatile marker compounds of common coffee roast defects. *Food Chemistry*, Vol. 211, p. 206–214.
46. Yoshinari O., Igarashi, K. (2010) Anti-diabetic effect of trigonelline and nicotinic acid, on KK- A^y Mice. *Current Medicinal Chemistry*, Vol. 17, p. 2196–2202.

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REVIEW

A REVIEW– EFFECT OF SALT ON THE SENSORY PERCEPTION OF SNACKS

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Abstract

Consumers have growing concerns regarding healthy eating habits, which can lead to healthier food choices in both choosing meals and snacking. With the growing understanding of healthy foods, changing the motivation of food preferences towards healthier, producers are obligated to transform their products with a balanced nutrition value. One of greatest worries is the salt intake as it is connected to increasing risk of cardiovascular diseases. The challenge for producers is to decrease the amount of salt while at the same time maintain the good taste of snacks or any other products, in order to make sure that consumer liking remains at the same level. The objective of the review was to analyse scientific literature, describe the possibilities of salt reduction of potato, corn or vegetable snacks and to understand how it could impact sensory characteristics of the snacks. The review outlines the current situation of progress that has been made in salt reduction and indicates the main priorities and problems in the salty snacks sector. Salt as well as spices makes a great impact on sensory profile because they determine the taste of product, which is directly linked to overall level of liking. Researchers have found that it is possible to substitute salt with different salt replacers (KCl, $MgCl_2$ and others) in combination with flavour enhancers. Salt reduction or replacing does not always directly affect consumer perception of products.

Keywords: salt reduction, snacks, sensory properties

Introduction

Salt is one of the most popular and most commonly used ingredients in the form of spices, additives and as well as food preservative in the food production sector. It ensures not only enhanced flavour and good texture but also contributes to extension of shelf-life (Mueller et al., 2016). Salt also can be used as an instrument to reduce taste of bitterness and decrease other off-tastes and enhance overall flavour intensity (Sinopoli, Lawless, 2012). However, salt is also one of causes that increase risks for high blood pressure, cardiovascular diseases and hypertension (He et al., 2013). The World Health Organization (WHO) recommends daily sodium intake for adults below 2 g (<5 g salt per day) (WHO, 2012b). The survey based on European Union (EU) salt reduction framework contains data collected from Member States from 1990s through 2012, where the main collection methods were: 24-hour dietary recall, collection of 24-hour urine samples and dietary records. The results showed that current daily salt consumption in Europe is estimated at 7–14 g per day, which is almost two times more than recommended daily intake (European Commission, 2013). These concerns regarding salt intake have led to the development of different strategies to reduce salt amount in food products – food reformulation by using salt replacers, salt mixtures, flavour enhancers (Desmond, 2006, Fellendorf et al., 2016); lowering salt amount gradually by stealth (Kilcast, Ridder, 2007; Liem et al., 2011); changing the size of salt (Desmond, 2006); changing the shape of salt, as well as different national initiatives like increasing awareness through public campaigns (Inguglia et al., 2017). As known, sodium's taste is primarily salty and sodium chloride is the saltiest sodium compound, whereas potassium and calcium has not only some component of saltiness, but they also have other flavours like "metallic" or "bitter" (Doyle, Glass, 2010). One of the most commonly used salt replacers is potassium chloride (KCl) because of its salty characteristics. It has also been researched that increased potassium consumption may reduce the risk of cardiovascular disease and reduce blood pressure, while having a beneficial effect on bone-mineral density. Therefore, WHO recommends to increase potassium consumption from food for adults (3.5 g potassium per day) in order to reduce risks of cardiovascular diseases (WHO, 2012a). The main food industry's concern regarding salt reduction in products is the impact on their sensory characteristics like texture and flavour, and overall acceptance of product.

Much of sodium intake comes from salt that is added to different snacks, such as chips, crackers, also dried and roasted nuts; in these cases, salt acts as flavour enhancer to snacks and it is consumed in particulate form as it isn't dissolved within the food (Chindapan et al., 2018). Potato chips and similar snacks from vegetables and wholegrains are very popular snack food which is consumed by various age groups between meals and during entertainment events. Therefore, it is also important to reduce salt in this food sector which could also partly contribute to global salt consumption reduction.

The aim of the review is to analyse scientific literature, describe the possibilities of salt reduction in potato, corn or vegetable snacks and to understand how it could impact sensory characteristics of the snacks. Review outlines the current situation of progress that has been made in salt reduction and indicates the main priorities and problems in the salty snacks sector.

of the day (Wang et al., 2018).

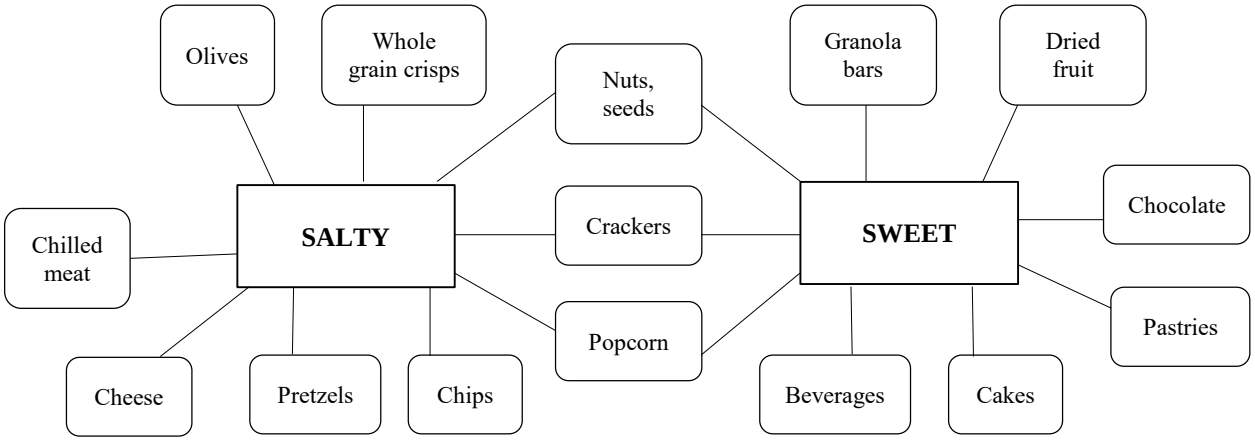

Figure 1. Examples of salty and sweet snacks

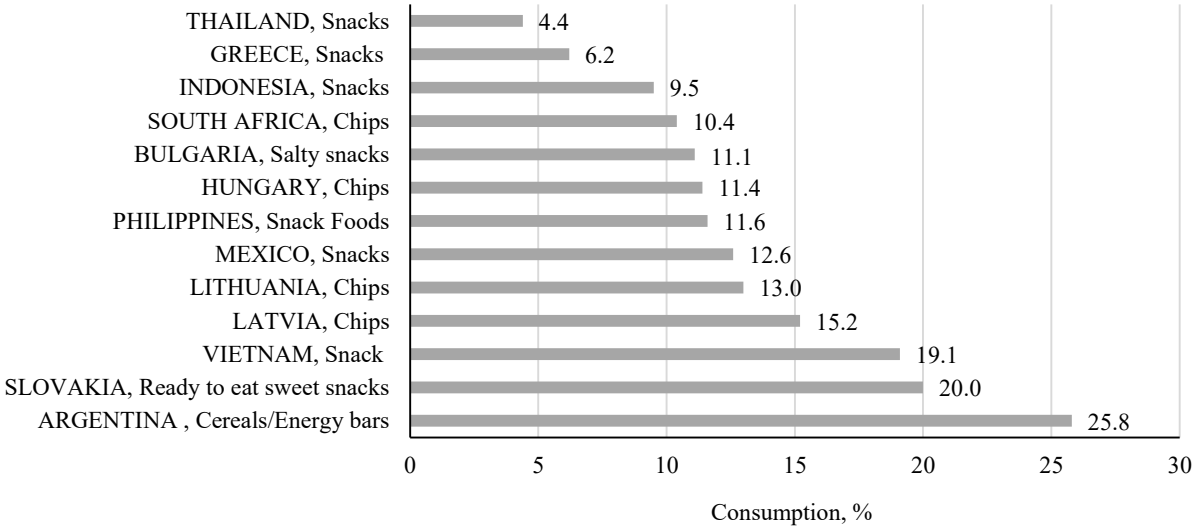

Figure 2. Countries where annual snack consumption is growing, %
(source: Nielsen Retail Measurement Service, 2014)

Classification and consumption of snacks

Snacking is eating in-between meals and it can be also described as any eating occasion outside of the main meals (breakfast, lunch or dinner).

The term "snacks" or "snack food" implies that these foods are energy dense and at the same time nutrient-poor, containing high amount of nutrients, consumption level of which should be limited, e.g., sugar, sodium, saturated fat; the most popular snacks are cookies, cakes, chips, nuts and other salty snacks as well as sweetened beverages (Hess, Slavin, 2018). The general classification of snacks from different scientific sources is summarised in Figure 1. Snacks can be divided in two groups – salty snacks and sweet snacks, where both categories include well-known products like chocolate and cookies as sweets and chips and pretzels as salty snacks. However, there are also products which can be included in either category depending and seasonings, e.g., nuts, crackers and popcorn. It is also important to remember that different consumers have different definitions for snacks (Hess, Slavin, 2018), it depends on society, friends, family, daily habits and eating motivations. Snacks usually are consumed daily between regular meals regardless of their healthiness and it is proved that access to free snacks and beverages at work is an important factor in employee satisfaction. Research by Baskin et al. (2016) showed that those employees who had access to free snacks at their workplace would report being very happy with their jobs by 20% more compared to those without available free snacks at work. It confirms once more that snacking is a part of daily routine, although snack consumption varies considering different eating motivations of each person. Snack choice is strongly linked to different geographical regions, the biggest sale contribution from overall snack category is confectionary, which includes sugary sweets like chocolate, candy, gum in Europe and Middle East/Asia (respectively €41.2 billion and €1.7 billion in 2014); salty snacks contribute more than one-fifth of snack sales in North America (€24.6 billion in 2014), refrigerated snacks comprise almost one third of snacks in Asia-Pacific (€12.1 billion in 2014) and cookies and snack cakes make up more than one-fourth of total snacks in Latin America (€7.6 billion in 2014) (Nielsen retail measurement service, 2014). Nielsen Holding

statistic data from year 2014 showed that there are growing sales of other snack categories like savoury snacks, which include crackers, rice cakes and pita chips, meat snacks, which include jerky and dried meat, refrigerated snacks, which include yogurt, cheese snacks and pudding.

Many emerging markets rise to the top of those with an increased appetite for snacks (Figure 2). Seeing sales growth in non-essential categories, like snacks, is a good indicator that consumers in these countries are ready and able to spend beyond the bare necessities, marking an incredible opportunity for fast-moving consumer goods companies in these markets (Nielsen retail measurement service, 2017).

Salt consumption in Europe

Europe Union (EU) strategy on nutrition, overweight and obesity related health issues sets out priorities to reduce ill health, where one of priorities is "making the healthier option available", which includes such initiatives as salt reduction campaigns (European Commission, 2013). In 2008 Member States agreed to create European Union Framework on voluntary national salt initiatives, where the overall aim on salt reduction was to contribute towards World Health Organisation (WHO) or national recommendations on salt intake level, and within the Salt Framework it was agreed to conduct a survey across the Member States collecting data from 2008 until 2012 (European Commission, 2013).

The salt intake among adults in most European countries ranges from 6.3 up to 13.6 g per day (Table 1). Table 1 shows mean values among adults both men and women. The lowest salt intake by survey data is in Germany, Cyprus, Bulgaria, Latvia and Sweden (6.3– 8.0 g per day), whereas the highest salt consumption was observed in Czech Republic, Slovenia, Hungary, Portugal and Poland (11.5–13.6 g per day).

Table 1

Estimated salt intake (g per day) of adults in EU
(source: European Commission, 2013)

Country	Adults	Country	Adults
Czech Republic	13.6	Switzerland	9.0
Slovenia	12.7	Netherlands	8.8
Hungary	12.5	Denmark	8.6
Portugal	12.3	France	8.6
Poland	11.5	Austria	8.5
Romania	11.1	Slovak Republic	8.2
Belgium	10.5	Finland	8.1
Estonia	10.0	United Kingdom	8.1
Norway	10.0	Sweden	8.0
Spain	10.0	Latvia	7.3
Ireland	9.8	Bulgaria	7.1
Italy	9.6	Cyprus	6.5
Luxembourg	9.1	Germany	6.3
Lithuania	9.0		

For now, there is not an international standard for labelling sodium versus salt; altering labels to display sodium may lead to consumer confusion because many do not understand difference between salt and sodium (Kloss et al., 2015). According EU Regulation No 1169/2011, the term salt instead of sodium must be used on food labels to ensure overall understanding. It is important to focus on ensuring consumer awareness of salt impact on health, especially because food recipe reformulation by the food industry is voluntary (Kloss et al., 2015).

Strategies of salt reduction

Current approaches in salt reduction are expressed in the following methods:

1. reducing salt amount in products gradually and at the same time changing society's awareness about healthy diet (Girgis et al., 2003; Liem et al., 2011);
2. salt reduction by partial replacement of sodium chloride (Liem et al., 2011; Paulsen et al., 2014; Fellendorf et al., 2016; Tamm et al., 2016);
3. additional application of flavour enhancers (Desmond, 2006; dos Santos et al., 2014) and use of aromas to enhance saltiness perception (Lawrence et al., 2011; Nasri et al., 2011);
4. changing physical characteristics of salt (Rama et al. 2013; Emorine et al., 2014).

However, it needs to be highlighted that salt reduction methods vary with different type of foods, it depends on various ingredients and also processing type used before consumption (Mitchell et al., 2011). In addition, different consumer age groups need to be taken into account because of their different sensory perceptions (Conroy et al., 2018).

Salt replacers and flavour enhancers

Salt enhancers do not have a salty taste themselves, but they enhance the salty taste when they are in combination with NaCl; they activate receptors in the mouth and throat in order to help compensate the salt reduction (Rodrigues et al., 2016). Nevertheless, more detailed studies are required to explore this interaction. Different strategies have been developed for using salt replacers with many different substitutes and ingredient proportions to achieve the best result. The main purpose of substitution is to duplicate salt properties and maintain the same or better taste and other sensory features of product (Inguglia et al., 2017). Most commonly used salt replacers are made of mineral salts like potassium chloride (KCl). Unfortunately, replacement of NaCl with KCl mostly has a negative impact on product flavour and texture, as well as colour. A research on fermented sausages showed that partial replacement of NaCl (above 40%) with blend of KCl and glycine or K-lactate and glycine created negative effects of taste and texture (Gelabert et al., 2003). One of the main restrictions for using salt replacers is the additional flavour, which mostly is described as bitter and "metallic" caused by KCl. Nevertheless, KCl is one of most widely used substitutes. In studies regarding salt reduction possibilities of potato chips, it was discovered

that it is possible to partly substitute NaCl with KCl, but it has significant effect on the acceptability of potato chips. Although reduced salt amount has a positive effect on purchase intent and in general partial KCl replacement maintains similar saltiness acceptability, but texture was significant aspect, which negatively affected overall product liking (Torrico et al., 2019). Magnesium sulphate also ensures salty taste similar to KCl but there is also a bitter aftertaste depending on the concentration.

Several studies on odour-induced saltiness enhancement (OISE) showed that it also is possible to reduce sodium content by using different aromas; although for the best result for sensory perception it would be better to combine this method together with other strategies, e.g., salt replacers and flavour enhancers, to maintain consumer acceptability (Nasri et al., 2011; Syarifuddin et al., 2016). OISE driving factors for solid food are odour quality and intensity, and in case of salt reduction, it is important to select aromas associated with saltiness like comté cheese and sardine odour, because odours, which are not associated with salty taste, e.g., carrot odour, do not enhance salt perception (Lawrence et al., 2011).

Flavour enhancers are another possibility, which can be used to reduce salt amount and improve product flavour. Some commonly used well-known flavour enhancers are yeast extracts, lactates, monosodium glutamate (MSG), nucleotides and hydrolysed vegetable protein (HVP) amongst others (Desmond, 2006). Studies have revealed that added MSG compounds that are responsible for a greater umami taste intensity enhance overall flavour intensity of potato chips (Zhang, Peterson, 2018). However, application of food enhancers also is limited – in case of MSG, it is classified as a food additive, which is generally not well appreciated by consumers; whereas in case of HVP and yeasts, they themselves have a salt content up to 40% and consequently the usage of them is limited. The study of Khetra et al. (2016) showed that it is possible to considerably reduce sodium amount in Cheddar cheese by using a combination of a salt replacer, HVP and adenosine-5'-monophosphate, which also in case of cheese reduced bitterness. Several studies found that one of best combinations for salt reduction is a mixture of NaCl, KCl and MSG (Pereira et al., 2015; Rodrigues et al., 2016). The optimum salt content for chips and snacks in the snack sector has been observed at approximately 1.6% NaCl, research about salt level reduction in shoestring potatoes has shown that it is possible to maintain the same level of salt perception and similar sensory acceptability by using a mix of NaCl (30%), MSG (30%) and KCl (40%) to provide salt taste, where optimal formulation in product would be 0.48% of sodium chloride, 0.92% of potassium chloride, and 0.43% of monosodium glutamate, which accordingly would equivalent in salty taste to 1.6% NaCl (Pereira et al., 2015). Several studies suggest that the amount of KCl as a partial salt replacer shouldn't exceed 40%, as when it's above 40% sensory evaluation regarding flavour tends towards negative results due to pronounced bitterness and metallic taste (Gelabert et al., 2003; Pereira et al., 2015; Torrico et al., 2019).

Change of salt size and shape

Salt particle shape and size also has important function and it can be a convenient tool to attain desirable result in salt reduction. Alongside changing size of salt crystals, it is possible to change also the physical form of crystals – from granular to flakes. Flaked form has a higher solubility in the saliva due to the structure and, therefore, it is possible to use this in salt reduction strategy. It has also been proved, that flaked shape has a better water and fat binding characteristics than granular salt when used on red meat (Desmond, 2006). The biggest advantage is that flaked salt is a "clean" salt and there aren't additional flavours or changes in sensory perception while the drawback is its costs (Inguglia et al., 2017). Combination of particle size and form can achieve results with higher saltiness intensity as smaller size particles with wider surface area (flaked) and with highly agglomerated structure rapidly dissolves upon consumption (Rama et al., 2013; Quilaqueo et al., 2015). So, the salt structure also is important for salt perception – the smaller size of salt particles, the higher salt perception, because of faster release of the sodium molecule the maximum salt intensity is achieved sooner (Kloss et al., 2015).

Research on fried potato crisps coated with different sized salt particles showed that it has a considerable impact on the sodium delivery rate into saliva where smaller salt (NaCl) crystals have quicker way to release sodium and thus, reach maximum perception of saltiness; it can be concluded that application of smaller or modified size salt crystals can be a way to reduce salt amount in potato chips (Rama et al., 2013). Studies about reducing the particle sizes combing with salt replacers (KCl) and enhancers (MSG) in shoestring potatoes found that a smaller size not only intensified the taste of sodium chloride but also of monosodium glutamate, though the bitter and metallic taste, which are characteristics of potassium chloride at high concentrations, was not detected; in result the overall conclusion was that the combination of NaCl, KCl and MSG with reduced particle size significantly promotes sodium reduction without a negative impact related to the sensory profile (Rodrigues et al., 2016).

Super small salt size can be obtained by mechanical grinding of larger salt crystals, anti-solvent crystallization or spray-drying, although particles gained from grinding or anti-solvent crystallization may display asymmetric morphology and a wide size distribution (Chindapan et al., 2018). Spray drying is a popular method, which is used to obtain micro and nano size particles with the possibility to control such properties as size, size distribution and morphology (Cho et al., 2015). From technological point of view, each of these methods are highly product dependent, for example, transforming salt crystal dissolution rate is beneficial for the dry product group (Busch et al., 2013).

Moncada et al. (2015) concluded that cheese crackers which surface was treated with nano spray-dried salt particles (average size of particle 1.5 μm) had significantly higher saltiness scores than the crackers treated with regular salt (average size of particle 1500 μm). The results of Chindapan et al. (2018) study showed that spray-dried salt particles, in both combination with KCl and without it, had a significantly higher saltiness than commercial salt, these salts can be used to reduce sodium, especially in dry foods such as French fries, nuts, snacks etc. The research about salt reduction possibilities for pizza crust found that coarse-grained salt with crystal size 0.4–1.4 mm and aqueous salt solution led to a higher saltiness perception due to faster sodium availability, which allowed to reduce sodium amount up to 25% while keeping the same taste quality (Mueller et al., 2016). Spray-dried salt can be also used in combination with chitosan and acid microparticles, which ensures similar results with lower sodium amount but the same salinity perception (Yi et al., 2017).

Sensory acceptability of snacks with reduced sodium content

The main challenge for food manufacturers who are inclined to reformulate recipes in order to reduce salt amount is the level of sensory acceptability of product after changes. Overall, our society is also changing their food choice habits due to more healthier motivations, which is the result of different campaigns to spread the awareness of product quality, its ingredients and how these substances impact health. Thereby, food producers are forced to modify their products due to both society's awareness on health issues related to eating habits and market demands of food rich in nutrients. Torrico et al. (2019) revealed that sodium reduction by partially replacing it with KCl had a significant effect on the acceptability of potato chips, while the perception of saltiness remained at a similar level, but KCl also had an impact on texture which negatively affected overall liking, nevertheless, sodium reduction still made a positive effect on purchase intent.

Conclusions

The purpose of this review was to analyse scientific literature and summarize some of the possibilities to reduce salt amount in potato, corn or vegetable snacks and evaluate how it could impact sensory perception of the snacks. Different salt reduction strategies have a positive effect on snack overall acceptability by maintaining equal salt perception and improving purchase intent by indicating information about salt reduction on packaging. However, there is still a limited amount of studies regarding salt reduction possibilities in potato and vegetable chips, and wholegrain snacks. It is suggested that more studies in this area should be carried out.

References

1. Baskin E., Gorlin M., Chance Z., Novemsky N., Dhar R., Huskey K., Hatzis M. (2016) Proximity of snacks to beverages increases food consumption in the workplace: A field study. *Appetite*, Vol. 103, p. 244–248.
2. Busch J. L.H.C., Yong F. Y.S., Goh S. M. (2013) Sodium reduction: Optimizing product composition and structure towards increasing saltiness perception. *Trends in Food Science and Technology*, Vol. 29, p. 21–34.
3. Chindapan N., Niamnuy C., Devahastin S. (2018) Physical properties, morphology and saltiness of salt particles as affected by spray drying conditions and potassium chloride substitution. *Powder Technology*, Vol. 326, p. 265–271.
4. Cho H. Y., Kim B., Chun J. Y., Choi M. J. (2015) Effect of spray-drying process on physical properties of sodium chloride/maltodextrin complexes. *Powder Technology*, Vol. 277, p. 141–146.
5. Conroy P. M., O'Sullivan M. G., Hamill R. M., Kerry J. P. (2018) Impact on the physical and sensory properties of salt-and fat-reduced traditional Irish breakfast sausages on various age cohorts acceptance. *Meat Science*, Vol. 143, p. 190–198.
6. Desmond E. (2006) Reducing salt: A challenge for the meat industry. *Meat Science*, Vol. 74, p. 188–196.
7. dos Santos B. A., Campagnol P. C. B., Morgano M. A. Ô., Pollonio M. A. R. (2014) Monosodium glutamate, disodium inosinate, disodium guanylate, lysine and taurine improve the sensory quality of fermented cooked sausages with 50% and 75% replacement of NaCl with KCl. *Meat Science*, Vol. 96, p. 509–513.
8. Doyle M. E., Glass K. A. (2010) Sodium reduction and its effect on food safety, food quality, and human health. *Comprehensive Reviews in Food Science and Food Safety*, Vol. 9, p. 44–56.
9. Emorine M., Septier C., Thomas-Danguin, T., Salles C. (2014) Ham particle size influences saltiness perception in flans. *Journal of Food Science*, Vol. 79 (4), p. 693–696.
10. European Commission (2013) *Survey on Members States' Implementation of the EU Salt Reduction Framework.* [accessed on 09.02.2019] Available: https://ec.europa.eu/health/sites/health/files/nutrition_physical_activity/docs/salt_report1_en.pdf
11. Fellendorf S., O'Sullivan M. G., Kerry J. P. (2016) Impact of ingredient replacers on the physicochemical properties and sensory quality of reduced salt and fat black puddings. *Meat Science*, Vol. 113, p. 17–25.
12. Gelabert J., Gou P., Guerrero L., Arnau J. (2003) Effect of sodium chloride replacement on some characteristics of fermented sausages. *Meat Science*, Vol. 65, p. 833–839.
13. Girgis S., Neal B., Prescott J., Prendergast J., Dumbrell S., Turner C., Woodward M. (2003) A one-quarter reduction in the salt content of bread can be made without detection. *European Journal of Clinical Nutrition*, Vol. 57, p. 616–620.
14. He F. J., Li J., Macgregor G. A. (2013) *Effect of longer-term modest salt reduction on blood pressure.* John Wiley & Sons, 104 p.
15. Hess J. M., Slavin J. L. (2018) The benefits of defining "snacks". *Physiology & Behavior*, Vol. 193, p. 284–287.
16. Inguglia E.S., Zhang Z., Tiwari B.K., Kerry P.J. (2017) Salt reduction strategies in processed meat products – A review. *Trends in Food Science & Technology*, Vol. 59, p. 70–78.
17. Khetra Y., Kanawjia S.K., Puri R. (2016) Selection and optimization of salt replacer, flavour enhancer and bitter blocker for manufacturing low sodium Cheddar cheese

using response surface methodology. *LWT-Food Science and Technology*, Vol. 72, p. 99–106.
18. Kilcast D., den Ridder C. (2007) *Reducing salt in foods. practical strategies.* Woodhead Publishing. p. 201–220.
19. Kloss L., Meyer J.D., Graeve L., Vetter W. (2015) Sodium intake and its reduction by food reformulation in the European Union – A review. *NFS Journal*, Vol. 1, p. 9–19.
20. Lawrence G., Salles C., Palicki O., Septier C., Busch J., Thomas-Danguin T. (2011) Using cross-modal interactions to counterbalance salt reduction in solid foods. *International Dairy Journal*, Vol. 21, p. 103–110.
21. Liem D. G., Miremadi F., Keast R.S.J. (2011) Reducing sodium in foods: The effect on flavour. *Nutrients*, Vol. 3, p. 694–711.
22. Mitchell M., Brunton N. P., Wilkinson M. G. (2011) Current salt reduction strategies and their effect on sensory acceptability: A study with reduced salt ready-meals. *European Food Research and Technology*, Vol. 232, p. 529–539.
23. Moncada M., Astete C., Sabliov C., Olson D., Boeneke C., Aryana K. J. (2015) Nano spray-dried sodium chloride and its effects on the microbiological and sensory characteristics of surface-salted cheese crackers. *Journal of Dairy Science*, Vol. 98, p. 5946–5954.
24. Mueller E., Koehler P., Scherf K.A. (2016) Applicability of salt reduction strategies in pizza crust. *Food Chemistry*, Vol. 192, p. 1116–1123.
25. Nasri N., Beno N., Septier C., Salles C., Thomas-Danguin T. (2011) Cross-modal interactions between taste and smell: Odour-induced saltiness enhancement depends on salt level. *Food Quality and Preference*, Vol. 22, p. 678–682.
26. Nielsen Company (2014) *Snack Attack. What consumers are reaching for around the world*? [accessed on 11.02.2019] Available: https://www.nielsen.com/content/dam/nielsenglobal/kr/docs/global-report/2014/Nielsen%20Global%20Snacking%20Report%20September%202014.pdf
27. Nielsen Company (2017) *Booming snack sales highlight a growth opportunity in emerging markets.* [accessed in 11.02.2019] Available: https://www.nielsen.com/ma/en/insights/news/2018/booming-snack-sales-highlight-a-growth-opportunity-in-emerging-markets.html
28. Paulsen M. T., Nys A., Kvarberg R., Hersleth M. (2014) Effects of NaCl substitution on the sensory properties of sausages: Temporal aspects. *Meat Science*, Vol. 98, p. 164–170.
29. Pereira H. C., de Souza V. R., Azevedo N. C., Rodrigues D. M., Nunes C. A., Pinheiro A. C. M. (2015) Optimization of low sodium salts mix for shoestring potatoes. *Journal of Food Science*; Vol. 80, p. 1399–1403.
30. Quilaqueo M., Duizer L., Aguilera J. M. (2015) The morphology of salt crystals affects the perception of saltiness. *Food Research International*, Vol. 76, p. 675–681.
31. Rama R., Chiu N., Carvalho Da Silva M., Hewson L., Hort J., Fisk I. D. (2013) Impact of salt crystal size on in-mouth delivery of sodium and saltiness perception from snack foods. *Journal of Texture Studies*, Vol. 44, p. 338–345.
32. Rodrigues D. M., de Souza V. R., Mendes J. F., Nunes C. A., Pinheiro A.C.M. (2016) Microparticulated salts mix: An alternative to reducing sodium in shoestring potatoes. *LWT – Food Science and Technology*, Vol. 69, p. 390–399.
33. Sinopoli D.A., Lawless H. T. (2012) Taste properties of potassium chloride alone and in mixtures with sodium chloride using a Check-All-That-Apply method. *Journal of Food Science*, Vol. 77 (9), p. 319–322.
34. Syarifuddin A., Septier C., Salles C., Thomas-Danguin, T. (2016) Reducing salt and fat while maintaining taste: An approach on a model food system. *Food Quality and Preference*, Vol. 48, p. 59–69.
35. Tamm A., Bolumar T., Bajovic B., Toepfl S. (2016) Salt (NaCl) reduction in cooked ham by a combined approach of high pressure treatment and the salt replacer KCl. *Innovative Food Science and Emerging Technologies*, Vol. 36, p. 294–302.
36. Torrico D.D., Nguyen P.T., Li T,m Mena B., Viejo C.D., Fuentes S., Dunshea F.R. (2019) Sensory acceptability, quality and purchase intent of potato chips with reduced salt (NaCl) concentrations. *LWT-Food Science and Technology*, Vol. 102, p. 347–355.
37. Wang D., Van Der Horst K., Jacquier E., Afeiche M., Eldridge A. (2018) Snacking patterns in children: A comparison between Australia, China, Mexico, and the US. *Nutrients*, Vol. 10, p. 1–13.
38. WHO (2012a) *Guideline: Potassium intake for adults and children.*
39. WHO (2012b) *Guideline: Sodium intake for adults and children.*
40. Yi C., Tsai M. L., Liu T. (2017) Spray-dried chitosan/acid/NaCl microparticles enhance saltiness perception. *Carbohydrate Polymers*, Vol. 172, p. 246–256.
41. Zhang L., Peterson D. G. (2018) Identification of a novel umami compound in potatoes and potato chips. *Food Chemistry*, Vol. 240, p. 1219–1226.

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REVIEW

MODERN DIETARY PATTERNS BASED ON TERRITORIAL ORIGIN – A REVIEW

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Abstract

There are different dietary patterns around the globe formed on the account of various anthropogenic factors: cultural, economic and globalization. Human food consumption patterns can be formulated and defined depending on habitat. For example, since Scandinavian countries are surrounded by seas, fishery was quite developed historically, and even today, seafood constitutes a large part of daily Scandinavian food plate. On the other hand, world globalization has led to the appearance of such unhealthy food consumption patterns as unbalanced nutrition or refined product excess in a daily diet. There is a strong association between unhealthy eating habits and diseases, which means that healthy eating habits could lower a wide range of such disease emergence possibility as metabolic syndrome, type 2 diabetes, and cardiovascular diseases. The review discusses types of modern dietary patterns around the globe – their origins, main principles and effects on health, comparison of nutrient ratios among the most popular dietary patterns (Nordic diet, Mediterranean diet, Okinawa diet) and the "unhealthy" Western diet.

Keywords: Nordic diet, Mediterranean diet, Okinawa diet, Western diet

Introduction

There are different dietary patterns around the globe formed on account of various anthropogenic factors: cultural, economic and globalization.

From the biological mechanism point of view, environment which conditioned human genetic makeup, i.e., where our ancestors survived, is more favorable for descendants (Cordain et al., 2005). It is recognized that industrial revolution and globalization occurred so recently on the time-scale of evolution, that human genome cannot be fully adapted to environment (Carrera-Bastos et al., 2011; Boyd, Eaton, 1985).

The review summarizes types of modern dietary patterns around the globe – their origins, main principles and effects on health, compares nutrient ratios among the most popular dietary patterns and the "unhealthy" Western diet.

1. Nordic diet

Geographical position of Scandinavian (Nordic) countries (i.e. Denmark, Finland, Norway, Sweden) provides unique coastal climate and special light conditions (lack of sunlight in the winter and plenty of light during the summer period) for plant growth (Nordic Council of Ministers, 2008).

1.1. Food pyramid

New Nordic Cuisine Manifesto was defined in 2003 with the purpose to popularize Nordic cuisine among the world (Nordic Council of Ministers, 2008). Principles and guidelines of the New Nordic Diet were widely described in the Guidelines for the New Nordic Diet in 2012. The diet is based on three main cornerstones:

- "more calories from plant foods and fewer from meat;
- more foods from the sea and lakes;
- more foods from the wild countryside" (Mithril et al., 2011).

Compared to the traditional food pyramid, the base of ND pyramid is given to high vegetable and fruit consumption (Figure 1). Nordic diet (ND) suggests to establish the daily diet on (in descending order): fruits and vegetables (including root vegetables, wild berries and potatoes); whole grains and legumes; nuts and fresh herbs; dairy products; seafood; seaweed; free-range meat (including game); sweets, beverages etc. (Mithril et al., 2013).

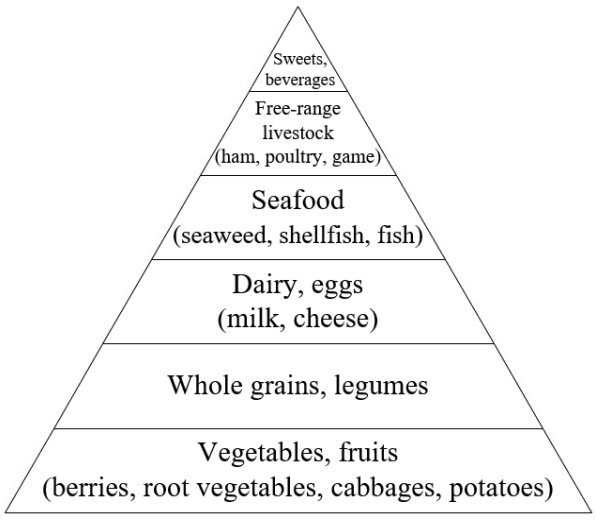

Figure 1. Nordic diet food pyramid
(made by author, based on Mithril et al., 2013)

1.2. Effect on health

High adherence to ND has been strongly associated with a positive influence on inflammation (De Mello et al., 2011; Uusitupa et al., 2013), endothelial dysfunction (De Mello et al., 2011), blood pressure reduction in people with metabolic syndrome (MetS) (Brader et al., 2014; Andersen et al., 2015).

A study in Denmark showed, that there is an evident connection between adherence to Nordic diet and risk of type 2 diabetes (T2D), which is explained by the high content of dietary fibre, which affect the level of glucose and insulin sensitivity due to low glycaemic index (GI) (Lacoppidan et al., 2015). However, two independent studies in Finland (Kanerva et al., 2014), a study in Germany (Galbete et al., 2018) and a study in Sweden (Shi et al., 2018) did not find association between Nordic diet score and T2D biomarkers.

2. Mediterranean diet

Mediterranean diet (MD) origins are found in olive tree growing areas of the Mediterranean basin, which are considered natural in all countries of the Mediterranean coast (Ighbareyeh et al., 2018; Sánchez-Villegas et al., 2018).

2.1. Food pyramid

Principles and guidelines of MD were widely described in Bach-Faig et al. (2011), focusing on nutritional aspects. Later, Dernini et al. (2017) characterized the benefits of MD in four thematic areas:

1) nutrition and health;
2) environment;
3) economy;
4) society and culture.

According to MD principles, ⅓ to ⅔ of every meal should consist of vegetables, cereals and fruits, providing macronutrients, low GI carbohydrates and antioxidants; whole grains, legumes and dairy products are considered as the main source of protein (Figure 2).

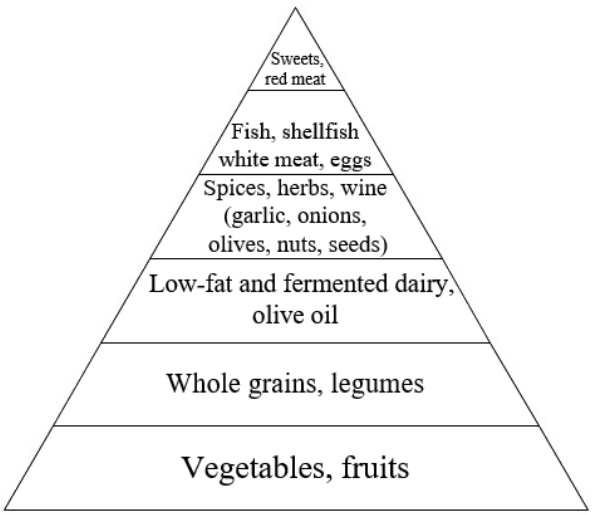

Figure 2. Mediterranean diet food pyramid
(made by author, based on Bach-Faig et al., 2011; Davis et al., 2015)

Olive oil is considered as the main source of lipids; wine and other fermented beverages are recommended as a polyphenol source (1 glass for women, 2 glasses for men daily) (Bach-Faig et al., 2011; Davis et al., 2015).

2.2. Effect on health

Numerous studies show positive effect of adherence to MD pattern regarding moderate alcohol consumption on the risk of CVD (e.g. 40% as found by Ndlovu, Van Jaarsveld, and Caleb (2019). A 12 year follow-up study even showed that daily alcohol consumption lowers cardiovascular disease (CVD) risk for 30 to 35% for men (Mukamal et al., 2003). As reported by Ndlovu et al. (2019) the type of alcohol is not of importance, as it is ethanol which affects the density of cholesterol. MD has been recognized as a dietary pattern with strong association with the improvement of MetS risk factors, body weight reduction in particular (Shai et al., 2008; Estruch et al., 2016). A randomized controlled trial on obese postmenopausal women (n=144) with at least one other MetS criterion, showed a loss of 6.6–7.6 kg on average in 16 weeks after energy restricted dietary intervention (Bajerska et al., 2018).
In the European case-cohort study high adherence to MD was found to lower possibility of T2D by 12% in comparison to individuals with low adherence to MD (Dora Romaguera, 2011). Effects of diet on T2D are affected by several factors, e.g., low-GI carbohydrates, low fat dairy, polyunsaturated fatty acids (PUFA) from vegetable oils, low red meat and processed meat intake (De Koning et al., 2011).

3. Okinawan diet

As stated by Rosenbaum et al. (2010), residents of Okinawa prefecture (most southern island chain of Japan) have a very high life expectancy compared to the rest of the world. Traditional Okinawan diet (OD) is a dietary pattern that existed in Okinawa prefecture before the globalization and westernization after World War II. It is known for a low-calorie and almost vegetarian dietary pattern, due to the specific climatic and terrain conditions (Willcox et al., 2007; Gavrilova, Gavrilov, 2012; Willcox, Willcox, 2014).

3.1. Food pyramid

OD mainly consists of vegetables and legumes, i.e. sweet potato, cabbages and soy in different variations (miso, tofu, soy milk etc.), serving as a carbohydrate and protein source (Figure 3) (Willcox et al., 2014).

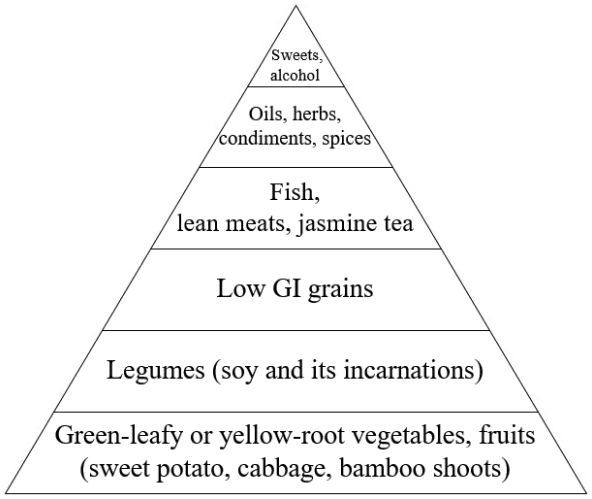

Figure 3. Okinawan diet food pyramid
(made by author, based on Willcox et al., 2014)

3.2. Effect on health

Although seemingly paradoxal, yet historically established caloric (dietary) restriction is considered to be a key factor of longevity (Gavrilova, Gavrilov, 2012). According to Willcox et al. (2007), Okinawan population have been consuming 11% less calories than it would be recommended in relation to body weight. There is a hypothesis that caloric restriction induces stress, which triggers the biological pathways that result in gene encoding which influence longevity, aiding in metabolism regulation (Willcox, Willcox, 2014).

4. Western diet

There are plenty of scientific papers connecting various chronic diseases to the so-called Western diet (WD), like cancer, CVD and diabetes that comprise MetS (Verboven et al., 2018; WHO, 2014), but what does the term Western diet actually imply?

According to WHO, overweight individuals composed a staggering 1.9 billion of all adults. The main cause of obesity is considered energy intake imbalance with energy consumption (WHO, 2018).

A study by Serra-Majem et al. (2009) showed correlation of WD with intake of red and processed meat, eggs, sauces, fast food, pre-cooked food, whole dairy products and potatoes. Several other studies associate WD with high fat and sugar consumption (Verboven et al., 2018). The breakdown of food groups in WD is given in Figure 4.

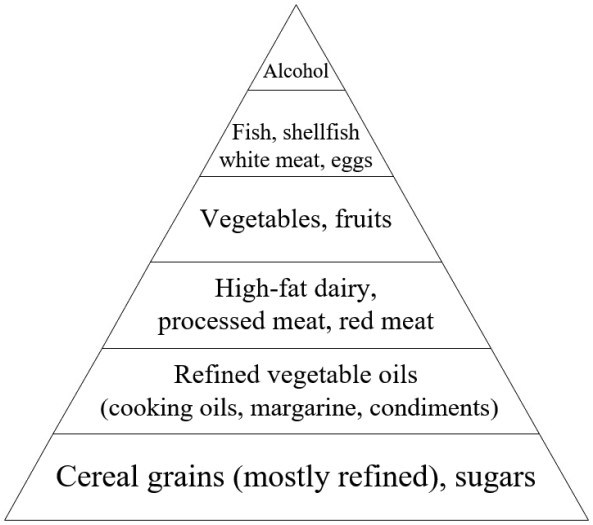

Figure 4. Typical Western diet food pyramid
(made by author, based on Cordain et al., 2005)

According to data presented by Cordain et al. (2005), based on scientific papers year from year 2001 to 2004, US diet energy intake constituted of 20.4% refined grains, 18.6% refined sugars, and 17.6% refined vegetable oils.

5. Comparison

According to previously mentioned studies, dietary patterns differ in many ways, e.g. culture of food consumption, its pattern, physical activity, but especially in nutrient intake ratios and their "signature" foods.

5.1. Energy intake

According to EFSA (2017), reference intake (RI) is the amount of macronutrients needed to maintain physiological functions, usually expressed as % of daily energy intake.

The comparison of average nutrient intakes (Table 1) indicates significant variations between types of diets. While Mediterranean diet has similar amounts of carbohydrates, protein and saturated fatty acids to Western diet, average intake of fat it significantly higher. In this case, however, the type of fat is of importance. Okinawa diet shows the greatest differences compared to the rest of diets.

Table 1

Average nutrient energy intake as a percentage of total energy among presented dietary patterns

Nutrients	Types of diets				Reference intake[4]
	ND[1]	MD[2]	OD[2]	WD[3]	
Carbohydrates, %	51	43	85[)]	49.9	45–60
Dietary fibre, g	41	n.d.	n.d.	n.d.	25
Protein, %	17	13	9	15.7	10–20
Fat, %	32	42	6	34.4	20–35
Saturated fatty acids, %	10	9	2	11.6	as low as possible[(d)]

ND – Nordic diet, MD – Mediterranean diet, OD – Okinawa diet, WD – Western diet
n.d. –not defined
[1]Mithril et al., 2013; [2]Willcox et al., 2014; [3]Paeratakul et al., 2003; [4]EFSA, 2017

With regards to protein reference intake, the data presented in Table 1 corresponds to intake 0.80 g per kg of body weight regardless of gender.

5.2. Signature foods

The term "signature" foods first occurred in (Biltoft-Jensen et al., 2015) and can be described as foods that are characteristic to the diet (Andersen et al., 2015; Biltoft-Jensen et al., 2015).

Signature foods for previously described dietary patterns are presented in Table 2. WD pattern presents itself as an unhealthy example of dietary pattern, typical for most of modern developed countries: fried potatoes, high amount of salt, refined grain products and simple sugars (sucrose, glucose) which drastically affect blood sugar levels. ND, MD and OD dietary patterns have common food group representatives, all of them describe whole grains as the main source of dietary fibre and low-GI carbohydrates. It is recommended to consider whole grains with legumes as the main source of protein; sea products as a source for essential amino acids and PUFAs, fruits and vegetables as the main source for polyphenols and carbohydrates.

The main differences for MD in comparison to ND and OD are olives and olive oil as the source of vegetable lipids, and daily consumption of wine as the source of polyphenols. ND philosophy implies focus on wild forest foods, e.g., wild berries, mushrooms and herbs considered as a source of polyphenols; root vegetables and cabbage as a source of carbohydrates.

Table 2

Signature food comparison of different dietary patterns

Food group	Types of diet			
	ND[1]	MD[2]	OD[3]	WD[4]
Carbohydrate sources	Root vegetables	Vegetables	Yellow-root vegetables	–
	Cereals	Cereals	Rice	–
	Whole grain	Whole grain	Whole grain	Refined grain
	Potatoes	–	Sweet potatoes	Potatoes (deep fried)
	–*	Fruits	–	Sucrose, fructose, glucose
Vegetables and herbs	Cabbage	–	Kale, collard	–
	Mushrooms	–	Shiitake mushrooms	–
	Wild plants	–	–	–
Legumes, nuts and seeds	Legumes	Legumes	Tofu, soy, legumes	–
	Nuts	Tree nuts	–	–
	Seeds	Seeds	–	–
Protein sources	Sea products	Sea products	Sea products	–
	Game meat	–	–	–
	Poultry	Poultry	–	–
	–	Eggs	–	–
Fat sources	Sea products	Olive oil	–	Margarine, butter, cooking oils
Polyphenol sources	Herbs	Herbs	–	–
	Berries	Wine	–	–
Other	–	–	–	Alcohol
	–	–	–	Salt

ND – Nordic diet, MD – Mediterranean diet, OD – Okinawa diet, WD – Western diet
[1]Andersen et al., 2015; [2]Bach-Faig et al., 2011; [3]Willcox, Willcox, 2014; [4]Cordain et al., 2005
* Not defined.

OD also implies cabbages and cruciferous vegetables, i.e., kale and collard, and yellow-root vegetables. The time and serves as the main source of protein today as well. OD is high in legumes, especially in soybeans and its by-product – soymilk. Soy was common in whole Asia throughout the time and serves as the main source of protein today as well.

Conclusions

Nutrition is an important aspect in staying healthy through one's life. Therefore, chosen dietary patterns greatly affect the resistance of the human body to those chronical diseases where diet is one of the main trigger factors.

Adherence to dietary patterns with higher complex carbohydrate, polyunsaturated fatty acid, fruit and vegetable content have shown the reduction of health problems in long term. Whereas, consumption of deep fried and refined foods, high amount of salt and simple sugars is associated with such chronical diseases as metabolic syndrome, type 2 diabetes, and cardiovascular disease. Even though the residents of Mediterranean terrain and Okinawa prefecture follow dietary patterns, which suggest higher longevity and health benefits, the globalisation has had a negative effect on their dietary patterns, which now have become closer to those of the Western diet.

In order to reap maximal health benefits from nutrition, consumers should try to incorporate signature foods from Nordic, Mediterranean and Okinawa diet into their daily lifestyle instead of traditional Western diet staple foods.

References

1. Andersen R., Biltoft-Jensen A., Andersen E. W., Ege M., Christensen T., Ygil K. H., Thorsen A. V., Damsgaard C. T., Astrup A., Michaelsen K. F., Tetens I. (2015) Effects of school meals based on the New Nordic Diet on intake of signature foods: A randomised controlled trial. The OPUS School Meal Study. *British Journal of Nutrition*, Vol. 114(5), p. 772–779.
2. Bach-Faig A., Berry E. M., Lairon D., Reguant J., Trichopoulou A., Dernini S., Padulosi S. (2011) Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutrition*, Vol. 14(12A), p. 2274–2284.
3. Bajerska J., Chmurzynska A., Muzsik A., Krzyżanowska P., Mądry E., Malinowska A. M., Walkowiak J. (2018) Weight loss and metabolic health effects from energy-restricted Mediterranean and Central-European diets in postmenopausal women: A randomized controlled trial. *Scientific Reports*, Vol. 8(1), p. 11170.
4. Biltoft-Jensen A., Damsgaard C. T., Andersen R., Hess Ygil K., Andersen E. W., Ege M., Christen T., Bergmann Sorensen L., Stark K. D., Tetens I., Thorsen A. V. (2015) Accuracy of self-reported intake of signature foods in a school meal intervention study: comparison between control and intervention period. *British Journal of Nutrition*, Vol. 114, p. 635–644.
5. Brader L., Uusitupa M., Dragsted L. O., Hermansen K. (2014) Effects of an isocaloric healthy Nordic diet on ambulatory blood pressure in metabolic syndrome: A randomized SYSDIET sub-study, *European Journal of Clinical Nutrition*, Vol. 68(1), p. 57–63.
6. Cordain L., Eaton S. B., Sebastian A., Mann N., Lindeberg S., Watkins B. A., O`Keefe J. H., Brand-Miller J. (2005) Origins and evolution of the Western diet: health implications for the 21st century. *The American Journal of Clinical Nutrition*, Vol. 81(2), p. 341–354.

7. Davis C., Bryan J., Hodgson J., Murphy K. (2015) Definition of the mediterranean diet: A literature review. *Nutrients*, Vol. 7(11), p. 9139–9153.
8. De Koning L., Chiuve S., Fung T. T., Willett W. C., Rimm E. B., Hu F. B. (2011) Diet-quality scores and the risk of type 2 diabetes in men. *Diabetes Care,* Vol. 34(5), p. 1150–1156.
9. De Mello V. D. F., Schwab U., Kolehmainen M., Koenig W., Siloaho M., Poutanen K., Mykkanen H., Uusitupa M., Poutanen K. (2011) A diet high in fatty fish, bilberries and wholegrain products improves markers of endothelial function and inflammation in individuals with impaired glucose metabolism in a randomised controlled trial: The Sysdimet study. *Diabetologia,* Vol. 54, p. 2755–2767.
10. Dernini S., Berry E. M., Serra-Majem L., La Vecchia C., Capone R., Medina F. X., Trichopoulou A. (2017) Med diet 4.0: The Mediterranean diet with four sustainable benefits. *Public Health Nutrition*, Vol. 20(7), p. 1322–1330.
11. Dora Romaguera (2011) Mediterranean Diet and type 2 diabetes risk in the European prospective investigation into cancer and nutrition (EPIC) study. *Diabetes Care*, Vol. 34(9), p. 1913–1918.
12. EFSA (2017) Dietary reference values for nutrients, Summary report. Available at: https://www.efsa.europa.eu/sites/default/files/2017_09_DRVs_summary_report.pdf
13. Estruch R., Martínez-González M. A., Corella D., Salas-Salvadó J., Fitó M., Chiva-Blanch G., Fiol M., Gomez-Gracia E., Aros F., Lapetra J., Serra-Majem L., Pinto X., Buil-Cosiales P., Sorli J. V., Munoz M. A., Basora-Gallisa A., Ros E. (2016) Effect of a high-fat Mediterranean diet on bodyweight and waist circumference: a prespecified secondary outcomes analysis of the PREDIMED randomised controlled trial. *The Lancet Diabetes and Endocrinology*, Vol. 4(8), p. 666–676.
14. Galbete C., Kröger J., Jannasch F., Iqbal K., Schwingshackl L., Schwedhelm C., Weikert C., Boeing H., Schulze M. B. (2018) Nordic diet, Mediterranean diet, and the risk of chronic diseases: The EPIC-Potsdam study. *BMC medicine*, Vol. 16(99), p. 1–13.
15. Gavrilova N. S., Gavrilov L. A. (2012) Clinical section / debate comments on dietary restriction, Okinawa Diet and Longevity. *Gerontology*, Vol. 58, p. 221–223.
16. Ighbareyeh M. H., Carmona C., Ighbareyeh M. M. H., Suliemieh A. A. A. (2018) Effect of bioclimate factors on olive (*Olea Europaea L.*) yield to increase the economy and maintaining food security in Palestine. *International Journal of Development Research*, Vol. 6(12), p. 10648–10652.
17. Kanerva N., Rissanen H., Knekt P., Havulinna A. S., Eriksson J. G., Mä Nnistö S. (2014) The healthy Nordic diet and incidence of type 2 diabetes — 10-year follow-up. *Diabetes Research and Clinical Practice*, Vol. 106, p. 34–37.
18. Lacoppidan S., Kyrø C., Loft S., Helnæs A., Christensen J., Hansen C., Dahm C., Overvad K., Tionneland A., Olsen A. (2015) Adherence to a healthy Nordic food index is associated with a lower risk of type-2 diabetes – The Danish diet, cancer and health cohort study. *Nutrients*, Vol. *7*(10), p. 8633–8644.
19. Mithril C., Dragsted L. O., Meyer C., Blauert E., Holt M. K., Astrup A. (2011) Guidelines for the new Nordic Diet. *Public Health Nutrition*, Vol. 15(10), p. 1941–1947.
20. Mithril C., Dragsted L. O., Meyer C., Tetens I., Biltoft-Jensen A., Astrup A. (2013) Dietary composition and nutrient content of the New Nordic Diet. *Public Health Nutrition*, Vol. 16(05), p. 777–785.
21. Mukamal K. J., Conigrave K. M., Mittleman M. A., Camargo C. A., Stampfer M. J., Willett W. C., Rimm E. B., Israel B., Mjs N. (2003) Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men from the divisions of general medicine and primary care. *The New England Journal of Medicine,* Vol. 348, p. 109–118.
22. Ndlovu T., Van Jaarsveld F., Caleb O. J. (2019) French and Mediterranean-style diets: Contradictions, misconceptions and scientific facts – A review. *Food Research International*, Vol. 116, p. 840–858.
23. Nordic Council of Ministers (2008) *New Nordic Cuisine.* [accessed on 12.02.2019.] Available: http://norden.diva-portal.org/smash/get/diva2:701317/FULLTEXT01.pdf
24. Paeratakul S., Ferdinand D. P., Champagne C. M., Ryan D. H., Bray G. A. (2003) Fast-food consumption among US adults and children: Dietary and nutrient intake profile. *Journal of the American Dietetic Association*, Vol. 103, p. 1332–1338.
25. Carrera-Bastos P., Fontes-Villalba M.,O'Keefe J. H, Lindeberg S., Cordain L. (2011) The western diet and lifestyle and diseases of civilization. *Research Reports in Clinical Cardiology,* Vol. 2, p. 15–35.
26. Rosenbaum M. W., Willcox B. J., Willcox D. C., Suzuki M. (2010) Okinawa: A naturally calorie restricted population. **In:** *Calorie Restriction, Aging and Longevity,* p. 43–53.
27. S. Boyd Eaton M. K. (1985) Paleolithic nutrition - A Cconsideration of its nature and current implications. *The New England Journal of Medicine*, Vol. 312(5), p. 283–289.
28. Sánchez-Villegas A., Sánchez-Tainta A., Sánchez-Villegas A., Zazpe I. (2018) A Healthy-eating model called Mediterranean Diet. *The Prevention of Cardiovascular Disease Through the Mediterranean Diet*, p. 1–24.
29. Serra-Majem L., Bes-Rastrollo M., Román-Viñas B., Pfrimer K., Sánchez-Villegas A., Martínez-González M. A. (2009) Dietary patterns and nutritional adequacy in a Mediterranean country. *British Journal of Nutrition*, Vol. 101(2), p. 21–28.
30. Shai I., Schwarzfuchs D., Henkin Y., Shahar D. R., Witkow S., Greenberg I., Golan R., Fraser D., Bolotin A., Vardi H., Tangi-Rozental O., Zuk-Ramot R., Sarusi B., Brickner D., Schwartz Z., Sheiner E., Marko R., Katorza E., Thiery J., Fiedler G. M., Bluher M., Stumvoll M., Stampfer M. J., Abra D. (2008) Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. The *New England Journal of Medicine*, Vol. 3(10), p. 229–270.
31. Shi L., Brunius C., Johansson I., Bergdahl I. A., Lindahl B., Hanhineva K., Landberg R. (2018) Plasma metabolites associated with healthy Nordic dietary indexes and risk of type 2 diabetes - A nested case-control study in a Swedish population. *American Journal of Clinical Nutrition*, Vol. 108(3), p. 564–575.
32. Uusitupa M., Hermansen K., Savolainen M. J., Schwab U., Kolehmainen M., Brader L., Mortensen L. S., Cloetens L., Johansson-Persson A., Onning G., Landin-Olsson M., Herzig K.-H., Hukkanen J., Rosqvist F., Iggman D., Paananen J., Pulkki K. J., Siloaho M., Dragsted L., Barri T., Overvad K., Bach Knudsen K. E., Hedemann M. S., Arner P., Dahlman I., Borge G. I. A.,

Baardseth P., Ulven S. M., Gunnarsdottir I., Jonsdottir S., Thorsdottir I., Oresic M., Poutanen K. S., Riserus U., Akesson B. (2013) Effects of an isocaloric healthy Nordic diet on insulin sensitivity, lipid profile and inflammation markers in metabolic syndrome – A randomized study (SYSDIET). *Journal of Internal Medicine*, Vol. 274(1), p. 52–66.

33. Verboven M., Deluyker D., Ferferieva V., Lambrichts I., Hansen D., Eijnde B. O., Bito V. (2018) Western diet given to healthy rats mimics the human phenotype of diabetic cardiomyopathy. *Journal of Nutritional Biochemistry*, Vol. 61, p. 140–146.
34. WHO (2014) Attaining the nine global noncommunicable diseases targets; a shared responsibility, *Global Status Report on Noncommunicable Diseases*.
35. WHO (2018) Obesity and overweight [accessed on 12.03.2019.]. Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
36. Willcox B. J., Willcox D. C. (2014) Caloric restriction, caloric restriction mimetics, and healthy aging in Okinawa: controversies and clinical implications. *Current Opinion in Clinical Nutrition and Metabolic Care*, Vol. 17(1), p. 51–58.
37. Willcox B. J., Willcox D. C., Todoriki H., Fujiyoshi A., Yano K., He Q., David J., Suzuki M. (2007) Caloric restriction, the traditional Okinawan Diet, and healthy aging the diet of the world's longest-lived people and its potential impact on morbidity and life span. *Annals of the New York Academy of Sciences*, Vol. 1114, p. 434–455.
38. Willcox D. C., Scapagnini G., Willcox B. J. (2014) Healthy aging diets other than the Mediterranean: A focus on the Okinawan diet. *Mechanisms of Ageing and Development*, Vol. 136–137, p. 148–162.

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ESSENTIAL ELEMENTS IN MATURE HUMAN MILK

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Abstract

Human milk is recognised as the best source of essential elements for the infant in the first months of life. The objective of research was to determine the content of calcium, iron, magnesium, potassium and selenium in mature human milk. To evaluate possible relation with maternal (age, parity, breastfeeding pattern, dietary intake) and infant's (age, birth weight, sex) attributes. In the study participated 65 lactating women from Latvia (at least one month postpartum). Elements' content in human milk was analysed using ICP-MS (Agilent 7700x, Japan). Dietary data (72-hour food diary) were evaluated using the Fineli Food Composition Database (https://fineli.fi). Data analysis were carried out using IBM SPSS Statistics, version 22.0. Spearman's correlation, partial correlation and Mann-Whitney U test were used to evaluate how maternal & infant characteristics influence essential elements' content in human milk. Average content for calcium, magnesium and potassium in human milk was 28.40±7.06, 3.85±0.64 and 59.60±8.29 mg 100 mL^{-1}, respectively. Iron content in 97% of samples (n=63) was below detection limit (<0.10 mg 100 mL^{-1}). Selenium content in all samples was below detection limit (<0.002 mg 100 mL^{-1}). Calcium content in human milk was affected by breastfeeding pattern – average content of 30.51±7.31 mg 100 mL^{-1} for exclusive breastfeeding and 25.41±5.43 mg 100 mL^{-1} for partial breastfeeding, p=0.003. Dietary intake of essential elements among participants was within recommendations. Our obtained values for calcium, magnesium and potassium in human milk were in accordance with other studies. Further evaluation should be done for the optimisation of the ICP-MS method for the detection of iron and selenium in human milk.

Keywords: human milk, essential elements

Introduction

For the first six months of life, human milk is the most suitable source of essential elements (calcium, potassium, magnesium, iron, selenium) necessary for infant's growth and development (World Health Organization, 2019). These elements are transported from maternal blood to lactocytes and then secreted in milk. This mechanism is closely controlled by mammary epithelial cells to match infant's requirements (Montalbetti et al., 2014; Wysolmerski, 2010).

Calcium is needed for normal development of the skeletal system (bones, teeth) (Bae, Kratzsch, 2018). Calcium content in human milk varies around 20 to 30 mg 100 mL^{-1} (EFSA, 2013). To maintain the necessary amount of calcium in milk, maternal bone resorption, intestinal absorption and renal retention of calcium increases. Yet, maternal diet has less impact on calcium level in milk (Bae, Kratzsch, 2018).

Magnesium supports normal muscle and nerve function and is a cofactor in several enzymatic reactions (de la Guardia, Garrigues, 2015; EFSA, 2013). Magnesium content in human milk broadly varies (from 15 to 64 mg 100 mL^{-1}) (EFSA, 2013).

Potassium is the major cation in the human cells, therefore important in maintaining homeostasis. Its content in human milk is ~50 mg 100 mL^{-1} (EFSA, 2013).

Iron is the oxygen carrier in the human body (de la Guardia, Garrigues, 2015; EFSA, 2013). The major source of iron during the first six months of life is infant's body stores accumulated during the prenatal period (Friel et al., 2018). Human milk contains a comparatively small amount of iron (0.04–0.10 mg 100 mL^{-1}) but its bioavailability is high (20 to 50%) (EFSA, 2013; Hale, Hartmann, 2017). Both sources of iron (body stores and human milk) ensure that infant's requirements for iron are satiated for the first six months of life (Friel at al., 2018; Hale, Hartmann, 2017; Montalbetti et al., 2014). After six months, it is recommended to ensure that an infant receives iron-containing complementary foods (Hale, Hartmann, 2017; World Health Organization, 2019). Maternal intake of iron does not affect iron content in human milk (Hale, Hartmann, 2017).

Glutathione peroxidase is a selenium-containing antioxidant enzyme that protects the body against oxidative stress (EFSA, 2013). Selenium is also needed for the synthesis of thyroid hormones and proper functioning of the immune system (Rayman, 2000). Selenium content in human milk is affected by maternal intake of selenium and it significantly varies among women in Europe – from 0.0003 to 0.0084 mg 100 mL^{-1} with an average content of 0.003 mg 100 mL^{-1} (EFSA, 2013; Hale, Hartmann, 2017). In Latvia's neighbouring country – Estonia, low selenium level in mature human milk samples has been observed (0.0008–0.0029 mg 100 mL^{-1}) (Kantola et al., 1997). Zachara and Pilecki (2001) have pointed out that infants in Poland also could receive a lower intake of selenium than recommended. It is potentially due to low selenium content in food products and therefore in the maternal diet (Zachara Pilecki, 2001). Soil fertilisation with sodium selenite in Finland have resulted in an increased intake of selenium among lactating women and therefore in a rise of selenium level in serum and milk, respectively (Kantola et al., 1997). Soils in Latvia are low in bioavailable selenium, therefore food products also could contain a low amount of selenium (Duma et al., 2011).

European Food Safety Authority's (EFSA) opinion as well as recommended daily intakes set by the Ministry

of Health of the Republic of Latvia on the dietary intake of essential elements for infants and toddlers are compiled in Table 1 (EFSA, 2013; Recommended total energy and nutrient intake among Latvian population, 2017).

Table 1

Dietary intake of essential elements for the infants and toddlers

Element	Adequate intake[1]	Recommended daily intake[2]
	mg per day	mg per day
Potassium	400 [1] (0 to>6 months old) 800 [1] (6 to<12 months old) 800 [1] (12 to<36 months old)	n.i. [3] (0 to 6 months old) 1100 [2] (7 to 12 months old) 1600 [2] (1 to 3 years old)
Calcium	200 [1] (0 to>6 months old) 400 [1] (6 to<12 months old) 600 [1] (12 to<36 months old)	n.i. [3] (0 to 6 months old) 550 [2] (7 to 12 months old) 600 [2] (1 to 3 years old)
Magnesium	25 [1] (0 to >6 months old) 80 [1] (6 to <12 months old) 85 [1] (12 to <36 months old)	n.i. [3] (0 to 6 months old) 60 [2] (7 to 12 months old) 100 [2] (1 to 3 years old)
Iron	0.3 [1,4] (0 to>6 months old) 8 [1] (6 to<12 months old) 8 [1] (12 to<36 months old)	n.i. [3] (0 to 6 months) 8 [2] (7 to 12 months) 8 [2] (1 to 3 years)
Selenium	0.0125 [1] (0 to >6 months old) 0.0150 [1] (6 to<12 months old) 0.0200 [1] (12 to<36 months old)	n.i. [3] (0 to 6 months old) 0.0150 [2] (7 to 12 months old) 0.0200 [2] (1 to 3 years old)

[1] – EFSA, 2013
[2] – Recommended total energy and nutrient intake among Latvian population, 2017
[3] – no information
[4] – breastfed infants

Different methods have been used for the detection of elements in human milk like atomic absorption spectrometry (AAE), flame atomic emission spectrometry (FAES), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS is the most suitable method to measure elements in human milk due to its ability to simultaneously measure several elements even if they are found in trace amounts (de la Guardia, Garrigues, 2015; Levi et al., 2018).

The majority of essential elements in human milk are independent of maternal intake, nutritional requirements for certain nutrients during lactation raises (Hale, Hartmann, 2017; Segura et al., 2016). Recommended intake of potassium, magnesium and iron among lactating women is the same as for non-lactating but guidelines developed by the Ministry of Health of the Republic of Latvia suggest that intake of calcium and selenium should be increased during lactation (see Table 2) (Recommended total energy and nutrient intake among Latvian population, 2017).

Table 2

Guidelines for daily essential elements' intake among women in Latvia[1]

Element	Non-lactating women	Lactating women
	mg per day	mg per day
Potassium	3100	3100
Calcium	800	900
Magnesium	280	280
Iron	15	15
Selenium	0.05	0.06

[1] – Recommended total energy and nutrient intake among Latvian population, 2017

Previously essential elements' content hasn't been sufficiently analysed in Latvia. Therefore, the aim of this study was to determine the content of calcium, iron, magnesium, potassium and selenium in mature human milk and to evaluate possible relation with maternal (age, parity, breastfeeding pattern, dietary intake) and infant's (age, birth weight, sex) attributes.

Materials and Methods

This cross-sectional study was conducted from November 2016 to December 2017. Overall, 65 lactating women from Latvia (at least one month postpartum) took part in the study. Maternal (age, parity, breastfeeding pattern) and infant's (age, birth weight, sex) characteristics were compiled using a questionnaire and are summarized in Table 3.

Table 3

Characteristics of the participants (n=65)

Characteristic	Average±SD [1]	Range
Maternal		
Age, years	31±4	23–39
Parity	2±1	1–4
Infants & toddlers		
Age, months	6±4	2–21
Sex	31 girls, 34 boys	
Birth weight, kg	3.51±0.59	1.60–5.36
Breastfeeding pattern according to infants & toddlers age		
Exclusively breastfed	36 infants (1 to 6 months old) 2 infants (6 to 12 months old)	
Partially breastfed	4 infants (1 to 6 months old) 19 infants (6 to 12 months old) 4 toddlers (>12 months old)	

[1] – standard deviation

Participating women filled a 72-hour food diary and obtained a pooled human milk sample in the next 24 hours (~10 mL). Mothers were allowed to choose the most convenient method for milk expression – 18 participants used manual method (by hand),

38 participants used breast pump but 9 participants combined both methods.

Participants expressed few millimetres of milk after the breastfeeding session. Sampling capacity was not specified but the pooled sample had to include morning, mid-day and evening feedings' milk. Milk was collected into non-sterile propylene container (Plastiques Gosselin, France). Participants were instructed to keep samples refrigerated (+4±2 °C) during sampling and afterwards place the container into a freezer (-18 °C). Then samples were transported to the laboratory (using a cooler with ice packs) and kept frozen until the analysis (-18±3 °C).

Elements' (^{39}K, ^{43}Ca, ^{24}Mg, ^{56}Fe, ^{78}Se) content in human milk was analysed using ICP-MS, according to manufacturer's (Agilent 7700x, Japan) instructions.

Dietary data (a 72-hour food diary) were evaluated using the Fineli Food Composition Database (https://fineli.fi) and exported to the MS Excel 2013. Unfortunately, we were unable to use the Latvian Food Composition Database to calculate essential elements' intake among participants. Taking into account that in the 1990s, soils in Finland were fertilised with sodium selenite (Kantola et al., 1997), food products from Finland should contain higher amounts of selenium than products from Latvia and our calculated results for selenium intake among participants from Fineli Food Composition Database could be overestimated.

Approximately one-third of the participants (n=20) noted the use of nutritional supplements containing at least one of the evaluated elements. Information about essential elements' content in nutritional supplements was taken from the Food and Veterinary Service's Register of Nutritional Supplements (https://registri.pvd.gov.lv/ub) and counted to the data calculated from the Fineli Food Composition Database.

Data analysis were carried out using IBM SPSS Statistics, version 22.0. Spearman's correlation, partial correlation and Mann-Whitney U test were used to evaluate how maternal & infant characteristics influence essential elements' content in human milk ($p<0.05$).

Prior to the study, approval from Riga Stradiņš Ethic Committee was received (No. 4/28.7.2016). All the women signed written consent.

Results and Discussion

This is one of the first reports determining essential elements' content in human milk along with the dietary assessment of essential elements' intake among women in Latvia. Average content for calcium, magnesium and potassium in human milk was 28.40±7.06, 3.85±0.64 and 59.60±8.29 mg 100 mL^{-1}, respectively. Iron content in 97% of samples (n=63) was below detection limit (<0.10 mg 100 mL^{-1}). Selenium content in all samples was below detection limit (<0.002 mg 100 mL^{-1}) (see Table 4).

Table 4

Essential elements' content in mature human milk according to infant's & toddler's age (n=65)

Element	Age	Average±SD	Detection limit [1]
	months	mg 100 mL^{-1}	mg 100 mL^{-1}
Potassium	0 to 6	60.83±7.54	2
	≥6	57.48±9.06	
Calcium	0 to 6	30.91±7.45	0.20
	≥6	25.31±5.66	
Magnesium	0 to 6	3.96±0.67	2
	≥6	3.69±0.59	
Iron	0 to 6	BDL [2]	0.10
	≥6		
Selenium	0 to 6	BDL [2]	0.002
	≥6		

[1] – based on 20 × standard deviation of method blanks, 2.5 mL analytical portion and 50 mL analytical solution.
[2] – below the detection limit

Our obtained average results for calcium (28.40 mg 100 mL^{-1}), potassium (59.60 mg 100 mL^{-1}) and magnesium content (3.85 mg 100 mL^{-1}) in human milk was within range reported from other studies (16–35 mg 100 mL^{-1} for calcium, 33–71 mg 100 mL^{-1} for potassium and 1.6–5.0 mg 100 mL^{-1} for magnesium) (de la Guardia, Garrigues, 2015).

Calcium content in human milk significantly correlated with potassium ($r=0.471$, $p=0.000$) and magnesium content ($r=0.307$, $p=0.013$). Calcium content in human milk was affected by breastfeeding pattern – average content of 30.51±7.31 mg 100 mL^{-1} for exclusive breastfeeding and 25.41±5.43 mg 100 mL^{-1} for partial breastfeeding, $p=0.003$. Other characteristics (maternal age, parity, infant's age, birth weight, sex) did not influence essential elements' content in milk ($p>0.05$).

World Health Organization recommends that infants should be breastfed till six months of age. After that, complementary foods should be introduced into an infant's diet but breastfeeding should be continued till two years of age and beyond (World Health Organization, 2019). To evaluate if exclusively breastfed infants till six months of age in Latvia receive sufficient intake of essential elements, we selected exclusively breastfed infants till six months of age among our participants (n=36), calculated average values for essential elements' content in human milk and multiplied it with the average amount of daily consumed milk (~780 mL, according to EFSA, 2013). Calculations were compared to the intake of essential elements which are considered adequate for the majority of infants according to EFSA (EFSA, 2013) (see Table 1). Results are summarized in Table 5 and show that exclusively breastfed infants in Latvia receive sufficient amounts of potassium, calcium and magnesium.

Partially breastfed infants from 7 to 12 months on average consume ~520 mL of human milk per day (World Health Organization, 1998). After six months of age, human milk is still an important source of essential elements, providing ~32% of magnesium, ~25%

calcium and ~28% of the recommended daily intake of potassium (Table 5).

Table 5

Calculated consumed amount of essential elements among participating infants (n=55)

Element	Intake via human milk	Recommendation
	mg per day	mg per day
Exclusively breastfed infants till 6 months of age (n=36)		
Potassium	474	400 [1]
Calcium	241	200 [1]
Magnesium	31	25 [1]
Partially breastfed infants from 7 to 12 months of age (n=19)		
Potassium	307	1100 [2]
Calcium	137	550 [2]
Magnesium	19	60 [2]

[1] – EFSA, 2013

[2] – Recommended total energy and nutrient intake among Latvian population, 2017

While advancement in technologies allows us to determine trace amounts of elements in human milk, researchers are still faced with some difficulties. Currently, there is no suitable reference material to check the accuracy for obtained results in human milk. An acid digestion preparation was used prior to the ICP-MS analysis in our study. Levi et al. (2018) have observed that the concentration of selenium in the human milk samples were higher (~15%) with the alkali dilution method in contrast to the acid digestion but a higher iron concentration (~28%) in human milk were obtained using acid digestion method, respectively. Therefore, the selection of sample preparation method for human milk should be chosen according to the selected elements (Levi et al., 2018). Our obtained detection limits for iron and selenium were significantly higher compared to Björklund et al. (2012), who reported 100 µg vs 0.16 µg 100 mL^{-1} for iron, 2 µg vs 0.06 µg 100 mL^{-1} for selenium. Although it should be noted that their limit of detection was based only on five blank samples while ours – on 20 blank samples.

Detection of selenium (^{78}Se) and iron (^{56}Fe) isotopes using ICP-MS could also be influenced by spectrometric interferences with other polyatomic ions, mostly argon ions (de la Guardia, Garrigues, 2015). Argon was used as a carrier gas for ICP-MS analysis in our study.

Further evaluation should be done for the optimisation of the ICP-MS method for the human milk analysis to decrease detection limits for iron and selenium isotopes.

During lactation, mothers should consume a well-balanced diet to restore nutrients lost due to milk synthesis (Hale, Hartmann, 2017). We asked our participants to complete a 72-hour food diary. Average daily intake of evaluated essential elements among participants was within recommended ranges (Table 6). Dietary potassium, calcium and magnesium intake did not influence the content of these elements in milk, respectively.

Table 6

Essential elements' intake among participating women (n=65)

Element	Average intake±SD	Recommended daily intake [1]
	mg per day	mg per day
Potassium	3862.42±2001.55	3100
Calcium	1002.25±570.13	900
Magnesium	398.90±187.13	280
Iron	16.92±15.16	15
Selenium	0.09±0.05	0.06

[1] – Recommended total energy and nutrient intake among Latvian population, 2017

Correlation coefficients between dietary intake and content of elements in human milk were r=-0.041 (p=0.747) for potassium, r=-0.012 (p=0.924) for calcium, r=0.117 (p=0.355) for magnesium. This is consistent with the data from other studies (Bravi et al., 2016; Hale, Hartmann, 2017). Dietary intake of iron and selenium among participants was also within recommended ranges but obtained values of these elements in milk were below detection limit. Therefore, we could not calculate correlations between maternal intake of iron and selenium and content of these elements in milk, respectively.

Conclusions

Exclusively breastfed infants till six months of age receive sufficient amount of potassium, calcium and magnesium. After the first half of year, human milk is still an important source of essential elements for the infant.

Dietary intake of evaluated essential elements among participating women was within recommendations developed by the Ministry of Health of the Republic of Latvia.

The Ministry of Health of the Republic of Latvia should develop recommendations regarding essential elements' intake for 0 to 6 months old infants.

Further evaluation should be done for the optimisation of the ICP-MS method for the detection of iron and selenium in human milk.

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References

1. Bae Y.J., Kratzsch J. (2018) Vitamin D and calcium in the human breast milk. *Best Practice & Research Clinical Endocrinology & Metabolism*, Vol. 32, p. 39–45.
2. Björklund K.L., Vahter M., Palm B., Grandér M., Lignell S., Berglund M. (2012) Metals and trace element concentrations in breast milk of first time healthy mothers: a biological monitoring study. *Environmental Health*, Vol. 11(92), p. 1–8.

3. Bravi F., Wiens F., Decarli A., Dal Pont A., Agostoni C., Ferraroni M. (2016) Impact of maternal nutrition on breast-milk composition: a systematic review. *American Journal of Clinical Nutrition*, Vol. 104, p. 646–662.
4. de la Guardia M., Garrigues S. (2015) *Handbook of Mineral Elements in Foods*. UK: John Wiley & Sons, 792 p.
5. Duma M., Alsina I., Dubova L., Stroksa L., Smiltina Z. (2011). The effect of sodium selenite and selenate on the quality of lettuce. **In:** *11th Baltic Conference on Food Science and Technology "Food science and technology in a changing world" FOODBALT 2017 Conference proceedings,* Jelgava, Latvia, p. 39–44.
6. European Food Safety Authority (EFSA) (2013). *Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union*. [accessed on 01.02.2019.]. Available at: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2013.3408
7. Food and Veterinary Service. *Register of Nutritional Supplements*. [accessed on 02.02.2019.]. Available at: https://registri.pvd.gov.lv/ub
8. Friel J., Qasem W., Cai C. (2018) Iron and the Breastfed Infant. *Antioxidants*, Vol. 7(54), p. 1–8.
9. Hale T.W., Hartmann P. (2017) *Textbook of Human Lactation*. New York: Springer Publishing Company, 661 p.
10. Kantola M., Mand E., Viitak A., Juravskaja J., Purkunen R., Vartiainen T., Saarikoski S., Pasanen M. (1997) Selenium contents of serum and human milk from Finland and neighbouring countries. *The Journal of Trace Elements in Experimental Medicine*, Vol. 10, p. 225–232.
11. Levi M., Hjelm C., Harari F., Vahter M. (2018) ICP-MS measurement of toxic and essential elements in human breast milk. A comparison of alkali dilution and acid digestion sample preparation methods. *Clinical Biochemistry*, Vol. 53, p. 81–87.
12. Montalbetti N., Dalghi M.G., Albrecht C., Hediger M.A. (2014) Nutrient transport in the mammary gland: Calcium, trace minerals and water soluble vitamins. *Journal of Mammary Gland Biology and Neoplasia*, Vol. 19, p. 73–90.
13. Rayman M.P. (2000) The importance of selenium to human health. *Lancet*, Vol. 356(9225), p. 233–241.
14. Segura S.A., Ansótegui J.A., Díaz-Gómez M., en representación del Comité de Lactancia Materna de la Asociación Espa˜nola de Pediatría (2016) The importance of maternal nutrition during breastfeeding: Do breastfeeding mothers need nutritional supplements? *Anales de Pediatría*, Vol. 84(6), p. 347.e1–347.e7.
15. The Ministry of Health of the Republic of Latvia. *Recommended total energy and nutrient intakes among Latvian population, 2017*. [accessed on 01.02.2019.]. Available at: http://www.vm.gov.lv/images/userfiles/Tava%20veseliba/Ieteicam%C4%81s_ener%C4%A3ijas_un_uzturvielu_devas.pdf in Latvian)
16. The nutrition unit of the National Institute for Health and Welfare. *The Fineli Food Composition Database Release 19 (March 3, 2018)*. [accessed on 02.02.2019.]. Available at: https://fineli.fi/fineli/en/index
17. World Health Organization (1998) *Complementary Feeding of young Children in Developing Countries: a Review of Current Scientific Knowledge*. [accessed on 04.02.2019.]. Available at: https://apps.who.int/iris/handle/10665/65932
18. World Health Organization. *Breastfeeding*. [accessed on 01.02.2019.]. Available at: https://www.who.int/topics/breastfeeding/en/
19. Wysolmerski J.J. (2010) Interactions between breast, bone, and brain regulate mineral and skeletal metabolism during lactation. *Annals of the New York Academy of Sciences*, Vol. 1192, p. 161–169.
20. Zachara B.A., Pilecki A. (2001) Selenium concentration in the milk of breast-feeding mothers and its geographic distribution. *Environmental Health Perspectives*, Vol. 108(11), p. 1043–1046.

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EVALUATION OF COLOUR IN SERRA DA ESTRELA CHEESE PRODUCED IN DIFFERENT DAIRIES ALONG THE MILKING SEASON

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Abstract

The objective of the present work was to compare the colour of Serra da Estrela Cheese manufactured in different dairies. The samples used for the study were obtained from 6 dairies situated in PDO region for Serra da Estrela cheese: Sabores & Ambientes (Oliveira do Hospital), Casa Agrícola dos Arais (Celorico da Beira), Casa da Ínsua (Penalva do Castelo), Queijaria de Germil (Penalva do Castelo), Quinta de São Cosme (Gouveia), Quinta da Lagoa (Nelas). The colour measurements were made using a colorimeter that registered the values of the Cartesian coordinates in CIELAB colour space: L* a* b*. To evaluate the colour of each producer along the milking season and the colour difference was then calculated. The results obtained in this work indicated that the colour characteristics of the Serra da Estrela Cheeses are slightly different between samples coming from different dairies where the cheeses were manufactured. Nevertheless, in the beginning of data collecting the values of the colour coordinates varied in a limited range, which means that the variations could be considered acceptable attending to the nature of the traditional manufacture process associated with this PDO product. Furthermore, the results showed that along the milking season very important changes in colour took place. However, these changes were found very much dependent of the dairy, as the results of total colour change demonstrated.

Keywords: colour properties, lightness, quality, traditional cheese

Introduction

The "Serra da Estrela" cheese is a traditional Portuguese cheese, with a very ancient history, being presently one of the most valued products, much appreciated due to its particular sensory characteristics like smooth texture. For its production, only three key ingredients are used: ewe's milk, salt and dried thistle flower (*Cynara cardunculus* L.); the last used for the coagulation of the milk. The manufacture process remains very similar to that used in the old days, being for that reason classified as a PDO (Protected Designation of Origin) product according to the European Union and Portuguese regulations (Carocho et al., 2016a, 2016b).

Serra da Estrela cheese is recognized as a unique high-quality type of cheese, produced with raw milk obtained from sheep of the autochthonous breed "Bordaleira Serra da Estrela". Nevertheless, some less conscious producers might use a mixture of Bordaleira Serra da Estrela ewe's milk with cheaper and / or low-quality milk from other more productive breeds, leading to increase cheese yield and lower products costs. This is why the certification of the producers is so important and allows controlling quality of the products along the entire chain, from raw-materials to the final cheese, also guarantying traceability (Cunha et al., 2016).

This Portuguese delicacy is the most famous cheese produced in the country, being also much appreciated worldwide, with exports rising by the year. Because it is preferentially consumed as a soft cheese, the usual time to achieve that texture is 4 weeks of ripening. Nevertheless, some prefer to consume the Serra da Estrela Cheese as a hard cheese after a minimum of 6 months of storage, being this called Old Serra da Estrela Cheese (Carocho et al., 2016b; Reis, Malcata, 2011).

The characteristic colour of Serra da Estela Cheese is light yellow, varying according to maturation time and storage conditions, as well as with manufacturing process and characteristics of the raw milk used, namely the homogenization of the casein micelles in the raw milk and the native microbiota. For this reason, the objective of the present work was to compare the colour of Serra da Estrela Cheese manufactured in different dairies and to evaluate the evolution of the colour along the milking season.

Materials and Methods

Samples used for the study

The samples used for the study were obtained from 6 dairies situated in PDO region for Serra da Estrela cheese production: Sabores & Ambientes (Oliveia do Hospital), Casa Agrícola dos Arais (Celorico da Beira), Casa da Ínsua (Penalva do Castelo), Queijaria de Germil (Penalva do Castelo), Quinta de São Cosme (Gouveia), Quinta da Lagoa (Nelas). At each of the 4 evaluation moments, 5 cheeses were collected from each dairy.

Milk production

The cheeses used for analyses were produced from ewe's raw milk, coagulated with an aqueous extract of dried thistle, and the maturation time was 45 days in all cases. The cheese manufacture followed the traditional process. The milk is heated to 28–32 °C and salted, then vegetable rennet for coagulation is added – the wild thistle flower (0.3%), previously grounded with salt. After 60 minutes, the curd is manually cut and a new filtration is done to remove the remaining serum. Once the steps of moulding, pressing and new salting have taken place, maturation follows.

The milk used for the Serra da Estrela cheese comes from manual milking and is filtered through a white cloth. Salt and vegetable rennet (dried wild thistle flower) is added to heated milk (30 °C). After 45 to 60 minutes the curd is manually cut and filtered to remove the remaining whey. Then follow the steps of moulding, pressing and salting of the surface, and finally the cheese is ready for maturation, being turned and

washed every day with water for the first 15 days and then more sporadically until the end of the ripening process. The maturation times were 45 days for all samples collected in the different dairies.

Colour measurement

Along the milking season the colour of the cheeses' rind was evaluated, on a monthly basis, with measurements made in March (month 1), April, May and June (month 4), i.e., with a 30 days' interval. For each dairy 5 cheeses were considered for evaluation and 20 colour measurements were made in each cheese (10 at top side and 10 at bottom side) to allow calculating the mean value and standard deviation.

The colour measurements were made with a colorimeter CR-400 (Konica Minolta) using the Cartesian coordinates in CIELAB colour space: L*=lightness, a*=green / red, b*=blue / yellow. While lightness ranges from 0 (black) to 100 (white), a* and b* are the opposing colour coordinates ranging from -60 (green / blue, respectively) to +60 (red / yellow, respectively) (Guiné et al., 2014; Guiné et al., 2015).

To evaluate the colour difference in relation to the reference, considered at the beginning of maturation, the colour difference (ΔE) was calculated by Equation (1) (Valdivia-López and Tecante, 2015):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}, \quad (1)$$

where each term is the difference between the colour coordinate of the reference and the material.

A larger ΔE denotes a greater colour difference from the reference material. A typical scale for evaluation of the colour difference is this: ΔE in the range [0.0–2.0] corresponds to unrecognizable differences, in the range [2.0–3.5] corresponds to differences possible to recognize by an experienced observer and over 3.5 corresponds to clear differences of colour (Valdivia-López, Tecante, 2015).

The Cartesian coordinates were also used to calculate the polar or cylindrical coordinates, in the Munsell colour system: Value (V), Hue angle (Hº) and Chroma or saturation (C), as defined by equations (2) to (4):

$$\begin{cases} H^o = arctg\,(b^*/a^*), \text{ for } a^*>0 \,;\, b^*>0 \\ H^o = 180^o + arctg\,(b^*/a^*), \text{ for } a^*<0 \,;\, b^*>0 \\ H^o = 270^o + arctg\,(b^*/a^*), \text{ for } a^*<0 \,;\, b^*<0 \\ H^o = 360^o + arctg\,(b^*/a^*), \text{ for } a^*>0 \,;\, b^*<0 \end{cases} \quad (2)$$

$$C = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$V = L^* / 10 \quad (4)$$

Statistical analysis

To evaluate whether the results obtained in terms of mean value were statistically different between samples a statistical analysis was applied. The Post-Hoc Tukey HSD (Honestly Significant Difference) test was used with coupled to an analysis of variance (ANOVA) for comparison between three or more groups. Tukey's is a statistical test to identify the differences among groups of data and consists of a single multi-step process for comparison, carried out in conjunction with ANOVA. The test identifies where the difference between two mean values is higher than the standard error which could be expected. For statistical analysis was used the statistical software SPSS version 25 (IBM, Inc.) and the level of significance considered was 5% ($p<0.05$).

Results and Discussion

Comparison between dairies

Table 1 shows the results obtained for Cartesian colour coordinates of the cheeses obtained from the six dairies included in the study, in March, the high season of Serra da Estrela cheese production. The values obtained for lightness (L*) are in the range 56.84–67.82, indicating that the samples have a clear colour, since the values are over 50 and therefore closer to the white (L*=100) than black (L*=0). When the samples were compared using ANOVA, significant differences were observed in lightness, with the sample QC being the darkest and sample SA the lightest. Furthermore, the results indicate that the differences in lightness explained almost 90% of the variance observed, which is very much relevant.

Table 1

Cartesian colour coordinates of the cheeses in March (first month of the evaluation period)

Dairy[1]	L*[3]	a*[3]	b*[3]
SA	67.82±2.99^{d}	-2.28±0.55^{b}	25.88±1.16^{c}
CA	64.87±1.11^{c}	-2.82±0.17^{a}	28.11±0.77^{d}
CI	57.21±1.69^{a}	-0.11±0.82^{d}	25.64±2.12^{bc}
QG	64.37±0.81^{c}	-2.08±0.35^{b}	24.30±1.38^{b}
QC	56.84±0.91^{a}	-2.29±0.99^{b}	26.88±4.11^{cd}
QL	58.46±1.91^{b}	-1.61±0.38^{c}	19.62±1.78^{a}
Statistics			
F	287.842	63.676	74.060
p-value	<0.0005	<0.0005	< 0.0005
VE[2] (%)	89.57	59.80	61.28

[1]SA: Sabores & Ambientes, CA: Casa Agrícola dos Arais, CI: Casa da Ínsua, QG: Queijaria de Germil, QC: Queijaria de São Cosme, QL: Quinta da Lagoa.
[2]VE: Variance explained.
[3]Values with the same superscript in the same column are not significantly different (p<5%).

Regarding the opposing colour coordinate a*, also significant differences were obtained ($p < 0.0005$), with the lowest value for the sample CA and the highest for sample CI (Table 2). For this parameter the values obtained were negative, which means that they stand in the green zone and not red (for which a* would be positive). Nevertheless, the values are quite near zero, and therefore the intensity of the green coloration is low, just a very slight tone. For a* the variance explained was a little lower, but still very relevant, approximately 60%. For coordinate b* the values were positive and high, meaning that the samples were yellow (blue would be for negative b*) and with a high intensity of the yellow colouration, varying from 19.62 (sample QL) to 26.88 (sample QC). Again these differences were statistically

significant, and explained more than 60% in the variability of the property.

Table 2 shows the cylindrical coordinates of the analysed cheeses also in March, the first month of the evaluation period. Hue corresponds to the distinction between colours when positioned around a colour wheel, denominated as the Hue wheel, varying from 0º to 360º. Hue changes according to the angle, i.e., as it is moved around the centre. For Hº=0º is red, for Hº=60º is yellow, Hº=120º is green, Hº=180º is cyan, Hº=240º is blue, Hº=300º is magenta, Hº=360º is red. The values obtained for Hue in the present work are near 180º, meaning they correspond the transition between green and cyan.

Table 2

Cylindrical coordinates of the cheeses in March (first month of the evaluation period)

Dairy[1]	Hº[3]	C[3]
SA	178.52±0.02[a]	25.99±1.15[c]
CA	178.53±0.01[a]	28.25±0.77[d]
CI	179.69±1.55[b]	25.64±2.11[bc]
QG	178.51±0.01[a]	24.39±1.39[b]
QC	178.68±0.67[a]	27.00±4.04[cd]
QL	178.51±0.02[a]	19.69±1.78[a]
Statistics		
F	16.534	76.684
p-value	< 0.0005	< 0.0005
VE[2] (%)	27.87	62.10

[1]SA – Sabores & Ambientes, CA – Casa Agrícola dos Arais, CI – Casa da Ínsua, QG – Queijaria de Germil, QC – Queijaria de São Cosme, QL – Quinta da Lagoa.
[2]VE: Variance explained.
[3]Values with the same superscript in the same column are not significantly different (p<5%).

The Chroma is the quality of the colour's purity, corresponding to its intensity or saturation, and changes when moving from the centre (where the saturation is minimum) in the direction of the periphery (where the saturation is maximum). There is no definite upper limit for the values of Chroma, and different areas of the colour space can have different maximum Chroma coordinates. For example, light yellow colours have considerably more potential Chroma than light purples, due to the nature of the eye and the physics of colour stimuli. As a consequence, a wide range of possible Chroma levels exist, up to the high 30 s for some hue–value combinations, although values of Chroma around 8 already correspond to vivid solid colours. For the samples at study, the values obtained for Chroma are very high, varying in the range 19.69–28.25 (Table 2). These values indicate that the cheeses evaluated showed purity colours.

The colour coordinate "Value" is exactly the same as L*, just divided by 10, varying from 1 to 10 and with the exact same interpretation as lightness, reason why it was not calculated.

Variation of colour along the cheese production season

Figure 1 presents the photos of the cheeses from different dairies between March and June (4 months).

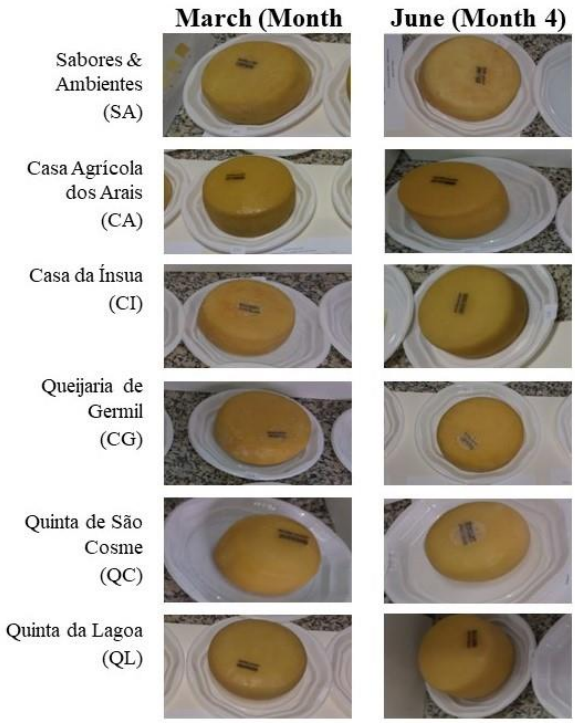

Figure 1. Cheeses between March and June

Figure 2 shows the variation along time of the colour coordinate L*, and the results for most dairies are not consistent with a unique trend along time.

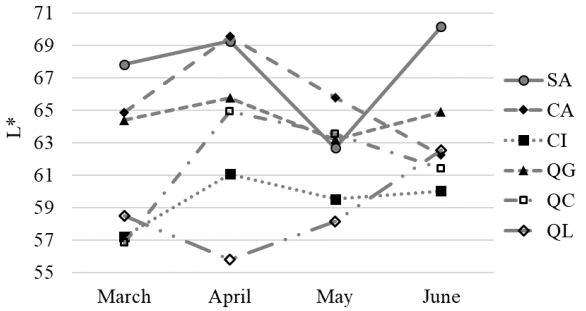

Figure 2. Variation of L* (lightness) along cheese production season

SA – Sabores & Ambientes, CA – Casa Agrícola dos Arais, CI – Casa da Ínsua, QG – Queijaria de Germil, QC – Queijaria de São Cosme, QL – Quinta da Lagoa

For example, for the sample QL the measurement in March is clearly distinct from the increasing trend observed for the other dairies. In fact, while for most samples the global trend is to increase lightness, for sample QL the lightness decreases after the first month. Globally it is possible to see that the dairies with more uniform cheeses in terms of colour along the evaluation period considered were QG and CI. The evaluation period is spring, in which the climate tends to turn from cold to hot, and this might influence the pastures that feed the sheep, and therefore also the characteristics of the cheese, which in turn depend on the milk and also the maturation process.

Regarding the variation of the opposing coordinate a* (Figure 3), there is a much higher concordance between the samples, just with exception of sample CI, whose values of greenness were excessive in the first two months of evaluation (March and April). For some samples the values of a* remained practically unchanged (samples QC and QL), while for other there was a slight increasing trend (samples SA, CA and QG). This increase in the value of a* means a decrease in the intensity of the green colouration towards an approximation of the limit of the red zone (a* positive).

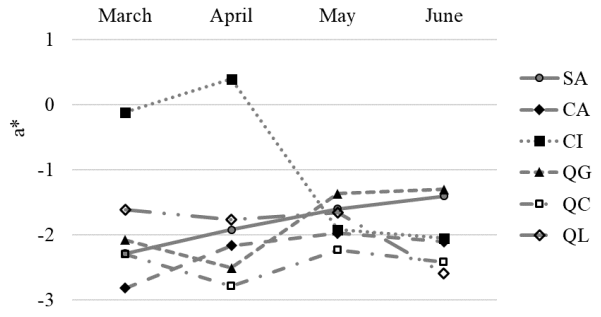

Figure 3. Variation of a* (greenness) along cheese production season

SA – Sabores & Ambientes, CA – Casa Agrícola dos Arais, CI – Casa da Ínsua, QG – Queijaria de Germil, QC – Queijaria de São Cosme, QL – Quinta da Lagoa

The results obtained for b* are presented in Figure 4, and they show that yellowness varied just slightly along the season, with the values for most samples being practically equal in March when compared with June (samples SA, QC, QG, CA). The sample QL was the one for which highest changes were observed, increasing the yellowness from around 20 to near 25.

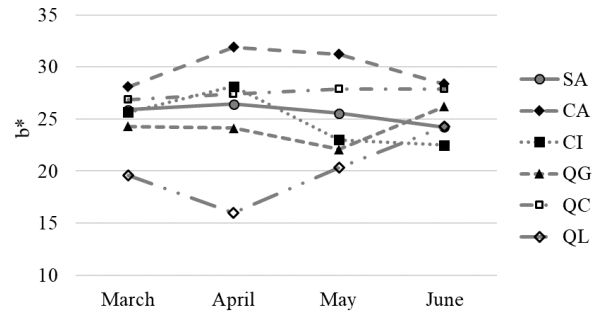

Figure 4. Variation of b* (yellowness) along cheese production season

SA – Sabores & Ambientes, CA – Casa Agrícola dos Arais, CI – Casa da Ínsua, QG – Queijaria de Germil, QC – Queijaria de São Cosme, QL – Quinta da Lagoa

Figure 5 reveals the change in the hue angle as comparing the beginning and the end of the evaluation period, respectively March and June. The results obtained indicate that the nature of the colour, chromaticity, remained practically unchanged for most samples, just with exception for sample SA whose value increased (changing in the direction green → cyan) and sample CI, whose value decreased (changing in the opposite direction (cyan → green).

The results in Figure 6 refer to the variation of Chroma evaluated between March and June. The results show that while for some samples the saturation or colour intensity decreased (SA, CA and CI) for sample QL it increased importantly while just slightly for samples QG and QC. This means that the cheeses produced in the dairies SA, CA and CI tend to develop a more faded colour while the others tend to become more vivid with time.

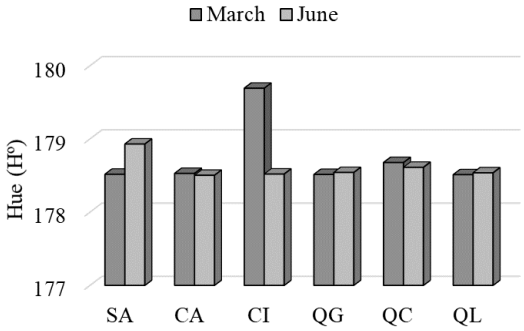

Figure 5. Changes in hue from the beginning to the end of the evaluation period

SA – Sabores & Ambientes, CA – Casa Agrícola dos Arais, CI – Casa da Ínsua, QG – Queijaria de Germil, QC – Queijaria de São Cosme, QL – Quinta da Lagoa

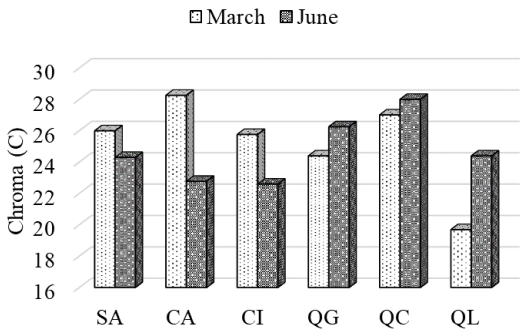

Figure 6. Changes in chroma from the beginning to the end of the evaluation period

SA – Sabores & Ambientes, CA – Casa Agrícola dos Arais, CI – Casa da Ínsua, QG – Queijaria de Germil, QC – Queijaria de São Cosme, QL – Quinta da Lagoa

Because it is difficult to assess the actual colour changes when analysing individually the different colour coordinates, the total colour difference was calculated (ΔE), from the Cartesian coordinates (L*a*b*). The values for colour difference are indicated in Figure 7, expressing the total variations in colour along the milking season, i.e., from March to June. The results indicate that globally the samples QL, QC and CI were those whose colour changes the most during time, while sample QG was that where the colour remained more similar to the initial colour.

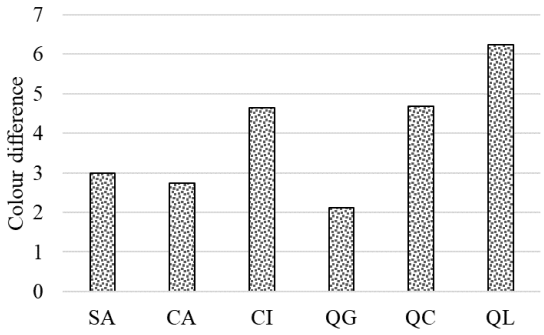

Figure 7. Total colour difference in the samples analysed after the evaluation period (June) in relation to the first month (March)

SA – Sabores & Ambientes, CA – Casa Agrícola dos Arais, CI – Casa da Ínsua, QG – Queijaria de Germil, QC – Queijaria de São Cosme, QL – Quinta da Lagoa

According to the classification the colour difference for sample QG was practically unrecognizable (value very near 2), the differences for samples SA and CA were possible to recognize by an experienced observer (values close to 3) while the differences in samples CI, QC and QL were clear (values over 3.5 in the three cases).

Conclusions

The results obtained in this work showed that the colour characteristics of the Serra da Estrela Cheeses are quite different depending on the dairy where they are manufactured, for example dairy SA as compared with CI. Nevertheless, the values of the colour coordinates varied in a limited range, which means that the variations were acceptable having in consideration the nature of the traditional manufacture process associated with this PDO product.

However, along the milking season very important changes in colour took place, which is tolerable having in mind the nature of the physical-chemical changes produced during the cheese manufacture. Still, these changes were very much dependent of the dairy, as the results of total colour difference demonstrated.

Acknowledgment

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References

1. Carocho M., Barreira J.C.M., Bento A., Fernández-Ruiz V., Morales P., Ferreira I.C.F.R. (2016) Chestnut and lemon balm based ingredients as natural preserving agents of the nutritional profile in matured “Serra da Estrela” cheese. *Food Chemistry*, Vol. 204, p. 185–193.
2. Carocho M., Barros L., Barreira J.C.M., Calhelha R.C., Soković M., Fernández-Ruiz V., Buelga C.S., Morales P., Ferreira, I.C.F.R. (2016) Basil as functional and preserving ingredient in “Serra da Estrela” cheese. *Food Chemistry*, Vol. 2017, p. 51–59.
3. Cunha J.T., Ribeiro T.I.B., Rocha J.B., Nunes J., Teixeira J.A., Domingues L. (2016) RAPD and SCAR markers as potential tools for detection of milk origin in dairy products: Adulterant sheep breeds in Serra da Estrela cheese production. *Food Chemistry*, Vol. 211, p. 631–636.
4. Guiné R.P.F., Henriques F., Barroca M.J. (2014) Influence of drying treatments on the physical and chemical properties of cucumber. *Journal of Food Measurement and Characterization*, Vol. 8(3), p. 195–206.
5. Guiné R.P.F., Almeida I.C., Correia A.C., Gonçalves F.J. (2015) Evaluation of the physical, chemical and sensory properties of raisins produced from grapes of the cultivar Crimson. *Journal of Food Measurement and Characterization*, Vol. 9(3), p. 337–346.
6. Reis P.J.M., Malcata F.X. (2011) Ripening-related changes in Serra da Estrela cheese: A stereological study. *Journal of Dairy Science*, Vol. 94(3), p. 1223–1238.
7. Valdivia-López M.Á., Tecant, A. (2015) Chia (*Salvia hispanica*): A Review of Native Mexican Seed and its Nutritional and Functional Properties. *Advances in Food and Nutrition Research*, Vol. 75, p. 53–75.

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LACTOSE HYDROLYSIS IN DIFFERENT SOLIDS CONTENT WHEY AND MILK PERMEATES

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Abstract

Dairy permeates contain almost original amount of lactose, small fraction of other solid compounds and water. For complete lactose hydrolysis and glucose-galactose syrup production, it is essential to ensure the optimal substrate concentration and conditions to improve product quality and save energy, water and material costs. The aim of this study was to investigate β-galactosidase preparates capability using concentrated whey and milk permeates with 20, 30 and 40% (w w^{-1}) of total solids. Commercial β-galactosidase preparates (Ha-Lactase 5200 produced by *Kluyveromyces lactis* and NOLA Fit5500 produced by *Bacillus licheniformis*, Chr.HANSEN; GODO-YNL2 produced by *Kluyveromyces lactis*, Danisco, Denmark) at dosage 500 NLU·L^{-1} (Ha-Lactase 5200, GODO-YNL2) and 500 BLU·L^{-1} (NOLA Fit5500) were used for lactose hydrolysis. The concentration of each permeate was 20, 30 and 40% (w w^{-1}) of total solids. 10% KOH was used to adjust optimal pH for fermentation media. Samples were monitored in incubator for 4 hours at temperature 42.5±0.5 °C. Lactose, glucose and galactose concentrations were determined by HPLC (Shimadzu LC–20 Prominence, USA). Ha-Lactase 5200 preparate was able to increase lactose hydrolysis rate more than 90% in all permeates at 20% and 30% solids concentration. GODO-YNL2 preparate showed the highest conversion of glucose in sweet whey permeate at 20% solids concentration and the lowest conversion of galactose in sweet whey permeate at 30% solids concentration. The results indicated that substrate type and solids concentration have an effect on the relative activity of commercial enzymes. The study results give a greater understanding about permeates suitability for glucose-galactose syrup production.

Keywords: lactose hydrolysis, glucose-galactose syrup, permeate, β-galactosidase

Introduction

In food industry, carbohydrate plays an important role in production, in nutritional value of product and is an important factor of sweet taste formation (Evdokimov et al., 2015). Hydrolysis of lactose into monosaccharides gives several advantages that have been appreciated by the food industry. Final product has higher sweetness and solubility, contains diverse types of monosaccharide at different concentration. Lactose can be enzymatically hydrolysed using β-galactosidase which produced from GRAS microorganisms or chemically hydrolysed with acids and ion-exchange resins (Illanes, 2016). Hydrolysis of lactose at range of 50 to 90% in concentrated whey and permeate syrup increases sweetness and prevents lactose crystallization during evaporation process, planning to reach 60–70% of solids in concentrated whey (Macwan et al., 2016). Products can be used for invention of innovative and low calorie syrups with high sweetness (Rhimi et al., 2010). One of the perspective applications of lactose is glucose-galactose syrup production by enzymatic hydrolysis. Syrup is viscous, thick sugar solution containing approximately 20% of water, 68% of glucose and galactose, 11% of lactose and 1% of minerals (Lindsay et al., 2018). Glucose-galactose syrup characterises with a sweet taste, dark yellow colour, transparency, good solubility (Budriene et al., 2005) and it might be used as sucrose substitute. Milk and whey permeates are valuable sources for enzymatical lactose hydrolyse (Ryan, Walsh, 2016). Industries prefer to hydrolyse lactose using free enzyme however, the immobilization of β-galactosidase begins to draw the attention of producers. The ability to efficiently hydrolyse whey lactose is one of the key factors determining overall process economy (Vasileva et al., 2016). The aim of this study was to investigate β-galactosidase preparates for fermentation capability using concentrated whey and milk permeates with 20, 30 and 40% (w w^{-1}) of total solids.

Materials and Methods

Chemicals and materials

Acetonitrile (HPLC grade, ≥99.93% purity), column SUPELCOSIL™ LC-NH_2, (250 mm × 4.6 mm × 5 µm), D-lactose monohydrate (≥99.5% purity), D(+) galactose (≥99% purity), D(+) glucose (≥99.5% purity), KOH (≥85%, pellets) were purchased from Sigma-Aldrich. Sweet whey permeate was donated by SC "Smiltenes piens", but whey and milk permeates were obtained from SC "Tukuma piens".

Enzymes

Three commercial β-galactosidase preparates Ha-Lactase 5200 produced by *Kluyveromyces lactis,* activity 5200 NLU·g^{-1} and NOLA Fit5500 produced by *Bacillus licheniformis*, activity 5500 BLU·g^{-1} (Chr.HANSEN, Denmark) and GODO-YNL2 produced by *Kluyveromyces lactis*, activity 5000 NLU·g^{-1} (Danisco, Denmark) were used in the present study.

Solids concentration

Solids concentration of permeates was performed by Chandrapala et al. (2016) method with some modifications. The concentration of permeates solids was accomplished using pilot scale evaporation FT 22 (Armfield, UK) under vacuum conditions to achieve approximately 20% (w w^{-1}) of solids. Evaporation conditions were as follows: flow rate 8 L h^{-1}, warming steam pressure 1 bar, permeate temperature 78±1 °C, cooling water rate 5 L h^{-1}, and vacuum 0.56 bar. Rotary vacuum evaporator Laborota 4000 efficient (Heidolph, Germany) was used to reach permeates solids concentration 30% (w w^{-1}) and 40% (w w^{-1}).

Refractometer 30GS Mettler (Toledo, Japan) was used for permeates solids determination.

Permeates hydrolysis

Experiments were carried out based on Dutra Rosolen et al. (2015) method with some modification. Commercial enzyme weighted into 100 mL conical flasks and 50 mL permeate added with an appropriate pH. All substrate pH for hydrolysis with Ha-Lactase 5200 enzyme was adjusted till 6.5–6.7, for NOLA Fit5500 enzyme 5.4–5.8 and for GODO-YNL2 enzyme 7.5–7.7. 10% KOH was used to adjust an optimal medium pH for enzymatic hydrolysis. Triplicate experiments were prepared for each type of permeate.

The dosage of commercial enzymes for permeate lactose hydrolysis is summarized in Table 1.

Table 1

Summary of added enzymes (mean values ± SD (n = 3))

Enzyme preparate	Weight, mg	Unit
Ha-Lactase 5200	53.2±2.5	292±13 NLU·g^{-1}
NOLA Fit5500	52.1±2.1	270±11 BLU·g^{-1}
GODO-YNL2	53.6±2.2	268±12 NLU·g^{-1}

Samples were fermented at temperature 42.5±0.5 °C 4 hours. Fermented samples were put in water bath and heated at 80 °C 5 min for enzyme inactivation. Samples were transferred into 50 mL test tubes and placed into freezer at -18 °C for further analyses.

HPLC analysis

Lactose, glucose and galactose was determined according to method which was used in Žolnere et al. (2018) work with some modifications.

Sample preparation: samples were transferred into 2 mL test tube and centrifuged 5 min at 10 000 rpm. Approximately 1.5 mL of filtered sample was placed into sampler vials and sealed for HPLC analysis. HPLC (Prominence HPLC system, Shimadzu LC-20, Torrance, CA, USA) was used for sugar determination, refractive index detector RID-10A; column SUPELCOSIL™ LC-NH2, (25 cm×4.6 mm) 5 µm column; 35 °C temperature; gradient mobile phase acetonitrile: deionized water (80 : 20); volume of the injected sample: 10 µL; total analysis time of up to 15 min; flow rate: 1.0 mL min^{-1}.

Data analysis

Results were expressed as mean ± standard deviation (SD) of three replicates for composition measurements. Statistical analyses were carried out using One-Way ANOVA and Tukey test. Differences were considered statistically significant when $p<0.05$.

Results and Discussion

Sweet whey permeate pH in all solids concentration was in range of 5.8–6.1, acid whey permeate 4.4–4.8 and milk permeate 5.5–5.8. According to specification of enzyme preparates for Ha-Lactase 5200 optimal medium pH is range of 6.5 to 8.0, NOLA Fit5500 of 5.4 to 7.0 and for GODO-YNL2 from 7.5 to 8.0 (Žolnere et al., 2018).

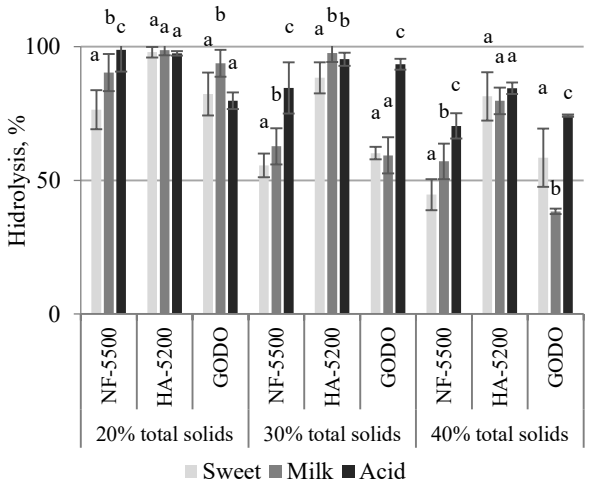

Figure 1. The comparison of analysed commercial enzymes activity in permeates at different solids concentration

*Results indicated with the same letter do not differ significantly ($p>0.05$)

NF-5500 - NOLA Fit5500 enzyme, HA-5200 - Ha-Lactase 5200 enzyme, GODO - GODO-YNL2 enzyme

The highest percentage of hydrolysis (97.5–98.8%) showed Ha-Lactase 5200 enzyme in all permeates at 20% (w w^{-1}) solids concentration. Similar results we noticed at 30% (w w^{-1}) and 40% (w w^{-1}) solids concentration (hydrolyse percentage ranged from 88.3 to 97.6% and 79.7–84.5%, respectively). Other enzymes had showed several results at different solids concentration. The solids concentration can be one of the factor, which influenced NOLA Fit5500 and GODO-YNL2 enzymes activity than for Ha-Lactase 5200 enzyme.

Permeate composition may affect the enzyme activity. Addition of potassium ions during substrate pH adjustment may influence enzyme activity as well. According Jurado et al. (2004) data, metal ions affect the stability and activity of β-galactosidase. The main monovalent ions activator is K^+ for *Kluyveromyces lactis* β-galactosidase but K^+ and Na^+ for *Bacillus licheniformis* β-galactosidase (Juajun et al., 2011; Jurado et al., 2004). For hydrolysis with Ha-Lactase 5200 and GODO-YNL2 was necessary to add 10% KOH to all permeates but for hydrolysis with NOLA Fit5500 it was required to add alkali only for acid whey permeate samples. Hydrolysis results with NOLA Fit5500 enzyme showed that acid whey permeate samples, where 10% KOH was added, performed the highest hydrolyse percentage in all 3 solid concentrations. Its approved K^+ ion positive influence on *Bacillus licheniformis* β-galactosidase activity. Evaluating Ha-Lactase 5200 and GODO-YNL2 enzymes, the lower optimal pH level is 6.5 and 7.0, respectively, which needs to be adjusted. The addition of 10% KOH differs, more alkali was added to optimized GODO-YNL2 enzyme activity in all

permeates samples which leads to increased K^+ ion concentration in substrate and influenced the enzyme activity and lactose hydrolysis capability.

Figure 2 illustrates the amount of glucose and galactose in permeates at 20% (w w^{-1}) solids concentration. Results showed that almost all hydrolyse reactions finished with higher glucose amount but as the exception were acid whey permeate sample with NOLA Fit5500 enzyme (glucose 65.4 g L^{-1}, galactose 70.1 g L^{-1}). Sweet whey and milk permeates hydrolysis results showed that there is no significant difference between galactose amount using different β-galactosidase preparates. Reported by Harju et al., (2012) lactose hydrolysis of 70% in dairy product increases sweetness by an amount corresponding to an addition of approximate 2% sucrose. The hydrolysis of lactose into glucose and galactose increase product sweetness because glucose has higher sweetness level than lactose (Pruksasri, 2015). It's reflected also on results where almost all samples showed higher glucose concentration which means samples sweetness at the end were higher than at the beginning of hydrolysis reaction.

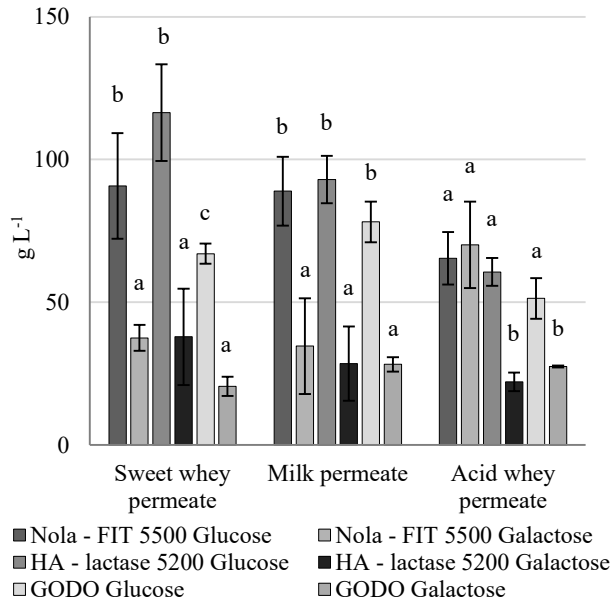

Figure 2. Amount of glucose and galactose in permeates at 20% (w w^{-1}) solids concentration

*Results indicated with the same letter between one type of monosaccharide in certain permeate do not differ significantly ($p>0.05$).

Glucose and galactose amount in permeates at 30% (w w^{-1}) solids concentration (Figure 3) was within results showed in Figure 2. The monosaccharides outcome from all enzyme preparates was quite close. Results indicated that activity of enzyme preparates had affected permeates composition and physical-chemical indices. The hydrolysis of lactose by NOLA Fit5500 enzyme did not show similar outcome results with the hydrolysis of other enzyme preparates. It should be highlighted that in the reaction was used preparates with β-galactosidase from different sources and were used three substrates, which were compared.

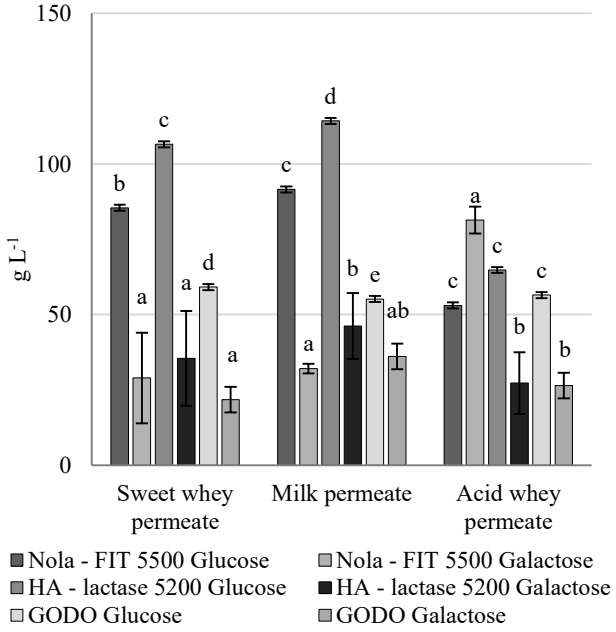

Figure 3. Amount of glucose and galactose in permeates at 30% (w w^{-1}) solids concentration

* Results indicated with the same letter between one type of monosaccharide in certain permeate do not differ significantly ($p>0.05$).

The hydrolysis of microbial enzyme *Bacillus licheniformis* properties has not been fully studied therefore, there is a need for numerous studies to obtain a better knowledge of gained results. As it was reported by Božanić et al. (2014) acid whey contains higher amount of the calcium, phosphate, lactic acid and lactate than it is in sweet whey. These could be considered as the main factors, which affects the effectiveness of this particular enzyme activity to hydrolyse lactose in acid whey.

The results in Figure 4 indicated that the amount of glucose and galactose was lower comparing with the results from Figure 2 and 3. It can be approved with the hydrolysis percentage of permeates at 40% (w w^{-1}) solids concentration (see in Figure 1). The cause of low lactose hydrolysis might be that β-galactosidase activity was decreased by the high lactose concentration in 40% (w w^{-1}) solids concentrate permeates and the presence of glucose and galactose. According to Demirhan et al. (2008) study glucose and galactose at concentration 10.32 g L^{-1} and 13.03 g L^{-1}, respectively, start acting as inhibitors slowing down lactose hydrolysis reaction. As an option for complete lactose hydrolysis of permeates at 40% (w w^{-1}) solids concentration would be time extension for reaction.

β-Galactosidase activity may affect several factors, such as temperature, pH, pressure, concentration of substrate and presence of metal ions (Bosso et al., 2016). The study results showed that addition of 10% KOH influenced lactose hydrolysis and new carbohydrates formation.

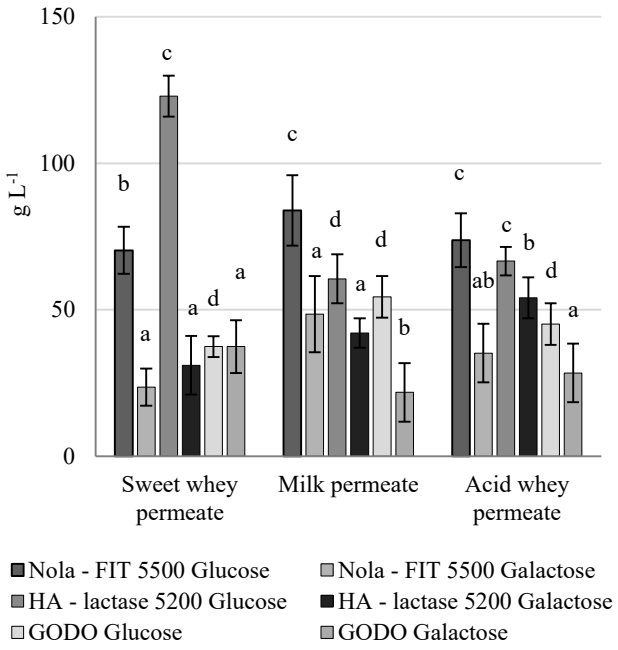

Figure 4. Amount of glucose and galactose in permeates at 40% (w w^{-1}) solids concentration

*Results indicated with the same letter between one type of monosaccharide in certain permeate do not differ significantly ($p>0.05$).

Mariyani et al. (2015) had reported that in the situation when after lactose hydrolysis the galactose concentration was lower than glucose that might be associated to the development of galacto-oligosaccharides. The substrate for hydrolysis containing highly concentrated lactose, β-galactosidase is able to produce galacto-oligosaccharides and affect the final concentration of glucose and galactose. As stated by Suárez et al. (2018), glucose and galactose are being produced equimolar, while lactose concentration decreases slowly and water (as a nucleophile) activity is high. However, when lactose concentration keeps be high, activity of water is low and lactose starts to act as nucleophile and transgalactosylation become more active and starts producing galactooligosaccharides. This statement can explain the relation between glucose and galactose results in this study.

Conclusions

These results provide more accurate information in which substrates the commercial enzyme is able to hydrolyse lactose to a maximal extent. Each substrate at certain concentration has different physical and chemical properties which influence enzyme activity and profile of final outcome. Use of KOH for medium pH control can be evaluated positively because it works as activator for enzyme. Almost all of the results showed that after lactose hydrolysis the dominant monosaccharide was glucose. The obtained results can be used in further research to work on glucose-galactose syrup production technology.

Acknowledgment

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References

1. Bosso A., Morioka L. R. I., Santos L. F., Suguimoto H. H. (2016) Lactose hydrolysis potential and thermal stability of commercial β-galactosidase in UHT and skimmed milk. *Food Science and Technology*, Vol. 36, p. 159–165.
2. Božanić R., Barukčić I., Lisak K. (2014) Possibilities of whey utilisation. *Journal of Nutrition and Food Sciences*, Vol. 2, p. 1–7.
3. Budriene S., Gorochovceva N., Romaskevic T., Yugova L. V., Miezeliene A., Dienys G., Zubriene A. (2005) β-galactosidase from *Penicillium* canescens. Properties and immobilization. *Central European Journal of Chemistry*, Vol. 3, p. 95–105.
4. Chandrapala J., Wijayasinghe R., Vasiljevic T. (2016) Lactose crystallization as affected by presence of lactic acid and calcium in model lactose systems. *Journal of Food Engineering*, Vol. 178, p. 181–189.
5. Demirhan I. N., Kilic D., Ozbek B. (2008) Product inhibition of whey lactose hydrolysis. *Chemical Engineering Communications*, Vol. 195, p. 293–304.
6. 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*, Vol. 2015, p. 1–7.
7. Evdokimov I., Somov V., Kurash Y., Perminov S., Knyazev S. (2015) Application of whey-derived syrups in dairy products. *Foods and Raw Materials*, Vol. 3, p. 89–95.
8. Harju M., Kallioinen H., Tossavainen O. (2012) Lactose hydrolysis and other conversions in dairy products : Technological aspects. *International Dairy Journal*, Vol. 22, p. 104–109.
9. Illanes A. (2016). *Lactose: Production and Upgrading. In: Lactose - derived prebiotics*. A. Illanes, C. Guerrero, C. Vera, L. Wilson, R. Conejeros, F. Scott (eds.). Elsevier, p. 1–33.
10. 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*, Vol. 89, p. 645–654.
11. Jurado E., Camacho F., Luzón G., Vicaria J. M. (2004) Kinetic models of activity for β-galactosidases: Influence of pH, ionic concentration and temperature. *Enzyme and Microbial Technology*, Vol. 34, p. 33–40.
12. Lindsay M. J., Walker T. W., Dumesic J. A., Rankin S. A., Huber G. W. (2018) Production of monosaccharides and whey protein from acid whey waste streams in the dairy industry. *Green Chemistry*, Vol. 20, p. 1824–1834.
13. Macwan S. R., Dabhi B. K., Parmar S. C., Aparnathi K. D. (2016) Whey and its utilization. *International Journal of Current Microbiology and Applied Sciences*, Vol. 5, p. 134–155.
14. Mariyani N., Faridah D. N., Khusniati T., Lioe, H. N. (2015) Hydrolysis of UHT milk lactose by partially purified crude enzyme of β - galactosidase obtained from Lactobacillus plantarum B123 indigenous strain. *International Food Research Journal*, Vol. 22, p. 2274–2279.
15. Pruksasri S. (2015). Dairy stream lactose fractionation/concentration using polymeric ultrafiltration

membrane. **In:** *Membrane processing for dairy ingredient separation*. K. Hu, J. Dickson (eds.). Wiley-Blackwell, p. 35–66.
16. Rhimi M., Boisson A., Dejob M., Boudebouze S., Maguin E., Haser R., Aghajari N. (2010) Efficient bioconversion of lactose in milk and whey: Immobilization and biochemical characterization of a β-galactosidase from the dairy Streptococcus thermophilus LMD9 strain. *Research in Microbiology*, Vol. 161, p. 515–525.
17. Ryan M. P., Walsh G. (2016) The biotechnological potential of whey. *Reviews in Environmental Science and Biotechnology*, Vol. 15, p. 1–20.
18. Suárez S., Guerrero C., Vera C., Illanes A. (2018) Effect of particle size and enzyme load on the simultaneous reactions of lactose hydrolysis and transgalactosylation with glyoxyl-agarose immobilized β-galactosidase from Aspergillus oryzae. *Process Biochemistry*, Vol. 73, p. 56–64.
19. Vasileva N., Ivanov Y., Damyanova S., Kostova I., Godjevargova T. (2016) Hydrolysis of whey lactose by immobilized β-galactosidase in a bioreactor with a spirally wound membrane. *International Journal of Biological Macromolecules*, Vol. 82, p. 339–346.
20. Žolnere K., Ciproviča I., Ķirse A., Cinkmanis I. (2018) A study of commercial β-galactosidase stability under simulated in vitro gastric conditions. *Agronomy Research*, Vol. 16, 1555–1562.

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RHEOLOGICAL PROPERTIES OF LACTOSE-FREE YOGHURT IN RELATION TO ENZYME CONCENTRATIONS

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Abstract

Lactose-free yoghurt is one of the main sources of useful nutrients for people who have got lactose intolerance. Therefore preparing lactose-free yoghurt is considered as an actual issue in the Republic of Uzbekistan. Many factors can affect the rheological and textural parameters of lactose-free yoghurt, including enzyme concentration, which is used to convert lactose in glucose and galactose. The aim of the study was to evaluate enzyme concentration influence on the rheological properties of lactose-free yoghurt. Yoghurt samples were made from pasteurized milk (fat content 2.5%, Tukuma piens Ltd., Latvia) using commercial frozen yoghurt starter culture FD-DVS YC-X11 (Danisco Ltd., Denmark) containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* strains, and different concentrations of enzyme NOLA™ Fit 5500 (Chr Hansen JSC., Denmark) – 0; 500; 1000; 1500 and 2000 BLU L^{-1}. Milk was pasteurized (95±1 °C, 5 min), cooled down (40±1 °C), inoculated with starter and enzyme, mixed and fermented till pH 4.8±0.1 then samples were mixed and cooled down till 5±1 °C. Textural properties and viscosity of yoghurt samples were analysed using texture analyser TA.HD Plus and DV-III Ultra Programmable Rheometer. Lactose, galactose, glucose contents were measured by HPLC (SHIMADZU, Prominence, USA). The textural properties (such as firmness, consistency, cohesiveness) and viscosity of analysed yoghurt samples were significantly ($p<0.05$) different for samples with higher enzyme concentration, which showed the lowest viscosity. Rheological properties denote a weaker network of yoghurt gel in samples with full lactose hydrolysis. Results from chromatography provided no lactose presence in any experimental sample with enzyme, the galactose was dominant monosaccharide in each sample treated with enzyme.

Keywords: lactose-free yoghurt, enzyme, viscosity, consistency

Introduction

Yoghurt is a dairy product produced by fermenting the milk using starter culture consisting of the mix of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Wolf et al., 2015). Yoghurt contains naturally an expressive content of Ca and K and it is a source of high quality protein, providing satiety and promoting muscle growth and bone health (Souza et al., 2018). Apart from this, daily intake of yoghurt is usually associated with beneficial health effect such as protection against cancer, enhancement of immune response and cholesterol reduction in the blood (Wolf et al., 2015). However, not all people can consume dairy products including yoghurt because of the high lactose content (3.5–3.7%). Lactose is a disaccharide presented in all type of milk with different quantities. It is hydrolysed by the intestinal brush-border enzyme, lactase, into absorbable sugars, namely glucose and galactose. People with disability of digestion of lactose due to the lack of enzyme – β-galactosidase in small intestines are called lactose intolerant. More than 70% of the world population suffers from lactose intolerance (Schmidt et al., 2017), but its occurrence largely depends on the population group: only approx. 10% of Northern Europeans but more than 90% of South-East Asians suffer from lactose intolerance (Jelen, Tossavainen, 2003). For this reason, majority of the world population encounters problems due to their consumption of foods containing high amount of lactose. One of the strategies more assayed to obtain reduced-lactose yoghurts is the lactose hydrolysis by the enzymatic treatment (Mlichová, Rosenberg, 2006).

But, lactose-free yoghurt with good technical quality parameters is still not available on the markets of Uzbekistan. Recently, Kasimov et al. (2015) has performed experiment by testing gene variants of the Uzbek population from Andijan region. Despite of the fact that people suffering from lactose intolerance in the Republic of Uzbekistan is under 20%, results showed that almost 81% of the volunteers participated in the experiment possess LNP C/C-13910 gene, which is characterized by a decrease in the level of lactase in adulthood. This means, producing yoghurt and dairy products with reduced lactose content and acceptable rheological properties is an actual task in the Republic of Uzbekistan.

β–galactosidase (lactase) is an enzyme that catalyses the hydrolysis of lactose to glucose and galactose. It can be isolated from plants, animals, yeasts, fungi and bacteria. It is potentially important in the production of lactose-reduced products in the food and dairy industry for lactose intolerant populations (Souza et al., 2018). Since it is possible and preferable to perform lactose hydrolysis simultaneously with fermentation in order to save extra processing time, co-hydrolysis (starter and enzyme) used in production. This technological step, as well selection of enzyme concentration and starter can influence the rheological parameters of the product. However there is lack of information about lactose– free fermented products' rheological properties, which can be influenced by lactose enzymatic hydrolysis. The distinctive texture, mouth feeling and flavour make yoghurt an appealing product that takes a great portion in the dairy industry.

The aim of the study was to evaluate enzyme concentration influence on the rheological properties of lactose-free yoghurt.

Materials and Methods

Yoghurt preparation

Pasteurized milk was bought from the local shop (fat content 2.5%, Tukuma piens Ltd., Latvia). Initially, cow milk (pH 6.70±0.05) was pasteurized (95.0±1.0 C for 5 min), rapidly cooled till 40 °C and divided into five beakers (triplicate) in order to add different concentrations of enzyme (NOLA™ Fit 5500, Chr. Hansen Holding JSC, Denmark). Enzyme concentrations used in the research was: 0 (A – control sample), 500 (B); 1000 (C); 1500 (D) and 2000 (E) BLU L^{-1}, manufacturer recommended dose range from 500 to 18000 BLU L^{-1}. Enzyme and commercial frozen starter culture FD-DVS YC-X11 (Danisco Ltd., Denmark; containing EPS producing cultures: *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus)* were added at the same time for each sample. All beakers were incubated in a thermostat (Incubator IN55, Memmert, Germany) at 40±1 °C during 5.0±0.5 hours until pH reached 4.8±0.1. After that, samples were mixed and cooled down till 5±1 °C. Then, beakers containing yoghurt were matured and stored at 5±1 °C for 24 hours before analysis.

Rheological analysis

Viscosity was measured using DV-III Ultra Programmable Rheometer (Brookfield Engineering Laboratories Inc., USA). Yoghurt after an overnight storage at 5 °C was transferred into the small sample holder. Rotation speed of the SC4-16 spindle was set at 10 min^{-1}. Data was recorded in the Rheocalc V2.6.: Rheometer program 10 s after beginning of the spindle rotation. Viscosity was measured 5 times using fresh yoghurt sample each time.

The rheological measurements were performed on a rheometer MCR-302 (Anton Paar GmH, Austria) with cone-plate geometry (50 mm, 1°), gap 0.5 mm at 4.0±0.1 °C. The flow behaviour of yoghurt samples was evaluated by shear rate sweep after 30 s of pre-shear at 5 s^{-1} for equilibration. Then shear rate was increased from 0.1 to 200 s^{-1} in 300 s (for upward curve). Before downward curve a shearing for 60 s at 200 s^{-1} was performed. In the next interval, shear rate was decreased from 200 to 0.1 s^{-1} in another 300 s. The flow curves were used to calculate thixotrophy area and yield point. In the oscillatory tests, storage (elastic) modulus G´ and loss (viscous) modulus G´´ were measured at frequencies between 0.1 and 100 Hz, with a constant strain 1% within the linear viscoelastic region determined in preliminary amplitude test (strain 0.01–10%, frequency 1 Hz). The storage modulus, loss modulus and the tangent of the phase angle (tan δ) at frequency 1 Hz were reported.

Textural analysis

TA.HD Plus (Stable Micro Systems, UK) was employed for texture properties of yoghurts, such as firmness, consistency, cohesiveness. A single compression test was performed using a back extrusion cell disc (A/BE; diameter 35 mm; distance 30 mm; pre-test speed 1.00 mm s^{-1}; test speed 1.00 mm s^{-1}; post-test speed 10.00 mm s^{-1}; trigger force 0.098 N) and extension bar with 50 kg load cell. All data were collected automatically on computer by Exponent 32 Software for further analysis.

Microstructure characterization

In order to learn and monitor the microstructure of yoghurt samples, the light microscope *LEICA DM 3000 LED* was applied. The microscope consisted of two lens with magnification 10 × and 20 × and was coupled with *LEICA DFC290 HD* camera to take digital pictures and save those in the computer. LAS V4.2 program of the computer was used to measure size of whey pockets on the surface of yoghurt samples from pictures.

Chromatography analysis

10 mL of yoghurt samples transferred into named tubes and mixed with 4 drops of CaCl (5%). Then samples were heated till 95 °C and poured through filter paper in order to split protein and obtain whey. To make the whey more transparent it was centrifuged (Centurion Scientific Limited - PrO-Research K243) for 10 min, at 13000 rpm. The content of individual sugars – glucose, galactose and lactose was determined by a high-performance liquid chromatograph LC 20 Prominence (Shimadzu, Japan). Chromatographic parameters were set as follows: detector – refractive index RID-10A; column – Alltech NH2, 4.6 mm×250.0 mm, 5 μm; temperature 25 °C; isocratic elution regime, mobile phase –A – acetonitrile, B – deionized water (A70 : B30); capacity of the injection sample – 10 μL; total time of the analysis – up to 15 min; flow rate – 1.0 mL min^{-1}. Acquired data were processed using Shimadzu LabSolutions software (LCsolution Version 1.21 SP1).

Data processing / Statistical analysis

Analysis of variance (ANOVA) was done using Statistical 10.0 software (Stat Soft. Inc., Tulsa, USA) and the mean comparisons of parameters were performed by t-test, with the level of significance at 0.05.

Results and Discussion

Rheological properties of yoghurts

The effect of lactose hydrolysis on the characteristics of the yoghurts during research was investigated. Significant differences ($p<0.05$) were detected regarding the viscosity results (see Fig. 1). The highest viscosity value was recorded for the control sample. Among the yoghurt samples hydrolysed by the enzyme showed consistent decrease as the amount of enzyme has increased in the samples. Viscosity of yoghurt samples were significantly different ($p<0.05$) from each other and depended on enzyme concentration.

All yoghurt samples presented a typical shear-thinning behaviour (Fig. 2) for the shear rates from 0.1 to

200 s^{-1}. Flow curves of yoghurts showing shear stress versus shear rate clearly demonstrate hysteresis loops. It has been reported that high viscosity is related with bigger hysteresis area. In the present study hysteresis area decreased with the enzyme concentration, showing opposite trend to viscosity.

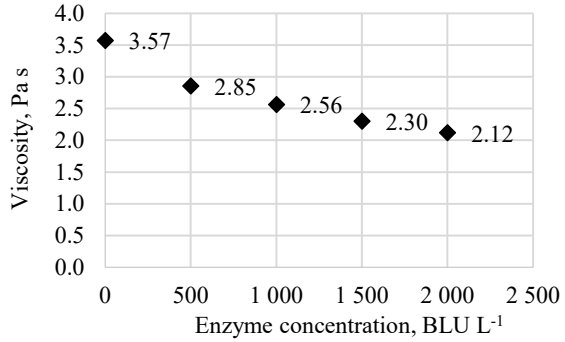

Figure 1. Yoghurt viscosity (n=5)

The largest hysteresis area was found for the control and yoghurt with enzyme 500 BLU L^{-1}. When enzyme was added to yoghurt its yield point was reduced, which can be associated to the changes in three-dimensional structures (Jaster et al., 2018).

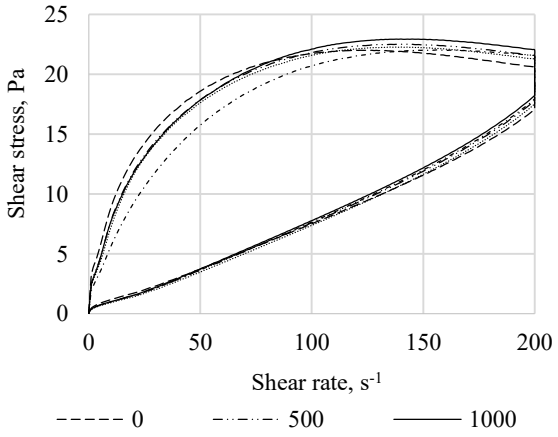

Figure 2. Flow curves of the yoghurt samples with different enzyme concentrations (0–2000 BLU L^{-1})

All samples with added enzyme had lower values of yield point compared to control yoghurt (Table 1), which also denotes a weaker network of yoghurt gel.

Table 1

Rheological properties of yoghurts with different enzyme concentrations

Enzyme concentration, BLU L^{-1}	Hysteresis area, kPa s^{-1}	Yield point, Pa
0	2.37±0.06^{a}	0.39±0.02^{a}
500	2.32±0.08^{a}	0.16±0.08^{b}
1000	2.23±0.05^{b}	0.14±0.10^{bc}
1500	2.20±0.09^{b}	0.11±0.09^{c}
2000	2.06±0.10^{c}	0.17±0.02^{b}

(n=3); values with different superscripts within the same column are significantly different (P< 0.05).

The apparent viscosity decreased with increasing shear rate (Fig. 3), which also showed that yoghurt was pseudoplastic. This shear-thinning behaviour may be attributed to the destroying weak bonds formed in yoghurt gels (Cui et al., 2014) and reduce hydrophobic interactions between molecules. This disruption is greater in the beginning of the process. The viscosity profiles for the samples almost overlapped at the most shear rates, except sample made with the enzyme concentration 2000 BLU L^{-1}, which exhibited lower apparent viscosity.

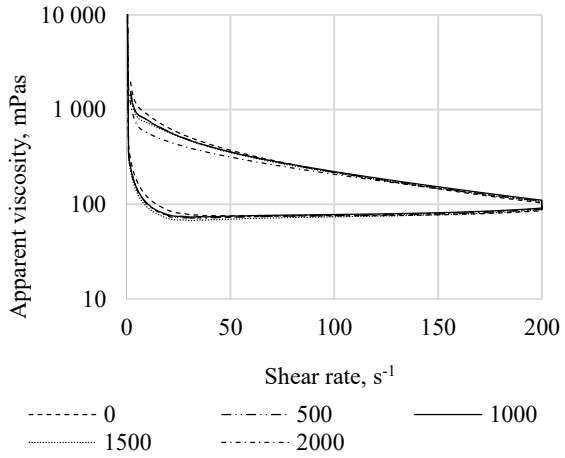

Figure 3 Viscosity curves of the yoghurt samples with different enzyme concentrations (0–2000 BLU L^{-1})

Table 2 shows the storage modulus (G′), loss modulus (G′′) and loss tangent (tan δ = G′′/G′) of the yoghurt samples with different enzyme concentrations. The lowest tan δ value of control sample indicate that the sample is more solid-like compared to the samples with enzyme additive.

Table 2

Viscoelastic properties of yoghurts with different enzyme concentrations from frequency sweeps (at 1 Hz)

Enzyme concentration, BLU L^{-1}	Storage modulus G′, Pa	Loss modulus G′′, Pa	Loss factor tan δ
0	12.07^{a}	3.38^{a}	0.281^{d}
500	10.93^{ab}	3.17^{b}	0.291^{cb}
1000	11.27^{a}	3.19^{b}	0.284^{d}
1500	9.29^{b}	3.12^{b}	0.338^{a}
2000	7.87^{c}	2.43^{c}	0.318^{b}

(n=3); values with different superscripts within the same column are significantly different (p< 0.05)

Small amplitude oscillatory tests revealed the decrease of storage modulus G′ with the enzyme concentration. The G′ values ranged between 7.87 and 12.07 Pa, with lower values for samples with higher enzyme concentration, indicating formation of weaker gel comparing to control sample or sample with

500 BLU L^{-1} enzyme. Similar trend was observed for loss modulus G´´.

Textural properties of yoghurts

Textural properties of yoghurts samples are presented in the Table 3. A highly significant firmness, consistency and cohesiveness were found for the control sample making standard sample significantly different from the other samples and reduced viscosity with the increased amount of enzyme for rest of the samples. Despite of the fact that textural results showed the tendency of decrease in relation to the enzyme concentration, statistical test provided no difference among the samples of 500, 1000 and 1500 BLU L^{-1}. Significant differences were detected between samples of the smallest and the highest concentrations regarding to the firmness, consistency and cohesiveness. There are several factors, which can directly affect the rheological properties of the yoghurt. For example, lipid content and globule size are important factors affecting the gel firmness. However, looking at our results from rheological studies, it was clear that lactose hydrolysis had a significant influence on the texture and viscosity of yoghurts.

Changes of lactose free yoghurt rheological properties can be explained by lactose enzymatic hydrolysis, changing rate of glucose and galactose (Table 4) consumed by starter cultures and differences in the production of derived compounds (Goff, 2002).

Table 4

Sugar profile in yoghurt samples

Enzyme concentration, BLU L^{-1}	Glucose, g L^{-1}	Galactose, g L^{-1}	Lactose, g L^{-1}
0	0.612	6.942	33.425
500	18.441	23.924	0.000
1000	19.419	27.202	0.000
1500	19.704	25.685	0.000
2000	18.977	26.436	0.000

It is supposed, that the concentration of exopolysaccharide was assumed high on the sample, where less enzyme was added corresponding to the higher firmness, higher cohesiveness, higher consistency and higher viscosity, respectively. It was also reported that the exopolysaccharide could improve the texture of yoghurt, still exopolysaccharide produced by LAB interacts with the free water in the gel-like structure (Han et al., 2016). In this study control sample and yoghurt hydrolysed with less amount of commercial enzyme showed higher viscosity and texture behaviour comparing to other samples.

Table 3

Textural properties of yoghurt samples

Enzyme concentration, BLU L^{-1}	Firmness, g	Consistency, g s	Cohesiveness, g
0	66.42 ± 5.05^{a}	1753.96 ± 162.86^{a}	-66.34 ± 5.29^{a}
500	55.69 ± 3.35^{bcd}	1453.86 ± 6.30^{bcd}	-59.16 ± 7.86^{bcde}
1000	55.36 ± 2.67^{cde}	1453.71 ± 70.75^{cde}	-56.02 ± 6.64^{cde}
1500	54.04 ± 3.95^{de}	1423.88 ± 110.61^{de}	-55.53 ± 5.03^{de}
2000	53.13 ± 3.33^{e}	1388.13 ± 82.61^{e}	-53.88 ± 3.37^{e}

(n=5); values with different superscripts within the same column are significantly different ($p< 0.05$)

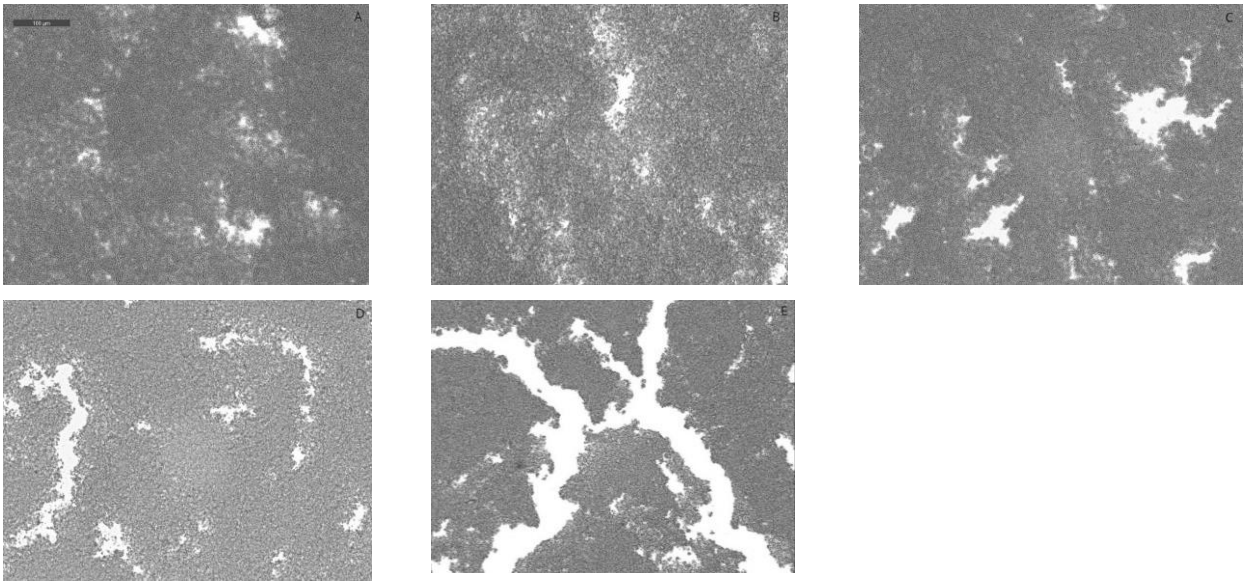

Figure 4. Microscopic images of yoghurt samples

A – control sample; B – 500 BLU L^{-1}; C – 1000 BLU L^{-1}; D – 1500 BLU L^{-1}; E – 1500 BLU L^{-1} (all images under 100 µm)

The possible explanation of obtained results was the activity of other enzymes incorporated in NOLA™ Fit 5500. According to the information obtained from the literature cellulases and proteases have been used for more efficient dispersion and extraction of proteins, particularly after heat treatment, used in yogurt production, making them denaturated (Aguilera, Stanley, 1999).

Microstructure of yoghurts

The results from the microstructural analyses of yoghurts are presented in Fig. 4. The microstructural observations showed that sample with less enzyme had smaller whey pockets rather than those with higher concentration of enzyme. Similar results had also been reported by other studies (Ibrahim, 2018; Schmidt et al, 2017). This might be due to the high water-binding capacity of EPS as well as modifications of yoghurt microstructure by EPS culture. Thus, the yoghurts made from EPS producing starters showed better textural characteristics. Furthermore, yoghurt cultures producing EPS may decrease the extent of syneresis (the lower whey separation). Syneresis is considered as a major defect in yoghurt and connected with extensive rearrangements of the gel network (Han et al., 2016). Whey pockets became wider depending on the enzyme concentration used on each sample due to the reason mentioned above.

According to the measurements, size of the whey pocked also increased: control sample (248 μm^2); 500 BLU L^{-1} (642 μm^2); 1000 BLU L^{-1} (2640 μm^2); 1500 BLU L^{-1} (4004 μm^2); 2000 BLU L^{-1} (101 494 μm^2). Sugar profile and concentrations in yoghurt samples are given in Table 4. Results from chromatography analysis provided no lactose presence in any sample with enzyme, the galactose was dominant monosaccharide in each sample treated with enzyme.

Complete lactose conversion to galactose and glucose in each sample (B, C, D, E) with NOLA™ Fit 5500 enzyme was detected in the research.

In order to achieve the highest conversion of lactose with less amount of commercial enzyme is beneficial from financial side.

Conclusions

Rheological properties of lactose-free yoghurt significantly depend on enzyme concentration used for lactose hydrolysis, reflected in a significantly lower viscosity, firmness, consistency, but higher size of whey pockets in yoghurt samples with increased amount of enzyme comparing to the control sample. Results show that rheological properties of the yoghurt strongly depend on the lactose hydrolysis level.

For obtaining lactose free yoghurt with acceptable rheological properties the lowest concentration of β-galactosidase (500 BLU L^{-1}) is recommended.

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References

1. Aguilera J., M., Stanley D.W., (1999) Microstructure and mass transfer: Solid-liquid extraction, In: *Microstructural Principles of Food Processing and Engineering*. 2nd ed. Aguilera J., M., Stanley D.W. (eds). Gaithersburg, Maryland, AN Aspen Publication, p. 325–340.
2. Cui B., Lu Y.M., Tan C.P., Wang G.Q., Li G.H. (2014) Effect of cross-linked acetylated starch content on the structure and stability of set yoghurt. *Food Hydrocolloids*, Vol. 35, p. 576–582.
3. Goff H.D. (2002) Formation and stabilisation of structure in ice-cream and related products. *Current Opinion in Colloid & Interface Science*, Vol. 7(5–6), p. 432–437.
4. Han X., Yang Z., Jing X., Yu P., Zhang Y., Yi H., Zhang L. (2016) Improvement of the texture of yogurt by use of exopolysaccharide producing lactic acid bacteria. *BioMed Research International*, Vol. 2016, p. 1–6.
5. Ibrahim A.H. (2018) Impact of hydrolyzed lactose by β-galactosidase enzyme on the physicochemical and organoleptic properties of fermented camel milk. *Emirate Journal of Food and Agriculture*, Vol. 30(9), p. 778–790.
6. Jaster H., Arend G. D., Rezzadori K., Chaves V. C., Flávio Reginatto H., Cunha Petrus J. C. (2018) Enhancement of antioxidant activity and physicochemical properties of yogurt enriched with concentrated strawberry pulp obtained by block freeze concentration. *Food Research International*, Vol. 104, p. 119–125.
7. Jelen P., Tossavainen O. (2003) Low lactose and lactose-free milk and dairy products - Prospects, technologies and applications. *Australian Journal of Dairy Technology*, Vol. 58(2), p. 161–165.
8. Kasimov S., Nazarova G., Kasimova D., Yunusova Z., Rakhmatova M. (2015) A single nucleotide polymorphism C/T - 13910 and consumption of dairy products in Uzbek population. *Journal of Evolution of Medical and Dental Sciences*, Vol. 4(86), p. 15042–15050.
9. Mlichová Z., Rosenberg M. (2006) Current trends of β-galactosidase application in food technology. *Journal of Food and Nutrition Research*, Vol 45(2), p. 47–54.
10. Schmidt C., Mende S., Jaros D., Rohm H. (2017) Fermented milk products: effects of lactose hydrolysis and fermentation conditions on the rheological properties. *Dairy Science and Technology*, Vol. 96(2), p. 199–211.
11. Souza S.O., Santos V.S., Santos E.S., Ávila D.V.L., Nascimento C.C., Costa S.S.L., Garcia A.B., Araujo R.G.O. (2018) Evaluation of the mineral content in milk and yogurt types using chemometric tools. *Microchemical Journal*, Vol. 143, p. 1–8.
12. Wolf I.V., Vénica C.I., Perotti M.C. (2015) Effect of reduction of lactose in yogurts by addition of β-galactosidase enzyme on volatile compound profile and quality parameters. *International Journal of Food Science and Technology*, Vol 50(5), p. 1076–1082.

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EVALUATION OF MICROBIOLOGICAL QUALITY OF COLOSTRUM

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Abstract

Bovine colostrum is an important source of different biologically active compounds: immunoglobulins, lactoferrin, lysozyme, lactoperoxidase etc., therefore vital for a dairy calf's ability to survive. There is the lack of information about microbiological quality of colostrum. Still it is very important parameter, which can be significant for calf mortality and antibodies absorption rate, the aim of the study was to evaluate microbiological quality of colostrum obtained from Latvian cows. Colostrum samples (n=20, 50 mL^{-1}) were collected in conventional farm with 500 cows (Zemgale, Latvia) during December 2018 to January 2019 one hour after calving. Samples were immediately frozen (-19±1 °C, within 30 min) and delivered to the laboratory. The colony forming units (LVS EN ISO 4833-1:2013) and presence of β-glucuronidase positive *Escherichia col* (LVS EN ISO 16649-2:2007) were detected in colostrum samples. Descriptive statistics were used for data analysis. The average total plate count of analysed colostrum samples was 5.62 log CFU mL^{-1}, colony forming units ranged from 4.97 to 5.90 log CFU mL^{-1}. In the current research β-glucuronidase positive *Escherichia coli* CFU ranged from >1 to 8300 mL^{-1} in colostrum sample. Research results associated with low antibodies absorption rate by calf and high risk of diarrhoea in the farm.

Keywords: colostrum, total plate count, Ig concentration

Introduction

Bovine colostrum is an important source of different biologically active compounds: immunoglobulins (Ig), lactoferrin, lysozyme, lactoperoxidase etc., therefore vital for a dairy calf's ability to survive. Studies show that the highest concentration of biologically active components in colostrum is collected in cows at the first milking after calving (Hurley, Theil, 2011), reporting maximum concentration in the first four hours (Sacerdote et al., 2013) to six hours (Borad, Singh, 2018) after calving.

The concentration of Ig in bovine colostrum affects the passive immunity acquisition in new-born calves (Swan et al., 2007; Quigley et al., 2013; Mann et al., 2016; Arsenopoulos et al., 2017). Therefore, accurate measurement of Ig is essential to provide healthy growth of the younger generation on the dairy farms, which will be able to provide new-born calves with necessary Ig concentration (>50 mg mL^{-1}) in colostrum (Lago et al., 2018). Quigley et al. (2013), Morrill et al. (2015), Yaylak, Yavuz, Özkaya (2017) and other authors used Brix refractometer to estimate Ig concentration in colostrum. Authors concluded that Brix measurement of total solids in fresh colostrum is a sufficiently accurate method for estimating IgG concentration, which is confirmed by the use of alternative methods in studies. Quigley et al. (2013) suggested, that predicted IgG concentration in colostrum based on Brix percentage is variable. Current industry recommendations designate discarding colostrum should contain <50 mg of IgG mL^{-1} and > 5 log10 CFU mL^{-1} total bacteria count (Morrill et al., 2012; Quigley et al., 2013; Morrill et al., 2015; Yaylak et al., 2017).

Colostrum bacterial contamination is another important quality parameter. Microbiologically contaminated colostrum can reduce animal performance as well as increase morbidity and mortality rates in the farms (Elizondo-Salazar et al., 2010; Mohammed et al., 2018). Microorganisms can bind to free immunoglobulins and block absorption of these molecules by enterocytes (Morrill et al., 2012; Santos et al., 2017).

Last data reported about microbiological quality of colostrum from Latvian dairy herds is from 2008, when microorganisms count ranged from 4.3 log to 6.0 log CFU mL^{-1} (Gala ziņojums, 2008).

Information about microbiological quality of colostrum in Latvia is limited and outdated; still it is very important parameter and can be significant factor detecting calf mortality rate. Therefore, the aim of the study was to evaluate microbiological quality of colostrum obtained from Latvian cows.

Materials and Methods

Colostrum was collected from Holstein Black cows, lactation period ranged from: 1st to 4th. Colostrum samples (n=20, 50 mL) were collected in conventional farm with 500 cows (Zemgale, Latvia). Colostrum samples were classified according lactation number 1st (n=4), 2nd (n=8), 3rd (n=5) and 4th (n=3). Colostrum samples were collected from December 2018 to January 2019 one hour after calving according LVS EN ISO 707:2011 Milk and milk products – Guidance on sampling. Samples at 20 °C were immediately after collection used for immunoglobulins and total solids content detection.

Samples for microbiological analysis were immediately frozen (-19±1 °C, within 30 min) and delivered to the laboratory, stored less than 30 days before analysis. Before microbiological tests, samples were removed from freezer, thawed and homogenized in a water bath (~45±2 °C), then prepared according the following standards: LVS EN ISO 6887-1 and 5:2011 Microbiology of food and animal feeding stuffs –

Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions, Part 5: Specific rules for the preparation of milk and milk products.
The total plate count (TPC) was detected in colostrum samples according to standard LVS EN ISO 4833-1:2013 Microbiology of the food chain. Horizontal method for the enumeration of microorganisms. Part 1: Colony count at 30 °C by the pour plate technique; presence of *Escherichia coli* (*E.coli*) according to LVS EN ISO 16649-2:2007 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of beta-glucuronidase positive *Escherichia coli*. Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.
The colostrometer (COLOSTROMETER^{tm} Biogenics, USA) was used for evaluation of Ig concentration in mg mL^{-1}. Percentage of Ig in colostrum was measured using an optical Brix refractometer (Model BX, UK) with a range of 0 to 34% Brix.
Descriptive statistics were used for data analysis.

Results and Discussion

One of the most important factors for bovine neonatal immune function is the first feeding of colostrum. Colony forming units is very important parameter, which indicate quality of many products, colostrum isn't exception. One of the quality criteria for bovine colostrum is the total plate count based on Commission Regulation lays down the same hygiene requirements for bovine colostrum quality as for raw bovine milk, TPC does not exceed 100 000 CFU mL^{-1} (>5 log CFU mL^{-1}) (European Commission, 2006). Previous studies have shown a wide range of microorganisms count in colostrum, ranged from 1.4 to 7.0 log CFU mL^{-1} (Gelsinger, Heinrichs, 2017), from 3.0 to 6.8 log CFU mL^{-1} (Morrill et al., 2012), from 5.4 to 7.2 log CFU mL^{-1} (Dunn et al., 2017), from 1.86 to 11.02 log CFU mL^{-1} (Swan et al., 2007).
During the research, samples were divided into two groups by TPC and its correspondence to Regulation. Colony forming units and distribution of analysed samples are shown in Table 1.

Table 1

Proportion of colostrum samples by colony forming units

Group	Proportion %	min	max
		Log CFU mL^{-1}	Log CFU mL^{-1}
1	5	3.97	3.97
2	95	5.08	5.90

Among the colostrum samples in the current study, there was a wide variation of TPC, from 3.97 to 5.90 log CFU mL^{-1}. Other authors reported more significant total plate count variations from 3 to 6.80 log CFU mL^{-1} (Morrill et al., 2012) and from 2.34 to 6.94 log CFU mL^{-1} (Quigley et al., 2013).

In this study only 5% of the colostrum samples meet microbiology safety criteria that matched <5 log CFU mL^{-1}, in 95% of samples total bacterial count ranged from 5.09 to 5.90 log CFU mL^{-1}. Similar results, high percentage of microbiologically contaminated colostrum, that exceed higher permissible limit of guidelines for microbial criteria, were obtained in other studies: overall 81% samples (n=1239) (Dunn et al., 2017).
Gelsinger & Heinrichs (2017) proved, that high TPC, detected in analysed dairy farm (average 5.62 log CFU mL^{-1}), can negatively influence Ig absorption by calves. Microbiological contamination is an indicator of colostrum quality. In the current study it was not satisfactory and according to Arsenopoulos et al. (2017) can be a reason for neonatal calf diarrhoea, still storage of colostrum practised in farms for the longest period of time can enhance negative influence, promoting bacteria growth. Morrill et al. (2012) reported about TPC reduction by 0.5 log in colostrum samples after freezing, in comparison with the samples that were stored chilled.
Authors (Elmoslemany et al., 2010; Zhao et al., 2010; Sacerdote et al., 2013; Mann et al., 2016) mentioned different factors influencing bacteria count in colostrum – stage of lactation is one of them. Average total plate count and *E. coli* distribution of cow's lactation are show in Table 2.

Table 2

Distribution of colostrum samples by total and *E. coli* bacteria count

Lactation period	Proportion %	Average TPC	Average *E.coli*
		Log CFU mL^{-1}	Log CFU mL^{-1}
mean	100	5.62	2.64
1st	20	5.76	3.32
2nd	40	5.49	1.43
3rd	25	5.63	1.52
4th	15	5.66	0.88

According to our results, the highest total bacteria count was in colostrum obtained after 1st lactation (5.76 log CFU mL^{-1}), followed by samples obtained from 3rd and 4th lactation period with average bacteria count ranged from 5.62 to 5.65 log CFU mL^{-1}, respectively. Lowest TPC was determined in colostrum after 2nd lactation – 5.49 log CFU mL^{-1}. Other authors presented contradictory results, higher bacteria count after 3rd lactation, were presented in Sacerdote et al. (2013) study, in colostrum after 2nd and 3th lactation period total bacteria count increased from 7.5 log to 7.8 log CFU mL^{-1}. Different tendency was detected by Morrill et al. (2012), who reported lower TPC in colostrum obtained after 2nd lactation, comparing to 3rd lactation colostrum. According to the research results bacteria count significantly increased after 1st lactation from 4.5 log to 4.7 log in 2nd lactation samples, but decreased till 4.3 log CFU mL^{-1} after 3th lactation, respectively (Morrill et al. 2012). Our results can be

explained with tissue damage in older animals and easier microorganism migration inside.

Enterobacteria presence was detected in 100% of the samples, results can be explained with poor hygiene rules adherence (Santos et al., 2017) in the farm. In the current study, used β-glucuronidase positive *Escherichia coli* count determination method allows to identify about 90% to 100% *E. coli* strains (Public Health England, 2014). *E. coli* is commonly used as alternative marker of poor sanitary practice, direct and indirect fecal bacteria contamination. Levels of these bacteria often act as an indirect measure of the potential for dangerous fecal pathogens to be present in food (Neeliah, Arlandoo, Kureemun, 2016).

Presence of β-glucuronidase positive *E. coli* was identified in 85% of colostrum samples analysed during research (Table 3).

Table 3

Proportion of colostrum samples by *E. coli* count

Group Log CFU mL^{-1}	Proportion %	min Log CFU mL^{-1}	max Log CFU mL^{-1}
No growth	15	–	–
<1	35	0.00	0.85
1–1.99	40	1.08	1.93
2–2.99	5	2.23	2.23
>3	5	3.92	3.92

Compared to other authors results proportion of colostrum samples by *E. coli* count is significantly higher ($p<0.05$) than those reported by Mohammed et al. (2018) – 12% and Phipps et al. (2016) – 37% study results. In the current research *E. coli* count ranged from 0.00 to 3.92 log CFU mL^{-1}, and corresponds to US standards of bacterial contamination of colostrum <4 log CFU mL^{-1}. Other authors data about total coliform count 0.00–4.87 log (Quigley et al., 2013), 3.78–6.91 log (Gelsinger, Heinrichs, 2017) and 0.00–9.48 log CFU mL^{-1} (Swan et al., 2007) confirm our research results.

In the current study, significant number of samples met the industry recommendations in terms of total coliform count (100%), however; only 5% of the analysed colostrum samples were within limits in terms to TPC. Colostrum microbiological contamination can occur in different ways: milk can be contaminated with commensal bacteria from the teat skin, epithelial lining of teat canal, the lactiferous duct while it is being excreted of milk during collection, processing, handling and storage (Alegbeleye et al., 2018). Some countries practise heat treatment as for improving microbiological quality of colostrum, as for reducing bacterial infection in neonatal calves and increasing Ig absorption (Elizondo-Salazar et al., 2010; Gelsinger, Heinrichs, 2017). For ensuring calves with high quality colostrum such experience can be recommended for Latvian dairy herds.

The level of Ig in bovine colostrum after first milking can be highly variable, from 60 to 100 mg mL^{-1} (Sanchez et al., 2004), from 1.4 to 204 mg mL^{-1} (Dunn et al., 2017), from <1 to 200 mg mL^{-1} (Morrill et al., 2012), from 12.8 to 154.3 mg mL^{-1} (Morrill et al., 2015). The concentration of immunoglobulins in analysed colostrum samples ranged from 40 to 118 mg mL^{-1}, mean value – 85 mg mL^{-1} (Figure 1).

Other authors reported significant ($p<0.05$) lower Ig concentration in colostrum – 63.6 mg mL^{-1} (Lago et al., 2018). Only in one analysed colostrum sample Ig concentration was less than 50 mg mL^{-1} – 40 mg mL^{-1}, but other 95% of samples contained above 53 mg mL^{-1} of Ig. Other studies showed lower Ig concentration (<50 mg mL^{-1}) in individual colostrum samples, overall 44% samples (Dunn et al., 2017), 30% (Morrill et al., 2012), 17% (Yaylak et al., 2017) were not according recommendations, but mean concentration of Ig still exceeds industry recommendations for IgG concentration in bovine colostrum.

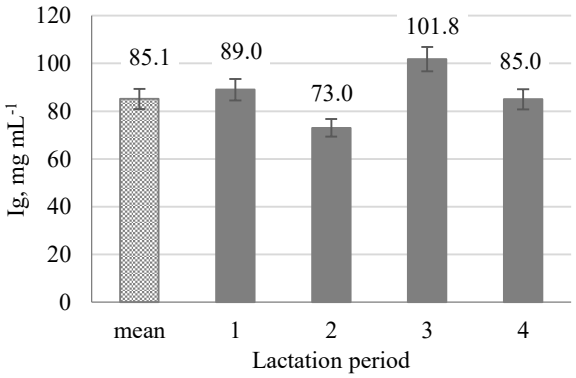

Figure 1. Mean Ig concentration in colostrum samples

Researchers found a relationship between lactation and Ig concentration in colostrum (Zhao et al., 2010; Morrill et al., 2012; Yaylak et al., 2017). The first lactation cows were found to have significantly lower IgG concentration in colostrum samples compared to cows in the second or higher lactation. This may be associated to increased tissue damage in older animals that increase the leakage of Ig from the serum in milk (as observed in somatic cells) (Sanchez et al., 2004). Colostrum production is often lower in cattle of the first lactation, which implies less development of the mammary glands and potentially reduces the transport ability of IgG to the mammary gland (Morrill et al., 2012).

Comparing Ig concentration after 1st and 2nd lactation, results were similar to Yaylak et al. (2017), higher Ig concentration was after 1st, comparing to 2nd lactation 94.1 and 88.03 mg mL^{-1}, respectively.

Contradictory results obtained by Morrill et al. (2012), Ig concentration (95.5 mg mL^{-1}) after 3rd lactations was higher compared with first two lactations – 1st (42.4 mg mL^{-1}) and 2nd (68.6 mg mL^{-1}). Latvian researchers' results detected the mean concentration of Ig tends to decrease with each subsequent lactation (from 1st to 4th) in the colostrum – 101.3 mg mL^{-1}, 89.6 mg mL^{-1}, 88.1 mg mL^{-1} and 89.7 mg mL^{-1} (Eihvalde et al., 2012).

In the current study, higher Ig concentration in colostrum after 1st lactation can be explained with high TPC and *E.coli* count in colostrum, which resulted in a mammary gland protection mechanism.
Obtained results did not reveal any tendency to reduce or increase Ig concentration depending on the lactation period as other authors reported. This may be related to a small number of samples, breed, nutrition, length of the dry period of cows, vaccination and other factors (Dunn et al., 2017).
Figure 2 shows the distribution of Brix refractometer readings for the analysed samples. The percentage of Ig in colostrum in the analysed farm ranged from 20 to 32%, mean 24% Brix.

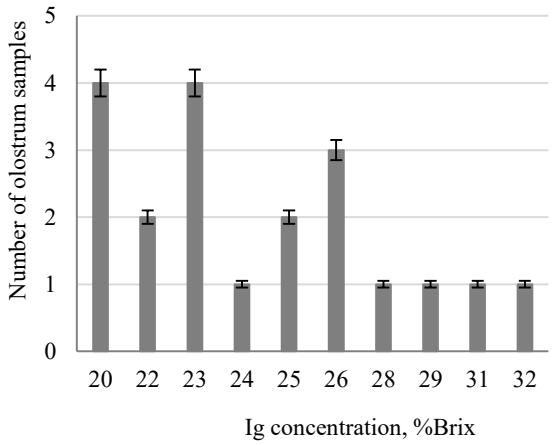

Figure 2. Distribution of Ig concentrations (% Brix) by the number of samples

The distribution of samples was the same as in a case with colostrometer measurements: higher concentration (28%) was in 3rd colostrum, then follow 1st (26%), 4th (22%), and 2nd (21%).
Recommended concentration 21% is considered the break point for high-quality (>50 mg mL^{-1}) bovine colostrum (Morrill et al., 2012; Quigley et al., 2013). Based on this recommendation, 80% of the samples should be included in a high-quality colostrum group.
Other authors reported lower mean Ig concentration – 21.24% (Morrill et al., 2015), – 23.8% Brix (Quigley et al., 2013), – 20.3% (Lago et al., 2018). Yaylak et al. (2017) report higher concentration of Ig – 24.61%.
Obtained research results showed poor microbiological quality of colostrum regarding total plate count in the colostrum from analysed dairy farm. Nowadays there is no control for microbiological quality of colostrum in Latvian dairy farms. However it can be a determinative factor in mortality rate of calves, as for passive immunity transfer, still bacteria can bind to free immunoglobulins in the intestinal lumen and block absorption of these molecules by enterocytes (Santos et al., 2017). For comprehensive assessment of Latvian colostrum quality, number of analysed samples should be increased and further research should be done.

Conclusions

Poor microbiological quality of colostrum obtained from analysed farm was proved through a high total plate count (average – 5.65 log CFU mL^{-1}) exceeding permissible level for such product and Enterobacteria presence in 100% of analysed samples.
Despite sufficient concentration of Ig in analysed samples (mean 85.1 mg mL^{-1} and 24% Brix) colostrum microbiological quality should be improved.
Research results can be associated with low Ig absorption rate by calves and high risk of diarrhoea in the farm.

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References

1. Alegbeleye O. O., Guimarães J. T., Cruz A. G., Sant'Ana A.S. (2018) Hazards of a 'Healthy' Trend? An appraisal of the risks of raw milk consumption and the potential of novel treatment technologies to serve as alternatives to pasteurization. *Trends in Food Science and Technology*, Vol. 82, p. 148–166.
2. Arsenopoulos K., Theodoridis A., Papadopoulos E. (2017) Effect of colostrum quantity and quality on neonatal calf diarrhoea due to *Cryptosporidium* Spp. infection. *Comparative Immunology, Microbiology and Infectious Diseases,* Vol. 53, p. 50–55.
3. Borad S.G., Singh A.K. (2018) Colostrum immunoglobulins: Processing, preservation and application aspects. *International Dairy Journal*, Vol. 85, p. 201–210.
4. *European Commission Regulation (EC) No. 1662/2006 Amending Regulation No. 853/2004,* Official Journal of the European Union, [accessed on 20.03.2019]. Available: https://eur-lex.europa.eu/legal-content/GA/TXT/?uri=CELEX:32006R1662
5. Dunn A., Ashfield A., Earley B., Welsh M., Gordon A., Morrison S.J. (2017) Evaluation of factors associated with immunoglobulin G, fat, protein, and lactose concentrations in bovine colostrum and colostrum management practices in grassland-based dairy systems in Northern Ireland. J*ournal of Dairy Science*, Vol. 100 (3), p. 2068–2079.
6. Eihvalde I., Kairiša D., Zagorska J. (2012) Analysis of factors influencing immunoglobulin concentration in colostrum of dairy cows. *Scientific Papers, Animal Science Series*, Vo. 57 (94), p. 256–259.
7. Elizondo-Salazar J.A., Jayarao B.M., Heinrichs A.J. (2010) Effect of heat treatment of bovine colostrum on bacterial counts, viscosity, and immunoglobulin G concentration. *Journal of Dairy Science*, Vol. 93 (3), p. 961–967.
8. Gala ziņojums (2008) *Latvijā iegūtā govju jaunpiena nekaitīguma kritēriju noteikšana.* [accessed on 20.03.2019]. Available: https://www.llu.lv/zps_projekti?page=20
9. Gelsinger S.L., Heinrichs A.J. (2017) Comparison of immune responses in calves fed heat-treated or unheated colostrum. *Journal of Dairy Science*, Vol. 100 (5), p. 4090–4101.
10. Hurley W.L., Theil P.K. (2011) Perspectives on immunoglobulins in colostrum and milk. *Nutrients,* Vol. 3(4), p. 442–474.

11. Lago A., Socha M., Geiger A., Cook D., Silva-del-Río N., Blanc C., Quesnell R., Leonardi C. (2018) Efficacy of colostrum replacer versus maternal colostrum on immunological status, health, and growth of preweaned dairy calves. *Journal of Dairy Science*, Vol. 101 (2), p. 1344–1354.
12. Mann S., Leal Yepes F.A., Overton A.L., Lock T.R., Lamb J.J., Wakshlag S.V., Nydam D.V. (2016) Effect of dry period dietary energy level in dairy cattle on volume, concentrations of immunoglobulin G, insulin, and fatty acid composition of colostrum. *Journal of Dairy Science*, Vol. 99 (2), p. 1515–1526.
13. Mohammed S.A., Marouf S.A., Erfana A.M., El-Haleem El-Jakee J.K., Hessain A.M., Dawoud T.M., Kabli S.A., Moussa I.M. (2018) Risk factors associated with *E. Coli* causing neonatal calf diarrhea. *Saudi Journal of Biological Sciences*, Article in press.
14. Morrill K.M., Robertson K.E., Spring H.D., Robinson M.M., Tyler A.L. (2015) Validating a refractometer to evaluate immunoglobulin G concentration in jersey colostrum and the effect of multiple freeze–thaw cycles on evaluating colostrum quality. *Journal of Dairy Science*, Vol. 98 (1), p. 595–601.
15. Morrill K.M., Conrad E., Lago A., Campbell J., Quigley J., Tyler H. (2012) Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. *Journal of Dairy Science*, Vol. 95(7), p. 3997–4005.
16. Neeliah S.A., Arlandoo J.D.L., Kureemun B. R. (2016) Ready to eat salads in selected retail outlets of Mauritius: An assessment of their hygiene status through enumeration of β-glucuronidase positive *Escherichia Coli*. *International Food Research Journal*, Vol 23 (6), p. 2661–2667.
17. Phipps A.J., Beggs D.S., Murray A.J., Mansell P.D., Stevenson M.A., Pyman M.F. (2016) Survey of bovine colostrum quality and hygiene on Northern Victorian dairy farms. *Journal of Dairy Science,* Vol 99(11), p. 8981–8990.
18. Public Health England (2014) *β-glucuronidase positive Escherichia Coli : pour plate method microbiology services food water and environmental microbiology standard method enumeration of β-glucuronidase positive Escherichia Coli: pour plate method enumeration of β-glucuronidase positi,* [accessed on 20.03.2019]. Available: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/342708/Ecoli_pour_plate.pdf
19. Quigley J.D., Lago A., Chapman C., Erickson P., Polo J. (2013) Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum. *Journal of Dairy Science*, Vol. 96 (2), p. 1148–1155.
20. Sacerdote P., Mussano F., Franchi S., Panerai A.E., Bussolati G., Carossa S., Bartorelli A., Bussolati B. (2013) Biological components in a standardized derivative of bovine colostrum. *Journal of Dairy Science*, Vol. 96(3), p. 1745–1754.
21. Sanchez J., Markham F., Dohoo I., Sheppard J., Keefe G., Leslie K. (2004) Milk antibodies against Ostertagia Ostertagi: Relationships with milk IgG and production parameters in lactating dairy cattle. *Veterinary Parasitology*, Vol. 120 (4), p. 319–330.
22. Santos G., Silva T.J., Santos F.H.R., Bittar C.M.M. (2017) Nutritional and microbiological quality of bovine colostrum samples in Brazil. *Revista Brasileira de Zootecnia,* Vol. 46 (1), p. 72–79.
23. Stewart S., Godden S., Bey R., Rapnicki P., Fetrow S., Farnsworth R., Scanlon M., Arnold Y., Muller K., Ferrouillet C. (2005) Preventing bacterial contamination and proliferation during the harvest, storage, and feeding of fresh bovine colostrum. *Journal of Dairy Science*, Vol 88 (7), p. 2571–2578.
24. Swan H., Godden S., Bey J., Wells R., Fetrow S., Chester-Jones H. (2007) Passive transfer of immunoglobulin G and preweaning health in holstein calves fed a commercial colostrum replacer. *Journal of Dairy Science,* Vol. 90 (8), p. 3857–3866.
25. Yaylak E., Yavuz M., Özkaya S. (2017) The effects of calving season and parity on colostrum quality of holstein cows. I*ndian Journal of Animal Research*, Vol. 51(3), p 594–598.
26. Zhao S., Zhang C., Wang J., Liu G., Bu D., Cheng J., Zhou L. (2010) Variations of immunoglobulins in colostrum and immune milk as affected by antigen releasing devices. *Asian-Australasian Journal of Animal Sciences*, Vol. 23 (9), 1184–1189.

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FORTIFICATION OF YOGURT WITH β-GLUCANS FROM OYSTER MUSHROOM

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Abstract

Mushrooms are a good source of biologically active substances. Basidiomycete *Pleurotus ostreatus* is not only widely used in food, but also have immunomodulating, antitumor, antiradical, anti-inflammatory, hypocholesterolic, hypoglycaemic and other beneficial medical effects. It is considered that β-glucans play significant role in the biological activities of the oyster mushroom. The aim of this research was to study the effect of the addition of various β-glucan containing preparations obtained from the submerged biomass of *P. ostreatus* on the process of milk fermentation in the production of yogurt and properties of the finished product. A total of three preparations were obtained by successive ethanolic and aqueous extractions. Preparations were added to milk in different concentrations before the introduction of starter culture. A starter containing *Streptococcus thermophilus* and *Lactobacillus delbuckii* subsp. *bulgaricus* cultures was used for fermentation. The titratable and active acidity was controlled during the fermentation. Physicochemical, structural-mechanical properties (dynamic viscosity, viscosity loss factor, mechanical stability factor, structure recovery ratio) of the obtained samples were studied. The sensory properties of the products were also evaluated. Results show that the addition of preparations does not adversely affect the fermentation process. Structural-mechanical properties of yogurt samples fortified by β-glucan-containing preparations depend on the preparation and its concentration. Sensory evaluation showed that experimental samples differed not only from the control sample but also from each other. In order to exclude slightly negative effect of some of the introduced components on the sensory characteristics of the product, it is advisable to use thickeners and structure-formers, for example, pectin.

Keywords: *Pleurotus ostreatus*, β-glucans, functional food, fermented milk product

Introduction

Fungi are a promising source of natural biologically active substances. For a large number of species and genera of various fungi, significant immunostimulant activity was detected (Vannucci et al., 2013). The polysaccharides of basidiomycetes, in particular β-glucans, are of considerable interest to researchers due to various useful properties (Bashir, Choi, 2017). The oyster mushroom *Pleurotus ostreatus* is widely cultivated throughout the world, and in addition to direct consumption it can also be an excellent source of various biologically active substances (Carrasco-González et al., 2017; Khan, Tania, 2012).

Polysaccharides obtained from *P. ostreatus* possess a wide range of biological activity *in vivo* and *in vitro* (Llauradó et al., 2015; Pasnik et al., 2017; Radzki et al., 2016). Thus, the hypolipidemic and hypocholesterol effect of oral consumption of dry biomass of *P. ostreatus* was shown (Shamtsyan at al., 2014).

Aqueous extracts from the fruit bodies of the *P. ostreatus* demonstrated a significant immunostimulatory effect: activating various immunocompetent cells, stimulating neutrophils of human peripheral blood, increasing production of interleukin 1-β, proinflammatory cytokines, etc. (Jesenak et al., 2013; Novak, Vetvicka, 2008).

Purified water-soluble heteropolysaccharides obtained by hot water extraction from the fruiting bodies of the *P. ostreatus* showed a strong antitumor effect on Hela cells and did not have direct cytotoxicity with respect to noncancerous cells (Tong et al., 2009). In other studies, the efficacy of water-insoluble fractions of *P. ostreatus* polysaccharides against Sarcoma 180 *in vivo* was studied. The studied polysaccharide preparations showed a decrease in the volume and mass of tumours (Facchini et al., 2014). It is believed that polysaccharides isolated from *P. ostreatus* can be used to develop new functional products (Giavasis, 2014; Lavelli et al., 2018). When incorporated into products, such preparation can provide additional prophylactic and functional properties, such as immunostimulant, hypoglycaemic, anti-radical, antitumor, anti-inflammatory and others (Khan et al., 2017).

Yogurt is a functional food product containing probiotic cultures and amino acids (Antontceva et al., 2018). Many manufacturers enrich yogurts with various vitamins, minerals and natural flavour additives (Vital et al., 2015). In recent years, the fortification of various food products, including pasta, flakes, cereals, bakery products, beverages, and dairy products with plant β-glucans as dietary fibre, has been actively studied (Bhaskar et al., 2017). Also, when giving additional functional properties to the products, it is important at least to preserve the initial physicochemical, rheological and sensory properties. Many studies have shown the possibility of using β-glucans from different sources as thickeners, texturizers and even fat replacers (Khorshidian et al., 2018; Raikos et al., 2018).

Hozova et al. (2004) established that the addition of pleuran (glucan isolated from *P. ostreatus*) hydrogel to yogurt did not inhibit the fermentation ability of yoghurt cultures and had no negative influence on the sensory acceptability (appearance, colour, consistency, taste) of the products. The pH of samples showed values typical

for this kind of product during a month-lasting storage. Authors suggested that the regular daily consumption of the innovative milk products with the application of an average dose of β-glucans, i.e. 5 mg per 150 mL yoghurt, would contribute to the reduction of the occurrence of relapsing or chronic infectious as well as autoimmune and oncological diseases.

P. ostreatus aqueous extract improved rheological properties and texture characteristics (lower firmness but higher cohesiveness, adhesiveness, springiness and less syneresis) of low-fat yogurt. Supplemented yogurts were darker, contained more polyphenols and exhibited higher antioxidant activity than controls in cold storage (Vital et al., 2015). In our previous study biomass of *P. ostreatus* treated with ethanol was introduced into low-fat yogurt. The addition of preparation had a positive effect on physical-chemical (titratable and active acidity) and rheological properties of the product (viscosity) during the storage period (Sorokin et al., 2016).

The aim of this research was to study the effect of the addition of various β-glucan containing preparations obtained from the submerged biomass of *P. ostreatus* on the process of milk fermentation in the production of yogurt and properties of the finished product.

Materials and Methods

β-glucan preparations from P. ostreatus

Choosing the type of added preparation and its quantity was based on the results of previous studies (Antontceva et al., 2018).

In the experiment, 3 different preparations obtained from the biomass of the culture *P. ostreatus (Jacq.: Fr.) P. Kumm* were used:

- preparation P1 is submerged biomass of *P. ostreatus*, treated twice with 80% ethanol during 3 hours at the temperature 80 °C to remove ethanol soluble compounds, such as lipids, triterpenes, and some low molecular weight compounds. The preparation contains 33.5% of water-soluble and water-insoluble β-glucans. The preparation P1 was introduced in a dosage of 0.1 and 0.25%;
- preparation P2 is a fraction of components released during water extraction during 3 hours at the temperature of 100 °C and insoluble in ethanol. The content of water-soluble β-glucans in the preparation is 23.8%. The preparation P2 was introduced in a dosage of 0.1% The effect of this preparation was studied only in lower concentration, as in contrary to other 2 crude preparations it is a purified preparation of water-soluble β-glucans;
- preparation P3 is a precipitate after successive ethanolic and water extraction containing a water-insoluble fraction: water-insoluble β-glucans, chitin or chitin-glucan complex and other water-insoluble high-molecular components. The content of β-glucans in the preparation is 41.2%. The preparation P3 was introduced in a dosage of 0.1 and 0.25%.

The detailed methods of obtaining the preparations has been described by Antontceva et al. (2018).

The content of β-glucans in preparations was determined using the β-glucan Assay Kit (Yeast & Mushroom) (Megazyme, USA) (McCleary, Draga, 2016).

Preparation of yogurt

Standardized milk (2.5% fat) and skimmed milk powder (1.5% fat) were mixed in such a proportion that obtained mixture had a mass fraction of dry substances of 9.45% and protein content of 3.27%. Fat content, mass fraction of dry substances and protein content in mixture and finished product was measured using milk analyser Lactoscan SA (Milkotronic Ltd., Bulgaria). For fermentation a lyophilized concentrated direct starter YO-MIX TM 305 LYO 250 DCU, which includes cultures *Streptococcus thermophilus*, *Lactobacillus delbuckii subsp. bulgaricus* (Danisco, Denmark) was used.

Preparations of β-glucans were introduced at the initial stage of preparing milk mixture in the amount of 0.1–0.25% of the total volume of the mixture (Table 1) at the temperature 30–35 °C with constant stirring. The mixture was left at rest for 30 minutes to swell, then stirred to a uniform consistency for 10–15 minutes, pasteurization was carried out in a water bath at 85 °C with 5 min holding time, then cooled to a temperature of 37 °C and then the starter was introduced. The obtained mixture was stirred for 10 minutes and fermented at 37±1 °C temperature for 6 hours until the clot titratable acidity was 100±2 °T. The control sample of yogurt was prepared without the addition of β-glucan preparations.

The titratable acidity was determined by titration of the samples with 1 N NaOH solution using phenolphthalein as the indicator. The pH value of samples was obtained by using an SG2-ELK SevenGo pH-meter (Mettler Toledo, Switzerland).

Table 1

Analysed yogurt samples

Yogurt samples	Type of preparation	Preparation concentration, %
Control	–	–
Experimental P1-0.1%	P1	0.10
Experimental P1-0.25%	P1	0.25
Experimental P2-0.1%	P2	0.10
Experimental P3-0.1%	P3	0.10
Experimental P3-0.25%	P3	0.25

Rheological studies were carried out to identify the effect of the added β-glucan containing preparations on the structure and consistency of yogurt using a rotational viscometer Rheotest-2 type RV 2 (VEB MLW PRUFGERATE-WERK, Germany). The mass of each test sample was 10 g, the measurement was carried out at a temperature of 20 °C. Samples were subjected to a uniform shear field with a constant gradient of the shear rate of 145.8 s^{-1} for 2 min, taking readings of the instrument every 15 seconds. According to the results of the measurements the coefficient of dynamic viscosity in the intact structure η_I (the first measurement), the destroyed structure η_D (after 2 min of mechanical impact) and the restored structure η_R (after 15 min of exposure) were determined.

Viscosity loss factor (VLF), mechanical stability factor (MSF) and structure recovery ratio (SRR) were calculated by the following equations 1–3, respectively:

$$VLF = \frac{(\eta_I - \eta_D)}{\eta_I} \times 100\% \quad (1)$$

$$MSF = \frac{\eta_I}{\eta_D} \quad (2)$$

$$SRR = \frac{\eta_R}{\eta_I} \times 100\% \quad (3)$$

where: VLF – viscosity loss factor,
MSF – mechanical stability factor,
SRR – structure recovery ratio,
η_I – coefficient of dynamic viscosity in the intact structure,
η_D – coefficient of dynamic viscosity of the destroyed structure,
η_R – coefficient of dynamic viscosity of the restored structure.

Samples were evaluated for such sensory properties as taste, odour, colour, consistency, and appearance by the blind taste test with participation of trained panel (8 panellists, 2 males and 6 females, aged 28–50 years), of the Faculty of Food Biotechnologies and Engineering of ITMO university. According to GOST R ISO (Russian State Standard) 22935-2-2011 "Milk and milk products. Sensory analysis. Part 2. Recommended methods for sensory evaluation" the sensory properties of the product must meet the following requirements: appearance, consistency – homogeneous, moderately viscous; taste and odour – clean, fermented milk without foreign tastes and odours; colour – milky white, uniform. The sensory analysis of the experimental samples was carried out in comparison with the control sample. Indicators were evaluated according to the degree of deviation from the sensory requirements on a five-point scale: 5 – no deviations, 4 – minimum deviations, 3 - noticeable deviations, 2 – significant deviations, 1 – very significant deviations.

All experiments and measurements were carried out at least in three replicates. Statistical processing of the data was performed using the software Statistica (version 10, Statsoft, USA). The level of confidence was chosen as 0.95. For the sensory characteristics average results of the sensory scoring evaluation were calculated.

Results and Discussion

The finished products had the following indicators: 2.5% fat content and 3.27% protein content.

The titratable (Table 2) and active (Table 3) acidity were measured during the fermentation process.

Table 2

Dynamics of changes in titratable acidity of different yogurt samples

Sample	Titratable acidity, °T			
	Duration, h			
	0	2	4	6
Control	18±1	27±2	82±2	103±2
P1-0.1%	18±2	30±2	82±2	103±1
P1-0.25%	18±1	28±1	82±1	103±2
P2-0.1%	21±1	28±2	79±1	105±2
P3-0.1%	21±2	28±3	82±2	100±2
P3-0.25%	21±2	27±2	75±2	102±1

According to the results of the experiment, the fermentation process of samples with the β-glucan preparations introduced is uniform and practically does not differ from the control ($p>0.05$). The required titratable acidity of the test samples (100 °T) was achieved in 6 hours.

Table 3

Dynamics of changes in active acidity of different yogurt samples

Sample	Active acidity (pH)			
	Duration, h			
	0	2	4	6
Control	6.58±0.01	6.09±0.01	4.65±0.01	4.08±0.01
P1-0.1%	6.58±0.02	6.02±0.02	4.66±0.01	4.18±0.02
P1-0.25%	6.58±0.02	6.03±0.01	4.57±0.02	4.18±0.01
P2-0.1%	6.56±0.02	6.07±0.01	4.65±0.03	4.22±0.01
P3-0.1%	6.57±0.01	6.13±0.01	4.70±0.01	4.20±0.02
P3-0.25%	6.52±0.01	6.14±0.02	4.72±0.01	4.31±0.01

Table 4

Structural-mechanical properties of yogurt samples

Sample	Viscosity loss factor, %	Mechanical stability factor	Structure recovery ratio, %
Control	25±1	1.4±0.1	71±1
P1-0.1%	29±2	1.5±0.1	69±2
P1-0.25%	11±1	1.1±0.1	79±2
P2-0.1%	19±18	1.2±0.1	77±1
P3-0.1%	26±1	1.4±0.1	75±1
P3-0.25%	26±2	1.5±0.2	70±3

Table 4 shows the values of the calculated viscosity loss factor, mechanical stability factor, and structure recovery ratio. These indicators characterize the stability of the structure of the product to mechanical stress and its ability to thixotropic recovery.

Analysing the data given in Table 4, it can be noted that the introduction of preparations P1 in the amount of 0.25% and P2 in the amount of 0.1% allows to obtain a product with structure more resistant to mechanical stress (mixing, pumping). Positive influence of addition of the preparations is evidenced by the smallest values of viscosity loss factor (11±1 and 19±1%, $p<0.05$) and

mechanical stability factor (1.1±0.1 and 1.2±0.1, $p<0.05$) with the most pronounced thixotropic properties (the structure recovery ratio was 79±2 and 77±1% ($p<0.05$) for the preparations P1 and P2, respectively). The samples with the addition of β-glucan-containing preparations P1 at a dosage of 0.25% and P2 at a dosage of 0.1% showed the highest structural and mechanical indicators. Therefore, we can conclude that the addition of the preparations P1 (0.25%) and P2 (0.1%) had a positive effect on the structure and consistency of the product ($p<0.05$), while the addition of the preparations P1 (0.1%) and P3 (0.1 and 0.25%) had no significant effect on the same parameters ($p>0.05$).

The results of the sensory evaluation of yogurt samples are shown in Table 5.

Table 5

Sensory evaluation of yogurt samples

Sample	Evaluation of the sample, points				
	Taste	Odour	Colour	Consis-tency	Appea-rance
Control	5.0	5.0	5.0	5.0	5.0
P1- 0.1%	4.9	5.0	3.9	4.5	4.8
P1- 0.25%	4.8	5.0	3.8	4.7	4.8
P2- 0.1%	5.0	5.0	4.7	4.9	5.0
P3- 0.1%	4.6	4.7	3.4	3.6	4.4
P3- 0.25%	4.5	4.7	3.2	3.5	4.1

According to the data obtained the addition of most preparations (except P2) negatively influenced the colour of the final products ($p>0.05$).

Addition of P2 in concentration of 0.1% resulted in a slight change in colour from white to light beige. The problem of coloration can be solved by masking it with fruit filling or food colouring.

When using water-insoluble β-glucans (P1 and P3) in high dosages, heterogeneity of consistency (especially for P3) was detected. Insoluble particles of the preparation were precipitating during the fermentation process. It is possible to eliminate this drawback by adding a thickener such as pectin, guar gum, agar-agar, both in pure form and in the mixtures.

Presence of water-soluble β-glucans in preparation P1, which, as it was shown in Tables 4 and 5 are improving rheological and sensory properties of yogurts, probably can explain better performance of P1 in comparison to preparation P3.

Our results correspond to the results of other authors. Results of using fungal β-glucans in fortification of yogurt showed that the pH value and titratable acidity were not affected by the addition of different levels of glucans (0.3%, 0.4% and 0.5%), when *Streptococcus salivarious subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* were used as yogurt cultures (Pappa et al., 2018). When incorporating powdered brewer's yeast β-glucans into yogurt at concentrations (0.2–0.8% wt/wt), the titratable acidity remained unaffected, textural properties showed a gradual increment of hardness, adhesive force, and cohesiveness with increasing β-glucan concentration (Raikos et al., 2018). In comparison with the non-fat yogurt without β-glucan (Mejri et al., 2014), the application of yeast β-glucan in yogurt improved the rheological properties and the physical stability of the product.

Conclusions

The results of the study show that the addition of β-glucan preparations obtained from the submerged biomass of the culture of the fungus *P. ostreatus (Jacq: Fr.) P. Kumm* did not have a significant effect ($p>0.05$) on the values of titratable acidity in the production of yogurt. The effect on the structural and mechanical parameters of the product depended on the form and dosage of the preparation. In this case, it is possible to assume improving the consistency of the product, since the structural and mechanical characteristics of the experimental samples exceeded the corresponding characteristics of the control sample. Samples of yogurt fortified with preparation P2 had the best sensory characteristics from the experimental samples, taking into account a complex of all tested indicators. Thus, from the point of view of obtaining a product with the highest consumer properties, preference should be given to preparation P2 in the amount of 0.1% (weight).

References

1. Antontceva E., Sorokin S., Shamtsyan M., Krasnikova L. (2018) Influence of *Pleurotus ostreatus* preparations on fermentation products of lactic acid cultures. *Journal of Hygienic Engineering and Design*, Vol. 22, p. 47–52.
2. Bashir K.M.I., Choi J.S. (2017) Clinical and physiological perspectives of β-glucans: The past, present, and future. *International Journal of Molecular Sciences*, Vol. 18(9), p. 2–48.
3. Bhaskar D., Khatkar S. K., Chawla R., Panwar H., Kapoor S. (2017) Effect of β-glucan fortification on physico-chemical, rheological, textural, colour and organoleptic characteristics of low fat dahi. *Journal of Food Science and Technology*, Vol. 54(9), p. 2684–2693.
4. Carrasco-González J.A., Serna-Saldívar S.O., Gutiérrez-Uribe J.A. (2017) Nutritional composition and nutraceutical properties of the *Pleurotus* fruiting bodies: Potencial use as food ingredient. *Journal of Food Composition and Analysis*, Vol. 58, p. 69–81.
5. Facchini J.M., Alves E.P., Aguilera C., Gern R.M.M., Silveira M.L.L., Wisbeck E., Furlan S.A. (2014) Antitumor activity of *Pleurotus ostreatus* polysaccharide fractions on Ehrlich tumor and Sarcoma 180. *International Journal of Biological Macromolecules*, Vol. 68, p. 72–77.
6. Giavasis I. (2014) Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Current Opinion in Biotechnology*, Vol. 26, p. 162–173.
7. Hozova B., Kuniak L., Kelemenova B. (2004) Application of beta-D-glucans isolated from mushrooms *Pleurotus ostreatus* (Pleuran) and *Lentinus edodes* (Lentinan) for increasing the bioactivity of yoghurts. *Czech Journal of Food Sciences-UZPI (Czech Republic)*, Vol. 22(6), p. 204–214.
8. Jesenak M., Majtan J., Rennerova Z., Kyselovic J., Banovcin P., Hrubisko M. (2013) Immunomodulatory effect of pleuran (β-glucan from *Pleurotus ostreatus*) in children with recurrent respiratory tract infections. *International Immunopharmacology*, Vol. 15(2), p. 395–399.

9. Khan A.A., Gani A., Masoodi F.A., Mushtaq U., Silotry Naik A. (2017) Structural, rheological, antioxidant, and functional properties of β–glucan extracted from edible mushrooms *Agaricus bisporus, Pleurotus ostreatus* and *Coprinus attrimentarius*. *Bioactive Carbohydrates and Dietary Fibre*, Vol. 11, p. 67–74.
10. Khan M.A., Tania M. (2012) Nutritional and medicinal importance of *Pleurotus* mushrooms: An overview. *Food Reviews International*, Vol. 28(3), p. 313–329.
11. Khorshidian N., Yousefi M., Shadnoush M., Mortazavian A.M. (2018) An overview of β-glucan functionality in dairy products. *Current Nutrition & Food Science*, Vol. 14(4), p. 280–292.
12. Lavelli V., Proserpio C., Gallotti F., Laureati M., Pagliarini E. (2018) Circular reuse of bio-resources: The role of: *Pleurotus spp.* in the development of functional foods. *Food and Function*, Vol. 9(3), p. 1353–1372.
13. Llauradó G., Morris H.J., Tamayo V., Lebeque Y., Beltrán Y., Marcos J., Bermúdez R.C. (2015) Haematopoiesis radioprotection in Balb/c mice by an aqueous mycelium extract from the Basidiomycete *Pleurotus ostreatus* mushroom. *Natural Product Research*, Vol. 29(16), p. 1557–1561.
14. McCleary B.V., Draga A. (2016) Measurement of β-glucan in mushrooms and mycelial products. *Journal of AOAC International*, Vol. 99(2), p. 364–373.
15. Mejri W., Bornaz S., Sahli A. (2014) Formulation of non-fat yogurt with β-glucan from sent brewer's yeast. *Journal of Hygienic Engineering and Design*, Vol. 8, p.163–173.
16. Novak M., Vetvicka V. (2008) β-glucans, history, and the present: Immunomodulatory aspects and mechanisms of action. *Journal of Immunotoxicology*, Vol. 5(1), p. 47–57.
17. Pappa E.C., Kondyli E., MacNaughtan W., Kakour A., Nesseris K., Israilides C. (2018) Quality and sensory properties of reduced fat yoghurt made with addition of β-glucans. *Food and Nutrition Sciences*, Vol. 9, p. 390–402.
18. Pasnik J., Ślemp A., Cywinska-Bernas A., Zeman K., Jesenak M. (2017) Preventive effect of pleuran (β-glucan from *Pleurotus ostreatus*) in children with recurrent respiratory tract infections - Open-label prospective study. *Current Pediatric Research*, Vol. 21(1), p. 99–104.
19. Radzki W., Ziaja-Sołtys M., Nowak J., Rzymowska J., Topolska J., Sławińska A., Michalak-Majewska M., Zalewska-Korona M., Kuczumow, A. (2016) Effect of processing on the content and biological activity of polysaccharides from *Pleurotus ostreatus* mushroom. *LWT-Food Science and Technology*, Vol. 66, p. 27–33.
20. Raikos V., Grant S. B., Hayes H., Ranawana V. (2018) Use of β-glucan from spent brewer's yeast as a thickener in skimmed yogurt: Physicochemical, textural, and structural properties related to sensory perception. *Journal of Dairy Science*, Vol. 101(7), p. 5821–5831.
21. Shamtsyan M., Antontceva E., Panchenko A., Petrischev N. (2014) Hyperlipidemic and hypocholesterolic action of submerge cultured mushrooms. *Journal of Hygienic Engineering and Design*, Vol. 7, p. 96–99.
22. Sorokin S., Tufeanu R., Shamtsyan M., Mironescu I. D., Antonceva E., Tița O. (2016) Physico-chemical and rheological properties of yogurt with added *Pleurotus ostreatus* biomass treated with ethanol. *Journal of EcoAgriTourism*, Vol. 12(2), p. 39–46.
23. Tong H., Xia F., Feng K., Sun G., Gao X., Sun L., Sun X. (2009) Structural characterization and in vitro antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus*. *Bioresource Technology*, Vol. 100(4), p. 1682–1686.
24. Vannucci L., Krizan J., Sima P., Stakheev D., Caja F., Rajsiglova L., Horak V., Saieh M. (2013) Immunostimulatory properties and antitumor activities of glucans (Review). *International Journal of Oncology*, Vol. 43(2), p. 357–364.
25. Vital A.C.P., Goto P.A., Hanai L.N., Gomes-da-Costa S.M., de Abreu Filho B.A., Nakamura C.V., Matumoto-Pintro P.T. (2015) Microbiological, functional and rheological properties of low fat yogurt supplemented with *Pleurotus ostreatus* aqueous extract. *LWT-Food Science and Technology*, Vol. 64(2), p. 1028–1035.

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COMPARISON OF FREE-RANGE, BARN AND CAGED HENS' EGGS COMMERCIALLY AVAILABLE IN LATVIA

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Abstract

Consumers often consider free-range eggs to be superior to eggs from caged hens. The aim of the study was to identify whether different hen housing systems had an influence on egg quality in Latvia. Free-range, barn and caged hens' eggs commercially available in Latvia were obtained at the production facility of Balticovo JSC. The average sample of forty eggs of each type were analysed at the laboratories of Faculty of Food Technology, Latvia University of Life Sciences and Technologies and J.S. Hamilton Baltic JSC. Such parameters as nutritional and energy value, fatty acid profile, cholesterol, vitamins A, E, D, B_1, B_2, B_3, B_6, B_7, B_9, B_{12}, minerals Na, K, Ca, Mg, Fe, Cu, Zn, Se, I, P, Cl, heavy metals Pb, Cd and microbiological quality were assessed. Comparison to reference intakes and possible indication of nutrient claims was evaluated according to published nutrient recommendations and EU regulations. The results show that depending on egg type it is possible to use such nutrient claims as "high protein", "high omega-3 fatty acids", "high monounsaturated fat", "source of vitamin A", "source of vitamin E", "high vitamin B2", 'high selenium", "source of phosphorus", "source of vitamin D" and "source of iodine". All egg samples were microbiologically safe and did not contain heavy metals. Nutrient content varied significantly within some of the parameters in eggs commercially available in Latvia ($p<0.05$); however, whether consumers choose free-range, barn and caged hens' eggs has an insignificant effect on their diet, based on daily egg consumption per capita.

Keywords: egg quality, nutritional value, population reference intakes

Introduction

Eggs are an excellent source of various macro- and micronutrients – easily digestible protein, including all essential amino acids, unsaturated fat, lecithin, fat-soluble and water-soluble vitamins, and minerals (Miranda et al., 2015; Kralik, Kralik, 2017). There are several factors which influence nutritional quality of hen eggs such as breed and age of hen, feed composition and nutrient density, in addition to the rearing system (Küçükyılmaz et al., 2012).

Depending on the hen housing system, in the EU eggs are coded using numbers 0–3 (Commission Regulation (EC) No 589/2008): 0 – organic eggs, 1 – free-range eggs, 2 – barn eggs, 3 – eggs from caged hens.

Scientific literature notes that there can be noticeable differences in some qualitative parameters between eggs from conventional and organic rearing systems (Küçükyılmaz et al., 2012). However, further research is needed in controlled settings, as egg quality is more directly associated with hen nutrition than hen housing within the conventional system (Karcher et al., 2015).

Several scientists (Karsten et al., 2010; Anderson, 2011) point out that free-range eggs contain more vitamin E and long chain ω3 fatty acids, have a significantly lower ω6 : ω3 ratio, whereas eggs from caged hens have a lower fat content and less mono- and polyunsaturated fats than free-range eggs and barn eggs. Recent data has shown a significant effect of hen diet with regards to ω3 fatty acid content in eggs (Coorey et al., 2015).

A research from 2012 pointed out that a significant part of consumers believe that organic and free-range eggs are more nutritious and tastier than conventional eggs (Küçükyılmaz et al., 2012), yet there is no consistent evidence in scientific literature. Whereas, data from 2008 indicated that consumers are mainly interested in egg freshness and price, showing little interest in layer rearing system (Sokołowicz et al., 2008).

As literature data demonstrates variable results on egg qualitative parameters and such research has not been carried out before, the aim of the study was to identify whether different hen housing systems had an influence on egg quality in Latvia.

Materials and Methods

Materials

Free-range, barn and caged hens' eggs commercially available in Latvia (Table 1) were obtained at the production facility of Balticovo JSC during the beginning of September 2018. Forty eggs of each type were analysed at the laboratories of Faculty of Food Technology, Latvia University of Life Sciences and Technologies and J.S. Hamilton Baltic JSC.

Table 1

Eggs used for the research

Sample code	Farming method (Commission Regulation (EC) No 589/2008)	Type of eggs on the market
O_11	Free range eggs	FREE RANGE eggs
O_22	Barn eggs	Farmer – Barn eggs
O_33	Eggs from caged hens	Eggs in boxes

Nutritional composition and qualitative parameters

Nutritional and energy value of eggs was determined according to EU Regulation No 1169/2011. Standard methods were used to assess such nutritional parameters as moisture (PN-A-86509:1994), protein (PB-116 ed. II of 30.06.2014), total fat (PN-A-86509:1994), fatty acids (PN-EN ISO 12966-1:2015, PN-EN ISO 12966-2:2011), ash (PN-A-79011-8:1988), sugar (PB-287 ed. Of 27.09.2014) and cholesterol (PB-75/GC ed. I of 20.01.2009); carbohydrates were calculated by difference (FAO, 2003).

The content of Na, K, Ca, Mg, P, Fe, Cu, Zn, Se was determined using flame atomic absorption spectrometry according to PB-223/ICP, ed. II of 12.01.2015. Chloride was assessed using potentiometry (PN-EN ISO 5943:2007), whereas iodine content was analysed according to DIN EN 15111:2007-06. Heavy metals were assayed according to PB-68/ICP ed. III of 18.09.2012.

The following methods were used to determine fat-soluble and B-group vitamins in eggs: PB-40/HPLC ed. III of 28.02.2009 for A and E vitamin, PN-EN 12921:2009 for cholecalciferol, PN-EN 14122:2014-07 for thiamine, PN-EN-14152:2014-07 for riboflavin, EN 12652:2009 for niacin, PN-EN 14164:2014-08 for pyridoxine, PB-244 ed. I 10.10.2013 for biotin, PB-372 ed. I 30.11.2015 for folic acid, and PB-328 ed. I 30.11.2015 for cobalamin.

In order to evaluate microbiological quality of eggs from different hen laying systems such parameters as the count of mesophilic aerobic and facultative anaerobic microorganisms (ISO 4833-1:2013) and *Enterobacteriaceae* (ISO 21528-2:2017) were assessed.

Comparison to reference intakes of vitamins and minerals, and eligibility to nutrient claims

Eligibility to nutrient claims (Table 2) was evaluated according to Regulation (EC) No 1924/2006 with regards to protein, vitamins and minerals, and Regulation (EC) No 116/2010 with regards to fatty acid content and profile.

Table 2

Nutrient claim guidelines for eggs (Regulation (EC) No 1924/2006, No 116/2010, No 1169/2011)

Nutrient claim	Explanation
high protein	at least 20% of the energy value of the food is provided by protein
source of [name of vitamin/s] and/or [name of mineral/s]	at least 15% of the nutrient reference values supplied by 100 g
high [name of vitamin/s] and/or [name of mineral/s]	at least twice the value of 'source of [name of vitamin/s] and/or [name of mineral/s]'
source of omega-3 fatty acids	at least 0.3 g ALA, or at least 40 mg of the sum of EPA and DHA per 100 g and per 100 kcal
high omega-3 fatty acids	at least 0.6 g ALA, or at least 80 mg of the sum of EPA and DHA per 100 g and per 100 kcal
high monounsaturated fat	at least 45% of the fatty acids present in the product derived from MUFA and MUFA provides more than 20% of energy of the product

ALA – α-linolenic acid, EPA – eicosapentaenoic acid, DHA – docosahexaenoic acid, MUFA – monounsaturated fatty acids

Comparison of the amount of micronutrients to population reference intakes of vitamins and minerals (incl. coverage (%) calculation) was carried out based on EU Regulation No 1169/2011 (Annex XIII) and the ordinance No 212 "Recommended allowance of energy and nutrients for Latvian citizens", issued by Ministry of Health of the Republic of Latvia on November 24, 2017 (Table 3). The second document was developed on the basis of the Nordic Nutritional Recommendations (Nordic Council of Ministers, 2012).

Table 3

Vitamin and mineral daily requirements

Vitamin or mineral	Population reference intake	
	EU Regulation No 1169/2011	Ordinance No 212
A, µg	800.0	900 ♂, 700 ♀
E, mg	12.0	10 ♂, 8 ♀
D, µg	5.0	10
B_1, mg	1.1	1.4 ♂, 1.1 ♀
B_2, mg	1.4	1.5
B_3, mg	16	n.d.
B_6, µg	1.4	1.5 ♂, 1.3 ♀
B_{12}, µg	2.5	2.0
B_9, µg	200.0	300 ♂, 400 ♀
B_7, µg	50.0	n.d.
K, mg	2000.0	3500 ♂, 3100 ♀
Ca, mg	800.0	800
Mg, mg	375.0	350 ♂, 280 ♀
P, mg	700.0	600
Fe, mg	14.0	9 ♂, 15 ♀
Cu, µg	1000.0	900
Zn, mg	10.0	9 ♂, 7 ♀
Se, µg	55.0	60 ♂, 50 ♀
I, µg	150.0	200

n.d. – no data, ♂ – male, ♀ – female

Data processing

The obtained data was processed with MS Excel v16 software; differences among results were considered significant at $p<0.05$. One-way ANOVA and Tukey's test were used to analyse the differences within data.

Results and Discussion

Nutritional composition of eggs from different hen laying systems

The results of nutritional value parameters of eggs (Table 4) from different hen housing systems show that eggs from caged hens (sample O_33) contain 9% less protein than free-range or barn eggs ($p>0.05$). Free-range chicken eggs have 10% less fat ($p>0.05$), but both free-range and barn eggs contain more cholesterol than caged eggs, respectively 32% and 64% more. Eggs from caged hens contain significantly less cholesterol compared to barn eggs ($p<0.05$).

The latest literature data shows that dietary cholesterol has minimal effect on blood cholesterol levels in practically healthy individuals (Benito-Vicente et al., 2018), thus, content of cholesterol in eggs should not be considered problematic.

The composition of fat was similar within all groups; free-range eggs (sample O_11) showed insignificantly

lower saturated and monounsaturated fatty acid content ($p>0.05$). With regards to long chain ω3 fatty acids, especially EPA and DHA, there were no differences among egg samples.

Table 4

Nutritional parameters and energy value of eggs from different hen housing systems per 100 g

Parameters	Egg samples		
	O_11	O_22	O_33
Moisture, %	77.5	76.3	77.3
Protein, g	12.3	12.4	11.3
Fat, g	8.73	9.66	9.62
− saturated (SAFA), g	2.4	2.7	2.7
− monounsaturated (MUFA), g	4.5	5.1	5.2
− polyunsaturated (PUFA), g	1.7	1.8	1.6
− ω3, g	0.3	0.3	0.3
− α-linolenic acid, mg	200	200	200
− eicosapentaenoic acid, mg	<100	<100	<100
− docosahexaenoic acid, mg	100	100	100
− ω6, g	1.3	1.5	1.3
− ω6 : ω3 ratio	4.3:1	5:1	4.3:1
− ω9, g	4.1	4.6	4.7
Trans fatty acids, g	<0.1	<0.1	<0.1
Cholesterol content, mg	290^{ab*}	360^{a}	220^{b}
Ash, g	0.84	0.86	0.88
Carbohydrates, g	0.6^{a}	0.8^{ab}	0.9^{b}
− sugars, g	<0.2	<0.2	<0.2
Energy value, kJ	541	583	563

* values within the same row sharing the same letter are not significantly different ($p>0.05$)

The ratio of ω6 : ω3 in food products has become fairly important when discussing the prevention of chronic diseases in humans. While previous research suggests that ω6 : ω3 ratio of 4 : 1 is optimum for healthy adults, latest data indicates ratio of 2 : 1 to 1 : 1 as the target for human nutrition (Bhardwaj et al., 2016). This ratio falls just above the previous recommendations in free-range, barn and caged hens' eggs (4.3 : 1 to 5 : 1). Taking into account that most Western dietary patterns provide the ratio of 15 : 1 up to 25 : 1 (Bhardwaj et al., 2016), this is nonetheless a significant decrease and eggs should be included in a healthy diet as a source of essential fats.

The ω6 : ω3 ratio in eggs from caged hens is significantly lower compared to other studies, where it varied from 11.03 : 1 to 66.29 : 1 (Petrović et al., 2012; Lešić et al., 2017).

Insignificant differences were observed for the rest of the tested nutritional parameters (Table 4). Miranda et al. (2015) have reported similar nutritional parameters previously.

Vitamin content in eggs showed differences among samples (Table 5). Free-range eggs contained significantly lower amounts of vitamin A and E, yet more vitamin D and folic acid ($p<0.05$) than other samples. Lowest thiamine content was found in barn eggs ($p<0.05$) and eggs from caged hens had the least amount of folic acid ($p<0.05$).

Table 5

Vitamin content in eggs from different hen housing systems per 100 g

Vitamins	Egg samples		
	O_11	O_22	O_33
A, µg	140.00^{a*}	163.00^{b}	160.00^{b}
E, mg	2.600^{a}	3.80^{b}	3.30^{b}
D, µg	0.77^{a}	0.48^{b}	0.46^{b}
B_1, mg	0.081^{a}	0.064^{b}	0.090^{a}
B_2, mg	0.51	0.44	0.44
B_3, mg	0.20	0.20	0.10
B_6, µg	0.10	0.08	0.10
B_{12}, µg	0.067^{a}	0.087^{b}	0.075^{ab}
B_9, µg	15.40^{a}	12.30^{b}	10.40^{c}
B_7, µg	4.62^{a}	3.88^{b}	3.58^{b}

* values within the same row sharing the same letter are not significantly different ($p>0.05$)

A study on vitamin content in egg yolk oil extracted from eggs obtained from different laying hen housing systems in Latvia (Kovalcuks, 2015) showed that free-range egg yolk oil contained significantly lower amounts of vitamin E ($p<0.05$) compared to caged hens' egg yolk oil, which is in agreement with the current results. Content of vitamin A was not affected by the housing system, yet especially low content on vitamin E was found in eggs of free-range hens with free diet (no supplementation).

The findings of Anderson (2013) showed differences in eggs from caged hens compared to the results of this study – higher level of cholesterol (330 mg), which is closer to barn eggs in this research, significantly less vitamin A (106.8 µg) compared to all tested samples, more vitamin E (4.8 mg), while content and composition of fatty acids was similar.

It has been reported that that free-range eggs contain more vitamin E and long chain ω3 fatty acids (Karsten et al., 2010), whereas eggs from caged hens have a lower fat content and less mono- and polyunsaturated fats than free-range eggs and barn eggs (Anderson, 2011). These data do not coincide with the results of eggs in Latvia; however, this could be explained by seasonal differences or genetics of hens (Küçükyılmaz et al., 2012). In addition, Karsten et al. (2010) reported that pastured free-range hens should be supplemented by commercial feed to meet optimal energy and protein needs.

Free-range eggs contained significantly more iodine than other egg samples ($p<0.05$) (Table 6). The highest iron content was observed in barn eggs ($p<0.05$), whereas the lowest copper, selenium and zinc levels were detected in eggs from caged hens ($p<0.05$).

Research from Thailand showed that iodine content in regular eggs was 75.96 µg per 100 gram and in eggs enriched with iodine – around 184 µg per 100 grams (Charoensiriwatana et al., 2014), while enriched eggs from Poland could accumulate up to 203 µg iodine per 100 grams (Lipiec et al., 2012).

Table 6

Content of minerals and heavy metals in eggs from different hen housing systems

Parameters	Egg samples		
	O_11	O_22	O_33
Na, mg 100 g^{-1}	170.00	160.00	160.00
K, mg 100 g^{-1}	134.00	127.00	137.00
Ca, mg 100 g^{-1}	45.20^{a*}	53.40^{b}	42.00^{a}
Mg, mg 100 g^{-1}	12.80^{a}	12.60^{a}	10.40^{b}
P, mg 100 g^{-1}	177.00	183.00	178.00
Fe, mg 100 g^{-1}	1.52^{a}	1.73^{b}	1.45^{a}
Cu, µg 100 g^{-1}	50.70^{a}	54.00^{a}	40.10^{b}
Zn, mg 100 g^{-1}	1.00^{ab}	1.10^{a}	0.84^{b}
Cl^{-}, %	0.30	0.30	0.30
Se, µg 100 g^{-1}	34.40^{a}	32.2^{ab}	30.30^{b}
I, mg kg^{-1}	1.26^{a}	<0.50^{b}	<0.50b
Pb, mg kg^{-1}	<0.010	<0.010	<0.010
Cd, mg kg^{-1}	<0.0010	<0.0010	<0.0010

* values within the same row sharing the same letter are not significantly different (p>0.05)

Attia et al. (2017) analysed mineral content in eggs from caged hens; the comparison shows that eggs in Latvia have a higher content of magnesium, iron, lower content of sodium and phosphorus and equal content of zinc, potassium and copper. Whereas the content of calcium, iron and zinc was higher in hen eggs from Kenya (Chepkemoi et al., 2017).

Kiczorowska et al. (2015) reported that free-range system hens produced eggs richest in micronutrients (K, Na, Ca, Mg, Zn, Se, Mn and Fe), as it allows hens to supplement their dietary ration. It is in partial agreement with our results.

Content of lead and cadmium in all egg samples was below the detection level, therefore, it can be concluded that all egg samples are free of heavy metals. A research of Egyptian scientists (Abdel-Hameid et al., 2017) established equally noticeable contamination with Pb and Cd in both free-range and caged hens' eggs.

In addition, the count of mesophilic aerobic and facultative anaerobic microorganisms and *Enterobacteriaceae* was below 10 colony forming units per gram, leading to conclusion that all egg samples are safe from microbiological point of view.

Eligibility of free-range, barn and caged hens' eggs to nutrient claims

Comparison of the amount of various nutrients to population reference intakes of vitamins and minerals (Table 3) allowed to evaluate the adherence of egg samples to nutrient claims (Table 2). Possible nutrient claims were quite similar for egg samples, yet there were several differences within various parameters (Table 7). All egg samples can be labelled as 'high protein', 'high omega-3 fatty acids' and 'high monounsaturated fat'. Free-range, barn and caged hens' eggs are eligible for such nutrient claims – "source of vitamin A", "source of phosphorus", "high vitamin B_2" and "high selenium" content. While free-range and caged hens' eggs are a "source of vitamin E", packaging of barn eggs can be labelled "high vitamin E".

Table 7

Compliance with requirements for nutrient content claims (EC No 1169/2011)

Macro or micro nutrient	Egg samples		
	O_11	O_22	O_33
Protein*	38%	35%	33%
MUFA$^{\times}$	41%	46%	47%
MUFA*	31%	33%	35%
ω3 (EPA + DHA)	>100 mg	>100 mg	>100 mg
Vitamin A$^{\infty}$	18%	20%	20%
Vitamin E$^{\infty}$	22%	32%	28%
Vitamin D$^{\infty}$	15%	10%	9%
Vitamin B_1^{∞}	7%	6%	8%
Vitamin B_2^{∞}	36%	31%	31%
Vitamin B_3^{∞}	1%	1%	1%
Vitamin B_6^{∞}	7%	6%	7%
Vitamin B_{12}^{∞}	3%	3%	3%
Vitamin B_9^{∞}	8%	6%	5%
Vitamin B_7^{∞}	9%	8%	7%
Potassium$^{\infty}$	7%	6%	7%
Calcium$^{\infty}$	6%	7%	5%
Magnesium$^{\infty}$	3%	3%	3%
Phosphorus$^{\infty}$	25%	26%	25%
Iron$^{\infty}$	11%	12%	10%
Copper$^{\infty}$	5%	5%	4%
Zinc$^{\infty}$	10%	11%	8%
Selenium$^{\infty}$	63%	59%	55%
Iodine$^{\infty}$	84%	>30%	>30%

* as % of energy

$^{\times}$ as % of fatty acids

$^{\infty}$ as % of nutrient reference (Table 3) supplied by 100 g

Free-range eggs are also eligible for "source of vitamin D" and "high iodine". Barn and caged hens' eggs are a 'source of iodine'.

Coverage of vitamins and minerals in comparison to reference intakes in Latvia

According to the data of the Latvian Central Statistical Bureau (CSB), the daily consumption in Latvia was 207 eggs per capita in 2016; people consume ca. ½ of egg daily on average. This does not, however, include bottled egg whites, egg powder, eggs added to pancakes, cake dough, salad with mayonnaise etc. (CSB, 2016).

Tested eggs cover various amounts of vitamins for Latvian consumers (Figure 1). Coverage of vitamin D, thiamine (B_1), pyridoxine (B_6), cyanocobalamin (B_{12}) and folic acid (B_9) for both males and females of an average egg (53 g) falls below 5% daily, therefore, have an insignificant effect on diet. Coverage of vitamin A accounts up to 12% depending on gender. The highest coverage can be observed for vitamin E (17–25% for females, 14–20% for males) and riboflavin (16–18%). The differences observed in vitamin content of eggs (Table 5) does not *a priori* indicate that it will have a

significant effect on the daily diet, based on vitamin coverage calculation (Figure 1).

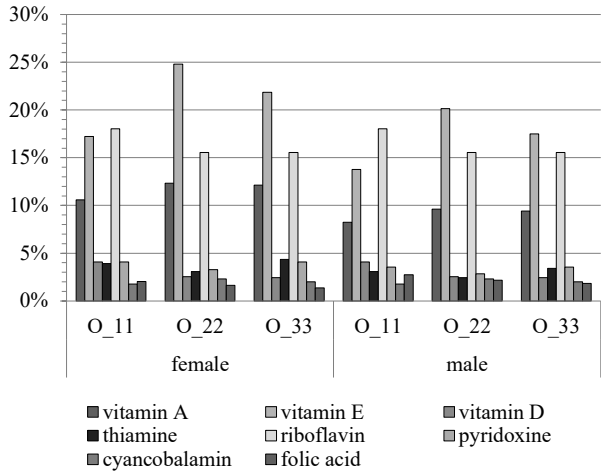

Figure 1. Coverage of vitamins (%) of an average egg (53 g) in comparison to reference intakes

O_11 – free-range eggs, O_22 – barn eggs, O_33 – eggs from caged hens

With regards to coverage of minerals (Figure 2), one average egg can provide below 5% of potassium, calcium, magnesium and cooper daily needs, whereas iron and zinc coverage is below 10%.

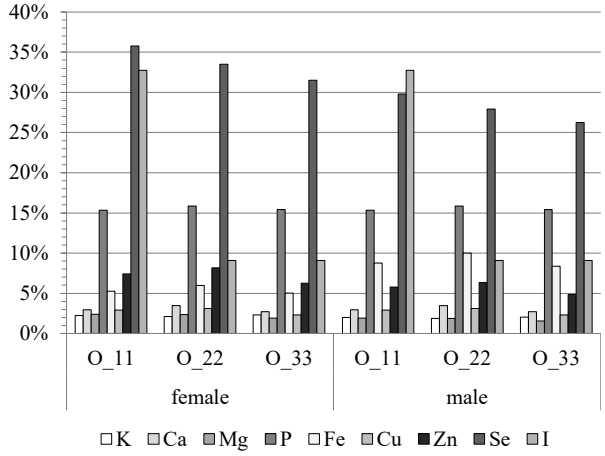

Figure 2. Coverage of minerals (%) of an average egg (53 g) in comparison to reference intakes

O_11 – free-range eggs, O_22 – barn eggs, O_33 – eggs from caged hens

Coverage of selenium ranges from 26 to 30% for males and 32 to 36% for females, which also does not indicate significant coverage differences.

Iodine is the only micronutrient that shows significant preference to consuming free-range eggs, as an average egg covers 33% of daily needs for Latvian adults. According to Konrāde et al. (2017), Latvian consumers, especially women, have insufficient iodine intake which could lead to brain development issues of foetus. It is suggested to increase daily iodine intake from seafood and milk products for women of childbearing age, and use iodine supplements during pregnancy.

Free-range eggs are 'high iodine', therefore, these eggs are also an excellent option to increase iodine content in daily diet.

Conclusions

The results indicate that different hen housing systems have an influence on egg nutritional composition in Latvia ($p<0.05$). Eggs are an excellent source of nutrients as shown by such nutrient claims – "high protein", "high omega-3 fatty acids", "high monounsaturated fat", "source of vitamin A", "source of vitamin E"/"high vitamin E", "high vitamin B2", "high selenium", "source of phosphorus", "source of vitamin D" and "source of iodine"/"high iodine". However, whether consumers choose free-range, barn and caged hens' eggs has an insignificant effect on their diet, based on daily egg consumption per capita.

These results also show the need for qualitative analyses of food produced in Latvia, as climatic conditions, soil and other factors indicate significant differences compared to other countries. In addition, further research is needed, in order to exclude seasonal effect on egg nutritional parameters.

References

1. Ahmed Abdel-Hameid A., Enas El-Prince M., Doha Y., Al-Shimaa M.F. (2017) Lead, cadmium and copper levels in table eggs. *Journal of Advanced Veterinary Research*, Vol. 7(3), p. 66–70.
2. Anderson K. E. (2011) Comparison of fatty acid, cholesterol, and vitamin A and E composition in eggs from hens housed in conventional cage and range production facilities. *Poultry Science*, Vol. 90(7), p. 1600–1608.
3. Anderson K. E. (2013) Comparison of fatty acid, cholesterol, vitamin A and E composition, and *trans* fats in eggs from brown and white egg strains that were molted or nonmolted. *Poultry Science*, Vol. 92(12), p. 3259–3265.
4. Attia Y.A., Mohammed A. Al-Harthi M.A., Shiboob M.M. (2014) Evaluation of Quality and Nutrient Contents of Table Eggs from Different Sources in the Retail Market. *Italian Journal of Animal Science*, Vol. 13(2), p. 369–376.
5. Benito-Vicente A., Uribe K.B., Jebari S., Galicia-Garcia U., Ostolaza H., Martin C. (2018) Familial hypercholesterolemia: The most frequent cholesterol metabolism disorder caused disease. *International Journal of Molecular Sciences*, Vol. 19(11), p. 3426.
6. Bhardwaj K., Verma N., Trivedi R.K., Bhardwaj S., Shukla N. (2016) Significance of ratio of omega-3 and omega-6 in human health with special reference to flaxseed oil. *International Journal of Biological Chemistry*, Vol. 10, p. 1–6.
7. Charoensiriwatana W., Srijantr P., Teeyapant P., Wongvilairattana J. (2014) Consuming iodine enriched eggs to solve the iodine deficiency endemic for remote areas in Thailand. **In:** *Functional Foods. The Connection Between Nutrition, Health, and Food Science*. L. Coles (ed.). Alberta: Apple Academic Press, p.279–288.

8. Chepkemoi M., Macharia J.W., Sila D., Oyier P., Malaki P., Ndiema E., Agwanda B., Obanda V., Ngeiywa K.J., Lichoti J., Ommeh S.C. (2017) Physical characteristics and nutritional composition of meat and eggs of five poultry species in Kenya. *Livestock Research for Rural Development,* Vol. 29(8), p. 1–11.
9. Commission Regulation (EC) No 589/2008 of 23 June 2008 laying down detailed rules for implementing Council Regulation (EC) No 1234/2007 as regards marketing standards for eggs (OJ L 163, 24.6.2008, p. 6–23.
10. Commission Regulation (EU) No 116/2010 of 9 February 2010 amending Regulation (EC) No 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims (OJ L 37/16, 10.2.2010, p.16–18)
11. Coorey R., Novinda A., Williams H., Jayasena V. (2015) Omega-3 fatty acid profile of eggs from laying hens fed diets supplemented with chia, fish oil, and flaxseed. *Journal of Food Science,* Vol. 80(1), p. 180–187.
12. CSB Statistics Database (2013) *Consumption of food products average per household member per year*. [accessed on 10.01.2019.]. Available at: http://data.csb.gov.lv/.
13. Food and Agriculture Organization of the United Nations (FAO) (2003) FAO Food and Nutrition Paper No 77. Food energy – methods of analysis and conversion factors: Report of a technical workshop. Food and agriculture organization of the United Nations, Rome, p. 93.
14. Karcher D. M., Jones D. R., Abdo Z., Zhao Y., Shepherd T. A., Xin H. (2015) Impact of commercial housing systems and nutrient and energy intake on laying hen performance and egg quality parameters. *Poultry Science*, Vol. 94(3), p. 485–501.
15. Karsten H.D., Patterson P.H., Stout R., Crews G. (2010) Vitamins A, E and fatty acid composition of the eggs of caged hens and pastured hens. *Renewable Agriculture and Food Systems*, Vol. 25(1), p. 45–54.
16. Kiczorowska B., Smolinsky W., Kwiecień M., Winiarska-Mieczan A., Rusinek – Prystupa E., Ridha A., Al-Yasiry M. (2015) Nutritional value and the content of minerals in eggs produced in large-scale, courtyard and organic systems. *Journal of Elementology,* Vol. 20(4), p. 887–895.
17. Konrāde I., Kalere I., Strēle I., Makrecka-Kūka M., Veisa V., Gavars D., Rezeberga D., Pīrāgs D., Lejnieks A., Gruntmanis U., Neimane L., Liepiņš E., Dambrova M. (2017) Iodine deficiency in Latvia: current status and need for national recommendations. *Proceedings of the Latvian Academy of Sciences. Section B*, Vol. 71(6), p. 401–407.
18. Kovalcuks A. (2015) Comparison of bioactive compound content in egg yolk oil extracted from eggs obtained from different laying hen housing systems. *International Journal of Biological, Food, Veterinary and Agricultural Engineering*, Vol. 9(6), p. 589–593.
19. Kralik G., Kralik Z. (2017) Poultry products enriched with nutricines have beneficial effects on human health. *Medicinski Glasnik*, Vol. 14 (1), p. 1–7.
20. Küçükyılmaz K., Bozkurt, M., Herken, E.N., Çınar M., Çatl A.U., Bintaş E., Çöven F. (2012) Effects of rearing systems on performance, egg characteristics and immune response in two layer hen genotype. *Asian-Australasian Journal of Animal Sciences*, Vol. 25(4), p. 559–568.
21. Lešić T., Krešić G., Cvetnić L., Petrović M., Pleadin J. (2017) The influence of hen age on fatty acid composition of commercial eggs. *Croatian Journal of Food Science and Technology*, Vol. 9(2), p. 158–167.
22. Lipiec, E., Warowicka, O., Ruzik, L., Zhou Y., Jarosz M., Połeć-Pawlak K. (2012) Investigation of iodine bioavailability from chicken eggs versus iodized kitchen salt with in vitro method. *European Food Research and Technology*, Vol. 234(5), p. 913–919.
23. Miranda J.M, Anton X., Redondo-Valbuena C., Roca-Saavedra P., Rodriguez J.A., Lamas A., Franco C.M., Cepeda A. (2015) Egg and Egg-Derived Foods: Effects on Human Health and Use as Functional Foods. *Nutrients*, Vol. 7(1), p. 706–729.
24. *Nordic Nutrition Recommendations 2012. Integrating nutrition and physical activity*. Nordic Council of Ministers, 2014. [accessed on 01.02.2019.]. Available at: https://norden.diva-portal.org/smash/get/diva2:704251/FULLTEXT01.pdf
25. Petrović M., Gačić M., Karačić V., Gottstein Ž., Mazija H., Medić H. (2012) Enrichment of eggs in n-3 polyunsaturated fatty acids by feeding hens with different amount of linseed oil in diet. *Food Chemistry*, Vol. 135, p. 1563–1568.
26. *Recommended allowance of energy and nutrition for Latvian citizens, ordinance No 212*. Ministry of Health of the Republic of Latvia, November 24, 2017. [accessed on 01.02.2019.]. Available at: http://www.vm.gov.lv/images/userfiles/Tava%20veseliba/Ieteicam%C4%81s_ener%C4%A3ijas_un_uzturvielu_devas.pdf
27. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods (OJ L 404, 30.12.2006, p. 9)
28. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 (OJ L 304, 22.11.2011, p. 18–63)
29. Sokołowicz Z., Krawczyk J., Dykiel M. (2008) Hen housing system and egg quality as viewed by consumers. *Annals of Animal Science*, Vol. 8(1), p. 71–80.

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CHANGES OF PHYSICAL PARAMETERS OF MEAT DURING WET AGEING

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Abstract

Fresh meat quality can be improved by maturation. Maturation is ageing of fresh meat under controlled temperature conditions during a certain period of time thereby improving its sensorial and textural parameters. Ageing is a natural process and there are two maturation methods: wet and dry. Dry ageing produces a more flavourful product but wet ageing is characterized by a higher outcome of the products and a lower risk of microbial spoilage during maturation. The aim of this research was to investigate the changes of quality parameters of pork, beef and lamb meat during wet ageing. In the current research, the longest lumbar muscle (*Musculus longissimus lumborum*) of pork, beef and lamb was used. Meat was aged at the following conditions: vacuum packed in polyethylene/ polyamide pouches and stored for 35 days at 2±1 °C. During wet ageing (on the 0, 7th, 14th, 21th, 28th, and 35th day) the following quality parameters were analysed: moisture content (LVS ISO 1442:1997), water activity (Novasina LabSwift-aw, Sweden), pH value (LVS ISO 2917:2004) and tenderness using TA.HD.Plus Texture Analyser (Stable Microsystems, UK). The obtained results indicated a pH value decrease in all meat samples during ageing ($p<0.05$). Observed results showed a very strong positive correlation between moisture content and water activity. The changes of water activity were not significant ($p>0.05$), it increased in pork and lamb meat, but decreased in beef meat. The apposite results were obtained regarding the hardness of meat samples, namely, pork hardness decreased during ageing, but increased for beef and lamb meat samples. Based on the results, wet ageing process is more suitable for pork meat.

Keywords: meat maturation, wet ageing, pork, lamb, beef

Introduction

The consumers of meat and practice in meat industry have identify the importance of developing technologies improve the appearance, nutrition, to extend shelf-life and animal products safety (Yang et al., 2016). Humans carry on with to scan for ways to effectively eliminate, control, or decelerate the process of ageing. The meat ageing process is shown positively, and the specialists of storing meat for increasing periods of time after the slauthering of an animal, in order to improve its tenderness, has been a practice followed by many for a long time (Toldra, 2010). Ageing is the natural process of making meat a supply is difficult. The development of tenderness depends on the skeletal muscle cell architecture and integrity as well as on events that transform these proteins and their interplay. Namely, degradation and oxidation of proteins have been identify as processes that change protein as well as meat softness (Lonergan, et al., 2010). For improving the sensory properties of meat the process of ageing is a one of the popular method used by meat industry. Ageing is still widely used commercially successful as a *post-mortem* interventional for softenig of meat. Ageing to make better the meat tenderness through disruption by intracellular proteolytic systems of the muscle structure (Bhat et al., 2018).

After slaughtering animal muscle undergoes molecular changes, what can be subdivided in three stages – first stage – *pre-rigor*, second stage – *rigor-mortis* and third or tenderization stage – *post-rigor*.

The latter mostly depends on ageing time and temperature, type of muscles, individual genotype and species of animal, but the common focal point is the activization of proteolytic enzymes driving the destruction of muscle fibres (Longo et al., 2015)

Meat muscles undergo *rigor-mortis* stage during the conversion of muscle to meat and increase in meat tenderness that is a results of this process (Lee et al. (2016). However, the Chen et al. (2015) and Cruzen et al. (2014) in scientific publications were concluded that the *rigor-mortis* can be resolved by cytoskeletal and myofibrillar proteins degradation in meat by proteinases such as cathepsin, caspase, calpain and proteasome while ageing, which the meat tenderness can be improved. Toldra (2010) in some scientific papers was mentioned that the “ageing” of *post-mortem* has been called “ripening” or “conditioning” and is a natural process. In this time of process meat is expose to controlled storage conditions in refrigerate. While any species from meat could be aged, *post-mortem* ageing is usually finite to beef and at the slaughter time the relative youth pork, lamb and veal. For a long time, meat ageing technologies such as dry ageing and wet ageing have been used to improve meat quality (Kim et al., 2017). Fresh meat ageing process is very inportant to meet the big requirements and hopefulness of an exceptional diet experience. Agening of fresh meat to increase the palatability of the products, to enlarge the tenderness over time and to evolve the aroma, taste, smell. The common ageing methods are dry and wet ageing. Dry ageing is a process wherewith carcasses, primal, and / or sub primal of animal are stored without preventative packaging for one to more weeks in refrigeration temperatures. But during wet ageing meat is packed in a sealed barrier film and stored at a temperature over the meat freezing point (Velotto et al. 2015).

Therefore, the aim of research was to investigate the changes of quality parameters of pork, beef and lamb meat during wet ageing.

Materials and Methods

Raw materials

In the researches was used the longest lumbar muscle (*Musculus longissimus lumborum*) from three different animal meat – pork, beef and lamb. The right and left lumbar muscles were not evaluated separately in the study. The meat was bought at specialized meat shops: pork meat – in meat market "Kunturi" Ltd., Riga; beef meat – "Jelgavas tirgus" Ltd., Jelgava; lamb meat – in "Bairons LBC" Ltd. market place "Iecenu delikateses", Ieceni.

Preparation of meat samples

The temperature of the meat during transportation did not exceed 4 °C, the transport time – 1.0–1.5 h. The meat samples were packed in vacuum bags of 200×250 mm, the sample size being 150–250 g. For the packaging material transparent PE / PA (polyethylene / polyamide) pouches (film thickness 60 μm) were chosen. The packaging material is impermeable to gases and moisture. Samples were vacuum-packed (20 mbar) using a vacuum packing machine *Multivac C300* (Sepp Haggenmüller GmbH&Co, Germany).

Maturation of meat and analysis of samples

Packaged meat samples were maturated in refrigerator 35 days at temperature 2±1 °C. Changes in physical parameters during maturation period were analysed on the 0, 7th, 14th, 21th, 28th and 35th day of storage.

Methods

Moisture content was established according to standard LVS ISO 1442:1997.

Water activity was determined using a water activity detector *Novasina LabSwift-a_w* (Novatron Scientific, Sweden).

pH value determined according to the standard LVS ISO 2917:2004.

Meat tenderness was determined using the texture analyser *TA.HD.Plus* (Stable Microsystems, UK). Warner-Bratzler shear device was used to investigate the shear test of meat hardness, which be composed of a blade and a slotted platform. Samples of meat was divided in strips of 2 cm width and set under shearing blade, which at a speed of 1 mm s^{-1}, sheared the test portion in half, parallel to the meat fibre. 10 independent measurements was finished for each sample of meat. Exponent software were used for data compose (Stable Microsystems Ltd., UK) (Sazonova et al., 2018).

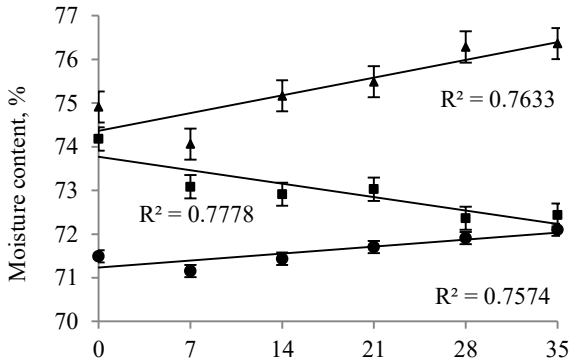
Data analysis

For the calculation of mean arithmetical values and standard deviations Microsoft Excel v16 software was used. To determine the significance of research results SPSS 20.0 software was used and the impact of factors and their interaction, the significance effect (p-value) to explore with ANOVA analyses.

Results and Discussion

Moisture content

Changes in moisture content in all meat samples during maturing are not significant (p=0.68) (Figure 1). Comparison of the 0-day sample with the 35-day matured sample showed a 0.61% increase in the moisture content of the pig meat and a decrease of 1.74% in the beef. In the lamb, fluctuations in moisture content during maturation were lower than in beef and pork meat. Comparison of the 0-day sample with 35 days of matured meat resulted in a 1.45% increase in the moisture content of the lamb at the end of the ripening. The decrease or increase in the moisture content at the end of the maturing period could be caused by the action of microorganisms and various enzymes, resulting in a change in the ratio of free to bound water,



which also affects changes in moisture content. The lamb and pork are characterized by a higher moisture content, which could be related to the age of the animal.

Figure 1. Moisture content change in the maturation period

● – pork meat; ■ – beef meat; ▲ – lamb meat

During the maturation process the greatest influence on the capacity of meat to preserve moisture is the activity of enzymes, especially calpains (Di Luca et al., 2011). Enzyme activity is affected by the lactic acid content that results from glycogen degradation. As shown by the results obtained, meat containing less lactic acid is characterized by higher proteolytic enzyme activity. Beef meat had a lower pH value, which decreased during maturation and which may be due to its age and higher glycogen content.

Water activity

During meat maturation, water activity changes (Figure 2) in all meat samples were not significant (p>0.05). In pork and lamb meat, water activity increased during the entire maturation period, but in beef decreased. By comparing the 0-day samples with 35 days of matured pork, beef and lamb meat, water activity changes were not significant (p=0.10).

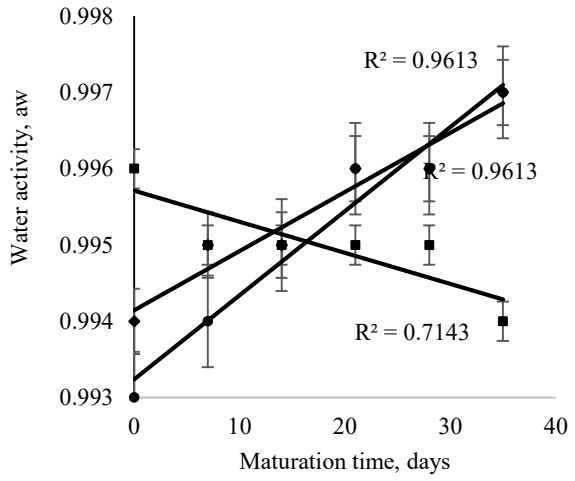


Figure 2. Water activity change in period of maturation

● – pork meat; ■ – beef meat; ▲ – lamb meat

Water activity is influenced by moisture content, amount of preservatives, and type of maturation. During dry maturation, moisture from the product evaporates faster than in the case of wet maturation and thus the water activity in the product decreases faster. As maturation was carried out in vacuum-packed meat in air- and moisture-tight material and no osmotically active substances or bacterial cultures are added to the raw materials used, the reduction in water activity is only possible in the meat and on the surface of the meat on the microflora.

In the present study a very strong positive correlation between moisture and water activity was determined. For different meat samples correlation coefficient was following: pork meat r=0.792; beef meat r=0.844; lamb meat r=0.813.

pH value

During period of maturation, all meat samples showed significant ($p<0.05$) changes in pH values (Table 1). In all meat samples, the pH value decreased during the maturation period. The lowest pH was observed in beef meat and during maturation period value change from 5.39 to 5.23. From the other hand, the highest pH value was detected in lamb (5.77) meat and in the time of maturation, it decreased and reached 5.72.

The one of the most important meat quality indicators is pH value. It is closely related to many other characteristics of meat, affecting the suitability of their processing and culinary applications, such as colour, tenderness and self-life (Daszkiewicz et al., 2016; Hamoen et al., 2013; Glamoclija et al., 2015). As reported by Rammouz et al. (2004) an easy-to-measure parameter of meat is pH that provides information on *post-mortem* muscle glycolysis, which makes it possible to detect meat quality defects. That is as pale, soft, exudative or PSE meat, and dark, firm, dry or DFD meat. Knox et al. (2008) mentioned that the rate of *post-mortem* glycolysis may be too rapid, leading to a rapid decrease in the pH value (typical for PSE meat) or too slow, resulting in a too high pH value (characteristic of DFD meat). In both cases, abnormal physico-chemical properties of meat (colour, water-holding capacity) are developed. The authors Adzitey and Nurul (2011) note: "Such meat has limited processing suitability and low consumer acceptance, which generates vast economic losses". The meat pH can markedly influence meat quality, such as softness (Bidner et al., 2004). The researcher Silva et al. (1999) was describe that softness had a linear relation with pH value in meat, while Pulford et al. (2009) was found low and high pH values beef to be gentle than intermediary pH samples. The experimental data reported that the toughest beef become in intermediary pH samples (Lomiwes et al., 2014). The initial pH value of the meat depends on the glycogen quantity in the muscle of animals. Because the glycogen content of the animals muscle tissue is higher, as its decomposition results in a higher lactic acid content and a lower pH value. As can be seen from the data in Table 1, the higher the glycogen content in the beef but the less it is in the pork and lamb meat. The high pH value in pigs and lambs could be explained by the age of the animal, because high levels of glycogen in the meat of these animals have not been able to accumulate and therefore the pH is higher.

Table 1

pH changes in period of maturation

Sample of meat	Maturation time, days					
	0	7	14	21	28	35
Pork meat	5.74±0.05	5.60±0.03	5.63±0.02	5.36±0.01	5.30±0.02	5.53±0.03
Beef meat	5.39±0.01	5.32±0.07	5.22±0.01	5.20±0.02	5.08±0.03	5.23±0.02
Lamb meat	5.77±0.02	5.87±0.04	5.77±0.04	5.67±0.03	5.55±0.02	5.72±0.04

Tenderness

During the maturation period, the pork tenderness (Figure 3) decreased, but the tenderness of beef and lamb increased and changes are not significant ($p=0.32$).

The tenderness changes and dynamics of changes were different and depending on the type of meat. Largest fluctuations in tenderness during maturation were observed in lamb and beef, but smaller in pork meat. During the 35 days of maturation, pork tenderness decreased from 159.78 to 119.51 N or fell by 25.21%. The opposite results were obtained by analysing the beef and lamb meat tenderness. Beef meat tenderness increased from 301.83 to 525.42 N or by 74.08%, and lamb increased more than 2 times or from 163.19 to 334.38 N.

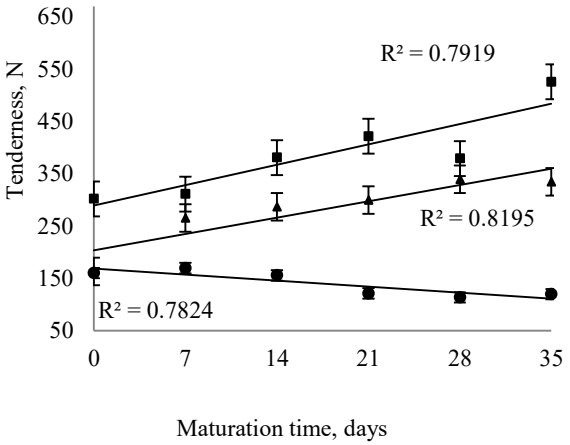


Figure 3. Tenderness change in the period of maturation

● – pork meat; ■ – beef meat; ▲ – lamb meat

The increase in tenderness could possibly be caused by temperature fluctuations during maturation, thus affecting enzyme activity and inhibiting myofibrillar protein degradation. Increase in tenderness could also be affected by an increase in lactic acid bacteria in beef and lamb (Kim et al., 2017). During the maturation process, the meat's tenderness is affected by pH value. In some cases (Wu et al., 2014) it is claimed that meat tenderness is linearly dependent on the pH of the meat. An increased meat tenderness indicates that meat has low proteolytic enzyme activity, especially calpan activity. In some literature sources was mentioned that the meat tenderness may be affected by the beakdown of myofibril protein by *post-mortem* period proteolytic activity. The authors Muroya et al. (2010) and Li et al. (2014) research have concentrate on determining the contribution of various myofibrillar proteins to meat softness. It has been systematically reported that tender meat compared to solid meat has a quicker and wide degradation of titin, desmin, troponin-T, nebulin. The degradation of these proteins during ageing process was observed. Therefore researchers have tried to prescribe the suitable ageing time to acquire acceptable softness. The results of the studies are consistent with Marino et al. (2013) who noted meat hardness is also affected by the fat contend of meat. Increased intramuscular fat during maturation helps reduce hardness. The amount of intramuscular fat in the meat depends on the animal sex, breed, age, quality and growing conditions. As we know, the fat content of beef and lamb is lower than that of pork. Therefore, pork meat may be softer and become softer during maturation.

Conclusions

The results showed a very strong positive correlation between moisture content and water activity. For different meat samples correlation coefficient was as follows: pork meat r=0.792; beef meat r=0.844; lamb meat r=0.813.

During meat maturation, the changes of moisture content, water activity and tenderness in all meat samples were not significant ($p>0.05$).

Pork, beef, lamb meat samples showed a significant ($p<0.05$) decrease in pH value during maturation period.

References

1. Adzitey F., Nurul H. (2011) Pale, soft, exudative (PSE) and dark, firm, dry (DFD) meats: causes and measures to reduce these incidences – A mini review. *International Food Research Journal*, Vol. 18 (1), p. 11–20.
2. Bhat Z.F., Morton D.J., Mason S.L., Bekhit Alaa El-Din A. (2018) Role of calpain system in meat tenderness: A review. *Food Science and Human Wellness*, Vol. 7(3), p. 196–204.
3. Binder B.S., Ellis M., Brewer M.S., Campion D., Wilson E.R., Mc Keith F.K. (2004) Effect of ultimate pH on quality characteristics of pork. *Journal of Muscle Foods*, Vol. 15 (2), p. 139–154.
4. Chen L., Feng X.C., Zhang Y.Y., Liu X B., Zhang W.G., Li C.B., Ullah N., Xu X.L., Zhou G.H. (2015) Effects of ultrasonic processing on caspase-3, calpain expression and myofibrillar structure of chicken during post-mortem ageing. *Food Chemistry*, Vol. 177, p. 280–287.
5. Cruzen S.M., Paulino P.V.R., Lonergan S.M., Huff-Lonergan E. (2014) Postmortem proteolysis in three muscles from growing and mature beef cattle. *Meat Science,* Vol. 96, p. 854–861.
6. Daszkiewicz T., Piaskowska N., Zapadka J., Kubiak D. (2016) Effect of different ultimate pH range on meat quality of crossbred Polish Holstein x Limousin heifers. *Polish Journal of Natural Science*, Vol. 31 (4), p. 533–543.
7. Di Luca A., Mullen A. M., Elia G., Davey G., Hamill R. M. (2011). Centrifugal drip is an accessible source for protein indicators of pork ageing and water-holding capacity. *Meat Science*, Vol. 88, p. 261–270.
8. Glamoclija N., Starcevic M., Janjic J., Ivanovic J., Boskovic M., Djordjevic J., Markovic L., Balticm Z. (2015) The effect of breed line and age on measurements of pH-value as meat quality parameter in breast muscles (m. pectoralis major) of broiler chickens. *Procedia Food Science*, Vol. 5, p. 89–92.
9. Hamoen J.R., Vollebregt H.M., Van Der Sman R.G.M. (2013) Prediction of the time evolution of pH in meat. *Food Chemistry*, Vol. 141 (3), p. 2363–2372.
10. Kim J.H., Kim D.H., Ji D.S., Lee H.J., Yoon D.K., Lee C.H. (2017) Effect of aging process and time on physicochemical and sensory evaluation of raw beef top round and shank muscles using an electronic tongue. *Korean Journal of Food Science of Animal Resource*. Vol. 37 (6), p. 823–832.
11. Kim Y.H.B., Meyers B., Kim H.W., Liceaga A.M., Lemenager R.P. (2016) Effects of stepwise dry/wet-aging and freezing on meat quality of beef loins. *Meat Science*, Vol. 123, p. 57–63.
12. Knox B.L., Van Laac K.R.L., Davidson P.M. (2008) Relationship between ultimate pH and microbial, chemical and physical characteristics of vacuum-packaged pork loins. *Journal of Food Science*, Vol. 73(3), p. 104–110.
13. Lee C.W., Lee J.R., Kim M.K., Jo C., Lee K.H., You I., Jung S. (2016) Quality improvement of pork loin by dry aging. *Korean Journal of Food Science of Animal Resources.* Vol. 36 (3), p. 369–376.
14. Li P., Wang T., Mao Y., Zhang Y., Niu L., Liang R., Zhu L., Luo X. (2014) Effect of ultimate pH on postmortem myofibrillar protein degradation and meat

quality characteristics of chinese yellow crossbreed cattle. *The Scientific World Journal,* Vol. 2014, p. 1–8.
15. Lomiwes D., Farouk M.M., Wu G., Young O.A. (2014) The development of meat tenderness is likely to be compartmentalised by ultimate pH. *Meat Science*, Vol. 96 (1), p. 646–651.
16. Lonergan E.H., Zhang W., Steven M.Lonergan S.M. (2010) Biochemistry of postmortem muscle – Lessons on mechanisms of meat tenderization. *Meat Science*, Vol. 84, p. 184–196.
17. Longo V., Lana A., Bottero M.T., Zolla L. (2015) Apoptosis in muscle-to-meat aging process: The omic witness. *Journal of Proteomics*, Vol. 125, p. 29-40.
18. Marino R., Albenzio M., della Malva A., Santillo A., Loizzo P., Sevi A. (2013). Proteolytic pattern of myofibrillar protein and meat tenderness as affected by breed and aging time. *Meat Science*, Vol. 95, p. 281–287.
19. Muroya S., Ertbjerg P., Pomponio L., Christensen M. (2010) Desmin and troponin T are degraded faster in type IIb muscle fibers than in type I fibers during postmortem aging of porcine muscle. *Meat Science*, Vol. 86 (3), p. 764–769.
20. Pulford D.J., Dobbie P., Vazques S.F., Fraser-Smith E., Frost D.A., Morris C.A. (2009) Variation in bull beef quality due to ultimate muscle pH is correlated to endopeptidase and small heat shock protein levels. *Meat Science*, Vol., 83 (1), p. 1–9.
21. Rammouz R.E., Babile R., Fernande Z X. (2004) Effect of ultimate pH on the physicochemical and biochemical characteristics of turkey breast muscle showing normal rate of post-mortem pH fall. *Poultry Science*, Vol. 83(10), p. 1750–1757.
22. Sazonova S., Galoburda R., Gramatina I., Straumite E. (2018) High pressure effect on the sensory and physical attributes of pork. *Research for Rural Development 2018*, Vol.1, p. 227–232.
23. Silva J.A., Patarata L., Martins C. (1999) Influence of ultimate pH on bovine meat tenderness during ageing. *Meat Science,* Vol. 52 (4), p. 453–459.
24. Toldra, F. (2010) *Handbook of meat processing*. USA: Publishing by Wiley-Blackwell. 566 p.
25. Velotto S., Pagano F., Barone C.M.A., Esposito M., Civale G., Crasto A. (2015) Effect of aging technologies on some qualitative characteristics of *Longissimus dorsi muscle* of Marchigiana beef. *Agronomy Research*, Vol. 13 (4), p. 1143–1151.
26. Wu G., Farouk M. M., Clerens S., Rosenvold K. (2014). Effect of beef ultimate pH and large structural protein changes with aging on meat tenderness. *Meat Science*, Vol. 98, p. 637–645.
27. Yang X., Niu L., Zhu L., Liang R., Zhang Y., Luo X. (2015) Shelf-Life extension of chill-stored beef Longissimus steaks packaged under modified atmospheres with 50% O_2 and 40% CO_2. *Journal of Food Science*, Vol. 81(7), p. 1–7.

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EFFECT OF FEEDING ON THE SENSORIAL QUALITY OF LAMB AND GOAT KID MEAT

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Abstract

Goat kid and lamb meat is quite well accepted by the consumers. The specific flavour is more expressed for sheep meat, also lamb meat has more intense aroma and it is more tender than that of goat kid meat. Several factors can affect the quality characteristics of goat kid and lamb meat, and the feeding of livestock is one of them. The aim of this study was to evaluate the effect of feeding on the lamb and goat kid meat physical and sensorial quality. In the samples of *M. semimembranosus* muscles from 8 Boer goat kids and 8 Latvian Dark head lambs (four animals per treatment) finished on diets varying in content of concentrated feed were analysed for the proximate composition, chemical and physical indices. Four Boer goat kids as feed supplement received oats, four kids – fodder beans, four lambs – a mix with fodder beans, four lambs – a mix with beans and peas. For sensory evaluation as control was used beef meat. For all five samples hardness and intensity of sensory properties (aroma, texture, taste and aftertaste) were analysed. Non-significant ($p>0.05$) influence was obtained on meat hardness according different additives of concentrated feed. There was no significant difference ($p>0.05$) in aroma and taste between samples, but there was significant difference in the texture and aftertaste ($p<0.05$). The aftertaste was weak for beef meat (BM) sample, but there were no significant differences ($p>0.01$) in the intensity of aftertaste with both goat kid meat samples. However, there was a significant difference ($p<0.05$) between both lamb meat samples.

***Keywords*:** lamb meat, goat kid meat, quality, sensory properties

Introduction

Goats and sheep in farmland were kept for centuries and were mainly used for milk, meat and wool production (Zervas et al., 2011). Small ruminants are the most efficient transformers of low-quality forage into high quality animal products (Lombardi, 2005) with distinguished chemical composition and organoleptic characteristics, and with the least use of fossil fuel.

The number of meat goats worldwide totals 464 million heads and meat sheep's – 567 million heads, and the estimated goat meat production is 5.8 million tons, sheep meat – 9.4 million tons (FAO, 2017). However, despite the large number of animals, consumers still prefer beef rather than sheep or goat meat. In Latvia the number of goats for meat production is 4 thousand heads and sheep's – 50.5 thousand heads, and the estimated sheep meat production is 0.9 thousand tons, but no production of goat meat for market (FAO, 2017). According to Teixeira et al. (2017) the most popular is meat from sheep and goats till 3 months of age and 5–8 kg carcass weight (cordeiro and cabrito) or from sheep and goats with 6 to 9 months age and more than 11 kg carcass weight (lamb and chivo). Nutritionally, goat is an important source of high-quality proteins, healthy fats, and with low calorie, intramuscular fat, saturated fat, and sodium contents. Besides, goat meat is rich in iron, potassium and essential amino acids, ensuring that goat meat is high quality meat (Horcada et al., 2012).

There are two main farming and feeding systems for lamb and goat kid meat production – intensive and extensive, with a great variation between those two (Joy et al., 2008). Feeding forage, which also includes grain feed, can alter carcass quality indices as well as internal and carcass fat levels (Goetch et al., 2011). The proportion of concentrated feed may have a few impacts on meat quality, but the total level of carcass fat was higher among diets higher in concentrates (Ryan et al., 2007). The content of fat in the sheep and goat carcass is very variable. In females fats are deposited in the carcass tissue relatively faster than in males, but in wethers intermediate (Mahgoub et al., 2005). These differences are more pronounced in the case of high levels of energy and concentrates in the feed. Restricted intake of nutrients increases lean meat production and reduces fat accumulation regardless of gender.

In recent years, consumers have been choosing healthier foods and are paying more attention to product quality. It has been found that milk and meat fat and cholesterol content can have a significant impact on human health (Zervas et al., 2011). Meat quality, as perceived by consumers, is a subjective, multidimensional and dynamic concept. Consumers around the world consume both sheep and goat meat, and in some cases can even replace mutton, lamb or beef. Gkarane et al. (2017) reported small sensory differences due to lamb sex. Ram meat has a more intense aroma compared to the castrate meat, more pronounced animal and farm smell, aroma of wool, aroma of manure and sweat. A similar assessment is also given to goat meat. Carcass weight and dressing percentage depends on feeding of lambs and goat kids. Feeding system also can have impact on animal's growth rate, meat quality indices (muscle and fat ratio, content of fatty acids), meat flavours, taste and tenderness, intensity of fat deposition. Carcass dressing percentage, conformation score and fatness degree also depends on feeding of lambs and kids (Carrasco et al., 2009; Toplu et al., 2013).

The quality of meat, which depends on the chemical, microbial and sensorial qualities of the meat, is important for consumers when buying meat (Madruga et al., 2010). In the past, the consumers have encountered a negative experience with the purchase of

older animal's meat with a pronounced sheep or goat taste and fiberiness. Nowadays, consumers are given the opportunity to buy the meat from young animals (chevon) (Brand et al., 2018), which haven't pronounced specific taste and aroma. Goat kid meat is preferred with less fat than beef or lamb. Therefore, it requires low-heat and slow cooking to preserve tenderness and juiciness (Madruga et al., 2010; Silva et al., 2011).

In recent years, the demand for low-fat meat and meat products has increased in order to avoid health risks associated with excessive fat intake. It is known that goats produce meat with a low fat content. Information on the characteristics of goat and sheep meat and its products in Latvia is still limited and needs more study. The present study attempts to evaluate the effect of feeding on the lamb and goat kid meat physical and sensorial quality.

Materials and Methods

Meat sampling

Samples of *Musculus semimembranosus* muscles were taken from 8 Boer goat kids (average age before slaughter 287 days) and 8 Latvian Dark head lambs (average age – 208 days) (four animals per treatment) finished on diets varying in content of concentrated feed. The goat kids and lambs were housed in a barn in four pens. Four Boer goat kids received oats (G1) as a feed supplement, four kids – oat mix with fodder beans (G2), four lambs – grains mix with fodder beans (L1), four lambs – grains mix with beans and peas (L2) (Table 1).

Table 1

Scheme of the research

Groups	Number of animals	Concentrated feed
G1	4 kids	100% oats
G2	4 kids	85% oats+15% fodder beans
L1	4 lambs	15% oats+20 %barley+15% wheat +50% fodder beans
L2	4 lambs	15% oats+20% barley+15% wheat +30% fodder beans+20% peas

L1; L2 – lamb meat, G1; G2 – goat kid's meat

The total sample of each kid and lamb group consisted of four samples from each animal left hind leg *Musculus semimembranosus,* but a sample of beef from *M. longissimus* was used for comparison. The samples were packed in the thick plastic bags to exclude as much air as possible, and stored at -20 °C. Before analyses the *M. semimembranosus* cuts were defrosted at 10 °C for a period of 24 h.

Physical and chemical analysis

For raw meat samples content of dry matter, protein, intramuscular-fat was determined using standard analytical methods. Dry matter content was determined by LVS ISO 1442:1997, fat content by LVS ISO 1443:1973, the protein content was calculated from nitrogen content multiplied by 6.25 using ISO 937:1978, pH value by LVS EN ISO 2917:2004.

Moisture-to-protein (W / P) ratio was calculated as an indication of physiological maturity (Brzostowski et al., 2008).

Texture analysis

A sample of 50×100 mm was prepared for muscle texture analysis, the longest edge being parallel to the muscle fibres. The sample of 15 mm thickness was analysed by Warner Bratzler blade (HDP/BSW) on a texture analyser TA.HDplus (Stable Microsystems, UK). Toughness was measured as the maximum force required for shearing a piece of fresh meat perpendicular to the fibre, at a speed of 2 mm s^{-1}. Four samples of each goat kid group and two samples from each lamb group were analysed in triplicate for the fibrousness of the muscle tissue. Trigger force was set at 0.098 N. A higher reading indicated a greater shear force and therefore tougher meat.

Sensory analysis

Meat from two goat kid (samples G1 and G2) and two lamb (samples L1 and L2) groups were sampled to evaluate the sensory properties of the meat, as well as a sample of beef meat from market as a control (BM). Meat samples for sensory evaluation were prepared as meatballs. For the meatballs, to 500 g of meat one egg was added, the meat was kneaded manually. Meatballs were formed into approximately 3.0 cm diameter and cooked in water at 90±5 °C for 6 min. The cooking process was carried out in two repetitions to ensure that each panellist had a hot, freshly cooked product.

Sensory tests were carried out at the Faculty of Food Technology, Latvia University of Life Sciences and Technologies. A total of 30 semi-trained panellists participated in the evaluation of meat sample sensory attribute intensity and overall liking. The panellists used computers equipped with FIZZ software (Biosystemes, France) to evaluate the intensity of sensory properties (aroma, flavour, texture and aftertaste) on unstructured 7-point line scales (1–weak; 7–very strong). For overall acceptability of meat samples, the ranking test (1–very dislike; 5–like very much) was applied. Between the samples, warm black tea was used for neutralization of taste.

Statistical analysis

Meat sample chemical composition and sensory properties were analysed with mathematical data processing methods – average, ANOVA. In all analyses, statistical significance was declared at $p<0.05$.

Results and Discussion

The quality of the meat is a relative concept and depends on the physical and chemical properties of the meat as well as on the consumer preferences.

Chemical composition and physical attributes of meat

According to the analyses of Boer goat crossbreed kids and Latvian Dark head lamb meat, was determined an average dry matter (27.0–32.1%), protein (18.2–19.6%)

and intramuscular fat content (6.7–13.9%) (Table 2). Our results are close to the findings obtained by Moawad et al. (2013) and Dhanda (2001), who reported 24.7–27.7%, 19.9–21.0% and 3.3–4.7% for dry matter, protein and fat contents, respectively.

Table 2

Meat quality traits of *M. semimembranosus* muscle of lamb and goat kids

Parameters	Samples			
	L1 (n=4)	L2 (n=4)	G1 (n=4)	G2 (n=4)
Dry matter, %	31.9±1.04	32.1±2.27	31.8±1.28	27.0±1.06
Protein, %	18.2±1.16	19.4±0.64	19.6±0.53	19.3±0.26
Intramuscular fat, %	12.05±0.86	13.9±1.97	7.9±0.34	6.7±1.52
pH	5.71±0.06	5.69±0.05	5.77±0.01	5.87±0.03
Moisture / Protein (W / P) ratio	3.79±0.26	3.49±0.08	3.48±0.04	3.78±0.08

L1; L2 – lamb meat, G1; G2 – goat kid's meat

Results of Table 2 indicated that lower dry matter and intramuscular fat content was observed in goat meat samples. These results confirmed the findings obtained by Brzostowski et al. (2008) who reported that goat meat from purebred French Alpine and Boer crossbred kids exhibited a desirable water-to-protein ratios (3.89 and 4.18), respectively. Generally, the lower the water/protein ratio, the better the quality of the meat (Pearson, 1991). In our research adding of fodder beans in feed ration increased the moisture/protein ratio in meat, the meat was with higher content of moisture and lower content of protein.

A key determinant of meat quality is pH. The ultimate pH values of goat kid's and lamb meat were found as 5.69–5.87 (Table 2), and higher values were found for goat meat. The pH is important to the chilled meat because it affects its shelf life, colour and quality. High pH (above 5.8) can indicate stressed animals during pre-slaughter handling and generally means lower quality of meat, and meat is dark, firm and dry (Dhanda et al., 2003; Hughes et al., 2017). More active animals are easily irritable and thus significantly reduce glycogen reserves in the liver. High pH in the meat also reflects a decrease in muscle glycogen due to stress or other factors (Muchenje et al., 2009). The lower meat pH is associated with the tenderness of the meat, which is characterized by longer sarcomeres after cooling and lower shear force value (Simela et al., 2004). However, it is still unclear why goats are so stressed before slaughter.

Meat texture

Tenderness can be evaluated as the maximum shear force necessary to cut the meat perpendicular to the fibres (Pena et al., 2009).

Table 3

Goat kid and lamb meat tenderness

Meat samples	Shear force, N	V, %
G1	301.90±34.12	39.15
G2	293.50±17.86	21.07
L1	207.00±7.79	9.22
L2	360.10±42.16	28.68

L1; L2 – lamb meat, G1; G2 – goat kid's meat
V – coefficient of variation

Average shear force values obtained in this study were from 207 to 360 N (Table 3) and were much higher to those observed by Moawad et al. (2013) on Egyptian Baladi goat breed goats' kids (58 N cm^{-2} for boiled meat), because our measurements were taken on fresh meat samples. Shear force is highly dependent on the preparation of individual meat samples, temperature, tendon and connective tissue inclusions and other factors, so that the results for each meat sample as well as for the repetition of one sample are not homogeneous. As we can see from the data in Table 3, the most tender meat was obtained from lambs fattened with a higher proportion of fodder beans in the feed, whereas the toughest and most difficult to prepare meat was presented by a lamb group, where the lambs were fed with fodder bean and peas mix. The statistical analysis of the data has shown that there were no significant differences between the groups. According to the research of Johnson et al. (1995), goat meat tenderness was reported to be lower than that of sheep and beef. According to Schenfeldt et al. (1993) study the sheep *M. semimembranosus* was significantly ($p<0.01$) more tender than the Angora and Boer goat cuts. In our study for all analysed meat samples we found weak positive correlation ($r=0.25$; $p>0.05$) between fat content and tenderness, but for lamb meat samples we found moderate positive correlation ($r=0.49$; $p>0.05$).

Sensorial attributes of meat

For five boiled meatball samples (G1, G2, L1, L2 and BM) the intensity of sensory properties (aroma, texture, flavour and aftertaste) were analysed.

The taste and flavour of the meat depend on the aldehydes, ketones, sugars, peptides and fatty acids that make up the specific flavour of a species (Madruga et al., 2011).

It has been found that increased intramuscular fat content increases the aroma and flavour of goat meat (Leick et al., 2012). As can be seen from Table 4, there was no significant difference ($p>0.05$) in aroma and flavour between samples, but there was a significant difference in the samples texture and aftertaste ($p<0.05$). In a study completed by Babiker et al. (1990), it was found that goat meat was leaner and darker compared to lamb. A softer, more chewable texture was found for a samples L2 and L1 (lamb) compared to samples G1, G2 (goat kid) and control (BM).

Table 4

Intensity of meatballs sensory properties

Attribute	Samples				
	L1	L2	G1	G2	BM
Aroma	3.63[a]	3.73[a]	4.13[a]	3.60[a]	4.10[a]
Texture	3.23[b]	3.53[ab]	4.10[a]	4.10[a]	4.07[a]
Flavour	4.33[a]	4.17[a]	3.90[a]	3.87[a]	3.80[a]
Aftertaste	3.77[a]	4.10[a]	3.70[b]	3.63[ab]	3.17[b]

the same letters in rows indicate no significant differences between samples ($p<0.05$)
L1; L2 – lamb meat, G1; G2 – goat kid's meat, BM – control, beef meat

The texture of the meat shared a positive strong correlation with meat protein content ($r=0.81$; $p<0.05$), and a strong negative correlation with meat fat content ($r=-0.88$; $p<0.05$) and flavour of the meat ($r=-0.98$; $p<0.05$). A weak aftertaste has been found for a control sample (BM), which was not significantly different ($p<0.05$) from the G1 and G2 samples. While, the samples G1 and G2 did not differ significantly from the L1 and L2 samples in the aftertaste intensity. In our research we found strong positive correlation with intramuscular fat content and aftertaste ($r=0.77$; $p<0.05$). It means that if meat is fattier, it will be more tender, less structured, and with more expressed flavour and aftertaste.

Table 5

Correlation between sensory attributes

Properties	Aroma	Texture	Flavour	Aftertaste
Aroma	1.00			
Texture	0.56	1.00		
Flavour	-0.59	-0.98	1.00	
Aftertaste	-0.52	-0.58	0.69	1.00

In this study we found a strong negative correlation ($r=-0.98$, $p<0.05$) between meat texture and flavour (Table 5) – the softer is meat texture, the more pronounced the flavour. According to Brand et al. (2018) the goat kid meat flavour showed a moderate correlation with meat aroma ($r=0.59$; $p<0.01$), but in our research meat flavour shown moderate negative correlation with meat aroma ($r=-0.59$; $p<0.05$) (Table 5). Also Brand et al. (2018) found that lamb had a more pronounced flavour and aroma, but the goat meat had no such pronounced taste.

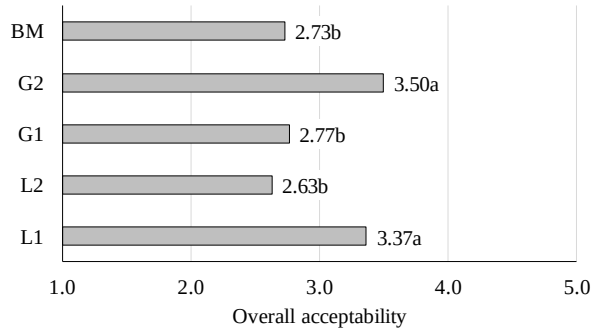

Figure 1. Overall acceptability of meatball samples
the same letters indicate no significant differences between samples ($p<0.05$)
L1; L2 – lamb meat, G1; G2 - goat kid's meat, BM – control, beef meat

One of the most important factors determining the quality of cooked meat is the tenderness of the meat. Similar results were achieved on Boer goats by Werdi Pratiwi et al. (2004), indicating that meat tenderness is an important factor in assessing the sensory properties of meat. Using the Ranking test, the degree of overall acceptability of meatball samples (Figure 1) was determined on the scale: 1–very dislike, 5–like very much.

Panellists more preferred samples L1 and G2, because they liked their texture and taste. For the control sample (BM), the overall acceptability level was the same as for samples L2 and G1. These samples panellists described as difficult to chew with weak taste and flavour. The results obtained suggest that the panellists did not have a negative attitude towards the specific taste and flavour of the samples, as described by the other scientific studies. Low consumption of lamb and sheep meat in European countries (OECD, 2019) is associated with this specific meat taste. Crouse (1983) indicated that consumers prefer beef or pork instead of lamb. The opinions of the panellists as to why the particular meat sample was liked or disliked differed drastically because each person has different preferences of what he likes or dislikes.

Southeast countries consumers prefer sheep meat instead of beef or pork meat, so the market generally lacks both lamb and mutton.

Conclusions

As indicated by chemical parameters, lower dry matter and intramuscular fat content was observed in goat meat samples. Adding of fodder beans in feed ration increased the moisture/protein ratio in meat; the meat was with higher content of moisture and lower content of protein. More tender meat was obtained from lambs fattened with a higher proportion of fodder beans in the feed, whereas tougher and the most difficult to prepare meat was presented by a lamb group, where the lambs were fed with fodder bean and peas mix. There was no significant difference ($p>0.05$) in aroma and flavour between samples, but there was significant difference in the texture and aftertaste ($p<0.05$). The aftertaste was weak for beef meat (BM) sample, but there were no significant differences ($p>0.05$) in the intensity of aftertaste in both goat kid meat samples. The most pronounced aftertaste was in lamb meat samples, and in our research we found strong positive correlation with intramuscular fat content ($r=0.77$; $p<0.05$). It means that

if meat is fattier, it will be more tender, less structured, and with more expressed flavour and aftertaste.
By physical properties, as well as eating qualities, it is concluded that goat kids meat from Boer crossbreed raised under Latvian conditions is an excellent source of healthy red meat alternative. The findings suggested that different diets can be varied to still produce chevon and lamb meat with uniform meat quality characteristics.

Acknowledgments

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References

1. Babiker S.A., El Khider I.A., Shafie S.A. (1990) Chemical composition and quality attributes of goat meat and lamb. *Meat Science*, Vol. 28, p. 273–277.
2. Brand T.S., Van Der Merwe D.A., Hoffman L.C., Geldenhuys G. (2018) The effect of dietary energy content on quality characteristics of Boer goat meat. *Meat Science,* Vol. 139, p. 74–81.
3. Brzostowski H., Niznikowoski R., Tanski Z. (2008) Quality of goat meat from purebred French Alpine kids and Boer crossbreeds. *Archiv fur Tierzucht-Archives of Animal Breeding*, Vol. 51 (4), p. 381–388.
4. Carrasco S., Ripoll G., Sanz A., Alvarez-Rodriquez J., Panea B., Revilla R., Joy M. (2009) Effect of feeding system on growth and carcass characteristics of Churra Tensina light lambs. *Livestock Science,* Vol. 121, p. 56–63.
5. Crouse J. D. (1983) The effects of breed, sex, slaughter weight, and age on lamb flavor. *Food Technology,* Vol. 39, p. 264–268.
6. Dhanda J.S. (2001) Evaluation of crossbred goat genotypes for growth, carcass and meat quality characteristics. Ph.D. Thesis, University of Queensland, Australia.
7. Dhanda J.S., Taylor D.G., Murray P.J. (2003) Growth, carcass and meat quality parameters of male goats: effects of genotype and live weight at slaughter. *Small Ruminant Research*, Vol. 50, p. 57–66.
8. Food and Agriculture Organization of the United Nations (FAO), 2017. Available: http://www.fao.org/faostat/en/#data/QL
9. Gkarane V., Allen P., Gravador S., Diskin M.G., Claffey N.A., Fahey A.G., Brunton N.P., Farmer L.J., Moloney A.P., Monahan F. J. (2017) Effect of castration and age at slaughter on sensory perception of lamb meat. *Small Ruminant Research,* Vol. 157, p. 65–74.
10. Goetsch A.L., Merkel R.C., Gipson T.A. (2011) Factors affecting goat meat production and quality. *Small Ruminant Research,* Vol. 101, p. 173–181.
11. Horcada A., Ripoll G., Alcalde M. J., Sanudo C., Teixeira A., Panea B. (2012) Fatty acid profile of three adipose depots in seven Spanish breeds of suckling kids. *Meat Science*, Vol. 92, p. 89–96.
12. Hughes J., Clarke F., Purslow P., Warner R. (2017) High pH in beef *longissimus thoracis* reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. *Food Research International*, Vol. 101, p. 228–238.
13. Johnson D. D., Eastridge J. S., Neubauer D. R., McGowan C.H. (1995) Effect of sex class on nutrient content of meat from young goat. *Journal of Animal Science*, Vol. 73, p. 296–301.
14. Joy M., Ripoll G., Delfa R. (2008). Effects of feeding system on carcass and non-carcass composition of Churra Tensina light lambs. *Small Ruminant Research,* Vol. 78, p. 123–133.
15. Leick C., Behrends J., Solaiman S., Broadway P., Min B., Mikel W., Schilling M. (2012) Sensory properties and instrumental texture analysis of chevon patties from intact male Boer and Kiko goats harvested at four endpoints. *Meat Science*, Vol. 91, p. 215–222.
16. Lombardi G. (2005) Optimum management and quality pastures for sheep and goat in mountain areas. In: Molina Alcaide E., Ben Salem H., Biala K., Morand-Fehr P. (eds.). *Sustainable grazing, nutritional utilization and quality of sheep and goat products*. Zaragoza: CIHEAM, p. 19–29.
17. Madruga M., Elmore J., Oruna-Concha M., Balagiannis D., Mottram D. (2010) Determination of some water-soluble aroma precursors in goat meat and their enrolment on flavour profile of goat meat. *Food Chemistry*, Vol. 123, p. 513–520.
18. Madruga M.S., Bressan M. C. (2011) Goat meats : Description, rational use, certification, processing and technological developments. *Small Ruminant Research*, Vol. 98(1–3), p. 39–45.
19. Mahgoub O., Lu C.D., Hameed M.S., Richie A., Al-Halhali A.S., Annamalai K. (2005) Performance of Omani goats fed diets containing various metabolizable energy densities. *Small Ruminant Research*, Vol. 58, p. 175–180.
20. Moawad R.K., Mohamed G.F., Ashour M.M.S., Enssaf M., El-Hamzy A. (2013) Chemical composition, quality characteristics and nutritive value of goat kids meat from Egyptian Baladi breed. *Journal of Applied Sciences Research,* Vol. 9 (8), p. 5048–5059.
21. Muchenje V., Dzama K., Chimonyo M., Strydom P.E., RaatsJ.G. (2009) Relationship between stress responsiveness and meat quality in three cattle breeds. *Meat Science*, Vol. 81, p. 653–657.
22. OECD (2019) Meat consumption (indicator). doi: 10.1787/fa290fd0-en (Accessed on 22 February 2019)
23. Pearson D. (1991) The Chemical Analysis of Food. Churchill: New York, London, p. 374–410.
24. Pena F., Bonvillani A., Freire B., Juarez M., Perea J., Gomez G. (2009) Effects of genotype and slaughter weight on the meat quality of Criollo Cordobes and Anglonubian kids produced under extensive feeding conditions. *Meat Science,* Vol. 83, p. 417–422.
25. Ryan S.M., Unruh J.A., Corrigan M.E., Drouillard J.S., Seyfert M. (2007) Effects of concentrate level on carcas traits of Boer crossbred goats. *Small Ruminant Research*, Vol. 73, p. 67–76.
26. Schenfeldt H.C., Naud R.T., Bok W., van Heerden S.M, Smit R. (1993) Flavour- and tenderness-related quality characteristics of goat and sheep meat. *Meat Science,* Vol. 34, p. 363–379.
27. Silva T.M., Oliveira R.L., Larissa Pires Barbosa L.P., Garcez Neto A.F., Bagaldo A.R., Duarte Lanna D.P., Alves da Silva M.C., Brito de Jesus I. (2011) Preliminary study on meat quality of goats fed levels of licury oil in the diet. Asian-Aust. *Journal of Animal Science*, Vol. 24 (8), p. 1112–1119.
28. Simela L., Webb E.C., Frylinck L. (2004) Effect of sex, age, and preslaughter conditioning on pH, temperature, tenderness properties and colour of indigenous *South African goats. Journal of Animal Science*, Vol. 34, p. 208–211.
29. Teixeira A., Fernandes A., Pereira E., Manuel A., Rodrigues S. (2017) Effect of salting and ripening on the

physicochemical and sensory quality of goat and sheep cured legs. *Meat Science*, Vol. 134, p. 163–169.
30. Toplu H.D.O., Goksov E.O., Nazligul A., Kahraman T. (2013) Meat quality characteristics of Turkish indigenous Hair goat kids reared under traditional extensive production system: effects of slaughter age and gender. *Journal of Tropical Animal Health and Production*, Vol. 45(6), p. 1297–1304.
31. Werdi Pratiwi N.M., Murray P.J., Taylor D.G. (2004) Meat quality of entire and castrated male Boer goats raised under Australian condition and slaughtered at different weights: physical characteristics, shear force values and eating quality profiles. *Animal Science*, Vol. 79, p. 213–219.
32. Zervas G., Tsiplakou E. (2011) The effect of feeding systems on the characteristics of products from small ruminants. *Small Ruminant Research,* Vol. 101, p. 140–149.

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INNOVATIVE STRUCTURED FISH MEAT PRODUCTS FROM BALTIC SPRAT (*SPRATTUS SPRATTUS BALTICUS*)

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Abstract

Fish is very important in the diet, providing complete protein, unsaturated ω3 fatty acids, calcium, phosphorus and vitamin D. However, Baltic sprat (*Sprattus sprattus balticus*), one of the most abundant fish stocks in the Baltic Sea, is underutilised due to its small size. The aim of the study was to develop structured fish forcemeat using whole fish from the Baltic Sea small *Clupeiformes* fish for further use in finished and semi-finished fish products. The testing of fish as raw material and developed structured fish forcemeat showed that all parts of fish can be used in the production as valuable ingredients. Due to the textural properties of the structured fish forcemeat, the possibilities of using it in finished and semi-finished fish products is wide. A total of seven basic product groups – sausages, terrines, frozen semi-finished products, fish meatballs and other culinary preparations, dumplings, pies, pasta and ravioli – were developed. Products made from structured fish forcemeat have a high nutritional value, particularly with regards to protein content and amino acid profile.

Keywords: structured fish forcemeat, semi-finished products, nutritional value, processing

Introduction

Fish is an important source of complete protein, unsaturated fatty acids, minerals (Ca, P) and vitamins (A, D) in the diet (Tilami, Sampels, 2017). One of the main catch in the Baltic sea is Baltic sprat (*Sprattus sprattus balticus*) (Ministry of Agriculture (2017), often underutilised due to its small size. Such minerals as calcium and phosphorus are concentrated in fish cartilage, scales, tail and bones (Stanek et al., 2013), thus suggesting the use of whole fish for production of high value products.

As shown by Proskina et al. (2018), the catch quotas available to Latvia (set by Council Regulation (EU) 2016/1903) were decreased for cod (-57%), Baltic herring (-1%) and salmon (-10%), while a significant increase of 20% was given to sprats in 2017. Economic consideration is one of the main reasons why the replacement of structured cod mincemeat with structured forcemeat from small *Clupeiformes* fish caught in the Baltic sea should be studied (Proskina et al., 2018).

The replacement of structured white fish mincemeat with small clupeid fish would also allow to reduce the production of traditional canned sprats, export of which have decreased by almost 25% due to Russian import ban on EU products (Russian embargo…, 2019).

The aim of the study was to develop structured fish forcemeat using whole fish from the Baltic Sea small *Clupeiformes* fish for further use in finished and semi-finished fish products.

Materials and Methods

Raw materials

Baltic sprat (*Sprattus sprattus balticus*) in two thermal conditions was provided by Piejura Ltd. for the preliminary research: chilled fish 12 hours after catch and frozen fish in a flash freezer.

Experimental design

The laboratories of Faculty of Food Technology, Latvia University of Life Sciences and Technologies were used for raw material testing and innovative product development and quality analyses.

The preliminary research was carried out to determine: a) the possibilities to use whole fish for the development of new products and b) the effect of freezing on fish quality. A total of three fish samples and two thermal conditions were tested: chilled and frozen whole sprats, headed and gutted sprats, and sprat heads. The main part of the research was to develop structured fish forcemeat recipe and test the possibilities for its applications in new product development.

Development of structured fish forcemeat

When developing structured fish forcemeat from whole fish, two opposite tasks must be completed. Firstly, fish must be homogenised to the level where fish structure is not completely destroyed and consumer can notice fish taste and texture, however, in this case, it is possible that fish bones and other undesirable fish parts are present. Secondly, with a more intense homogenisation, a fine, highly homogeneous and paste-like texture is obtained, which becomes less viscous (overly fluid) due to the destruction of the muscles. To solve this problem, the addition of textured soy is suggested to substitute the lost texture from the intense homogenisation.

The main operations of structured fish forcemeat production are given in Figure 1.

For structured fish forcemeat production fresh, chilled or frozen fish can be used. When using frozen fish, there is no thawing, but frozen fish blocks are chopped in cylindrical or guillotine industrial blades. This allows to

reduce cutter-mixer blade load and speed up homogenisation. It also ensures a low temperature in the coarse-ground fish mincemeat, which has a positive effect on the colour and safety of the product.
Emulsification in a high-speed cutter-mixer is performed until a rough consistency is obtained.

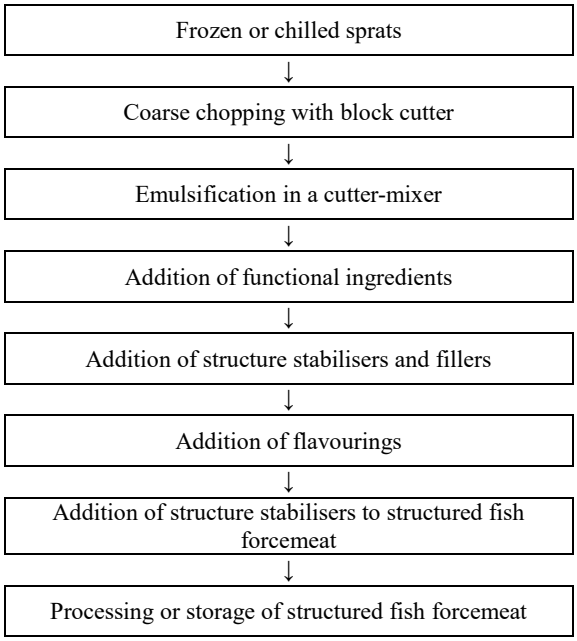

Figure 1. General process scheme of structured fish forcemeat production

Such functional ingredients as food-grade sodium phosphate (E339), salt and titanium dioxide (E171) are added. Titanium dioxide works as the colourant (EFSA, 2016) and should be added before other ingredients. Fish is crushed whole without heading and gutting, and fillet rinsing, therefore, such preservatives as microorganism inhibitors (e.g. potassium sorbate) can be used (Bashir et al., 2017).
Structure stabilizers and fillers of plant origin allow to stabilise low viscous consistency of emulsified fish forcemeat from sprats. According to the recipe, starch is added initially, followed by textured flour or semolina, then necessary consistency of the product is achieved by the addition of pea flour and food fibre.
Dried, fried or fresh onions are used as flavour additives. Before use, dried onions should be hydrated with water in a ratio 1: 2.
Coarse texture of sprat structured forcemeat is given by the addition of hydrated textured soy.
Structured fish forcemeat is then forwarded to production of new products or stored up to 24 h at 2–6 °C temperature.

Development of semi-finished products from structured fish forcemeat

The production technology and recipes of co-extruded fish meatballs with filling already implemented at the factory was adapted to substitute traditional fish minced meat (which is more expensive) with structured fish forcemeat. As shown by Silovs and Dmitrijeva (2018), co-extrusion allows to obtain product, which is a combination of fish with sauce – structured fish products with various fillings. Such parameters as nutritional and amino acid profile were assessed in semi-finished products from structured fish forcemeat.

Quality analysis

Standard methods were used to assess nutritional composition of samples: protein content (PB-116 ed. II of 30.06.2014), fat content (PN-A-86509:1994), ash content (PN-A-79011-8:1988), amino acids (PB-53/HPLC ed. II of 30.12.2008), dietary fibre (AOAC 991.43:1993), fatty acid profile (ISO 12966-4:2015), salt content (PB-318/FAAS, ed. I of 27.07.2015). Carbohydrates were calculated by difference (FAO, 2003). Energy value was calculated according to EU Regulation No 1169/2011.

Data processing

MS Excel *v16* software was used to process the obtained data; differences were considered significant at $p<0.05$. All parameters were analysed in triplicate, mean values are given in tables. ANOVA and Tukey's test were applied to evaluate the results.

Results and Discussion

Quality of chilled and frozen sprats

The comparison of nutritional parameters of chilled and frozen sprats showed that there are not significant differences in protein, fat, ash content and energy value (Table 1). Literature studies show that there are several advantages of using frozen fish (Vanhaecke et al., 2010) if the production site is not close to water bodies: high microbiological quality, reduction of raw material costs, reduction of seasonal effect, increase in economic performance of production.

Table 1

Chemical composition of chilled and frozen sprat parts per 100 g

Samples	Composition parameters			
	Protein, g	Fat, g	Ash, g	Energy, kcal
AC*	14.9[a]	5.0[a]	2.0[b]	104.2[a]
BC	16.0[a]	5.1[a]	1.7[b]	110.3[a]
DC	10.8[b]	4.9[a]	3.8[a]	87.3[b]
AF	15.0[a]	4.8[a]	2.2[b]	103.2[a]
BF	15.5[a]	5.2[a]	1.7[b]	108.8[a]
DF	10.3[b]	4.7[a]	3.6[a]	83.5[b]

Values within the same column sharing the same letter are not significantly different ($p>0.05$).
* A – whole sprats, B – headed and gutted sprats, D – sprat heads, C – chilled, F – frozen.

In addition, testing of separate fish parts – whole sprats, headed and gutted sprats and sprat heads – established that sprat heads have a significantly higher content of ash ($p<0.05$) which represents total minerals. Fish heads make up 10–15% of the fish carcass (Smárason et al., 2014) and contains around 10 g of protein (Table 1), therefore showing potential as a raw

material for structured fish forcemeat. Based on these results, whole sprats were used in the further experiments.

Development of structured fish forcemeat

Ground fish mincemeat is characterised by viscous consistency which must be stabilised with raw materials with good water binding properties. For this purpose, structural stabilizers (soy, carrot, wheat, beet, etc.), native starch (tapioca, potato, rice), textured flour (pea, rice, buckwheat, corn), semolina etc. can be used (Wild et al., 2014).

The flavour of the structured fish forcemeat depends on the percentage of fish in the finished product. By increasing the concentration of fish above 50%, the organoleptic quality indicators – taste and colour – decreased, whereas reduction of fish concentration to 40% improved the taste and colour. It is also possible to add various spices and herbs, onions (dried, fried, fresh) during structured fish forcemeat preparation, which greatly improves the taste of the finished product. The most effective additive that significantly improves the flavour of sprat structured forcemeat is onions and, with proper degree of shredding provides the product with a suitable texture. The final recipe of structured fish forcemeat from sprats is summarised in Table 2.

Table 2

Recipe of structured fish forcemeat

Ingredients	Amount, %
Frozen sprats	45.45
Wheat fibre	3.64
Water needed for wheat fibre	7.27
Titanium dioxide	0.91
Salt	0.45
Water-soluble food-grade sodium phosphate	0.45
Textured soy	4.55
Water needed for textured soy	10.91
Fresh onions	5.45
Semolina	3.64
Pea flour	3.64
Native rice starch	2.73
Water needed for fillers	9.09
Seasonings	1.82

Possibilities for structured fish forcemeat application in new product development

Due to the textural properties of structured fish forcemeat, its applications in food are wide. It can be used in at least seven product groups based on production specifics (Table 3, Figure 2). Sensory evaluation showed that new products from structured fish forcemeat were of good sensory quality and could compete with similar products (analogues from fillet minced meat) on the market (Straumite et al., 2018).

Quality comparison of raw sprats, structured fish forcemeat and semi-finished products

The comparison of nutritional and energy value of raw sprats, structured fish forcemeat and semi-finished breaded fish meat balls showed significant differences in several parameters (Table 4).

Table 3

Use of structured fish forcemeat in production of various products

Product group	Developed products
Sausages (emulsified fish products)	Cooked sausage Frankfurters Cooked bratwurst
Pates (chilled fish culinary products)	Pâté Terrine (classic, in layers, with filling)
Semi-finished products (frozen)	Dumplings
Frozen and breaded / battered fish culinary products	Fish fingers Fish meatballs Burger patties
Frozen culinary products without breading	Cutlet Cutlet in sauce Meatballs
Pies (baked pastry with structured fish stuffing)	Puff pastry pies Yeast dough pies
Pasta (portion of pasta dough is replaced with structured fish forcemeat)	Pasta Noodles

Figure 2. Products developed from structured fish forcemeat

Protein content in raw sprats, structured fish forcemeat and breaded fish meat balls is high, providing 30.8%, 41.5% and 26% of energy value, respectively. All tested products are eligible for nutrient claim ‘high protein’ based on Regulation (EC) No 1924/2006.

All three samples also have a content of ‘high omega-3 fatty acids’ (Commission Regulation (EU) No 116/2010). The claim ‘high omega-3 fatty acids’ can be used when 100 g and 100 kcal of products contain at least 80 mg of the sum of long chain ω3 fatty acids – eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Raw sprats contain 2100 mg EPA and DHA per 100 g and 1220 mg per 100 kcal, structured fish forcemeat – 1000 mg per 100 g and 567 mg per 100 kcal and breaded fish meat balls – 800 mg per 100 g and 329 mg per 100 kcal.

In addition, structured fish forcemeat and semi-finished breaded fish meat balls have a ‘high monounsaturated fat’ content, providing over 46% of fatty acids from

MUFA, and MUFA provides over 22% of energy (Commission Regulation (EU) No 116/2010).

Table 4

Nutritional parameters and energy value of sprats and sprat products per 100 g

Parameters	Samples		
	RS*	SF	FMB
Protein, g	13.20[c]	18.30[a]	15.80[b]
Fat, g	12.10[b]	9.40[c]	14.00[a]
Saturated (SAFA), g	3.40[a]	2.00[b]	2.70[ab]
Monounsaturated (MUFA), g	4.40[b]	4.40[b]	7.30[a]
Polyunsaturated (PUFA), g	3.70[a]	2.80[b]	3.80[a]
− ω3, g	3.20[a]	1.80[b]	1.80[b]
− eicosapentaenoic acid, mg	0.90[a]	0.40[b]	0.30[b]
− docosahexaenoic acid, mg	1.20[a]	0.60[b]	0.50[b]
− ω6, g	0.50[c]	1.00[b]	2.00[a]
− ω9, g	3.10[b]	3.50[b]	6.40[a]
Trans fatty acids, g	< 0.10[a]	< 0.10[a]	< 0.10[a]
Ash, g	1.89[b]	4.00[a]	3.83[a]
Carbohydrates, g	2.60[b]	1.30[c]	13.00[a]
Dietary fibre, g	0.00[b]	1.30[a]	0.80[a]
Salt, g	0.12[b]	2.05[a]	1.88[a]
Energy value, kJ	716[c]	737[b]	1014[a]

Values within the same row sharing the same letter are not significantly different (p>0.05).
* RS – raw sprats, SF – structured fish forcemeat, FMB – semi-finished breaded fish meat balls

The rest of the differences in nutritional parameters can be explained by the recipes of sprat products; higher content of protein in structured fish forcemeat is due to the addition of textured soy, while higher content of carbohydrates in breaded fish meat balls is due to the coating and filling of the product.

One of the important parameters to verify the quality of new products is amino acid profile, as additional ingredients and the coating and filling can reduce the qualitative and quantitative amino acid content.

Amino acid composition of raw sprats shows (Table 5) that the total amount of amino acids per gram of protein is 806.4 mg, and based on the essential amino acid profile sprat protein is complete. A study by Mohanty et al. (2014) reviewed the amino acid profile of 27 different food fish and their results on sprat amino acid content is comparable to our results.

The results of amino acid content in structured fish forcemeat and breaded fish meat balls in mg per g protein indicates (Table 5) that the total amount of amino acids in fish balls per 1 gram does not differ significantly from the content of amino acids in 1 g protein of structured forcemeat (p>0.05).

The added ingredients for optimum structured fish forcemeat production have a positive effect on the amino acid profile, and structured fish forcemeat protein retains its full value after these technological improvements. It can also be concluded that the coating and the filling of fish balls has an insignificant effect on total amino acid content, especially with regards to essential amino acids.

Table 5

Content of amino acids in sprats and sprat products, mg g^{-1} protein

Amino acids	Samples			MAAP (FAO, 2013)
	RS*	SF	FMB	
Ala	58.3	51.4	48.1	–**
Arg	47.0	68.3	63.3	–
Asp	72.7	96.7	92.4	–
Glu	129.5	177.0	188.6	–
Gly	53.8	49.2	46.2	–
Cys	5.6	12.6	10.8	–
Pro	34.1	49.2	52.5	–
Ser	30.3	45.9	44.3	–
Tyr	26.5	35.0	30.4	38
Phe[×]	38.6	45.9	43.7	
His[×]	18.2	25.1	24.1	15
Ile[×]	35.6	39.9	38.0	30
Leu[×]	68.2	74.9	72.8	59
Lys[×]	76.5	75.4	61.4	45
Met[×]	28.0	20.8	19.6	16
Thr[×]	34.1	38.8	37.3	23
Trp[×]	n.d.	n.d.	n.d.	6
Val[×]	49.2	43.7	43.0	39
Total	806.4	949.7	916.5	–

* RS – raw sprats, SF – structured fish forcemeat, FMB – semi-finished breaded fish meat balls, MAAP – maintenance amino acid pattern.
** Pattern not defined by FAO.
[×] Essential amino acids.
n.d. – no data

Based on the FAO recommendations for maintenance amino acid pattern, protein of structured fish forcemeat and breaded fish meat balls is also complete, indicating that processing and additional ingredients do not affect the protein quality of new products.

Conclusions

The results of the study show that is it possible to use whole sprats in structured sprat forcemeat production, however, it is suggested to use no more than up to 50% of sprats in total forcemeat composition. Structured sprat forcemeat can be labelled 'high protein', 'high omega-3 fatty acids' and 'high monounsaturated fat', in addition, products made from it could be eligible for these claims as well. Protein of sprats and structured forcemeat is complete based on essential amino acid profile. Semi-finished breaded fish meat balls made from structured sprat forcemeat have a high nutritional value, particularly with regards to protein content and amino acid profile.

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References

1. Bashir K.M.I, Kim J-S., An J.H., HakSohn J., Choi J.S. (2017) Natural food additives and preservatives for fish-paste products: A Review of the past, present, and future states of research. *Journal of Food Quality*, Vol. 2017, p. 1–32.
2. Commission Regulation (EU) No 116/2010 of 9 February 2010 amending Regulation (EC) No 1924/2006 of the European Parliament and of the Council with regard to the list of nutrition claims (OJ L 37/16, 10.2.2010, p.16-18).
3. Council Regulation (EU) 2016/1903 of 28 October 2016 fixing for 2017 the fishing opportunities for certain fish stocks and groups of fish stocks applicable in the Baltic Sea and amending Regulation (EU) 2016/72 *(*OJ L 295, 29.10.2016, p. 1–10).
4. EFSA (2016) *Food colours: titanium dioxide marks re-evaluation milestone*. [accessed on 27.02.2019.]. Available: https://www.efsa.europa.eu/en/press/news/160914
5. FAO (2013) *Dietary protein quality evaluation in human nutrition*: *Report of an FAO Expert Consultation.* FAO Food and Nutrition Paper No 92. Rome: Food and Agriculture Organization of The United Nations. 66 p.
6. Kirse A., Karklina D., Muizniece-Brasava S., Galoburda R. (2017) Influence of sous vide treatment and high pressure processing on nutritional value and overall acceptance of pulse spreads. *Proceedings of the Latvian Academy of Sciences. Section B*, Vol. 71, No. 6 (711), p. 474–480.
7. Ministry of Agriculture (2017) Action Programme for the Development of Fisheries 2014-2020, version 2.2, p. 132. [accessed on 12.03.2019.]. Available: https://www.zm.gov.lv/public/files/CMS_Static_Page_Doc/00/00/01/11/39/fs-01usersLinda.BirinaDesktopAAProgramme_2014LV14MFOP001_2_2_lv.pdf
8. Mohanty B., Mahanty A., Ganguly S., Sankar T.V., Chakraborty K., Rangasamy A., Paul B., Sarma D., Mathew S., Asha K.K., Behera B., Aftabuddin M., Debnath D., Vijayagopal P., Sridhar N., Akhtar M.S., Sahi N., Mitra T., Banerjee S., Paria P., Das D., Das P., Vijayan K.K., Laxmanan P.T., Sharma A.P. (2014) Amino acid compositions of 27 food fishes and their importance in clinical nutrition. *Journal of Amino Acids*, Vol. 2014, p. 1–7.
9. Proskina L., Pilvere I., Nipers A., Silovs M. (2018) Characteristics of the fishing industry in Latvia. **In:** *Proceedings of the 2018 International Conference "Economic Science for Rural Development"*, Jelgava, Latvia, p. 56–64.
10. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods (OJ L 404, 30.12.2006, p. 9)
11. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 (OJ L 304, 22.11.2011, p. 18–63)
12. Russian embargo badly hurts Latvian fish canners. [accessed on 12.03.2019.]. Available: https://iegvu.agribusinessintelligence.informa.com/CO224125/Russian-embargo-badly-hurts-Latvian-fish-canners
13. Silovs M., Dmitrijeva O. (2018) Fish processing industry modernization and co-extrusion method in fish product assortment diversification. **In:** *Proceedings of the 2018 International Conference "Economic Science for Rural Development"*, Jelgava, Latvia, p. 305–311.
14. Smárason B. Ö., Viðarsson J.R., Þórðarson G., Magnúsdóttir L. (2014) *Life Cycle Assessment on fresh Icelandic cod loins. Report summary: Skýrsla Matís Resources & Products*. [accessed on 12.03.2019.]. Available: http://www.matis.is/media/matis/utgafa/24-14_AVS-LCA-greining_Lokaskyrsla.pdf
15. Stanek M., , Peter E., Janicki B. (2013) Content of the calcium and phosphorus in the meat of Prussian carp (*Carassius auratus gibelio* Bloch, 1783) from the Lake Gopło (Poland). *Journal of Central European Agriculture*, Vol. 14(1), p. 1–10.
16. Straumite E., Gorbatovskiy A., Sazonova S., Gramatina I., Muizniece-Brasava S., Kruma Z., Kirse A., Ciprovica I., Sabovics M. (2018) Sensory evaluation of structured fish mass products from Baltic sprat (*Sprattus sprattus balticus Schneider*). **In**: *Abstract book of 12th Baltic Conference on Food Science and Technology "Food R&D in the Baltics and Beyond" FOODBALT 2018 Conference*, Kaunas, Lithuania, p. 108.
17. Tilami S.K., Sampels S. (2017) Nutritional Value of Fish: Lipids, Proteins, Vitamins, and Minerals. *Reviews in Fisheries Science & Aquaculture*, Vol. 26(2), p. 1–11.
18. Vanhaecke L., Verbeke W., De Brabanderac H.F. (2010) Glazing of frozen fish: Analytical and economic challenges. *Analytica Chimica Acta*, Vol. 672 (1–2), p. 40–44.
19. Wild F., Czerny M., Janssen A.M., Kole A.P.W., Zunabovic M., Domig K.J. (2014) The evolution of a plant-based alternative to meat. *Agro FOOD Industry Hi Tech*, Vol. 25(1), p. 45–49.

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ECONOMIC BASE SCENARIO ASSESSMENT OF INNOVATIVE PRODUCT MANUFACTURING FROM BALTIC SEA SPRATS (*SPRATTUS SPRATTUS BALTICUS*)

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Abstract

Fisheries have a long history and tradition in Latvia. In recent years, the fish processing subsector has been challenged by the development and introduction of new and innovative products manufactured from Baltic Sea sprats (*Sprattus sprattus balticus*), which serve as an alternative to canned sprat production. Therefore, the aim of the present research was to perform an economic assessment of the establishment of a potential factory for the production of innovative fish products from sprats caught in the Baltic Sea. To achieve the aim, two specific research tasks were set: 1) to perform a financial and efficiency analysis of the investment project for the industrial production of the newly developed innovative products; 2) to determine the economic effect from the establishment of the factory. The research base scenario established the minimum amount of necessary investment to start up a factory and estimated processing capacity at 408 tons of sprats a year to pay back the investment in time by producing two new innovative products from Baltic Sea sprats: fish buns and fish meatballs in jelly. The research established main economic and financial performance indicators for the investment in the factory project, calculated the number of employees needed for the factory and identified the economic benefits the factory would provide. The research found that setting up a factory within one year could reach the planned fish processing capacity in the third year of operation, reaching 4.7 million euro in turnover and 2 million euro in net profit with a 44% net margin in year 3 and onwards.

Keywords: sprats, innovative products, economic assessment

Introduction

Growing demand for fish has promoted a major expansion of European fleets in size and fishing range. According to European Commission data, more than 140 000 people are employed as fishers in the European Union (EU) (Schroeer, Sakai et al., 2011). The EU catches on average approximately 6.4 mln. t of fish a year, while the fisheries sector (which is divided into three subsectors: fishing, aquaculture and fish processing) employs about 350 thousand people. However, the pronounced local resource depletion underlines the need for close cooperation between industry and the public sector to counter uncertainties that act as a constraint on investment in utilization schemes (Catchpole, Ribeiro-Santos et al., 2017). Despite the mixed performance (Da Rocha et al, 2012; Khalilian et al, 2010; Sissenwine, James, 2007), the EU Common Fisheries Policy, which sets targets for as well as series of restrictions on the EU fisheries sector, is an important instrument for the sector growth.

In Latvia, the fisheries sector has a long history and tradition. This is both an advantage, as the country has developed an extensive infrastructure for fisheries and built up considerable experience, and a challenge. Since 2014 when the Russian Federation imposed restrictions on imports of fish products from Latvia, the market has made the fisheries sector go beyond the comfort area for the large-scale production of conventional fish products. As pointed out by Laugalis (2009), a number of significant problems, which need political solutions at the national and European levels, exist in the fisheries sector of Latvia. The most significant is the available resources of fish for catching and processing. Since 2005, the key initiative in regulating fishing and in setting fishing quotas in the Baltic Sea belongs to the European Commission, as the volume of catches by the European Union Member States in the Baltic Sea accounts for 95% of the total. The Baltic Sea sprat is the most significant fish species in terms of percentage of the total fishing quota for Latvia. In 2017, Latvian fishermen caught 35.7 thou.t of sprats, which was 7.6 thou.t more than in 2016. The total allowable catch for 2017 was increased by 29%. In 2017, Latvia's fishing quota for sprats equalled 36 107 t, which was exhausted by 99%. In 2017, the total catch of sprats by all the EU Member States equalled 285.7 thou.t or 39.2 thou.t more than in 2016. Overall, the EU Member States exhausted their quotas for sprats by 94.6 % (Kornilovs, Ustups, 2018).

In the period 2012–2016, according to the Central Statistical Bureau (2019), the fish processing subsector of Latvia sharply decreased the processing of sprats (-41%), and this input was imported significantly less (-50%), while the volume of catches remained at the same level. Because of the restrictions on fish product imports imposed by the Russian Federation, the market for products made from sprats considerably shrank, and fish processors were forced to significantly decrease their output. Consequently, a considerable quantity of this raw material was not required, and the exports of sprats from Latvia rose by 51%, reaching as many as 27.7 thou. t in 2017.

Ankvica (2018) points out that an analysis of the results of 2017 reveals that Latvian fish processors, working under the sanctions of the Russian Federation, actively sought new sales partners in various countries. Nevertheless, there are several reasons of the slow expansion of the market. One of the key reasons is the fact that in Latvia, canned sprat and Baltic herring still dominate in the production of prepared and canned fish.

Benga and Hazners (2018), however, point out that in Latvia, the fish product segment has been always mainly export-oriented. The capacity of the domestic market is limited, and the domestic consumption has been slightly declining for many years. Approximately 85% of fish production is exported. Fishing quotas for a number of significant fish species have been reduced or will be reduced in the future too; consequently, fishermen and fish processors have to consider this trend already now. New ways have to be sought to process the very limited fish resources and generate maximum value-added so that funds are accumulated for the further development of this sector.

As indicated by Neiva et al. (2010) fish mince production is equally beneficial in terms of economic profit for producers since it allows utilisation of low value species (i.e. due to its small shape) and it also diminishes the volume of processing by-products promoting rational use of the limited catch. According to the estimations provided by the Food and Agriculture Organization of the United Nations (2001) the introduction of fish mince processing can increase the yield from 8% to up to 50% in comparison to traditional filleting alone. However, despite the fact that for certain fish species minced fish production is a well-established process (Neiva et al., 2010), the present market for the minced fish products meant for human consumption is not saturated despite the growing demand. The expansion of the market is significantly hindered by technological and production challenges that should be addressed virtually for each fish species individually - mainly obtaining preferable colour of the final product, its structure as well as organoleptic features acceptable for the final user. In addition, fish mince manufacturing requires increased hygienic and processing care due to mince's rapid spoilage in comparison to traditional products. Currently, the minced fish (various species) is used for manufacture of fish four, fish cakes, (cheaper) fish fingers, surimi, kemaboko, and as a filler in laminated filler blocks with up to 15% mince. There also were attempts to produce fish crackers from minced fish and starch, fish patties from minced rockfish meat (Destura, Haard, 1999) and from minced Indian major carp (Seghal et al., 2008).

In Latvia, as pointed out by the Ministry of Agriculture (Ministry of Agriculture, 2018), very diverse fish products are produced: frozen, salted and smoked fish, unsterilized preserves and ready-to-serve products as well as sterilized canned fish. The fish-processing subsector mainly processes the fish caught in the Baltic Sea. Of the entire assortment of processed fish, 66% is canned fish, the sale of which is hindered in eastern markets because of trade restrictions and lower purchasing power there. The most important raw material for the canning industry produced in Latvia are Baltic sprats. They make up about 70% of the amount of sterilized canned food. Besides the restricted market opportunities, the processing of canned sprats is known for a large amount of waste, and the resulted processed products, in fact, provide little significant added value when comparing raw fish and fish fillets. To increase the level of processing, reduce the amount of by-products and losses of valuable food raw materials, a significant number of, for example, cod processors are producing block minced cod from mechanically dumped bones using separators and neopresses. This process allows valuable fish raw materials involvement in food production, which otherwise requires either expensive disposal or, at best, results in fish flour production. The existing technology suggests that the fish mince production could be an option for the Baltic sprats processing. This introduces an important priority to create new, innovative processed products out of Baltic sprats that are demanded in currently open and prospective foreign markets in order to diversify and increase fish product exports.

Within the European Maritime and Fisheries Fund (EMFF) project No. 16-00-F01101-000005 "Production of structured fish forcemeat from Baltic sea fish and its use in fish products" an innovative structured fish forcemeat was produced out of Baltic sprats along with new, customer-ready products, the introduction of which in production requires an economic analysis.

The project developed recipes and technological know-how for the production of seven new, innovative products from structured fish forcemeat made from Baltic sprats: fish spaghetti, fish dumplings, fish sausage, fish fingers (breaded), fish buns, fish terrine (filled with egg) and fish meatballs (in jelly). The base scenario of the research analyses the establishment of a factory for the production of two products (fish buns and fish meatballs in jelly), as the production of all the seven products requires a considerably larger initial investment, yet the available resources are limited. Therefore, the research aim was to perform an economic assessment of the establishment of a potential factory for the production of innovative fish products from sprats caught in the Baltic Sea. To achieve the aim, two specific research tasks were set:

1) to perform a financial and efficiency analysis of the investment project for the industrial production of the newly developed innovative products;
2) to determine the economic effect from the establishment of the factory.

Materials and Methods

The present research used the performance results, acquired by means of a financial model, for an investment project – the establishment of a fish processing factory producing two products from unprocessed Baltic sprats (fish buns and fish meatballs in jelly). The production process in the factory will comprise a full cycle from raw Baltic sprats to market-ready products of structured fish forcemeat.

The base scenario estimated processing capacity at 408 tons of sprats per year, selling the products in the market of the Baltic States. The research developed a set of detailed assumptions based on the information provided by researchers from Faculty of Food Technology of Latvia University of Life Sciences and

Technologies – S. Muizniece-Brasava, A. Kirse-Ozolina, I. Gramatina, I. Ciprovica, S. Sazonova, E. Straumite, Z. Kruma, M. Sabovics, D. Kunkulberga, J. Kivite, T. Kince, J. Zagorska. Sales prices were assumed, taking into account the price level of similar products and wholesaler and retailer mark-ups. The research has assumed that the entire quantity of products is sold at EXW (ex-works) prices, as the wholesaler is responsible for marketing and distribution, and the wholesaler mark-up accounts for 15% of the retailer purchase price. In the most optimistic case, the sales prices are planned to be lower than the prices in the Baltic market, depending on product group, in order to have an opportunity to sell larger quantities in low purchasing-power markets. However, the price formation could be complicated because the products are innovative and no analogues are available in the market. For this reason, the research used the price level of the most similar products as a benchmark. The financial model assumed that the investment project for the fish processing factory would be implemented without public financial assistance. It is also assumed that the factory operates in a one-shift mode and the factory establishment project begins in January 2019, lasting for 18 months.

The financial model simulated a situation for a period of 10 years. The model simulated the cash flow by year and by month, performed profit or loss account, balance sheet, net present value and payback period calculations and cost and profit margin calculations for each product type and produced a project implementation schedule and a summary of key financial performance indicators for investors. The model also has a set of entry fields for detailed project assumptions.

The key financial performance indicators presented in the research were acquired by employing some of the calculation methods provided by DuPont analysis. DuPont analysis is a common form of financial statement analysis, which indicates that the DuPont components represent an incremental and viable form of information about the operating characteristics of a firm (Houmes, et al., 2018; Bauman, 2014; Soliman, 2008). The mentioned indicators are as follows:

1) EBIDTA – earnings before interest, taxes, depreciation and amortization, in EUR and as a % of net turnover;

2) net margin, %: net profit divided by net turnover and multiplied by 100;

3) ROE – return on equity, %: net profit divided by equity capital and multiplied by 100;

4) ROA – return on assets, %: net profit divided by total assets and multiplied by 100;

5) NPV – net present value: a discounted net present value of future cash flows over the entire period, EUR;

6) IRR – internal rate of return: it is the expected compound annual rate of return that will be earned on a project or investment. Mathematically, the IRR can be found by setting the net present value (NPV) equation (1) equal to zero (0) and solving for the rate of return (IRR) (Schmidt, 2014):

$$0 = NPV = \sum_{n=0}^{N} \frac{\text{CF n}}{(1+IRR)^{n}} \quad (1)$$

where: CF_n – cash flow n^{th} period;
N – holding period;

7) gross value added of the enterprise and its associated enterprises or a group of enterprises, which is determined by the following equation (2):

$$BPV = PVR - Nn + S \quad (2)$$

where PVR – value added at factor cost, EUR;
Nn – indirect taxes, EUR;
S – any subsidies, EUR;

8) value added at factor cost is computed by the following equation (3):

$$PVR = A + KP + Icsd + KI - Ipp - Nc - Nr \quad (3)$$

where A – net turnover, EUR;
KP – capitalised output, EUR;
Icsd – income from other economic activities, EUR;
KI – change in stock, EUR;
Ipp – purchases of goods and services, EUR;
Nc – other taxes on products, which relate to turnover but are not deductible, EUR;
Nr – production-related duties and taxes, EUR.

Results and Discussion

Analysis of financial and investment efficiency indicators for establishing a fish processing factory.

To attract an investor being able to implement the project for the establishment of the factory using the technology developed by the EMFF project and to begin producing the products, it is important to indicatively demonstrate the investment efficiency indicators of the project. The application of financial models for investment projects is practised at the early stage, as the models serve as an instrument for making economically feasible decisions on the projects in principle and for making decisions during the design and implementation stages. Such an approach contributes to a result-focused process, in which financial and investment efficiency indicators are employed as criteria.

According to the base scenario (Table 1), investments in fixed assets are estimated at EUR 6.23 mln., among them EUR 3.32 mln. is necessary for the purchase of equipment. This scenario intends to install equipment for production of structured fish forcemeat as an intermediate product and two production lines, one for fish buns and one for fish meatball in jelly which will be the end products of the factory. The simple and discounted payback periods for the base scenario are equal to five years (r=0.05). The internal rate of return equals 0.35. The direct production cost of restructured and structured fish forcemeat (which is made of sprats, a mixture of food additives, semolina and starch) is

estimated at approximately 0.5 EUR kg^{-1}. For comparison, the cost of minced white fish meat that is currently used to produce competing products is 1.3–1.6 EUR kg^{-1}. A significant cost reduction gives an opportunity to earn a sufficient net margin to arouse interest in potential investors and implement the project. It has to be understood that the greatest risk for an investor is created by the market factor – the real success in selling the new products in the markets of the Baltic States. To achieve the sales volume planned, the new products have to gain a market share of at least 5% in the Baltic market. It is a challenge to supply high-quality innovative products, and this target could be achieved by a highly professional marketing team.

Table 1

Key financial and economic performance indicators (base scenario) for the establishment of the fish-processing factory and the production of fish buns and fish meatballs in jelly from Baltic Sea sprats

Indicator/year	2019	2020	2021 - 2028*
Net turnover, thou. EUR	0	2 359	4 717
EBITDA, thou. EUR	-106	1 591	2 906
EBITDA, %	x	67	62
Net profit or loss, thou. EUR	-106	1 169	2 061
Net margin, %	x	50	44
ROE, %	-4	16	14
ROA, %	-4	15	14
Gross value added, thou. EUR	-38	1 856	3 357
Number of employees, full-time equivalent	2	8	22
Sprat consumption, t	0	204	408
Net present value, thou. EUR	8 636		
IRR	0.35		
Total investment in fixed assets, thou. EUR	6 227		
Investment in current assets, thou. EUR	213		

* Annual average.

Source: authors' calculations (Latvia University of …, 2018)

To reduce the market risk, it is advised to initially implement the base scenario, considering an opportunity to expand the factory in the future and installing additional production lines at the factory for the production of other innovative products, developed by the project, from structured fish forcemeat. The most important practical result from the economic perspective is the fact that the projected investment efficiency level of the project is sufficiently high (Table 1) to arouse interest in investors and continue implementing the project, considering all the market risks. At the same time, it is important to be aware that the implementation of the project could be affected by industry-specific and general factors of the investment environment. For this reason, the implementation of resource-intensive business ideas might require more time. Nevertheless, creating a broad assortment of new, innovative products to be introduced in production that are appropriate for the fish processing subsector of Latvia is a new strength of the fisheries sector of Latvia that could contribute to tackling problems in the sector. New products being ready for introduction in production and support by competent scientists who have developed the products are available for the enterprises of the sector that can cope with challenges in the market, change and grow.

Potential economic effect from the establishment of the fish-processing factory.

Even though the positive effect of establishing the factory on the national economy of Latvia is not a significant factor from the perspective of an investor in this project, it is important from the viewpoint of the society of Latvia. If it is obvious that the factory can considerably contribute to the fisheries sector and the national economy as a whole, this project is worth supporting from the perspective of general public interests. It is advantageous for the investor to use financial assistance provided by the EMFF to establish the factory. Previously, large-scale fish processing projects in Latvia were implemented with the support of the SAPARD programme and, after joining the EU, with the support of the EU Funds. However, no such significant projects have been implemented after 2010, as the global financial crisis lowered purchasing power in significant target markets and the trade restriction imposed by the Russian Federation decreased the exports of sprat products from Latvia, thereby causing a crisis the fish-processing subsector of Latvia. That is why it is useful to project the potential economic effect of the project for the purpose of substantiating the benefit of granting financial assistance by the government.

Baltic sprats represent a strategic resource of Latvia that has to be processed in Latvia, manufacturing finished products for sale. The establishment of the factory and the new products manufactured by it could make an extra contribution to the national economy and diversify the assortment supplied by the fish-processing subsector, while at the same time causing no resource shortage threat to the other fish processors using sprats as an input. The production process at the factory is not labour intensive, as it is automated to a high extent, yet the project is expected to create 22 competitively remunerated jobs.

Table 2

Effect of the fish-processing factory establishment project on the foreign trade balance of Latvia

Indicator	Previous practice	Base scenario
Exports of raw sprats, t	27 735	27 327
Revenue from raw sprat exports, mln. EUR	5.55	5.47
Revenue from exports of structured fish forcemeat products, mln. EUR*	0.00	3.15
Total revenue from exports, mln. EUR	5.55	8.62

* Excluding the part of products sold in the domestic market; it was assumed the price of sprats 0.2 thou. EUR t^{-1} and exports remained at the 2017 level.

Source: authors' calculations (Latvia University of …, 2018)

The establishment of the factory will improve the foreign trade balance of Latvia, as total revenue from fish product exports are expected to rise (Table 2). Exporting unprocessed sprats and assuming that their sales price is 0.2 EUR kg^{-1}, total revenue from exports would be EUR 5.5 mln. In reality, enterprises can generate higher revenue per unit of output, e.g. if freezing sprats by means of individual quick freezing equipment. Implementing the project for manufacturing structured fish forcemeat products slightly decreases revenues from raw sprat exports, whereas the revenues from processed fish products are expected to increase considerably. This economic activity will make a qualitative contribution to the assortment of processed fish products manufactured in Latvia and create new opportunities for entering western markets, mainly offsetting the loss caused by a decrease in the output of sprat products observed since 2014.

Establishing the factory for producing structured fish forcemeat products, compared with manufacturing conventional canned sprat products, will positively affect the national economy of Latvia, contributing to the GDP and foreign trade balance and, to a lesser extent, the environment. The factory establishment project has potential for further processing of sprats and generating high value-added for part of the Baltic sprats currently exported by Latvia. For this reason, other alternatives to Baltic sprat processing have to be sought, thereby taking advantage of generating higher value-added and giving an opportunity for fishing enterprises to earn more revenue.

Conclusions

In the post-crisis period, the fish processing subsector of Latvia needs to manufacture new, innovative products from Baltic sprats that generate higher value-added than conventional canned sprat products do.

Investing EUR 6.23 mln. in establishing the fish processing factory gives an opportunity to manufacture new, innovative products from sprats caught in the Baltic Sea. In the third year of operation, the factory is expected to achieve the planned fish processing capacity – 408 t of fish, a turnover of EUR 4.7 mln. and a net profit of EUR 2 mln. with a 44% net margin, employing 22 full-time employees.

Establishing the factory is financially beneficial not only to the investor of the project but also to the national economy, as the foreign trade balance of Latvia is expected to improve owing to an increase in total revenue from fish product exports.

Creating a broad assortment of new, innovative products to be introduced in production that are appropriate for the fish processing subsector of Latvia is a new strength of the fisheries sector of Latvia that could contribute to tackling problems in the sector. In the future, it is advised to broaden the diversity of new fish products available for introduction in production in terms of kinds of the products, taking into account the specifics of prospective markets and market segments and the aspect of investment capacity. The new products available for introduction and the growing capacity of Latvian scientists will contribute to the development of the fish-processing subsector.

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References

1. Ankvica L. (2018) Marketing of fish products in Latvia in 2017 (in Latvian). **In:** *Latvian Fisheries Yearbook 2018*. Publisher: Latvian Rural Advisory and Training Centre, p. 66–75.
2. Bauman M. P. (2014) Forecasting operating profitability with DuPont analysis: Further evidence, *Review of Accounting and Finance*, Vol. 13 (2), pp.191–205.
3. Benga E., Hazners J. (2018) Fish production and marketing opportunities (in Latvian). **In:** *Latvian Fisheries Yearbook 2018*. Publisher: Latvian Rural Advisory and Training Centre, p. 82–86.
4. Catchpole Th. L., Ribeiro-Santos, A., Mangi, St.C., Hedley, Ch., Gray, T.S. (2017) The challenges of the landing obligation in EU fisheries. *Marine Policy*, Vol. 82, p. 76–86.
5. *Central Statistical Bureau of Latvia (CSP) (2019) RUG020. Sale of manufactured industrial products (PRODCOM at 10-digits), (quantity, thsd euro)*. [accessed on 22.12.2018.]. Available at: http://data1.csb.gov.lv/pxweb/lv/rupnbuvn/rupnbuvn__rupn__ikgad/RUG020.px/?rxid=a39c3f49-e95e-43e7-b4f0-dce111b48ba1
6. Da Rocha J. M., Cervino S., Villasante S. (2012) The common fisheries policy: an enforcement problem. *Marine Policy*, Vol. 36(6), p.1309–1314.
7. Destura F. I., Haard N. F. (1999) Development of intermediate moisture fish patties from minced rockfish meat (*Sebastes* sp.). *Journal of aquatic food product technology*, Vol. 8(2), p. 77–94.
8. Houmes R., Jun CC., Capriotti K., Wang D. (2018) Evaluating the long-term valuation effect of efficient asset utilization and profit margin on stock returns: Additional evidence from the DuPont identity. **In:** *Meditari Accountancy Research*, Emerald Publishing Limited, Vol. 26 (1), pp.193-210.
9. Khalilian S., Froese R., Proelss A., Requate T. (2010) Designed for failure: a critique of the common fisheries policy of the European Union. *Marine Policy*, Vol. 34(6), p. 1178–1182.
10. Kornilovs G., Ustups D. (2018) State of fish stocks and regulation of fishing in the Baltic Sea in 2017–2018 (in Latvian). **In:** *Latvian Fisheries Yearbook 2018*. Publisher: Latvian Rural Advisory and Training Centre, p. 43–57.
11. Laugalis A. (2009) Latvian Fisheries Policy after joining the European Union (in Latvian). Bachelor's work. University of Latvia.
12. Latvia University of Life Sciences and Technologies in cooperation with the Society for the Greatest World (2018) Final report of project No 16-00-F01101-000005 "Production of structured fish forcemeat from Baltic sea fish and its use in fish products" (in Latvian). Jelgava, May 2018, 224 p.

13. Neiva C. R. P., Machado T. M., Tomita R. Y., Furlan É. F., Lemos Neto M. J., Bastos D. H. M. (2011) Fish crackers development from minced fish and starch: an innovative approach to a traditional product. *Food Science and Technology*, Vol. 31(4), p. 973–979.
14. Schmidt, R. (2014) What is IRR and How Does it Work? JUNE 9, 2014. [accessed on 2.01.2019.]. Available at: https://www.propertymetrics.com/
15. Schroeer A., Sakai C., Vulperhorst V., Białaś A. (2011) The European Union and Fishing Subsidies. September 2011, p. 16. [accessed on 3.01.2019.]. Available at: http://baltic.oceana.org/sites/baltic.oceana.org/files/EU_Subsidies_Report_FINAL_FINAL.pdf
16. Sehgal H., Shahi M., Sehgal G. K., Thind S. S. (2008) Some quality aspects of fish patties prepared from an Indian major carp, *Labeo rohita* (Ham.). *International journal of food sciences and nutrition*, Vol. 59(3), p.192–201.
17. Sissenwine M., Symes D. (2007) Report to the general directorate for fisheries and maritime affairs of the European Commission [accessed on 01.02.2019.]. Available at: https://www.researchgate.net/publication/255631911_Reflections_on_the_Common_Fisheries_Policy
18. Soliman T. M. (2008) The use of DuPont analysis by market participants. *The accounting Review*, Vol. 83(3), p. 823–853.
19. Ministry of Agriculture (2018) Agriculture of Latvia in 2018 (in Latvian). Riga, 180 p. [accessed on 01.02.2019.]. Available at: https://www.zm.gov.lv/public/files/CMS_Static_Page_Doc/00/00/01/33/19/Gadazinojums.pdf.

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DETERMINING IMPORTANT FACTORS ON FISH CONSUMPTION WITH CONJOINT ANALYSIS IN TEKIRDAG, TURKEY

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Abstract

In the world, there is an increasing consumption trend from red meat to white meat, especially to the fish. Despite the known benefits of fish consumption on human health, it is still not at the desired level in Turkey. The average fish consumption is 5.58 kg per capita in Turkey, and it is below the World (14.9 kg) and the EU (16.89 kg) average fish consumption. The aim of the study was to determine the importance level given to the factors that respondents consider when purchasing fish. In this study, surveys were conducted with 248 respondents. Conjoint analysis was used to determine the most important factors that influence respondents while purchasing fish. According to the results, fish consumption per capita in Tekirdag is 14.69 kg per year. In conjoint analysis results for all respondents, the most important factors in fish consumption are price (34.2%), form (31.4%), production method (26.9%) and supply channel (7.5%). The factors that influence respondents are changing according to the income groups. While price is the most important factor for respondents with a monthly income 1210 EUR or less, respondents with monthly income 1210 EUR and above pay attention to the processing type factor. Fish price and form are the most important factors for respondents. Retailers should aim to deliver fresh fish to consumers with a low-price policy. Due to this, it is needed to establish fish markets where necessary food controls and inspections are made, consumers relied, complying with European Union standards, fresh and cheap fish is sold.

Keywords: conjoint analysis, fish consumption, consumer behaviour, income levels

Introduction

In recent years, growing world population causes insufficiency of animal protein sources which have an important role in human nutrition. Fish is one of the important sources of animal protein. Fish consumption depends on several factors such as economic factors, the way of the product releasing to the market and consuming seafood habit. Fish which provides important benefits especially with the consumption during the development period of individuals, is consumed in different ways and in different amounts in different regions. The most important reason for this situation is cultural differences and different eating habits between different regions.

Turkey has rich fisheries resources. The average fish consumption per capita is 5.58 kg in Turkey and it is lower than the world and the EU average. The average fish consumption per capita is 14.9 kg per year in world and 16.89 kg per year in EU (FAO, 2018). It is a fact that increasing fish consumption, which is quite important in terms of health, will have a positive effect on future generations.

The aim of the study is to determine the importance level given to the factors that respondents consider when purchasing fish. In this study, the factors that respondents give importance when purchasing fish are determined by using conjoint analysis.

In previous studies using conjoint analysis about fish consumption preferences, price was determined as one of the most important attributes (Halbrent et al., 1992; Harrison et al., 1998; Boughanmi et al., 2007; Akpınar et al., 2009; Claret et al., 2012; Hanis et al., 2013; Geslani et al., 2015). Most of previous studies compared preference of wild-caught and farm-raised fish and wild-caught created higher utility than farm-raised (Akpınar et al., 2009; Claret et al., 2012; Musa et al., 2012; Geslani et al, 2015; Tomic et al., 2017). Halbrent et al. (1992), Harrison et al. (1998) and Boughanmi et al. (2007) measured consumers' fish form preferences. Ariji (2010) determined that the information process is important for marketing and that labelling will be an effective marketing method. Izzhati et al. (2018) reported that the most important criteria in consumers' choice of packaged fish are brand, additional information, colour, material, shape and size.

Materials and Methods

In this study, data was collected from respondents living in Tekirdag province. Also, macro data are from Food and Agriculture Organization of the United Nations, local and international studies, projects and reports.

Tekirdag is located in north western Turkey, in the Marmara Region. Tekirdag is also on the both Marmara Sea and Black Sea coast. Tekirdag is a developed province in terms of agriculture and industrial sectors.

According to the sampling calculated from limited population formula (Miran, 2002) given below (N=48 000 households, 90% confidence interval, 5% margin of error and p=q=0.5 to achieve maximum sample size), face to face surveys were applied to the randomly selected respondents representing 248 different households between January 2015 and May 2015. The number of households included in the sample were proportional to the number of households in the neighbourhoods (1):

$$n = \frac{Npq}{(N-1)\sigma_p^2 + pq} \quad (1)$$

where: n – sample size,
N – population size,
p – expected proportion who consume fish,
q – (1-p),
σ_p^2 – rate variance.

Conjoint analysis is one of the methods used to determine consumer preferences according to the different characteristics of any product. Conjoint

analysis is based on the fact that the complexity of choices depends on more than one factor and each factor depends on two or more levels. The preference degrees given to products by respondents are the basis of the method. In the method, the utility level of each feature discussed are obtained by separating general evaluations for products with different properties offered to the respondent to the qualifications of these products (Green, Srinivsan, 1978).

The basic concept, which is measured as the personal choice judgment of individuals, is called utility. The aim of conjoint analysis within the framework of utility concept is estimation of relative contributions of independent variables to total utility and determination of variables that have the highest utility. Conjoint analysis is a statistical analysis conducted following the steps given below.

a. Determination of Factors and Factor Levels. Each variable used in the conjoint analysis includes various features of the product or service and their actual levels (Saraçlı, Şıklar, 2005).
b. Determination of Combinations. One of the variables is determined as a dependent variable and the effect of other variables on the selection of dependent variable levels is examined. The number of combinations is determined by crossing the levels of all factors involved in the study (full design). However, the very high number of combinations creates problems in terms of both applicability and predictability. For this reason, a subset of all theoretically possible combinations should be selected (orthogonal design). The selected combinations are asked to the respondents to score them.
c. Estimation of Utility Values. Conjoint analysis is used to decide partial utilities (partworths) (β) for all factors based on scored data. In addition, the total utility (y) can be calculated for each combination with these partial utilities. Thus, the relative importance of the combinations is determined.

The additive model of the conjoint analysis (Prelec, 2002) is defined as (2):

$$y_k = \sum_{j=1}^{j} \sum_{m=1}^{m_j} \beta_{jm} x_{jm} \qquad (2)$$

where: y_k – estimated total utility for incentive k
β_{jm} – partial utility for value (category) m of factor j

$$x_{jm} = \begin{cases} 1; if\ incentive\ k\ has\ value\ m\ of\ factor \\ 0; else \end{cases}$$

The partial utilities (β) are estimated by the Ordinary Least Square (OLS) method. In the conjoint analysis, the most preferred combination can be determined by calculating the total utility value for each combination that the respondents scored.

In this study, Conjoint analysis was used in order to determine the importance of the factors of fish purchase and the importance level of these factors.

Table 1

Factors and factor levels used in conjoint analysis

Factors		Factor Levels
Production Method	1	Farm-raised
	2	Wild-caught
Price	1	20% decrease
	2	Current price
	3	20% increase
Supply Channel	1	Restaurant
	2	Fishmonger
	3	Supermarket
Form	1	Canned/Pickled
	2	Fresh
	3	Battered/Breaded

When the factor levels in Table 1 are examined, it is seen that there are 2×3×3×3=54 possible combinations. However, it is not possible for respondents to score 54 combinations in a healthy way.

Table 2

Combinations that respondents scored

Combinations	Production method	Price	Supply channel	Form
1	Farm-raised	20% increase	Fishmonger	F
2	Farm-raised	20% decrease	Restaurant	C / P
3	Wild-caught	Current price	Fishmonger	C/P
4	Wild-caught	20% decrease	Supermarket	F
5	Wild-caught	20% increase	Restaurant	B / B
6	Farm-raised	20% decrease	Fishmonger	B / B
7	Farm-raised	Current price	Restaurant	F
8	Farm-raised	Current price	Supermarket	B / B
9	Farm-raised	20% increase	Supermarket	C / P

F – Fresh, C / P – Canned / Pickled, B / B – Battered / Breaded

9 combinations (the highest number obtained using two multipliers of 54 (3×3=9) as it is seen above) were chosen with orthogonal design from 54 combinations and respondents were asked to score these combinations from 1 to 10. 9 combinations that respondents scored are shown in Table 2.

Results and Discussion

According to the results of the survey, the demographic structures of 248 respondents are shown in Table 3. A 44% of the respondents are female and 56% are male. Majority of respondents, around 59%, are between

26 and 40 years old. Majority of the respondents (around 42%) have bachelor's degree. 64% of the respondents are married and 36% are single. According to the average household income, 13% of the respondents are between 345–689 EUR, 34% are between 690–1209 EUR, 27% are between 1210–1724 EUR and 23% are 1725 EUR and above. According to the average household food expense, 23% of the respondents are between 85–174 EUR, 24% are between 175–259 EUR, 27% are between 260–344 EUR and 21% are 345 EUR and above.

Table 3

Demographic structure of surveyed respondents

Demographic parameters	Groups	Proportion, %
Gender	Female	43.7
	Male	56.3
Age	18–25	10.3
	26–40	58.9
	41–55	26.7
	55+	4.1
Marital status	Married	64.1
	Single	35.9
Education	Primary	5.6
	Secondary	1.8
	High school	21.1
	Associate degree	8.5
	Undergraduate	41.9
	Postgraduate	21.1
Average monthly household income (EUR)	<344	2.9
	345–689	13.0
	690–1209	34.1
	1210–1724	27.0
	1725<	23.0
Average monthly household food expense (EUR)	<85	4.4
	85–174	23.0
	175–259	24.4
	260–344	26.7
	345<	21.5

According to the survey results, fish consumption per capita in Tekirdag is 14.69 kg per year. Conjoint analysis was applied to all respondents without any grouping and respondents' preference criteria are determined. In order to examine whether the income levels of the respondents affect fish purchase preferences, the respondents were divided according to the income groups and the conjoint analysis was repeated.

Conjoint analysis results for all respondents

The utility coefficients and relative importance values obtained for each level of all factors are shown in Table 4. According to the results, the most important factor for respondents when purchasing fish is price with 34.22%. 20% reduction in prices has 1.061 utility coefficient and it provides the most utility. 20% reduction in prices is followed by current prices with 0.225 utility coefficient and 20% mark-up in prices with -1.287 utility coefficient. Also, Halbrent et al. (1992), Harrison et al. (1998), Boughanmi et al. (2007), Akpınar et al. (2009), Claret et al. (2012), Hanis et al. (2013) and Geslani et al. (2015) determined that price is the most important factor in fish consumption.

The second factor is form with 31.36%. The most preferred forms are fresh, canned / pickled and battered / breaded fish with 1.385, -0.619 and -0.767 utility coefficients respectively. Production method is the third factor with 26.96%. Wild-caught fish is preferred with 0.925 utility coefficients when compared farm-raised fish with -0.925 utility coefficient. According to previous studies, most of the consumers prefer wild-caught fish instead of farm-raised fish (Akpınar et al., 2009; Claret et al., 2012; Musa et al., 2012; Geslani et al, 2015; Tomic et al., 2017).

Table 4

Conjoint analysis results

Factor	Factor level	Utility	Relative importance
Constant		3.994	
Prod. Method	Farm-raised	-0.925	26.96
	Wild-caught	0.925	
Price	Current price	0.225	34.22
	20% increase	-1.287	
	20% decrease	1.061	
Supply Channel	Restaurant	-0.269	7.46
	Supermarket	0.243	
	Fishmonger	0.026	
Form	Fresh	1.385	31.36
	Canned/Pickled	-0.619	
	Battered/Breaded	-0.767	

The fourth factor is the supply channel with 7.46%. The first one is the supermarket with 0.243 utility coefficient, the second one is the fishmonger with 0.026 utility coefficient of 0.026 and the third one is restaurant with -0.269 utility coefficient.

When approached in terms of marketing, retailers should aim to deliver fresh fish to consumers with a low-price policy. For this purpose, products must be delivered from production department to the market in the fastest way and the cold chain should not be broken. In order to reduce fish price, it should be aimed to eliminate the intermediaries in the process that passes from the fish production centres or from the ports to the consumers. For this purpose, producers and fishmongers should be able to sell their own products by founding marketing cooperatives.

The utility scores of each combination were calculated by using the utility coefficients given in Table 5. According to the calculated utility scores, the most preferred combination is number 4 with a score of 7.609. In other words, respondents prefer fresh, wild-caught fish sold at the supermarket with 20% reduction price. The second preferred combination is number 3 with 4.552 score. Respondents' second choice is canned / pickled fish done by using wild-caught fish. They prefer purchasing this product from the fishmonger and with current prices.

Table 5

Combination scores used in conjoint analysis

Combination	Production method	Price	Supply channel	Form	Utility score
1	Farm-raised	20% increase	Fishmonger	Fresh	3.194
2	Farm-raised	20% decrease	Restaurant	Canned / Pickled	3.243
3	Wild-caught	Current price	Fishmonger	Canned / Pickled	4.552
4	Wild-caught	20% decrease	Supermarket	Fresh	7.609
5	Wild-caught	20% increase	Restaurant	Battered / Breaded	2.597
6	Farm-raised	20% decrease	Fishmonger	Battered / Breaded	3.390
7	Farm-raised	Current price	Restaurant	Fresh	4.411
8	Farm-raised	Current price	Supermarket	Battered / Breaded	2.771
9	Farm-raised	20% increase	Supermarket	Canned / Pickled	1.407

Table 7

Respondent preferences according to income groups

Factors and factor levels		Income groups*				
		1	2	3	4	5
Production method	Farm-raised	-0.702	-0.871	-1.019	-1.083	-0.671
	Wild-caught	0.702	0.871	1.019	1.083	0.671
Price	Current price	0.032	0.414	0.029	0.373	0.285
	20% increase	-1.968	-1.701	-1.323	-1.120	-1.140
	20% decrease	1.937	1.287	1.294	0.747	0.854
Supply channel	Restaurant	-0.302	-0.207	-0.354	-.104	-0.364
	Supermarket	0.127	0.310	0.264	0.085	0.366
	Fishmonger	0.175	-0.103	0.090	0.019	-0.002
Form	Fresh	-0.016	1.494	1.131	1.644	1.630
	Canned / Pickled	0.127	-0.667	-0.486	-0.607	-0.916
	Battered / Breaded	-0.111	-0.828	-0.645	-1.038	-0.715

*Income groups are given in Table 6

The least preferred combination was the 9th combination with the score 1.407. The least preferred by respondents was canned / pickled products done with farm-raised fish, sold at the supermarket with 20% increased price. When the combinations with calculated utility score are examined in Table 5, it is seen that respondents would buy canned / pickled products from fishmonger if the price is same. Respondents may also prefer to consume farm-raised fish in the restaurant with the condition that the price is same.

Conjoint Analysis Results by Income Groups

Distribution of surveyed respondents by monthly income groups per household is given in Table 6.

Table 6

Distribution of respondents by income groups (EUR)

Income groups	Group code	Number	Ratio (%)
<344	1	8	3.2
344–689	2	29	11.7
690–1209	3	88	35.5
1210–1724	4	65	26.2
1725<	5	58	23.4
Total		**248**	**100.00**

35.48% of the respondents are in the 3rd income group. Other income groups are the 4th group with 26.21%, the 5th group with 23.39%, the 2nd group with 11.69% and the 1st group with 3.23% respectively. When production method is examined, the utility coefficients of all groups are positive for wild-caught fish. In other words, wild-caught fish is priority for all groups. The 4th income group is the most reacting group for production method (Table 7). While 20% increase in prices has negative utility coefficient for each group, in case of a 20% decrease or no change in the prices, the utility coefficients are positive in each group. While the 1st income group is giving the most importance to the 20% decrease in prices, the 4th income group gives the least importance. The 1st income group gives the most reaction in case of a 20% increase in prices. It is observed that there is no significant difference between the reactions to the prices in 4th and 5th income groups. All income groups react to purchase fish from the restaurant. The 5th income group is the most reacting group to purchase fish from the restaurant. The income group that most prefer the supermarket to purchase fish is the 5th income group and the income group that most prefer the fishmonger is the 1st income group. The reasons for this are thought that fish from fishmonger is more affordable, and high perception of prices due to the variety of fish sold in the supermarket. It is seen that supermarkets have high priority in fish purchasing place. It was determined that all groups have negative utility coefficient for battered / breaded fish. The most reactive income group for battered / breaded fish is the 4th income group. Although there is not much difference between the 4th and 5th group in fresh fish, the

respondents in the 4th group preferred fresh fish. While canned / pickled fish is most preferred by the 1st income group, it is least preferred by the 5th income group. The importance levels of factors according to income groups are shown in Table 8.

Table 8

Relative importance of factors according to income groups

Factors	Income levels				
	1	2	3	4	5
Production method	23.31	23.02	28.91	31.37	20.29
Price	**64.82**	**39.48**	**37.13**	27.04	30.16
Supply channel	7.92	6.83	8.77	2.74	11.04
Form	3.95	30.68	25.19	**38.85**	**38.51**

Accordingly, the 1st, the 2nd and the 3rd income groups give priority to price, while the 4th and the 5th income groups give priority to form. Although the form of the fish is the second most important factor for respondents, the demand for processed fish products is quite low. The main reason for this, fresh fish consumption is at the forefront in the Turkish culture. However, living conditions are changing in Turkey as all over the world. It is needed in order to save time and string out the fish consumption to 12 months, fish should be offered to consumers in different forms. While there is a wide range of canned fish in Europe, canned fish products are quite limited in Turkey. Therefore, the products can be diversified and extended to increase the canned fish consumption and thus total fish consumption.

Conclusions

According to the results the two most important factors that respondents consider in purchasing preferences are the price of fish (34%) and the form of fish (31%). Accordingly, it is seen that the price is an important competitive element and respondents prefer to buy fresh fish.

When the factors that respondents give importance to according to income level are examined, low-income group focuses directly on the price regardless whether the fish is canned / pickled, fresh or battered / breaded. The high-income group is more selective and prefers to consume fresh fish.

While fishmonger is preferred as a purchase place by the low-income group, supermarkets are preferred by rest of the income groups

Based on the most important reasons for respondents, fish markets where necessary food controls and inspections are made, consumers relied, complying with European Union standards, fresh and cheap fish is sold, need to be established. Furthermore, these fish markets should be easily accessible and centrally located.

References

1. Akpınar M.G., Dağıstan E., Mazlum Y., Gül M., Koç B., Yılmaz Y. (2009) Determining household preferences for fish consumption with conjoint analysis in Turkey. *Journal of Animal and Veterinary Advances*, Vol. 8(11), p. 2215–2222.
2. Ariji M. (2010) Conjoint analysis of consumer preference for bluefin tuna. *The Japanese Society of Fisheries Science*, Vol.76, p. 1023–1028.
3. Boughanmi H., Al-Musalami J., Al-Oufi H., Zaibet L. (2007) Estimating consumer preferences for value-added fish products in Oman. *Journal of Food Products Marketing*, Vol. 13(2), p. 47–68.
4. Claret A., Guerrero L., Aguirre E., Rincon L., Hernandez M.D., Martinez I., Benito Peleteiro J., Grau A., Rodriguez-Rodriguez C. (2012) Consumer preferences for sea fish using conjoint analysis: Exploratory study of the importance of country of origin, obtaining method, storage conditions and purchasing price. *Food Quality and Preference*, Vol. 26(2), p. 259–266.
5. *FAO Food and Agriculture Organization of the United Nations, Fishery Statistical Databases*. [accessed on 21.12.2018.]. Available: at: http://www.fao.org/ faostat/en/?#data/CL
6. Geslani C., Loke M.K., Barnes-Mauthe M., Leung P. (2015) Seafood purchasing preferences of Hawaii Chefs: Comparing actual purchase to stated preferences from conjoint choice experiment. *Journal of International Food & Agribusiness Marketing*, Vol. 27(1), p. 50–63.
7. Green P.E., Srinivasan V. (1978). Conjoint analysis in consumer research: Issues and outlook. *Journal of Consumer Research*, Vol. 5(2), p. 103–123.
8. Halbrendt C., Bacon R.J., Pesek J. (1992) Weighted least squares analysis for conjoint studies: The case of hybrid striped bass. *ProQuest Agricultural Science Collection*, Vol. 8 (2), p. 187–198.
9. Hanis A., Jinap S., Mad Nasir S., Alias R. (2013) Eliciting Malaysian consumer preferences for marine fish attributes by using conjoint analysis. *World Applied Sciences Journal*, Vol. 28 (12), p. 2054–2060.
10. Harrison R.W., Özayan A., Meyers A.P. (1998). A conjoint analysis of new food products processed from underutilized small crawfish. *Journal of Agricultural and Applied Economics*, Vol. 30 (2), p. 257–265.
11. Izzhati D.N., Mastrisiswadi H., Jazuli T.T. (2018) Consumer preferences for the design of smoked fish packaging using conjoint Analysis. *Jurnal Ilmiah Teknik Industri*, Vol. 17 (2), p. 177–185.
12. Miran B. (2002) Temel İstatistik. Ege Üniversitesi, 314p, İzmir.
13. Musa S.M., Aura C.M., Kundu R. (2012). Wild-caught fish versus aquaculture fish products: a fish marketing concept for aquaculture quality improvement, A case study of Nyanza Region, Kenya. *International Journal of Science and Research*, Vol. 3(9), p. 1972–1978.
14. Prelec D. (2002) Strategic Marketing Measurement. Fall 2002. Massachusetts Institute of Technology: MIT OpenCourseWare, https://ocw.mit.edu.
15. Saraçlı S., Şıklar E. (2005) Bireysel emeklilik şirketlerinin tercihinde etkili olan faktörlerin konjoint analizi ile incelenmesi. *Anadolu Üniversitesi Sosyal Bilimler Dergisi*, Vol. 5 (2), p. 1–12.
16. Tomic M., Luceviz Z., Tomljanovic T., Matulic D. (2017) Wild-caught versus farmed fish – consumer perception. *Croatian Journal of Fisheries*, Vol. 75, p. 41–50.

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EFFECT OF NITROGEN FERTILIZATION ON PROTEIN CONTENT AND RHEOLOGICAL PROPERTIES OF WINTER WHEAT WHOLEMEAL

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Abstract

Winter wheat (*Triticum aestivum* L.) occupies a significant part of the agricultural land in Latvia. High quality winter wheat production is a newsworthy problem nowadays. Growing consumer demand for healthier food is mostly related to wholegrain products in which wholemeal wheat is utilized. The aim of this work is to evaluate the effect of nitrogen fertilization on winter wheat protein content and rheological properties of dough prepared with wholemeal. A field trial was carried out in the study and research farm "Peterlauki" of the Latvia University of Life Sciences and Technologies for three years (2009–2012). The trial included two winter wheat cultivars, like ′Bussard′ and ′Zentos′ (originating from Germany). Nitrogen was applied in spring after the resumption of vegetative growth. Nitrogen top-dressing rates were as follows: 60, 90, 120, and 150 kg ha^{-1}. Grain crude protein content (%N×5.7) was determined by Kjeldahl method. Rheological properties of wholemeal wheat dough were assessed using farinograph, such parameters as water absorption, dough development time, dough stability, degree of softening were tested. Nitrogen fertilization significantly affected protein content and water absorption ($p<0.05$). Wholemeal flour prepared with cv ′Bussard′ exhibited high stability and can be considered as strong flour. In addition, wholemeal flour prepared with cv ′Zentos′ was medium strength flour which can be used in bread baking. The results indicated that application of 120 kg ha^{-1} nitrogen fertilizer for cultivar ′Bussard′ and 150 kg ha^{-1} for cv ′Zentos′ can be recommended, to achieve high-quality wholemeal.

***Keywords*:** winter wheat, nitrogen fertilization, wholemeal, protein content, rheological properties

Introduction

Winter wheat *(Triticum aestivum* L.) is the main cereal crop cultivated in many areas worldwide. Traditional wheat flour is obtained from the endosperm part of the kernel, whereas, wholemeal is obtained by grinding the whole grain. Contrary to endosperm, wholemeal also contains fibre-rich bran, outer coating of the kernel, and wheat germ, the sprouting part of the seed. These parts give wholemeal its distinct flavour, texture and colour, but they also increase the nutritional value of the product because of its natural antioxidants, dietary fibre, B group vitamins etc. (Akhtar et al., 2009).

Climate, fertilization, and cultivar affect baking quality of wheat flavour (Cesevičiené et al., 2012; Koppel, Ingver, 2010; Knapowski, Ralcewicz 2004; Preston et al., 2001). Protein content in wheat grain is related to the strength of dough and it affects bread baking potential (Ahmed, 2015; Linina et al., 2014; Jablonskite-Raškė et al., 2012; Krejčirova et al., 2006; Miš, 2005). Protein accumulation in bread wheat is mostly affected by the climate peculiarities of the particular year and way how nitrogen rates have fluctuated during the growing season (Chope et al., 2015; Krejčirova et al., 2006; Varga et al., 2003). The differences among cultivars are the factors that affect the content of grain protein (Cesevičiené et al., 2012; Linina, Ruza, 2012) as during maturation wheat needs sunlight, moderate humidity and warmth. If the conditions mentioned above are observed, then biological maturity and sufficient rheological and technological properties of the grain will follow (Krejčirova et al., 2006).

Characterization of the rheological properties of dough is a way, which assists in the prediction of processing behaviour and the quality control of baked goods (Koga et al., 2015; Vaicuilute-Funk et al., 2015; Liatukas, Ruzgas, 2012). Lengthy development time, high stability with a small level of softening characterises strong flours, but low stability, short development time, which results in high degree of softening is characteristic for weak flours (Koppel, Ingver, 2010).

Therefore, the goal of the paper is to assess the effect of nitrogen fertilization on the protein content of winter wheat and rheological properties of dough prepared with wholemeal.

Materials and Methods

A field trial was carried out in the study and research farm 'Peterlauki' (latitude: 56º 30.658' longitude: 23º 41.580') of the Latvia University of Life Sciences and Technologies (LLU) during a three-year period from 2009/2010 to 2011/2012. Soil at the site was Endocalcaric Abruptic Luvisol (Word Reference Base) silt loam. The content of organic matter in the soil was 27–31 g kg^{-1}, pH KCl 6.6–7.0.

Winter wheat bread cv ′Bussard′ and ′Zentos′ were sown after black fallow. Sowing was performed in the second ten-day period of September. These cultivars were sown in a plot size of 36 m^2 at the rate of 400 germinating seeds per m^2. Sowing was performed as a quadruple and treatments were arranged in a randomized block design. Phosphorus (P_20_5) and potassium (K_20) fertilizers were applied as 72 kg ha^{-1} and 90 kg ha^{-1} in autumn, respectively. Nitrogen was applied after resumption of vegetative growth in spring. Nitrogen (N) top-dressing rates were as follows: 60, 90, 120 and 150 kg ha^{-1}, and coded as N60, N90, N120 and N150. All plant protection requirements were fulfilled. Winter wheat was harvested on August 4 in 2010, August 5 in 2011 and August 3 in 2012. Sampling procedure for grain quality evaluation was conducted in accordance with the ICC 101/1 standard.

Protein content and rheological properties of wheat wholemeal were determined each year at the laboratories of Latvia University of Life Sciences and

Technologies, Faculty of Food Technology. Protein content (PC, %N×5.7) was determined by Kjeldahl method as described in the standard of ICC 105/2 using Kjeltec System 1002 (Foss Tecator AB, Sweden). Grains were milled to wholemeal using a hammer-mill (Laboratory Mill 3100, Perten, Finland) equipped with 0.8 mm sieve. Farinograph water absorption (WA), dough development time (DDT), dough stability time (ST) and degree of softening (DS12) were determined according to ICC 115/1 standard using Brabender farinograph (Brabender, Germany) equipped with a mixer for 300 g flour sample.

Experimental data were evaluated using two–factor analysis of variance (ANOVA), Fisher's criterion ($p<0.05$), the least significant difference ($LSD_{0.05}$) and the influence of impact factors (η^2) was also determined. Differences of wholemeal rheological properties between both winter wheat cultivars were determined by t-Test: Two-Sample Assuming Unequal Variance. Correlation analysis between nitrogen fertilization rates and wholemeal rheological properties was carried out.

Results and Discussion

Protein content is the main quality criterion that influences the properties of baking quality of flour (Koppel, Ingver, 2010). Protein content of 12–13% is suitable for bread making. The amount of wet gluten, which can be used as an indicator, also affects the baking quality of grains used for bread (Linina, Ruza, 2012).

Protein content in cv ′Bussard′ increased from 139.2 to 147.5 g kg^{-1} during the tree year period, while in cv ′Zentos′ it increased from 113.2 to 131.2 g kg^{-1}, as a result – being much lower ($p<0.05$) (Table 1).

Table 1

Protein content (g kg^{-1}) in winter wheat wholemeals depending on nitrogen fertilizer rate

Nitrogen (N) fertilization rate	Cultivars	
	′Bussard′	′Zentos′
N60	139.2[a]	113.2[a]
N90	139.8[a]	118.5[b]
N120	147.2[b]	127.5[c]
N150	147.5[b]	131.2[d]

The means marked with the same letter in the same column are not significantly different ($p>0.05$).

Grain protein content significantly varies depending on the cultivar and nitrogen fertilization rate as reported previously (Marti et al., 2015; Cesevičiene, 2012; Jablonskité-Raščé et al., 2012; Kučerova, 2005). Marti et al. (2015) reported that the content of albumin, globulin and glutenin proteins in grain gradually increased with the increase of amount of nitrogen. In the current research, nitrogen fertilization significantly ($p<0.05$) increased grain protein content for both cultivars. The highest protein content was found in the grains of both cultivars grown in the plots treated with N150. Protein content in cv ′Bussard′ fertilized at all doses was high and the treatment can be used to improve the quality of grains. The cv ′Zentos′ fertilized with the treatments of N120 and N150 could be suggested for bread wheat cultivars.

Dough quality is one of the most significant factors letting to foresee the ultimate bread-making value of winter wheat cultivars (Liatukas, Ruzgas, 2012). The rheological properties of wholemeals were tested by assessing water absorption (g kg^{-1}), dough development time (min), its stability time (min) and its extent of softening (FU-farinograph unit).

Water absorption is an important characteristic for wheat flour (Cesevičiené et al, 2012; Koppel, Ingver, 2010). Water must be mixed into the flour at an optimal stage for the dough to be able to reach the stage, where it has 'optimum development'. In comparison to weak flours, strong wheat flours are able to absorb and preserve more water (Miš, 2005). Therefore, if the dough development time is short, the dough mixing time will also be shorter (Sabovics, Straumite, 2012). According to Kopel and Ingver (2010) an appropriate water absorption value for yeast bread is 550–650 g kg^{-1}.

In the present investigation, the water absorption ranges from 707 to 732 g kg^{-1} for cv ′Bussard′ wholemeal and from 677 to 706 g kg^{-1} for cv ′Zentos′ (Table 2), thus suggesting that both wholemeals are strong.

Table 2

Water absorption and dough development time in winter wheat wholemeals in relation to nitrogen fertilization rate

Nitrogen (N) fertilization rate	Cultivars			
	′Bussard′		′Zentos′	
	WA	DDT	WA	DDT
N60	707[a]	4.56[a]	677[a]	5.25[a]
N90	716[b]	4.47[a]	675[a]	5.85[b]
N120	727[c]	4.83[b]	690[b]	5.98[c]
N150	732[c]	5.16[c]	706[c]	6.39[d]

WA – water absorption (g kg^{-1}); DDT – dough development time (min).

The means marked with the identical letter in the same column do not differ significantly ($p>0.05$).

Water absorptions in wheat flours varies depending on the cultivars (Marti et al., 2015), and in our investigation, wholemeal prepared from cv ′Bussard′ had a much higher water absorption level ($p<0.05$). In the report of Vaiciulyte-Funk et al. (2015), water absorption of refined wheat flour for cv ′Zentos′ was 607 g kg^{-1}, and for cv Portal – 656 g kg^{-1}. Kalnina et al. (2015) showed that wholemeal flour absorbs a higher amount of water, the tested cv ′Zentos′ showed a water absorption of 692 g kg^{-1} as similar results can also be seen in this study. Cesevičiené et al. (2012) found that wheat flour obtained from winter wheat grown in conventional production (nitrogen fertilization of 120 kg ha^{-1}) had water absorption values range from 594 to 627 g kg^{-1}, while water absorption in flours from organic production (without nitrogen fertilization) were within the range of 573 to 603 g kg^{-1}. An increase in nitrogen fertilization significantly increases protein content and water absorption

(Miš et al., 2005; Wooding et al., 2000) and these data are in agreement with our findings.

Dough development time (DDT) shows the relative strength of the dough and may also reflect on the degree of water absorption. Dough development time of the two cultivars was high, DDT values of cv ′Bussard′ and cv ′′Zentos′ ranged from 4.56 to 5.16 min and 5.25 to 6.39 min, rescpectively (Table 2). Kalnina et al. (2015) had similar results by discovering that DDT value of wholemeal cv ′Zentos′ was 6.30 min, while Cesevičiené et al. (2012) showed 2.4 min DDT for refined flour of the same cultivar. The excessively high DDT could be caused by the presence of higher moisture content of the bran particles in wholemeal, where bran particles may have delayed dough gluten development (Haridas Rao et al., 1989).

When wheat was treated with higher doses of nitrogen fertilization, its wholemeal significantly extended DDT as reported by Cesevičiene et al., (2012). Dough development time is also influenced by protein content of wheat flour (Vaiciulyte-Funk et al., 2015). This study also proved it.

Stability time of the dough (ST) is a very important factor which affects the possible level of fermentation and mechanical stress the dough can be exposed to. Good quality dough has a stability from 4 to 12 min which is defined as a strong flour (Kopel, Ingver, 2010). Dough stability is a significant indicator in determining flour strength by observing the quantity and quality of dough protein content (Kučerova, 2005). In our investigation, it was found that dough stability of cv ′Bussard′ and cv ′Zentos′ wholemeal was within the range of 9.31–10.41 min and 6.89–7.08 min, respectively, when an increased dose of nitrogen fertilization (N120 and N150) was applied (Table 3).

Similar outcomes were found in the study by Varga et al. (2003). Wholemeal obtained from cv ′Bussard′ had a significantly higher dough stability time than that of the cv ′Zentos′ ($p<0.05$), however, both cultivar wholemeals were strong. Flour stability is related with cultivar properties, which confirms the reports of Kopell and Ingver (2010) published before. Water absorption and mixing properties of wholemeal dough could be improved by increasing wheat protein content. Softening degree (DS12) is the distance from the centre of the curve to 500 FU (farinograph units) consistency line after 12 minutes from the end of the dough development time. Dough mixing quality is taken as satisfactory when the softening value is less than 70 FU (Williams, 1997). Degree of softening of cv ′Bussard′ ranges from 21.18 to 26.78 FU and that of wholemeal cv ′Zentos′ ($p<0.05$), however, both cultivar wholemeals were ′ cultivar from 37.4 to 50.2 FU (Table 3).

Genetic characteristics of cultivar influence dough softening (Liatukas, Ruzgas, 2012), it was also confirmed in our study. When nitrogen application was increased during the growth of cv ′Zentos′, ($p<0.05$), however, both cultivar wholemeals were the degree of softening values of its wholemeal tended to decline, this was in agreement with earlier reports (Cesevičiene et al., 2012). Conversely, wholemeal cv ′Bussard′ fluctuated with the increase of nitrogen fertilization level.

Table 3

Dough stability time and degree of softening of winter wheat wholemeals depending on nitrogen fertilization rate

Nitrogen Fertilizer (N)	Cultivars			
	′Bussard′		′Zentos′	
	ST	DS12	ST	DS12
N60	7.84^{a}	21.18^{c}	6.34^{a}	50.2^{c}
N90	8.49^{b}	25.13^{b}	6.77^{b}	44.6^{b}
N120	9.31^{c}	22.28^{a}	6.89^{c}	40.1^{a}
N150	10.41^{d}	26.78^{b}	7.08^{d}	37.4^{a}

ST – dough stability time (min), DS12 – dough degree of softening value after 12 min (FU – farinograph units).
The means marked with the identical letter in the same column are not significantly different ($p>0.05$).

By Fisher's criteria, nitrogen fertilizer and year (weather conditions in trial years), and interaction of nitrogen fertilizer × year significantly ($p<0.05$) impacted (η^2) the protein content of wholemeal and such rheological parameters as water absorption, time of dough development and stability as well as softening degree (Table 4).

Table 4

Impact factors (η^2) of winter wheat wholemeal quality indices, (%)

Source of variation	PC	WA	DDT	ST	DS12
	cv ′Bussard′				
N	7.6	39.9	14.8	28.5	10.6
Y	87.3	39.1	77.3	55.9	58.1
N×Y	4.4	17.9.	7.1	15.2	29.1
	cv ′Zentos′				
N	19.8	38.5	38.3	3	22.8
Year	78.8	43.5	45.2	94	53.1
N×Y	1.1	10.7*	15.1	2.9	20.5

N – nitrogen fertilizer, Y – year, N×Y – nitrogen and year interaction, PC – protein content, WA – water absorption, DDT – dough development time, ST – dough stability, DS12 – degree of softening, * – not significant

Winter wheat grain protein content depended on year by 87.3% (cv ′Bussard′) and 78.8% (cv ′Zentos′), while the influence of nitrogen fertilizer was also remarkable – 7.6% for cv ′Bussard′ and 19.8% for cv ′Zentos′. The lowest impact was found in the interaction of nitrogen fertilizer × year.

Nitrogen fertilizer significantly affected water absorption values of wholemeals. Conversely, the lower impact was found on dough mixing properties. Koppel and Ingver (2010) found influence of the year on protein content, water absorption and dough stability time as the most remarkable result, a total of 15 winter and 14 spring wheat cultivars were harvested and tested in Estonia in the years between 2004–2007. The accumulation of protein in wheat kernels is better if the

weather is warmer, there is more sunlight and less moisture (Cesevičiene et al., 2012).

In 2010 and 2011, the average temperature from April to July was >+14 °C, which led to more favourable conditions for protein synthesis in grain, while cool and rainy weather caused adverse effect during grain filling and maturation period in the year of 2012. The two cultivars had the lowest protein content sin 2012.

A statistically important moderate positive correlation was observed between nitrogen fertilizer and water absorption value of wholemeal cv ′Bussard (r=0.633) and cv Zentos′ (r=0.592) (n=12, $r_{0.05}$=0.576). Dough development time for cv Zentos exhibited moderate positive relationship with nitrogen fertilizer (r=0.608).

Conclusions

By increasing nitrogen fertilizer rate, the grain technological properties of both winter wheat cultivars were improved significantly. ′Bussard′ cultivar had higher values of crude protein content than cv ′Zentos′. Wholemeal of cv ′Bussard′ can be considered as strong flour, with a high mixing value with weaker flour, but the wholemeal of cv ′Zentos′ was medium strong and it could be used for direct bread baking. In order to achieve good grain quality for producing wholemeal, 120 kg ha^{-1} of nitrogen fertilizer should be used for cv ′Bussard′, and 150 kg ha^{-1} for cv ′Zentos.′

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References

1. Ahmed R., Ali R., Khan M.S., Moin M., Kazmi S.I., Sayeed S. A. (2015) Comparative study on the physicochemical and rheological parameters of soft wheat flour obtained from three countries. *American Journal Food Science and Nutrition Research*, Vol. 2 (3), p. 89–93.
2. Akhtar S., Anjum F.M., Rehman S.U., Sheikh M.A. (2009) Effect or mineral fortification on rheological properties of whole wheat flour. *Journal of Texture Studies,* Vol. 40, p. 51–65.
3. Cesevicienė J., Slepetiene A., Leistrumaite A., Ruzgas V. (2012) Effects of organic and conventional technological properties. *Journal of the Science of Food and Agriculture,* Vol. 92 (14), p. 2811–2818.
4. Chope G.A., Wan Y., Penson S.P., Bhandari D.G., Powers S.J., Shewry P.R., Hawkesford M.J. (2015) Effect of genotype, season, and nitrogen nutrition on gene expression and protein accumulation in wheat grain. *Journal of Agriculture and Food Chemistry,* Vol. 62, p. 4399–4407.
5. Haridas Rao P., Leelavathi K., Shurpalekar S.R. (1989) Effect of damaged starch on the chapatti–making quality of whole wheat flour. *Cereal Chemistry*. Vol. 66 (4), p. 329–333.
6. Jablonskité-Raščé D., Maikštenienė S., Mankevičienė A. (2012) Evaluation of productivity and quality of common wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.) in relation to nutrition conditions. *Zemdirbyste-Agriculture,* Vol. 100 (1), p. 45–56.
7. Kalnina S., Rakcejeva T., Kunkulberga, D., Galoburda R. (2015) Rheological properties of whole wheat and whole triticale flour blends for pasta production. *Agronomy Research,* Vol. 13 (4), p. 948–955.
8. Knapowski T., Ralcewicz M. (2004) Evaluation of qualitative features of Mikon cultivar winter wheat grain and flour depending on selected agronomic factors. *Electronic Journal of Polish Agricultural Universities,* 7 (1). [accessed on 08.01.2019.]. Available: http://www.ejpau.media.pl/volume7/issue1/agronomy/art-01.html
9. Koga S., Böcker U., Moldestad A., Tosi P., Shewry P.R., Mosleth E.F., Uhlen A.K. (2015) Influence of temperature on the composition and polymerization of gluten proteins during grain filling in spring wheat (*Triticum aestivum* L). *Journal of Cereal Science,* Vol. 65, p. 108–113.
10. Koppel R., Ingver, A. (2010). Stability and predictability of baking quality of winter wheat. *Agronomy Research,* Vol. 8 (III), p. 637–644.
11. Krejčirova L., Capouchova I., Petr J., Bicanova E., Kvapil R. (2006) Protein composition and quality of winter wheat from organic and conventional farming. *Zemdirbyste-Agriculture,* Vol. 93 (4), p. 285–296.
12. Kučerova J. (2005). The effect of sites and years on the technological quality of winter wheat grain. *Plant, Soil and Environment*, Vol. 51 (3), p. 101–109.
13. Liatukas Ž., Ruzgas V., Razbadauskienė K., Brazauskas G., Koppel R. (2012) Winter wheat cultivars ‘Kovas DS’, ‘Zunda DS’, ‘Vikaras DS’, ‘Kaskada DS’ for high input farming: development and characterization. *Zemdirbyste-Agriculture,* Vol. 99 (3), p. 225–264.
14. Linina A., Ruza A. (2012) Cultivar and nitrogen fertilizer effects on fresh and stored winter wheat grain quality indices. *Proceedings of Latvian Academy of Sciences, Section B,* Vol. 66 (4/5), p. 177–184.
15. Linina A., Kunkulberga D., Ruza A. (2014) Influence of nitrogen fertiliser on winter wheat wholemeal rheological properties. *Proceedings of Latvian Academy of Sciences, Section B,* Vol. 68 (3/4), p.158–165.
16. Marti A., Ulrici A., Foca G., Quaglia L., Pagani, M.A. (2015) Characterization of common wheat flours (*Triticum aestivum* L) through multivariate analysis of conventional rheological parameters and gluten peak test indices. *LWT–Food Science and Technology,* Vol. 64, p. 95–103.
17. Miš A. (2005) Influence of chosen factors on water absorption and rheological properties of gluten of bread wheat (*Triticum aestivum* L), *Acta Agrophysica,* Vol. 128 (8), p. 1–120.
18. Preston K.R., Hucl P., Townley-Smith T.F., Dexter J.E., Williams P.C., Stevenson S.G. (2001) Effects of cultivar and environment on farinograph and Canadian short process mixing properties of Canada western red spring wheat. *Canadian Journal of Plant Science,* Vol. 81 (3), p. 391–398.
19. Sabovics M., Straumite E. (2012) Rheological properties of triticale (Triticosecale Wittmack) flour blends dough. **In:** *Annual 18 International conference*: *Research for Rural Development,* Conference Proceeding. Vol. 1, p. 143–148.
20. Vaiciulute-Funk L., Joudeikiene G., Bartkiene, E. (2015) The relationship between wheat baking properties, specific

high molecular weight glutenin components and characteristic of varieties. *Zemdirbyste-Agriculture*, Vol. 102 (2), p. 229–238.
21. Varga B., Svecnjak Z., Jurkovic Z., Kovacevic J., Jukic, Z. (2003) Wheat grain and flour quality as affected by cropping intensity. *Food Technology and Biotechnology*, Vol. 41 (4), p. 321–329.
22. Williams P. (1997) Variety development and quality control of wheat in Canada: Characterization by functionality. **In:** *International Japanese conference on Near-Infrared Reflectance, Proceedings of International Conference*, Japan, p. 1020–1044.
23. Wooding A.R., Kavale S., MacRitche, Stooddard F. L., Wallace A. (2000) Effect of nitrogen and sulfur fertilizer on protein composition, mixing requirements, and dough strength of flour wheat cultivars. *Cereal Chemistry*, Vol. 77 (6), p. 798–807.

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EFFECT OF ORGANIC AND CONVENTIONAL PRODUCTION SYSTEMS ON THE WINTER WHEAT GRAIN QUALITY

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Abstract

Cereal crops are cultivated worldwide in diverse environments. In Latvia wheat (*Triticum aestivum* L.) is the most common cereals. One of the most important wheat quality indices are gluten quantity and quality. The aim of this experiment was to assess the effect of different farming systems (organic and conventional) on wet gluten and quality of four winter wheat cultivars and one grain line. Field investigation with winter wheat cultivars 'Fredis', 'Edvins', 'Skagen', 'SW Magnific' and line '94-5-N' were carried out within the framework of value for cultivation and use of plant variety testing system (VCU) at the Research Institute of Agronomy (in Skriveri) of Latvia University of Life Sciences and Technologies, in 2017/2018. Gluten content, index and water-binding capacity were significantly ($p<0.05$) affected by the agricultural production systems and cultivars. In our trial were found statistically significant differences among agricultural production systems. Significantly lower gluten content and water binding capacity had in organic winter wheat grains, while gluten was significantly stronger, compared to the conventional. A statistically significant ($p<0.01$) positive correlation was found between winter wheat gluten content and water-binding capacity ($r=0.999$) for both production systems. The cultivars 'Fredis' and 'Edvins' had better gluten content and water-binding capacity that make them more suitable for the organic production systems, compared to other cultivars.

***Keywords*:** winter wheat, gluten content, gluten index, water-binding capacity

Introduction

Winter wheat (*Tritticum aestivum* L.) is one of significant and the most productive cereal species in Latvia used for food grain production, especially for bread preparation (Linina, Ruza, 2018). Researcher Jaskulska et al. (2018) describe that the cereal of wheat is the most important crop, next to corn, rice and soybean, is allocated for human consumption and for animal feed. They continue that after processing, grain is used for pastas, groats, flour and bread or added to other food and feed products. Husenov (2018) explains that wheat grain is rich in carbohydrates and has higher protein content than other major cereals, such as rye, maize and rice. It also contains substantial amounts of vitamins, minerals (e.g., Fe, Zn) and phytochemicals, making it a good source of nutrition. Wheat bread is an important component of human diet as a source of energy due to the high content of proteins as well as carbohydrates. Different combinations of proteins and carbohydrates in the wheat flour allow the production of different types of bread. Husenov (2018) also found that the bread making quality varies with backing technologies, type of bread as well as cultural traditions, it is not straight-forward to define the universal criteria for bread making quality.

Food wheat must be of good quality. Grain quality significantly varies depending on the various among cultivars (Linina, Ruza, 2012; Dekic et al., 2018; Litke, Gaile, 2018; Šekularac et al., 2018). Researchers Cesevičienė et al. (2009) and Kreičirova et al. (2006) in their study emphasizes that during ripening wheat needs moderate moisture and sunny and warm weather. Such circumstances secure biological maturity and acceptable technological properties of cereal.

To a large extent, cereal quality depends on the content of vitamins, antioxidants and nutritional compounds, also mineral nutrients, content of organic compounds (carbohydrates, fat and protein and its fractions) (Jaskulska et al., 2018).

Mis (2005) describe that gluten quality and quantity are important indices for technological processing of wheat. He also emphasizes that content of gluten is commonly used as a predictor of baking quality. Gluten index has frequently been used as a parameter of technological quality, having in mid that it is determined faster and requires a smaller amount of flour when compared to farinographic parameters (Oikonomou et al., 2015).

Jaskulska et al. (2018) describe that a special biological and performance function is played by protein, especially the gluten fraction. Glutenin and gliadin ensure dough elasticity and extensibility. Gluten determines elasticity, softness and cohesion of bread both fresh and after storage. The baking value of cereal and flour describes many traits, most importantly those, which characterize its protein complex (the content of total protein and wet gluten) and enzymatic complex (falling number).

Some researchers found that the value of the gluten parameter is affected by genotype and crop-years weather conditions. Temperature and precipitation of grain filling have a significant impact on the gluten content and index (Skudra, Ruža, 2016).

Curic et al. (2001) determined that gluten is capable of forming cohesive and adhesive masse, films and three-dimensional networks, all essential to baking performance and gluten content increases with the amount of total protein content. Researchers emphasizes that gluten proteins can be categorized based on their solubility into gliadins (alcohol-water soluble) and

glutenins (insoluble). Both glutenins and gliadins had impact to the properties of gluten. The glutenins provide strength and elasticity of dough, while gliadins create viscosity requirea for dough development. As defined in the trials, then the optimum ratio between gliadin and glutenin for high quality of gluten is found to be 1:1:1 (Curic et al., 2001). Hussain et al. (2009) describe that sunny weather and low amount of precipitation after the postanthesis stage increases the content of gluten and protein content. Jaskulska et al. (2018) found out that nitrogen fertilisation makes a similar effect. It is closely connected with obtained grain yield and quality. Krejčirova et al. (2006) confirms that the relative low nitrogen availability in organic production systems limits cereal and plant nitrogen; that way, both are influenced by crop year weather condition and cultivar. Researcher from Lithuania Cesevičienė et al. (2009) describe that it is known that cereal grown under organic production system has lower gluten and protein content than the conventional. Therefore it is important to have cultivars well adapted to organic production system, to get good quality.

The aim of research was to assess the effect of different farming systems (organic and conventional) on wet gluten and quality of four winter wheat cultivars and one grain line.

Materials and Methods

Field study

Field experiment in conventional and organic farming systems was carried out (2017/2018) at the Research Institute of Agronomy (Latvia University of life Sciences and Technologies) in Skriveri (56º 19′ N and 11º 24′ E). Winter wheat (*Triticumaestivum*L.) cultivars 'Fredis', 'Edvins', '94-5-N' (all from Latvia), 'Skagen' (Germany) and 'SW Magnific' (Sweden) were sown on 27th September (conventional) and on 2nd October (organic) in 2017 after black fallow in four replications (rate of 500 germinating seeds per m^2), a plot size of 16 m^2, field layout – randomised.

Soilatthe site was silty clay loam/clay. Soil agrochemical characteristics were as follows: in conventional production system (CON) – organic mater 3.3%, pH KCl 5.9, in organic production system (ORG) – organic mater 2.5%, pH KCl 6.7 and medium phosphorus and potassium content easily utilized by plants (in both production system).

In conventional production system (before sowing) plots were fertilized with complex NPK fertilizer 8:19:29 300 kg ha^{-1} nitrogen (N) 24 kg ha^{-1}, phosphorus (P) 57 kg ha^{-1} and potassium (K) 87 kg ha^{-1}.The first dose of nitrogen 85 kg ha^{-1} was given in spring at the beginning regrowth - starting of tillering (BBCH 20-25), the second time at the stem elongation (BBCH 32-35) N 60 kg ha^{-1} and the third time – at the beginning of heading (BBCH 51-53) (40 kg ha^{-1}). All the necessary plant protection measures were performed. Winter wheat was harvested at the growth stage GS 88-91, on 27th July in organic and on 6th August in conventional system. Harvested grain of each cultivar and plot was put into separate bags for analyses. The grain exceeding 14% moisture content was dried.

Weather data collection

Autumn of 2017 was long and wet. Winter was mild and favourable for good wheat overwintering. The air temperature in April 2018 was by 4.1 °C higher compared with long-term average observations. In Mai was by 5.1 °C warmer, which hat affected plant growth and development (Table 1).

Table 1

Weather conditions during the field investigation

	2018	LTM*	+ or –from LMT
Month	Average temperature, ºC		
April	9.0	4.9	+4.1
May	16.5	11.4	+5.1
June	16.9	15.0	+1.9
July	20.6	16.6	+4.0
Average	15.8	12.0	+3.8
	Sum of precipitation, mm		
April	43.3	47.0	-3.7
May	23.7	55.0	-31.3
June	36.1	69.0	-32.9
July	61.6	88.0	-26.4
Sum	164.7	259.0	-94.3

*LTM–long term mean

In June average daily temperature was by 1.9 °C which contributed to the accumulation of gluten and protein content. Temperature in the grain filling period (July), which is particularly important for grain quality formation, was by 4.0 °C warmer than the long- term average mean data. Precipitation in 2018 was by 94 mm less than long-term means data.

Technological properties of wheat

The winter wheat grain wet gluten index, gluten content and water-binding capacity were determined at the Latvia University of Life Sciences and Technologies, Institute of Soil and Plants Research in the Grain and Seed Research Laboratory according to Perten using Standard ICC 155.

Laboratory Mill 3100 (Perten Instruments, Sweeden) was using for grains milled to wholemeal at a particle size of 8.0 mm. Wet gluten was washed from wholemeal by an automatic gluten washing equipment (Glutomatic) and centrifuged through a specially constructed sieve under standardized conditions. The weight of the wet gluten was the weight of the gluten was passed.

The weight of the wet gluten was the weight of the gluten was passed. The total wet gluten was then dried (Glutork 2020) and weighed. The difference between the weights of total wet gluten and total dry gluten was calculated, which gave the weight of water bound in the wet gluten and this was water-binding capacity. Total wet gluten contents were expressed as percentage. The gluten index is the ratio of the wet gluten on the sieve (after gluten centrifugation) to the total wet gluten.

Statistical analysis

Two-factor analysis was used to determine significant differences. The Fisher's criterion was applied to

estimate the effects of production systems and cultivars. Component of variance ANOVA for each Quality characteristic was expressed as percentage to illustrate the relative impact of each source to the total variance. One-factor of variance by Fisher's criteria and least significant difference ($LSD_{0.05}$) were applied toassess the effect of conventional and organic production system to each of cultivar separately. Significance of the gluten content and quality between conventional and organic production systems determined by t-test: Two Sample Assuming Unequal variance. Correlation analysis between wet gluten, gluten index and water-binding capacity was also carried out.

Results and Discussion

Winter wheat grain gluten content, gluten index and water-binding capacity were significantly ($p<0.05$) affected by agricultural production system and cultivars. In conventional system, mean gluten content was 30.2% (V=8.0%) and it was higher than in organic system 21.8% (V=7.4%).The least variation of the grain gluten index (average 86.1) was noticed in the organic production system, with coefficient of variation of 10.1%.

Table 2

Winter wheat grain quality indices mean for all cultivars

Quality indices	Mean ±standard error	min	max	V%
Conventional production system (CON)				
WG,%	30.2±1.1	27.3	33.5	8.0
GI	67.5±7.9	47.0	86.0	26.0
WB,%	202.1±10.9	173.0	235.0	12.0
Organic production system (ORG)				
WG,%	21.8±0.7	20.3	24.3	7.4
GI	86.1±7.9	71.0	93.0	10.1
WB%	117.8±7.2	103.0	143.0	13.7

WG–gluten content, %; GI–gluten index,
WB–water binding capacity, %.

Scientists Mikos and Podolska (2012) from Polish reported that Gluten content is an important indicator of grain quality for the bread making industry. In Latvia, grain processing companies classify wet gluten into four classes. The first class is called to as very good with wet gluten content above 28.0%. The Second class is called to as good with gluten above 26.0%. In turn the third class is considered when wet gluten is above 24.0% and the fourth class is called to as low – containing wet gluten below 20.0%. The grain analyses in our investigation suggested that gluten content in conventionally grown grains was significantly ($p<0.05$) higher than in organically grown grains.

The gluten content in conventional production system ranged from 27.3% (line '94-5-N') to 33.5% ('Fredis') (Figure 1). Gluten content of harvested grains corresponded to criterion stated for food wheat (first and second class). The differences in gluten content among different samples can be explain by genetic variation in the winter wheat cultivars.

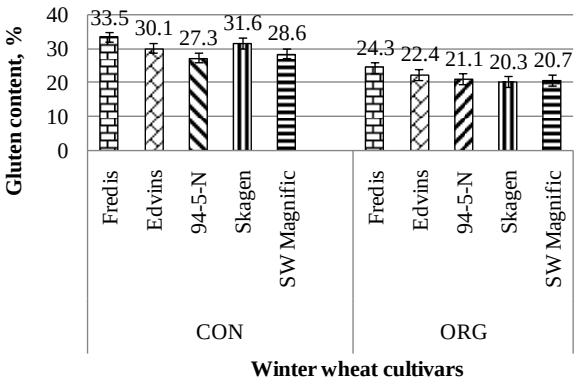

Figure 1. Winter wheat wholemeal gluten content in conventional (CON) and organic (ORG) production systems

Cesevičienė and co-authors (2012) in Dotnuva (Lithuania) found similar results (2012). Scientists concluded that warmer weather was more beneficial for the formation of protein (also gluten content) in winter wheat grains. Litke and Gaile (2018) reported that gluten content in winter wheat cultivar 'Skagen' in conventional system was from 14.7% (without nitrogen fertilization N0) to 25.9% (N180). The gluten content in organic production system ranged from 20.3% ('Skagen') to 24.3% ('Fredis') (Figure 1). Gluten content corresponds to food grain demands (fourth class). Konvalina et al. (2011) reported that gluten content in organic farming was from 18.1 to 23.6% in winter wheat cultivars grown in Czech Republic, because plants lack nutrients. These results agree with the findings of our study.

Gluten index is an indicator of gluten strength (Šekularac et al., 2018). Weak gluten has a gluten index value <30, the normal gluten index from 30 to 80 and strong gluten index >80 (Oikonomou et al., 2015). In both production systems gluten index varied from normal to strong (Figure 2). The highest gluten index showed 'Skagen' (in CON 86.0 units) and 'Edvins' (in ORG 93 units), while the lowest gluten index was measured for line '94-5-N' (in CON 52.8 units, in ORG 71.4 units). The gluten index in organic production system wassignificantly ($p<0.05$) higher compared to conventional. With the decrease in gluten content (ORG), the gluten index increased (Cesevičienė et al., 2012). Similar results obtained in our study.

Vaiciulute-Funk et al. (2015) showed that the gluten content (for six cultivars of winter wheat) was from 13.8% to 32.8% and gluten index from 11 to 94 units. Vaiciulute-Funk et al. (2015) also confirmed that the gluten index and gluten content of different winter wheat cultivars may vary in the same growing conditions, similar results we obtained in our experiment too.

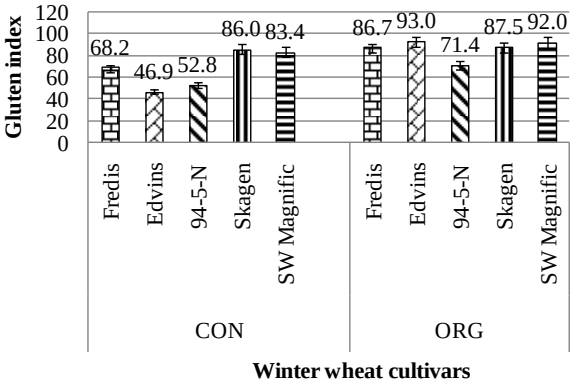

Figure 2. Winter wheat wholemeal gluten index in conventional (CON) and organic (ORG) production systems

Water-binding capacity is the ability of the gluten to attract and retain a certain amount of water. Water-binding capacity has significant influence on the rheological properties of the dough (Mis, 2005). In our investigation, water-binding capacity in organic production system was from 107% ('SW Magnific') to 143% ('Fredis'), and in conventional from 173% ('94-5-N') to 235% ('Fredis') (Figure 3). Water binding in wet gluten depended on cultivars.

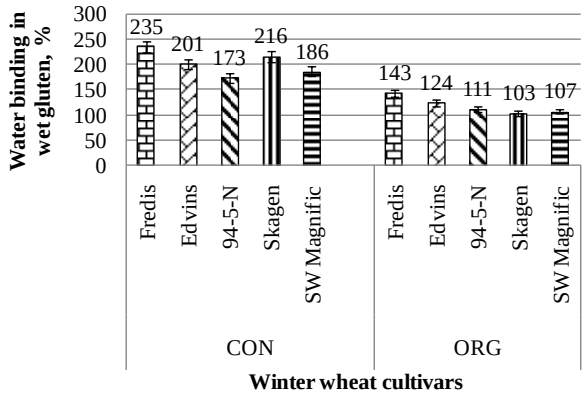

Figure 3. Winter wheat wholemeal water-binding capacity in conventional (CON) and organic (ORG) production systems

Data in Figure 1, 3 present a significant positive correlation was found between gluten content and water-binding capacity in both production systems: CON r=0.999, R^2=0.996, a regression equation y=1.532x+25.61; ORG r=0.999, R^2=0.893, a regression equation y=0.967x+18.87. Cultivars 'Fredis' and 'Edvins' had better gluten content and water-binding capacity that make them more suitable for the organic production systems, compared to other cultivars (Figure 1, 3). Researchers Sterna et al. (2017) reported that the choice of cultivar is a critical factor in efficient organic farming.

The production system in investigation year, cultivars and production system×cultivar interaction had a significant ($p<0.05$) impact on the gluten content, gluten index and water-binding capacity (Table 3).

Table 3

Impact factors of winter wheat quality indices

Source of variation	WG	GI	WB
Production system	83.1*	35.7*	83.1*
Cultivar	12.5*	39.4*	12.5*
Production system×cultivar	3.4*	23.6*	3.4*

WG–gluten content, %; GI–gluten index,
WB–water binding capacity, %. *significant ($p<0.05$)

Wet gluten and water-binding capacity were most affected by production system (83.1%) but influence of a cultivar was also remarkable (12.5%), while the influence production system×cultivar was small 3.4%

Our results showed that gluten index mostly depends on cultivar (39.4%) and production system (35.7%) and less on factor interaction (23.6%).

Conclusions

Winter wheat gluten content, gluten index and water binding capacity were significantly ($p<0.05$) affected by the agricultural production systems and cultivars. In our trial, statistically significant differences were found between agricultural production systems. Organic winter wheat grain had significantly ($p<0.05$) lower gluten content and water-binding capacity, while gluten was significantly stronger, compared with the conventional. Cultivars 'Fredis' and 'Edvins' had better gluten content and water-binding capacity that make them more suitable for the organic production system, compared to other cultivars. This paper analyses only the results of one year, the trial will be continued.

References

1. Cesevičienė J., Slepetiene A., Leistrumaite A., Ruzgas V. (2012) Effects of organic and conventional production systems and cultivars on winter wheat technological properties. *Journal of the Science of Food and Agriculture,* Vol. 92(14), p. 2811–2818.
2. Cesevičienė J., Leistrumaite A., Paplauskiene V. (2009) Grain yield and quality of winter wheat varieties in organic agriculture. *Agronomy Research,* Vol. **7**(1), p. 217–223.
3. Curic D., Karlovič D., Tušak D., Petrovic B., Dugum J. (2001) Gluten as a standard of wheat flour quality. *Food Technology and Biotehnology*, Vol. 39, p. 355–361.
4. Dekic V., Miliovojevic J., Popovic V., Brankovic S., Terzic D., Ugrenovic V. (2018). Effects of fertilization on production traits of winter wheat. **In**: *International GEA conference: Green room session. Conference proceedings*. Podgorica, Montenegro, p. 25–32.
5. Husenov B. (2018) *Towards an impact on food security for Tajikistan. Improved wheat grain and seed quality through plant breeding as a key.* Doctoral Thesis No. 2018:5. Faculty of Landscape Architecture, Horticulture and Crop Production Science Department of Plant Breeding Alnarp, 60 p.
6. Hussain A., Larsson H., Kuktaite R., Prieto-Linde M.L., Johansson E. (2009) Protein content and composition in organically grown wheat: influence of genotype.

Agronomy Research, Vol. 7 (Special issue II), p. 599–605.
7. Jaskulska I., Jaskulski D., Gałezewski L., Knapowski T. (2018) Mineral composition and baking value of the winter wheatGrain under varied environmental and agronomic conditions. *Journal of Chemistry,* Vol. 2018, p. 1–7.
8. Konvalina P., Stehno Z., Capuchova I., Moudry J. (2011) Wheat growing and quality in organing farming. In: Open Access books *Research in Organing Farming*, p. 105–122.
9. Krejčirova L., Capouchova I., Petr J., Bicanova E., Kvapil R. (2006) Protein composition and quality of winter wheat from organic and conventional farming. *Zemdirbyste-Agriculture,* Vol. 93(4), p. 285–296.
10. Linina A., Ruza A. (2018) The influence of cultivar, weather conditions and nitrogen fertilizer on winter wheat grain yield. *Agronomy Research,* Vol. 16(1), p. 147–156.
11. Linina A., Ruza A. (2012) Cultivar and nitrogen fertilizer effects on fresh and stored winter wheat grain quality indices. **In:** *Proceedings of the Latvian Academy of Sciences, Section B: Natural, Exact and Applied science*. Vol. 66(4/5), p. 177–184.
12. Litke L., Gaile Z. (2018) Effect of nitrogen fertilization on winter wheat yield and yield quality. *Agronomy Research,* Vol. 16(2), p. 500–509.
13. Mašauskienė A., Cesevičienė J. (2007) Tręšimo azoto trąšomis ir oro sąlygų poveikis žieminių kviečių glitimo savybėms grūdų laikymo metu (Impact of nitrogen fertilization and weather conditions on winter wheat gluten properties during the grain storage period). *Maisto Chemija ir Technologija*, Vol. 41(issue 1), p. 46–53.
14. Mikos M., Podolska G. (2012) Bread–making quality of old common bread (*Triticum aestivum* ssp.vulgare L.) and spelt (*Triticum aestivum* ssp. spelta L.) wheat cultivars. *Journal of Food, Agriculture and Environment,* Vol. 10(3, 4), p. 221–224.
15. Mis A. (2005) Influence of chosen factors on water absorption and rheological properties of gluten of bread wheat (*Triticuma estivum* L), *Acta Agrophysica*, Vol. 128(8), p. 1–12.
16. Oikonomou N. A., Bakalis S., Rahman M. S., Krokida M. K (2015) Gluten index for wheat products: main variables in affecting the value and nonlinear regression model. *International Journal of Food Properties*, Vol. 18, p. 1–11.
17. Šekularac A., Torbica A., Živančev D. (2018) The influence of wheat genotype and environmental factors on gluten index and the possibility of its use as bread quality predictor. *Genetica*, Vol. 50(1), p. 85–93.
18. Skudra I., Ruža A. (2016) Winter wheat grain baking quality depending on environmental conditions and fertilizer. *Agronomy Research*, Vol. 14 (2), p. 1460–1465.
19. Sterna V., Strazdina V., Kronberga A., Sturite I., Assveen M. (2017). Evaluation of the composition of winter wheat grains grown in Latvia and Norway. *AGROFOR International Journal*, Vol. 2(I3), p. 157–164.
20. Vaiciulute-Funk L., Joudeikiene G., Bartkiene E. (2015) The relationship between wheat baking properties, specific high molecular weight glutenin components and characteristic of varieties. *Zemdirbyste-Agriculture*, Vol. 102(2), p. 229–238.

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NAKED BARLEY INFLUENCE ON WHEAT BREAD QUALITY

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Abstract

Naked barley is a rich source of dietary fibre, non-starch polysaccharides, especially β-glucans and other functional ingredients. Therefore, there is a growing interest to include naked barley in products to increase their nutritional value. Barley is not popular for bakery products because its poor baking properties. High barley amount leads to decrease in bread loaf volume, storage time and consumer acceptability. Since it is known that using sourdough fermentation could be a possibility to obtain more acceptable products with higher nutrition value, producers interest to include barley flour in bread making increased. Therefore the aim of research was to evaluate nutritional characteristics, overall acceptability and storage possibility of bread enriched with naked barley grains or flour. Bread from 100% wheat flour type 812 was used as a control. There were prepared 7 samples of bread, where wheat bread was enriched with flour or grain of naked barley variety 'Kornelija' in the amount of 30 or 40% from total flour amount. Panellists were asked to evaluate intensity of bread sensory attributes – colour, aroma, porosity, hardness and sour taste – using 7-point unstructured line scale. Moisture and hardness of breads were evaluated after 24, 60 and 72 h of storage. Finally, bread samples enriched with barley grains had higher volume and porosity and lower hardness than samples enriched with barley flour. Samples enriched with barley flour prepared using sourdough method had sour taste and higher hardness. It was concluded, that all samples have maintained quality after 72 h of storage.

Keywords: naked barley, dietary fibre, β-glucans, sensory properties, wheat bread

Introduction

Increased interest in barley as a human food ingredient has been results from studies which have shown barley as an excellent source of dietary fibre, in particular, β-glucans (Arendt, Zannini, 2013; Baik, Ullrich, 2008). Including of barley grains in human nutrition is particularly valuable for diabetic patients and humans of risk group because its ability to lower glycemic index (Meija et al., 2019; Fulgoni et al., 2015; Jenkins et al., 2002). Barley (*Hordeum vulgare L.*) grain with high β-glucan content in the soluble dietary fibre fraction may be useful as a specialty crop for human food (Guler, 2003; Kalra, Jood, 2000).

Naked barley cultivars compared to hulled have increased nutritional value, especially content of protein, β-glucans and soluble dietary fibre and are free from the pales (Sterna et al., 2017; Baik, Ullrich, 2008) therefore it could be excellent raw material for functional food production.

One of main products used in daily diet is bread, unfortunately, barley is not popular for bakery products due to its poor baking properties and lower sensory acceptance of the products (Pejcz et al., 2016; Skendi et al., 2010; Jacobs et al., 2008), it does not contain gluten, which is necessary in bread making process. Bread producers are increasingly returning to simple raw materials and older technologies. Old processes, such as fermentation, are perceived as natural, and easier to understand than modern processes. The use of sourdough in the baking technology has been established as a traditional and potential process worldwide. Most of the positive effects on the texture and shelf life of the bread attributed to sourdough fermentation are caused by the produced metabolites (Su et al., 2019; Arendt et al., 2007). It has been demonstrated that sourdough improved the dough texture and bread quality of breads containing whole grain barley due to softening effect on bran particles during fermentation (Rieder et al., 2012) and increased arabinoxylans solubility (Pejcz et al., 2016; Gänzle 2014). The incorporation of naked barley in wheat bread and using different bread production methods strongly affects the quality of bread and is a possibility to increase bread nutritional value. Therefore, the aim of research was to evaluate nutritional characteristics, overall acceptability and storage possibility of bread enriched with naked barley grains or flour.

Materials and Methods

Raw materials

Grains and whole flour of a new naked barley variety 'Kornelija' (Stende, Latvia) characterised by high protein and β-glucans content and wheat flour Type 812 (Ltd Rigas Dzirnavnieks, Latvia) were used as main the raw materials. The chemical composition of raw material - naked barley grains of variety 'Kornelija' and wheat flour is summarised in the Table 1.

Table 1

Chemical composition of raw materials

Nutrients, g per 100 g	Naked barley grain	Wheat flour Type 812
Protein	20.80	13.40
Fat	2.11	1.30
Ash	2.63	0.70
Starch	47.90	66.0
β-glucans	6.15	0.60

Dough formulations and bread making

Bread was made using different methods: direct method was used for a control – wheat bread (sample C) and

samples D30 and D40, where wheat flour was replaced with barley flour as showed in Table 2. For samples G30 and G40 wheat flour was replaced with 300 g and 400 g soaked barley grain, respectively.

The added sourdough used in samples S30 and S40 was made from barley flour using Böcker (Germany) sourdough starter. Böcker sourdough starter work for all sourdough fermentation and contain the valuable lactic acid bacteria culture *Lactobacillus sanfranciscensis*. Additional ingredients for all bread recipes were 30 g sugar, 30 g salt and 30 g fresh baker's yeast.

Table 2

Dough composition

Sample code	Ingredients, g			
	Wheat flour type 812	Naked barley flour	Naked barley grains	Water
C	1000	–	–	705
D30	700	300	–	700
D40	600	400	–	695
G30	700	–	300	690
G40	600	–	400	690
S30	700	150[1]/150[2]	–	690
S40	600	200[1]/200[2]	–	670

[1] – naked barley flour in sourdough,
[2] – naked barley flour in dough.
C – 100% wheat bread; D30 – 30% naked barley flour; D40 – 40% naked barley flour; G30 – 30% naked barley grains; G40 – 40% naked barley grains; S30 – 30% naked barley flour in sourdough; S40 – 40% naked barley flour in sourdough.

The dough was mixed by means of a Varimixer Bear AR10 (Ltd Wodschow & Co., Denmark) mixer for 7 minutes (3 minutes at a speed of 1; 4 minutes at a speed of 2). The fermentation duration was 10 minutes at a room temperature 22±2 °C temperature. Then the dough was divided into pieces of 400±5 g, placed into a container (150×70×100 mm in size) and fermented for 40 minutes at a 35±3 °C temperature. The bread was baked in convective oven Sveba Dahlen S8 for 30 minutes at a 200±10 °C temperature, with steam for first 3 seconds. The bread baking experiments were repeated twice.

After cooling, the bread samples were packaged in the polyethylene bags and stored at room temperature (20±2 °C) for 5 days.

Determination of loaf volume

Loaf volume was measured by small seeds displacement method (Al-Saleh, Brennan, 2012). A container was used to measure the volume using rapeseeds. Seeds were poured into container of known volume until the bottom was covered. The loaf was placed inside the container which was then filled to the top with more seeds. The extra rapeseeds, which equal the loaf volume, were measured in a graduated cylinder. Samples were measured in triplicate, and the average data was recorded.

Determination of weight loss

Weight loss (WL) was calculated based on the dough weight before baking and after baking. The weight loss (%) of the test bread samples was calculated using the following equation (1):

$$WL\ (\%) = \frac{W_{dough} - W_{hot\ bread}}{W_{hot\ bread}} \times 100 \qquad (1)$$

where:

W_{dough} – weight of dough, g;
$W_{hot\ bread}$ – weight of bread after baking, g.

Bread moisture content determination

Moisture content of breadcrumb was determined using air-oven (Memmert GmbH, Germany) according AACC method 44-15.02 24 h, 72 h and 96 h after baking.

Determination of bread hardness during storage

Hardness (N) of samples' breadcrumb was tested 24 h, 72 h and 96 h after baking using Texture Analyser – TA.HD.plus (Stable Micro Systems, UK) according to modified AACC method 74-09.01.

Calculation of nutritional and energy values

Nutrition and energy values were calculated using a specific Excel spreadsheet according Regulation (EU). No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers.

Sensory evaluation

Wheat bread with naked barley flour and grain was evaluated by 39 panellists, with an average age of 38 years. A 7-point unstructured line scale was used for rating bread sensory attributes – colour, aroma, porosity, hardness and sour taste – intensity. Samples for panellists were assigned 3-digit codes and presented in a Williams' Latin Square design. Warm black tea was used as palate cleansers between samples. The format of the evaluation sheet, data collection and interpretation of data were processed with FIZZ Aquistion Ver.2.51 software (Biosystemes, France).

Data statistical analysis

Statistical data analysis was performed using SPSS 17.0. Sensory evaluation data were analysed by ANOVA using Fisher's least significant difference test (LSD) with a significance level of 95% ($p<0.05$).

Results and Discussion

Bread loaf volume is an important attribute of baked product quality as increased loaf volume leads to softer crumb, improved eating quality and higher consumer preference. High loaf volume of bread can be achieved by several parameters including flour quality, different ingredients, the baking process and addition of enzymes. Results of loaf volume measurements confirmed that high barley concentration led to decrease in loaf volume (Pejcz et al., 2016; Jacobs et al., 2008). Volume of samples D30 with 30% naked barley flour was determined higher – 811±11 mL than volume of samples D40 – 711±9 mL (Table 3 and Figure 1).

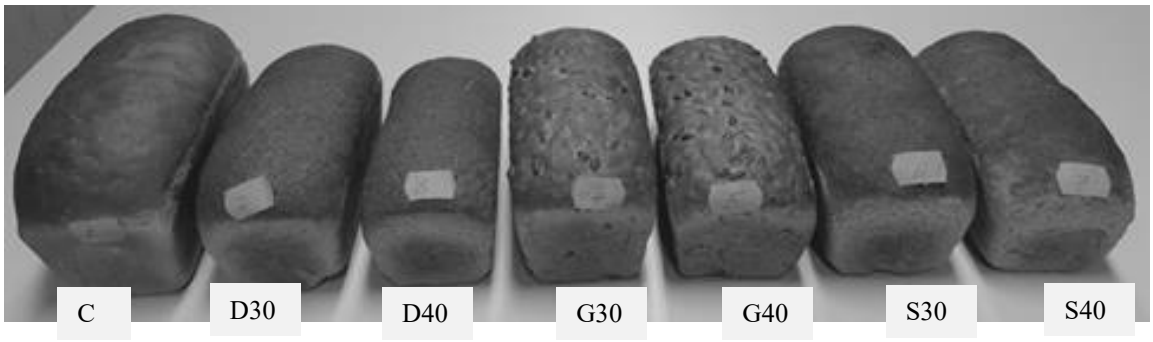

Figure 1. Samples of bread

C – 100% wheat bread; D30 – 30% naked barley flour; D40 – 40% naked barley flour; G30 – 30% naked barley grains; G40 – 40% naked barley grains; S30 – 30% naked barley flour in sourdough; S40 – 40% naked barley flour in sourdough

The negative changes of bread volume could be the result of an increased amount of dietary fibre and the presence of non-starch polysaccharides affecting gluten network weakening and lowering gas retention in the dough in consequence (Gänzle, 2014). Results of tested loaf volume, moisture and weight loss are summarised in the Table 3. Decrease in loaf volume in samples with higher barley content G40 versus G30 observed also when barley included as grains. The volume of bread samples with naked barley flours, grains and sourdough decreased compared to control (Figure 1). Bread samples with soaked naked barley grain on the crust were evident. The crust for bread samples with barley was not as smooth as the control sample. Al-Attabi et al. (2017) also found that crust sensory texture which was connected to the smoothness or roughness, decreased with the increasing substitution of barley flour or grain.

Table 3

Moisture, weight losses and loaf volume of fresh bread samples

Samples	Moisture, %	Weight loss, %	Loaf volume, mL
C	58.0±2.2	11.3±0.6	1178±22
D30	55.6±0.3	10.3±0.4	811±11
D40	55.6±0.2	10.5±1.0	711±9
G30	58.6±3.3	9.8±0.9	880±20
G40	55.9±0.2	9.2±0.7	781±20
S30	54.6±0.2	10.2±0.1	814±11
S40	55.2±0.3	10.4±0.2	764±30

C – 100% wheat bread; D30 – 30% naked barley flour; D40 – 40% naked barley flour; G30 – 30% naked barley grains; G40 – 40% naked barley grains; S30 – 30% naked barley flour in sourdough; S40 – 40% naked barley flour in sourdough

Comparing samples with the same barley content D40, G40 and S40 concluded that higher loaf volume determined if barley included in dough as grains. Using sourdough method (samples S30 and S40) contributed to higher volume of bread 814 mL and 764 mL respectively, than using direct method (loaf volumes 811±11 mL and 711±9 mL). These results are in agreement with Pejcz et al. (2016) study where loaf volume using direct method was determined 530 cm^3, but using sourdough method 557 cm^3.

The results showed that moisture content was not influenced by amount of barley flour amount in dough, because in samples D30 and D40 determined the same moisture content, same as in bread samples S30 and S40 (Table 3). The smallest weight loss was determined in bread samples where included barley grains – G30, G40. This trend persistent in all storage time. Obtained results of moisture content (Figure 2) in storage time showed higher values in samples C, G30 and G40, it is in the wheat bread and in the bread with barley grains.

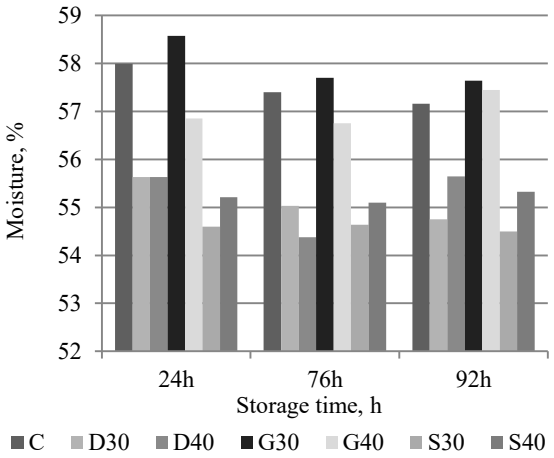

Figure 2. Changes of bread moisture during storage

C – 100% wheat bread; D30 – 30% naked barley flour; D40 – 40% naked barley flour; G30 – 30% naked barley grains; G40 – 40% naked barley grains; S30 – 30% naked barley flour in sourdough; S40 – 40% naked barley flour in sourdough

Grinding may affect the hydration properties, in particular, the kinetics of water uptake as the result of the increase of surface area, the fibres hydrate more rapidly (Dhingra et al., 2012). As it was observed comparing moisture content of the samples where used barley grains (D30, D49) and moisture content of the samples where used barley flour (D30, D40, S30, S40). The hardness increased within 96 h storage in all samples (Figure 3). The smallest increase calculated for samples G30 (4.0 N) and C wheat bread (4.5N) while in samples D30, D40, S30, S40 increase of hardness was similar 12.9N; 12.6N; 12.5N and 13.5N respectively.

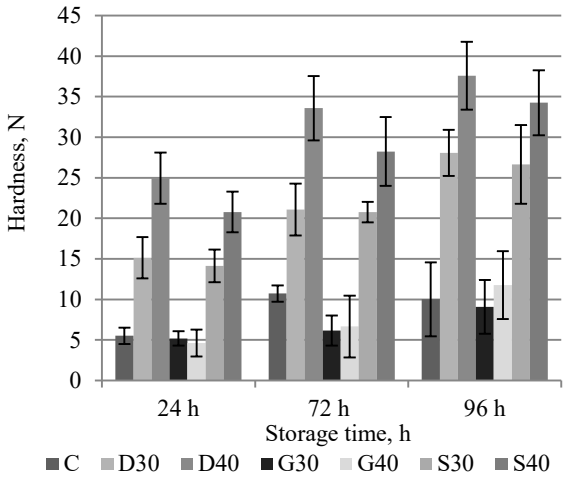

Figure 3. Hardness of bread samples in storage time
C – 100% wheat bread; D30 – 30% naked barley flour; D40 – 40% naked barley flour; G30 – 30% naked barley grains; G40 – 40% naked barley grains; S30 – 30% naked barley flour in sourdough; S40 – 40% naked barley flour in sourdough

Results of hardness showed are similar for samples C, G30, G40. Hardness of samples with barley flour was significantly higher than hardness of samples with barley grains or wheat flour ($p<0.05$). Comparison of samples with the same amount of barley flour showed advantages of sourdough method because hardness of samples D30 determined higher (15.1±2.6 N) than hardness of samples S30 (14.1±2.0 N) similar in samples with 40% barley flour content. Sheikholeslami et al. (2018) also concluded that effect of naked barley amount with guar gum on increasing the volume and decreasing the hardness of breadcrumb. The guar gum additive plays an important role here. With barley flour alone, it is difficult to reach a large volume of bread and good porosity.
Calculated nutritional value of bread samples assumed in Table 4.

Nutrition value and energy value of bread samples

Parameters	Bread samples		
	C	D30; G30; S30	D40; G40; S40
Energy value, kcal	218	212	210
Energy value, kJ	923	898	889
Protein content, g 100 g^{-1}	8.4	9.8	10
Fat content, g 100 g^{-1}	0.8	0.95	1.00
Carbohydrates, g 100 g^{-1}	43	39	38
Dietary fibre, g 100 g^{-1}	2.9	3.5	3.6
– including β-glycans	0.4	1.7	2.1

C – 100% wheat bread; D30 – 30% naked barley flour; D40 – 40% naked barley flour; G30 – 30% naked barley grains; G40 – 40% naked barley grains; S30 – 30% naked barley flour in sourdough; S40 – 40% naked barley flour in sourdough

Bread enriched with barley grains had significantly higher dietary fibre content including β-glycans content ($p<0.05$) and insignificantly higher protein and fat content ($p>0.05$). Increase of barley content in flour composition results in decrease in carbohydrates content and total energy value.

Sensory evaluation of bread

Sensory evaluation results showed, that the addition of naked barley (flour, grains or sourdough) did not affect ($p=0.0513$) the bread aroma intensity (Figure 4). There were no significant differences ($p>0.05$) in the intensity of bread crumb colour intensity between control (C) and bread with 30 and 40% barley grains (samples G30 and G40), but the crumb colour intensity influenced naked barley flour and sourdough – it became darker. Pejcz et al. (2017) reported, that a darker crumb colour of wheat bread with barley, influence consumers overall liking of bread samples. Kinner et al. (2011) found that addition of barley flour had a negative influence on bread volume and crumb texture.

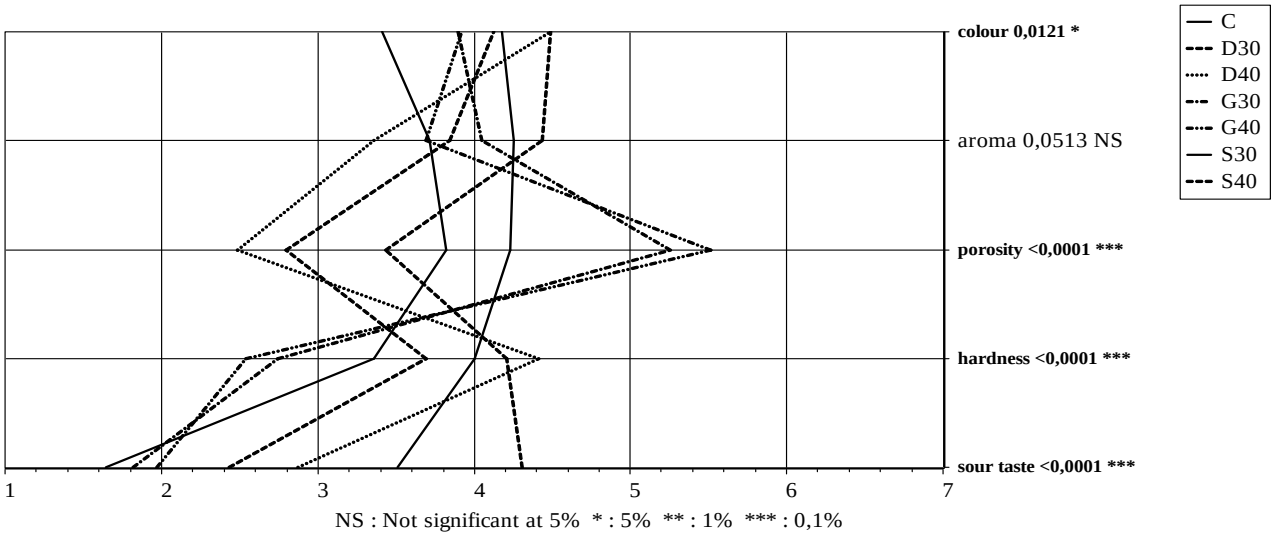

Figure 4. Sensory attributes intensity of bread with naked barley
C – 100% wheat bread; D30 – 30% naked barley flour; D40 – 40% naked barley flour; G30 – 30% naked barley grains; G40 – 40% naked barley grains; S30 – 30% naked barley flour in sourdough; S40 – 40% naked barley flour in sourdough

Similar results have been obtained in our research – samples D30 and D40 had less pronounced porosity. Though adding naked barley grains (30% and 40%), it is possible to obtain bread with good porosity. In this study a strong negative correlation (r=-0.85) was found between bread porosity and hardness – bread has smaller pores in crumb, thus increasing the hardness of bread. The addition of sourdough from naked barley has a significant effect ($p<0.05$) on the sour taste of the analysed bread samples, resulting from the action of lactic acid bacteria of starter.

Conclusions

Bread enriched with barley grains has significantly higher content of dietary fibre, including β-glycans, higher protein and fat. Increase of barley content in flour composition results in decrease in carbohydrates and total energy value.

Results confirmed that high barley amount in dough led to decrease in loaf volume of bread - higher loaf volume of bread determined when barley included in dough as grains. Comparison of samples with same amount of barley flour showed advantages of sourdough method – higher loaf volume and smaller hardness in storage time. Though adding naked barley grains to 30% and 40%, it is possible to obtain bread with good porosity, but addition of sourdough increased the sour taste of bread.

Acknowledgment

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References

1. Al-Attabi Z.H., Merghani T.M., Ali A., Rahman M.S. (2017) Effect of barley flour addition on the physic-chemical properties of dough and structure of bread. *Journal of Cereal Science*, Vol. 75, p. 61–68.
2. Al-Saleh A., Brennan C.S. (2012) Bread wheat quality: some physical, chemical and rheological characteristics of Syrian and English bread wheat samples. *Foods*, Vol. 1, p. 3–17.
3. Arendt E.K., Zannini E. (2013) Barley. **In***: Cereal grains for the food and beverage industries*. ed. S.Hughes. Cambridge: Woodhead Publishing. p. 155–200.
4. Arendt E. K., Ryan L. A., Dal B. F. (2007) Impact of sourdough on the texture of bread. *Food Microbiology*, Vol. 24(2), p. 165–174.
5. Baik B.K., Ullrich S.E. (2008) Barley for food: Characteristics, improvement, and renewed interest. *Journal of Cereal Sci*ence, Vol. 48, p. 233–242.
6. Dhingra D., Michael M., Rajput H. Patil R.T. (2012) Dietary fibre in foods: A review. *Journal of Food Science and Technology*, Vol. 49 (3), p. 255–266.
7. Fulgoni V. L., Chu Y., O'Shea M., Slavin J. L., DiRienzo M. A. (2015) Oatmeal consumption is associated with better diet quality and lower body mass index in adults: the National Health and Nutrition Examination Survey (NHANES), 2001-2010, *Nutrition Research*, Vol. 35(12), p. 1052–1059.
8. Guler M. (2003) Barley grain beta-glucan content as affected by nitrogen and irrigation. *Field Crops Research*, Vol. 84, p. 335–340.
9. Gänzle M.G. (2014) Enzymatic and bacterial conversions during sourdough fermentation. *Food Microbiology,* Vol 37, p. 2–10.
10. Jacobs M.S., Izydorczyk M.S., Preston K.R., Dexter J.E. (2008) Evaluation of baking procedures for incorporation of barley roller milling fractions containing high levels of dietary fibre into bread. *Journal of the Science of Food and Agriculture,* Vol.88, p. 558–568.
11. Jenkins A.L., Jenkins D.J.A., Zdravkovic U., Wursch P., Vuksan V. (2002) Depression of the glycaemic index by high levels of beta-glucan fiber in two functional foods tested in type 2 diabetes. *European Journal of Clinical Nutrition*, Vol. 56, p. 622–628.
12. Kalra S., Jood S. (2000) Effect of dietary barley β-glucan on cholesterol and lipoprotein fractions in rats. *Journal of Cereal Science,* Vol. 31, 141–145.
13. Kinner M., Nitschko S., Sommeregger J., Petrasch A., Linsberger-Martin G., Grausgruber H. (2011) Naked barley optimized recipe for pure barley bread with sufficient beta-glucan according to the EFSA health claims. *Journal of Cereal Science*, Vol. 53(2), p. 225–230.
14. Meija L., Havensone G., Lejnieks A. (2019) Postprandial glycaemic and insulinaemic responses after consumption of activated wheat and triticale grain flakes. *Journal of Nutrition and Metabolism,* Vol. 2019, p. 1–7.
15. Pejcz E., Czaja A., Wojciechowicz-Budzisz A., Gil Z. (2017) The potential of naked barley sourdough to improve the quality and dietary fibre content of barley enriched wheat bread. *Journal of Cereal Science,* Vol. 77, p. 97–101.
16. Pejcz E., Wojciechowicz-Budzisz A., Gil Z., Czaja A., Radoslav S. (2016) Effect of naked barley enrichment on the quality and nutritional characteristic of read – Part I. The effect on wheat bread. *Engeneering Sciences and Technologie,* Vol. 2(21), p. 38–45.
17. Rieder A. Holtekjolen A.K., Sahlstrom S., Moldestad A., (2012) Effect of barley and oat flour types and sourdoughs on dough rheology and bread quality of composite wheat bread. *Journal of Cereal Science*, Vol. 55, p. 44–52.
18. Sheikholeslami Z., Karimi M., Komeili H. R., Mahfouzi M. (2018) A new mixed bread formula with improved physicochemical properties by using hull-less barley flour at the presence of guar gum and ascorbic acid. *LWT-Food Science and Technology*, Vol. 93, p. 628–633.
19. Skendi A., Biliaderis C. G., Papageorgiou M., Izydorczyk M.S., (2010) Effects of two barley β-glycan isolates on wheat flour dough and bread properties, *Food Chemistry*, Vol. 115, p. 1159–1167.
20. Sterna V., Zute S., Jansone I., Kantane I. (2017) Chemical composition of covered and naked spring barley varieties and their potential for food production. *Polish Journal of Food and Nutrition Sciences,* Vol. 67 (2), p. 151–158
21. Su X., Wu F., Zhang Y., Yang N., Chen F., Jun Z., Xu X. (2019) Effect of organic acids on bread quality improvement. *Food Chemistry*, Vol. 278, p. 267–275.

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INFLUENCE OF GERMINATION TEMPERATURE AND TIME ON PHENOLIC CONTENT AND ANTIOXIDANT PROPERTIES OF CEREALS

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Abstract

Germination improves nutritional quality of cereals due to synthesis of various phytochemicals. The aim of the current research was to evaluate influence of the applied germination conditions on the changes in total phenolic compounds (TPC) and antioxidant activity of triticale, hull-less barley, hull-less oat, wheat, and rye grains. Triticale (cv. Inarta), hull-less barley (cv. Irbe), hull-less oat (cv. Lizete), rye (cv. Kaupo), and wheat (cv. Ellvis) grains were cleaned, washed and steeped in water at the ratio of 1:2 (grains to water) for 24 h at 22±2 °C. After steeping, grain germination was performed for 12, 24, 36 and 48 h in the dark at 17 °C, 25 °C, 35 °C and the total phenolic content and antioxidant activity have been analysed by DPPH and ABTS assays. During germination, TPC in all analysed cereal types increased, but dynamics was cereal type dependent and it is not possible to give general tendency or the best parameters applicable for cereals in general. Most significant changes were observed in the analysed grains during germination at 25 °C and 35 °C, with the highest increase in hull-less barley. The current study indicates that germination is effective tool for improvement of different grain nutritional value, suggesting their potential use in new product development.

Keywords: triticale, hull-less barley, hull-less oats, DPPH, ABTS

Introduction

Cereals are a very important part of human diet worldwide. Consumers prefer cereal-based products with improved functionality, and it can be reached by modifying technological processes (Henry et al., 2015). Selection of valuable, untraditional raw materials and technological processes resulting in increased nutritional quality are important factors in new product development. Triticale (*Triticosecale Wittmack*) is a promising substitute to wheat (*Triticum* spp.) in functional processed flour products such as bread, pasta etc. Triticale contains higher amounts of protein, ash and non-starch content of lipids, starch, lignin and bioactive compounds as polyphenols (Fraś et al., 2016). Applications of barley (*Hordeum vulgare* L.) in industrial processing has been widely evaluated, especially for possible use in food and feed due to its nutritional value. Hull-less barley has technological benefits due to the lack of hull, but also it differs in chemical composition resulting in higher content of insoluble and soluble fractions of dietary fibre and other bioactive compounds (Blandino et al., 2015; Kruma et al., 2016a). Oats (*Avena sativa* L.) also are divided in two groups – hulled and hull-less. The main application of hull-less oats in the world are for feed purposes. However, nutritional value of hull-less oats are characterised by higher content of proteins, lipids, fibres (Sterna et al., 2016) and similar content of phenolic compounds (Kruma et al., 2016a) compared to hulled samples. Rye grains are source of starch, dietary fibre and protein in the diet. Additionally, Pihlava et al. (2018) indicated that whole rye grain is a valuable source of diverse groups of phenolic compounds. Wheat is considered as a good source of energy and irreplaceable nutrients. However white flour has a low antioxidant capacity (Dziki et al., 2014).

Antioxidant activity of cereals is provided by phenolic compounds, as a major group of radical scavengers. These molecules are secondary metabolites of plants, which possess antioxidant activity and reduce damages caused by free radicals for human DNA, RNA etc. Recent investigations are focused on evaluating commonly consumed foods including cereals for their antioxidant properties. Several techniques are developed to reduce losses of phenolic compounds in foods and technologies are developed to increase their level (Wu et al., 2013). Germination process could increase their content resulting in food with improved nutritional value (Cevallos-Casals, Cisneros-Zevallos, 2010).

Germination is a competitive and economical approach to increase the nutritive quality and amount of bioactive compounds in cereal grains (Singh et al., 2015). Steeping is the first step in germination process with the aim to accelerate water penetration, which transforms the inactive tissue into living tissue, and grains metabolism is activated. Germination activates endogenous cereal enzymes, when proteins and carbohydrates are hydrolysed into smaller molecules as mono-, disaccharides, dextrin's, peptides, free amino acids, and additionally new bioactive compounds are synthetized (Wu et al., 2013). At the initial germination stages phenolics may serve as radical scavengers or antioxidants, while later they could become part of the structure for new growing plant and lose some of their antioxidant efficiency (Cevallos-Casals, Cisneros-Zevallos, 2010).

The aim of the current research was to evaluate influence of the applied germination conditions on the changes in total phenolic compounds (TPC) and antioxidant activity of triticale, hull-less barley, hull-less oat, wheat, and rye grains.

Materials and Methods

Plant material

Triticale cv. Inarta, hull-less barley cv Irbe, hull-less oat cv. Stendes Emilija, rye cv. Kaupo, and wheat cv. Elvis grains were cultivated conventionally at the Institute of Agricultural Resources and Economics in 2015. The germination experiments were carried out at the laboratories of the Faculty of Food Technology at Latvia University Life Sciences and Technologies.

Grain germination

Cereals included in experiment were cleaned, washed and steeped in water at the ratio of 1:2 (grains to water) for 24±1 h at 22±2 °C. After steeping, water was drained and grains were allowed to germinate for 12, 24, 36 and 48 hours in the dark at controlled temperature (17, 25 and 35±2 °C with relative humidity (RH) 95±2%. The climatic chamber ICH110 (Memmert, Germany) was used for germination process.

Extraction and determination of total phenolic compounds and antioxidant activity

Extraction procedure was applied as described by Kruma et al. (2016b). The total phenolic content (TPC) of the germinated cereal extracts was analysed in accordance with Folin-Ciocalteu spectrophotometric methodology (Singleton et al., 1999). Stable 2,2-diphenyl-1-picrylhydraziyl (DPPH) radical and 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic) acid ($ABTS^{+}$) radical cation assay was used to evaluate antioxidant activity. All analytical methods outlined in details by Kruma et al. (2016b).

Statistical analysis

The obtained data were analysed by analysis of variance (ANOVA); significance was defined at $p<0.05$. Relationship between TPC and antioxidant activity such as $DPPH^{-}$ and $ABTS^{+}$ scavenging activity was estimated by a linear correlation analysis. The averages of triplicate extractions are reported.

Results and Discussion

Total phenolic compounds (TPC)

Comparing TPC between tested grains, hull-less barley showed the highest content and it is in agreement with the previous study (Kruma et al., 2016a). Also other authors emphasize benefits of barley, showing their antioxidant, antiradical, and antiproliferative effects. Incorporation of barley into the daily diet provide health benefits (Madhujith et al., 2007). During steeping process, TPC increased significantly (Fig. 2) and the highest increase was observed in hull-less barley sample (63%).

The results indicate that physical modifications and enzymatic activity during steeping process increase phenolic content. Cereals contain both free and bound phenolic compounds and they are soluble-extractable polyphenols and insoluble-non-extractable (Arranz, Saura Calixto, 2010; Koletta et al., 2014). In the current experiment, extractable phenolic compounds were determined and increase could be explained by the increase in the fraction of soluble phenolic compounds due to hydrolysis of lignin and hemicelluloses bound forms from cell walls (Thammapat et al., 2015). Steeping was performed at 22 °C temperature, but in further experiments higher temperatures for hull less cereals and triticale were tested, because for rice the highest TPC has achieved by steeping at 50 °C (Thammapat et al., 2015).

During germination TPC in all analysed cereal types increased (Fig. 1–5) but the dynamics were cereal type dependent and it is not possible to give general tendency or the best germination parameters applicable for all cereals. As shown by Cevallos-Casals and Cisneros-Zevallos, (2010), phenolic compounds posing antioxidant activity are synthesised during germination which can result in germinated cereals with improved nutraceutical properties. Generally, germination at 17 °C temperature resulted in lower TPC. For triticale, no significant differences of TPC between germination at 25 °C and 35 °C were observed and the highest TPC after 24 h was reached. For hull-less oats the highest results were for samples germinated at 35 °C, only at the end of the experiment (after 48 hours) no differences between 25 °C and 35 °C were observed. For hull-less barley the highest TPC was obtained after germination at 35 °C for 24 hours. The same content is possible to reach also after 36 hours at 25 °C and 35 °C temperature. For wheat and rye, the highest results were for samples germinated at 25 °C after 48 hours. Similar results about significant influence of germination time and temperature was obtained by several authors. Investigation of kiwicha sprouts indicated that germination temperature had more significant effect on the increase of phenolic content than time and their interaction (Paucar-Menacho et al., 2017), for brown rice the highest TPC was found in samples germinated at 28 °C for 96 h (Cáceres et al., 2014). In triticale the initial TPC was the lowest but during germination, it increased 2.5-fold, while for oats the results were even higher – a 2.6-fold increase was observed. However, hull-less barley which had the highest initial TPC out of all tested cereals, the increase during germination was the lowest (0.7-fold). Results can be associated with different controlling mechanism of enzymatic activity of hydrolases and polyphenol oxidases during germination (Mamilla, Mishra, 2017). Similar explanation was found also for brown rice showing activation of phenylpropanoid pathway and also hydrolyses of phenolic compounds incorporated into cell walls (He et al., 2011). Also, one of the explanations for the increase of TPC is a liberation of phenolic acids during germination process by enzymes that hydrolyses starch (Tian et al., 2010).

Comparing to raw materials in all germinated samples an increase of TPC was detected. Chen et al. (2016) investigated canary seeds germination and stated that the highest content of total phenolics was in germinated seeds followed by raw seeds and soaked seeds. Another study also confirmed this conclusion by testing different seeds and accumulated phenolics and antioxidant

activity was the highest in 7-day sprouts and lowest in steeped seeds (Cevallos-Casals, Cisneros-Zevallos, 2010).

In literature a contrary trend with TPC decrease during germination has also been reported for purple corn and that can be explained by the activation of polyphenol oxidases that are involved in degradation of phenolic compounds in food matrixes (Paucar-Menacho et al., 2017). Germination conditions influenced TPC of the tested samples differently and it can be explained by physiological structure and chemical composition of cereals, but even together with temperature effect of cultivar was observed resulting in different TPC content (Cáceres et al., 2014). Temperature 26 °C and time 63 h were identified as the optimal conditions by Response Surface Methodology for enhancing the γ-aminobutyric acid and TPC content as well as the antioxidant activity of purple corn (Paucar-Menacho et al., 2017).

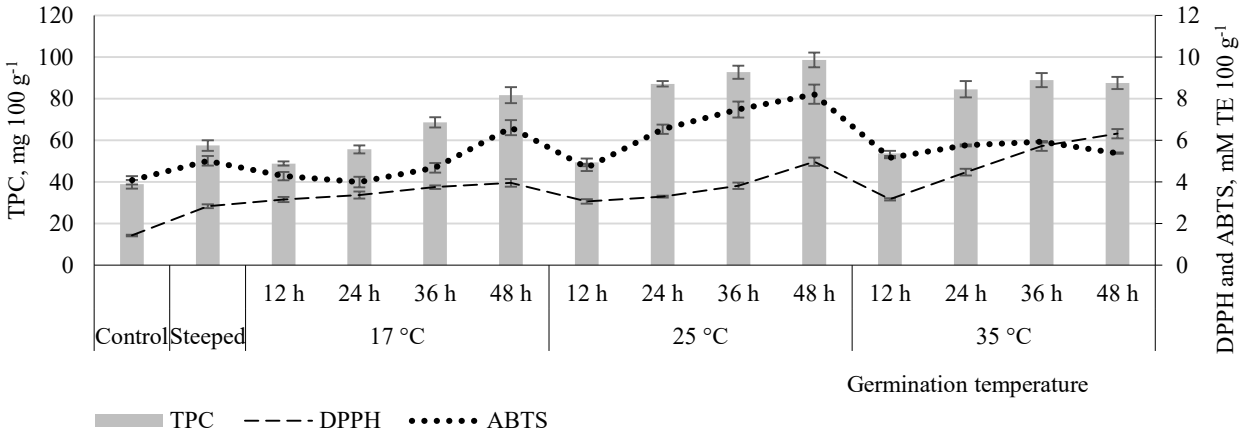

Figure 1. Dynamics of TPC, DPPH, and ABTS during germination process in triticale

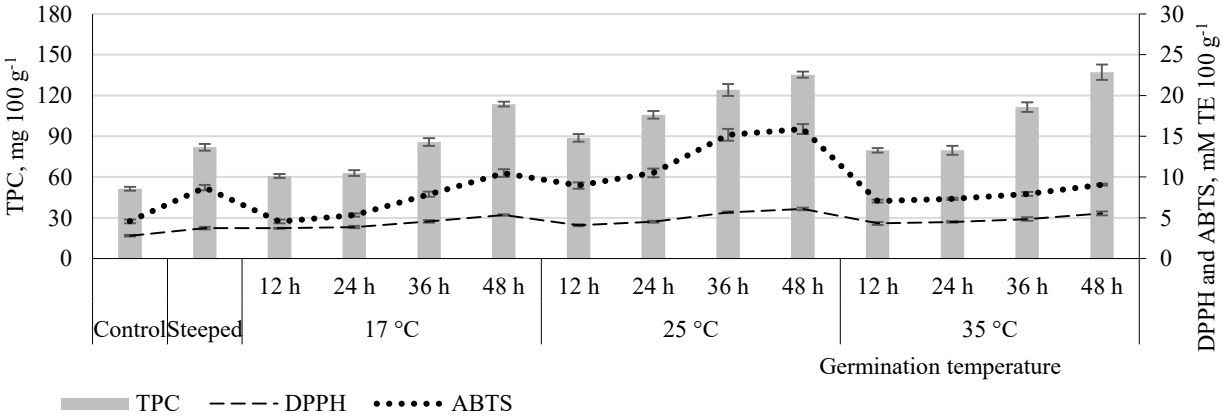

Figure 2. Dynamics of TPC, DPPH, and ABTS during germination process in hull-less oats

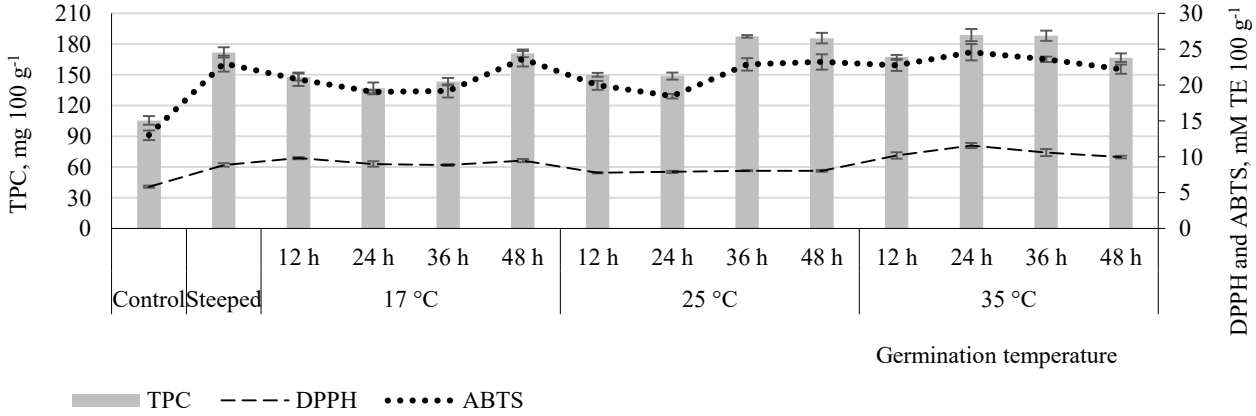

Figure 3. Dynamics of TPC, DPPH, and ABTS during germination process in hull-less barley

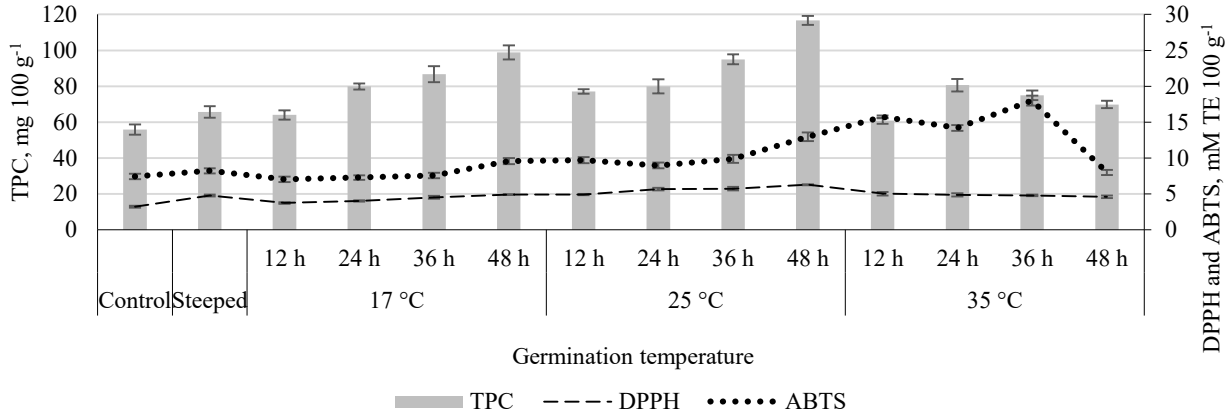

Figure 4. Dynamics of TPC, DPPH, and ABTS during germination process in rye

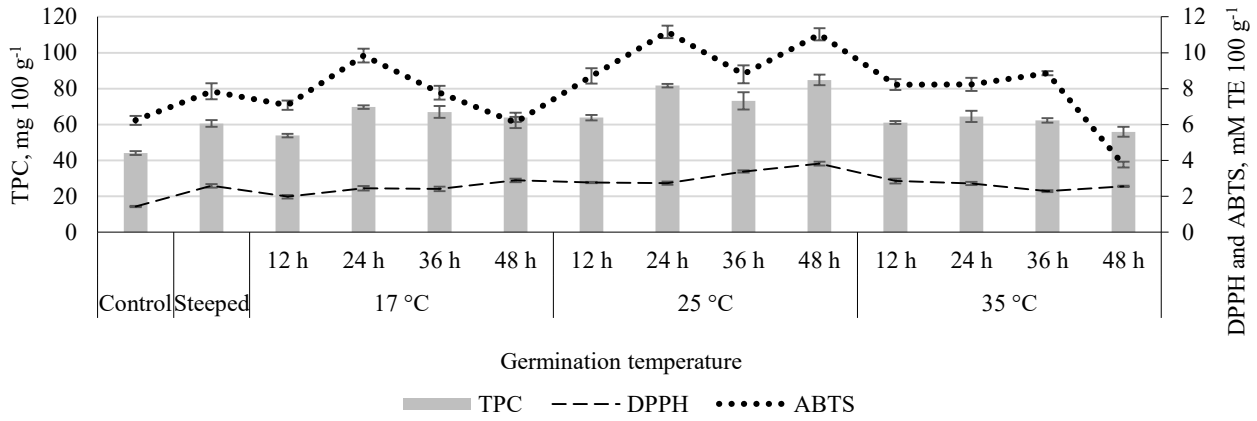

Figure 5. Dynamics of TPC, DPPH, and ABTS during germination process in wheat

Radical scavenging activity

Assays of DPPH and $ABTS^{+}$ scavenging activity are widely used for evaluation the antioxidant activity of plant matrixes. Different antioxidant assays give different results due to radicals' activity (Aguilera et al., 2015). During steeping process, antioxidant activity increased significantly (Figures 1–5). The highest increase in ABTS scavenging activity was observed in steeped hull-less barley sample – by 77%. Whereas the highest increase of DPPH activity was in triticale, reaching 97%, although initial TPC and antioxidant activity in triticale was the lowest comparing all tested grains. Among the tested cereals steeped hull-less barley had the highest DPPH scavenging activity.

Also, Žilić et al. (2011) detected the higher DPPH radical scavenging activity in hull-less barley, which is in line with our study. DPPH activity during germination is time and temperature dependent. For triticale the highest increase was observed at 35 °C temperature and reached the maximal activity after 48 hours. Whereas for wheat, rye and hull-less oats the highest results were reached after germination for 48 hours at 25 °C. Both time and temperature affected antioxidant activity positively but response of temperature was more intense (Paucar-Menacho et al., 2017). Whereas antioxidant activity results of other experiment showed up to 4-fold higher activity after 96 h germination of brown rice, and activity was cultivar specific (Cáceres et al., 2014).

For scavenging of ABTS cations temperature is significant factor. ABTS radical is widely applied for antioxidant assays due to its reactivity with different classes of antioxidants. But comparing to DPPH, its stability is lower and also preparation process is more time and chemicals consuming (Mareček et al., 2017). In triticale the best results were obtained during germination at 25 °C, and by increasing germination time, activity also increased. Also, for hull-less oats 25 °C showed the best results, and the highest activity after 36 hours was reached. For hull-less barley the highest results after 24 hours were reached and the same tendency that was observed for TPC and DPPH scavenging activity. Results showed that ABTS determination more emphasised the effect of the variety on the total antioxidant capacity whereas DPPH scavenging activity showed the effect of the harvest year (Mareček et al., 2017).

Differences in antioxidant activity could also be explained by the results from experiments about β-glucan as antioxidant. Hull-less barley and oat cultivars compared to the hulled cultivars contains higher level of β-glucans, resulting in better nutritional value (Tiwari, Cummins, 2012). Antioxidant activity pathway of β-glucans is not well understood and

β-glucan extracted from germinated barley showed higher antioxidant capacity comparing to ungerminated sample (Ronda et al., 2015). Also antioxidant activity can be affected by protein content and according to Mareček et al. (2017) higher protein content results in lower antioxidant activity

To understand the role of phenolic compounds in the radical scavenging activity of cereals, correlation analyses were performed. In Table 1 the Pearson`s coefficients between the levels of phenolic compounds and antioxidant activity in tested cereal types are presented.

Table 1

The correlations between TPC and radical scavenging activity in germinated cereals

Cereal	TPC / DPPH	TPC / ABTS	DPPH / ABTS
Triticale	0.77	0.86	0.47
Hull-less barley	0.21	0.57	0.75
Hull-less oats	0.93	0.83	0.83
Wheat	0.81	0.79	0.46
Rye	0.71	0.13	0.42

Triticale, hull-less oats, wheat and rye showed a highly positive correlation between the content of phenolic compounds and antioxidant activity. Lower values were obtained for hull-less barley. In cereals weak or moderate correlation between DPPH scavenging activity and TPC are observed because one of the main cereal phenolic acid – ferulic acid has weak antioxidant properties (Đorđević et al., 2010). It could be also explained by the contribution of other compounds to antioxidant activity. These compounds could be β-glucan, proteins etc.

Strong correlation between phenolic compounds and antioxidant capacity that was found in investigations of different cereals (Chen et al., 2016; Deng et al., 2012) showing that the phenolic compounds provide antioxidant activity of water-soluble fraction.

Conclusions

Germination process increased total phenol content and antiradical activity of cereal grains, and the most significant changes were observed in the analysed grains during germination at 25 °C and 35 °C. Hull-less barley grains exhibited the highest changes in chemical composition among all tested cereals. During germination TPC in all analysed cereal types increased, but the dynamics were cereal type dependent, therefore, it is not possible to give general tendency and the best parameters applicable for all cereals. As phenolic compounds posing antioxidant activity are synthesised during germination, it can result in germinated cereals with improved nutraceutical properties, thus, suggesting potential use in new product development.

Acknowledgment

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References

1. Aguilera Y., Herrera T., Benítez V., Arribas S. M., López de Pablo A. L., Esteban R. M., Martín-Cabrejas M. A. (2015) Estimation of scavenging capacity of melatonin and other antioxidants: Contribution and evaluation in germinated seeds. *Food Chemistry*, Vol. 170, p. 203–211.
2. Arranz S., Saura Calixto F. (2010) Analysis of polyphenols in cereals may be improved performing acidic hydrolysis: A study in wheat flour and wheat bran and cereals of the diet. *Journal of Cereal Science*, Vol. 51(3), p. 313–318.
3. Blandino M., Locatelli M., Gazzola A., Coïsson J. D., Giacosa S., Travaglia F., Bordiga M., Reyneri A., Rolle L. Arlorio M. (2015) Hull-less barley pearling fractions: Nutritional properties and their effect on the functional and technological quality in bread-making. *Journal of Cereal Science*, Vol. 65, p. 48–56.
4. Cáceres P.J., Martínez-Villaluenga C., Amigo L., Frias J. (2014) Maximising the phytochemical content and antioxidant activity of Ecuadorian brown rice sprouts through optimal germination conditions. *Food Chemistry*, Vol. 152, p. 407–414.
5. Cevallos-Casals B. A., Cisneros-Zevallos L. (2010) Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. *Food Chemistry*, Vol. 119(4), p. 1485–1490.
6. Chen Z., Yu L., Wang X., Gu Z., Beta T. (2016) Changes of phenolic profiles and antioxidant activity in canaryseed (*Phalaris canariensis* L.) during germination. *Food Chemistry*, Vol. 194, p. 608–618.
7. Deng G.-F., Xu X.-R., Guo Y.-J., Xia E.-Q., Li S., Wu S., Chen F., Ling W.H., Li H.-B. (2012) Determination of antioxidant property and their lipophilic and hydrophilic phenolic contents in cereal grains. *Journal of Functional Foods*, Vol. 4(4), p. 906–914.
8. 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*, Vol 119(3), p. 957–963.
9. Dziki D., Rózyło 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 and Technology*, Vol. 40(1), p. 48–61.
10. 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*, Vol. 71, p. 66–72.
11. He D., Han C., Yao J., Shen S., Yang P. (2011) Constructing the metabolic and regulatory pathways in germinating rice seeds through proteomic approach. *Proteomics*, Vol. 11, p. 2693–2713.
12. Henry R. J., Rangan P., Furtado A. (2015) Developing cereals acceptable to consumers for production in new and variable climates. *Procedia Environmental Sciences*, Vol. 29, p. 9–10.
13. 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*, Vol. 60(3), p. 561–568.
14. Kruma Z., Tomsone L., Galoburda R., Straumite E., Kronberga A., Åssveen M. (2016a) Total phenols and

antioxidant capacity of hull-less barley and hull-less oats. *Agronomy Research*, Vol. 14(Special Iss. 2), p. 1361–1371.
15. Kruma Z., Tomsone L., Ķince T., Galoburda R., Senhofa S., Sabovics M., Straumite E., Sturite I. (2016b) Effects of germination on total phenolic compounds and radical scavenging activity in hull-less spring cereals and triticale. *Agronomy Research*, Vol. 14(2), p. 1372–1383.
16. Madhujith T., Shahidi F. (2007) Antioxidative and antiproliferative properties of selected barley (*Hordeum vulgarae* L.) cultivars and their potential for inhibition of low-density lipoprotein (LDL) cholesterol oxidation. *Journal of Agricultural and Food Chemistry*, Vol. 55(13), p. 5018–5024.
17. Mamilla R. K., Mishra V. K. (2017) Effect of germination on antioxidant and ACE inhibitory activities of legumes. *LWT - Food Science and Technology*, Vol. 75, p. 51–58.
18. Mareček V., Mikyška A., Hampel D., Čejka P., Neuwirthová J., Malachová A., Cerkal R. (2017) ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. *Journal of Cereal Science*, Vol. 73, p. 40–45.
19. Paucar-Menacho L. M., Peñas E., Dueñas M., Frias J., Martínez-Villaluenga C. (2017) Optimizing germination conditions to enhance the accumulation of bioactive compounds and the antioxidant activity of kiwicha (*Amaranthus caudatus*) using response surface methodology. *LWT-Food Science and Technology*, Vol. 76, p. 245–252.
20. Pihlava J. M., Hellström J., Kurtelius T., Mattila P. (2018) Flavonoids, anthocyanins, phenolamides, benzoxazinoids, lignans and alkylresorcinols in rye (*Secale cereale*) and some rye products. *Journal of Cereal Science*, Vol. 79, p. 183–192.
21. Ronda F., Perez-Quirce S., Lazaridou A., Biliaderis C.G. (2015) Effect of barley and oat β-glucan concentrates on gluten-free rice-based doughs and bread characteristics. *Food Hydrocolloids*, Vol. 48, p. 197–207.
22. Singh A.K., Rehal J., Kaur A., Jyot G. (2015) Enhancement of attributes of cereals by germination and fermentation: A Review. *Critical Reviews in Food Science and Nutrition*, Vol. 55(11), p. 1575–1589.
23. Singleton V.L., Orthofer R., Lamuela-Raventos R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, Vol. 119(25), p. 152–178.
24. Sterna V., Zute S., Brunava L. (2016) Oat grain composition and its nutrition benefice. *Agriculture and Agricultural Science Procedia*, Vol. 8, p. 252–256.
25. Thammapat P., Meeso N., Siriamornpun S. (2015) Effects of NaCl and soaking temperature on the phenolic compounds, α-tocopherol, γ-oryzanol and fatty acids of glutinous rice. *Food Chemistry*, Vol. 175, p. 218–224.
26. Tian B., Xie B., Shi J., Wu J., Cai Y., Xu T., Xue S.J., Deng Q. (2010) Physicochemical changes of oat seeds during germination. *Food Chemistry*, Vol. 119(3), p. 1195–1200.
27. Tiwari U., Cummins E. (2012) Dietary exposure assessment of β-glucan in a barley and oat-based bread. *LWT-Food Science and Technology*, Vol. 47(2), p. 413–420.
28. 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*, Vol. 53(5). p. 451–463.
29. Žilić S., Hadži-Tašković Šukalović V., Dodig D., Maksimović V., Maksimović M., Basić Z. (2011) Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. *Journal of Cereal Science*, Vol. 54(3), p. 417–424.

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FERMENTATION WITH *LACTOBACILLUS* STRAINS FOR ELIMINATION OF GLUTEN IN WHEAT *(TRITICUM AESTIVUM)* BY-PRODUCTS

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Abstract

Recently there is an increase in the number of consumers with gluten intolerance that causes expanding of the demand for gluten-free products. Gluten-free diet is unbalanced and usually has a higher percentage of calories from fat, less of carbohydrates, as well as low intake of non-starch polysaccharides. To solve this problem, new strategies are looked for to eliminate gluten in products of wheat and other cereals and to make them more balanced. Fermentation with lactic cultures and/or enzymes enables to reduce the gluten content in wheat flour. However, this process takes a long time, it is complicated to control, and hydrolysed gluten loses its technological properties. The purpose of this work was to find another way of removing gluten residues: first remove gluten from wheat by wet fractionation, then hydrolyse gluten residues in the remaining fractions by using biotechnological measures. The fractions of starch, fiber and bran had an initial gluten concentration of 85–33750 mg kg^{-1}. For eliminating the gluten residues, they were fermented with four probiotic strains separately: *Lactobacillus plantarum* P-1, *Lactobacillus brevis* R-1, *Lactobacillus acidophilus* 308, *Lactobacillus acidophilus* 336. Short (12 hours) and long fermentation (24 hours) at 30 and 37 °C was used. Gluten was degraded in wheat starch to below 20 mg kg^{-1} using *Lactobacillus plantarum* in short time, other strains performed better using long fermentation. In conclusion, it could be stated that sourdough-based biotechnology could eliminate the immunogenicity of wheat by-products and to improve the quality of life of celiac patients.

Keywords: wheat, gluten, hydrolysis, sourdough, *Lactobacillus*

Introduction

Wheat is one of the most popular cereals in the world, however, gluten proteins of wheat are responsible for very common allergic reactions in populations, leading to immune disorder and non-celiac gluten sensitivity (Gujral et al., 2012; Kang et al., 2013; Catassi et al., 2014). Currently, the only therapy is a strict, lifelong gluten-free diet (GFD). Compliance with a GFD is an extremely challenging task, given a number of problems related to poor quality of gluten-free products compared to their gluten-rich counterpart (Do Nascimento et al., 2017) as well as these products are more expensive (Stevens, Rashid, 2008). Patients with celiac disease are looking for alternatives and are using products from gluten free materials such as corn, rice, millet, buckwheat, amaranths and potatoes. The diet of these patients is unbalanced and have a higher percentage of calories from fat and less from carbohydrates, also in GFD was obtained low intakes of non-starch polysaccharides (Thompson et al., 2005; Wild et al., 2010). Products made from naturally gluten-free raw materials resulted in breads often having inferior textural and sensory properties compared to the corresponding gluten-containing products (Hager et al., 2012; Miranda et al., 2014; Pellegrini, Agostoni, 2015). To resolve this socioeconomic problem, new strategies are looked for to eliminate harmful gluten from wheat and other cereals and to produce balanced products with good sensory properties (Greco et al., 2011; Nionelli, Rizzello, 2016).

Wheat gluten fragments (peptides) remain intact during digestion. They penetrate through the small intestine wall and initiate antigenic cellular immune responses. There is no immune response if the gluten is hydrolysed to peptides, which contain less than nine amino acid residues. Research on the use of biological measures in wheat products to eliminate or reduce the immune toxicity of gluten proteins is being actively pursued in the last decade. Numerous studies (Di Cagno et al., 2008; Giuliani et al., 2016; Gerez et al., 2012; Loponen et al., 2007; Romanová, Urminská, 2017; De Palma et al., 2010; Stefanska et al., 2016) were carried out using lactic cultures - their individual strains or various combinations. They focus on probiotic strains' possibilities to decrease the toxicity of wheat flour, but there is a lack of informationon on biological measures to completely eliminate gluten from wheat processing products.

Sourdough fermentation with lactic acid bacteria (LAB) can improve the texture, palatability, aroma, shelf life and nutritional value of wheat breads (Guerzoni et al., 2011), texture and palatability of whole grain, fiber-rich or gluten-free products, stabilise or increase levels of various bioactive compounds, retard starch bioavailability and improve mineral bioavailability (Katina et al., 2005; Moroni et al., 2009). LAB degrade celiac active gluten peptides, because some species of LAB produce specific peptidases during growth, which are capable to hydrolyse hardly cleavable bonds between amino acids in proline-rich peptides (Vukotić et al., 2016). Selecting strains of LAB with targeted proteolytic effects is vital important (Stefańska et al, 2016).

The use of sourdough LAB was at first proposed with the aim of eliminating traces of gluten epitopes in 2002. Di Cagno et al. (2002) showed that selected LAB, possessing proteolytic activities, could efficiently hydrolyse the toxic peptides of gliadin in wheat sourdough. The pool of *L. alimentarius* 15M, *L. brevis* 14G, *L. sanfranciscensis* 7A, and *L. hilgardii* 51B has a

pattern of specialized peptidases capable of hydrolysing different peptide bonds that potentially include the proline (Di Cagno et al., 2004). Study of Di Cagno et al. (2008) highlighted the use of selected LAB consisted of *Lactobacillus sanfranciscensis* LS40 and LS41 and *Lactobacillus plantarum* CF1 to eliminate risks of contamination by gluten and to enhance the nutritional properties of GF bread.

Several studies were carried out using individual strains of LAB also. Sourdough fermentation using *Lactobacillus sanfranciscensis* (Thiele et al, 2004; Vermeulen et al, 2006) or *Lactobacillus plantarum* (Yin et al, 2015) showed a decrease in pH and resulted in hydrolysis and solubilization of wheat proteins.

De Angelis et al. (2006) showed the capacity of probiotic VSL#3 preparation to hydrolyse extensively wheat flour. Probiotic product VSL#3 including *Streptococcus thermophilus, L. plantarum, L. acidophilus, L. casei, L. delbrueckii spp. bulgaricus, Bifidobacterium breve, B. longum and B. infantis* strains was used in the fermentation of a mass with wheat flour in order to hydrolyse gliadin peptides and promoted almost complete hydrolysis of gliadin peptides. Patent Application WO2006/097415 (2006) describes a process for gluten degradation by means of the use of a complex mixture consisting of at least six lactic acid bacteria and/or bifidobacteria and long fermentation times (24–31 hours) also. After hydrolysis some gliadins were partially hydrolysed, but others were not susceptible to hydrolysis process. *Lactobacillus plantarum* CRL 775 and *Pediococcus pentosaceus* CRL 792 also hydrolysed gliadins during wheat dough fermentation (Gerez et al., 2012).

Romanová, Urminská (2017) described growth characteristics and intracellular aminopeptidases activities of *Lactobacillus plantarum* CCM 3627 and *Lactobacillus brevis* CCM 1815. The results confirm production of active proline aminopeptidase, which is important for cleavage of proline rich-peptides. Two strains: *Enterococcus mundtii* and *Wickerhamomyces anomalus* exhibited the potential to be used as probiotic for sourdough fermentation: they have shown the ability to tolerate low pH, bile salt properties and hydrophobicity compared to other gluten-degrading yeast and bacterial strains (Sakandar et al., 2018).

Stefańska et al. (2016) have selected 11 LAB strains capable of hydrolysing gliadin in bakery sourdoughs. However, in all sourdoughs were found some polypeptides with IgE-reactive epitopes. Previous research has shown that fermentation with LAB reduces the amount of reactive gluten fragments, but does not reach the safe limit for gluten free products, which is 20 mg kg^{-1} (Standard 118-1979, 2015).

Fermentation with mixtures of selected lactic acid cultures in combination with fungal enzymes enable to reduce the gluten content in wheat flour to gluten free limit (Rizzello et al., 2007; 2014). However, this process takes a long time, it is necessary to control it in several stages, but hydrolysed gluten still loses its technological properties: enzymatic hydrolysis destroys the gluten network, reduces the elasticity of the dough and baked goods (Van Den Broeck et al., 2009).

In this work, it would be advisable to combine physical and biotechnological measures for the preparation of raw materials for the production of gluten-free wheat products: at first remove gluten from wheat by wet fractionation, then hydrolyse gluten residues in the remaining fractions by using LAB fermentation. Whereas the levels of gluten are low in the wheat by-products, it can be expected that the effect of LAB will be sufficient to eliminate it.

Wet fractionation of wheat could be done by centrifuging of the flour-water mixture in the laboratory according to Czuchajowska and Pomeranz (1993) as well as at industrial plants producing gluten and starch from wheat that are widely used in the food industry, meanwhile the fractions of fiber and bran are diverted to feed production.

Eliminating of gluten residue in wheat processing products allows produce gluten free starch and gluten free fraction of arabinoxylan and other non-starch polysaccharides, suitable for flour mixtures or bakery production for users intolerant to gluten or celiac sufferers.

The aim of the research was to use fermentation with *Lactobacillus* strains for elimination of gluten in wheat by-products.

Materials and Methods

Materials

Investigations were carried out at the Kaunas University of Technology, Food Institute, Lithuania. Samples of wheat fractions after dry and wet fractionation: starch, fiber and bran were provided by Roquette Amilina, AB, Lithuania.

A fraction of wheat bran was obtained as a by-product during the dry milling of wheat grain and was composed of outer layers of wheat kernel, mainly pericarp. A fraction of fiber was obtained as a by-product in the wet processing of the flour for starch and gluten separation, and was mainly composed of seed coat and aleurone residues.

Physical-chemical analysis of composition of wheat by-products

Moisture content of wheat by-products was measured by humidity measuring device Kern MLS 50-3HA 160N.

pH was measured by pH-meter ORION 3STAR.

Determination of protein content was done by Kjeldahl method (LST EN ISO 20483).

Research on the selection of Lactobacillus strains

Research to remove wheat gluten was carried out experimenting with microorganisms of the collection from the KTU Food Institute. Four probiotic strains: *Lactobacillus plantarum* P-1, *Lactobacillus brevis* R-1, *Lactobacillus acidophilus* 308, *Lactobacillus acidophilus* 336 were used.

The LAB cultures were stored for the study at –72–74 °C in the VIABANK (MWE medical wire) system. Cultures revived in MRS broth (Biolife, Italy):

an initial LAB suspension was prepared by seeding of the initial culture on MRS agar (Biolife, Italy) and incubating at 30 °C *(L. plantarum* P-1*, L. brevis* R-1) and at 37 °C (*L. acidophilus* 308, *L. acidophilus* 336) for 24 h. Each LAB culture was transformed then into sterile milk and incubated at an appropriate temperature for 72 h under anaerobic conditions (aerostat with oxygen sorbent).

The number of lactic acid bacteria was determined by the method of seeding in Petri dishes by incubation on MRS agar under anaerobic conditions for 72 h at 30 or 37 °C.

Total plate count of samples performed according to standard procedures LST EN ISO 4833:2003, found 2.1×10^2 CFU g^{-1}, count of yeast and mold 1.2×10^1 CFU g^{-1}, aerobic and anaerobic spore forming bacteria was not found. The amounts of these microorganisms in the samples were small and the samples were suitable for consumption and biotechnological work without sterilization from the microbiological safety point of view.

Wheat starch or fiber samples (5–10 g) were weighed into glass tubes (30 mL), distilled water (10 mL) and LAB suspension (7 mL) at an active concentration of $1.7–2.8\times10^6$ was added (Table 1) and mixed well.

Table 1

Preparing of samples of wheat product with different LAB strains

Sample	Wheat by-product		LAB strains	Active concentration, CFU g^{-1}
	Starch, g	Fibre, g		
SLA1	5	–	*L. acidophilus* 308	2.0×10^6
SLA2	5	–	*L. acidophilus* 336	2.1×10^6
SLB	5	–	*L.brevis* R-1	1.7×10^6
SLP	5	–	*L.plantarum* P-1	2.8×10^6
FLA1	–	10	*L. acidophilus* 308	2.0×10^6
FLA2	–	10	*L. acidophilus* 336	2.1×10^6
FLB	–	10	*L.brevis* R-1	1.7×10^6
FLP	–	10	*L.plantarum* P-1	2.8×10^6

LAB – lactic acid bacteria, SLA1 – starch with *L. acidophilus* 308*;* SLA2 – starch with *L. acidophilus* 336*;* SLB – starch with *L. brevis* R-1*;* SLP – starch with *L. plantarum* P-1*;* FLA1 – fiber with *L. acidophilus* 308*;* FLA2 – fiber with *L. acidophilus* 336*;* FLB – fiber with *L. brevis* R-1*;* FLP – fiber with *L. plantarum* P-1

Samples were incubated at 30 and 37 °C, pH and gluten content in sourdough were measured after 12 and 24 hours.

Gluten quantitation by ELISA

Gluten residues in wheat products were quantitated by competitive ELISA using G12 antibody AACCI 38-52.01 (Romer Labs, UK Ltd) according to the manufacturer's instructions. Gluten concentrations were established based on calibration function provided by Romer Labs. Multiscan EX microplate reader with a 450 nm filter was used for the reading of the strips.

Results and Discussion

The fractions of starch, fiber and bran had different moisture content (10.78–72.01%), different amount of total protein (0.32–16.9%) an initial gluten concentration of 85–33750 mg kg^{-1} (Table 2).

For eliminating of gluten residues starch and fiber were fermented with four probiotic strains separately: *Lactobacillus plantarum* P-1, *Lactobacillus brevis* R-1, *Lactobacillus acidophilus* 308, *Lactobacillus acidophilus* 336. Short (12 hours) and long fermentation (24 hours) at 30 and 37 °C was used.

Table 2

Characteristics of samples

Wheat by-products	Moisture, %	Total protein, %	Gluten content, mg kg^{-1}
Fiber*	72.01±0.20	3.80±0.02	7800.00±218.00
Bran**	13.43±0.20	16.90±0.02	33750.00±945.00
Starch	10.78±0.20	0.32±0.02	85.00±2.00

* Outer layers of wheat kernel, mainly pericarp.
** Seed coat and aleurone residues.

The reducing of gluten content depending on the decrease of pH was observed. Gerez et al. (2008), Rollan et al. (2016) demonstrated also that protein hydrolysis in sourdough were partially caused by pH-dependent activation of cereal enzymes according to change in proteolytic activity. Di Cagno et al. (2002) proved also, that primary proteolysis is exerted by wheat endogenous enzymes, which are activated by the low pH. Among the selected LAB cultures, the lowest pH was achieved by using *L. acidophilus* 308, the least acidic sourdough was obtained with *L. brevis* R-1. The pH of the fermentation of starch was also lower than sourdough of fiber (Figure 1).

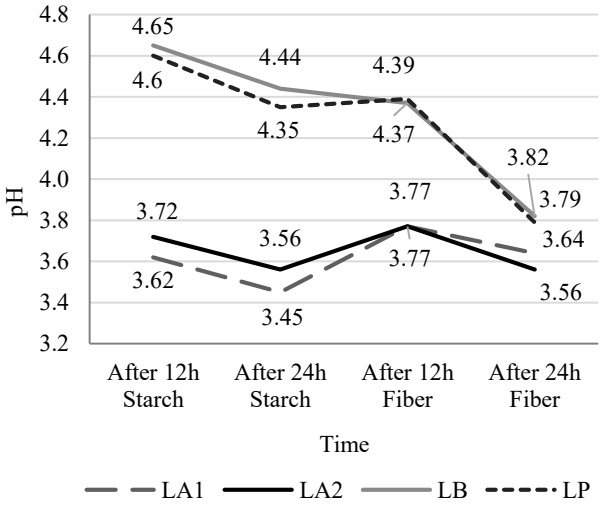

Figure 1. Decreasing of pH in different sourdough
LA1 – *L. acidophilus 308*; LA2 – *L. acidophilus 336;* LB – *L. brevis R-1*; LP – *L. plantarum P-1*

Further hydrolysis of peptides was exerted by intracellular peptidases of LAB in a strain-specific manner: the type and amount of released amino acids depend on the fermenting strain (Di Cagno et al., 2002).

Table 3

pH and gluten content in samples of wheat starch after fermentation

Samples	Temperature, °C	Duration of fermentation, h			
		12		24	
		pH	Gluten content, mg kg^{-1}	pH	Gluten content, mg kg^{-1}
SLA1	37	3.62±0.02	25.00±0.60	3.45±0.02	12.00±0.30
SLA2	37	3.72±0.02	28.00±0.70	3.56±0.02	26.00±0.70
SLB	30	4.65±0.02	36.00±0.90	4.44±0.02	30.00±0.80
SLP	30	4.60±0.02	20.00±0.50	4.35±0.02	15.00±0.40

SLA1 – starch with *L. acidophilus* 308*;* SLA2 – starch with *L. acidophilus* 336*;* SLB – starch with *L. brevis* R-1*;* SLP – starch with *L. plantarum* P-1

Table 4

pH and gluten content in samples of wheat fiber after fermentation

Samples	Temperature, °C	Duration of fermentation, h			
		12		24	
		pH	Gluten content, mg kg^{-1}	pH	Gluten content, mg kg^{-1}
FLA1	37	3.77±0.02	4500.00±126.00	3.64±0.02	2450.00±67.00
FLA2	37	3.77±0.02	4650.00±130.00	3.56±0.02	2200.00±62.00
FLB	30	4.37±0.02	5100.00±143.00	3.82±0.02	2810.00±79.00
FLP	30	4.39±0.02	4700.00±132.00	3.79±0.02	2500.00±70.00

FLA1 – fiber with *L. acidophilus* 308*;* FLA2 – fiber with *L. acidophilus* 336*;* FLB – fiber with *L. brevis* R-1*;* FLP – fiber with *L. plantarum* P-1

The selected strains exhibited different proteolytic activity in this research, which leads to a reduction of gluten content in wheat sourdoughs. The amount of non-digestible gluten peptides decreased in all fermented starch samples after 12 h, but the most pronounced proteolytic effect was observed in sourdough with *L. Plantarum* P-1 (Table 3).

After 24 hours the lowest amount of immunoreactive gluten peptides was found in sourdough with *L. acidophilus* 308 and *L. Plantarum* P-1. Sourdough fermentation decreases the disulphide bonds in gluten network, which influence its digestibility in people with gluten sensitivity (Gänzle et al, 2008). Although fermentation of starch with *L. acidophilus* 336 most reduced pH, failed to reduce the gluten content to 20 mg kg^{-1}. Fiber of wheat (after wet fractionation) had a high initial content of gluten (up to 33750 mg kg^{-1}).

Despite a significant decrease in pH after fermentation with LAB the amount of gluten was reduced but remained still high (Table 4). The results showed that LAB fermentation can only eliminate small amounts of gluten while content of gluten at high concentrations still remains high after hydrolysis. Similar results were obtained by hydrolysis of fermentation of wheat flour. In the wheat flour 74590–80127 mg kg^{-1} of gluten were found (Greco et al., 2011). Results achieved in the studies (De Angelis et al., 2006; Stefańska et al., 2016) demonstrate that the proteolytic activity of the selected LAB strains is not high enough to allow their use for the degradation of allergenic proteins in bakery products made from wheat flour intended for patients with food allergy to gluten. Fermentation with LAB, however, could be used in production from raw materials with low gluten content and, at high concentrations, their effects must be combined with proteolytic effects of the enzymes.

Conclusions

The role of a fermentation process for improving the quality of GF products and developing a new concept of GF products is very important, however, this method is not suitable to completely degrade gluten, when its initial amount in the raw material is high.

Gluten could be degraded in wheat starch to levels below 20 mg kg^{-1} by application of *Lactobacillus plantarum* P-1 in a short time period, and *L. acidophilus* 308 after a long fermentation. These selected LAB strains may be applied as the specific starter cultures to prepare bakery products of special nutritional use from wheat starch, but do not reduce the amount of gluten in fiber and bran to a safe limit for gluten-free diet.

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References

1. Catassi C., Gatti S., Fasano A. (2014) The new epidemiology of celiac disease. *Journal of Pediatric Gastroenterology and Nutrition,* Vol. 59, p. 7–9.
2. Czuchajowska Z., Pomeranz Y. (1993) Protein concentrates and prime starch from wheat flours. *Cereal Chemistry,* Vol. 70 (6), p. 701–706.
3. De Angelis M., Rizzello C.G., Fasano A., Clemente M.G., De Simone C., Silano M., De Vincenzi M., Losito I., Gobbetti M. (2006) VSL# 3 probiotic preparation has the capacity to hydrolyze gliadin polypeptides responsible for celiac sprue probiotics and gluten intolerance. *Biochimica et Biophysica Acta (BBA) Molecular Basis of Disease*, Vol. 1762(1), p. 80–93.
4. De Palma G., Cinova J., Stepankova R., Tuckova L., Sanz Y. (2010) Pivotal Advance: Bifidobacteria and gram-negative bacteria differentially influence immune

responses in the proinflammatory milieu of celiac disease. *Journal of Leukocyte Biology,* Vol. 87(5), p. 765–778.
5. Di Cagno R., De Angelis M., Auricchio S., Greco L., Clarke C., De Vincenzi M., Giovannini C., D'Archivio M., Landolfo F., Parrilli G., Minervini F., Arendt E. Gobbetti M. (2004) Sourdough bread made from wheat and nontoxic flours and started with selected *Lactobacilli* is tolerated in celiac sprue patients. *Applied and Environmental Microbiology,* Vol. 70, p. 1088–1096.
6. Di Cagno R., De Angelis M., Lavermicocca P., De Vincenzi M., Giovannini C., Faccia M., Gobbetti M. (2002) Proteolysis by sourdough lactic acid bacteria: effects on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance. *Applied and Environmental Microbiology,* Vol. 68(2), p. 623–633.
7. Di Cagno R., Rizzello C.G., De Angelis M., Cassone A., Giuliani G., Benedusi A., Gobbetti M. (2008) Use of selected sourdough strains of *Lactobacillu*s for removing gluten and enhancing the nutritional properties of gluten-free bread. *Journal of Food Protection,* Vol. 71(7), p. 1491–1495.
8. Do Nascimento A.B., Fiates G.M.R., Teixeira E. (2017) We want to be normal! Perceptions of a group of Brazilian consumers with coeliac disease on gluten-free bread buns. *International Journal of Gastronomy and Food Science,* Vol. 7, p. 27–31.
9. Gänzle M. G., Loponen J., Gobbetti M. (2008) Proteolysis in sourdough fermentations: mechanisms and potential for improved bread quality. *Trends in Food Science & Technology,* Vol. 19 (10), p. 513–521.
10. Gerez C. L., Dallagnol A., Rollán G., de Valdez G. F. (2012) A combination of two lactic acid bacteria improves the hydrolysis of gliadin during wheat dough fermentation. *Food Microbiology,* Vol. 32(2), p. 427–430.
11. Giuliani G., Benedusi A., Di Cagno R., Rizzello C. G., De Angelis M., Gobbetti M., Cassone A. (2016) U.S. Patent No. 9,386,777. Washington, DC: U.S. Patent and Trademark Office.
12. Greco L., Gobbetti M., Auricchio R., Di Mase R., Landolfo F., Paparo F., Terrone G. (2011) Safety for patients with celiac disease of baked goods made of wheat flour hydrolyzed during food processing. *Clinical Gastroenterology and Hepatology,* Vol. 9 (1), p. 24–29.
13. Guerzoni M. E., Gianotti A., Serrazanetti D. I. (2011) Fermentation as a tool to improve healthy properties of bread. **In:** *Flour and breads and their fortification in health and disease prevention.* London: Academic Press, p. 385–393.
14. Gujral N., Freeman H., Thomson A. (2012) Celiac disease: Prevalence, diagnosis, pathogenesis and treatment. *World Journal of Gastroenterology,* Vol. 18 (42), p. 6036–6059.
15. Hager A. S., Wolter A., Czerny M., Bez J., Zannini E., Arendt E. K., Czerny M. (2012). Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts. *European Food Research and Technology*, Vol. 235 (2), p. 333–344.
16. Kang J. Y., Kang A. H. Y., Green A., Gwee K. A., Ho K. Y. (2013) Systematic review: worldwide variation in the frequency of coeliac disease and changes over time. *Alimentary Pharmacology & Therapeutics,* Vol. 38(3), p. 226–245.
17. Katina K., Arendt E., Liukkonen K. H., Autio K., Flander L., Poutanen K. (2005) Potential of sourdough for healthier cereal products. *Trends in Food Science & Technology,* Vol. 16 (1–3), p. 104–112.
18. Loponen J., Sontag-Strohm T., Venäläinen J., Salovaara H. (2007) Prolamin hydrolysis in wheat sourdoughs with differing proteolytic activities. *Journal of Agricultural and Food Chemistry,* Vol. 55 (3), p. 978–984.
19. Miranda J., Lasa A., Bustamante M. A., Churruca I., Simon E. (2014). Nutritional differences between a gluten-free diet and a diet containing equivalent products with gluten. *Plant Foods for Human Nutrition,* Vol. 69 (2), p. 182–187.
20. Moroni A. V., Dal Bello F., Arendt E.K. (2009) Sourdough in gluten-free bread-making: an ancient technology to solve a novel issue? *Food Microbiology*, Vol. 26(7), p. 676–684.
21. Nionelli L., Rizzello C. G. (2016) Sourdough-based biotechnologies for the production of gluten-free foods. *Foods,* Vol. 5(3), p. 65–79.
22. Pellegrini N., Agostoni C. (2015) Nutritional aspects of gluten-free products. *Journal of the Science of Food and Agriculture*, Vol. 95 (12), p. 2380–2385.
23. Rizzello C. G., De Angelis M., Di Cagno R., Camarca A., Silano M., Losito I., Gianfrani C. (2007) Highly efficient gluten degradation by lactobacilli and fungal proteases during food processing: new perspectives for celiac disease. *Applied and Environmental Microbiology,* Vol. 73 (14), p. 4499–4507.
24. Rizzello C. G., Curiel J. A., Nionelli L., Vincentini O., Di Cagno R., Silano M., Coda R. (2014) Use of fungal proteases and selected sourdough lactic acid bacteria for making wheat bread with an intermediate content of gluten. *Food Microbiology,* Vol. 37, p. 59–68.
25. Rollan G., De Angelis M., Gobbetti M., De Valdez G. F. (2005) Proteolytic activity and reduction of gliadin-like fractions by sourdough *lactobacilli. Journal of Applied Microbiology,* Vol. 99(6), p. 1495–1502.
26. Romanová K., Urminská D. (2017) Potential of *Lactobacillus plantarum* CCM 3627 and *Lactobacillus brevis* CCM 1815 for fermentation of cereal substrates. *Potravinarstvo Slovak Journal of Food Sciences*, Vol. 11 (1), p. 544–549.
27. Sakandar H.A., Usman K. and Imran M. (2018) Isolation and characterization of gluten-degrading *Enterococcus mundtii* and *Wickerhamomyces anomalus*, potential probiotic strains from indigenously fermented sourdough (Khamir). *LWT Food Science and Technology*, Vol. 91, p. 271–277.
28. Standard 118-1979 (2015) Codex Alimentarius Commission. Revision 1, Amendment 2. [accessed on 04.12.2018.]. Available at: http://www.codex alimentarius.net/download/standards/291/cxs_118e.pdf
29. Stefańska I., Piasecka-Jóźwiak K., Kotyrba D., Kolenda M., Stecka K. M. (2016) Selection of lactic acid bacteria strains for the hydrolysis of allergenic proteins of wheat flour. *Journal of the Science of Food & Agriculture,* Vol. 96 (11), p. 3897–3905.
30. Stevens L. and Rashid M. (2008) Gluten-free and regular foods: a cost comparison. *Canadian Journal of Dietetic Practice and Research,* Vol. 69 (3), p. 147–150.
31. Thompson T., Dennis M., Higgins L. A., Lee A. R., Sharrett M. K. (2005) Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods? *Journal of Human Nutrition and Dietetics,* Vol. 18(3), p. 163–169.
32. Thiele C., Grassl S., Gänzle M. (2004) Gluten hydrolysis and depolymerization during sourdough fermentation. *Journal of Agricultural and Food Chemistry,* Vol. 52(5), p. 1307–1314.
33. Van Den Broeck H. C., Van Herpen T. W., Schuit C., Salentijn E. M., Dekking L., Bosch D., Van Der Meer I. M. (2009) Removing celiac disease-related gluten proteins from bread wheat while retaining technological

properties: a study with Chinese spring deletion lines. *BMC Plant Biology,* Vol. 9(1), p. 41.
34. Vermeulen N., Kretzer J., Machalitza H., Vogel R. F., Gänzle M. G. (2006) Influence of redox-reactions catalysed by homo-and hetero-fermentative *lactobacilli* on gluten in wheat sourdoughs. *Journal of Cereal Science,* Vol. 43 (2), p. 137–143.
35. Vukotić G., Strahinić I., Begović J., Lukić J., Kojić M., Fira D. (2016) Survey on proteolytic activity and diversity of proteinase genes in mesophilic *Lactobacilli. Microbiology,* Vol. 85 (1), p. 33–41.
36. Wild D., Robins G. G., Burley V. J., Howdle P. D. (2010) Evidence of high sugar intake, and low fibre and mineral intake, in the gluten-free diet. *Alimentary Pharmacology & Therapeutics,* Vol. 32 (4), p. 573–581.
37. Mixture of at least 6 species of lactic acid bacteria and/or bifidobacteria in the manufacture of sourdough (2006). [accessed on 08.11.2018.]. Available at: https://patents.google.com/patent/WO2006097415A1/en
38. Yin, Y., Wang, J., Yang, S., Feng, J., Jia, F., Zhang, C. (2015) Protein degradation in wheat sourdough fermentation with *Lactobacillus plantarum M616*. *Interdisciplinary Sciences: Computational Life Sciences,* Vol. 7 (2), p. 205–210.

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EFFECT OF TEMPERATURE CHANGES DURING TRANSPORTATION ON MUESLI QUALITY

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Abstract

Muesli is often packed in pre-made stand-up pouches and flowpacks. There are different storage and packaging solutions based on the composition of the storage atmosphere. Modified atmosphere packaging (MAP) extends shelf-life of product as it affects biochemical, enzymatic and microbiological changes. Nevertheless, during food product transportation around 9% of complaints are related to wet cargo. It is the second most common problem in freight transportation and causes greatest losses during logistics. A sudden drop in temperature during transportation can cause condensation, increasing moisture migration in the product, reducing its quality and promoting deterioration. The aim of this study was to determine optimal muesli packaging solutions to eliminate the possibility of product quality deterioration due to changes in temperature during muesli transportation. Muesli samples were packed in three different types of packaging – 2-layer (PET/PE) and 3-layer (PAP/AL/PE) stand-up pouches, and biodegradable PLA pouches in air ambience and MAP environment. Samples were stored at ambient temperature (20±1 °C) for 14 days. After two-week ambient storage, samples were stored at -5±1 °C, 0±1 °C and 30±1 °C temperature for 5 days, followed by storage at ambient temperature for 2 days. The results of water activity, moisture content, pH value, texture and microbiological parameters showed no significant changes in muesli quality.

Keywords: muesli, packaging, MAP, temperature changes

Introduction

Cereals and their products are an important source of energy, carbohydrates, proteins and dietary fibre, which also contain number of micronutrients such as vitamin E, vitamin B, magnesium and zinc, and several bioactive compounds that play an important role in the human diet (McKevith, 2004). Cereals are one of the most important food sources which are used to produce different grain products and animal feed (Wrigley, 2019). Cereal-based food products have been the basis of the human diet since ancient times (Borneo, Leon, 2011). One of the growing grain-based food trends are muesli. Muesli is product made from a mixture of oats and other cereals, dried fruits and nuts and eaten with milk (Complete Oxford Dictionary, 2011).

One of the main factors playing an important role in food quality is packaging. The principal function of packaging is protection and preservation from external contamination. This involves retarding deterioration, extending shelf life and maintaining the quality and safety of packaged food. The package protects food from such environmental influences as heat, light, the presence and absence of moisture, oxygen, pressure, enzymes, undesirable odours, microorganisms etc. (Kour et al., 2013). It is crucial to minimize the rates of biochemical, enzymatic and microbial degradation reactions to extend product shelf life. Commonly it is achieved by using proper sanitation conditions during production. Modified atmosphere packaging (MAP) is defined as the enclosure of packed food with an optimal gas composition that is specially designed to extend its shelf life (Kirtil, Oztop, 2016). Food quality is highly dependent on temperature management. Storage and transportation temperature are the most variable factors influencing product changes which lead to quality deterioration (Rozman et al., 2008).

The aim of this study was to determine optimal muesli packaging solutions to eliminate the possibility of product quality deterioration due to changes in temperature during muesli transportation.

Materials and Methods

Experiments were carried out at the laboratories of the Faculty of Food Technology, Latvia University of Life Sciences and Technologies.

Raw materials and packaging solutions

All ingredients used for muesli sample preparation were supplied by Felici Ltd. (Table 1).

Table 1

Recipe of muesli used for the research

Ingredients	Amount, %	Country of origin
Oat wholegrain flakes	40	Latvia
Toasted triticale wholegrain flakes	30	United Kingdom
Roasted, diced peanuts	8	Argentina
Whole flaxseeds	6	Latvia
Chocolate shavings	6	Germany
Raisins	6	Turkey
Sunflower seeds	4	Bulgaria

Muesli samples were packed in three different packaging materials – 3-layer stand-up pouches made from paper, aluminium and polyethylene layers (PAP/AL/PE) (thickness 148±2 μm), 2-layer stand-up pouches made from polyethylene terephthalate and polyethylene layers (PET/PE) (thickness 132±2 μm) and biodegradable material made from polylactic acid and silicon dioxide layers (PLA/SiO_2) (thickness 50±2 μm). Two types of packaging environment were used: air ambience and modified air packaging (MAP) (30% CO_2, 70% NO_2). The sample weight was

250±1 g. A total of 24 different samples were packed and tested (Table 2).

The prepared samples were stored at ambient temperature (20±1 °C) for 14 days. After a two-week storage at ambient temperature, samples were moved to different temperature regimes and stored at -5±1 °C, 0±1 °C and 30±1 °C temperature for 5 days, followed by storage at ambient temperature for 2 days before analysis.

Table 2

Sample codes used in the research

Packaging material	Packaging environment	Storage temperature, °C	Sample codes
Without any	Air ambience	20±1	Control*
PAP/AL/PE	Air ambience	0±1	1F0
		-5±1	1F5
		20±1	1F20
		30±1	1F30
	MAP	0±1	2F0
		-5±1	2F5
		20±1	2F20
		30±1	2F30
PET/PE	Air ambience	0±1	1P0
		-5±1	1P5
		20±1	1P20
		30±1	1P30
	MAP	0±1	2P0
		-5±1	2P5
		20±1	2P20
		30±1	2P30
Biodegradable PLA/SiO_2	Air ambience	0±1	1B0
		-5±1	1B5
		20±1	1B20
		30±1	1B30
	MAP	0±1	2B0
		-5±1	2B5
		20±1	2B20
		30±1	2B30

* initial sample (before packaging)

Physicochemical quality analysis

Muesli was grinded in a laboratory mill for moisture, water activity and pH analysis. Three replicates were tested per analysis for each sample.

Moisture content (%) was determined by drying at 110±1 °C for 2 h using Memmert drying chamber (GmbH Memmert, Germany).

Water activity (a_w) was determined using Novasina LabSwift-aw (Novatron Scientific, UK) equipment.

pH of muesli was assessed using Jenway 3510 pH-meter (Cole-Parmer, UK) after mixing it with distilled water (10:1).

Hardness (N) was assessed with Texture Analyser TA.HD Plus (Stable Micro Systems, UK) using back extrusion rig with 45 mm disc. Two types samples were analysed – a dry muesli sample (20.0±0.1 g) and a muesli sample (20.0±0.1 g) soaked in milk (50±1 mL) for 1 minute (Medina et al., 2013). The following parameters were used for the measurements of dry muesli sample: test speed 10 mm s^{-1}, distance 10 mm, trigger force 0.049 N. Test speed of 15 mm s^{-1} was used to test muesli sample (20.0±0.1 g) soaked in milk.

Microbiological parameters

Preparation of decimal dilutions for microbiological analysis was carried out according to ISO 6887-4:2017 standard. For yeast and mould determination Malt extract agar medium (Malt extract agar, Ref. 01-111, Scharlau, Spain) was used with incubation at 27±1 °C for 48 hours (Kirse et al., 2017). Total microorganism colony count was assessed according to ISO 4833-1:2013 standard. Colonies of microorganisms were counted using automatic counter (aColyte, Topac Inc., USA). Number of colonies was expressed as colony forming units per gram of product (CFU g^{-1}). Two parallel repetitions per dilution for reliable data were performed.

Data processing

The obtained data processing was performed using MS Excel 2013 software. ANOVA analysis were performed to determine differences within the samples. Samples were tested in triplicate. Factors were defined as significant if p-value was below 0.05.

Results and Discussion

Changes in water activity, moisture content, pH value

Changes in water activity, moisture content and pH value after storage in different temperatures are shown in Table 3. Water activity influences various biochemical reactions and microbial growth in food products (Syamaladevi et al., 2016). Results show that water activity changes in all samples was insignificant, a_w ranged between 0.330 to 0.420. It shows that environment is not beneficial for microbial growth (Madriz-Sanch, 2003). Most noticeable water activity changes were found in sample 2P20 which was packed in 2-layer stand-up pouch (PET/PE).

All muesli ingredients have different moisture content which affects the total moisture level in the mixed muesli. As reported by Kince et al. (2018) toasted grain flakes have up to 4.2 times lower moisture content than rolled grain flakes, therefore suggesting that moisture content of whole muesli should be low. Total moisture content in all samples ranged between 2.95–3.99%. It was 4 times lower than found in the study by Senhofa et al. (2015), in which the studied muesli did not contain toasted flakes but had a higher proportion of dried fruits. The highest determined moisture content was for 1P30 sample which for dried food products is still low, yet, there were differences of only borderline significance in moisture content among all samples ($p<0.1$). Temperature changes did not affect moisture of muesli samples, which could be associated with barrier properties of packaging materials – moisture migration between environment outside and inside the packaging was not confirmed.

pH value affects food product quality, mostly colour, sensory parameters and microbial growth (Andres-Bello et al., 2013). Results showed that pH value in muesli samples ranged between 5.700 to 6.129.

Overall, there were no significant changes in water activity, moisture content and pH value ($p>0.05$) of all tested muesli samples during temperature abuse testing and with regards to the initial muesli sample.

Table 3

Water activity, pH and moisture changes in muesli after storage at different temperatures

Samples	Water activity, a_w	pH	Moisture, %
Control	0.362±0.02	5.567±0.070	3.04±0.23
1F0	0.373±0.02	5.989±0.098	3.22±0.10
1F5	0.387±0.01	5.888±0.069	3.80±0.33
1F20	0.378±0.01	6.129±0.064	3.85±0.12
1F30	0.378±0.01	6.009±0.115	3.31±0.16
2F0	0.374±0.02	5.993±0.064	3.43±0.25
2F5	0.380±0.02	5.973±0.032	3.99±0.33
2F20	0.338±0.01	5.762±0.132	2.95±0.27
2F30	0.370±0.01	5.932±0.017	3.71±0.20
1P0	0.365±0.01	5.948±0.031	3.46±0.10
1P5	0.363±0.02	5.817±0.019	3.77±0.04
1P20	0.346±0.01	5.734±0.066	3.37±0.02
1P30	0.345±0.01	5.700±0.033	3.18±0.24
2P0	0.368±0.02	6.080±0.027	3.15±0.42
2P5	0.364±0.02	5.925±0.048	3.18±0.07
2P20	0.420±0.01	5.738±0.074	3.13±0.25
2P30	0.365±0.02	5.827±0.091	3.80±0.32
1B0	0.378±0.02	5.832±0.107	3.60±0.41
1B5	0.374±0.02	5.815±0.057	3.56±0.36
1B20	0.389±0.01	5.991±0.034	3.97±0.46
1B30	0.373±0.01	5.765±0.046	2.89±0.04
2B0	0.366±0.01	5.657±0.164	3.15±0.02
2B5	0.369±0.01	5.962±0.073	3.56±0.15
2B20	0.392±0.01	5.655±0.048	3.52±0.30
2B30	0.363±0.02	5.900±0.027	3.08±0.46

Changes in muesli hardness

Changes in hardness for dry muesli samples and muesli samples soaked in milk are shown in Figure 1 and Figure 2, respectively. The results indicated that the highest hardness of dry muesli samples was found in sample 2F0, results ranged between 486−1020 N. Whereas, the highest hardness for muesli samples soaked in milk was found in sample 1P30, results ranged between 28−98 N.

One of the main factors affecting the results is the heterogeneous structure of muesli samples (Roos, Drusch, 2016), as the investigated muesli consists of crunchy flakes, seeds, chocolate and soft raisins.

Based on the heterogenous structure, the results both for dry and soaked in milk muesli hardness were inconsistent and scientifically sound conclusions can not be drawn. Therefore, each muesli ingredient should be tested separately. Results show that dry muesli samples were 10 to 17 times harder than muesli samples soaked in milk on average.

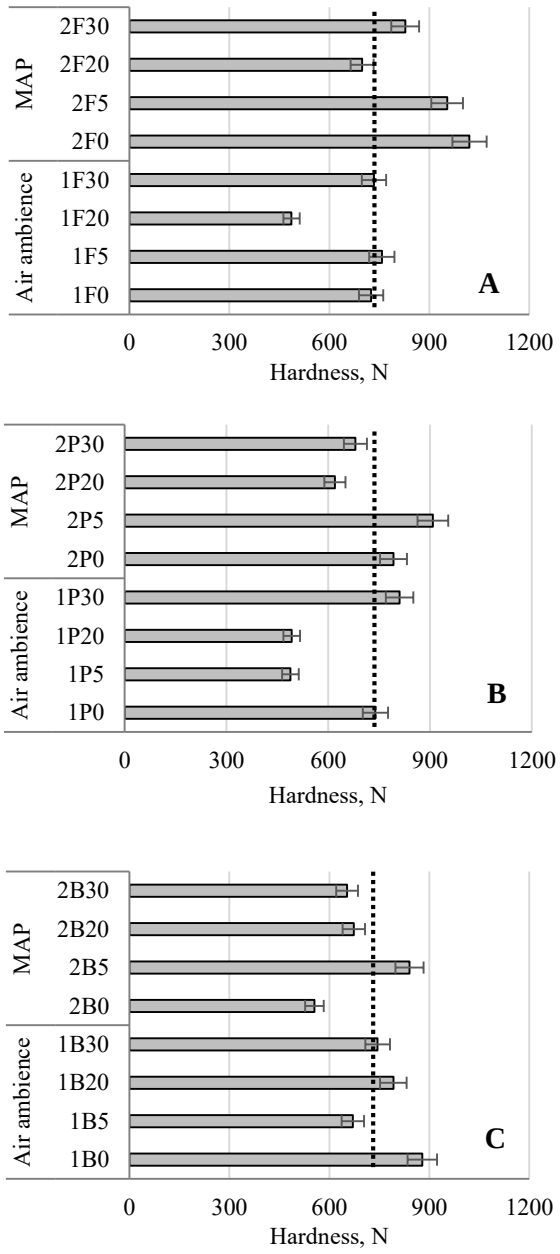

Figure 1. Hardness of dry muesli samples

A – 3-layer stand-up pouches (PAP/AL/PE), B – 2-layer stand-up pouches (PET/PE), C – biodegradable material (PLA/SiO_2), ------ initial hardness

Changes in microbiological parameters

Changes in total microbial count are shown in Table 4. For each package type the lowest amount of mesophilic aerobic and facultative anaerobic microorganisms was detected in samples which were stored at -5±1 °C temperature. For 3-layer stand-up pouches (samples F) and biodegradable material (samples B), the highest total microbial count was determined in samples which were stored at 30±1 °C temperature. The results show that in all samples microbial level was low.

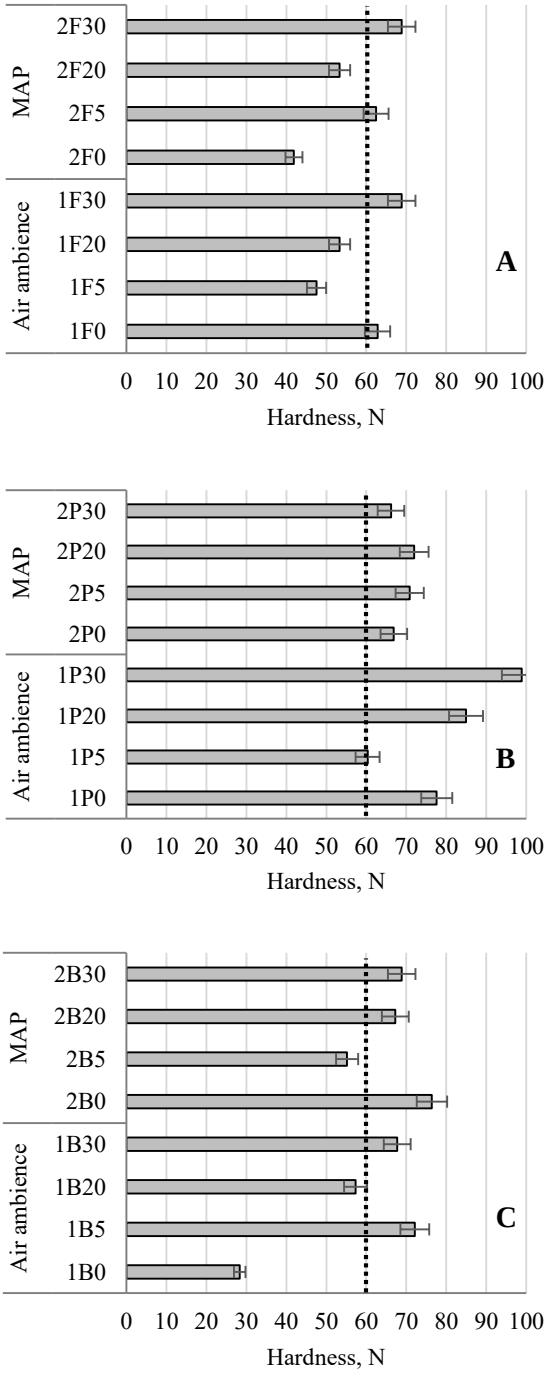

Figure 2. Hardness of muesli samples soaked in milk

A – 3-layer stand-up pouches (PAP/AL/PE), B – 2-layer stand-up pouches (PET/PE), C – biodegradable material (PLA/SiO_2), ------ initial hardness

Microorganism growth is affected by moisture content and water activity in food products (Bullerman, Bianchini, 2008) which in all samples was low, thus, we can conclude that packaging environment was not a significant factor for microbial growth in muesli ($p>0.05$). With regards to admissible level of total plate count, Nerbrink (2007) reported the levels of 10^4 to 10^5 CFU g^{-1} as an acceptable limit.

Yeasts were not found in any of the muesli samples. The highest contamination with moulds was detected in samples 2P20 and 1P5, however, there were not significant differences among samples ($p>0.05$), and mould count did not exceed 10^2 CFU g^{-1}. According to ICMFS (2005), the admissible level of yeast and mould count in cereals and similar dry products varies between 10^2 to 10^4 CFU g^{-1}. There were not significant differences between initial muesli sample and samples after temperature abuse. Senhofa et al. (2015) reported similar results on muesli microbial contamination.

Table 4

Microorganism levels in muesli samples after storage at different temperatures

Samples	Total plate count, CFU g^{-1}	Mould count, CFU g^{-1}
Control	1.96×10^1	2.07×10^1
1F0	1.26×10^2	3.84×10^1
1F5	5.77×10^1	4.96×10^1
1F20	1.26×10^2	2.38×10^1
1F30	1.79×10^2	5.52×10^1
2F0	4.21×10^1	6.51×10^1
2F5	2.15×10^1	3.44×10^1
2F20	8.91×10^1	3.16×10^1
2F30	1.47×10^2	2.84×10^1
1P0	1.09×10^2	4.21×10^1
1P5	5.80×10^1	9.61×10^1
1P20	8.93×10^2	5.58×10^1
1P30	1.10×10^2	7.05×10^1
2P0	2.74×10^2	2.78×10^1
2P5	4.77×10^2	5.64×10^1
2P20	1.70×10^2	9.63×10^1
2P30	1.39×10^2	5.79×10^1
1B0	1.26×10^2	2.15×10^1
1B5	4.21×10^1	2.31×10^1
1B20	1.05×10^2	4.03×10^1
1B30	2.02×10^2	9.50×10^1
2B0	5.25×10^1	5.01×10^1
2B5	5.80×10^1	8.01×10^1
2B20	1.42×10^2	4.89×10^1
2B30	1.63×10^2	3.84×10^1

CFU g^{-1} – colony forming units per gram

It can be concluded that all muesli samples are safe for consumption.

Conclusions

The results of water activity, moisture content, pH value, texture and microbiological parameters did not show significant changes for each of the tested parameters. It is suggested to subject muesli samples to additional time-temperature abuse during storage in order to evaluate quality changes throughout extended logistics chain.

Acknowledgment

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References

1. Andres-Bello A., Barreto-Palacios V., Garcia-Segovia P., Mir-Bel J., Martinez-Monzo J. (2013) Effect of pH on

color and texture of food products. *Food Engineering Reviews*, Vol. 5(3), p. 158–170.
2. Borneo R., Leon A. E. (2011) Whole grain cereals: Functional components and health benefits. *Food & Function*, Vol. 3(2), p. 110–119.
3. Bullerman L. B., Bianchini A. (2008) Food safety issues and the microbiology of cereals and cereal products. **In:** *Microbiologically safe foods. Chapter – 15.* p. 315–335.
4. Complete Oxford Dictionary (2011) Complete Oxford Dictionary 11. Oxford: Bukupedia. p. 3748.
5. *ICMFS (International Commission on Microbiological Specifications for Foods)* (2005) Cereals and cereal products. Micro-Organisms in foods 6; Microbial ecology of food commodities. New York: Kluwer Academic & Plenum Publishers, p. 392–439.
6. Kince T., Straumite E., Klava D., Kruma Z., Abelniece K., Balgalve A. (2018) Effects of processing conditions on physical parameters of triticale flakes. *Agronomy Research*, Vol. 16(2), p. 1417–1424.
7. Kirse A., Skudra L., Karklina D., Galoburda R., Muizniece-Brasava S. (2017) Improvement of microbiological safety and shelf-life of pulse spreads through sous vide and high-pressure processing. *Agronomy Research*, Vol. 15(2), p. 1304–1315.
8. Kirtil E. Oztop M.H. (2016) Controlled and modified atmosphere packaging. *Food Science*, Vol. 8(1), p. 1–22.
9. Kour H., Ahmad N., Wani T., Malik A., Kanl R., Chauhan H., Gupta P., Bhat A. (2013) Advances in food packaging – A review. *Stewart Postharvest review*, Vol. 9(4), p.1–7.
10. Madriz-Sancho M.F. (2003) *Encyclopedia of food sciences and nutrition* (Second edition). ASV: Academic press, p. 6000.
11. McKevith B. (2004) Nutritional aspects of cereals. *Nutrition Bulletin*, Vol. 29, p. 11–142.
12. Medina T. W., Quevedo R. A., Aguilera J. M. (2013) Changes on image texture features of breakfast flakes cereals during water absorption. *Food Science and Technology International*, Vol. 19(1), p. 45–57.
13. Nerbrink E. (2007) Guidelines for product safety; Analyzes and maximum levels. Version 6
14. Roos Y.H., Drusch S. (2016) *Phase Transitions in foods.* New York: Academic Press. 380 p.
15. Rozman V., Liška A., Volenik M., Kalinovič I., Šmič B. (2008) Influence of relative humidity and temperature to the changes in grain temperature in stored wheat and maize. *Agriculture Conspectus Scientificus*, Vol. 72(3), p. 215–219.
16. 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*, Vol. 13(4), p. 1064–1073.
17. Syamaladevi R. M., Tang J., Villa-Rojas R., Sablani S., Carter B., Campbell G. (2016) Influence of water activity on thermal resistance of microorganisms of low-moisture foods: a review. *Comprehensive Reviews in Food Science and Food Safety*. Vol. 15(1), p. 353–370.
18. Wrigley C. W. (2019) *Swaison`s handbook of technical and quality management for the food manufacturing sector*. 18 – Cereals. USA: Woodhead publishing. p. 582.

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BIOCONVERSION OF WASTE BREAD TO GLUCOSE FRUCTOSE SYRUP AS A VALUE-ADDED PRODUCT

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Abstract

Bread is one of the most wasted products of all food in many countries around the world. Bread waste is a resource of carbohydrates, proteins and lipids which can be reused in order to get value-added products. In this study, bread residues were converted into a glucose syrup via two stage enzymatic hydrolysis. The optimization process and response surface methodology were used to find the optimal substrate, water and enzyme ratio to produce the highest yields of fermentable sugars. The effects of bread (11.34–28.66 g 100 g^{-1}), α-amylase (0.013–0.047 KNU g^{-1} bread) and glucoamylase (0.23–0.57 AGU g^{-1} bread) loadings were investigated at liquefaction and saccharification stages. Results indicated that the amount of both enzymes was significant and determines the final glucose yield. Isomerization was performed for glucose conversion to fructose using optimal amounts of materials determined in hydrolysis experiments. Another optimization process was performed to determine the most effective amount of glucose isomerase enzyme (2.59–5.41 IGIU g^{-1} syrup) and pH (6.79–8.21). The increase in enzyme loading and pH up to 8 accelerated isomerization reaction. With the optimal process parameters, the highest fructose yield was achieved (40.32%). Hydrolysis and isomerization processes show bread waste potential of being a resource for the bioproduction of higher value products.

Keywords: bread waste, bioconversion, enzymatic hydrolysis, isomerization

Introduction

Bread is one of the main carbohydrate sources in human diet. Because of today's new technologies and attempt to satisfy consumers' needs, large quantities of bakery products are produced every day. Bread has a relatively short shelf life and about one-third of all that is produced goes to waste. It was estimated that approximately 1.2 million tonnes of bread are thrown away annually (Melikoglu, Webb, 2013).

Bread residues mostly consist of polysaccharides and small amounts of disaccharides. It is a potential biosource for production of fermentable sugars. Compared with traditional waste treatment methods, such as composting, incineration, landfill or other waste disposals, bioconversion to sugars is an environmentally friendly technology (Haroon et al., 2016; Hudeckova et al., 2017). Starch can be converted into glucose by using enzymatic hydrolysis. This process contains two stages. Firstly, enzyme α-amylase is used to liquefy bread slurry and obtain dextrins as well as small amounts of glucose. In the second stage, enzyme glucoamylase breaks down dextrins into glucose molecules (Demirci et al., 2017; Mojovic et al., 2006). To produce fructose syrup, hydrolyzed bread is further processed by the addition of enzyme glucose isomerase, which converts glucose into its isomer – fructose. This reaction is equilibrium limited therefore the final product is glucose-fructose syrup (Gaily et al., 2010; Gaily et al., 2012).

Sugar hydrolysates, obtained from conversion of glucose, can be used for various purposes: as sucrose substitute for production of bread and bakery products, as sweeteners or bulking agents in confectionery as well as further processed into high fructose syrup (HFS). HFS can be further converted to hydroxymethylfurfural and used for biofuel production (Dehkordi et al. 2009; Kwan et al., 2018).

The aim of this research is to create a multi-enzymatic composition and optimize the operating conditions of waste bread bioconversion to glucose-fructose syrup that ensures economical production and high yields of the final product.

Materials and Methods

Raw materials

Three loaves of wheat-rye bread were obtained from a local supermarket in Kaunas, Lithuania. Wheat-rye bread was prepared with sourdough. The ingredients used in preparation were rye and wheat flour, water, sugar, salt, yeast and caraway. After shelf life, bread slices were dried in ambient conditions, powdered by a laboratory mill, an average sample was made for analysis and stored at -20 °C until use. The moisture of bread (8.77±0.04%) was determined by the standard drying method in an oven at 105 °C to constant mass. Starch content was measured spectrophotometrically using commercial Megazyme test kit based on AACC International Approved Method 76-13.01 and it was 85.58±0.69% of bread dry matter.

Enzymes

A thermostable α-amylase (Liquoflow®) is produced by a genetically modified strain of *Bacillus*. The enzyme activity is 240 KNU g^{-1} bread (the amount of α-amylase which breaks down 5.26 g of starch per hour).

Glucoamylase (Saczyme® Plus 2X) is produced by selected strains of *Aspergillus niger*. The enzyme activity is 1500 AGU g^{-1} bread (the amount of enzyme which hydrolysis 1 μmol maltose per minute).

Glucose isomerase (Sweetzyme® IT) is an immobilized enzyme, produced from a selected strain of *Streptomyces murinus*. A typical activity is 400 IGIU g^{-1} bread (the amount of enzyme which converts glucose to fructose at an initial rate of 1 μmol min^{-1} at standard conditions). All enzymes are produced by Novozyme JSC, Denmark.

Bread waste hydrolysis and isomerisation

The optimization process of bread waste hydrolysis was investigated by using response surface methodology (RSM) and central composite design (CCD). Design-Expert 7.0.0 software (Stat–Ease Inc., Minneapolis, USA) was used for studying the effect of three independent variables (bread, α-amylase and glucoamylase loadings). Complete experiment consisted of 18 runs and all runs were performed in duplicate. Results were analysed and a mathematical model that fitted the best by one-way analysis of the variance (ANOVA) was selected.

Bread waste hydrolysis was conducted according to modified Demirci et al. (2017) method. The enzymatic reaction was performed in 250 mL conical flasks. Various weight ratios of powdered bread (11.34–28.66 g 100 g^{-1}) were mixed with distilled water. pH was adjusted to 5.6 using 1 mol L^{-1} concentration NaOH solution. Conical flasks were covered with aluminium foil and placed in a 45 °C water bath for 20 min with 2.5 Hz stirring speed. The temperature was raised to 60 °C, various concentrations of thermostable α-amylase (0.013–0.047 KNU g^{-1} bread) were added and the total sample weight was adjusted to 100 g by the addition of distilled water. Samples were further heated to 90 °C for 60 min in the drying oven and cooled to 50 °C.

In the saccharification stage pH of the samples was adjusted to 4.3 with 1 mol L^{-1} concentration H_2SO_4 solution and various amount of glucoamylase (0.23–0.57 AGU g^{-1} bread) were added to the mixture. Flasks were placed in a 65 °C water bath for 24 hours with 2.5 Hz stirring speed. Samples were withdrawn during the experiment and stored at -20 °C until analysis.

One solution for obtaining a high yield of glucose was selected based on Design-Expert software. Bread loading (18.64 g 100 g^{-1}), the amount of α-amylase (0.03 KNU g^{-1} bread) and glucoamylase (0.43 AGU g^{-1} bread) were chosen for process optimization. The amount of glucoamylase and pH were optimized using RSM and CCD. The experiment had 12 runs in total and all runs were performed in duplicate. Results were analysed using methodology described previously.

Glucose conversion to fructose was conducted according to modified Gaily et al. (2010) method. Bread slurry was filtered using a sieve and 100 mL of filtrate was poured into 250 mL conical flasks. pH was adjusted to 6.79–8.21 and various amounts of glucose isomerase enzyme (2.59–5.41 IGIU g^{-1} syrup) were added (Table 2). Flasks were covered with aluminium foil and placed into a 65 °C water bath with 2 Hz stirring speed.

Determination of glucose and fructose

Glucose and fructose content in the samples was determined by enzymatic K-FRUGL 05/17 test (Megazyme International Ireland Limited, Ireland). The concentrations of D-glucose and D-fructose were measured spectrophotometrically using commercial test kit. D-glucose and D-fructose yields were calculated according to equations in the Megazyme assay procedure.

Statistical Analysis

Mean values and standard deviations were calculated using MS Excel. IBM SPSS Statistics 23 (SPSS, Inc., Chicago, IL) was used for statistical data analysis. One-way analysis of variance (ANOVA) with Tukey's posthoc test was performed and significant differences were determined at α=0.05.

Results and Discussion

Glucose yield was investigated to optimize waste bread hydrolysis. Although the optimal amount of enzymes is recommended by the producer, the interval of the values is quite wide and adapted to the hydrolysis of starch rather than bread. Bread loading should be optimal because small amount of bread will result in low glucose yield and too high concentration of bread will give a very thick slurry, what makes harder for enzymes to break down all starch (Ebrahimi et al., 2008; Zhang et al., 2004).

Table 1

Optimization of bread waste hydrolysis

No	Hydrolysis parameters			Glucose yield, %	
	Bread loading, g 100 g^{-1}	α-amylase loading, KNU g^{-1} bread	Glucoamylase loading, AGU g^{-1} bread	Experimental	Predicted
1	15.00	0.020	0.30	66.52±1.22^{a}	66.29
2	25.00	0.020	0.30	57.08±1.03^{bc}	56.84
3	15.00	0.040	0.30	77.82±1.77^{h}	77.58
4	25.00	0.040	0.30	64.60±1.92^{fg}	64.37
5	15.00	0.020	0.50	73.49±0.44^{bc}	73.25
6	25.00	0.020	0.50	66.00±2.40^{b}	65.77
7	15.00	0.040	0.50	80.19±0.61^{bc}	79.96
8	25.00	0.040	0.50	71.01±1.92^{fg}	70.77
9	11.34	0.030	0.40	68.99±1.52^{a}	69.31
10	28.66	0.030	0.40	65.39±1.09^{gh}	65.70
11	20.00	0.013	0.40	62.57±0.86^{bc}	62.89
12	20.00	0.047	0.40	83.38±0.14^{gh}	83.70
13	20.00	0.030	0.23	56.40±1.58^{de}	56.72
14	20.00	0.030	0.57	77.50±2.86^{h}	77.81
15	20.00	0.030	0.40	83.53±2.58^{ef}	83.45
16	20.00	0.030	0.40	84.82±1.87^{h}	83.45
17	20.00	0.030	0.40	83.96±0.43^{h}	83.45
18	20.00	0.030	0.40	81.52±0.29^{cd}	83.45

Different superscript letters within a column indicate significant differences (one-way ANOVA and Tukey's test, $p < 0.05$).

Process parameters, experimental and predicted values of glucose yields are presented in Table 1. Analysis of response surface showed that the model itself was significant according to the Student test ($p = 0.0005$) and the "lack of fit" was not significant ($p = 0.5191$). The significant independent variables in the model ($p<0.05$) were α-amylase and glucoamylase loadings. Obtained results revealed that glucoamylase loading with the

F value=128.86 had a slightly larger effect on the total glucose yield than α-amylase (F=125.38). The determination coefficient (R^2=0.9955), which indicates the ability of the model to predict results, confirmed the adequacy of the model. An adequate ratio, which measures the signal to noise ratio, was 23.28. A ratio greater than 4 is desirable and indicates an adequate signal. More than 5 times higher value proved that this model can be used to navigate the design space.

3D and 2D response surface plots illustrate the effect of hydrolysis parameters on the total glucose yield (Figure 1). Plots were created considering one of the independent variables as a constant meanwhile the other two were changing and predicting response. Picture 1A shows the effect of bread versus α-amylase loading at a constant glucoamylase loading (0.40 AGU g^{-1} bread). Results indicate that the amount of both bread and α-amylase determines the final glucose yield. The highest glucose yield was obtained when bread loading ranges from 15 to 22 g 100 g^{-1} and α-amylase loading is at least 0.032 KNU g^{-1} bread. Bread loading greater than 22 g 100 g^{-1} had a negative effect on glucose yield. This effect can be explained by the formation of a thicker bread slurry before hydrolysis, which is less accessible to enzymes. To obtain the highest glucose yield, the lowest amount of enzyme α-amylase is used when bread loading is between 18.5 and 20.5 g 100 g^{-1}. A plot (Fig. 1B) illustrates the effect on bread and glucoamylase loadings at a constant α-amylase value of 0.03 KNU g^{-1} bread. Results show that both independent variables were relevant to the final glucose concentration. The highest yield of glucose was obtained when glucoamylase loading was greater than 0.38 AGU g^{-1} bread and bread loading ranged from 16.5 to 23 g 100 g^{-1}. Bread loading at less than 16.5 and over 23 g 100 g^{-1} decreased glucose output. 1C plot represents the effect on α-amylase and glucoamylase loadings at a constant bread loading of 20 g 100 g^{-1}. The increasing concentrations of enzymes increase glucose yield. The highest yield of glucose was obtained when α-amylase loading was greater than 0.027 KNU g^{-1} bread and glucoamylase loading – more than 0.36 AGU g^{-1} bread. The final glucose yield ranged from 56.40 to 84.82 %. Compared with Demirci et al., 2017 research where glucose yield was 62–86% obtained yields during the experiment were slightly lower. The difference between results may be due to different type of bread and lower enzymes loadings.

After all results were analysed, a tendency was observed that increasing the amount of enzyme (α-amylase up to 0.047 KNU g^{-1} bread and glucoamylase to 0.55 AGU g^{-1} bread) increases glucose yield. Higher loadings of α-amylase and glucoamylase did not have a significant effect on a total glucose yield. It was also noticed that optimal bread content is 15–20 g 100 g^{-1}. The optimal hydrolysis conditions were determined based on the obtained mathematical model: bread loading – 18.64 g 100 g^{-1}, α-amylase – 0.035 KNU g^{-1} bread and glucoamylase – 0.43 AGU g^{-1} bread. Under these conditions, 86.10% yield of glucose was predicted by the software. When the experiment was repeated with suggested values, the total glucose yield of 84.95±0.47% was obtained. Similar actual and predicted values confirm that this model is sufficiently accurate and can be adapted for the optimization of hydrolysis process.

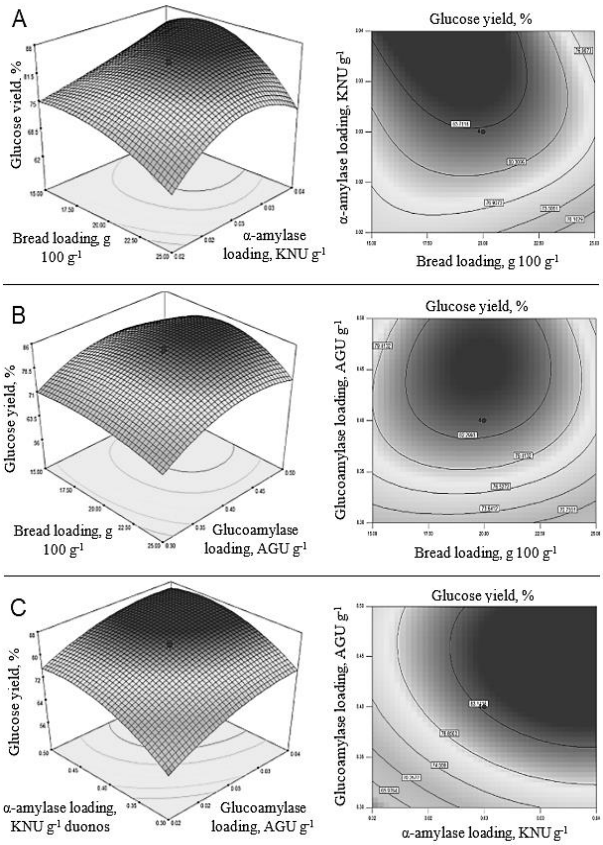

Figure 1. Response surface of the total glucose yield

In order to produce glucose-fructose syrup, the glucose syrup is further processed by adding another enzyme – glucose isomerase. This conversion effectiveness mainly depends on the glucose isomerase loading and pH. Isomerization parameters were optimized and the total yield of fructose was selected as the response factor. Process parameters, experimental and predicted values of fructose yields are presented in Table 2.

Results showed that fructose yield ranges from 32.68 to 40.32%. Compared with data presented in Gaily et al. (2010) research the highest obtained fructose yield was almost the same as the fructose yield (40.9%) produced in similar conditions. Fructose reached the highest yield at pH 7.5 and at the maximum enzyme loading of 5.41 IGIU g^{-1} of syrup. A high fructose yield (39.86%) was also obtained using a slightly lower enzyme loading (5.0 IGIU g^{-1} syrup) but at a higher pH (pH 8). However, the results showed that by further increasing the pH, the fructose yield starts to decrease.

ANOVA was used to identify adequacy of the quadratic regression model. The evaluated "lack of fit" coefficient was insignificant with a p=0.6375. Student test showed that the model itself was significant (p<0.0001, F=131.84). The determination coefficient (R^2=0.9910)

confirmed the adequacy of the completed model. An adequate ratio was 32.71 which proved that this model can be used to navigate the design space. Both independent variables are significant and have the effect on fructose yield in glucose conversion process. Considering these independent variables, glucose isomerase loading had a greater effect on fructose yield than pH. Their F values are respectively 288.03 and 227.60.

Table 2

Optimization of glucose isomerization

No	Parameters		Fructose yield, %	
	Glucose isomerase loading, IGIU g^{-1} syrup	pH	Experimental	Predicted
1	3.00	7.00	32.68±0.27^{de}	32.40
2	5.00	7.00	37.02±0.89^{cd}	36.92
3	3.00	8.00	36.45±0.71^{f}	36.47
4	5.00	8.00	39.86±0.44^{de}	40.07
5	2.59	7.50	34.31±0.18^{c}	34.48
6	5.41	7.50	40.32±0.80^{a}	40.23
7	4.00	6.79	32.77±1.07^{de}	33.02
8	4.00	8.21	38.31±0.10^{c}	38.13
9	4.00	7.50	38.73±0.71^{ef}	38.68
10	4.00	7.50	39.02±0.27^{a}	38.68
11	4.00	7.50	38.82±0.18^{b}	38.68
12	4.00	7.50	38.15±0.11^{b}	38.68

Different superscript letters within a column indicate significant differences (one-way ANOVA and Tukey's test, $p < 0.05$).

3D and 2D response surface plots show the relationship between independent variables (glucose isomerase loading and pH) and response (fructose yield) (Figure 2).

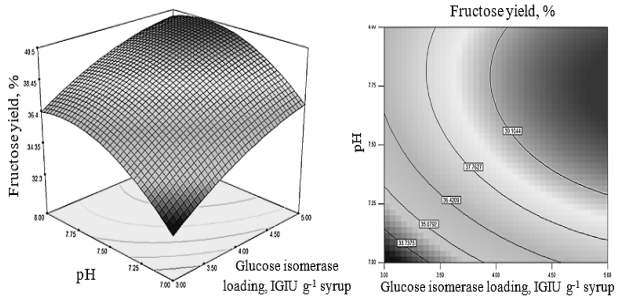

Figure 2. Response surface of the total fructose yield

The results presented in the response surface plots confirmed that the enzyme content and pH are significant parameters. The highest fructose yield is obtained when enzyme loading is greater than 4.0 IGIU g^{-1} syrup and pH is more than 7.3. Considering suggested solutions from Design-Expert 7.0.0 software for fructose yield optimization, the optimal conditions were the following: glucose isomerase loading from 4.84 to 5.0 IGIU g^{-1} syrup, pH – from 7.69 to 7.87. Within these optimization parameter values, the theoretical fructose yields should reach 40.32–40.42%. Isomerization is considered effective because glucose conversion to fructose is equilibrium limited to 42% fructose (Gaily et al., 2010; Zhang et al., 2004).

Conclusions

Bread waste hydrolysis and isomerization showed that biotechnological methods can be used for glucose-fructose syrup production. The final technological scheme for optimized bioconversion of waste bread consisted of the following steps:

I) preparation of aqueous suspension of bread waste (18.64 g 100 g^{-1});
II) liquefaction (α-amylase loading 0.035 KNU g^{-1} bread);
III) saccharification (glucoamylase loading 0.43 AGU g^{-1} bread);
IV) isomerization (glucose isomerase loading 4.84–5.0 IGIU g^{-1} syrup, pH 7.69–7.87).

Using this scheme, it is possible to produce glucose-fructose syrup of 45.27±0.55% glucose and 40.32±0.80% fructose.

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References

1. Dehkordi A.M., Tehrany M.S., Safari I. (2009) Kinetics of glucose isomerization to fructose by immobilized glucose isomerase (Sweetzyme IT), *Industrial & Engineering Chemistry Research*, Vol. 48(7), p. 3271–3278.
2. Demirci A.S., Palabiyik I., Gumus T., Ozalp S. (2017) Waste bread as a biomass source: optimization of enzymatic hydrolysis and relation between rheological behavior and glucose yield, *Waste and Biomass Valorization*, Vol. 8(3), p. 775–782.
3. Ebrahimi F., Khanahmadi M., Roodpeyma S., Taherzadeh M.J. (2008) Ethanol production from bread residues, *Biomass and Bioenergy*, Vol. 32(4), p. 333–337.
4. Gaily M.H., Elhassan B.M., Abasaeed A.E., Al-Shrhan M. (2010) Isomerization and kinetics of glucose into fructose, *International Journal of Engineering & Technology*, Vol. 10(3), p. 1–10.
5. Gaily M.H., Sulieman A.K., Abasaeed A.E. (2013) Kinetics of a three-step isomerization of glucose to fructose using immobilized enzyme, *International Journal of Chemical Engineering and Applications*, Vol. 4, p. 31–34.
6. Haroon S., Vinthan A., Negron L., Das S., Berenjia A. (2016) Biotechnological approaches for production of high value compounds from bread waste, *American Journal of Biochemistry and Biotechnology*, Vol. 12(2), p. 102–109.
7. Hudeckova H., Supinova P., Babak L. (2017) Optimization of enzymatic hydrolysis of waste bread before fermentation, *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, Vol. 65(1), p. 35–40.
8. Kawa-Rygielska J., Pietrzak W., Czubaszek A. (2012) Characterization of fermentation of waste wheat-rye bread mashes with the addition of complex enzymatic preparations, *Biomass and Bioenergy*, Vol. 44, p. 17–22.

9. Kwan T.H., Ong K.L., Haque M.A., Kwan W.H., Kulkarni S., Lin C.S.K. (2018) Valorisation of food and beverage waste via saccharification for sugars recovery, *Bioresource technology*, Vol. 255, p. 67–75.
10. Melikoglu M., Webb C. (2013) Use of waste bread to produce fermentation products. **In:** *Food Industry Wastes: assessment and recuperation of commodities.* Academic Press, p. 63–76.
11. Mojovic L., Nikolic S., Rakin M., Vukasinovic M. (2006) Production of bioethanol from corn meal hydrolyzates, *Fuel*, Vol. 85(12–13), p. 1750–1755.
12. Zhang Y., Hidajat K., Ray A.K. (2004) Optimal design and operation of SMB bioreactor: production of high fructose syrup by isomerization of glucose, *Biochemical Engineering Journal*, Vol. 21(2), p. 111–121.

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CHANGES OF BIOLOGICALLY ACTIVE COMPOUND LEVEL IN POTATOES DURING STORAGE UNDER FLUORESCENT LIGHT

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Abstract

Potatoes (*Solanum tuberosum* L.) are globally grown and consumed crop and contains many vitally important elements benefiting human diet that makes them actual subject from scientific and food market standpoint. Potatoes naturally contain active element combination named phenolic compounds that are secondary metabolites and enrich potatoes with antioxidant, anticarcinogenic, antibacterial, etc. qualities. The aim of current study was to evaluate changes of biologically active compound level in potatoes during storage under fluorescent light. In present research potatoes of four varieties ('Imanta', 'Magdalena', 'Blue Congo', and 'Lenora') were tested. For the experiment samples were kept under fluorescent light of 1000 lux for 3 and 7 days in climate chamber with controlled temperature of 20 °C and relative humidity of 40% that way imitating real shop shelf conditions. Total phenolic content, antioxidant activity (DPPH˙ and ABTS assays), moisture and firmness was determined in potatoes. It was established that during all storage period potato firmness did not change significantly, while moisture decreased in some cases. Phenolic compounds and antioxidant activity showed variety dependent variations.

***Keywords*:** potatoes, phenolic compounds, antioxidant activity, storage, fluorescent light

Introduction

Currently, we are experiencing a growing interest in antioxidant rich products and additives (Huang, 2018). Potato (*Solanum tuberosum* L.) is in the first line of world's popular crops, and being rich in antioxidants, it is a great addition to the consumer diet (Leo et al., 2008).

One of the most important bioactive compound group is phenolics that are also present in potatoes, showing highly beneficial impact on human health (Velioglu et al., 1998; Espin et al., 2000; Manach et al., 2004). Potatoes are ranged as a third consumed crop in terms of phenolic compound source (Chun et al., 2005).

Numerous studies show that phenolic compounds present antioxidant, antibacterial, anticarcinogenic, anti-inflammatory, antiglycemic, antiviral and vasodilatory features (Mattila, Hellstrom, 2006; Leo et al., 2008; Berghe, 2012; Kazeem et al., 2012; Konaté et al., 2012). Studies also show that human longevity, eyes and mental health as well as cardiovascular system are positively impacted by potato phenolic compounds (Parr, Bolwell, 2000; Manach et al., 2004; Scalbert et al., 2005).

Correlation between potato phenolics and total antioxidant capacity has been established, and that means that higher phenolics level provides higher antioxidant capacity (Andre et al., 2007). Potato analyses show that phenolics can be found in whole tuber, although the skin presents highest phenolics level (Lewis et al., 1999; Nara et al., 2006).

Speaking of variety dependent phenolics level, studies show that all potatoes regardless of variety contain phenolics, but the highest level (almost twice as much) can be found in purple-fleshed (also red-fleshed) varieties in contrast to white-fleshed and yellow-fleshed ones (Ezekiel et al., 2013) and it may be explained with high level of anthocyanins that are pigments in such varieties (Im et al., 2008; Al-Weshahy, Rao, 2009; Zarins et al., 2018).

Environmental stress is the factor that might force phenolics to accumulate in potatoes tubers and other parts of potato plant as natural protection reaction (Chalker-Scott, 1999; Percival et al., 2000; Sakihama et al., 2000; Grace et al., 2014). Studies presents that artificial light may negatively influence quality of potatoes accelerating formation of glycoalkaloids but also it may have positive effect triggering raise of phenolic compound level and antioxidant activity (Arezki et al., 2001; Izquierdo et al., 2011; Nascimento et al., 2013; Ballester, Lafuente, 2017).

The aim of the current study was to evaluate changes of biologically active compound level in potatoes during storage under fluorescent light.

Materials and Methods

Raw materials

Potatoes were planted in May and harvested in September 2018. The soil type in the field was sod-podzolic (PVv), sandy loam, pH KCl 5.3, organic matter 1.8%, contained P_2O_5 120 mg kg^{-1}, K_2O 143 mg kg^{-1}. Harvested tubers were kept in the storage facility at air temperature of 4 °C and relative humidity of 80±5%. In the experiment, four potato (*Solanum tuberosum* L.) cultivars with white, yellow and purple coloured flesh were evaluated, namely 'Lenora', 'Imanta', 'Blue Congo', 'Magdalena'. A total of two kilograms of table potato tubers per cultivar were used.

Treatment conditions

Potatoes were exposed to conditions that are based on mean data obtained from 30 shops during winter period: light – 1000 lux, relative air humidity – 40% and air temperature – +20 °C. In those conditions, on

shop shelf, potatoes are stored shortly (during approximately one-week period) before they are purchased by consumer. During all short storage period on the shop shelf, potatoes are exposed to constant light during all working hours of the shop, increased (room) temperature and decreased air humidity. In the current study imitation of real retail shelf conditions were achieved by using climate chamber ICH110 (Memmert GmbH + Co. KG, Germany) with constant and even pre-set humidity and temperature. Luminescent lamp Vagner SDH Circular (32 W) was used as artificial light source.

Firmness measurement

The changes of potato firmness were determined using TA.HD Plus Texture Analyser (Stable Micro Systems Ltd, United Kingdom) measuring force (Newton) that is needed to penetrate potato skin and flesh. Potato skin and flesh penetration parameters were as follows – probe: cylinder, 2 mm diameter, type P/2; contact area: 3.00 mm^2; test mode: compression; pre-test speed: 1.00 mm s^{-1}; test speed: 1.00 mm s^{-1}; post-test speed: 10.00 mm s^{-1}; distance (penetration depth): 5 mm; trigger type: force; trigger force: 0.049 N.

Chemical analysis

For extraction of phenolic compounds from potatoes the homogenized potato samples were mixed with ethanol (80/20 v/v) in a conical flask with a magnetic stirrer at room temperature as described in previous experiments (Kampuse et al., 2016). The extraction process was done in triplicate. Determination of total phenolic content (TPC) was done according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999). Antioxidant activity of potato extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS+) radical cation as described in previous experiments (Kampuse et al., 2016).

Statistical analysis

Experimental results are means of three replications and were analysed by Microsoft Excel 2010 (descriptive statistics) and SPSS 17.00 (ANOVA and correlation analysis). Differences were considered as significant at $p<0.05$.

Results and Discussion

Moisture

In the analysed potato flesh and peels no significant changes in moisture during all storage period were observed, yet a tendency of slight decrease was established (Fig. 1). Measured data presents all potato variety ability to almost retain all moisture in fluorescent lighting storage conditions, although longer exposure to light and longer storage time may show wider variations of moisture changes.

Firmness

These data allow to understand the changes of potato firmness during storage period. 'Imanta' showed the highest initial (before treatment) firmness of 12.81 N while 'Blue Congo' and 'Magdalena' presented the lowest amount of force needed – 9.54 N and 9.66 N, respectively (Fig. 2).

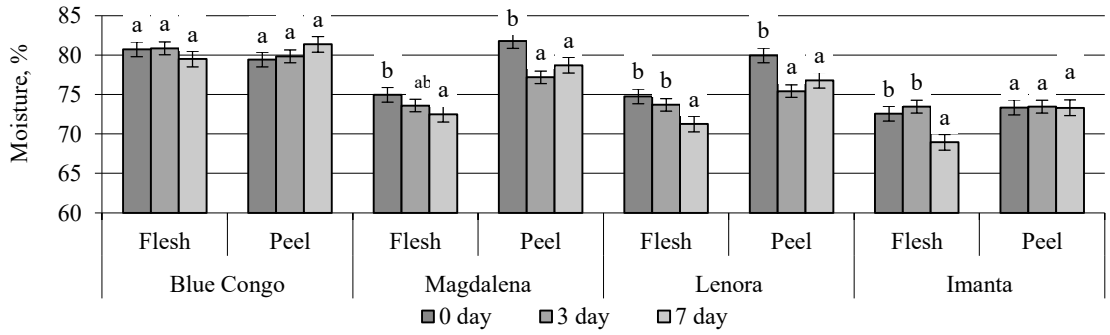

Figure 1. Moisture of potato flesh and peel

The same letters indicate no significant differences between samples ($p>0.05$).

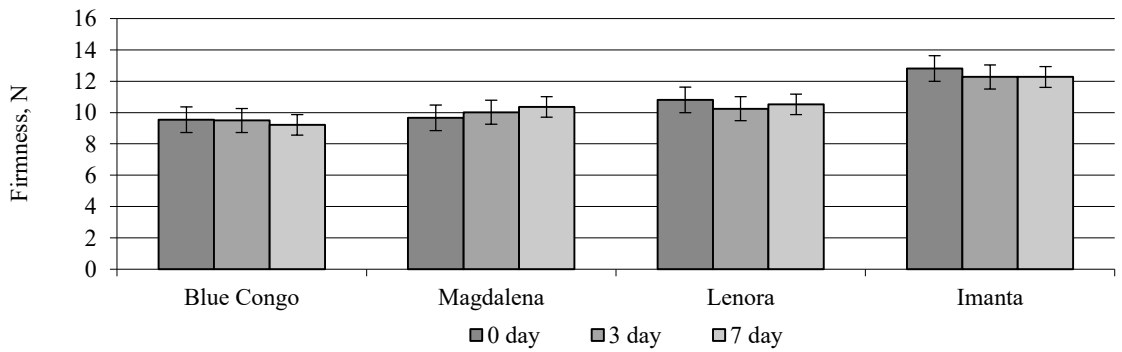

Figure 2. Potato firmness

Data shows that there was not significant ($p>0.05$) variation in firmness during storage, and that allows to conclude that current study conditions for these specific potato varieties did not significantly affect potato firmness. Resource map of potatoes showed that during retail, potato mass losses are only 1.5–3.0% that also confirms potato stability under shop conditions in terms of moisture and firmness. The most important factors creating losses during storage on shop shelves are caused by potato tuber greening / sprouting (Terry et al., 2011)

Total phenolics

'Blue Congo' showed the highest initial (before treatment with light) total phenolic content of 265.44 mg 100 g^{-1} while 'Imanta' presented the lowest amount of 115.92 mg 100 g^{-1} (Fig. 3). 'Magdalena' and 'Lenora' both has no significant differences ($p>0.05$) in initial total phenolic content of 154.60 mg 100 g^{-1} and 154.80 mg 100 g^{-1}, respectively. After treatment with light, only variety 'Blue Congo' potatoes showed significant decrease of total phenolic content on day 3, while other potato varieties showed increase of total phenolic content. On day 7 all varieties experienced increase of total phenolic content, except 'Imanta' that retained its phenolics content without changes. The highest initial level of total phenolic content in peels was presented by 'Blue Congo' (226.52 mg 100 g^{-1}) followed by 'Magdalena' (166.97 mg 100 g^{-1}), 'Lenora' (134.41 mg 100 g^{-1}) and 'Imanta' (123.02 mg 100 g^{-1}) (Fig. 3). After treatment with light on day 3 all potato varieties showed different tendencies: 'Blue Congo' peels presented increase, 'Magdalena' peels – decrease and for 'Lenora' and 'Imanta' peels no significant differences were observed. After 7 days of treatment, similar tendencies remained. Different studies showed that higher content of phenolics is located in peels (Lewis et al., 1999; Nara et al., 2006), but our results suggest that content is similar and, in some cases, even higher in flesh (varieties 'Blue Congo' and 'Lenora'). Measured data shows that artificial lighting forces variation of total phenolic content making it to increase or decrease depending on variety (Wang et al., 2015). Despite that phenolics changes under artificial lighting are moderate (Sun et al., 2017), longer storage might present more sufficient differences. Other studies showed that under blue light intensive synthesis of phenolic compounds starts from third day (Ballester, Lafuente, 2017).

Antioxidant activity

It is a natural ability of redox molecules in potatoes to suppress free radicals (Puchau et al., 2010), and studies show that it might be linked to total phenolic content (Leo et al., 2008), however, connection between those two still depends on tested material and conditions.

DPPH scavenging activity: the highest initial 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (before treatment with light) was determined in 'Blue Cong', i.e., 14.97 mmol TE 100 g^{-1} while 'Imanta' presented the lowest activity of 8.44 mmol TE 100 g^{-1} (Fig. 4).

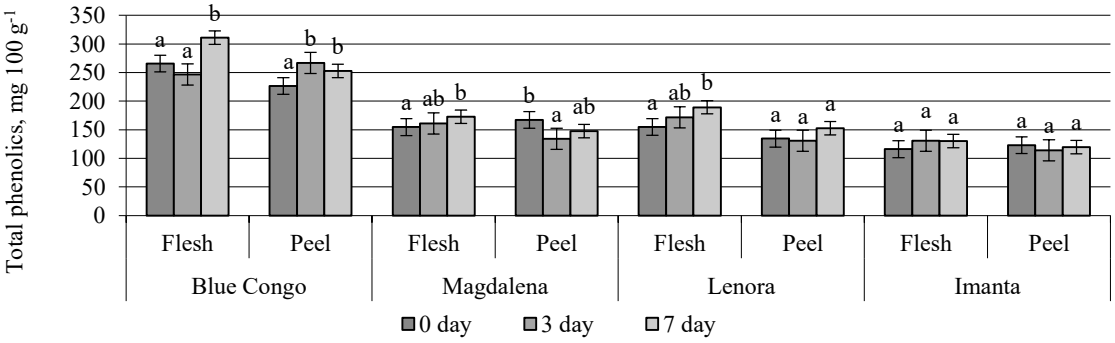

Figure 3. Total phenolic content of potato flesh and peel as the gallic acid equivalent

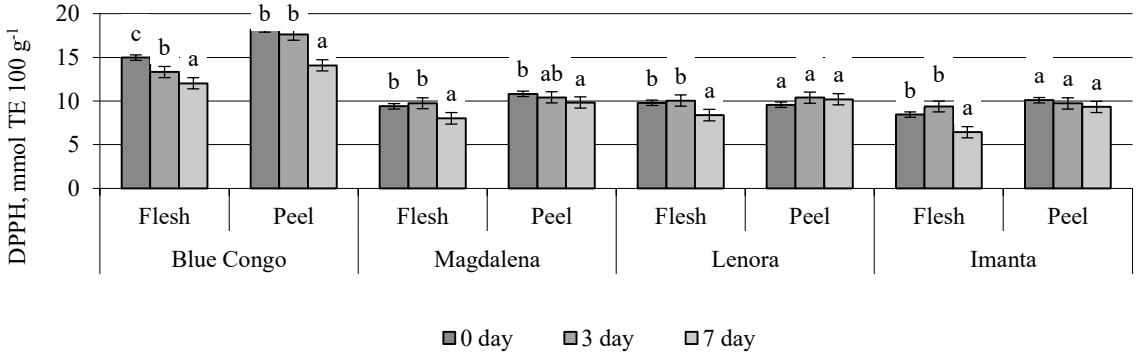

Figure 4. DPPH scavenging activity of potato flesh and peel

The same letters indicate no significant differences between samples ($p>0.05$).

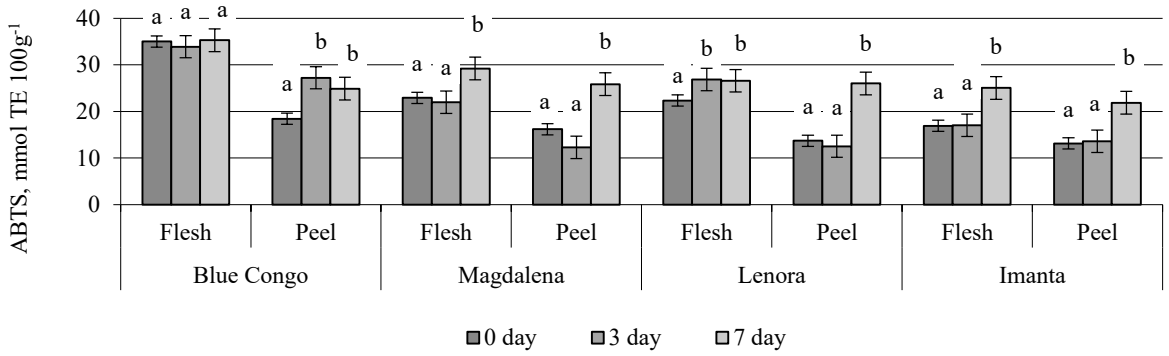

Figure 5. ABTS scavenging activity of potato flesh and peel

The same letters indicate no significant differences between samples (p>0.05).

'Magdalena' and 'Lenora' both has insignificant differences in initial DPPH scavenging activity of 9.40 mmol TE 100 g^{-1} and 9.78 mmol TE 100 g^{-1} respectively. After treatment with light all potato varieties showed insignificant changes in scavenging activity on day 3, except 'Blue Congo' that presented a decrease. Although day 7 comes with DPPH scavenging activity decrease in all cases compared to day 3, i.e., 'Blue Congo' shows 12.02 mmol TE 100 g^{-1}, 'Magdalena' (8.01 mmol TE 100 g^{-1}), 'Lenora' (8.38 mmol TE 100 g^{-1}) and 'Imanta' (6.42 mmol TE 100 g^{-1}). 'Blue Congo' showed the highest initial DPPH scavenging activity of potato peels presenting 18.18 mmol TE 100 g^{-1} while 'Lenora' had the lowest activity of 9.37 mmol TE 100 g^{-1} (Fig. 4) 'Magdalena' and 'Imanta' both has insignificant differences in initial DPPH scavenging activity of 10.80 mmol TE 100 g^{-1} and 10.09 mmol TE 100 g^{-1} respectively. After 7 days treatment DPPH scavenging activity decreased in all peels compared to day 3, i.e., 'Blue Congo' showed 14.08 mmol TE 100 g^{-1}, 'Magdalena' (9.81 mmol TE 100 g^{-1}), 'Lenora' (10.18 mmol TE 100 g^{-1}) and 'Imanta' (9.32 mmol TE 100 g^{-1}).

Measured data gives reason to suggest that storage under fluorescent lighting generally reduces potato DPPH scavenging activity and longer storage might give more reduction (Xu et al., 2014). Experiments on strawberries showed that blue light increased 1,1-diphenyl-2-picrylhydrazy (DPPH) radical-scavenging activity as well as ascorbic acid, total sugar content, titratable acidity content (Xu et al., 2014).

ABTS scavenging activity. The highest initial 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging activity (before treatment with light) was found in 'Blue Cong', i.e., 35.01 mmol TE 100 g^{-1} while 'Imanta' presented the lowest activity of 16.91 mmol TE 100 g^{-1} (Fig. 5). 'Magdalena' and 'Lenora' both has insignificant differences in initial ABTS scavenging activity of 22.92 mmol TE 100 g^{-1} and 22.32 mmol TE 100 g^{-1} respectively. After treatment with light potatoes of variety 'Lenora' showed slight increase of ABTS scavenging activity on day 3. Although day 7 came with ABTS scavenging activity increase compared to day 3, i.e., 'Magdalena' (29.19 mmol TE 100 g^{-1}), 'Imanta' (25.05 mmol TE 100 g^{-1}) except in case of 'Lenora' that presented insignificant changes presenting 26.57 mmol TE 100 g^{-1}. 'Blue Congo' potato peels showed the highest initial ABTS scavenging activity – 18.41 mmol TE 100 g^{-1} while 'Lenora' and 'Imanta' had insignificant differences in initial ABTS scavenging activity and at same time the lowest activity 13.69 mmol TE 100 g^{-1} and 13.13 mmol TE 100 g^{-1}, respectively (Fig. 5). 'Magdalena' showed 16.16 mmol TE 100 g^{-1} initial activity. After treatment with light variety 'Magdalena' showed a decrease and 'Lenora' insignificant decrease of ABTS scavenging activity on day 3, except 'Blue Congo' that presented an increase and 'Imanta' that presented insignificant increase. Day 7 came with significant ABTS scavenging activity increase in all cases compared to day 3, i.e., 'Magdalena' (25.83 mmol TE 100 g^{-1}), 'Lenora' (25.99 mmol TE 100 g^{-1}) and 'Imanta' (21.83 mmol TE 100 g^{-1}) except in case of 'Blue Congo'.

Current study shows that ABTS scavenging activity experiences significant increase during potatoes storage under fluorescent light in period from day 3 to 7 except in case of one variety where slight decrease was experienced. Data suggest that longer storage might lead to greater activity changes.

Correlation analysis

Studies suggest that there is a correlation between phenolics and antioxidant capacity, and higher phenolic level might present also a stronger antioxidant effect (Andre et al., 2007). While the current study shows negative correlation between total phenolic content (TPC) and DPPH scavenging activity (Fig. 6) and between ABTS scavenging activity and DPPH scavenging activity (Fig. 8), there is still a positive correlation between total phenolic content and ABTS scavenging activity (Fig. 7).

Correlation of TPC and DPPH. 'Blue Congo' (A) and 'Lenora' (C) presented a strong negative correlation while 'Magdalena' (B) and 'Imanta' (D) showed a moderate negative correlation. Data suggests that changes of one parameter will substantially trigger changes of the other one in opposite direction.

Correlation of TPC and ABTS. 'Magdalena' (B) and 'Imanta' (D) presented a moderate positive correlation (Fig. 7), 'Blue Congo' (A) showed a strong positive correlation while 'Lenora' (C) had a very strong positive correlation. Obtained information allows putting forward

the assumption that changes of one value will substantially force changes of other one in same direction, i.e., increase of one value will promote to increase other value.

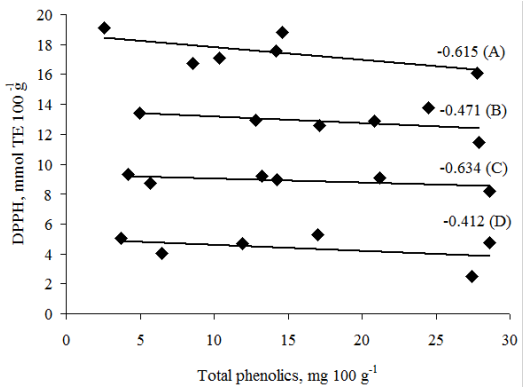

Figure 6. Correlation between total phenolic content and DPPH scavenging activity of potato
A – 'Blue Congo', B – 'Magdalena', C – 'Lenora', D– 'Imanta'

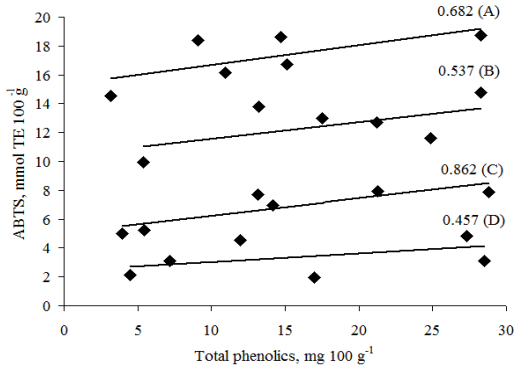

Figure 7. Correlation between total phenolic content and ABTS scavenging activity of potato
A – 'Blue Congo', B – 'Magdalena', C – 'Lenora', D– 'Imanta'

Correlation of DPPH and ABTS. 'Magdalena' (B) and 'Imanta' (D) presented a very strong negative correlation (Fig. 8), 'Blue Congo' (A) showed a strong negative correlation while 'Lenora' (C) had a weak negative correlation.

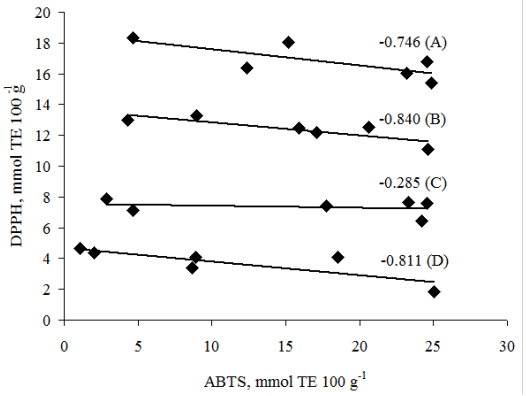

Figure 8. Correlation between DPPH scavenging activity and ABTS scavenging activity of potato
A – 'Blue Congo', B – 'Magdalena', C – 'Lenora', D– 'Imanta'

From the obtained information we can see that parameters are negatively linked, i.e., one will substantially trigger changes of other in opposite direction, except in case of 'Lenora' that will experience only slight interaction of parameters.

Conclusions

During all storage period, potato firmness changed insignificantly, and in some cases a decrease in moisture was detected. Correlation between total phenolic content and DPPH scavenging activity, and between ABTS scavenging activity and DPPH scavenging activity was negative in all cases and ranged from a weak negative to a very strong negative correlation. Correlation between total phenolic content and ABTS scavenging activity was positive in all cases and ranged from a moderate positive to a very strong positive correlation. After exposure to fluorescent light phenolic compound level and antioxidant activity changed significantly, and those changes were dependent on potato variety.

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References

1. 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*, Vol. 42(8), p. 1062–1066.
2. 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*, Vol. 55(2), p. 366–378.
3. Arezki O., Boxus P., Kevers C., Gaspar T. (2001) Changes in peroxidase activity, and level of phenolic compounds during light-induced plantlet regeneration from Eucalyptus camaldulensis Dehn. nodes in vitro. *Plant Growth Regulation*, Vol. 33(3), p. 215–219.
4. Ballester A.R., Lafuente M.T. (2017) LED blue light-induced changes in phenolics and ethylene in citrus fruit: Implication in elicited resistance against *Penicillium digitatum* infection. *Food Chemistry*, Vol. 218, p. 575–583.
5. Berghe W.V. (2012) Epigenetic impact of dietary polyphenols in cancer chemoprevention: Lifelong remodeling of our epigenomes. *Pharmacological Research*, Vol. 65(6), p. 565–576.
6. Chalker-Scott L. (1999) Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology*, Vol. 70(1), p. 1–9.
7. 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*, Vol. 85(10), p. 1715–1724.
8. 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*, Vol. 48(5), p. 1588–1592.
9. Ezekiel R., Singh N., Sharma S., Kaur A. (2013) Beneficial phytochemicals in potato – A review. *Food Research International*, Vol. 50(2), p. 487–496.
10. Grace M.H., Yousef G.G., Gustafson S.J., Truong V.D., Yencho G.C., Lila M.A. (2014) Phytochemical changes in phenolics, anthocyanins, ascorbic acid, and carotenoids associated with sweet potato storage and impacts on bioactive properties. *Food Chemistry*, Vol. 145, p. 717–772.
11. Huang D. (2018) Dietary antioxidants and health promotion. *Antioxidants*, Vol. 7(1), p. 1–3.
12. 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*, Vol. 56(9), p. 3341–3349.
13. Izquierdo A.M., Torres M.P.N., Jiménez G.S., Sosa F.C. (2011) Changes in biomass allocation and phenolic compounds accumulation due to the effect of light and nitrate supply in Cecropia peltata plants. *Acta Physiologiae Plantarum*, Vol. 33, p. 2135–2147.
14. Kampuse S., Tomsone L., Kruma Z., Sabovics M., Skrabule I. (2016) Effect of lovage phenolics to formation of acrylamide in French fries. *Agronomy Research*, Vol. 14(S2), p. 1315–1327.
15. 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*, Vol. 2(9), p. 727–732.
16. Konate K., Hilou A., Mavoungou J.F., Lepengue A.N., Souza A., Barro N., Datte 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*, Vol. 11(1), p. 1–6.
17. 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*, Vol. 56(11), p. 4154–4163.
18. Lewis C.E., Walker J.R.L., Lancaster J.E. (1999) Changes in anthocyanin, flavonoid and phenolic acid concentrations during development and storage of coloured potato (*Solanum tuberosum* L.) tubers. *Journal of the Science of Food and Agriculture*, Vol. 79(2), p. 311–316.
19. Manach C., Scalbert A., Morand C., Remesy C., Jimenez, L. (2004) Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, Vol. 79(5), p. 727–747.
20. Mattila P., Hellstrom J. (2006) Phenolic acids in potatoes, vegetables, and some of their products. *Journal of Food Composition and Analysis*, Vol. 20 (3–4), p. 152–160.
21. Nara K., Miyoshi T., Honma T., Koga H. (2006) Antioxidative activity of bound form phenolics in potato peel. *Bioscience, Biotechnology, and Biochemistry*, Vol. 70(6), p. 1489–1491.
22. Nascimento L.B., Leal-Costa M.V., Coutinho M.A., Moreira N.S., Lage C.L., Barbi N.S., Costa S.S., Tavares E.S. (2013) Increased antioxidant activity and changes in phenolic profile of *Kalanchoe pinnata* (Lamarck) Persoon (Crassulaceae) specimens grown under supplemental blue light. *Photochemistry and Photobiology*, Vol. 89(2), p. 391–399.
23. 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*, Vol. 80(7), p. 985–1012.
24. Percival G.C., Baird L. (2000) Influence of storage upon light-induced chlorogenic acid accumulation in potato tubers (*Solanum tuberosum* L.). *Journal of Agricultural and Food Chemistry*, Vol. 48, p. 2476–2482.
25. 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*, Vol. 26(5), p. 534–541.
26. Sakihama Y., Mano J., Sano S., Asada K., Yamasaki H. (2000) Reduction of phenoxyl radicals mediated by monodehydroascorbate reductase. *Biochemical and Biophysical Research Communications*, Vol. 279, p. 949–954.
27. 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*, Vol. 45(4), p. 287–306.
28. Singleton V.L., Orthofer R., Lamuela-Raventos R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, Vol. 299, p. 152–178.
29. Sun H.N., Mu T.H., Xi L.S. (2017) Effect of pH, heat, and light treatments on the antioxidant activity of sweet potato leaf polyphenols. *International Journal of Food Properties*, Vol. 20(2), p. 318–332.
30. Terry L.A., Mena C., Williams A., Jenney N., Whitehead P. (2011) Fruit and vegetable resource maps: Mapping fruit and vegetable waste through the wholesale supply chain. *Portland State University Business Faculty Publications and Presentations*, Vol. 6, p. 1–92.
31. 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*, Vol. 46(10), p. 4113–4117.
32. Wang Q., Cao Y., Zhou L., Jiang C.Z., Feng Y., Wei S. (2015) Effects of postharvest curing treatment on flesh colour and phenolic metabolism in fresh-cut potato products. *Food Chemistry*, Vol. 169, p. 246–254.
33. Xu F., Shi L., Chen W., Cao S., Su X., Yang Z. (2014) Effect of blue light treatment on fruit quality, antioxidant enzymes and radical-scavenging activity in strawberry fruit. *Scientia Horticulturae*, Vol. 175, p. 181–186.
34. Zarins R., Kruma Z., Tomsone L., Kampuse S., Skrabule I., Konosonoka I. H. (2018) Comparison of phenolic compounds and antioxidant activity of fresh and freeze-dried potatoes, *Agronomy Research*, Vol. 16(2), p. 1546–1554.

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BIOLOGICALLY ACTIVE COMPOUND STABILITY IN THE INDUSTRIAL POTATO PROCESSING BY-PRODUCTS

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Abstract

The biologically active compound extraction from potato peel by-products is an actual topic that is targeted not only on the new product development but also provides an important insight into industrial by-product recycling possibilities. As actual information on the extractable biologically valuable compound stability during the industrial potato peel by-products storage is scarce, the aim of present research had been to analyse phenolics and glycoalkaloids degradation tendencies in potato processing by-products (peels form different abrasion peeling lines), imitating the actual industrial storage conditions. In the present study, α-solanine and α-chaconine were the most stable among the analysed compounds. Chlorogenic acid concentration showed slower decrease rates in large and middle size peel pieces during the first days of storage, when compared to small size samples (possibly due to ongoing chlorogenic acid synthesis form phenylalanine). The peel material that does not contain whole cells (ground peel) is not recommended for extracting purposes due to the rapid phenolic compound oxidation and degradation. Alternatively, peel material that consists of large and middle size peels can be used for the extraction purposes during the first two days of peel storage under industrial storage conditions, without major decreases in the yield. Deeper studies on biologically active compound degradation dynamics are necessary to maximise the extraction yield of phenolics and glycoalkaloids from the industrial potato processing wastes.

Keywords: potato peel, by-product recycling, glycoalkaloids, phenolics.

Introduction

One of the most important crops for human consumption are potatoes (Solanum tuberosum L.), with an approx. 32 kg per capita worldwide. As a result, industrial potato production waste management is rising a concern European for the potato industry. On our road to the sustainable economy, an environmentally friendly solution should be found. A number of studies were conducted showing that potato peel, one of the major potato processing waste (Al-Weshahy, Rao, 2012), is a potential source for methane (Liang, McDonald, 2015), biofuel (Liang, McDonald, 2014), lactic acid (Liang et al., 2014), pullulan (Göksungur et al., 2011), and another valuable compound production (Matharu et al., 2016). For the food industry, biologically active compound extraction and further application in food production is one of the main recycling possibilities, and the number of researches have been evaluating possible processing methods (Al-Weshahy, Rao, 2012; Amado et al., 2014; Cardoso et al., 2013; Hossain et al., 2014; Luthria, 2012; Samarin et al., 2012; Wijngaard et al., 2012). Despite that, not much research had been conducted to understand the actual raw material (peel) storage time before the recycling/extraction process, to ensure maximal biologically active compound yield and minimal degradation rates.

Two types of potato by-products (peeling wastes) can be distinguished based on the peeling method: steam and abrasion peeling. Depending on parameters, steam peeling can result in almost 90% losses in the total glycoalkaloid content, and also reduces total phenolic compound content by 67% (Mäder et al., 2009). As those are main biologically active compounds that potato peel is known for (Schieber, Saldaña, 2009), abrasion peeling wastes are more suitable as a raw material for extraction purposes as it provides higher yields. Phenolics and glycoalkaloids are distributed unevenly between different parts of potato tuber, with most of them being located exactly in the peel (Lopez-Cobo et al., 2014), which makes abrasion peeling wastes even more perspective source for extraction purposes than the whole tuber itself. It is also important to take in the consideration that content, composition, and distribution of biologically active compounds in potato tuber is highly dependent on the potato variety (Navarre et al., 2011).

Phenolic compounds (a secondary plant metabolites) form a heterogeneous class of organic compounds in plants, providing pigmentation and protection against various external factors (ultraviolet light, pathogenic microorganisms, another) (Williams, Grayer, 2004). In potato peels, they are represented mostly by an ester of caffeic and quinic acids – chlorogenic acid. And it can form up to 90% of all phenolics in potato tuber (Friedman, 1997; Im et al., 2008). Another important group of secondary metabolites in plants is glycoalkaloids. These compounds are toxic to viruses, microorganisms, insects, animals, and even to humans. In potatoes they are represented mostly by α-solanine and α-chaconine, both containing the same aglycone – solanidine (Rayburn et al., 1994). Their content is influenced by many post-harvest factors and can vary greatly between tubers, depending on the potato variety itself, exposure to light and irradiation, presence of mechanical injuries, and another storage (Friedman, 2004).

As a result, due to its composition, the potato peel extract is known for exhibiting antioxidative and antiradical activities (Friedman et al., 2017). It was able to prevent lipid oxidation in oil-in-water emulsions and plant oils (Habeebullah et al., 2010), minced mackerel meat (Sabeena Farvin et al., 2012), and ground beef patties (Mansour, Khalil, 2000). In addition, it showed

the ability to reduce the toxicity of cholesterol oxidation products (Hsieh et al., 2016) and to protect erythrocytes against oxidative damage (Singh, Rajini, 2008), and other health-positive effects.

To ensure the best quality and maximal yield of produced extracts from potato peel wastes on the industrial scale, it is important to ensure the quality of the raw material. The aim of the present study was to analyse biologically active compound degradation tendencies in potato processing wastes (peels) from the abrasion peeling lines, during the storage at ambient conditions.

Materials and Methods

Potato samples

As the present study is discussing biologically active compound degradation in order to determine the optimal storage conditions for industrial potato processing by-products, so they could be further recycled, a new potato (*Solanum tuberosum* L., cultivar 'Magdalena', pedigree 82-28.9/15876.41) genotype specially developed for industrial processing purposes had been used. Potato tubers were donated by the State Priekuli Plant Breeding Institute (currently Institute of Agricultural Resources and Economics, Latvia). Sample preparation, storage, and extract preparation were carried out in the Department of Food Technology, Latvia University of Life Sciences and Technologies, in Fall of 2015. LC-HRMS analysis was conducted in the same year in the Department of Chemistry, University of Latvia.

Chemicals and reagents

Agricultural origin ethanol (96.6%) had been obtained from 'Stumbras' (Kaunas, Lithuania). All standards (α-solanine, α-chaconine, chlorogenic acid), acetonitrile, and formic acid were purchased from Sigma-Aldrich (currently Merck KGaA, Darmstadt, Germany).

Extract preparation

The preliminary study on industrial potato processing by-products in Latvia (data is not shown) showed that three main potato peel types are available as wastes: large peel pieces (approx. 5 cm), small peel pieces (approx. 5 mm), and finely shredded peel. Based on the acquired data, similar three types of peel samples had been produced. They were stored in the open plastic container at ambient conditions for 6 days at room temperature (21±2 °C), without additional aeration. Once a day, each sample type was collected, freeze-dried using Armfield FT33 (Ringwood, UK) with a final moisture content of ca. 5%, ground in the laboratory scale mill Foss KN 195 Knifetec (Hillerod, Denmark), and were immediately used for the extraction purposes. The whole experiment was repeated in three consecutive batches. The remaining moisture content was determined gravimetrically at 105 °C.

Biologically active compound extraction

80% ethanol had been used as a solvent (Mane et al., 2015). In short, 2 g of prepared potato peel powder was dispersed in 20 mL of the solvent. Extractions were conducted under the ultrasound treatment using YJ 5120-1, Citizen Scale (Mumbai, India), for 15 min at 33±1 °C. Supernatants were separated by a centrifuging, ELMI CM (Riga, Latvia), for 15 min at 2,500 × g. The extraction of the same sample had been repeated three times and acquired supernatants were combined and used for further analyses.

Liquid chromatography and mass spectrometry

Liquid chromatography - high-resolution mass spectrometry (LC-HRMS) had been performed on a Agilent 1290 Infinity UHPLC system, Agilent Technologies (Santa Clara, USA). Compound separations were performed on the Atlantis dC18 3.5 µm column (2.1×150 mm), Waters (Dublin, Ireland). The elution solvents consisted of 0.1% formic acid solution (A) and acetonitrile (B). The flow rate was 0.3 mL min^{-1}, with the 22 min gradient. The program was as follows: 5% A in 2 min, 5-95% A in 10 min, keep 95% of A for 5 min, 95-5% A in 2 min, keep 5% of A for 3 min. The HRMS had been performed on the Agilent 6230 TOF LC/MS system, Agilent Technologies (Santa Clara, USA), using both positive and negative electrospray ionization. Applied parameters: drying gas flow 10.0 L min^{-1}, 325 °C, ionisation 130 V, internal reference mass 121.050873 m/z and 922.009798 m/z.

The acquired data had been processed using *MassHunter 7.00* software, Agilent Technologies (Santa Clara, USA). The identification of separated compounds in extracted ion chromatograms was based on the [M+H]+ ion determination.

Chlorogenic acid, α-solanine and α-chaconine were identified based on used standards. The relative standard deviation was determined to be less than 2.0%. Phenylalanine was identified based on the retention time and mass-to-charge ratio, acquired from the literature (Lopez-Cobo et al., 2014).

Statistical analysis

Results are presented as mean values on a dry weight (dw) of peels. The compound degradation data approximation was conducted in the *MS Office Excel 2015* from Microsoft (Redmond, USA). Correlation coefficients for calibration curves were calculated using *MS Office Excel 2015* ($p<0.001$). The statistical analysis of the half-life values of the identified compounds had been conducted using the one-way analysis of variance (ANOVA, $p\leq0.05$).

Results and Discussion

Graphs of α-chaconine and α-solanine degradation dynamics can be seen in Figure 1.

Friedman et al. (2017) conducted a research on potato peels from several conventional and organic plant varieties and showed that α-chaconine levels could vary for conventional potato varieties for 424 to 1,297 mg kg^{-1} and 610 to 2,830 mg kg^{-1} for organic varieties. For α-solanine, in the same study, values varied from 215 to 412 mg kg^{-1} and from 239 to 750 mg kg^{-1} respectively. In the present study, α-solanine levels for all three peel

types before storage were ca. 360 mg kg^{-1}, and all of them showed similar degradation profiles.

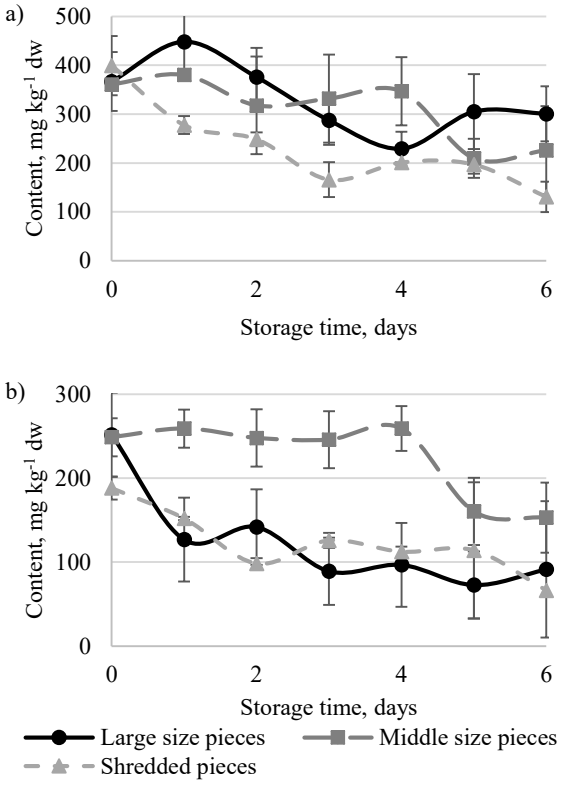

Figure 1. The decline of α-solanine (a) and α-chaconine (b) in potato peel wastes during the storage under ambient conditions

The linear approximation of acquired data showed that α-solanine degradation rates were higher in the peel of smaller size. Middle size peel samples showed similar results, but with a bigger rate. The fastest α-solanine degradation rates were observed in shredded peel samples. α-solanine degradation in three types of soil had been reported to have a half-life period of 1.8 to 4.1 days at 15 °C. α-solanine degradation in the soil had been reported previously, and it appeared to be related to the presence of different sorbents in low amounts (Jensen et al., 2009). In the present study, biologically active compounds in a greater content were exposed to the environmental impacts and enzymatic activity due to the cell disruption in peels (the bigger was the shredding rate – the bigger exposure). As a result, identified compounds showed lower degradation rates in samples with a higher rate of unbroken cells. For α-chaconine, large and shredded peel samples showed similar degradation rates except on the first day, making a similar declining picture. In the case of middle-size peel samples, α-chaconine levels were mostly constant during the first four days of storage, with an intensive recession on a subsequent day. Several explanations are possible. First of all, glycoalkaloids are distributed unevenly in the potato peel, as exposure to the light (including fluorescent) rapidly stimulates their production (Rocha et al., 2015; Romanucci et al., 2016). As a result of sample storage in the ambient conditions without being sheltered from the light beams (being sheltered from the direct sunlight, there is still a possibility of reflected light beam reaching samples), additional glycoalkaloid formation could occur during the storage. This fact addresses the increase in α-solanine content after the second day of storage. These results mean that potato peel wastes (by-products) should be stored while protected from the direct and reflected light.

Table 1 is showing half-life periods of four identified compounds.

Table 1

The half-life of the identified biologically active compounds based on a linear data approximation, in days

Identified compound	Value	LSP	MSP	SP
α-solanine	Ac.	n.a.	n.a.	2.5[a]
	Ca.	9.5	8.1	3.9[b]
	R^2	0.46	0.69	0.78
α-chaconine	Ac.	1.1[a]	n.a.	5.5[a]
	Ca.	2.9[b]	8.9	4.9[b]
	R^2	0.64	0.61	0.72
Chlorogenic acid	Ac.	2.5[a]	1.9[a]	n.a.
	Ca.	2.7[a]	2.4[b]	n.a.
	R^2	0.81	0.91	n.a.
Phenylalanine	Ac.	3.4[a]	1.1[a]	1.3[a]
	Ca.	3.5[a]	3.1[b]	2.8[b]
	R^2	0.75	0.60	0.81

* Lowercase letters indicate the difference between actual and calculated half-life values for each compound and peel type. LSP – large size peel, MSP – middle size peel, SP – shredded peel, Ac. – actual half-life, Ca. – calculated half-life, R^2 – coefficient of determination of calculated values, n.a. – not applicable.

Higher glycoalkaloid content can be preferred for the pharmaceutical industry as recent studies show that, despite their high toxicity, glycoalkaloids can exhibit health-promoting effects, including the ability to lower blood cholesterol (Friedman et al., 2000) and even exhibit anti-cancer activity (Friedman et al., 2007). Regarding the current study, glycoalkaloid degradation rates did not play a significant role, because (how it will be stated further) overall results showed that it is not advisable to store large and middle size peel pieces for longer than two days, while shredded peels are not applicable for proposed processing purposes. The degradation of α-solanine and α-chaconine during this time had been minimal, except α-chaconine that showed a decline in large peel pieces (Figure 1). Existing guidelines limit the glycoalkaloid content in food products to 200 mg kg^{-1} on fresh weight. It was shown that these limits may be too high (Friedman et al., 2007), as a clinical study on human volunteers resulted in the gastrointestinal disturbance of one participant after the consumption of food with a permitted glycoalkaloid content (Mensinga et al., 2005). Additionally, it was reported that the actual frequency of glycoalkaloid poisoning is unknown, as many poisoning incidences of may be underreported, because following symptoms include vomiting that more likely accepted as a result of

ingesting foodborne pathogens, general viral infections, or gastrointestinal illness (Jaspreet, Lovedeep, 2009). In case of the present study, consumption even of pure potato peel samples should not cause a toxic reaction, as recalculation on fresh peel weight will decrease average glycoalkaloid concentration in the samples till ca. 90 mg kg^{-1}. In case of application of the produced extract in food production, it will be very important to make sure not to exceed safety limits.

Figure 2 is showing the dynamics of chlorogenic acid concentration. It can be seen that middle and large size peels exhibit similar tendencies. At the same time, chlorogenic acid degraded almost fully in shredded samples already during the peeling process. It can be a result of the cell structure disruption, and the straight contact of plant metabolites and enzymes, that can undergo several possible reaction pathways that had been discussed previously in detail by another authors (Fifen et al., 2011; Galano et al., 2016; Tošović et al., 2017).

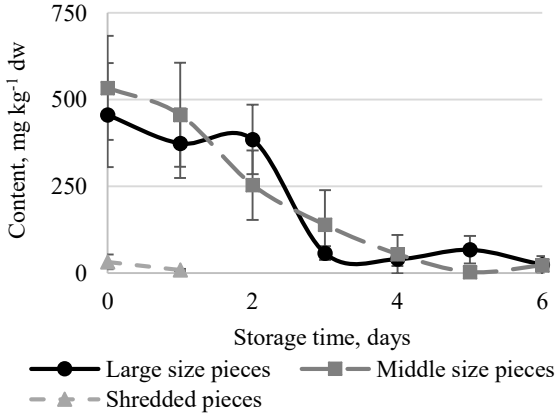

Figure 2. The decline of chlorogenic acid degradation in potato peel wastes during the storage under ambient conditions

Additionally, the samples did not undergo any additional treatment in order to deactivate polyphenol oxidase, a major cause of the enzymatic browning. As an additional plant protection mechanism, polyphenol oxidase catalyses monophenols transformation to *o*-diphenols and further *o*-dihydroxyphenols to *o*-quinones, with further polymerisation. In addition, reaction with amino acid groups of cellular proteins are taking place, that is a reason for brown and/or black pigment formation (Thygesen et al., 1995). In combination with exposed cell components, these processes significantly increase reaction speed and decrease chlorogenic acid content. As a result, in general, it is not reasonable to process shredded peel for phenolic compound extraction. Alternatively, large and medium-sized peel by-products could be used for this purpose during the first two days of storage at ambient conditions, showing half-life time of 2.7 (R^2=0.81) and 2.4 (R^2=0.91) days, respectively.

In addition, it was possible to identify one of the essential amino acids, phenylalanine, based on the mass-to-charge ratio, acquired from the literature. Degradation graphs are presented in Figure 3. Taking in consideration time when compound concentration decreased in half, phenylalanine had faster degradation rate in comparison with glycoalkaloids, with a half-life period of 3.5 days for large, 3.1 days for middle size peel, and 2.8 days for shredded peel samples. But starting with the second day of storage, phenylalanine concentration becomes almost constant.

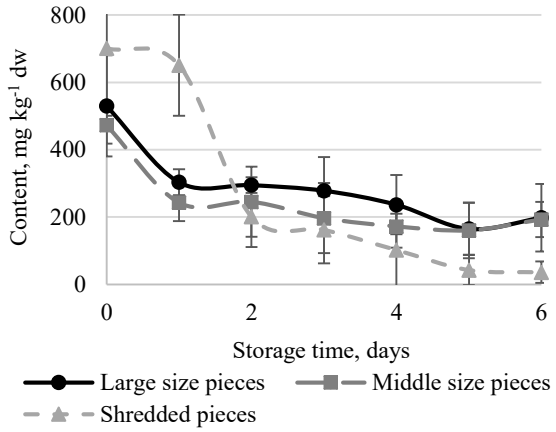

Figure 3. The decline of phenylalanine in potato peel wastes during the storage under ambient conditions

Phenylalanine, after the forming of p-coumaroyl-coenzyme A (p-coumaroyl-CoA), is known to take a part in the chlorogenic acid synthesis through 'core phenylpropanoid pathway' (Valiñas et al., 2017).

Comparing Figures 2 and 3, it can be seen that samples containing whole cells (large- and middle-sized peels) exhibit slower chlorogenic acid degradation rates during first days of storage, but a followed rapid decrease on a third day. During the same timeframe, phenylalanine degradation had been most active, but linear for the remaining storage time. Unfortunately, it was not possible to determine a wide range of metabolites that were present in extracts. As a result, it only can be assumed that chlorogenic acid synthesis could still occur during this first days, while later some of the reaction components could run out or become inactive, triggering the end of chlorogenic acid synthesis. The opposite situation can be seen in the case of shredded peel samples (as chlorogenic acid had been practically absent), where phenylalanine had been stable for the first several days, showing a rapid decrease only on a third. This could be a result of microbial activity, as the unpleasant smell shortly followed. At the same time, in addition to the chlorogenic acid pathway, the p-coumaroyl-CoA can take a core anthocyanin pathway, forming flavan-3-ols: catechin and epicatechin (Valiñas et al., 2017). As a result, taking in consideration also parallel degradation processes, rapid phenylalanine consumption does not result in the spikes in the chlorogenic acid content (synthesis). Unfortunately, there is not enough available published information to make definitive conclusions about exactly what type of processes are taking place in peels

that are separated from the potato tuber during the abrasion peeling process.

After the storage time, the surface of large and middle size peel pieces had been visually dry, but shredded peel represented a homogeneous mass with a pronounced microbiological decomposition signs. This can be a result of differences in cell integrities, that influenced the moisture evaporation and biologically active compound (including those with antimicrobial properties) degradation as had been discussed previously.

Conclusions

α-solanine and α-chaconine were the most stable among the analysed biologically active compounds in the present study. Chlorogenic acid concentration showed slower decrease rates in the large and middle size peel pieces during the first days of storage, when compared to small size samples; possibly due to ongoing chlorogenic acid synthesis from phenylalanine. For biologically active compound extraction from industrial potato peel wastes, it is recommended to use only potato peels that still contain whole cells, and their storage should not exceed two days at ambient conditions. Deeper studies on the biologically active compound degradation dynamics are necessary to maximise the yield of extractable target compounds from the industrial potato processing wastes.

Acknowledgement

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References

1. Al-Weshahy A., Rao V.A. (2012) Potato peel as a source of important phytochemical antioxidant nutraceuticals and their role in human health–A Review. **In:** *Phytochemicals as Nutraceuticals - Global Approaches to Their Role in Nutrition and Health*. V. Rao (ed). Shanghai: InTech, p. 207–224.
2. Amado I.R., Franco D., Sanchez M., Zapata C., Vazquez J.A. (2014) Optimisation of antioxidant extraction from *Solanum tuberosum* potato peel waste by surface response methodology. *Food Chemistry*, Vol. 165, p. 290–299.
3. Cardoso L.C., Serrano C.M., Quintero E.T., Lopez C.P., Antezana R.M., de la Ossa, E.J.M. (2013) High pressure extraction of antioxidants form *Solanum stenotomun* peel. *Molecules*, Vol 18, p. 3137–3151.
4. Fifen J.J., Nsangou M., Dhaouadi Z., Motapon O., Jaidane N. (2011) Solvent effects on the antioxidant activity of 3,4-dihydroxyphenylpyruvic acid: DFT and TD-DFT studies. *Computational and Theoretical Chemistry*, Vol. 966(1–3), p. 232–243.
5. Friedman M. (1997) Chemistry, biochemistry, and dietary role of potato polyphenols. A Review. *Journal of Agricultural and Food Chemistry*, Vol. 45(5), p. 1523–1540.
6. Friedman M. (2004) Analysis of biologically active compounds in potatoes (*Solanum tuberosum*), tomatoes (*Lycopersicon esculentum*), and jimson weed (*Datura stramonium*) seeds. *Journal of Chromatography A*, Vol. 1054, p. 143–155.
7. Friedman M., Fitch T.E., LevinN C.E., Yokoyama W.H. (2000) Feeding tomatoes to hamsters reduces their plasma low-density lipoprotein cholesterol and triglycerides. *Journal of Food Science*, Vol. 65(5), p. 897–900.
8. Friedman M., Kozukue N., Kim H.J.J., Choi S.H.H., Mizuno M. (2017) Glycoalkaloid, phenolic, and flavonoid content and antioxidative activities of conventional nonorganic and organic potato peel powders from commercial gold, red, and Russet potatoes. *Journal of Food Composition and Analysis*, Vol. 62(March), p. 69–75.
9. Friedman M., McQuistan T., Hendricks J.D., Pereira C., Bailey G. S. (2007) Protective effect of dietary tomatine against dibenzo[a,l]pyrene (DBP)-induced liver and stomach tumors in rainbow trout. *Molecular Nutrition & Food Research*, Vol. 51(12), p. 1485–1491.
10. Galano A., Mazzone G., Alvarez-Diduk R., Marino T., Alvarez-Idaboy J.R., Russo N. (2016) Food antioxidants: chemical insights at the molecular level. *Annual Review of Food Science and Technology*, Vol. 7(1), p. 335–352.
11. Göksungur Y., Uzunoullari P., Dağbağli S. (2011) Optimization of pullulan production from hydrolysed potato starch waste by response surface methodology. *Carbohydrate Polymers*, Vol. 83, p. 1330–1337.
12. Hossain M.B., Tiwari B.K, Gangopadhyay N., O'Donnell C.P., Brunton N.P., Rai D.K. (2014) Ultrasonic extraction of steroidal alkaloid from potato peel waste. *Ultrasonic Sonochemistry*, Vol 21, p. 1470–1476.
13. Hsieh Y.L., Yeh Y.H., Lee Y.T., Huang C.Y. (2016) Dietary potato peel extract reduces the toxicity of cholesterol oxidation products in rats. *Journal of Functional Foods*, Vol. 27, p. 461–471.
14. 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*, Vol. 56(9), p. 3341–3349.
15. Jaspreet S., Lovedeep K. (2009) *Advances in Potato Chemistry and Technology*. Academic Press. 528 p.
16. Jensen P.H., Pedersen R.B., Svensmark B., Strobel B.W., Jacobsen O.S., Hansen H.C.B. (2009) Degradation of the potato glycoalkaloid α-solanine in three agricultural soils. *Chemosphere*, Vol. 76(8), p. 1150–1155.
17. Habeebullah K.S.F., Nielsen N.S., Jacobsen C. (2010) Antioxidant activity of potato peel extracts in a fish-rapeseed oil mixture and in oil-in-water emulsions. *Journal of the American Oil Chemists' Society*, Vol. 87(11), p. 1319–1332.
18. Liang S., McDonald A.G. (2014) Chemical and thermal characterization of potato peel waste and its fermentation residue as potential resources for biofuel and bioproducts production. *Journal of Agricultural and Food Chemistry*, Vol. 62(33), p. 8421–8429.
19. Liang S., McDonald A.G. (2015) Anaerobic digestion of pre-fermented potato peel wastes for methane production. *Waste Management*, Vol. 46, p. 197–200.

20. Liang S., McDonald A.G., Coats E.R. (2014) Lactic acid production with undefined mixed culture fermentation of potato peel waste. *Waste Management*, Vol. 34(11), p. 2022–2027.
21. Lopez-Cobo A., Gomez-Caravaca A.M., Cerretani L., Segura-Carretero A., Fernandez-Gutierrez A. (2014) Distribution of phenolic compounds and other polar compounds in the tuber of *Solanum tuberosum* L. by HPLC-DAD-q-TOF and study of their antioxidant activity. *Journal of Food Composition and Analysis*, Vol. 36(1–2), p. 1–11.
22. Luthria D.L. (2012) Optimization of extraction of phenolic acids form a vegetable waste product using a pressurized liquid extraction. *Journal of Functional Foods*, Vol. 4, p. 842–850.
23. Mäder J., Rawel H., Kroh L.W. (2009) Composition of phenolic compounds and glycoalkaloids alpha-solanine and alpha-chaconine during commercial potato processing. *Journal of Agricultural and Food Chemistry*, Vol. 57(14), p. 6292–6297.
24. 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*, Vol. 27, p. 509–514.
25. Mansour E.H., Khalil A.H. (2000) Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties. *Food Chemistry*, Vol. 69(2), p. 135–141.
26. Matharu A.S., de Melo E.M., Houghton J.A. (2016) Opportunity for high value-added chemicals from food supply chain wastes. *Bioresource Technology*, Vol. 215, p. 123–130.
27. Mensinga T.T., Sips A.J.A.M., Rompelberg C.J.M., van Twillert K., Meulenbelt J., van den Top H.J., van Egmond H.P. (2005) Potato glycoalkaloids and adverse effects in humans: an ascending dose study. *Regulatory Toxicology and Pharmacology*, Vol. 41(1), p. 66–72.
28. Navarre D.A., Pillai S.S., Shakya R., Holden M.J. (2011) HPLC profiling of phenolics in diverse potato genotypes. *Food Chemistry*, Vol. 127(1), p. 34–41.
29. Rayburn J.R., Bantle J.A., Friedman M. (1994) Role of carbohydrate side chains of potato glycoalkaloids in developmental toxicity. *Journal of Agricultural and Food Chemistry*, Vol. 42(7), p. 1511–1515.
30. Rocha A.B.O., Honório S.L., Messias C.L., Otón M., Gómez P.A. (2015) Effect of UV-C radiation and fluorescent light to control postharvest soft rot in potato seed tubers. *Scientia Horticulturae*, Vol. 181, p. 174–181.
31. Romanucci V., Pisanti A., Di Fabio G., Davinelli S., Scapagnini G., Guaragna A., Zarrelli A. (2016) Toxin levels in different variety of potatoes: Alarming contents of α-chaconine. *Phytochemistry Letters*, Vol. 16, p. 103–107.
32. Sabeena Farvin K.H., Grejsen H.D., Jacobsen C., Farvin S.K.H., Grejsen 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*, Vol. 131(3), p. 843–851.
33. Samarin A.M., Poorazarang H., Hematyar N., Elhamirad A. (2012) Phenolics in potato peels: extraction and utilization as natural antioxidants. *World Applied Science Journal*, Vol. 18(2), p. 191–195.
34. Schieber A., Saldaña M. (2009) Potato peels : A source of nutritionally and pharmacologically interesting compounds – A Review. *Food*, Vol. 3(2), p. 23–29.
35. Singh N., Rajini P.S. (2008) Antioxidant-mediated protective effect of potato peel extract in erythrocytes against oxidative damage. *Chemico-Biological Interactions*, Vol. 173(2), p. 97–104.
36. Thygesen P., Dry I., Robinson S. (1995) Polyphenol oxidase in potatoe. *Plant Physiology*, Vol. 109, p. 525–531.
37. Tošović J., Marković S., Dimitrić Marković J.M., Mojović M., Milenković D. (2017) Antioxidative mechanisms in chlorogenic acid. *Food Chemistry*, Vol. 237, p. 390–398.
38. Valiñas M.A., Lanteri M.L., ten Have A., Andreu A.B. (2017) Chlorogenic acid, anthocyanin and flavan-3-ol biosynthesis in flesh and skin of Andean potato tubers (*Solanum tuberosum* subsp. andigena). *Food Chemistry*, Vol. 229, p. 837–846.
39. Wijngaard H.H., Ballay M., Brunton N. (2012) The optimisation of extraction of antioxdants form potato peel by pressurised liquids. *Food Chemistry*, Vol. 133, p. 1123–1130.
40. Williams C.A., Grayer R.J. (2004) Anthocyanins and other flavonoids. *Natural Product Reports*, Vol. 21(4), p. 539–573.

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THE INFLUENCE OF PROCESSING AND STORAGE CONDITIONS ON QUALITY PARAMETERS OF PUMPKIN PUREE

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Abstract

The aim of this research was to investigate the influence of processing technology and storage conditions on the quality parameters of organic pumpkin 'Uchiki Kuri' puree. Pumpkin puree was produced industrially from organically grown cultivar 'Uchiki Kuri' by heating it in a heat exchanger, and treating through sieves. Obtained puree was used to prepare four different samples: sterilized sample, frozen sample, vacuum-cooked stored at room temperature, and vacuum-cooked stored at 4±2 ℃. Samples were stored for 26 weeks. During the storage period microbiological (yeasts, moulds, Lactic acid bacteria, and *Enterobacteriaceae*), and chemical (total carotenes, vitamin C, total phenols, DPPH˙, $ABTS^{\cdot+}$, water activity, and pH) parameters were analysed. Obtained results indicated that it is possible to store pumpkin purées up to six months, if those are sterilized at 110 ℃ for 15 minutes; frozen and stored at -20±2 ℃; and vacuum-cooked, packed in a bag-in-box and stored at 4±2 ℃ temperature, preserving their microbiological safety. The total carotenes content in all samples (except samples stored at 22±2 ℃ in the bag-in-box packaging) were stable during storage for 26 weeks, while vitamin C content decreased twice already after four-week storage. Significant decrease of antiradical activity DPPH˙ and $ABTS^{\cdot+}$ after 26-week storage was observed as well.

Keywords: frozen, sterilized, vacuum-cooked, microbial quality, total carotenes, ascorbic acid, total phenols

Introduction

Pumpkin (*Cucurbita maxima*) fruit can be a healthy and valuable ingredient in a number of dishes and fruit products (Nawirska-Olszańska et al., 2014). One of the most popular pumpkin products – pumpkin puree is used as an ingredient for production of purees, jellies, jams, juices (Biesiada et al., 2011), and even confectionery and pastry products. This vegetable is particularly valuable due to high content of carotenoids, including β-carotene, lutein, and violaxantine (Biesiada et al., 2009), vitamin C (Biesiada et al., 2009), phenols (Dini et al., 2013), and carbohydrates (Chen, Huang, 2018). It is established that pumpkin regulates metabolism, lowers glucose level in blood, and possesses detoxicating effect (Song et al., 2018). Another attributed function of pumpkin species is defence against cancer (Nawirska-Olszańska et al., 2014). Therefore, it is very important to work on the development of technologies preserving valuable compounds.

Pumpkin products have low acidity. According to Nawirska-Olszańska et al. (2014) pH value of pumpkin cultivar 'Uchiki Kuri' was 7.17–7.46, which according to Jay et al. (2005) is favourable for growth of moulds, yeasts, *Clostridium botulinum, Bacillus cereus, Salmonella* spp., *Listeria monocytogenes*. Low-acid foods (pH>4.6) need to be sterilized (>100 °C) because inhibiting effect of acidity on microbial flora is not present (Boz, Erdogdu, 2016). Pumpkin products possess very short shelf life. Therefore, processing methods such as – pasteurization, sterilization, freezing – are applied to extend the shelf-life. The choice of sterilization / pasteurization time is determined by temperature, chemical composition of the product, type of micro-organisms. However, application of high temperature treatment to fruit and vegetable products leads to degradation of many bioactive compounds (Taoukis et al., 1998).

As a good alternative to the high temperature treatment is frozen storage, which becomes more popular also for different semi-finished products (Skrupskis et al., 2017). Stability of physical, chemical and nutritional quality of foods is affected by various factors such as storage conditions and food matrix, including the initial microbial load and enzyme activity. Many studies have been conducted on storage stability of fruit products (Djaoudene, Louaileche, 2016), including stability of bioactive compounds (Skrovankova et al., 2015). However only few studies deal with storage stability of pumpkin puree.

Scientific researches are focusing on the use of various packaging materials and technological processes, with the aim extending the storage life without significantly altering the sensory properties of the product. One of such new packaging technologies is bag-in-box technology, which is very suitable for delivering bulk amounts of fruit and vegetable products for fast-paced institutional environments. There are very few or almost no investigations on the microbial safety and stability of bioactive compounds of fruit and vegetable products packed using bag-in-box technology.

The challenge for processing of vegetable products like pumpkins is to find a processing method which ensures a microbially safe product and as minimum 6 months shelf life with the highest contents of bioactive compounds. Therefore, the aim of this research was to investigate the influence of processing and storage conditions on the quality parameters of pumpkin 'Uchiki Kuri' puree.

Materials and Methods

Pumpkin puree preparation

Pumpkin puree from cultivar 'Uchiki Kuri' was produced industrially by washing, cutting, de-seeding, shredding in the cutter RM 2,2 (Voran Maschinen GmbH, Austria), heating in the heat exchanger

SCOOK10 (Tecmon Srl., Italy) at 90–96 °C for 2–5 min, and treating through a fruit puree extraction unit TPULP50 (Tecmon Srl., Italy). The obtained puree was divided into four batches, and further processed.

Frozen puree. Packaged in bag-in-box, cooled to room temperature, then frozen and stored in a freezer at -20±2 °C.

Sterilized puree. Heated till 75±2 ℃, filled in 250 mL glass jars, covered with caps and sterilized at 110 ℃ for 15 minutes in a pilot scale autoclave HST 50/100 (Zirbus technology, Germany). Stored in a dark place at 22±2 °C.

Bag-in-box 22 ℃. Heat-treated at 76–80 °C for 25 min in a vacuum cooker EV-150 at 0.6 bar, with additional heating before filling (93–94.6 °C, 0.9 bar, holding time 10 min). Stored in a dark place at 22±2 °C.

Bag-in-box 4 ℃. Heat-treated as described above. Stored in a dark place at 4±2 °C.

Microbiological analyses

Determination of the yeasts and moulds was completed according to ISO 21527-1:2008 (n=5). Total plate count (TPC) was performed according to the standard LVS EN 4833:2003 (n=5). Analysis of *Enterobacteriaceae* was performed according to the standard LVS ISO 21528-2 (n=5). Lactic acid bacteria (LAB) was detected in conformity with standard ISO 9332:2003 (n=5).

Physical and chemical analyses

Determination of moisture content was done according to ISO 6496:1999. Samples were dried using Universal Oven UF55 (Memmert, Germany), at 105±1 °C until constant weight (n=3).

Water activity (a_w) was determined using LabSwift-aw (AG Novasina, Switzerland) equipment (n=3).

pH was measured (n=3) by pH-meter (JENWAY 3510, Baroworld Scientific Ltd., UK) using standard method LVS ISO 5542:2010.

Colour analysis were performed (n=5) using colour analyser ColorTec-PMC (Accuracy Microsensors, Inc., USA). The CIE L* a* b* colour system version 1976 was used.

The total carotenes content was detected by spectrophotometric method using UV/VIS spectrophotometer Jenway 6705 (Bibby Scientific Ltd., UK) and method described by Kampuse et al. (2015) with modifications. A sample of 2 g was mixed with 20 mL of ethanol on a magnetic stirrer for 15 min. Further, 25 mL of petroleum ether were added and stirred for an hour. After mixing, samples were placed into darkness one more hour. The absorption of petroleum ether layer was measured at 440 nm (n=3).

The detection of ascorbic acid content. This method determines L-ascorbic acid, which is the reduced form of ascorbic acid, corresponding to the specimen after iodine method T-138-15-01:2002 (Segliņa, 2007) (n=4).

The total phenol content was detected according to the Folin-Ciocalteu method (Yu et al., 2003) with modifications. A sample of 2 g mixed with ethanol: water solution (80 : 20 v / v) was used for extract preparation (Priecina, Karklina, 2014). Absorption was recorded at 765 nm (n=3) using a spectrophotometer JENWAY 6300 (Baroworld Scientific Ltd., UK). The obtained data were expressed as mg of gallic acid equivalent (mg GAE kg^{-1} of sample).

Determination of antiradical activity (DPPH˙ and $ABTS^+$). The antiradical activity of extracts was measured on the basis of scavenging activities of the stable 2,2-diphenil-1-picrylhydrazyl (DPPH) free radical Yu et al. (2003) with modifications. To 0.5 mL of extracted sample, 3.5 mL freshly made DPPH˙ solution was added; the mixture was shaken and kept in the dark place at 18±1 °C for 30 min; absorbance was measured at 517 nm using JENWAY 6300 spectrophotometer (n=3). The antiradical activity was expressed as mg Trolox equivalent (TE) kg^{-1} of sample (Priecina, Karklina, 2014).

The radical scavenging activity of extracts was measured by $ABTS^+$ radical cation assay as described by Re et al. (1999). A stock solution of 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic) acid ($ABTS^+$) (2 mM) was diluted with phosphate buffered saline to obtain an absorbance of (0.800±0.030) at 734 nm. Then 5 mL of $ABTS^+$ solution was mixed with 0.05 mL of extract. The absorbance was read at ambient temperature 18±1 °C after 10 min. For both determinations of antiradical activity the obtained data were expressed as mM Trolox equivalents per kg of sample (Kruma et al., 2016).

All analyses were done at beginning and after 4, 7, 17, 24 and 26 weeks of storage, except ascorbic acid content, which was analysed up to 7th week of storage, and DPPH antiradical activity, which was done up to 24th week of storage. Each time two separate samples of puree were taken, and analyses were carried out in three to four replications.

Statistical analysis

The differences between the results were analysed using one-factor analysis of variance (ANOVA) followed by Tukey-Kramer method. The obtained results were presented as their means with standard errors. Differences among results were considered to be significant if $p<0.05$.

Results and Discussion

The microbial quality

The selected processing methods were effective to reduce counts of lactic acid bacteria, moulds, and *Enterobacteriaceae* under the detection limit; and their growth was not observed in any of samples during the storage. As shown in Fig. 1, rapid increase of yeast counts was observed in a pasteurized pumpkin purée, which was stored at 22±2 °C.

The development of yeast cells was delayed, when samples were stored at low temperatures, providing at least 6 months shelf life. When stored at 4±2 °C and frozen at –20±2 °C, yeast cells in pasteurised pumpkin purée are in anabiosis. In turn, the sterilized product is shelf-stable at room temperature 22±2 °C due to

inactivating spores rather than only vegetative microorganisms (Knockaert et al., 2012).

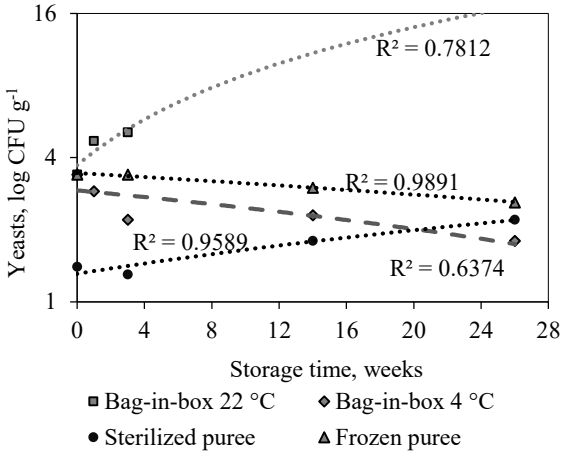

Figure 1. Yeast count in pumpkin puree during storage

An evaluation of total plate count in products (Fig. 2) showed a similar trend to yeasts.

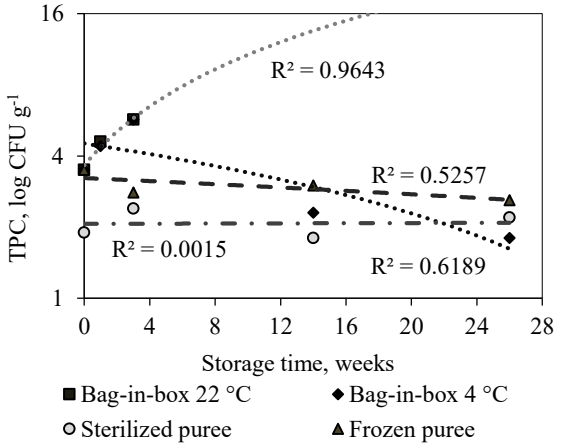

Figure 2. Total plate count in pumpkin puree during storage

Summarizing the obtained results of microbiological analysis, it was determined that the process of pasteurization and storage at room temperature 22±2 °C allows short-term storage - up to three weeks. In order to store the pumpkin puree up to six months and longer it is necessary to use the sterilization method, pasteurisation combined with refrigerated storage 4±2 °C or freezing.

Water activity (aw)

For a number of foods, it is important to perform tests on the amount of water activity (Fig. 3) in order to predict their stability and shelf life.

The water activity of the studied pumpkin samples was very high immediately after heat treatment (from 0.997–0.999), which testifies having good environment for the development of microorganisms. During storage of sterilized sample and sample stored at temperature 4±2 °C in the bag-in-box package, the water activity a_w slightly decreased (up to 0.992 and 0.993, respectively), although it did not have a significant effect on the growth of microorganisms.

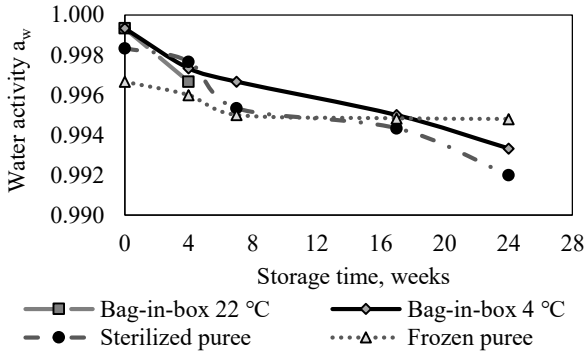

Figure 3. Water activity changes in pumpkin puree during storage

Changes of pH

pH is the second most important indicator behind water activity, which characterizes the potential development of microorganisms. If the pH of the product is above 4.5, it is already considered as a potentially good environment for the development of spore-forming and pathogenic microorganisms (Boz, Erdogdu, 2016). The pH of the pumpkin pure was relatively high: from 6.2 to 6.6 at the beginning of storage to 5.9–6.2 after 6 months of storage (Fig. 4).

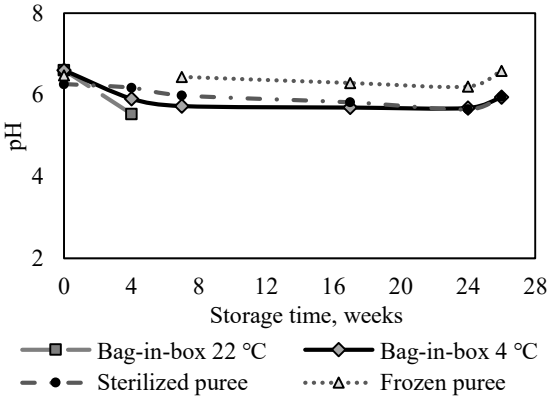

Figure 4. Changes of pH in pumpkin puree during storage

pH value of pumpkin cultivar 'Uchiki Kuri' in other investigations was mentioned even higher (7.17–7.46) (Nawirska-Olszańska et al., 2014). Such pH of the product is already close to the neutral pH, thus it is a good environment for the development of various spore-forming microorganisms.

The changes of the pH during the storage of product can often indicate the onset of microbial deterioration, which was also observed at 22±2 °C in a bag-in-box package stored sample – after 4 weeks of storage the pH of the product had dropped till 5.5.

Decrease in pH was observed also for samples without microbial spoilage what could be explained by non-enzymatic oxidation processes and formation of other phenolic compounds (Castro-López et al., 2016) such as

phenolic acids which could decrease the pH of product during storage.

Colour (CIE L a* b*) changes*

Comparing the colour of the pumpkin purée during storage, it was found that the colour of the sterilized purée had become lighter after 6 months of storage (L* value increased), while the purées stored at 4±2 °C had become slightly darker, but the colour intensity L* of frozen purees did not change significantly ($p<0.05$). The intensity of red colour for sterilized and frozen puree did not change significantly, but for puree stored at 4±2 °C, slight increase in red colour intensity a* was observed that could be explained with non-enzymatic oxidation reactions which can influence such colour pigments as carotenes, flavones, and flavonones. According to Chisari et al. (2007), the oxidation of phenolic compounds to o-quinones may subsequently polymerize yielding dark pigments. The intensity of yellow colour component b* for all samples remained practically unchanged.

The slightest colour difference ΔE (last day comparing to the first day) was for the frozen sample (2.6), but the biggest – for purée stored at 4±2 °C in the bag-in-box package (5.4), colour difference for sterilized puree was 3.8. The colour changes in the bag-in-box 4±2 °C packaging were considered as significant.

Total carotenes

The total carotenes content in all samples was stable during storage for 26 weeks except for samples stored at 22±2 °C in the bag-in-box packaging, which after 4 weeks of storage indicated a significant decrease ($p<0.05$) of total carotenes (Fig. 5), what could be related to the microbiological spoilage.

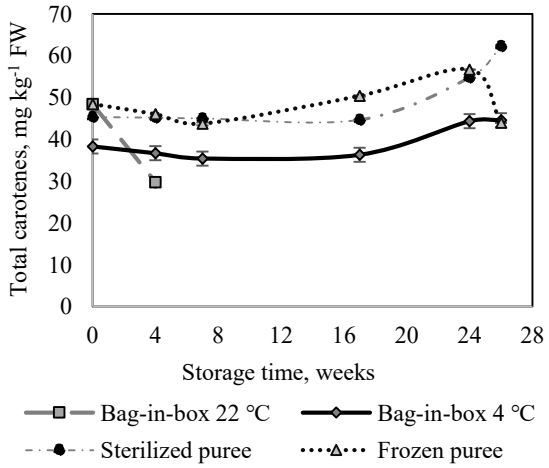

Figure 5. Changes of total carotenes content in pumpkin puree during storage

Provesi et al. (2011) in their research on carotenoid stability after processing and storage of pumpkin puree also revealed the stability of the major carotenoids. Heat processing is sufficient for the inactivation of enzymes and micro-organisms, which could degrade these compounds. Moreover, there is a partial vacuum inside the bottle because oxygen is removed from it and that allows to delay oxidation reactions. Storage at temperatures lower than 30 °C and protection from light are also important factors for the stability of carotenoids (Provesi et al., 2011). However, in other studies the losses of carotenoids after processing and storage were higher (Lin, Chen, 2005). Provesi et al. (2011) summarise that the stability of carotenoids in foods is variable. This happens not only because of extrinsic factors, such as the severity of heat treatment, presence or absence of light, temperature of storage, packaging, but also because of the characteristics of the food matrices, such as their chemical composition, the oxygen dissolved in the samples, size of the particles, and the physical state of the carotenoid in the food (Provesi et al., 2011).

Ascorbic acid content

After four weeks of storage, the content of vitamin C in all samples was reduced at least by half and practically reached the lowest limit of detection (36–54 mg per kg of product), therefore the evaluation of this parameter was discontinued after 7th week of storage.

As it is known from many investigations, vitamin C is one of the most unstable vitamins. In experiments with different vegetables, blanching resulted in big ascorbic acid loss, but retention remained stable after freezing of broccoli and green beans. Green beans lost >90% ascorbic acid after 16 days of storage. Linear decreases in ascorbic acid were found in most fresh-refrigerated and frozen vegetables during storage (Howard, 2008).

Our experiment showed that none of the storage conditions tested was sufficient for preserving the ascorbic acid in pumpkin puree packed in bag-in-box type packages.

The total phenol content

All studied samples showed a significant increase ($p<0.05$) in total phenol content (TPC) during storage (Fig. 6). TPC increased by 63.7%, on average, during 26-week storage. During the first eight weeks of storage a significant increase of TPC was observed in all samples, which was followed by a gradual increase with the extension of storage time. Ghirardello et al. (2016) also observed increase of TPC in hazelnuts stored at 5 °C and relative air humidity 55%. Unlike, Zhao et al. (2019) established TPC reduction in cherries stored at "near-freezing temperature (NFT) 0 °C". Also authors Shiri et al. (2011) indicated the changes in the total phenolic content of fresh-cut grapes during storage. As their results showed, the phenolic content gradually increased during storage in both PET and PVC packaging. According to findings of these authors, phenolic compounds are generally synthesized by the shikimate pathway in which phenylalanine ammonialyase (PAL) is the key enzyme. The physical damage of plant tissue can increase PAL activity, which leads to an increase in phenolic compounds (Shiri et al., 2011). In an investigation of Castro-López et al. (2016) with processed juice beverages during storage (4, 8 and 11 °C) for 20 days, the TPC values in all samples also showed a tendency to increase from

day 12. The authors have explained this phenomenon by a possible increment of polyphenolic compounds associated to the microbial growth or to reactions between oxidized polyphenols and formation of new compounds of antioxidant character during juice storage. They considered the possibility that during juice storage, some compounds could be formed and react with the Folin–Ciocalteu's reagent and significantly enhance the phenolic content as well.

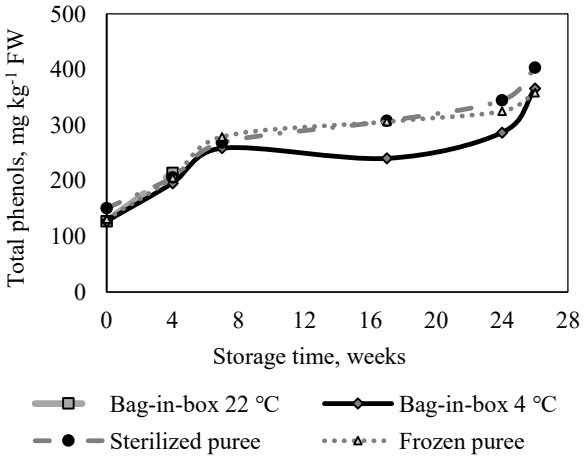

Figure 6. Changes of total phenol content in pumpkin puree during storage

There was no significant difference between TPC in samples, irrespective of storage conditions. Gradual increase was determined in frozen puree and sterilized puree after the week eighth. TPC in pumpkin puree packed in bag-in-box and stored at 4 °C did no change significantly between week 7 and week 17, which was followed by more rapid increase starting from week 18. The content of total phenols at the beginning of storage was 127.48 to 150.84 mg per kg of sample. After 26 weeks or 6 months of storage, the total phenol content was 358.5–403.27 mg per kg of sample.

Antiradical activity / radical scavenging activity

The DPPH˙ antiradical activity decreased slightly along the storage time. The most stable DPPH˙ antiradical activity was in the bag-in-box sample stored at 4±2 °C (Fig. 7).

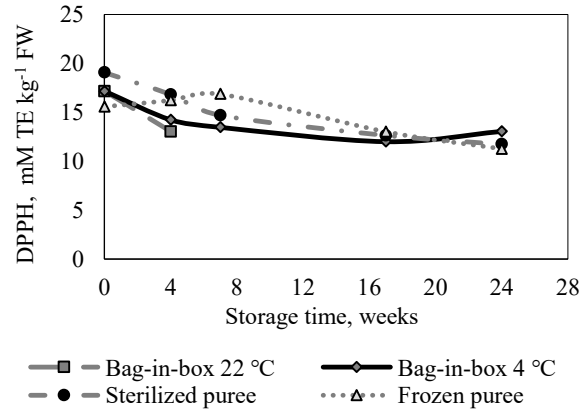

Figure 7. DPPH˙ antiradical activity content in pumpkin puree during storage

The fastest decrease of DPPH˙ antiradical activity was in samples packed in bag-in-box bags after four weeks of storage at 22±2 °C. After 24 weeks of storage, DPPH˙ antiradical activity decreased by 30.0%, on average, and it ranged from 19.78 to 23.43 mM TE per kg fresh weight. Our results are in line with findings of Zhao et al. (2019), who reported the highest DPPH˙ antiradical activity reduction rate in cherries stored at 5 °C. Ghirardello et al. (2016) observed opposite trend in hazelnuts stored at 5 °C for 8 months. Storage conditions had significant effect on $ABTS^{\bullet+}$ antiradical activity. The highest reduction of $ABTS^{\bullet+}$ antiradical activity was observed in a sterilized sample that was stored at room temperature (a decrease by 57.3%). The most stable $ABTS^{\bullet+}$ antiradical activity during the whole storage time was again in bag-in-box packed puree, which was stored at 4±2 °C (a decrease by 1.9%) (Fig. 8).

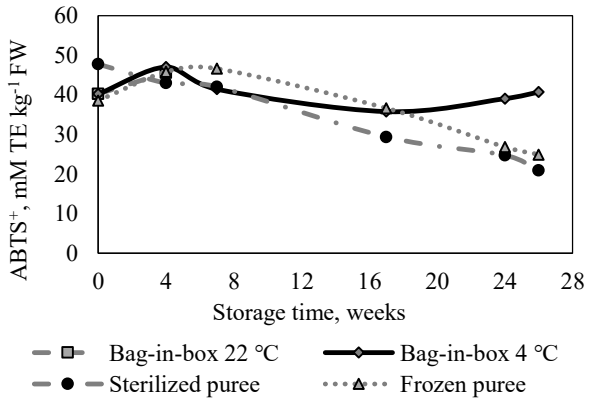

Figure 8. $ABTS^{\bullet+}$ antiradical activity in content in pumpkin puree during storage

Similar trend was revealed by Ghirardello et al. (2016), who studied hazelnut storage at –25 °C. Contrary, $ABTS^{\bullet+}$ antiradical activity increased after eighth months of hazelnut storage at 5 °C and relative air humidity 55%.

Conclusions

It is possible to provide microbial safety for pumpkin purées up to six months, if those are sterilized at 110 °C for 15 minutes, frozen and stored at -20±2 °C, or packed in bag-in-box after vacuum-cooking and stored at 4±2 °C. The total carotenes content was stable during storage for 26 weeks. None of the storage conditions tested was sufficient for preserving the ascorbic acid in pumpkin puree packed in bag-in-box type packages. All studied samples showed a significant increase in total phenol content (TPC) during storage. The DPPH˙ antiradical activity decreased slightly along the storage time. The fastest decrease of DPPH˙ antiradical activity was in samples packed in bag-in-box bags after four weeks of storage at 22±2 °C. The most stable $ABTS^{\bullet+}$ antiradical activity during the whole storage time was in bag-in-box packed puree, which was stored at 4±2 °C. The biggest total colour difference was for puree stored at 4±2 °C in the bag-in-box package. Based on the

results, the best methods for packaging and storage pumpkin puree are sterilization in glass jars and storage at room temperature and packaging in bag-in-box type packages and storage at 4 °C temperature.

Acknowledgment

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References

1. Biesiada A., Nawirska A., Kucharska A., Sokół-Łętowska A. (2009) The effect of nitrogen fertilization methods on yield and chemical composition of pumpkin (*Cucurbita Maxima*) fruits before and after storage. *Vegetable Crops Research Bulletin*, Vol. 70(1), p. 203–211.
2. Biesiada, A., Nawirska, A., Kucharska, A., Sokół-Łętowska, A. (2011) Chemical composition of pumpkin fruit depending on cultivar and storage. *Ecological Chemistry and Engineering A*, Vol. 18(1), p. 9–18.
3. Boz Z., Erdogdu F. (2016) Thermal processing: canning and aseptic packaging. In Y. H. Hui E. Özgül Evranuz (Eds.), *Handbook of Vegetable Preservation and Processing* (pp. 157–199). CRC Press.
4. Castro-López C., Sánchez-Alejo E. J., Saucedo-Pompa S., Rojas R., Aranda-Ruiz J., Martínez-Avila G. C. G. (2016). Fluctuations in phenolic content, ascorbic acid and total carotenoids and antioxidant activity of fruit beverages during storage. *Heliyon*, Vol. 2(9), p. 1–16.
5. Chen L., Huang G. (2018) Extraction, characterization and antioxidant activities of pumpkin polysaccharide. *International Journal of Biological Macromolecules Biomac*, Vol. 118, p. 770–774.
6. Dini I., Tenore G. C., Dini A. (2013) Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp. *LWT - Food Science and Technology*, Vol. 53(1), p. 382–385.
7. Djaoudene O., Louaileche H. (2016) Impact of storage conditions on the bioactive compounds and antioxidant capacity of commercial orange jam. *Journal of Analytical, Bioanalytical and Separation Techniques*, Vol. 1(1), p. 8–11.
8. Ghirardello D., Bertolino M., Belviso S., Dal Bello B., Giordano M., Rolle L., Vincenzo G., Antonucci M., Spigolon N., Zeppa G. (2016) Phenolic composition, antioxidant capacity and hexanal content of hazelnuts (Corylus avellana L.) as affected by different storage conditions. *Postharvest Biology and Technology*, Vol. 112, p. 95–104.
9. Howard L. (2008). Processing techniques and their effect on fruit and vegetable phytochemicals. In F. A. Tomás-Barberán, M. I. Gil (Eds.), *Improving the Health-Promoting Properties of Fruit and Vegetable Products* (pp. 449–472). Woodhead Publishing.
10. Jay J. M., Loessner M. J., Golden D. A. (2005) *Modern Food Microbiology*. Springer: New York, 790 p.
11. Kampuse S., Ozola L., Straumite E., Galoburda R. (2015) Quality parameters of wheat bread enriched with pumpkin (*Cucurbita Moschata*) by-products. *Acta Universitatis Cibiniensis. Series E: Food Technology*, Vol. 19(2), p. 3–14.
12. Knockaert G., Pulissery S. K., Colle I., Van Buggenhout S., Hendrickx M., Loey A. Van. (2012) Lycopene degradation, isomerization and in vitro bioaccessibility in high pressure homogenized tomato puree containing oil: Effect of additional thermal and high pressure processing. *Food Chemistry*, Vol. 135(3), p.1290–1297.
13. Kruma Z., Tomsone L., Galoburda R., Straumite E., Kronberga A., Åssveen M. (2016) Total phenols and antioxidant capacity of hull-less barley and hull-less oats. *Agronomy Research*, Vol. 14(Special Issue 2), p. 1361–1371.
14. Lin C. H., Chen B. H. (2005) Stability of carotenoids in tomato juice during storage. *Food Chemistry*, Vol. 90(4), p. 837–846.
15. Nawirska-Olszańska A., Biesiada A., Sokół-Łętowska A., Kucharska A. Z. (2014) Characteristics of organic acids in the fruit of different pumpkin species. *Food Chemistry*, Vol. 148, p. 415–419.
16. Priecina L., Karklina D. (2014) Natural antioxidant changes in fresh and dried spices and vegetables. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, Vol. 8(5), p. 492–496.
17. Provesi J. G., Dias C. O., Amante E. R. (2011) Changes in carotenoids during processing and storage of pumpkin puree. *Food Chemistry*, Vol. 128(1), p. 195–202.
18. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, Vol. 26, p. 1231–1237.
19. Segliņa D. (2007) Sea buckthorn fruits and their processing products. *Summary of promotion work for acquiring the Doctor's degree in Engineering Sciences in the Food Science*. [accessed on 13.03.2019] Available at: http://llufb.llu.lv/dissertation-summary/food/Kopsa vilkums-2007-LLU-Dalija-Seglina.pdf
20. Shiri M. A., Ghasemnezhad M., Bakhshi D., Dadi M. (2011) Changes in phenolic compounds and antioxidant capacity of fresh-cut table grape (*Vitis vinifera*) cultivar "Shahaneh" as influence by fruit preparation methods and packagings. *Australian Journal of Crop Science*, Vol. 5(12), p. 1515–1520.
21. Skrovankova S., Sumczynski D., Mlcek J., Jurikova T., Sochor J. (2015) Bioactive compounds and antioxidant activity in different types of berries. *International Journal of Molecular Sciences*, Vol. 16(10), p. 24673–24706.
22. Skrupskis I., Blija A., Beitane I., Boca S., Aboltins A. (2017) Research of half-finished frozen berry products. **In:** *11th Baltic Conference on Food Science and Technology "Food science and technology in a changing world" FOODBALT 2017*, p. 102–105.
23. Song J., Wei Q., Wang X., Li D., Liu C., Zhang M., Meng L. (2018) Degradation of carotenoids in dehydrated pumpkins as affected by different storage conditions. *Food Research International*, Vol. 107, p. 130–136.
24. Taoukis P. S., Panagiotidis P., Stoforos N. G., Butz P., Fister H., Tauscher B. (1998) Kinetics of vitamin C degradation under high pressure–moderate temperature processing in model systems and fruit juices. *High Pressure Food Science, Bioscience and Chemistry*, p. 310–316.
25. 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*, Vol. 51(6), p. 1566–1570.
26. Zhao H., Wang B., Cui K., Cao J., Jiang W. (2019) Improving postharvest quality and antioxidant capacity of sweet cherry fruit by storage at near-freezing temperature. *Scientia Horticulturae*, Vol. 246, p. 68–78.

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THE EVALUATION OF ORGANICALLY GROWN APPLE CULTIVARS FOR SPECIAL DIET PUREE PRODUCTION

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Abstract

The aim of this research was to evaluate organically grown Latvian extensive apple cultivars for production of puree for special diets with high content of bioactive compounds. Five organically grown extensive cultivars were selected for evaluation within the project: 'Antonovka', 'Filippa', 'Nicnera Zemenu', 'Rudens Svitrotais', 'Sipolins'. All these apple cultivars were evaluated fresh and after processing into puree. The content of soluble solids, titratable acids, vitamin C, total carotenes, total phenols, total flavonoids, antiradical activity (DPPH and $ABTS^+$), and pH were determined. The cultivars with the highest soluble solids content both fresh and puree were 'Nicnera Zemenu' and 'Sipolins' with 12.7 to 13.5 Brix%, but the lowest pH value and the highest titratable acids content was detected in apple cultivar 'Antonovka'. Fresh apples of this cultivar showed also the highest vitamin C content (14.5 mg 100 g^{-1}), but after processing into puree the vitamin C content significantly decreased and did not exceed 7.2 mg 100 g^{-1}. Fresh apples and apple puree from cultivar 'Antonovka' had the highest content of total phenolics, total flavonoids and DPPH radical scavenging activity, whereas the lowest results showed fresh apples and puree of cultivar 'Sipolins'. Apples are not a source of caroteinoids therefore the total carotenes content in fresh apples was not higher than 0.13 mg 100 g^{-1} (cultivar 'Filippa') and after processing in puree it significantly ($p<0.05$) decreased. Apple processing had a significant ($p<0.05$) influence on the bioactive compounds in product.

Keywords: total carotenes, vitamin C, total phenols, antiradical activity

Introduction

Apples (*Malus domestica* L.) are one of the most consumed fruits in European Union (Fernández-Jalao et al., 2018) and are key ingredient of many traditional desserts due to their availability and versatility (Keenan et al., 2012). They are rich in phenol compounds, pectin, sugar, insoluble and soluble dietary fibre, macro- and microelements, which possess various health benefits (Kalinowska et al., 2014; Boyer, Liu, 2004). Many of health benefits associated with apples are due to their chemical composition, especially, rich polyphenol content. It has been well documented that the main polyphenols in apple fruits are flavan-3-ols (catechin, epicatechnin, proanthocyanidins) hydrocinnamic acis (chlorogenic acid, p-coumaroylquinic aid) dihydrochalcones and flavonols (quercetin, kaempferol), two glycosides of phloretin and anthocyanins (Archivio et al., 2007; Song et al., 2007; Weichselbaum et al., 2010). Health benefits associated with apple polyphenols are a risk reduction of cardiovascular disease, some types of cancer, diabetes, Alzheimer's disease (Laaksonen et al., 2017; Fernández-Jalao et al., 2018) and have a positive influence on blood lipid parameters and blood pressure in human beings (Weichselbaum et al., 2010). The presence of ascorbic acid in apples also contributes to the total antioxidant capacity of polyphenols (Karaman et al., 2013). However according to Drogoudi et al. (2008) vitamin C accounts only for 0.4% of antioxidant potential in apples.

When assessing the benefits of apples, it should be taken into account that the composition of natural phytochemicals varies notably depending on the variety, region and corresponding weather conditions, ripeness, agricultural practice, and post-harvest conditions (Keenan et al., 2012; Kalinowska et al., 2014; Jakobek, Barron, 2016; Fernández-Jalao et al., 2018). Polyphenols in apple fruit play important role in the flavour and colour of apples and their processing products. When selecting apples for processing there are many other important fruit quality attributes such as soluble solids content, total titratable acidity, pH, firmness (Bonany et al., 2014). The type of processing also will affect the quality of final product; therefore, it is important to evaluate the performance of the specific apple varieties in processed products. Apple purees are concentrated plant food dispersions where soft insoluble particles composed of cell particles from parenchyma are dispersed in an aqueous solution of sugars, organic acids and pectic substances (Espinosa et al., 2011). The composition of puree and its rheological properties greatly depend on the apple variety used.

Worldwide, dysphagia affects approximately 590 million people (Cichero et al., 2017), who need modified texture diets such as pureed foods to allow safe swallowing. Preparation of pureed foods requires disintegration of regular texture into smaller pieces which may result in a reduced nutritional value and appeal (Keller et al., 2012). This has practical implications for older adults with generally lower food intake (Pfisterer et al., 2018) and therefore malnutrition prevalence reached 80% among patients with dysphagia (Ercilla et al., 2012). Keller and Duizer (2014) discovered that limited variety can lead to sensory fatigue, and therefore variety should be increased through different ingredients, spices, sauces. Additionally, the thickness of puree has safety implications (Ilhamto et al., 2014).

The aim of this research was to evaluate organically grown Latvian extensive apple cultivars for production of puree for special diets with high content of bioactive compounds.

Materials and Methods

Fresh apples

Five organically grown extensive cultivars from the farm Kurpnieki located in Aizpute region Laza parish were chosen for evaluation within the study – 'Antonovka', 'Filippa', 'Nicnera Zemenu', 'Rudens Svitrotais', 'Sipolins'. All these apple cultivars were evaluated fresh, fully ripened within 1 to 2 weeks after harvesting and storage in refrigerator at 4–6 ℃, and after processing into puree.

Apple puree preparation

Apple puree from all 5 cultivars was produced by washing, cutting into 2×2 cm pieces, heating in the steam cooker Philips HD 9126 for 25 min, blending with blender PHILIPS (30–40 s) into puree, and treating through sieves. The obtained apple puree was filled into 250 mL glass jars, covered and pasteurized at 90±2 ℃ for 5 min.

Sample preparation for chemical and physical analyses

An average sample from five fresh apples was homogenized for fresh analysis in addition to apple puree from the same cultivars.

Determination of total carotenes content

The total carotenes content was detected by spectrophotometric method with UV/VIS spectrophotometer Jenway 6705 (Bibby Scientific Ltd., UK) and method described by Kampuse et al. (2015) with modifications. Apple and apple puree sample of 4 g was mixed with 20 mL of ethanol on a magnetic stirrer for 15 min. 25 mL of petroleum ether were added and stirred for an hour. After mixing, samples were left for half an hour for dividing of layers. The absorption at 440 nm of petroleum ether layer was measured. Analyses were done in two repetitions and the results were expressed as mg 100 g^{-1} fresh weight.

Detection of ascorbic acid content

This method determines L-ascorbic acid, which is the reduced form of ascorbic acid. The titration with 0.5 n iodine solution was used according to method T-138-15-01:2002 (Segliņa, 2007). Analyses were done in two replications and two repeated measurements. The results were expressed as mg 100 g^{-1} fresh weight.

Determination of pH

pH was measured (n=3) by pH-meter (JENWAY 3510, Baroworld Scientific Ltd., UK) using standard method LVS ISO1842:1991.

Determination of apple and apple puree soluble solids content

The soluble solids content (Brix%) was measured with digital refractometer Refracto 30GS (Mettler Toledo, Japan) using standard method ISO 2173:2003. Measurements were carried out in five replications.

Determination of titratable acidity

Total titratable acids were determined by titration with 0.1 N NaOH (ISO 750:1998) in fresh apples and apple puree. The results were expressed in g 100g^{-1} fresh weight.

Extraction of phenolic compounds

For extraction of phenolic compounds five fresh apples were homogenised (combined sample containing pulp and peel). 5 g of homogenised fresh apples and apple purees were extracted with 50 mL ethanol / water solutions (80%) in an ultrasonic bath YJ5120-1 (Oubo Dental, USA) at 35 kHz. Extraction parameters: temperature 20 °C; solid to liquid ratio 1 : 10, time – 20 minutes.

Determination of total phenolic compounds

The total phenolic content (TPC) of the fresh apples and apple purees 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} fresh weight.

Determination of total flavonoid compounds

The total flavonoid content (TFC) was measured by a spectrophotometric method (Kim et al., 2003). The absorbance was measured at 415 nm and total flavonoids were expressed as catechin equivalents (CE) 100 g^{-1} fresh weight.

Determination of antioxidant activity

Antioxidant activity of the extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) radical as outlined by Yu et al. (2003). The absorbance was measured at 517 nm. Activity also was measured by 2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid ($ABTS^{\cdot+}$) radical cation assay (Floegel et al., 2011). The absorbance was measured at 734 nm. Antioxidant activity was expressed as mmol TE 100 g^{-1} fresh weight.

Statistical analysis

Experimental results are means of three replications (if not stated different) and were analysed by Microsoft Excel 2010 and SPSS 17.00. Analysis of variance (ANOVA) and Tukey's test were used to determine differences among samples. Differences were considered as significant at $p<0.05$. A linear correlation analysis was performed in order to determine relationship between TPC, TFC, antioxidant activity such as $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ antioxidant activity.

Results and Discussion

Ascorbic acid content

The ascorbic acid content of five organically grown old apple cultivar fresh apples was between 7.71 to 14.51 mg 100 g^{-1} FW (Table 1). As it is mentioned also in literature apples are not an important source of ascorbic acid. In evaluation of 71 Danish apple cultivars, authors Varming et al. (2013) found that ascorbic acid content ranged from less than 1 to 27 mg 100 mL^{-1}.

After production of puree the ascorbic acid content significantly ($p=0.000$) decreased by 11.5–59.3% in almost all cultivars. The biggest losses of ascorbic acid

content were detected for cultivars with the highest content of this vitamin in fresh stage (Table 1). Therefore, the differences of ascorbic acid content in fresh apples were significant (p=0.000) but after processing puree the ascorbic acid content in puree of all cultivars was similar (p=0.22). As it is mentioned in investigations with strawberries of Hartmann et al. (2008) the content of ascorbic acid, in comparison to that in the frozen strawberries, decreased significantly during the processing of the fruit to puree by 77%. As these authors noticed the pasteurization of purees at 85 °C for 2 min was the processing step causing the highest losses for the most parameters (Hartmann et al., 2008).

Table 1

Ascorbic acid and total carotenes content in fresh apples and purees

Cultivar	Ascorbic acid content, mg 100 g^{-1}	Total carotenes content, mg 100 g^{-1}
Fresh apples		
'Antonovka'	14.51±0.35^{d}	0.077±0.001^{b}
'Filippa'	12.70±0.68^{c}	0.132±0.001^{d}
'Nicnera Zemenu'	8.84±0.58^{b}	0.056±0.006^{a}
'Rudens Svitrotais'	7.71±0.48^{a}	0.104±0.002^{d}
'Sipolins'	8.99±0.38^{b}	0.094±0.002^{c}
Apple puree		
'Antonovka'	6.17±0.47	0.066±0.003^{b}
'Filippa'	5.16±0.40	0.068±0.005^{b}
'Nicnera Zemenu'	7.21±0.40	0.042±0.001^{a}
'Rudens Svitrotais'	6.82±0.77	0.065±0.003^{b}
'Sipolins'	6.00±1.21	0.076±0.002^{c}

Different letters in the same column represents significant differences between values (Tukey's test, p<0.05) for fresh apples and apple purees separately.

Total carotenes

The total carotenes content in all apple cultivars was low, 0.056–0.132 mg 100 g^{-1} FW (Table 1) and after processing similarly to ascorbic acid significantly decreased (p=0.000). The content of total carotenoids in other studies was from 26.16±0.99 to 37.02±0.69 μg g^{-1} DW (or calculating to fresh weight about 0.52–0.74 mg 100 g^{-1}) (Delgado-Pelayo et al., 2014).

The cultivar had significant effect on the amount of total carotenes content both in fresh and processed apples (p=0.000). The highest content of total carotenes was detected in 'Filippa' fresh apples and 'Sipolins' apple puree (Table 1).

Changes of pH

pH is an important factor influencing both product acidity and safety of product. If the product is considered for special diets of people after operations or old people it is not recommended to consume products with low pH value. pH value of fresh apples of different evaluated cultivars was in the range of 2.97–3.33 (Table 2). After processing of puree slight increase of pH in cultivars 'Nicnera Zemenu' and 'Sipolins' was detected. The cultivar 'Nicnera Zemenu' had the highest pH value both in fresh apples and puree. The pH of cultivar 'Antonovka' was even lower than 3.0 and therefore this cultivar is not recommended for puree of special diets.

Titratable acids content

The titratable acids content characterising the total amount of organic acids in the product gives also the sensory acid taste feeling. This parameter has also negative correlation with pH value therefore the best sample for special diet purpose is the cultivar with the lowest titratable acids content which again was cultivar 'Nicnera Zemenu' both for fresh apples and also puree (Table 2). There were significant differences (p=0.000) between cultivars in titratable acids content what proves the importance of cultivar for choosing raw material for processing puree for special diet purposes.

Table 2

Titratable acid, pH value and soluble solid content in fresh apples and purees

Cultivar	Titratable acids, g 100 g^{-1}	Soluble solids content, Brix%	pH value
Fresh apples			
'Antonovka'	1.11±0.003^{c}	10.65±0.06^{a}	2.97
'Filippa'	0.70±0.006^{b}	10.63±0.05^{a}	3.10
'Nicnera Zemenu'	0.61±0.007^{a}	13.50±0.22^{c}	3.33
'Rudens Svitrotais'	0.68±0.001^{ab}	12.25±0.24^{b}	3.26
'Sipolins'	0.71±0.004^{b}	12.78±0.31^{b}	3.22
Apple puree			
'Antonovka'	1.028±0.001^{d}	10.87±0.05	2.97
'Filippa'	0.67±0.000^{c}	10.90±0.08	3.15
'Nicnera Zemenu'	0.55±0.009^{a}	13.48±0.36	3.38
'Rudens Svitrotais'	0.64±0.007^{b}	11.80±0.45	3.26
'Sipolins'	0.74±0.005^{d}	13.40±0.29	3.31

Different letters in the same column represents significant differences between values (Tukey's test, p<0.05) for fresh apples and apple purees separately.

Soluble solids content

The soluble solids content is another factor, which influences the overall sensory profile of each product responsible mostly for sweet taste of product. The minimal allowed soluble solids content in apple juices and purees according to norms is 11.2% (according to the regulation of the Cabinet of Ministers of Latvia no. 1113, October 15, 2013). Cultivars 'Antonovka' and 'Filippa' had lower soluble solids content and therefore they are less suitable for processing puree. The cultivar 'Nicnera Zemenu' both for fresh apples and also puree had the highest soluble solids content. This cultivar significantly (p=0.000) differed from all other cultivars except puree of 'Sipolins' (Table 2).

Phenolic compounds and antioxidant activity

Comparing total phenols content of different cultivar apples, the highest content was detected in the cv. 'Antonovka', followed by 'Filippa' and 'Nicnera Zemenu' (Table 3). Francini and Sebastiani (2013) summarised different studies about phenolic compounds in apples and concluded that it ranges from 68.29 to 73.96 mg GAE 100 g^{-1} FW, on average, depending on the pulp colour. Latvian apples showed higher TPC

except cultivar 'Sipolins', and significantly higher content was found in cv. 'Antonovka' 115 mg GAE 100 g^{-1} FW.

Also, high phenolic content in 'Antonovka' apples grown in Georgia was detected (Gogia et al., 2014). In apple puree content of phenolics was 1.7 up to 2.4 times higher than in apples, possibly due to change in sample concentration during puree preparation. In apple purees the highest content was in samples made from 'Antonovka' and 'Nicnera Zemenu'. There are no data about purees of analysed cultivars, but comparing ciders from dessert apples, the highest TPC was detected for the cultivar 'Antonovka' sample, showing similar tendency as our results (Riekstina-Dolge et al., 2014).

There are no general guidelines for consumption of phenolics, because it depends on the structure of each compound and as the health claim with scientifically proved efficiency, only for olive polyphenols is confirmed. In literature, it is possible to find different studies about general consumption of phenolics in diet. For comparison, data from Poland was selected because there are more similar eating habits to Latvia, and daily dietary intake was 989 mg day^{-1}. Results showed that apples could significantly contribute to the total consumption of phenolics in diet, especially for people with special diet requirements.

Table 3

Total phenols and total flavonoids content in fresh apples and apple purees

Cultivars	TPC, mg GAE 100 g^{-1}	TFC, mg CE 100 g^{-1}	$DPPH^{\cdot}$, mmol TE 100 g^{-1}	$ABTS^{+}$, mmol TE 100 g^{-1}
Fresh apples				
'Antonovka'	115.25±4.61^{d*}	78.41±2.58^{b}	9.22±0.58^{c}	6.21±0.33^{cd}
'Filippa'	89.65±4.14^{c}	82.06±2.75^{c}	3.26±0.18^{ab}	6.72±0.41^{d}
'Nicnera Zemenu'	85.84±1.11^{c}	83.93±3.31^{c}	3.55±0.14^{b}	5.80±0.28^{c}
'Rudens Svitrotais'	68.21±2.05^{a}	36.70±2.71^{a}	3.09±0.18^{a}	3.36±0.11^{a}
'Sipolins'	79.10±2.98^{b}	76.01±2.83^{b}	2.88±0.11^{a}	4.01±0.18^{b}
Apple purees				
'Antonovka'	193.22±4.25^{e}	140.59±3.53^{d}	9.81±0.60^{c}	16.83±0.23^{c}
'Filippa'	152.25±2.07^{b}	102.74±3.21^{c}	8.09±0.28^{a}	13.73±0.15^{b}
'Nicnera Zemenu'	179.55±5.11^{d}	99.74±1.19^{c}	9.58±0.07^{c}	16.85±0.20^{c}
'Rudens Svitrotais'	165.31±5.93^{c}	48.91±3.08^{a}	8.96±0.09^{b}	14.14±0.42^{b}
'Sipolins'	133.85±1.20^{a}	91.69±2.12^{b}	7.96±0.29^{a}	12.82±0.32^{a}

* Different small letters in the same column represents significant differences between values (Tukey's test, $p<0.05$) for fresh apples and apple purees separately.

Flavonoids is an important class of phenolic compounds showing different health benefits. The highest content was detected in 'Filippa' and 'Nicnera Zemenu' apples, whereas in purees the highest content again was detected in 'Antonovka', showing higher stability of compounds presented in these apples. In apples flavonoids proportion decreased, except 'Antonovka'. 'Rudens Svitrotais' had the lowest proportion of flavonoids in their phenolic content that could explain also following lower antioxidant activity. Radical scavenging activity was the highest for cultivar 'Antonovka' apples and in apple puree the highest activity was in two cultivars – 'Antonovka', 'Nicnera Zemenu' purees.

Table 4

Correlation matrix between TPC, TFC and radical scavenging activity

	TPC	TF	DPPH	ABTS
TPC	1.00			
TF	0.61	1.00		
DPPH	0.91	0.48	1.00	
ABTS	0.97	0.60	0.83	1.00

Several authors reported positive correlation between phenolic content and antioxidant activity in apples (Fernández-Jalao et al., 2018; Ferrentino et al., 2018) and in the current study similar results were obtained (Table 4). Total phenolic content had a strong positive correlation with radical scavenging activity by both tests – DPPH and ABTS. Whereas there was a moderate correlation between total flavonoids and antioxidant activity. Generally, phenolic compounds are important factors influencing antioxidant activity of different fruits (Park et al., 2015)

Conclusions

Apples are a good source of some bioactive compounds as phenolic compounds and influence of cultivar on the quality parameters is significant. 'Antonovka' is rich in ascorbic acid, phenolic compounds and shows antioxidant activity, but other parameters as soluble solids are lower. The pH of cultivar 'Antonovka' was even lower than 3.0 and, therefore, this cultivar is not recommended for puree of special diets. The cultivar 'Nicnera Zemenu' has the highest ascorbic acid content in processed puree, the highest soluble solids content and also the lowest pH and titratable acids content. Therefore, data obtained in this study are essential for selecting of raw materials for development of new products for special diets.

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References

1. Archivio M. D., Filesi C., Benedetto R. Di, Gargiulo R., Giovannini C., Masella R. (2007) Polyphenols, dietary sources and bioavailability. *Annali dell'Istituto Superiore di Sanità*, Vol. 43(4), p. 348–361.
2. Bonany J., Brugger C., Buehler A., Carbó J., Codarin S., Donati F., Echeverria G., Egger S., Guerra W., Hilaire C., Höller I., Iglesias I., Jesionkowska K., Konopacka D., Kruczynska D., Martinelli A., Petiot C., Sansavini S., Stehr R., Schoorl F. (2014) Preference mapping of apple varieties in Europe. *Food Quality and Preference*, Vol. 32(PC), p. 317–329.
3. Boyer J., Liu R. (2004) Apple phytochemicals and their health benefits: Review. *Nutrition Journal*, Vol. 3(5), p. 1–15.
4. Cichero J.A.Y., Lam P., Steele C.M., Hanson B., Chen J., Dantas R. O., Duivestein J., Kayashita J., Lecko C., Murray J., Pillay M., Riquelme L., Stanschus S. (2017) Development of international terminology and definitions for texture-modified foods and thickened fluids used in dysphagia management: The IDDSI framework. *Dysphagia*, Vol. 32(2), p. 293–314.
5. Delgado-Pelayo R., Gallardo-Guerrero L., Hornero-Méndez D. (2014) Chlorophyll and carotenoid pigments in the peel and flesh of commercial apple fruit varieties. *Food Research International*, Vol. 65(PB), p. 272–281.
6. Drogoudi P. D., Michailidis Z., Pantelidis G. (2008) Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. *Scientia Horticulturae*, Vol. 115(2), p. 149–153.
7. Ercilla M., Ripa C., Gayan M., Arteche J.M., Odriozola B., Bello M.C., Barral I. (2012) Prevalence of dysphagia in the older using 'Eating Assessment Tool-10.' *European Journal of Hospital Pharmacy: Science and Practice*, Vol. 19(2), p. 205–206.
8. Espinosa L., To N., Symoneaux R., Renard C.M.G.C., Biau N., Cuvelier G. (2011) Effect of processing on rheological, structural and sensory properties of apple puree. *Procedia Food Science*, Vol. 1, p. 513–520.
9. Fernández-Jalao I., Sánchez-Moreno C., De Ancos B. (2018) Effect of high-pressure processing on flavonoids, hydroxycinnamic acids, dihydrochalcones and antioxidant activity of apple 'Golden Delicious' from different geographical origin. *Innovative Food Science and Emerging Technologies*, Vol. 51, p. 20–31.
10. Ferrentino G., Morozova K., Mosibo O. K., Ramezani M., Scampicchio M. (2018) Biorecovery of antioxidants from apple pomace by supercritical fluid extraction. *Journal of Cleaner Production*, Vol. 186, p. 253–261.
11. Floegel A., Kim D.O., Chung S. J., Koo S.I., Chun O.K. (2011) Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, Vol. 24(7), p. 1043–1048.
12. Francini A., Sebastiani L. (2013) Phenolic Compounds in Apple (*Malus* x *domestica* Borkh.): Compounds characterization and stability during postharvest and after processing. *Antioxidants*, Vol. 2(3), p. 181–193.
13. Gogia N., Gongadze M., Bukia Z., Esaiashvili M.V., Chkhikvishvili I. (2014) Total polyphenols and antioxidant activity in different species of apples grown in Georgia. *Georgian Medical News*, Vol. 232–233, p. 107–112.
14. Hartmann A., Patz C.D., Andlauer W., Dietrich H., Ludwig M. (2008) Influence of processing on quality parameters of strawberries. Journal of Agricultural and *Food Chemistry*, Vol. 56(20), p. 9484–9489.
15. Ilhamto N., Anciado K., Keller H.H., Duizer L.M. (2014) In-house pureed food production in long-term care: perspectives of dietary staff and implications for improvement. *Journal of Nutrition in Gerontology and Geriatrics*, Vol. 33(3), p. 210–228.
16. Jakobek L., Barron A.R. (2016) Ancient apple varieties from Croatia as a source of bioactive polyphenolic compounds. *Journal of Food Composition and Analysis*, Vol. 45, p. 9–15.
17. Kalinowska M., Bielawska A., Lewandowska-Siwkiewicz H., Priebe W., Lewandowski W. (2014) Apples: Content of phenolic compounds vs. variety, part of apple and cultivation model, extraction of phenolic compounds, biological properties. *Plant Physiology and Biochemistry*, Vol. 84, p. 169–188.
18. Kampuse S., Ozola L., Straumite E., Galoburda R. (2015) Quality parameters of wheat bread enriched with pumpkin (*Cucurbita Moschata*) by-products. *Acta Universitatis Cibiniensis. Series E: Food Technology*, Vol. 19(2), p. 3–14.
19. Karaman Ş., Tütem E., Başkan K.S., Apak R. (2013) Comparison of antioxidant capacity and phenolic composition of peel and flesh of some apple varieties. *Journal of the Science of Food and Agriculture*, Vol. 93(4), p. 867–875.
20. Keenan D.F., Valverde J., Gormley R., Butler F., Brunton N.P. (2012) Selecting apple cultivars for use in ready-to-eat desserts based on multivariate analyses of physico-chemical properties. *LWT-Food Science and Technology*, Vol. 48(2), p. 308–315.
21. Keller H., Chambers L., Niezgoda H., Duizer L. (2012) Issues associated with the use of modified texture foods. *The Journal of Nutrition, Health & Aging*, Vol. 16(3), p. 195–200.
22. Keller H.H., Duizer L.M. (2014) What do consumers think of pureed food? Making the most of the indistinguishable food. *Journal of Nutrition in Gerontology and Geriatrics*, Vol. 33(3), p. 139–159.
23. Kim D.O., Jeong S.W., Lee C.Y. (2003) Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*, Vol. 81(3), p. 321–326.
24. Laaksonen O., Kuldjärv R., Paalme T., Virkki M., Yang B. (2017) Impact of apple cultivar, ripening stage, fermentation type and yeast strain on phenolic composition of apple ciders. *Food Chemistry*, Vol. 233, p. 29–37.
25. MK noteikumi nr. 1113 Prasības attiecībā uz augļu sulām un tām līdzīgiem produktiem [The regulation of the Cabinet of Ministers of Latvia no. 1113, October 15, 2013 regarding fruit juices and similar products] (in Latvian).
26. Park Y.S., Im M.H., Ham K.S., Kang S.G., Park Y.K., Namiesnik J., Leontowicz H., Leontowicz M., Trakhtenberg S., Gorinstein, S. (2015) Quantitative assessment of the main antioxidant compounds, antioxidant activities and FTIR spectra from commonly consumed fruits, compared to standard kiwi fruit. *LWT-Food Science and Technology*, Vol. 63(1), p. 346–352.
27. Pfisterer K.J., Amelard R., Chung A.G., Wong A. (2018) A new take on measuring relative nutritional density: The feasibility of using a deep neural network to assess commercially-prepared puréed food concentrations. *Journal of Food Engineering*, Vol. 223, p. 220–235.
28. Riekstina-Dolge R., Kruma Z., Dimins F., Straumite E., Karklina D. (2014) Phenolic composition and sensory properties of ciders produced from Latvian apples.

Proceedings of Latvia University of Agriculture, Vol. 31(326), p. 41–45.
29. Segliņa D. (2007). Smiltsērkšķu augļi un to pārstrādes produkti [Sea buckthorn fruits and their processing products]. Summary of promotion work for acquiring the Doctor's degree in Engineering Sciences in the Food Science. (in Latvian)
30. Singleton V.L., Orthofer R., Lamuela-Raventos R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, Vol. 119(25), p. 152–178.
31. Song Y., Yao Y. X., Zhai H., Du Y. P., Chen F., Wei S. W. (2007) Polyphenolic compound and the degree of browning in processing apple varieties. *Agricultural Sciences in China*, Vol. 6(5), p. 607–612.
32. Varming C., Petersen M.A., Toldam-Andersen T.B. (2013) Ascorbic acid contents in Danish apple cultivars and commercial apple juices. *LWT-Food Science and Technology*, Vol. 54(2), p. 597–599.
33. Weichselbaum E., Wyness L., Stanner S. (2010) Apple polyphenols and cardiovascular disease - A review of the evidence. *Nutrition Bulletin*, Vol. 35(2), p. 92–101.
34. 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*, Vol. 51(6), p. 1566–1570.

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MINERAL AND BIOACTIVE COMPOUND CONTENT IN PLANT-BASED PROTEIN - ENRICHED PUREES

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Abstract

Adequate, well balanced, diverse diet together with regular physical activity is considered to be the key elements for good health. The intake of macro and microelements has an important role in normal function of our bodies, ensuring enzymatic reactions, nerve impulses and other processes. However overly increased amounts of certain vitamins and minerals in the diet can be toxic. Therefore, the amount of certain nutrients needs to be consumed according to the age, gender and health condition. The aim of the research was to create new plant-based protein-enriched purees with increased protein amount, detect bioactive compound and mineral content and compare the mineral content with the recommended daily mineral intake for Latvians. For this research three recipes of new plant-based purees were created and experimentally made using organically grown ingredients from Latvia. Additional ingredients as whey-protein isolate, cod liver oil and sugar were added. Products were processed using cook-vide. All samples were tested on their mineral compound content (Na, K, Ca, P, Mg, Fe, Zn, Cu, Se), total carotene (TC) and total phenol content (TPC), antiradical activity (DPPH). The obtained data in all samples showed only trace amounts of Se per 100 g of product, on average the content of Na was only 1.2% from recommended daily intake (RDI) for Latvians per 100 g of product. The highest RDI was obtained for Fe in sample Sp1 for men 11.8%, however for women only 7.1% of RDI. Overall the highest content of bioactive compounds was found in sample Sp2.

Keywords: total carotene, total phenols, DPPH, cook-vide

Introduction

The key role of sufficient nutrition is to provide living organisms with quantitatively and qualitatively appropriate nutrients to improve health, well-being and prevent from diseases (Cilla et al., 2018). Micronutrients are components that are needed for us in comparatively small amounts and include vitamins and minerals. Minerals are divided into major (macro) mineral compounds – Ca, Mg, K, Ma, Cl, P, S and trace (micro) minerals – I, Zn, Se, Fe, Mn, Cu, Co, Mo, F, Cr, B. These elements can be consumed by balanced nutrition of plant and animal sources (Mohammad et al., 2017).

Increased consumption of fruits and vegetables is recommended in dietary guidelines worldwide and fruits and berries are considered to be rich in nutrients and phytochemicals (Nile, Park, 2014).

Fruit and vegetable biochemical content differs depending on their variety and several environmental factors, however some similarities can be connected among the most widely found specimens of each particular crop.

Agricultural crops as beetroot, carrots and pumpkins are widely grown and used in Latvia. Beetroot (*Beta vulgaris* L.) can be a source of several minerals, for example 100 g of edible root part can contain 77 mg of Na, 16 mg – Ca, 0.79 mg – Fe, 38 mg – P, 305 mg – K, 23 mg of Mg and 0.35 mg of Zn. Beetroot is also a source of highly active pigments – betalains and carotenoids, polyphenols and flavonoids, saponins, but some bioactive compounds found at low levels – glycine, betaine and folate (Chhikara et al., 2019).

Consumption of carrots (*Daucus carota L.*) has increased in recent years due to their recognition as an important source of natural antioxidants, such as β-carotene, an average amount of 5.33 mg per 100 g of fresh carrot. It is also a good source of mineral compounds, where 100 g on average can contain Ca (34 mg), Fe (0.4 mg), P (25 mg), Na (40 mg), K (240 mg), Mg (9 mg), Cu (0.02 mg), and Zn (0.2 mg) (Sharma et al., 2012).

Pumpkins (*Cucurbitaceae*) have been traditionally eaten both by people and animals and it has several different varieties all with different chemical composition. But overall it is known to be a source of carotenoids, also vitamins like K, several complex B vitamins as well as for minerals – K, P, Mg, Fe and Se (Ozola, Kampuse, 2018).

Jerusalem artichoke (*Helianthus tuberosus*) is an economically important plant with wide use of application both in food production and biofuel production in the world (Yang et al., 2015), however not as widely used in Latvia although grown here as long as potatoes. Overall as a crop Jerusalem artichoke has high yield and wide adaptation to climatic and soil conditions (Yang et al., 2015). The tubers on average contain 80% of water, 15% of carbohydrates and 1 to 2% of protein. Carbohydrates in cells are mostly stored in the form of inulin. Some research shows that boiled Jerusalem artichoke tubers contain 30 mg of Ca, 0.4 mg of Fe, 420 mg of K, 3 mg of Na and also 20.0 μg of carotenoids (Kays, Nottingham, 2007).

Apples are considered to be of moderate energy and nutritional value among common fruits, however they are consumed in rather large quantities therefore can have a significant contribution (Lee, 2012).

Berries are typically considered to be a good source of several vitamins and bioactive compounds as polyphenols, antioxidants, also minerals and fibres in various concentrations (Nile, Park, 2014).

Strawberries (*Fragaria x ananassa*) are one of the most popular and widely used berry for juicing due to its taste, rich essential nutrients, bioactive compounds, fibre, minerals and phenolic compounds. On average 100 g of strawberries contain 7.68 g of carbohydrate, 2.0 g of fibre and up to 4.89 g of sugar and 58.8 mg of vitamin C.

100 g of fruit can contain K (153 mg), P (24 mg), Ca (16 mg), Mg (13 mg) (Wang et al., 2018). Red raspberries (*Rubus ideaus L.*) are usually grown as perennial crop for their flavour and red colour. Much like previously described berries raspberry contains high levels of phenolic acids, flavonoids and also anthocyanins (Jin et al., 2012). Sea buckthorn (*Hippophae rhamnoides L.*) is also a very common in Europe, Asia and North America. Berries have high contents of vitamin C, E and K, carotenoids, flavanols and sugars, and are used in food industry, medicine, cosmetics (Lukša et al., 2018). But lingonberry is a berry from Nordic countries rich in polyphenols and vitamins C and E (Kivimäki et al., 2013). Some additional mineral composition of lingonberries, apples, pumpkin and red raspberries can be seen in Table 1.

Table 1

Mineral content in lingonberry, raspberry, pumpkin and apple (DTU Fodevareinstituttet, 2019)

Minerals per 100 g of product	Lingon-berry	Rasp-berry	Pump-kin	Apple
Na (mg)	2.0	2.0	2.0	3.0
K (mg)	89.0	228.0	243.0	120.0
Ca (mg)	20.0	19.7	20.1	3.9
P (mg)	16.0	38.0	33.0	17.4
Mg (mg)	9.0	17.0	11.2	4.4
Fe (mg)	0.40	0.55	0.27	0.12
Zn (mg)	0.18	0.34	0.20	0.03
Cu (µg)	0.07	0.11	0.08	0.03
Se (µg)	0.00	0.19	-	0.30

Both bioactive compounds and minerals play an important role to maintain the basic functions of human body. Various bioactive compounds protect the body against diseases and disorders and the damaging effects of free radicals (Nile, Park, 2014), however minerals help to build strong bones and are a part for transmitting nerve impulses among many other functions (Mohammad et al., 2017).

A sufficient nutrition also means to maintain stable energy levels, this is especially important for people with regular increased physical activity such as athletes. However, no matter of what kind of physical activity each of us is doing, the most important thing is to eat different foods that can provide sufficient and regular energy intake of carbohydrates, proteins, fats and microelements (Potgieter, 2013). Products with increased protein content have become very popular with the athlete community. Protein consumption is necessary for muscle protein synthesis thus ensuring positive net muscle protein balance and for athletes engaged in resistance exercise can benefit from this with time by allowing muscle protein accretion and subsequent hypertrophy – growth of tissues or organs (Phillips et al., 2011). For this reason, in the last few years a growing interest in new product market has been noticed for well-balanced products and products, which are high in protein content. And sports nutritionists are more likely to develop individual nutrition plans depending on the needs of a single athlete and following a plan that athletes should consume diets that provide at least the recommended dietary allowance / adequate intake for all micronutrients (Nutrition and Athletic Performance, 2016).

Traditional cooking can lead to a loss of nutritional compounds and components responsible for flavour due to the required temperature and cooking time. Alternative cooking technologies such as vacuum treatments could help to decrease these losses (Iborra-Bernad et al., 2014; Ozola, Kampuse, 2017). The main advantage of vacuum cooking is the absence of oxygen that allows product cooking under 100 °C that is less harmful for thermolabile compounds (Iborra-Bernad et al., 2014; Ozola, Kampuse, 2017). The most common vacuum treatment is *sous-vide* that allows product cooking inside heat-stable vacuumized pouches. However, in this research a different method, called *cook-vide* or vacuum boiling was used. *Cook-vide* is used to prepare products in hermetically closed cooking kettle by lowering pressure with continuous function of vacuum pump, that decreases the amount of oxygen in kettle and lowers pressure thus allowing to reach product / water boiling point below 100 °C (Iborra-Bernad et al., 2014; Ozola, Kampuse, 2017).

For the purpose of this research the evaluated mineral compound content should ensure a minimum of 10% per compound of recommended daily mineral intake for Latvians per portion (200 g) of created product.

The aim of the research was to create new plant-based protein-enriched purees with increased protein content, detect bioactive compound and mineral content and compare the mineral content with the recommended daily mineral compound intake for Latvians.

Materials and Methods

Three plant-based purees enriched with protein were developed. Most of the plant ingredients were grown organically in Latvia and industrially processed into semi-finished purees, juices or pulp juices. The plant material input in percent for each prepared sample is shown in Table 2. Additional ingredients were used in puree preparation: the main protein source – whey protein isolate in all samples (6%), sugar (1%) in samples Sp1 and Sp2, and cod liver oil (0.5%) in all samples.

Table 2

Plant material input in recipes (%) of protein-enriched plant-based purees

Ingredients	Sample		
	Sp1	Sp2	Sp3
Sea buckthorn pulp juice	5.0	-	-
Apple puree	25.5	12.0	24.5
Carrot puree	15.0	19.0	-
Strawberry pulp juice	13.0	-	13.0
Jerusalem artichoke puree	34.0	-	-
Lingonberry pulp juice	-	16.5	-
Apple juice	-	20.0	-
Red beetroot puree	-	25.0	-
Red beetroot pulp juice	-	-	12.0
Pumpkin puree	-	-	28.0
Red raspberry pulp juice	-	-	16.0

Prepared recipes were vacuum cooked using cook-vide method at 0.06 MPa pressure, with boiling temperature at 79±2 °C for 15 min followed by hot filling in glass jars and pasteurization in hot water bath at 95±2 °C for 20 min. Jars were cooled to room temperature in cold water (7±3 °C) for 1 hour and stored refrigerated till testing.

Samples were tested on their total protein amount and afterwards analysed on their content of total carotenes, total phenols, antiradical activity and mineral content.

Total protein content in samples were detected by Kjeldahl method according to ISO 20483:2013.

The content of *total carotenes (TC)* was detected by spectrophotometric method described by (Полюдек-Фабини, Бейрих, 1981) and analysed using UV/VIS spectrophotometer Jenway 6705 (Bibby Scientific Ltd., UK) at 440 nm and expressed as mg 100 g^{-1} per product (Ozola et al., 2017). *Total phenol content (TPC)* was detected according to the Folin-Ciocalteu method described by (Yu et al., 2003) with modifications, where to 0.5 mL of sample extract 2.5 mL of 0.2 N Folin-Ciocalteu reagent was added, after 5 minutes 2.0 mL of 7.5% $NaCO_3$ was added and the resulting solution was mixed and left to stand for 30 minutes at 18±1 °C in a dark place (Ozola et al., 2017) and shortly before reading absorption at 760 nm was centrifuged (ELMI Centrifuge CM-6MT, LTF Labortechnik GmbH&Co, Germany) for 2 minutes at 3500 rpm. TPC was analysed using spectrophotometer JENWAY 6300 (Banworld Scientific Ltf., UK) and the obtained data were expressed as mg GAE 100 g^{-1} per product.

The *antiradical activity (DPPH)* was detected using 2,2-diphenil-1-picrylhydrazyl (DPPH) method described by Yu et al. (2003) with modifications where to 0.5 mL of extracted sample 3.5 mL of freshly made DPPH solution was added. The mixture was mixed and kept in the dark place at 18±2 °C for 30 minutes and centrifuged respectively to TPC detection and the absorbance was measured at 517 nm and the obtained data were expressed as mM TE 100 mL^{-1} of product (Ozola et al., 2017).

Samples were tested on their content of *minerals* such as Na, K, Ca, P, Mg, Fe, Zn, Cu, Se. The amount of Ca, Mg, Fe, Zn, Cu in tested samples was detected by Institue of Biology of University of Latvia using atomic absorption spectroscopy method, but the amount of K and Na detected by flame photometer Jenway PFP7 (Cole-Parmer, UK) and content of P was detected calorimetrically with ammonium molybdate. The amount of Se in samples was detected by Institute of Food Safety, Animal Health and Environment (BIOR) and their standardised BIOR-T-012-148-2013 method. The amount of minerals was expressed in mg kg^{-1} per dry sample, but recalculated to mg 100 g^{-1} per product.

Total protein content, TC, TPC and antiradical scavenging activity was measured in three repetitions, but mineral content was determined in single repetition.

Statistical analysis was done using 'Microsoft Office Excel' 2007 version. Differences between the obtained results on bioactive compound content were analysed using ANOVA: Single factor analysis with Tukey-Kramer post hoc test. The data is presented as their mean and differences among results were considered to be significant if p value $<\alpha_{0.05}$.

Results and Discussion

The average total protein content in all samples ranged from 6.2 to 6.5 g per 100 g of sample. Sample Sp1 contained 6.5±0.02 g 100 g^{-1} of product, Sp2 6.2±0.05 g 100 g^{-1} and sample Sp3 6.5±0.04 g 100 g^{-1} of product. These amounts are comparable to the expectations during sample preparation, due to the fact that majority of the used ingredient total protein content did not exceed 1 g per 100 g of sample (DTU Fodevareinstituttet, 2019). Also, the obtained protein content was appropriate for this type of product, as the average protein intake for adults should not exceed 10-20% of daily caloric value according to recommended energy and nutrient intakes for Latvian residents. These directions foresee ca. 75 g protein intake for adults on a 2000 kcal diet (*Ieteicamās enerģijas un uzturvielu devas Latvijas iedzīvotājiem*, 2017), in addition to the prepared purees are used as supplements to nutrition.

The obtained data on the prepared sample bioactive compound content showed some differences. Evaluation of the data on total carotene content (Fig. 1.) showed that sample Sp3 had by around 40% lower content of total carotenes than sample Sp2, which is a significant difference according to Tukey-Kramer post hoc test.

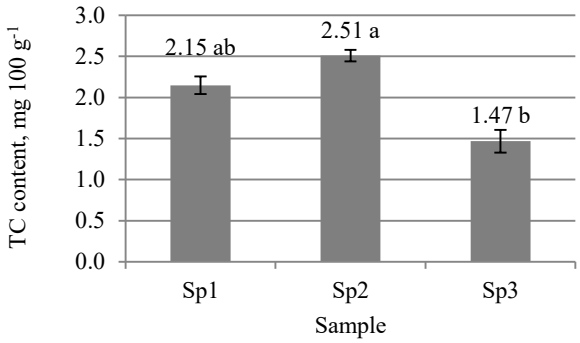

Figure 1. Total carotene content in prepared plant-based purees

Different letters indicate a significant difference in the mean at p<0.05 according to Tukey-Kramer test.

Differences between samples Sp1 and Sp2 were not as significant to be considered relevant in this case. The difference was only 14%. These results are directly influenced by the sample ingredient content, where samples Sp1 and Sp2 contained carrot as the major carotene source and also sea buckthorn in Sp1. The highest content of total phenols was also detected in sample Sp2 (Fig.2), however, contrary to TC content, sample Sp1 had 20% lower TPC, therefore significant differences between these samples were noticed.

Although TC and TPC showed some differences between separate samples, no such data was noticed after antiradical activity evaluation.

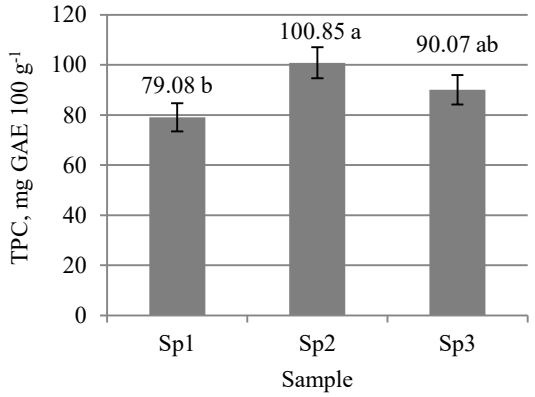

Figure 2. Total phenol content in prepared plant-based purees

Different letters indicate a significant difference in the mean at $p<0.05$ according to Tukey-Kramer test.

Similarly, to previous observations, sample Sp2 also showed slightly higher antiradical activity (Fig.3) compared to other samples. In comparison a research on high-pressure processed smoothies containing orange juice, papaya juice, melon juice, carrot puree and skimmed milk (Andrés et al., 2016), showed a total carotenoid content of 20.43±0.47 mg 100 mL^{-1} in thermally processed (80°C, 3 min) samples.

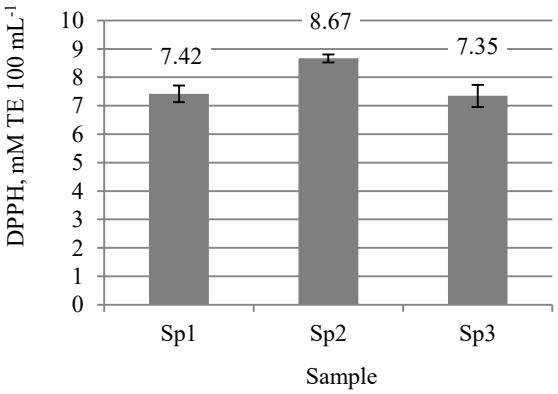

Figure 3. Antiradical activity in prepared plant-based purees

Total phenol content of 45.40±0.70 mg GAE 100 mL^{-1} and DPPH 53.9±0.7 mM TE 100 mL^{-1} with a gradual bioactive compound deterioration during 45 day refrigerated storage period was also established (Andrés et al., 2016). Overall, plant-based puree samples showed higher total carotene and antiradical activity levels, but did not exceed total phenol content. These results could suggest that plant-based purees made for this research contain different phenol compounds in rather high concentrations due to variety of berry juices used in the sample preparation. However, they do not have strong antiradical activity, which could shorten the shelf life of these products.

The data from mineral compound content (Table 3) analysis showed only trace amounts of Se (<0.20 mg kg^{-1}) present, levels were not high enough for precise amount declaration in prepared samples. This information does coincide with typical sources of Se nutritionally provided by intake of organ meats, cereals and other grains, dairy products, rice, sea food, eggs and other similar products (Selenium, 2018). Other tested mineral compounds were detected in higher and more substantial amounts.

Table 3

Mineral content in experimental samples and average RDI of minerals in Latvia (mg)

Minerals	Sample (mg 100 g^{-1})			RDI*
	Sp1	Sp2	Sp3	
K	230.5	180.9	213.1	3500 (m) 3100 (w)
Ca	40.3	36.6	36.6	800
P	34.5	25.6	39.5	600
Mg	18.5	17.1	23.2	350 (m) 280 (w)
Fe	1.1	0.4	0.4	9 (m) 15 (w)
Zn	0.2	0.2	0.2	9 (m) 7 (w)
Cu	0.1	0.1	0.1	0.9
Na	25.4	28.2	21.2	2000

RDI – average values of recommended daily mineral compound intakes for adults, m – adult men, w – adult women

According to the recommended energy and nutrient intakes for Latvian residents (*Ieteicamās enerģijas un uzturvielu devas Latvijas iedzīvotājiem*, 2017) the prepared plant-based protein-enriched purees with increased protein content showed that 100 g of sample Sp1 can provide a potassium daily intake of 7.4% for women and 6.6% for men and 5% of RDI of Ca. Sample Sp1 also showed a comparatively higher amount of iron, which is comparable to 7.1% of RDI for women and 11.8% for men, these amounts are approximately 60% higher than in sample Sp2 and Sp3. Sp1 contains 9.7% of RDI of Cu for both genders. Between the three experimental samples mineral compounds such as P, Mg and Zn were found to be in higher amounts in sample Sp3 than in other samples. The detected amount of Mg in Sp3 could possibly provide 8.3% of RDI for women and 6.6% of nutritional needs for men. This sample also provides the lowest amount of Na, approximately 1.1% of RDI, which is about 24% less than in sample Sp2.

Although the evaluation of some bioactive compounds overall showed higher amounts of total carotenes, phenols and antiradical activity in sample Sp2, the content of mineral compounds was relatively low

Conclusions

The chosen ingredients for sample preparation were able to ensure an appropriate amount of some mineral compounds per 200 g serving. However, it would be advisable to search for plant-based products with higher concentrations of selenium or adding a selenium complex to ensure partial coverage of recommended daily intake.

Also, low levels of zinc were detected in all samples, on average ensuring 4% of RDI per serving.

Sample Sp2 containing apple puree, carrot puree, lingonberry pulp juice, apple juice, red beetroot puree, overall had the lowest mineral compound coverage of RDI. The 10% margin per sample serving of mineral compounds was met by content of potassium (K) and copper (Cu) for both men and women, and of magnesium (Mg) for men.

The evaluation of bioactive compound content did not show substantial differences between the samples, slightly larger differences in content of total carotene were detected between samples Sp2 (apple puree, carrot puree, lingonberry pulp juice, apple juice, red beetroot puree) and Sp3 (apple puree, strawberry pulp juice, red beetroot juice, pumpkin puree, red raspberry pulp juice).

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References

1. Andrés V., Villanueva M. J., Tenorio M. D. (2016) The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chemistry*, Vol. 192, p. 328–335.
2. Chhikara N., Kushwaha K., Sharma P., Gat Y., Panghal A. (2019) Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry*, Vol. 272, p. 192–200.
3. Cilla A., Bosch L., Barberá R., Alegría A. (2018) Effect of processing on the bioaccessibility of bioactive compounds – A review focusing on carotenoids, minerals, ascorbic acid, tocopherols and polyphenols. *Journal of Food Composition and Analysis*, Vol. 68, p. 3–15.
4. DTU Fodevareinstituttet. (2019) [accessed on 11.02.2019]. Available at: https://frida. fooddata.dk/?lang=en
5. Iborra-Bernad C., Tárrega A., García-Segovia P., Martínez-Monzó J. (2014) Comparison of vacuum treatments and traditional cooking using instrumental and sensory analysis. *Food Analytical Methods*, Vol.7(2), p. 400–408.
6. Ieteicamās enerģijas un uzturvielu devas Latvijas iedzīvotājiem (2017) [accessed on 11.022019]. Available at: http://www.vm.gov.lv/images/userfiles/Tava veseliba/Ieteicamās_enerģijas_un_uzturvielu_devas.pdf
7. ISO 20483:2013 - Cereals and pulses - Determination of the nitrogen content and calculation of the crude protein content - Kjeldahl method. [accessed on 31.01.2019] Available at: https://www.iso.org/standard/59162.html
8. Jin P., Wang Y., Gao H., Chen H., Zheng Y., Wang C. Y. (2012) Effect of cultural system and essential oil treatment on antioxidant capacity in raspberries. *Food Chemistry*, Vol. 132, p. 399–405.
9. Kays S. J., Nottingham S. F. (2007) **In:** *Biology and Chemistry of Jerusalem Artichoke Helianthus tuberosus L.* Chemical Composition and Inulin Chemistry. CRC Press, Taylor & Francis Group, p. 53–96.
10. Kivimäki A. S., Siltari A., Ehlers P. I., Korpela R., Vapaatalo H. (2013) Lingonberry juice lowers blood pressure of spontaneously hypertensive rats (SHR). *Journal of Functional Foods*, Vol. *5*(3), p. 1432–1440.
11. Lee C. Y. (2012) Common nutrients and nutraceutical quality of apples. *New York Fruit Quarterly*, Vol. 20(3), p. 8–8.
12. Lukša J., Vepštaitė-Monstavičė I., Yurchenko V., Serva S., Servienė, E. (2018) High content analysis of sea buckthorn, black chokeberry, red and white currants microbiota – A pilot study. *Food Research International*, Vol. 111, p. 597–606.
13. Mohammad S., Gharibzahedi T., Jafari S. M. (2017) The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends in Food Science & Technology*, Vol. 62, p. 119–132.
14. Nile S. H., Park S. W. (2014) Edible berries: Bioactive components and their effect on human health. *Nutrition*, Vol. 30(2), p. 134–144.
15. Nutrition and Athletic Performance (2016) *Medicine & Science in Sports & Exercise*, Vol. 48(3), p. 543–568.
16. Ozola L., Kampuse S. (2017) The effect of vacuum cooking on enteral food made from fresh and semi-finished ingredients. **In:** *Research for Rural Development,* Vol. 1, p. 208–214.
17. Ozola L., Kampuse S. (2018) Influence of heat treatment methods on bioactive compound concentrations in pumpkin-guelder rose (*Viburnum opulus*) sauces. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences*, Vol. 72(2), p. 97–102.
18. Ozola L., Kampuse S., Galoburda R. (2017) The effect of high-pressure processing on enteral food made from fresh or semi-finished ingredients. **In:** *11th Baltic Conference on Food Science and Technology,* p. 80–85.
19. Phillips S. M., Luc, Van Loon J. C. (2011) Dietary protein for athletes: From requirements to optimum adaptation. *Journal of Sports Sciences*, Vol. *29*(1), p. 29–38.
20. Potgieter S. (2013) Sport nutrition: A review of the latest guidelines for exercise and sport nutrition from the American College of Sport Nutrition, the International Olympic Committee and the International Society for Sports Nutrition. *South Africa Journal of Clinical Nutrition*, Vol. *26*(1), p. 6–16.
21. Selenium. (2018) [accessed 06.02.2019] Available: from https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/
22. Sharma K. D., Karki S., Thakur N. S., Attri S. (2012) Chemical composition, functional properties and processing of carrot-a review. *Journal of Food Science and Technology*, Vol. 49(1), p.22–32.
23. Wang J., Wang J., Ye J., Kranthi Vanga S., Raghavan V. (2018) Influence of high-intensity ultrasound on bioactive compounds of strawberry juice: Profiles of ascorbic acid, phenolics, antioxidant activity and microstructure. *Food Control*, Vol. *96*, p.128–136.
24. Yang L., He Q. S., Corscadden K., Udenigwe C. C. (2015) The prospects of Jerusalem artichoke in functional food ingredients and bioenergy production. *Biotechnology Reports*, Vol. *5*(1), p. 77–88.
25. 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*, Vol. *51*(6), p. 1566–1570.
26. Полюдек-Фабини Р., Бейрих Т. (1981) Органический анализ. В: *Химия* (p. 499–500). Ленинград.

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EVALUATION OF QUALITY INDICATORS FOR DRIED MELONS FROM UZBEKISTAN

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Abstract

Melon is one of the most valuable and useful products, and it forces us to look for ways of processing these seasonal fruits for long-term storage. The easiest, cheapest and least time-consuming method of preservation is drying. The aim of the work was to study the effective methods of drying local varieties of melons and determine their quality indicators. As well as to study the quality indicators of dried melon obtained by the method of solar and artificial convective drying. The acceptance of dried melon samples, the content of total phenols, ascorbic acid (vitamin C), antioxidant activity, and the indicators of food safety and the microbiological background of dry products for several local melon varieties were studied. The highest total phenols content (TPC) in dried melon was detected in melon variety 'Oq uruq', but the highest antioxidant activity – in melon variety 'Obinavvot Samarqand' (DPPH˙) and 'Qundalang tur' ($ABTS^{\cdot+}$). The melon variety as well as the drying method had a significant effect on TPC and antioxidant activity. The artificial convective drying productivity was higher: microbiological, sensory and chemical analysis of food safety showed that the products obtained by the artificial convective drying are better in all indicators than in the solar drying.

Keywords: melon, drying method, quality

Introduction

Uzbekistan is the world's leading producer of fruits and vegetables with annual production of more than 11 million tons. Melon, watermelon and pumpkin crops have extensive processing capabilities (Strategy of actions …, 2018). More than 160 varieties of melons are currently grown in Uzbekistan, distinguished by precocity, yield, resistance to diseases and pests, and external environmental stress factors such as taste, preservation of quality, transportability, suitability for processing and drying. To date, 54 varieties of melons are included in the State Register of the Republic of Uzbekistan, of which 22 are early ripening, 19 are mid-season, 13 are late-ripening varieties (The state register…, 2018), the principal of which have been cultivated for several decades. Therefore, Uzbekistan is the leading melon-growing zone in Central Asia and 6 oases have been formed there – Khorezm, Bukhara, Samarkand, Tashkent, Fergana and Southern – for the cultivation of melons (Mavlyanova, 2005). Melon fruits have an original taste and beneficial properties. They contain 85–92% water, 8–20% dry matter, including, 0.8% protein, 1.8% fibre and 6.2% other carbohydrates, 0.9% fat, 0.6% ash, 20–30 mg 100 g^{-1} of vitamin C, 0.03–0.07 mg 100 g^{-1} of other vitamins, zinc, iron, calcium, magnesium, potassium, phosphorus and other trace elements, organic and mineral salts, and 31 kcal energy (Solval et al., 2012; Amiri et al., 2014; Ostonakulov et al., 2016). Sugar content of individual melon varieties of Central Asia reaches 14–16% (Ostonakulov et al., 2016). It is well known that melon peels and its seed oil are a good source of phenolic compounds. In addition, melon variety has various biological activities, such as antioxidant, anti-inflammatory, anti-diabetic, antibacterial, as well as others that fully justify the presence of biologically active compounds (Silva et al., 2018).

Part of the biologically active substances is not stable due to technological processes applied, resulting in a reduction in the biological value of products (Tomsone et al., 2014). The method of drying also affects the composition and activity of biologically active substances in plants (Tomsone et al., 2013). The antioxidant activity and the content of phenolic compounds in mulberry leaves, after air-drying at 60 °C or below, did not differ significantly from that of mulberry leaves after freeze-drying. At the same time these parameters in mulberry leaves decreased significantly after being air-dried at 70 °C (Katsube et al., 2009). Dried melon is a healthy snack that contains great amount of minerals, antioxidants and vitamins (Berdıyev et al., 2009). Several scientists have been studying melon drying processes (Rodrigues, Fernandes, 2007; Chayjan et al., 2012; Solval et al., 2012; Darvishi et al., 2015). Therefore, experienced solutions have shown that the best way to preserve the taste and useful properties of a melon is to dry it – the easiest, cheapest and least laborious method of preservation. In addition, dried melon is successfully transportable and has a longer shelf-life. One of the fundamental principles that shape the quality of food products is to ensure their safety and preserve quality, as unhealthy chemical and biological compounds accumulated in products during storage, processing and sale can enter human body with food. Packaging materials have an importance in solving the problems of preserving the nutritional value and the biological safety of food throughout the entire way from production to sale to the consumer.

The aim of the research was to determine the effect of drying on the chemical, microbiological quality and safety of local melons varieties. As well as to study the quality indicators of dried melon obtained by the method of solar and artificial convective drying.

Materials and Methods

The research was carried out in conjunction of the Samarkand Institute of Veterinary Medicine and Faculty of Food Technology, Latvia University of Life Sciences and Technologies.

Characterisation of raw materials and drying process

The following summer varieties (harvested in 2017–2018) with soft and firm mesocarps (edible part), early and medium ripe melons were tested:

- A – 'Qundalang tur' (*Cucumis melo* L ssp. Rigidus (Pang.) Fil. Var. Bucharici);
- B – 'Oq uruq' (*Cucumis melo* L ssp. Rigidus (Pang.) Fil. Var. Aestivales);
- C – 'Obinavvot Samarqand' (*Cucumis melo* L ssp. Rigidus (Pang.) Fil. Var. Bucharici);
- D – 'Kukcha-588' (*Cucumis melo* L ssp. Rigidus (Pang.) Fil. Var. Aestivales);
- E – 'Ichi qizil' (*Cucumis melo* L ssp. Rigidus (Pang.) Fil. Var. Aestivales).

Sugar content was determined in fresh melons with a PAL-1 ATAGO refractometer (ATAGO Co Ltd., Japan).

After harvest, melon fruit was selected for cleaning from peel and seeds without any signs of spoilage, then the fruit was cut into 3–4 cm thick slices and dried. Two drying methods were used: solar (helio) drying in special open ventilated areas from 8 to 12 days and artificial convective drying (first at t=38–40 °C, for 4–6 hours, and then at t=75–80 °C, for 6–8 hours) All samples were dried to achieve dry matter in the product not less than 18.2–20.0%.

The acceptance of dried melon samples

The hedonic evaluation method was used based on ISO 4121:2003 standard. The 9-point hedonic scale (9 – extremely like, 1 – extremely dislike) was used to determine the acceptance rate of dried melon samples. The acceptance of dried melon samples was evaluated by 8 trained panellists.

Microbiological and radionuclide analysis

Microbiological studies were carried out at the accredited laboratory of the production association "Agromir". The number of mesophilic aerobic and facultative anaerobic microorganisms (total plate count) was determined according to GOST 10444.15-94 and expressed as colony forming units per gram.

The total content of radionuclides Cs-137, Sr-90 was determined according to radioactive substances in environmental objects (accreditation certificate No. UZ.AMT.07MAI-187 dated September 17, 2007).

Chemicals

Folin-Ciocalteu phenol reagent, gallic acid and 2,2-diphenyl-1-picrylhydraziyl (DPPH$^{\bullet}$) and were purchased from Sigma-Aldrich (Switzerland).

Na_2CO_3, 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) (ABTS$^{\bullet+}$) were obtained from Acros Organic (USA). L(+)-Ascorbic acid (176.13 g mol^{-1}), petroleum ether 80/110 were purchased from Chempur (Poland), but iodine concentrate (0.05 mol L^{-1}) FIXANAL was obtained from Fluka Analytical, Sigma-Aldrich (Poland). Additional solvents as ethanol (96%) and ultra-pure water were provided by Latvia University of Life Sciences and Technologies (Latvia, Jelgava).

Extraction procedure for total phenolic content (TPC) and antioxidant activity (AA) assay

The homogenized sample, 2.00±0.01 g was extracted with 10 mL ethanol: water 80:20 (v/v) in an ultrasonic bath YJ5120-1 (Oubo Dental, USA) at 35 kHz for 30 minutes at 20±1 °C. The extracts were then centrifuged in a centrifuge CM-6MT (Elmi Ltd., Latvia) at 3 500 min^{-1} for 5 min. Residues were reextracted using the same procedure. After centrifugation, extracts were collected in a volumetric flask and filled to the mark with fresh solvent. The extraction process was carried out in triplicate. JENWAY 6300 spectrophotometer (Baroworld Scientific Ltd., UK) was used to determine TPC, scavenging activity of DPPH$^{\bullet}$ and ABTS$^{\bullet+}$.

Determination of total phenolic content (TPC)

TPC of melon extracts was determined according to the method presented by Singleton et al. (1999). TPC was expressed as the gallic acid equivalents (GAE) 100 g^{-1} of sample.

Determination of antioxidant activity (AA)

AA was determined using the method presented by Yu et al. (2003) and Re et al. (1999). AA was expressed as mM TE 100 g^{-1} of sample.

The determination of ascorbic acid and total carotene content

Ascorbic acid content in dried melons was detected by iodine method T-138-15-01:2002 (Seglina, 2007) in four replications. The content of ascorbic acid was calculated according to formula (1):

$$C=5000\times\frac{V_{sample}}{m\times V_{standard}} \quad (1)$$

where:

V_{sample} - volume of the iodine solution titrated in a sample, mL;

$V_{standard}$ - volume of the iodine solution titrated in a standard solution, mL;

m - the amount of sample, g.

Total carotene content was detected by spectrophotometric method using UV/VIS spectrophotometer Jenway 6705 (Bibby Scientific Ltd., UK) (Kampuse et al., 2015) with modifications. A sample of 2 g was mixed with 20 mL of ethanol and 10 mL of water. After 15 min 25 mL of petroleum ether was added and stirred for another hour. After mixing, samples were placed into darkness for the formation of two separate layers. The absorption of petroleum ether layer was measured at 440 nm (n=3).

Statistical analysis

Experimental results were analysed by Microsoft Excel 2010. Statistical analysis were performed by Tomsone et al. (2014).

Results and Discussion

The data obtained indicate (Table 1) that the yield of the studied melon varieties ranged from 25.0 to 32.8 tons per hectare, and an average of 28.9 tons.

Table 1

Sugar content and productivity of melon variety

Sample	Sugar content, %	Productivity of fresh melons, t ha^{-1}	Productivity after drying, t ha^{-1}	
			solar drying	convective drying
A	14.9^{b*}	27.5^{b}	3.7^{c}	4.0^{c}
B	12.2^{a}	25.0^{a}	2.7^{a}	2.9^{a}
C	11.9^{a}	26.3^{a}	2.7^{a}	2.9^{a}
D	13.5^{b}	29.1^{b}	3.3^{b}	3.6^{b}
E	10.8^{a}	32.8^{c}	3.0^{a}	3.4^{b}

* The mean values in the same column, followed by different letters, vary significantly ($p<0.05$).

A – 'Qundalangi tur'; B – 'Oq uruq'; C – 'Obinavvot Samarqand'; D – 'Kukcha-588'; E – 'Ichi qizil'

Melon varieties differed in the sugar content, which varied from 10.8 to 14.9%. The productivity of dried melons depended on the variety and methods of drying. The higher productivity of fresh melons was observed for sample D and E, but after drying the highest productivity was for sample A (3.7–4.0 t ha^{-1}) and D (3.3–3.6 t ha^{-1}) were productivity decrease for 86.5% compare to fresh melon productivity. Comparing both drying methods, the convective drying gives by 0.6–1.3% higher productivity for all samples, compared to solar drying.

According to the hedonic scale, trained panellists evaluated samples of dried melon (Fig. 1) ranging from 7 (like moderately) to 9 (like extremely). The results showed that the panellists liked all dried melon samples which were dried by convective drying method ($p>0.05$). The quality of dried melon products during solar drying was estimated at 7.1–8.0 points, while convective drying – 7.9–8.9 points, which is 0.8–1.2 points higher than in the solar drying.

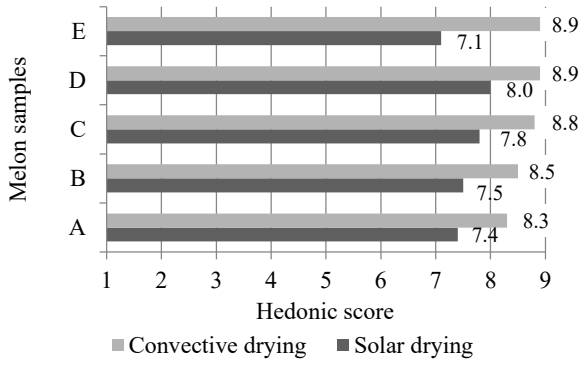

Figure 1. Evaluation results of dried melons using 9-point hedonic scale

A – 'Qundalangi tur'; B – 'Oq uruq'; C – 'Obinavvot Samarqand'; D – 'Kukcha-588'; E – 'Ichi qizil'

The panellists noted that the samples dried by convective drying method had retained a stronger melon aroma and taste compared to sun-dried samples. All convective dried samples and samples C and D from solar drying, degree of liking of which ranged from 7.8 to 8.9 (like very much to like extremely), were used for further analysis – microbiological and radionuclide testing, determination of total phenolic content, antioxidant activity and ascorbic acid content.

It was found that the total radionuclides content in the studied samples was below the acceptable concentration of Cs^{137} equal to 600 Bq kg^{-1}; Sr^{90} – 200 Bq kg^{-1}, respectively. The total radionuclides content in Cs^{137} samples ranged from 38.0 to 67.0 Bq kg^{-1}, but Sr^{90} – from 20.0 to 50.0 Bq kg^{-1}, which indicates that the radionuclides were below the acceptable concentration.

Results of the microbiological evaluation of dried melon samples is shown in Table 2.

Table 2

Microbiological indicators of dried melon samples

Melon sample	Drying method	Total plate count	Moulds
		CFU g^{-1}	CFU g^{-1}
A	Convective	2×10^4	1×10^2
B	Convective	2×10^4	1×10^2
C	Convective	2×10^4	2×10^2
	Solar	4×10^4	3×10^2
D	Convective	3×10^4	2×10^2
	Solar	4×10^4	3×10^2
E	Convective	2×10^4	1×10^2

A – 'Qundalangi tur'; B – 'Oq uruq'; C – 'Obinavvot Samarqand'; D – 'Kukcha-588'; E – 'Ichi qizil'

The acceptable concentration for total plate count is 5×10^4 CFU g^{-1}, while acceptable concentration for moulds is 5×10^2 CFU g^{-1} (Sanitary norms …, 2010). The obtained results in the analysed samples show that the microbiological indicators do not exceed the norm of acceptable concentration therefore the samples can be used in the diet. Only samples dried using the solar drying method had a higher total number concentration (4×10^4 CFU g^{-1}) of microorganisms, which indicates that the product is more exposed to microbiological contamination by drying in the sun.

The TPC and other biologically active compounds in plants are influenced by variety, climate, precipitation, stage of development, harvest time (Marrelli et al., 2012; Tomsone et al., 2012). The environmental stress conditions such as extreme temperatures, ozone, dehydration etc. positively affect the TPC in fruits and vegetables (Capanoglu, 2010). As well as the technological processes used for the further processing, the chemical composition of fruits and vegetables is greatly influenced (Katsube et al., 2009; Tomsone et al., 2013).

Results of Tukey's test showed that variety and drying method has significant ($p<0.05$) influence on the TPC (Table 3). In general, the highest TPC was fixed in the melon samples after artificial drying. Samples of dried melons by artificial drying can be arranged depending on TPC as follows (starting with the largest): B > E > C > D > A. New compounds can be formed in the drying process. This is due to the oxidation reactions of

glycoside hydrolysis or connection release cell of malfunction (Diaz-Moroto et al., 2002).

Table 3

Total phenolic content in dry melon depending on drying method and variety

Melon sample	Drying method	TPC mg GAE 100g^{-1}
A	Convective	88.11±4.82^{b*}
B	Convective	111.09±6.13^{d}
C	Convective	96.52±5.88^{b,c}
	Solar	90.26±5.28^{b}
D	Convective	90.34±3.17^{b}
	Solar	72.99±4.47^{a}
E	Convective	107.64±3.54^{cd}

* The mean values in the same column, followed by different letters, vary significantly ($p<0.05$).
A – 'Qundalangi tur'; B – 'Oq uruq'; C – 'Obinavvot Samarqand'; D – 'Kukcha-588'; E – 'Ichi qizil'

There are contradictory data in the literature on the effect of drying on the content of phenolic compounds in plants. Some fruits, berries and vegetables that have been studied after drying have a higher TPC than fresh samples (Arslan, Musa Özcan, 2010; Yang et al., 2010). But in other plants, after drying, the TPC was lower than in fresh samples (Miean, Mohamed, 2001; Erbay, Icier, 2009). In the process of drying, plant tissues become fragile, causing rapid destruction of the cell walls and accelerating enzyme activity (Hossain et al., 2010). High enzyme activity can cause release of bound phenolic compounds. Some researchers have noticed a decrease in phenolic acid content due to drying and this is mainly due to enzymatic oxidation (Del Caro et al., 2004). Elevated temperatures and the effect of oxygen can cause rapid degradation of phenolic compounds in plant material (Igual et al., 2012).
Literature data show that the drying and freezing affect not only the content of biologically active substances in plants, but also the activity of antioxidants in fruits, berries and vegetables (Pinelo et al., 2004; Chan et al., 2009; Siriamornpun et al., 2012; Chan et al., 2013). ANOVA analysis of variance showed that the variety and the drying method have significant effect on antioxidant activity ($p<0.05$). The results of Tukey's test (Table 4) showed significant differences between the varieties. Samples of dried melons by artificial drying can be arranged depending on DPPH$^{\cdot}$ as follows (starting with the largest): C > E > D > B > A.
By contrast, samples of dried melons by artificial drying can be arranged depending on ABTS$^{\cdot+}$ as follows (starting with the largest): A > E > C > B > D. The highest DPPH$^{\cdot}$ antioxidant activity was fixed for the sample C, while ABTS$^{\cdot+}$ assay showed higher results for the sample A. It was observed that the binding capacity of ABTS$^{\cdot+}$ cations is not related to the phenolic compounds present in the dried melons. This is especially evident in the sample A. Both the TPC and the activity of antioxidants are higher in artificial drying samples, except for ABTS$^{\cdot+}$ activity for sample D ('Kukcha-588').

Comparing the results of TPC and antioxidant activity, it can be concluded that convective drying is a better drying method for preserving phenolic compounds with antioxidant activity.

Table 4

Analysis of antioxidant activity in dry melon depending on drying method and variety

Melon sample	Drying method	DPPH$^{\cdot}$ mM TE 100 g^{-1}	ABTS$^{\cdot+}$ mM TE 100 g^{-1}
A	Convective	4.89±0.30^{bc*}	6.24±0.74^{c}
B	Convective	4.99±0.06^{bcd}	4.93±0.24^{bcd}
C	Convective	5.42±0.22^{d}	5.70±0.47^{bc}
	Solar	4.71±0.01^{b}	5.44±0.19^{bcd}
D	Convective	5.12±0.05^{bcd}	4.22±0.49^{a}
	Solar	4.20±0.20^{a}	4.87±0.38^{bc}
E	Convective	5.35±0.13^{cd}	6.14±0.64^{bc}

* The mean values in the same column, followed by different letters, vary significantly ($p<0.05$).
A – 'Qundalangi tur'; B – 'Oq uruq'; C – 'Obinavvot Samarqand'; D – 'Kukcha-588'; E – 'Ichi qizil'

Temperature is the most important factor influencing the drying rate of fruit and vegetables (Timoumi et al., 2004). Using solar drying method, the water evaporation process proceeds slower. Therefore, more time is available to continue internal biochemical reactions. As a result, various metabolic enzymes degrade biologically active compounds, including phenolic compounds. Therefore, solar dried melon samples contain lower TPC.

Table 5

The ascorbic acid and total carotenes content in dried melons

Melon sample	Drying method	Ascorbic acid content mg 100 g^{-1}	Total carotenes content mg 100 g^{-1}
A	Convective	86.95±15.87^{d*}	1.070±0.062^{e}
B	Convective	66.00±3.63^{b}	0.089±0.030^{c}
C	Convective	96.17±7.53^{d,e}	0.101±0.005^{c}
	Solar	56.60±5.91^{b}	0.219±0.194^{cd}
D	Convective	88.34±6.40^{d}	0.095±0.004^{c}
	Solar	30.15±1.83^{a}	0.026±0.002^{a}
E	Convective	72.71±2.07^{c}	0.037±0.003^{ab}

* The mean values in the same column, followed by different letters, vary significantly ($p<0.05$).
A – 'Qundalangi tur'; B – 'Oq uruq'; C – 'Obinavvot Samarqand'; D – 'Kukcha-588'; E – 'Ichi qizil'

The amount of ascorbic acid in dried melons was from 30.15 to 96.17 mg 100 g^{-1}. The highest ascorbic acid content was in sample C ('Obinavvot Samarqand') by convective drying (Table 5). According to the literature data the ascorbic acid content in fresh melons is from 20 to 30 mg 100 g^{-1} FW (Lester et al., 2005; Ostonakulov et al., 2016), which means that, using artificial drying technologies ascorbic acid is quite stable while after solar drying of the same samples the ascorbic acid content was significantly ($p<0.05$) lower (Table 5). The total carotenes content (Table 5) in dried melons was in range from 0.026 to 1.070 mg 100 g^{-1} and

the evaluated varieties significantly differed. Evaluating different varieties of fresh melons, authors Laur and Tian (2011) also found large fluctuations of β-carotene content among samples (from 0.063 to 3.138 mg 100 g^{-1}) which proves the importance of variety. The drying technology did not show any clear tendency to changes of total carotenes content.

Correlation analysis (Table 6) was performed to determine connection between these parameters – TPC, ascorbic acid content (AAC), total carotenes content (TCC) and antioxidant activity ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$).

Table 6

Pearson`s coefficients between total phenolic content and antioxidant activity for dried melon

	TPC	$DPPH^{\cdot}$	$ABTS^{\cdot+}$	AAC	TCC
TPC	1				
$DPPH^{\cdot}$	0.744**	1			
$ABTS^{\cdot+}$	0.335	0.397	1		
AAC	0.464*	0.855**	0.332	1	
TCC	-0.148	-0.045	0.477*	0.338	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

In our study all correlations between analysed parameters are positive except total carotenes content with TPC and $DPPH^{\cdot}$ (Table 6). For dried melons, the correlation between TPC and AA ($DPPH^{\cdot}$) was moderate (r=0.744), but a closer correlation was observed between ascorbic acid content and antioxidant activity ($DPPH^{\cdot}$). Consequently, it can be concluded that phenol compounds and ascorbic acid in dried melons provide the ($DPPH^{\cdot}$) antioxidant activity.

Conclusions

From the results it can be obtained that the best drying method for melons is artificial convective drying, which showed that the products obtained by artificial convective drying are better in all indicators than in the solar drying. Artificial convective drying method is better for preserving biologically active compounds and antioxidants in dried melons compared to solar drying method. In dried melons, phenolic compounds successfully act as antioxidants ($DPPH^{\cdot}$ method) for radical scavenging.

According to the results of the analysis, it is difficult to indicate which varieties of melons would be most suitable for drying, as the results for each melon variety are different. But obtaining the results of dried samples, a better result was given by variety – 'Qundalang tur' and 'Kukcha-588'.

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References

1. Amiri M., Tavakolipour H., Gharehyakheh S. (2014) Modeling of melon drying by application of microwave using mamdani fuzzy inference system. *European Journal of Experimental Biology*, Vol. 4 (1), p. 44–52.
2. Arslan D., Musa Özcan M. (2010) Study the effect of sun, oven and microwave drying on quality of onion slices. *LWT-Food Science and Technology*, Vol. 43, p. 1121–1127.
3. Berdıyev M., Arslan D., Özcan M. M. (2009) Nutritional composition, microbiological and sensory properties of dried melon: a traditional Turkmen product. *International Journal of Food Science and. Nutrition*, Vol. 60 (1), p. 60–68.
4. Capanoglu E. (2010) The potential of priming in food production. *Trends in Food Science and Technology,* Vol. 21, p. 399-407.
5. Chan E. W. C., Lim Y. Y., Wong S. K., Lim K. K., Tan S. P., Lianto F. S., Yong M. Y. (2009) Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chemistry*, Vol. 113, p. 166–172.
6. Chan E. W. C., Lye P. Y., Eng S. Y., Tan Y. P. (2013) Antioxidant properties of herbs with enhancement effects of drying treatments: A synopsis. *Free Radicals and Antioxidants*, Vol. 3, p. 2–6.
7. Chayjan R.A., Agha-Alizade H.H., Barikloo H., Soleymani B. (2012) Modeling some drying characteristics of cantaloupe slices. *Cercetari Agronomice Moldova XLV*, Vol. 2 (150), p. 5–14.
8. Darvishi H., Khodaei J., Azadbakht M. (2015) The parameters of mass transfer of convective drying in sliced melon. *Philippine Agricultural Scientist*, Vol. 98 (1), p. 360–372.
9. Del Caro A., Piga A., Pinna I., Fenu P. M., Agabbio M. (2004) Effect of drying conditions and storage period on polyphenolic content, antioxidant capacity and ascorbic acid of prunes. *Journal of Agricultural and Food Chemistry*, Vol. 52, p. 4780–4784.
10. Diaz-Moroto M. C., Perez-Coello M. S., Cabezudo M. D. (2002) Effect of different drying methods on the volatile compounds of parsley (*Petroselium crispum* L.). *European Food Research and Technology*, Vol. 215, p. 227–230.
11. Erbay Z., Icier F. (2009) Optimization of drying of olive leaves in a pilot-scale heat pump dryer. *Drying Technology*, Vol. 27(3), p. 416–427.
12. Hossain M. B., Barry-Ryan C., Martin-Diana A. B., Brunton N. P. (2010) Effect of drying method on the antioxidant capacity of six *Lamiaceae* herbs. *Food Chemistry*, Vol. 123, p. 85–91.
13. Igual M., García-Martínez E., Martín-Esparza M. E., Martínez-Navarrete N. (2012) Effect of processing on the drying kinetics and functional value of dried apricot. *Food Research International*, Vol. 47, p. 284–290.
14. Kampuse S., Ozola L., Straumite E., Galoburda R. (2015) Quality parameters of wheat bread enriched with pumpkin (*Cucurbita* Moschata) by-products. *Acta Universitatis Cibiniensis. Series E: Food Technology*, Vol. 19(2), p. 3–14.
15. Katsube T., Tsurunaga Y., Sugiyama M., Furuno T., Yamasaki Y. (2009) Effect of air-drying temperature on antioxidant capacity and stability of polyphenolic compounds in mulberry (*Morus alba* L.) leaves. *Food Chemistry*, Vol. 113(4), p. 964–969.
16. Laur L. M., Tian L. (2011) Provitamin A and vitamin C contents in selected California-grown cantaloupe and

honeydew melons and imported melons. *Journal of Food Composition and Analysis*, Vol. 24 (2), p. 194–201.
17. Lester G. E., Jifon J. L., Rogers G. (2005) Supplemental foliar potassium applications during muskmelon fruit development can improve fruit quality, ascorbic acid, and beta-carotene contents. *Journal of the American Society for Horticultural Science*, Vol. 130(4), p. 649–653.
18. Marrelli M., Menichini F., Statti G. A., Bonesi M., Duez P., Menichini F., Conforti F. (2012) Changes in the phenolic and lipophilic composition, in the enzyme inhibition and antiproliferative activity of *Ficuscarica* L. cultivar Dottato fruits during maturation. *Food and Chemical Toxicology*, Vol. 50, p. 726–733.
19. Mavlyanova R.F. (2005) Melons of Uzbekistan. Tashkent: PGRI www.ipgri.cgiar.org ISBN 978-92-9043-711-6 ISBN 92-9043-711-6. 206 p.
20. Miean K. H., Mohamed H. (2001) Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry*, Vol. 49(6), p. 3106–3112.
21. Ostonakulov T.E., Sanaev S.T., Khamdamova E.I. (2016) *Samarkand melons*. Samarkand: Navruz. 39 p.
22. Ostonakulov, T.E., Zuev V.I., Kodirkhuzhaev O.K. (2016). *Vegetable*. Tashkent: Navruz. 552 p.
23. Pinelo M., Rubilar M., Sineiro J., Nunez M. J. (2004) Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*). *Food Chemistry*, Vol. 85(2), p. 267–273.
24. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, Vol. 26, p. 1231–1237.
25. Rodrigues S., Fernandes F. A. N. (2007) Dehydration of melons in a ternary system followed by air-drying. *Journal of Food Engineering*, Vol. 80, p. 678–687.
26. Sanitary norms, rules and hygienic standards of the Republic of Uzbekistan. (2010) Hygienic requirements for food safety. SanPin RUz № 0283-10. Official Edition. Tashkent.
27. Seglina D. (2007) Sea buckthorn fruits and their processing products. *Summary of promotion work for acquiring the Doctor's degree in Engineering Sciences in the Food Science*. Jelgava, Latvia, 48 p.
28. Silva M. A., Albuquerque T. G., Alves R. C., Oliveira M. B. P. P., Costa H. S. (2018) Melon (*Cucumis melo* L.) by-products: Potential food ingredients for novel functional foods? *Trends in Food Science and Technology*, In press, Corrected Proof, Available online 26 July 2018.
29. Singleton V. L., Orthofer R., Lamuela-Raventos R. M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, Vol. 29, p. 152–178.
30. Siriamornpun S., Kaisoon O., Meeso N. (2012) Changes in colour, antioxidant activities and carotenoids (lycopene, b-carotene, lutein) of marigold flower (*Tagetes erecta* L.) resulting from different drying processes. *Journal of Functional Foods*, Vol. 4, p. 757–766.
31. Solval K.M., Sundararajan S., Alfaro L., Sathivel S., (2012) Development of cantaloupe (*Cucumis melo*) juice powders using spray drying technology. *LWT-Food Science and Technology*, Vol. 46, p. 287–293.
32. *Strategy of actions in five priority directions of development of the Republic of Uzbekistan in 2017 – 2021*. Presidential Decree –5308 of 22.01.2018. On the State Program National database of legislation, 01.23.2018, No. 06/18/ 5308/0610; 05/25/2018, No. 06/18/5447/1269. [accessed on 02.01.2018.]. Available at: http://lex.uz/docs/3516841
33. The state register of crops recommended for sowing in the territory of the Republic of Uzbekistan. (2018) Ministry of Agriculture and Water Resources of the Republic of Uzbekistan. The State Commission for the Testing of various varieties of crops. Tashkent. p. 54–56.
34. Timoumi S., Mihoubi D., Zagrouba F. (2004) Simulation model for a solar drying process. *Desalination*, Vol. 168, p. 111–115.
35. Tomsone L., Kruma Z. (2014) Influence of freezing and drying on the phenol content and antioxidant activity of horseradish and lovage. **In:** *9th Baltic conference on food science and technology "Food for consumer well-being" FOODBALT 2014 Conference proceedings*. Jelgava, Latvia, p. 192–197.
36. Tomsone L., Kruma Z., Galoburda R., Dimins F., Kreicbergs V. (2013) Influence of technological processes on the phenol content and antioxidant properties of horseradish roots (*Armoracia rusticana* L.). **In:** *2nd International conference on Nutrition and Food Sciences "Chemical, Biological and Environmental Engineering" International Conference proceedings*, Moscow, Russia, Vol. 53. p. 6–10.
37. Tomsone L., Kruma Z., Lepse L. (2012) Influence of genotype and harvest time on the phenolic content of horseradish (*Armoracia rusticana* L.) roots. **In:** *Research for rural development 2012: annual 18th international scientific conference proceedings*, Latvia University of Agriculture. Jelgava: LLU, 2012. Vol.1, p. 124–130.
38. Yang J., Chen J., Zhao Y., Mao L. (2010) Effects of drying processes on the antioxidant properties in sweet potatoes. *Agricultural Sciences in China*, Vol. 9(10), p. 1522–1529.
39. Yu L., Haley S., Perret J., Harris M., Wilson J., Haley S. (2003) Antioxidant properties of bran extracts from Akron wheat grown at different locations. *Journal of Agriculture and Food Chemistry*, Vol. 51, p. 1566–1570.

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COMPARISON OF CHEMICAL COMPOSITION OF FRESH AND FERMENTED CABBAGE JUICE

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Abstract

White cabbage (*Brassica oleracea* var. *capitata*) is an affordable and available vegetable in local markets around the globe. It is a source of vitamins, micro and macro nutrients. The aim of this study was to compare the chemical composition of fresh and fermented cabbage juices from three different varieties. In this study vitamin C content, antiradical activity, total phenolic content, and total carotenes were determined. Results show a significant ($p<0.05$) influence of cabbage variety on vitamin C content. Fermentation process decreased vitamin C content in the variety 'Selma' but increased in 'Ramkila' and 'Kilpatons'. There were no significant differences between varieties ($p>0.05$) in the antiradical activity (by DPPH method) of fresh cabbage juice while fermentation process slightly increased it in varieties 'Ramkila' and 'Kilpatons' but significantly increased it in 'Selma' (from 96.66 for fresh to 189.54 mg 100 g^{-1} on dry weight (DW) for fermented). There was no consistency in the antiradical activity by $ABTS^{+}$ method. Fermentation process slightly decreased it in variety 'Ramkila', significantly decreased it in variety 'Selma' (805.72 for fresh to 356.76 mg 100 g^{-1} DW for fermented) but significantly increased in 'Kilpatons' (517.09 for fresh to 845.48 mg 100 g^{-1} DW for fermented). Fermentation process significantly influenced contents of total phenolic compounds in two varieties – 'Ramkila' (1176.1 for fresh to 1637.7 mg 100g^{-1} DW for fermented) and 'Kilpatons' (1106.3 for fresh to 1872.9 mg 100g^{-1} DW for fermented). Results showed that white cabbage or sauerkraut is not a beneficial source of carotenes.

Keywords: fermented cabbage, antiradical activity, total phenolic content

Introduction

White cabbage (*Brassica oleracea* var. *capitata*) is an affordable and available vegetable in local markets around the globe. Annually cabbage and brassica vegetables are consumed approximately 6.3 kg worldwide (Rokayya et al., 2013) and more than 8 kg of white cabbage in Latvia per capita (Gailīte, 2018).

It is a vegetable of low caloric value (24–36 kcal 100 g^{-1}), low protein (1.4 g 100 g^{-1}), low fat (0.2 g 100 g^{-1}) content, but high in minerals (such as potassium: 208 mg 100 g^{-1}, calcium: 46 mg 100 g^{-1}, magnesium: 12 mg 100 g^{-1}), vitamin C (329.5 mg 100 g^{-1}), vitamin A (31 µg 100 g^{-1}), dietary fibre (3.0 g 100 g^{-1}) and water content (92 g 100 g^{-1}). Cabbage contains moderate carbohydrate (glucose: 2.0 g 100 g^{-1}, fructose: 1.8 g 100 g^{-1}) amounts, and have a high level of phenolic compounds including polyphenols (Rodriguez-Amaya, 2015). β-carotene content in cabbage ranged from 0.009–0.124 mg 100 g^{-1} fresh weight, total phenolics: 12.58–34.41 mg 100 g^{-1} fresh weight (Singh et al., 2006). Total phenolic contents, antioxidant capacity, flavonoid content are influenced by many factors, including growing developmental stages (Šamec, 2011). Most common way to use cabbage is either fresh in soups and salads, or fermented – sauerkraut.

Sauerkraut fermentation is a dynamic biochemical system, where chemical composition and microbial ecology of the system are continuously changing (Lu et al., 2003). To ferment cabbage, varieties with the highest carbohydrate content with a sugar content of at least 4% are selected. Fermentation process using lactic acid bacteria in cabbage increases the content of vitamins, free amino acids and other physiologically active substances in the product, though the choice of starter and cabbage variety has a noticeable impact on chemical composition (Vatansever, 2017; Martinez-Villaluenga et al., 2012). Spontaneous sauerkraut fermentation relies on a small population of lactic acid bacteria (LAB) (which are naturally present on fresh vegetables) and their metabolites. The process is divided into heterofermentative and homofermentative stages. In the first stage, the activity of heterofermentative *Leuconostoc mesenteroides* determines the quality of sauerkraut. Acid-tolerant *Lactobacillus* species takes over second homofermentative stage with decrease in pH (4.5–3.5) and accumulation of lactic acid. (Yoon et al., 2002; Lu et al., 2003). Consumption of sugars, pH reduction and acid production is very rapid in the first stage (~ first 2 weeks) and slows thereafter and remains unchanged after day 30 (Yoon et al., 2002). Strains isolated during the first stage (day 1 to day 3) belong to genus *Leuconosto*c or *Weisella*, after that it is Lactobacillus strains (*L. plantarum* and *L. brevis*) (Yoon et al., 2002).

Biochemical tests showed that *Leuconostoc* and *Weisella* strains produce carbon dioxide from glycose as well as characteristic slime of dextran from sucrose. None of *Lactobacillus* (*L. plantarum* nor *L. brevis*) strains produce slime colonies on sucrose agar, and only *L. brevis* strain produces carbon dioxide (Lu et al., 2003). To ensure the correct sequence of LAB species, which is essential to achieve a stable product with typical flavour and aroma, producers choose to use LAB starter culture (*Leuconostoc mesenteroides*) as well as to reduce NaCl content as low as 0.6% (Viander et al., 2003).

Bacterial growth is involved in the metabolism of phenolic compounds and is compound dependent (Rodriguez et al., 2009; Alberto et al., 2012).

Fermentation process leads to formation of bioactive compounds (Palani et al., 2016). The influence of fermentation process on chemical composition in white cabbage has been done by Spanish researchers. It states

that fermentation process increases ascorbic acid content, antioxidant activity and nitric oxide production inhibitory activity, though the choice of starter has a noticeable impact (Martinez-Villaluenga et al., 2012). For ascorbic acid formation, initial glucobrassicin levels in vegetables are important and is a reason why the amount of ascorbigen can significantly vary between different varieties (Wagner, Rimbach, 2009). The titratable acidity in sauerkraut ranges between 0.9–1.5% (Trail et al., 1996).

Consumption of fresh and fermented cabbage in Latvia is considerable. However little or no research was done to compare cabbage varieties grown in Latvia and how fermentation process affects their chemical composition.

The aim of this study was to compare chemical composition of fresh and fermented cabbage juices from three different varieties.

Materials and Methods

Experimental work was carried out in Latvia University of Life Sciences and Technologies, Faculty of Food technology.

Sample preparation

Three varieties of fresh cabbage heads were delivered by a farmer. Three 1 L bottles of sauerkraut juice from the same varieties were delivered by the same farmer. The varieties were: 'Selma' (with a light green firm head), 'Kilpatons' (with a greener, not so firm, head; sweet to taste), and 'Ramkila' (a big, very light, and very firm head with some spoiled leaves in between).

Cabbage heads were cleaned from outer, not-fresh leaves. The spoiled inner leaves of variety 'Ramkila' were removed. Segments (3 cm vide) were cut out of each head and juice was extracted with masticating slow juicer (Easyline, Villa-Verucchio model ELCJE6203235M).

Chemical and physical analyses

Vitamin C was determined using iodine method T-138-15-01:2002 (Segliņa, 2007) which determines reduced form of ascorbic acid (L-ascorbic acid). Experimental samples were mixed with 100 mL 6% $H_2C_2O_4$ solution blended and filtered. 10 mL of filtrate was mixed with 2 ml of 1% starch solution and titrated with 0.05 m iodine solution.

Preparation of extracts. Samples of fresh and fermented juices (~ 10 g) of each variety were extracted with 20 mL of 80% ethanol by stirring on the magnetic stirrer for 45 minutes. Two repetitions were made. Ethanolic extracts were then filtered into 25 mL flasks and stored at 4±2 °C till further analysis. Extracts were used for the estimation of total phenolic contents and antiradical activities (DPPH; $ABTS^+$) (Rokayya et al., 2013).

Total phenolic content in extracts was estimated spectrophotometrically using Follin-Ciocalteu reagent according to Prasad et al. (2013) with some modifications. Three repetitions of 0.5 mL of extract were mixed each with 2.5 mL Follin-Ciocalteu reagent (diluted ten times with deionized water), left to react for 5 min. Then 2 mL 7.5% Na_2Co_3 were added and mixtures were left to react for another 30 min. The absorption was read spectrophotometrically at 765 nm on Jennway 6300 (Baroworld Scientific Ltd., UK). The total phenolic content was determined using standard gallic acid calibration curve and results were expressed as milligrams of gallic acid equivalent (mg GAE 100 g^{-1} DW).

Antiradical activity was determined using $ABTS^+$ (2,2-azino-di-3-ethylbenzothialozine-sulphonic acid) decolouration method and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The $ABTS^+$ stock solution was made with addition of potassium persulfate as an oxidation agent (Rokayya et al., 2013) and left to react in the darkness for 12 h. To obtain the working solution of $ABTS^+$, the stock solution was diluted with phosphate buffered saline (PBS) to the absorption of 0.800±0.030 at 734 nm (against the blank) on JENWAY 6300. Three repetitions of 0.05 mL sample extract were mixed with 5 mL of $ABTS^+$ solution, left to react for 10 min and absorptions were determined as had been described previously.

The DPPH radical scavening assay was done according to the method of Kriengsak et al. (2006) with some modifications. The stock solution was made by mixing 0.004 g DPPH with 96% ethanol to the absorption of 1.000±0.02 units against the blank on spectrophotometer at 517 nm. Three repetitions of 0.5 mL sample extracts were mixed with 3.5 mL freshly made DPPH stock solution, left to react in the dark for 30 min and then absorption had been measured.

Total carotenes were determined using method described by Kampuse et al. (2015) with modifications. Samples of 5 g (with precision of 0.0002 g) of fresh cabbage and sauerkraut juices were mixed with 20 mL 96% ethanol and stirred magnetically, after 15 min 25 mL of petrol ether (80–110) was added and stirring continued for another 60 min. Samples were left to settle and results were read using UV/VIS spectrophotometer Jenway 6705.

Statistical analysis

The differences between results were analysed using two-factor analysis of variance (ANOVA) followed by Tukey-Kramer method. The obtained results were presented as means with standard errors. Differences among results were considered to be significant if $p<0.05$.

Results and Discussion

Chemical composition of fresh and fermented cabbage juices varied among cabbage varieties which might be due to different initial physical characteristics (Thakur et al., 2017) as well as it is influenced by growing conditions, storing, fermentation process and many more factors (Kusznierewicz et al., 2008; Wagner, Rimbach, 2009; Palani et. al., 2016).

Vitamin C. Analysing obtained results, it was determined that there is a significant ($p<0.05$) influence of cabbage variety on vitamin C (Fig. 1) content, also fermentation process affected it differently in studied

varieties. In two of analysed cabbage varieties vitamin C content increased after fermentation process – 'Ramkila' (511.8 for fresh to 591.9 mg 100 g^{-1} DW for fermented) and 'Kilpatons' (522.6 for fresh to 671 mg 100 g^{-1} DW for fermented), while variety 'Selma' had a decrease in vitamin C after fermentation (809.7 for fresh to 672.4 mg 100 g^{-1} DW for fermented). Differences in results can be explained by enzymatic reactions of chemical compounds in varieties as described by Wagner et al. (2009) and Martinez-Villaluenga et al. (2009). According to Thakur et al. (2017), ascorbic acid content increases during fermentation up to day 21, after which it gradually decreases in all varieties.

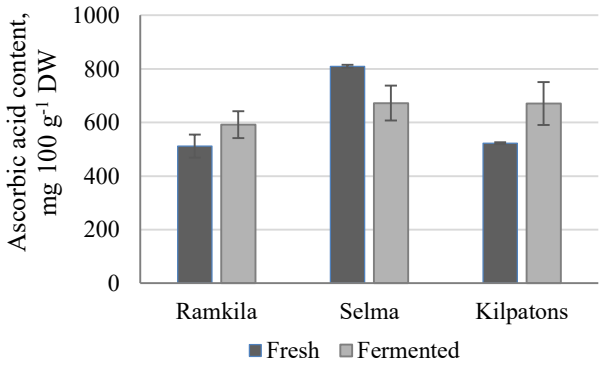

Figure 1. Ascorbic acid content in fresh and fermented cabbage juice, mg 100 g^{-1} DW

The loss of vitamin C due to cabbage fermentation partially may be explained by ascorbic acid involvement in ascorbigen formation. It also may be influenced by the production process of sauerkraut – vitamin C depletion occur when vegetables are severely cut or shredded. Trimming of outer leaves, that contain more vitamin C than inner leaves, results in greater decrease in vitamin C than enzymatic breakdown by ascorbate oxidase, autoxidation and so on (Martinez-Villaluenga et al., 2009). To avoid decrease of vitamin C in fermentation process, it is advisable to choose fresh cabbage heads with little or no damaged outer leaves and to control the fermentation process, time and temperature.

Total phenolic contents

Fermentation process significantly influenced total phenolic contents (Fig. 2) in two varieties – 'Ramkila' (1176.1 for fresh to 1637.7 mg 100 g^{-1} DW for fermented) and 'Kilpatons' (1106.3 for fresh to 1872.9 mg 100 g^{-1} DW for fermented). However, total phenolic content in the variety 'Selma' was not significantly ($p<0.05$) influenced by fermentation process (1603.5 for fresh to 1632.4 mg 100 g^{-1} DW for fermented). One of the reasons, as Kusznierewicz et al. (2007) have come to conclusion that antiradical activity initially increases during wounding or shredding for spontaneously fermented sauerkraut. Fermentation and chemical processes incurred by lactic acid can induce formation of novel compounds that can neutralize free radicals. However, metabolic pathways of biosynthesis or degradation of phenolic compounds by lactic acid bacteria have not been completely described (Rodriguez, 2009).

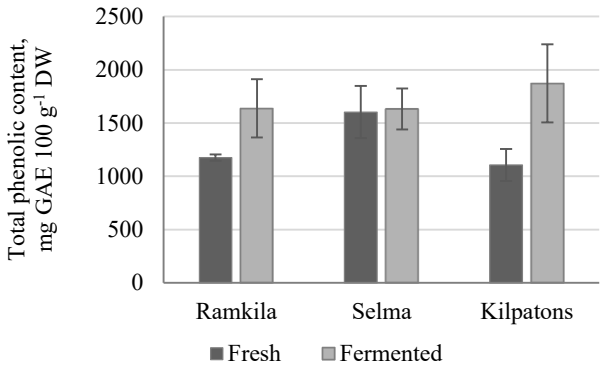

Figure 2. Total phenolic content in fresh and fermented cabbage juice, mg GAE 100 g^{-1} DW

Antiradical activity

Antiradical activity by $ABTS^{+}$ decolouration method showed significant ($p>0.05$) variations (Fig. 3). Variety 'Ramkila' (704.7 for fresh to 611.1 mg 100 g^{-1} DW for fermented) and 'Selma' (805.7 for fresh to 356.8 mg 100 g^{-1} DW for fermented) showed decreasing results, whereas in variety 'Kilpatons' antiradical activity increased (517.1 for fresh to 845.5 mg 100 g^{-1} DW for fermented).

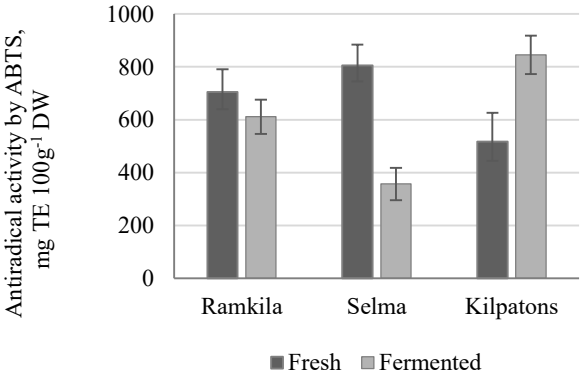

Figure 3. Antiradical activity by $ABTS^{+}$ in fresh and fermented cabbage juice, mg TE 100 g^{-1} DW

There were no significant differences between varieties ($p>0.05$) in DPPH radical scavenging assay in fresh cabbage juice, (95.9–98.5 mg 100 g^{-1} DW). Fermentation process significantly increased the antiradical activity (138.7–189.5 mg 100 g^{-1} DW) and there were significant differences between varieties (Fig. 4), too ($p < 0.05$). DPPH method is widely used to determine antiradical / antioxidant activity of purified phenolic compounds (Shalaby, Shanab, 2012). The overall content of phenols tends to increase which also can explain increase of DPPH antiradical activity. As concluded by Kusznierewicz et al. (2007), it is not

always the case that fermenting rises antiradical activity and vitamin C content.

Different antiradical activity results can be explained by differences in radical assay methods. DPPH is sensitive to acidic pH, samples react very slowly not reaching steady state after 8 hours, whereas $ABTS^+$ method has the extra flexibility to be used at different pH levels, samples react rapidly, reaching steady state within 30 minutes.

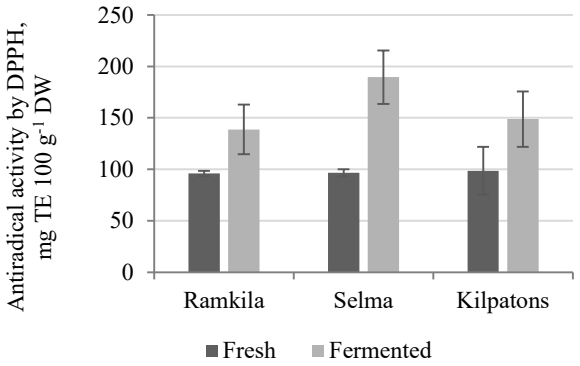

Fig. 4. Antiradical activity by DPPH in fresh and fermented cabbage juice

$ABTS^+$ assay measures the relative ability of antioxidant to scavenge the $ABTS^+$ generated in aqueous phase as compared with a water-soluble vitamin E analogue standard (Trolox) (Shalaby, Shanab, 2012) so it's radicals react with different compounds and show higher antioxidant capacity in cabbage juice (Šamec, 2011). The rise of $ABTS^+$ antiradical activity in variety 'Kilpatons' could be explained by different and more water-soluble compounds.

Total carotenes

Results showed that white cabbage and sauerkraut are not a beneficial source of carotenes, which is in agreement with the information reported by Singh (2006). Variety 'Ramkila' showed a decrease in total carotenes form 9.45 (for fresh) to 3.77 mg 100 g^{-1} DW (for fermented) and 'Selma' from 8.28 (for fresh) to 0.27 mg 100 g^{-1} DW (for fermented), while variety 'Kilpatons' had an increase from 4.36 (for fresh) to 10.35 mg 100 g^{-1} DW (for fermented).

Conclusions

Fermentation process positively influenced DPPH radical scavenging activity in tested cabbage varieties, however there is no such trend in the $ABTS^+$ activity. Fermentation process positively influenced variety 'Kilpatons' – total phenolic contents, vitamin C and antiradical activity ($ABTS^+$; DPPH) increased significantly. In variety 'Selma', fermentation process had little influence on total phenolic contents, but antiradical activity by $ABTS^+$ method and vitamin C content decreased. In variety 'Ramkila' – antiradical activity by $ABTS^+$ method decreased but total phenolic content and vitamin C increased.

References

1. Alberto M.R., Manca-deNadra M.C., Arena M.E. (2012) Influence of phenolic compounds on the growth and arginine deiminases system in a wine lactic acid bacterium. *Brazilian Journal of Microbiology,* Vol. 1517-8382, p. 167–176.
2. Gailīte M. (2018) Viss par labiem kāpostiem. Šķirnes, audzēšana, ražas, *Agrotops*. [accessed on 12.12.2018.]. Available at http://www.la.lv/jaunumi-kapostu-hibridu-piedavajuma
3. Kampuse S., Ozola L., Straumite E., Galoburda R. (2015) Quality parameters of wheat bread enriched with pumpkin (*Cucurbita Moschata*) by-products. *Acta Universitatis Cibiniensis, Series E: Food Technology*, Vol. 19(2), p. 3–14.
4. Kriengsak T., Boonprakob U., Crosby K., Cisneros-Zevallos L., Byrne D.H. (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis.* Vol. 19 (6–7), p. 669–675.
5. Kusznierewicz B., Smiechowska A., Bartoszek A., Namiesnik J. (2007) The effect of heating and fermenting on antioxidant properties of white cabbage. *Food Chemistry,* Vol. 108, p. 853–861.
6. Kusznierewicz B., Bartoszek A., Wolska L., Drzewiecki J., Gorinstein S., Namiesnik J. (2008) Partial characterization of white cabbages (*Brassica oleracea* var. *capitata* f. *alba*) from different regions by glucosinolates, bioactive compounds, total antioxidant activities and proteins. *LWT-Food Science and Technology*, Vol. 41 (1), p. 1–9.
7. Lu Z., Breidt F., Plengvidhya V., Fleming H.P. (2003) Bacteriophage ecology in commercial sauerkraut fermentations. *Applied and Environmental Microbiology,* Vol. 69(6), p. 3192–3202.
8. Martinez-Villaluenga C., Peñas E., Frias J., Ciska E., Honke J., Piskula M.K., Kozlowska H., Vidal-Valverde C. (2009) Influence of fermentation conditions on glucosinolates, ascorbigen, and ascorbic acid content in white cabbage (*Brassica oleracea* var. *capitata* cv. Taler) cultivated in different seasons. *Journal of Food Science*, Vol. 74 (1), p. 62–67.
9. Martínez-Villaluenga C., Peñas E., Sidro B., Ullate M., Frias J., Vidal – Valverde C. (2012) White cabbage fermentation improves ascorbigen content, antioxidant and nitric oxide production inhibitory activity in LPS-induced macrophages. *LWT-Food Science and Technology*, Vol. 46 (1), p. 77–83
10. Palani K., Harbaum-Piayda B., Meske D., Keppler J.K., Bockelman W., Heller K.J., Schwarz K. (2016) Influence of fermentation on glucosinolates and glucobrassicin degradation products in sauerkraut. *Food Chemistry*, Vol. 190, p. 755–762.
11. Prasad N., Yang B., Kong K.W., Khoo H.E., Sun J., Azlan A., Ismail A., Romli Z.B. (2013) Phytochemicals and antioxidant capacity from *Nypa fruticans* Wurmb fruit. *Evidence-Based Complementary and Alternative Medicine*, Vol. 2013, p. 1–9.
12. Rodriguez-Amaya D.B. (2015) Vegetables as sources of nutrients and bioactive compounds: Health benefits. **In:** *Handbook of Vegetable Preservation and Processing*. 2nd ed. Y. H. Hui, E. O. Evranuz (eds). Boca Raton: CRC Press, p. 1–22.
13. Rodriguez H., Curiel J.A., Landete J.M., Rivas B., Lopez F., Gomez-Cordoves C., Mancheno J.M., Munoz R. (2009) Food phenolics and lactic acid bacteria.

International Journal of Food Microbiology, Vol. 132(2–3), p. 79–90.
14. Rokayya S., Li C., Zhao Y., Li Y., Sun C. (2013) Cabbage (*Brassica oleracea* L. var. Capitata) Phytochemicals with antioxidant and anti-inflammatory potential. *Asian Pacific Journal of Cancer Prevention*, Vol. 14, p. 6657–6662.
15. Segliņa D. (2007) Sea buckthorn fruits and their processing products. *Summary of promotion work for acquiring the Doctor's degree in Engineering Sciences in the Food Science*. [accessed on 30.01.2019.]. Available at: summary/food/Kopsavilkums-2007-LLU-Dalija-Seglina.pdf
16. Shalaby E.A., Shanab S.M.M. (2012) Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis, Indian Journal of Geo-Marine Sciences,* Vol. 42(5), p. 556-564.
17. Singh J., Upadhyay A.K., Bahadur A., Singh B., Singh K.P., Mathura Rai (2006) Antioxidant phytochemicals in cabbage (*Brassica oleracea* L. var. capitata). *Scientia Horticulturae*. Vol. 108 (3), p. 233–237.
18. Šamec D., Piljac-Žegarac J., Bogovič M., Habjanič K., Gruz J. (2011) Antioxidant potency of white (*Brassica oleracea* L. var. *capitata*) and Chinese (*Brassica rapa* L. var. *pekinensis* (Lour.)) cabbage: The influence of development stage, cultivar choice and seed selection. *Scientia Horticulturae,* Vol.128 (2), p.78–83.
19. Thakur P. K., Panja P., Das A., Kabir J. (2017) Varietal response to sauerkraut preparation. *Journal of Crop and Weed*, Vol. 13(2), p. 90–94.
20. Trail A.C., Fleming H.P., Young C.T., McFeeters R.F. (1996) Chemical and sensory characterization of commercial sauerkraut. *Journal of Food Quality,* Vol. 19, p. 567–568.
21. Vatansever S., Vegi A., Garden-Robinson J., Hall C.A.III (2017) The effect of fermentation on the physicochemical characteristics of dry-salted vegetables. *Journal of Food Research,* Vol. 6 (5), p. 32–40.
22. Viander B., Maki M., Palva A. (2003) Impact of low salt concentration, salt quality on natural large-scale sauerkraut fermentation. *Food Microbiology*, Vol. 20 (4), p. 391–395.
23. Wagner E.A., Rimbach G. (2009) Ascorbigen: chemistry, occurrence, and biologic properties. *Clinics in Dermatology*. Vol. (2), p. 217–224.
24. Yoon S. S., Barrangou-Poueys R., Breidt F., Jr., Klaenhammer T. R., Fleming H. P. (2002) Isolation and characterization of bacteriophages from fermenting sauerkraut. *Applied and Environmental Microbiology,* Vol. 68(2), p. 973–976.

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EFFECT OF *NATURESEAL® AS5* AND PACKAGING MATERIALS ON THE MICROBIOLOGICAL QUALITY OF SHREDDED CARROTS DURING STORAGE

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Abstract

The aim of the study was to investigate microbiological quality changes of shredded carrots processed with *Natureseal® AS5* solution during storage in several packaging materials. Before packaging, shredded carrots were processed with commercial 2.5% *Natureseal® AS5* solution at temperature +19±1 °C for 5 min±2 s. Processed products were packed in different packaging materials: cellulose based biodegradable packaging *Polilactid BIO-PLA* containers and *NatureFlex NVS*, hermetically closed by breathing polymer film *BOPP PropafilmTM P2GAF*. Carrot samples were stored at the temperature +4±1 °C for 12 days. Quality indicators using standard methods were determined O_2 and CO_2 concentration inside several packaging and microbiological analysis of carrots.

Analysis of the carrot samples was tested out before and after packaging 3, 5. 8, 10 and 12 days of storage. Treatment of carrots with 2.5% *NatureSeal® AS5* water solution and subsequent storage for 12 days in biologically degradable and conventional packaging materials ensured a significant decrease of total count and yeast count ($p<0.05$). This indicates that 2.5% *NatureSeal® AS5* water solution can be successful agent towards a ample range of spoilage microorganisms and makes it attractive for food preservation.

Keywords: carrots, *NatureSeal® AS5*, storage, treatment

Introduction

The carrot (*Daucus carrota* L.) is a root vegetable that has world-wide distribution. Carrots are amply consumed in the diet either treated in beverages and meals or fresh (Augspole et al., 2014; Hag, Prasad, 2018; Scarano et al., 2018; Wang et al., 2015). The consumption of carrots, both fresh and processed, has increased due to the introduction of new carrot-obtained health and nutritional goodness (Gamboa-Santos et al., 2012). In the last years the demand for fresh-cut and natural products has noticeably increased. They are vegetables with good dietary and taste properties. Fresh carrot products such as shredded carrots, carrot disks, batons an important part of the fresh-cut vegetable industry Wang et al., 2015). Scientist Yu et al. (2018) reported that carrot is well known vegetable used to make home cooked meals, salads and convenient ready to eat products. Treated carrots are prepared by peeling off the outer layer of the carrot root and cutting into slices or small cubes, shredding and keeping refrigerated in packaging. Processed carrots oft suffer from excessive microbial growth, carrot enzymatic browning, increasing of respiration, tissue softening and carrot enzymatic browning, limits storage time and its market value of products (Yu et al., 2018). Minimal processing for fresh-cut carrots production usually includes – shredding, slicing, peeling and dicing before packaging and storage. Processed carrots have a major effect on the quality of the prepared product, amount of nutrients and storage time. To retain valuable carrot nutrients and protect from spoilage, proper packaging and storage conditions play an important role. Most of the producers and suppliers give importance to the vegetables packaging and storage conditions of their product (Islam et al., 2018). Minimal processing for fresh-cut carrots production include grading, washing, sorting, peeling, cutting and shredding, packaging and their storage (Wang et al., 2015). Due to a high respiration rate, loss of firmness, microbial quality and discoloration, minimally processed carrots has shelf life limitation from 4 to 5 days (Augspole et al., 2014).

Wang et al. (2015) informed that minimal processing of shredded carrots causing undesirable microbiological quality, physiological and biochemical changes to treated vegetables, even decreasing the products nutritional quality and shortening shelf life. Saranraj et al. (2016) highlights that water used for rinsing vegetables and sprinkling is a supply of different potential sources of microorganisms embrace soil, water, handling of the merchandise, gathering and process instrumentation and transport. Researcher Oliveira (2017) reported that microbial risk is found during treated carrots, mostly during the washing and shredding steps. In turn cutting operation releases nutrients which can promote microbiological growth. Literature data suggest that packaging is an integral part of the commercial industrial and food supply part. Various packaging technologies can be used for shelf life prolongation of processing products. Each packaging film has a specific O_2 and CO_2 permeability (Krasnova et al., 2012). Packaging not only acts as a barrier to protect the product from environmental obstacles and harmful germs and insects, but also it works as a trade mark of product (Islam et al., 2018).

Krasnova et al. (2012) previously described that *Innovia films Limited* non-perforated and breathable biaxially oriented *BOPP* films *PropaFresh P2GAF and P2G* for fresh vegetable produce packaging are tailor made to fulfil the specific needs of food packaging industry. In turn *BOPP* film a longer shelf life can be ensured with the assurance for freshness being intact and preserved. Some researchers found that inhibitors of browning in processed fruits include using ascorbic acid, citric acid

or their derivates (Rahman et al., 2011). Abubakr (2016) in his study has shown that *NatureSeal® AS1* and *NatureSeal® AS5* products can reduce browning in fresh cut fruit slice. Many authors (Augspole et al., 2017; Saha et al., 2009; Rössle et al., 2009) in their studies emphasize that the N*atureSeal™* is a calcium ascorbate powder used in the food industry. Asrey et al. (2008) in their studies described that *NatureSeal® AS5* is the first antioxidant product of its kind, which doesn't have a bad aftertaste or product residue. In turn researcher Rößle et al. (2009) accented that *NatureSeal® AS5* is already used commercially in Europe and elsewhere, thus adding practical value to the research. Researcher Abubake (2006) described that application of *NatureSeal™* agents is a popular approach for prevention enzymatic browning in fresh-cut vegetables. In her study Augspole et al. (2017) showed that *NatureSeal® AS5* water solution has antimicrobial activity, thus it may be possible that it has a essential effect on the microbiological quality of fresh-cut carrots during storage.

Therefore, the aim of the research was to investigate microbiological quality changes of shredded carrots treated with *NatureSeal® AS5* during storage in several packaging materials.

Materials and Methods

Materials

The object of the research was serotinous ′Nante′ carrot (*Daucus carota* L.) hybrid ′Nante/Forto′ harvested in Latvia, Jelgava region (the GPS-coordinates: 56° 39' 3.992" N 23° 43' 16.874" E).

'Nante/Forto' carrots were rinsed under running tap water and peeled. Then carrots were washed under water. Carrots were shredded using (Philips Comfort HR 7605, Austria). Carrots were shredded (cross-section 1.3×4.0 mm, length 30-45 mm) using shredder (Philips Comfort HR 7605, Austria). *NatureSeal® AS5* powder *AgriCoat NatureSeal™* is a commercial product – blend of dry ascorbic acid and calcium compound patented (No 1011342) by the Ministry of Agriculture the USA (*AgriCoat NatureSeal™* Ltd, England). Prepared *NatureSeal® AS5* solution used for treatment of shredded carrots. Carrots were immersed on prepared solution for 5 min±2 s. Treated carrots were placed on non-corrosive steel sieve (grid diameter 0.2±0.1 mm) to draw of the accumulated water (3 min ± 2 s) then packaged by 70±1 g in conventional and cellulose based biodegradable packaging materials. Polypropylene *DuniForm PP* boxes (thickness: δ=35±1 µm; size: 80×120×42 mm; capacity: 400 mL; barrier properties: not known) were placed in prepared pouches (size 255×245 mm) made of biodegradable *NatureFlex™ NVS INNOVIA* material (thickness: δ=35±1 µm; barrier properties: OTR (oxygen transmission rate): 3000 cm^3 m^{-2} 24 h^{-1}; CO_2TR (carbon dioxide permeability tester through films): 12000 cm^3 m^{-2} 24 h^{-1} and hermetically sealed using the packaging equipment (EUROMATIC, Italia). Packaging boxes *DuniForm PP* were hermetically thawed with *BOPP PropafilmTM P2GAF* "breathable" conventional polymer film covered with an anti-dewing coating (thickness: δ=38±1 µm; barrier properties: OTR (oxygen transmission rate): 3000 cm^3 m^{-2} 24 h^{-1}; CO_2TR (carbon dioxide permeability tester through films): 12000 cm^3 m^{-2} 24 h by means of SEAL 300 Faverani (Italia) equipment. *Nature Works® PLA P–360* boxes with non–hermetically sealed PLA cover (thickness: δ=30.0 µm; barrier properties: WVTR (water vapour transmission rate): 55 g (m^{-2} 24 h^{-1}±10% at 23±2 °C and HR (relative humidity) 85% (ASTM F 1249), OTR (oxygen transmission rate): 500 cm^3 m^{-2} 24 h at 23±2 °C and RH (relative humidity) 50%: (DIN 53380-3). Samples were stored for 12 days in a Commercial Freezer-Cooler "Elcold" at the temperature 4±1 °C.

The headspace gas composition and the content of microorganisms – mesophilic aerobic and facultative anaerobic (total bacteria), yeasts at each time of measurement were tested on sampling day and 3rd, 5th, 8th, 10th, and 12th day of storage. Four identical packs for each packaging mode were randomly selected for testing. Oxygen (O_2) and carbon dioxide (CO_2) was measured using a gas O_2 and CO_2 analyser OXYBABY®V.

Microbiological analysis

Microorganisms in treaded shredded carrots were tested according to the standard method LVS EN ISO 6887-1:1999 and 6887-4:2004. Total plate count (TPC) was determined in conformity standard LVS EN ISO 4833A. Yeast plate count determined in conformity with standard method LVS ISO 21527-2:2008. Microbiological data were expressed as log colony forming units per gram of product (log CFU g^{-1}).

Statistical analyses

The investigation results were processed by statistical and mathematical methods. Two-way analyses of variance ($p \leq 0.05$) were used to determine the significance of differences between various parameters. Statistics of a completely randomized desing were determined using the GLM (General Linear Model) and SPSS 21 (Statistical Package for Social Sciences).

Results and Discussion

Microbiological quality is the main quality parameter of food. Microbiological parameters determining shelf-life of shredded carrots were evaluated as the main quality parameters. Carrots washing, peeling and shredding damages tissue results in the growth of microorganisms. In the present research 2.5% *NatureSeal® AS5* was used as the agent for the processing of fresh shredded carrots. The results demonstrated that treatment was an effective method to hinder microbiological growth during storage and improving the shelf life of products (Fig.1.). Treatment of shredded carrots with 2.5% *NatureSeal® AS5* water solution had a positive effect on total plate count; after treatment it was possible to significantly

($p<0.05$) decrease TPC count by 1.34 log CFU g^{-1} compared to untreated shredded carrots.

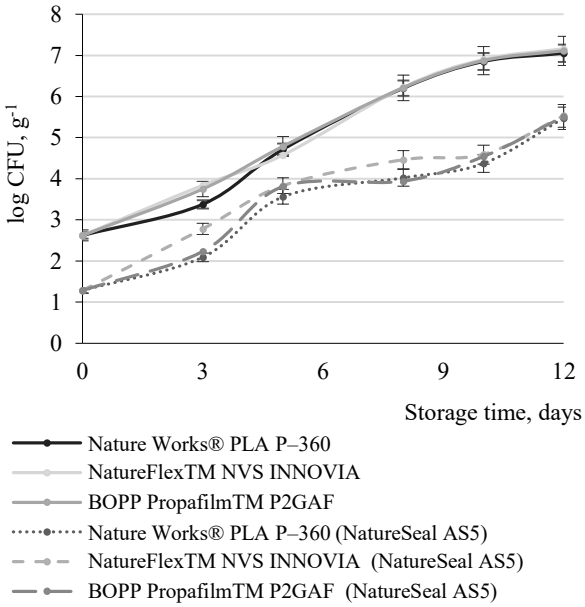

Figure 1. Dynamics of TPC count in shredded carrots treated with *NatureSeal® AS5*

Treated shredded carrots were microbial spoiled by mesopholic aerobic and facultative anaerobic microorganisms after 12 day. Microbial growth increased due to the increase in surface by cutting and peeling. The effect of shelf-life on the TPC was significant ($p<0.05$). Different packaging materials didn't significantly ($p=0.131$) influence changes of microbial results (TPC) in shredded carrots during storage. Researcher Karabagias (2018) reported that yeasts are able to grow in vegetables with a neutral or slightly acid pH environment and in the presence of organic acids, carbohydrates and other easily metabolized carbon sources. He explain that during their growth, yeasts metabolize some produce metabolic end-products and food components. This causes chemical and physical properties of a vegetable to change and the food is spoiled. Literature data suggest that *NatureSeal™* commercial product used by vegetables and fruits industry according to the technology to achieve effective preservation of fresh-cut vegetables. Good microbial quality (microbial loads <5 log CFU g^{-1}) of all processed carrot during 10 day storage, low storage temperature may be connected to the combination of antimicrobial compounds calcium and ascorbic acid (Giacalone, Chiabrando, 2013). In the present research (Fig. 2) from the beginning in the treated carrots yeasts count was 1.31 log CFU g^{-1} and in the non-treated carrots was 2.59 log CFU g^{-1}. However, independently form packaging material, yeast count of treated carrots increased 2.2 times after 8 day, which was significant ($p=0.023$). The results showed that it was possible to ensure microbial quality of carrots by preservation with 2.5% *NatureSeal® AS5* and reduce yeast count by 1.28 log CFU g^{-1} ($p<0.05$) compared to untreated shredded carrots. The current research showed that all used pacing materials didn't significantly ($p=0.158$) affect changes of yeast count in shredded carrots 12 days during storage.

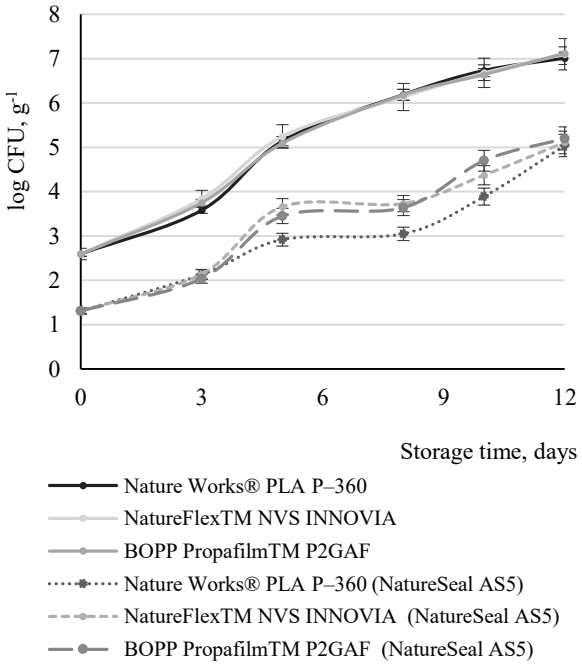

Figure 2. Dynamics of yeast count in shredded carrots treated with *NatureSeal® AS5*

Packaging containers *Nature Works® PPLA P-360* with non-hermetically sealed conditions and conventional breathable *BOPP Propafresh™ P2GAF*, conventional *NatureFlex™ NVS INNOVIA* film and *BOPP Propafilm™ P2GAF* film packing could be characterized as the best environment for respiration and quality maintenance of minimally processed (fresh-cut) produce (Dukalska et al., 2013). One of the tasks of this work was to assess O_2 and CO_2 composition dynamics in packaging with carrots treated with 2.5% *NatureSeal® AS5* (Fig. 3).

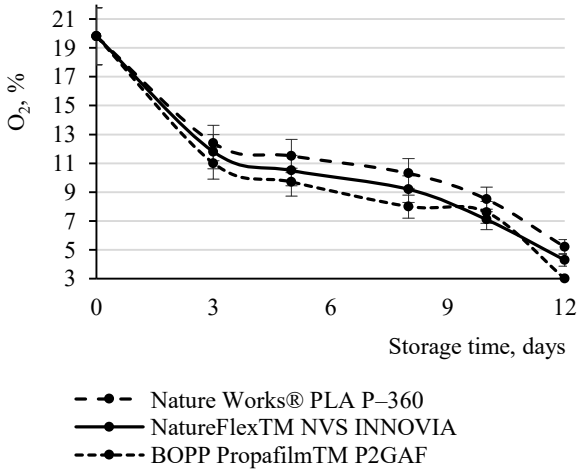

Figure 3. Oxygen content in the packages of shredded carrots treated with *NatureSeal® AS5*

During 12 days of storage an increase of CO_2 and decrease of O_2 was observed depending on the barrier properties of used lidding films. Oxygen content decreased by 19.8% during storage. The decrease of O_2 in different packaging was not significantly different ($p=0.153$). The obtained results showed that observed analysing content of O_2 inside packing with treated carrots. In BIO PLA non-hermetically sealed containers the decrease of O_2 was significant ($p<0.05$) – on the 3rd, 5th and 8th day of storage by 11.6, 4.3 and 11.3%, respectively (fig. 3). Oxygen content inside PLA containers after 10 day storage decreased from 12.7 to 11.3%. The content of oxygen after 12 days inside packaging decreased to 3% on average.

Researcher Augspole et al. (2014) in her study noticed that the increase of CO_2 inside packaging could indicate the beginning of spoilage process and elevated amounts of CO_2 indicate non acceptable quality of packaged shredded carrots. The amount of CO_2 increased 11.3 to 15.8 times (Fig. 4) after 12 day storage.

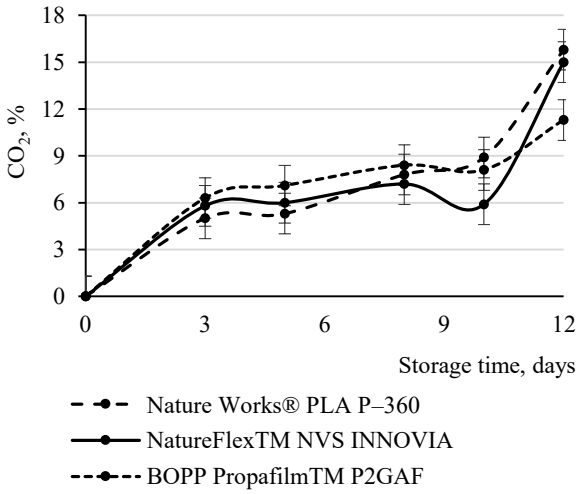

Figure 4. Carbon dioxide content in the packages of shredded carrots treated with *NatureSeal® AS5*

Food scientists have proved that observations prove that both conventional breathable and biodegradable packaging films with an appropriate O_2 transmission rate play an important role in development of equilibrium modified atmosphere and in quality in packages of fresh shredded carrots during storage (Dukalska et al., 2013; Augspole et al., 2012).

Conclusions

Biodegradable packaging materials help to protect microbiological quality of shredded carrots during storage due to their specific barrier properties. During storage, the increase of CO_2 and decrease O_2 was depended on barrier properties of used packaging. The treatment of shredded carrots with 2.5% *NatureSeal® AS5* water solution negatively influenced microbial growth. The results obtained in the current study that cellulose based biodegradable packaging materials can be an alternative to the conventional polymer films for treated fresh shredded carrots.

References

1. Abubakr M.A.S. (2016) Antibrowning activity of bioactive peptides from lab-cultured skim milk hydrolysate. *International Journal of Current Microbiology and Applied Sciences,* Vol. 5(10), p. 212–228.
2. Asrey R., Sasikala C., Barman K., Koley T.K. (2008) Advances in post-harvest treatments of fruits – A review. *Annals of Horticulture,* Vol. 1(1), p. 1–10.
3. Augspole I., Kince T., Skudra L., Dukalska L. (2017) The effect of hydrogen peroxide, ozonised water and Natureseal AS5 solution on the microbiological parameters of fresh-cut carrots. **In:** *FoodBalt 2017: 11th Baltic conference on food science and technology in a changing world": conference proceedings,* Jelgava, p. 112–116.
4. Augspole I., Rakcejeva T., Dukalska L., Skudra L. (2014) Providing quality of shredded carrots during storage by treatment with ozonated water. *Material Science and Applied Chemistry,* Vol. 30, p. 10–17.
5. Augspole I., Dukalska L., Rakcejeva T., Muizniece-Brasava S., Sabovics, M. (2012). Physicochemical quality evalution of feesh-cut carrots packed and stored in biodegradable film packaging. **In:** *Proceedings of 18th International Conference for Renewable Resources and Plant Biotechnology,* NAROSSA, Magdeburg, Germany, in CD.
6. Dukalska L., Ungure E, Augspole I., Muizniece-Brasava S., Levkane V., Rakcejeva T., Krasnova I. (2013) Evaluation of the influence of various biodegradable packaging materials on the quality and shelf life of different food products. *Proceedings of the Latvia* University *of Agriculture,* Vol. 30(325), p. 20–34.
7. Gamboa-Santos J., Soria A.C., Perez-Mateos M., Carrasco J.A., Montilla A., Villamiel M. (2012) Vitamin C content and sensorial properties of dehydrated carrots blanched conventionally or by ultrasound. *Food Chemistry,* Vol. 136(2), p.782–788.
8. Giacalone G., Chiabrando V., (2013) Effect of different treatments with calcium salts on sensory quality of fresh-cut apple. *Food and Nutrition Research*, Vol. 52(2), p. 79–86.
9. Hag R., Prasad K. (2018) Role of various coloring pigments within different varieties of carrot (*Daucus carota*). *Advances in Plants & Agriculture Research,* Vol. 8(5), p. 386–387.
10. Islam S., Hasan M., Salahuddin, Hafiz A.R., Rana S., Sultana R., Mahdi A.A., Mohammed J.K., Begum R., Haque A., Wang H. (2018) Effect of packaging materials & vitamin-C on various storage condition of fresh cut carrot. *International Journal of Life Science and Engineering,* Vol. 3(3), p. 46–58.
11. Karabagias I.K. (2018) Investigation of yeast and mould growth rate in chopped lamb meat packaged under different systems during refrigerated storage. *Acta Scientific Nutritional Health,* Vol. 2(8), p. 47–50.
12. Krasnova I., Dukalska L., Seglina D., Juhnevica K., Sne E., Karklina D. (2012) Effect of passive modified atmosphere in different packaging materials on fresh-cut mixed fruit salad quality during storage. *World Academy of Science, Engineering and Technology*, Vol. 6, p. 7–22.
13. Oliveira A.C.F. (2017) *Effect of abiotic stresses on bioactive contents of vegetables and high-pressure technology in related functional beverages. Doctoral Thesis,* Department of Food Engineering at Universidad Politécnica de Cartagena, 204 p.

14. Rahman S.M.E., Jin Y.G., Oh D.H. (2011) Combination treatment of alkaline electrolyzed water and citric acid with mild heat to ensure microbial safety, shelf life and sensory quality of shredded carrots. *Food Microbiology,* Vol. 28, p. 484–491.
15. Rößle C., Gormley T.R., Butler F. (2009) Efficacy of Natureseal® AS1 browning inhibitor in fresh–cut fruit salads applications, with emphasis on apple wedges. *Horticultural Science and Biotechnology*, Vol. 84(6), p. 62–67.
16. Saha K., Paulsingh S., Harte B. (2009) Effect of transport vibration on quality of minimally processed and packaged fresh-cut cantaloupe. *Applied Packaging Research,* Vol. 3(1), p. 15–26.
17. Saranraj P., Sivasakthivelan P., Sivasakthi S., Kavi Karunya S. (2016) Microbial spoilage and pathogens in vegetables collected from vegetable market. *Weekly Science International Journal,* Vol. 4(3), p. 1–9.
18. Scarano A., Gerardi C., D'Amico L., Accogli R., Santino A. (2018) Phytochemical analysis and antioxidant properties in colored Tiggiano carrots. *Agriculture,* Vol. 8 p. 1–8.
19. Wang X., Kong D., Ma Z., Zhao R. (2015) Effect of carrot puree edible films on quality preservation of fresh-cut carrots. *Irish Journal of Agricultural and Food Research*, Vol. 54(1), p. 64–71.
20. Yu Y., Jiang X., Ramaswamy H.S., Zhu S., Li H. (2018) High pressure processing treatment of fresh-cut carrots: Effect of presoaking in calcium salts on quality parameters. *Journal of Food Quality,* Vol. 4, p. 1–9.

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EVALUATION OF BEETROOT QUALITY DURING VARIOUS STORAGE CONDITIONS

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Abstract

To produce high quality processed products, it is necessary to not only grow new, promising beetroot varieties, but also to preserve their quality during storage. This requires advanced storage technologies, such as storage in a controlled and ultra-low oxygen atmosphere. In these conditions, beetroots retain their quality until the new harvest. This not only maximizes the quality of vegetables, reduces storage losses, but also extends the use of vegetables, which is very important for farmers to profitably of their produce. The aim of the work was to evaluate and optimize the process of storing various varieties of beetroot and evaluate their suitability for processing. Beetroot varieties 'Detroit 2', 'Boro H', 'Boltardy', 'Kestrel H',' Pablo H', 'Bona', 'Wodan H', 'Rhonda H', 'Subeto H', 'Action H', and 'Joniai' were investigated. Increasing the carbon dioxide content from 0.03% up to 3%, 5% or 8% and reducing oxygen content from 21% to 10% or 5% positively impacts the chemical composition of the stored beetroots. Some of the chemical composition parameters after storage in one or another composition in the controlled atmosphere were unchanged, but others remained stable and the degradation of betacyanins was clearly reduced. After the evaluation of various varieties of beetroots after storage, the following varieties were found to be the most suitable for storage and subsequent processing: 'Kestrel H', 'Joniai', 'Pablo H', and 'Rhonda H'. The optimal composition of controlled atmosphere for beetroot storage is 5% carbon dioxide, 5% oxygen and 90% nitrogen.

Keywords: beetroot; chemical composition; controlled atmosphere; storage

Introduction

Beetroots (*Beta vulgaris* L.) is grown and appreciated for its valuable nutritional properties, easy cultivation and good storage. The considerable advantage of beetroots is that they can be used fresh all year round, they can be stored until the fresh harvest. Beetroots are also very good for processing. Due to its valuable nutritional, dietary properties, beetroot demand and consumption has increased in recent years (Wroblewska et al., 2011; Esatbeyoglu et al., 2015; Moding et al., 2018). Betanine strengthens the walls of vascular capillaries, lowers blood pressure, improves fat metabolism and liver function, protects the cells of our body from the harmful effects of free radicals, as well as cancer, cardiovascular diseases, stops aging processes, also has beneficial effect on health with second-degree diabetes (Gilchrist et al., 2014). The biochemical composition of beetroots is determined by the complex of the genetic characteristics of the variety and the growth conditions, on average they accumulate about 11–17% of dry matter, 5–10% of sugars, vitamins C, PP, B_1, B_2, B_6, lots of potassium (Petronienė, Viškelis, 2004). Beetroots accumulate large amounts of nitrates and may exceed 2000 mg kg^{-1}. The beetroot quality can vary significantly from agrometeorological and growth conditions (Zalatorius et al., 2014).

Storage of beetroot varieties has not been investigated enough. Previous studies have shown that beetroot storage depends on storage conditions, variety, genotype, agroclimatic cultivation conditions, fertilization (Tucker et al., 1977; Henze, Baumann, 1979; Badelek et al., 2002; Bundinienė et al., 2007).

To produce high quality processed products, it is necessary not only to grow new, promising beetroot varieties, but also to preserve their quality during storage. This requires advanced storage technologies, such as storage in a controlled and ultra-low oxygen atmosphere. In these conditions, beetroots retain their quality until the new harvest. This not only maximizes the quality of vegetables, reduces storage losses, but also extends the use of vegetables, which is very important for farmers to profitably of their produce. Most of the studies are long overdue, some beetroot varieties and hybrids have been renewed, it is not clear which varieties with their biochemical composition and technological properties are most suitable for processing, it is unclear whether it is worth storing beetroots in a controlled atmosphere and, if so, under what conditions. Storing vegetables in a controlled atmosphere is universally recognized as a perfect storage technology that allows to store not only most of the production but also preserve biologically active components (Simson, Straus, 2010; Viškelis et al., 2012; Lepse et al., 2014). But for vegetables it is an expensive technology. Somehow there is a belief that it is worth storing only expensive products in a controlled atmosphere, such as berries or fruits, but it is not worth storing cheap vegetables. The aim of the work was to evaluate and optimize the process of storing various varieties of beetroot and evaluate their suitability for processing.

Materials and Methods

Research object

Beetroots (*Beta vulgaris* L.) were grown on the test fields of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in 2017. The following varieties were tested: ‘Detroit 2’, ‘Boro H’, ‘Boltardy’, ‘Kestrel H’, ‘Pablo H’, ‘Bona’, ‘Wodan H’, ‘Rhonda H’, ‘Subeto H’, ‘Action H’, and

'Joniai'. Beetroot chemical composition, physico-chemical properties and storage parameters were investigated. Beetroots were cultivated in light loam soil according to the integrated plant cultivation technology adopted by the Institute of Horticulture. Beetroots were harvested in end of September 2017. For analysis, similar sized, raw beetroots were selected.

Quality analysis

Ascorbic acid was measured by titration using 2,6-dichlorophenolindophenol sodium salt solution (AOAC, 1990[a]) by a slight modification as by Viskelis et al. (2010).

Soluble solids were quantified with a digital refractometer PR-32 (Atago Co., Ltd., Japan).

Dry matter content was determined gravimetrically by drying samples to a constant weight at 105 °C.

Monosaccharides, sucrose and total sugars were determined by the Bertrand method (AOAC 1990[b]).

The quantitative and qualitative composition of betalain was determined spectrophotometrically by Wruss et al. (2015) method.

Titratable acidity was estimated by titrating with 0.1 N NaOH solution to pH 8.0 and was expressed as a percentage of citric acid equivalent (Ермаков et al., 1987).

Active acidity (pH) was measured with inoLab pH Level 1 pH meter using SenTix 81 (WTW) electrode.

The amount of nitrates was determined potentiometrically with a selective electrode (Methodological instructions, 1990).

Beetroot colour indexes in the space of even contrast colours were measured with a spectrophotometer MiniScan XE Plus (Hunter Associates Laboratory, Inc., USA) as described in paper of Urbonaviciene et al. (2018). Parameters calculated were chroma (1) and hue angle (2):

$$(C = (a^{*2} + b^{*2})^{1/2}) \quad (1)$$

$$(h^{\circ} = \arctan(\frac{b^*}{a^*})) \quad (2)$$

where: C – chroma;
h° – hue angle;
a* – a colour value (redness);
b* – b colour value (yellowness).

Beetroot firmness was determined by the TA.XTPlus texture analyser (Stable Micro Systems, United Kingdom) using the P/2 probe (2 mm in diameter) (Luksiene et al., 2013).

Storage

All beetroot varieties storage has been carried out in three repositories under optimum conditions, at 1±1 °C and 90–95% relative humidity (RH). Selected varieties for best perspectives for processing and storage ('Kestrel H', 'Pablo H', 'Rhonda H' and 'Joniai') were also stored in Besseling CA Systems (Besseling Group B.V., The Netherlands) controlled atmosphere chambers maintaining a different composition of controlled atmosphere (Table 1). Gas composition, humidity, and storage temperature were selected according to the recommended conditions for storing vegetables in a controlled atmosphere (Thompson, 2010; Simson, Straus, 2010; Lepse et al., 2014). The weight of the roots in the controlled atmosphere was accurately weighed and averaged 15 kg. Quality was was studied during 7-month storage.

Table 1

Storage conditions in controlled atmosphere

No.	CO_2, %	O_2, %	Temperature, °C	RH, %
1	0.03	21	2±1	95
2	3.00	10	2±1	95
3	5.00	10	2±1	95
4	8.00	10	2±1	95
5	5.00	5	2±1	95
6	0.03	21	1±1	95
7	0.03	21	5±1	95

The statistical methods of data processing

All the experiments were carried out in triplicate. Means and standard deviations were calculated with STATISTICA 10 StatSoft, Inc., USA) and Excel (Microsoft, USA) software. One-way analysis of variance (ANOVA) along with the posthoc Tukey's HSD test was employed for statistical analysis. Differences were considered to be significant at $p<0.05$.

Results and Discussion

Changes in dry matter during storage were observed but were not significant (Table 2). After storage, the dry matter content in some beetroot varieties increased, in others decreased. The loss of dry matter occurs due to breathing because it consumes sugars (Viškelienė et al., 2017), but on the other hand, transpiration and water evaporation from the roots are taking place also. That is how two opposing / contradictory physiological processes take place and their relationship is hard to predict. However, in any case, these changes were not statistically reliable. Similar results were obtained with soluble solids (Table 2). During storage, monosaccharide, sucrose and total sugar content varied slightly and results were not statistically significant (Table 2).

Exceptionally high levels of betacyanins and betaxanthin were found in 'Detroit 2' and 'Joniai' beetroots, the highest amount of betalain was in 'Kestrel H' beetroots (Table 3). After storage, the amount of betacyanins and betaxanthin in the 'Detroit 2' and 'Joniai' beetroots decreased significantly, but still the highest amount of betalains remained in these beetroots, 1278 mg kg^{-1} and 1326 mg kg^{-1}, respectively.

The amount of organic acids during storage was reduced or did not change compared to fresh beetroots of the same variety (Table 3). These changes in most varieties were statistically unreliable due to the very low acidity of beetroots.

Statistically reliable post-storage decrease in organic acids was observed only in 'Boltardy', 'Rhonda H' and 'Subeto H' varieties (from 0.10% to 0.08%) ($p<0.05$).

Table 2

Changes in dry matter, soluble solids, monosaccharides, sucrose and total sugars during beetroot storage

Beet variety	Dry matter, %		Soluble solids, %		Monosaccharides, %		Sucrose, %		Total sugars, %	
	Before storage	After storage	Before storage	After storage	Before storage	After storage	Before storage	After storage	Before storage	After storage
Detroit 2	17.1 [a]	15.9 [a]	13.6 [a]	12.6 [a]	1.41 [a]	1.31 [a]	8.23 [a]	8.34 [a]	9.64 [a]	9.65 [a]
Boro H	13.7 [a]	14.6 [a]	11.2 [a]	11.5 [a]	1.16 [a]	1.09 [a]	8.07 [a]	8.18 [a]	9.23 [a]	9.27 [a]
Boltardy	16.0 [a]	17.2 [a]	14.0 [a]	13.9 [a]	1.48 [a]	1.35 [a]	8.30 [a]	8.40 [a]	9.78 [a]	9.75 [a]
Kestrel H	15.4 [a]	15.9 [a]	14.0 [a]	14.0 [a]	1.41 [a]	1.35 [a]	9.12 [a]	9.22 [a]	10.53 [a]	10.57 [a]
Pablo H	13.4 [a]	14.1 [a]	12.0 [a]	12.9 [a]	1.54 [a]	1.44 [a]	8.89 [a]	8.94 [a]	10.43 [a]	10.38 [a]
Bona	15.9 [a]	14.5 [a]	13.8 [a]	11.8 [a]	1.22 [a]	1.22 [a]	7.77 [a]	7.85 [a]	8.99 [a]	9.07 [a]
Wodan H	13.0 [a]	14.1 [a]	11.7 [a]	12.0 [a]	1.22 [a]	1.22 [a]	8.79 [a]	8.22 [a]	10.01 [a]	9.44 [a]
Rhonda H	15.1 [a]	14.0 [a]	12.0 [a]	12.8 [a]	1.22 [a]	1.32 [a]	8.29 [a]	8.09 [a]	9.51 [a]	9.41 [a]
Subeto H	13.1 [a]	13.9 [a]	11.0 [a]	11.5 [a]	1.35 [a]	1.22 [a]	8.29 [a]	8.09 [a]	9.64 [a]	9.31 [a]
Action H	13.8 [a]	14.7 [a]	12.0 [a]	12.6 [a]	1.09 [a]	1.12 [a]	8.14 [a]	8.01 [a]	9.23 [a]	9.13 [a]
Joniai	14.8 [a]	13.6 [a]	13.1 [a]	12.5 [a]	1.28 [a]	1.34 [a]	8.87 [a]	8.75 [a]	10.15 [a]	10.09 [a]

Different letters in "before storage" and "after storage" columns represent statistically significant differences in chemical composition parameters before and after storage.

Table 3

Changes in betacyanin, betaxanthin, acidity, pH and ascorbic acid content during beetroot storage

Beet variety	Betacyanins, mg kg^{-1}		Betaxanthins, mg kg^{-1}		Acidity, %		pH		Ascorbic acid, mg 100 g^{-1}	
	Before storage	After storage	Before storage	After storage	Before storage	After storage	Before storage	After storage	Before storage	After storage
Detroit 2	967 [a]	883 [a]	423 [a]	395 [a]	0.09 [a]	0.08 [a]	5.70 [a]	5.75 [a]	17.6 [a]	15.2 [b]
Boro H	474 [b]	589 [a]	259 [a]	275 [a]	0.08 [a]	0.07 [a]	5.78 [a]	5.78 [a]	16.8 [a]	14.0 [b]
Boltardy	562 [b]	671 [a]	271 [b]	347 [a]	0.10 [a]	0.08 [b]	5.70 [a]	5.77 [a]	16.1 [a]	13.2 [b]
Kestrel H	642 [a]	695 [a]	363 [a]	324 [a]	0.09 [a]	0.09 [a]	5.77 [a]	5.75 [a]	19.5 [a]	16.8 [b]
Pablo H	397 [b]	584 [a]	231 [b]	301 [a]	0.08 [a]	0.08 [a]	5.78 [a]	5.79 [a]	18.2 [a]	15.9 [b]
Bona	589 [a]	648 [a]	309 [b]	371 [a]	0.08 [a]	0.07 [a]	5.72 [a]	5.74 [a]	15.8 [a]	13.5 [b]
Wodan H	595 [a]	674 [a]	327 [b]	401 [a]	0.09 [a]	0.08 [a]	5.72 [a]	5.74 [a]	16.4 [a]	12.2 [b]
Rhonda H	505 [b]	681 [a]	300 [a]	344 [a]	0.10 [a]	0.08 [b]	5.75 [a]	5.76 [a]	18.3 [a]	15.7 [b]
Subeto H	403 [b]	592 [a]	258 [a]	251 [a]	0.10 [a]	0.08 [b]	5.71 [a]	5.74 [a]	13.2 [a]	10.4 [b]
Action H	412 [b]	528 [a]	246 [b]	311 [a]	0.09 [a]	0.09 [a]	5.70 [a]	5.74 [a]	13.4 [a]	10.8 [b]
Joniai	1030 [a]	887 [b]	608 [a]	439 [b]	0.09 [a]	0.08 [a]	5.71 [a]	5.74 [a]	17.8 [a]	15.6 [b]

Different letters in "before storage" and "after storage" columns for each parameter represent statistically significant differences in chemical composition parameters before and after storage.

Table 4

Changes in nitrates, skin and flesh firmness and colour indices during beetroot storage

Beet variety	Nitrates, mg kg^{-1}		Skin firmness, N cm^{-2}		Flesh firmness, N cm^{-2}		Colour a* value		Colour b*value	
	Before storage	After storage	Before storage	After storage	Before storage	After storage	Before storage	After storage	Before storage	After storage
Detroit 2	805 [a]	758 [a]	735 [a]	648 [b]	446 [a]	392 [b]	7.07 [a]	5.16 [b]	0.97 [b]	1.49 [a]
Boro H	717 [a]	705 [a]	549 [a]	532 [a]	338 [a]	322 [a]	8.41 [a]	7.21 [b]	1.61 [a]	1.74 [a]
Boltardy	751 [a]	722 [a]	597 [a]	520 [b]	333 [a]	308 [a]	7.77 [a]	7.89 [a]	1.22 [b]	1.85 [a]
Kestrel H	685 [a]	589 [b]	623 [a]	612 [a]	401 [a]	388 [a]	6.63 [b]	9.92 [a]	1.20 [a]	1.29 [a]
Pablo H	751 [a]	623 [b]	529 [a]	532 [a]	337 [a]	339 [a]	5.99 [b]	8.18 [a]	1.09 [b]	1.88 [a]
Bona	701 [a]	602 [b]	625 [a]	584 [a]	401 [a]	384 [a]	6.08 [a]	6.26 [a]	0.80 [b]	1.52 [a]
Wodan H	701 [a]	741 [a]	594 [a]	578 [a]	355 [a]	275 [b]	6.47 [a]	6.67 [a]	1.01 [b]	2.12 [a]
Rhonda H	701 [a]	680 [a]	663 [a]	655 [a]	416 [a]	394 [a]	9.41 [a]	8.25 [b]	1.24 [b]	2.33 [a]
Subeto H	734 [a]	771 [a]	548 [a]	551 [a]	354 [a]	348 [a]	6.42 [b]	10.91 [a]	0.94 [b]	2.10 [a]
Action H	769 [a]	797 [a]	567 [a]	551 [a]	357 [a]	326 [a]	4.90 [b]	10.71 [a]	0.52 [b]	2.93 [a]
Joniai	670 [a]	642 [a]	766 [a]	611 [b]	478 [a]	385 [b]	8.62 [a]	7.33 [b]	1.39 [b]	1.80 [a]

Different letters in "before storage" and "after storage" columns for each parameter represent statistically significant differences in beetroot quality parameters before and after storage.

During storage, pH tended to increase, but the results of the pH increase were statistically insignificant (Table 3). The amount of ascorbic acid in beetroots decreased statistically reliably (Table 3). Average loss of vitamin C during storage was about 15–20% from its original content in fresh beetroots. After 7 months of storage, the least ascorbic acid decrease was observed in 'Kestrel H', 'Pablo H', 'Rhonda H' and 'Joniai' varieties. Changes in nitrate content in the stored beetroots was not statistically significant in most varieties, except in ''Kestrel H', 'Pablo H', and 'Bona' varieties (Table 4).

Colour measurement also showed degradation of some compounds during storage. Beetroots of all varieties tested darkened during storage, i.e., their brightness coordinate L* decreased. The redness coordinate (a*) in most cases increased, especially in 'Subeto H', 'Action H', 'Kestrel H', 'Pablo H' varieties. However, the yellowness value (b*) increased significantly during storage in all varieties (Table 4). This indicates that biochemical and certain degradation processes occur during the storage of beetroots. In the case of beetroot storage, the colour purity (C*, chroma) varied unevenly: in some varieties it decreased, but in the majority of varieties increased. After storage, 'Subeto H', 'Action H', 'Kestrel H', 'Pablo H' varieties had the purest colour.

After 7 months of storage, the firmness of the beetroots reduced for both the skin and flesh (Table 4). The decrease in the firmness of both skin and flesh can be explained by the fact that beetroots still mature and age, hydrolysis of insoluble protopectin to soluble pectin takes place etc. The most important indicator for storage is the percentage of production after storage. The average production of beetroots was 67% after storage, hence, storage losses averaged 33%, similar as storage of onions (Cizauskas, Viskelis, 2002). The beetroots of 'Kestrel H' variety remained up to 78%. Other good varieties for storage were 'Pablo H', 'Bona', 'Subeto H' and 'Joniai'. Although storage losses seem great, but stored beetroots were quite acceptable. Storage loss consists of natural loss of mass due to respiration, transpiration and rotting. After storage the beetroot ringing had a clear tendency to decrease. During storage, due to the decrease in the firmness, betalains diffused into the lighter layers of the flesh, so in the overall assessment the internal ringing were increased. The 'Joniai' beetroots were the only one of the studied varieties to have no ringing after storage. After the total evaluation of the various varieties of beetroots after storage, the following varieties were found to be the most suitable for storage and processing: 'Kestrel H', 'Joniai', 'Pablo H' and 'Rhonda H'.

Changes in chemical composition and physical properties such as firmness, colour, etc. during storage are determined not only by the genotype of the variety, but also by the agrotechnical tools used (Petronienė, Viškelis, 2004; Bundinienė et al., 2007).

Beetroot is susceptible to boron deficiency, and the use of boron fertilizers not only increases yield but also reduces storage losses due to physiological damage related to the lack of boron (Bundinienė et al., 2017).

Table 5

Changes in betacyanin, betaxanthin, nitrates, flesh firmness and redness colour in beetroots during storage in controlled atmosphere chambers

Variant*	Beet variety	Betacyanins, mg kg^{-1}		Betaxanthins, mg kg^{-1}		Nitrates, mg kg^{-1}		Flesh firmness, N cm^{-2}		Colour a* value	
		Before storage	After storage	Before storage	After storage	Before storage	After storage	Before storage	After storage	Before storage	After storage
1	Kestrel H	643 ^{a}	678 ^{a}	364 ^{a}	328 ^{b}	685 ^{a}	584 ^{b}	401 ^{a}	375 ^{a}	6.63 ^{b}	8.97 ^{a}
	Pablo H	397 ^{b}	574 ^{a}	232 ^{b}	298 ^{a}	751 ^{a}	611 ^{b}	337 ^{a}	324 ^{a}	5.99 ^{b}	7.24 ^{a}
	Rhonda H	505 ^{b}	687 ^{a}	300 ^{a}	321 ^{a}	701 ^{a}	644 ^{a}	416 ^{a}	384 ^{a}	9.41 ^{a}	8.35 ^{b}
	Joniai	1031 ^{a}	866 ^{b}	608 ^{a}	558 ^{a}	670 ^{a}	621 ^{a}	478 ^{a}	359 ^{b}	8.62 ^{a}	7.66 ^{b}
2	Kestrel H	643 ^{a}	655 ^{a}	364 ^{a}	378 ^{a}	685 ^{a}	579 ^{b}	401 ^{a}	395 ^{a}	6.63 ^{b}	7.87 ^{a}
	Pablo H	397 ^{a}	324 ^{b}	232 ^{b}	378 ^{a}	751 ^{a}	688 ^{a}	337 ^{a}	331 ^{a}	5.99 ^{a}	6.19 ^{a}
	Rhonda H	505 ^{b}	621 ^{a}	300 ^{a}	304 ^{a}	701 ^{a}	624 ^{b}	416 ^{a}	395 ^{a}	9.41 ^{a}	8.99 ^{a}
	Joniai	1031 ^{a}	977 ^{a}	608 ^{a}	558 ^{a}	670 ^{a}	655 ^{a}	478 ^{a}	395 ^{b}	8.62 ^{a}	8.33 ^{a}
3	Kestrel H	643 ^{a}	635 ^{a}	364 ^{a}	354 ^{a}	685 ^{a}	644 ^{a}	401 ^{a}	391 ^{a}	6.63 ^{b}	8.92 ^{a}
	Pablo H	397 ^{b}	484 ^{a}	232 ^{b}	322 ^{a}	751 ^{a}	711 ^{a}	337 ^{a}	321 ^{a}	5.99 ^{a}	6.18 ^{a}
	Rhonda H	505 ^{b}	601 ^{a}	300 ^{a}	304 ^{a}	701 ^{a}	688 ^{a}	416 ^{a}	402 ^{a}	9.41 ^{a}	9.25 ^{a}
	Joniai	1031 ^{a}	924 ^{b}	608 ^{a}	589 ^{a}	670 ^{a}	649 ^{a}	478 ^{a}	426 ^{b}	8.62 ^{a}	8.35 ^{a}
4	Kestrel H	643 ^{a}	652 ^{a}	364 ^{a}	358 ^{a}	685 ^{a}	642 ^{a}	401 ^{a}	396 ^{a}	6.63 ^{a}	6.91 ^{a}
	Pablo H	397 ^{a}	414 ^{a}	232 ^{a}	244 ^{a}	751 ^{a}	678 ^{b}	337 ^{a}	339 ^{a}	5.99 ^{a}	6.18 ^{a}
	Rhonda H	505 ^{b}	642 ^{a}	300 ^{a}	289 ^{a}	701 ^{a}	689 ^{a}	416 ^{a}	421 ^{a}	9.41 ^{a}	9.38 ^{a}
	Joniai	1031 ^{a}	977 ^{a}	608 ^{a}	587 ^{a}	670 ^{a}	655 ^{a}	478 ^{a}	447 ^{a}	8.62 ^{a}	7.98 ^{a}
5	Kestrel H	643 ^{a}	681 ^{a}	364 ^{a}	351 ^{a}	685 ^{a}	587 ^{b}	401 ^{a}	405 ^{a}	6.63 ^{a}	6.85 ^{a}
	Pablo H	397 ^{a}	391 ^{a}	232 ^{a}	244 ^{a}	751 ^{a}	713 ^{a}	337 ^{a}	322 ^{a}	5.99 ^{a}	6.25 ^{a}
	Rhonda H	505 ^{a}	491 ^{a}	300 ^{a}	311 ^{a}	701 ^{a}	710 ^{a}	416 ^{a}	399 ^{a}	9.41 ^{a}	9.11 ^{a}
	Joniai	1031 ^{a}	977 ^{a}	608 ^{a}	588 ^{a}	670 ^{a}	688 ^{a}	478 ^{a}	459 ^{a}	8.62 ^{a}	8.58 ^{a}
6	Kestrel H	643 ^{a}	654 ^{a}	364 ^{a}	333 ^{a}	685 ^{a}	628 ^{a}	401 ^{a}	389 ^{a}	6.63 ^{b}	8.12 ^{a}
	Pablo H	397 ^{a}	441 ^{a}	232 ^{b}	288 ^{a}	751 ^{a}	623 ^{b}	337 ^{a}	328 ^{a}	5.99 ^{b}	7.18 ^{a}
	Rhonda H	505 ^{a}	521 ^{a}	300 ^{b}	352 ^{a}	701 ^{a}	694 ^{a}	416 ^{a}	391 ^{a}	9.41 ^{a}	8.67 ^{a}
	Joniai	1031 ^{a}	977 ^{a}	608 ^{a}	576 ^{a}	670 ^{a}	652 ^{a}	478 ^{a}	378 ^{b}	8.62 ^{a}	7.98 ^{a}
7	Kestrel H	643 ^{b}	721 ^{a}	364 ^{a}	387 ^{a}	685 ^{a}	541 ^{b}	401 ^{a}	358 ^{b}	6.63 ^{b}	9.98 ^{a}
	Pablo H	397 ^{b}	598 ^{a}	232 ^{b}	322 ^{a}	751 ^{a}	601 ^{b}	337 ^{a}	301 ^{b}	5.99 ^{b}	8.48 ^{a}
	Rhonda H	505 ^{b}	699 ^{a}	300 ^{b}	358 ^{a}	701 ^{a}	621 ^{b}	416 ^{a}	358 ^{b}	9.41 ^{a}	7.25 ^{b}
	Joniai	1031 ^{a}	799 ^{b}	608 ^{a}	421 ^{b}	670 ^{a}	611 ^{a}	478 ^{a}	342 ^{b}	8.62 ^{a}	7.01 ^{b}

Different letters in "before storage" and "after storage" columns for each parameter represent statistically significant differences in beetroot quality parameters before and after storage.

* Storage conditions are given in Table 1

Beetroots were stored not only under optimal conditions, i.e., at +1±1° C and 90–95% relative humidity, but also selected varieties for best perspectives for processing and storage ('Kestrel H', 'Pablo H', 'Rhonda H' and 'Joniai') were also stored in Besseling CA Systems controlled atmosphere chambers maintaining a different composition of controlled atmosphere (Table 1). During storage, the controlled atmosphere chambers were monitored not only for temperature, humidity, oxygen and carbon dioxide concentrations, but also for the amount of endogenous ripening and aging hormone ethylene. Ethylene was permanently removed catalytically.

While analysing changes in chemical composition during storage (Table 5), it can be concluded that increasing the carbon dioxide content from 0.03% up to 3%, 5% or 8% and reduced oxygen content from 21% to 10% or 5% positively impacts chemical composition of the stored beetroots. Some of the chemical composition parameters after storage in one or another composition in the controlled atmosphere remained stable and the degradation of betacyanins was clearly reduced. Beetroots kept in a controlled atmosphere retained a more stable colour, had lower colour changes after storage compared to fresh beetroots. Similarly, beetroot firmness remained closer to the firmness of the fresh beetroots.

From the results of chemical composition, colour coordinates and texture (Table 5), it can be concluded that the best regime to store the beetroots is in atmosphere containing 5% of carbon dioxide and 5% of oxygen. At higher oxygen levels, both chemical and physical indicators suffer a little more during storage. This optimal composition in the controlled atmosphere increases around 10% of standard production compared to storage in simple storage chambers. Beetroot 'Joniai' which was rated very well by its chemical composition and physical properties, while stored in basic storage, they kept only moderately well, but it was excellent in a controlled atmosphere, with storage losses of just 24%.

Conclusions

After examining the chemical composition, texture and colour coordinates of various varieties of beetroots, the optimal varieties for processing are 'Joniai', 'Rhonda H', 'Kestrel H', and 'Pablo H'. However, it is difficult to select the best varieties because each variety has its own advantages and disadvantages, and each variety needs to be selected for a particular product or specific function. The optimal composition of the controlled atmosphere storage for beetroots is 5% CO_2, 5% O_2 and 90% N_2. It increases up to 10% of standard production compared to storage in simple storage chambers.

References

1. AOAC. (1990[a]) Vitamin C (ascorbic acid) in vitamin preparations and juice. Helrich K. (ed.) *Official Methods of Analysis*, 15th edn, AOAC Inc., Arlington, VA, p. 1058.
2. AOAC. (1990[b]) Sucrose in fruits and fruit products. Helrich K. (ed.), *Official Methods of Analysis*. 15th end, AOAC Inc., Arlington, VA, 922 p.
3. Badelek E., Adamicki F., Elkner K. (2002) The effect of temperature, cultivar and root size on quality and storage ability of red beet. *Vegetable Crops Research Bulletin,* Vol. 56, p. 67–76.
4. Bundinienė O., Viškelis P., Zalatorius V. (2007) Papildomo tręšimo per lapus įtaka raudonųjų burokėlių derliui ir šakniavaisių kokybei. *Sodininkystė ir daržininkystė*, Vol. 26(1), p. 108–118.
5. Bundinienė O., Zalatorius V., Starkutė R. (2017) Naujos kartos trąšų įtaka perdirbti skirtų burokėlių derliui ir jo kokybės rodikliams. *Sodininkystė ir daržininkystė*, Vol. 36(2-4), p. 36–46.
6. Cizauskas A., Viskelis P. (2002) Influence of nitrogenous fertilizers on onion yield, quality, and storability. **In***: Plant Nutrition: Growth and Diagnosis*. Editors R. Dris, F.H. Abdelaziz, S.M. Jain. Science Publishers, Inc., Enfield, USA, Plymouth, UK, p. 129–135.
7. Esatbeyoglu T., Wagner A.E., Schini-Kerth V.B., Rimbach G. (2015) Betanin-A food colorant with biological activity. *Molecular Nutrition and Food Research*, Vol. 59, p. 36–47.
8. Gilchrist M., Winyard P.G., Fulford J., Annin, C., Shore A.C., Benjamin N. (2014) Dietary nitrate supplementation improves reaction time in type 2 diabetes: Development and application of a novel nitrate-depleted beetroot juice placebo. *Nitric Oxide*, Vol. 40, p. 67–74.
9. Henze J., Baumann. H. (1979) Quality of red beet (*Beta vulgaris* L.) as affected by storage conditions. *Acta Horticulturae*, Vol. 93, p. 59–66.
10. Lepse L., Viskelis P., Lepsis J., Bimsteine G. (2014) Influence of controlled atmosphere on the carrot storage quality. *Acta Horticulturae*, Vol. 1033, p. 59–64.
11. Luksiene Z., Buchovec I., Viskelis P. (2013) Impact of high-power pulsed light on microbial contamination, health promoting components and shelf life of strawberries. *Food Technology and Biotechnology,* Vol. 51(2), p. 284–292.
12. Methodological instructions for the determination of nitrates in crop production. (In Lithuanian). (1990) Vilnius, 41 p.
13. Moding K.J., Ferrante M.J., Bellows L.L., Bakke A.J., Hayes J.E., Johnson S.L. (2018) Variety and content of commercial infant and toddler vegetable products manufactured and sold in the United States. *American Journal of Clinical Nutrition*, Vol. 107, p. 576–583.
14. Petronienė O.D., Viškelis P. (2004) Biochemical composition and preservation of various red beet cultivars. *Sodininkystė ir daržininkystė*, Vol. 23(3), p. 89–97.
15. Simson S. P. Straus M. C. (2010) Post-harvest technology of horticultural crops. Oxford, 315 p.
16. Thompson A. K. *Controlled atmosphere storage of fruits and vegetables, 2nd edition*. CABI, 2010, 272 p.
17. Tucker W.G., Ward C.M., Davies A.C. (1977) An assessment of the long-term storage methods for beetroot, *Acta Horticulturae*, Vol. 62, p. 169–180.
18. Urbonavičienė D., Bobinaitė R., Trumbeckaitė S., Raudonė L., Janulis V., Bobinas Č., Viškelis P. (2018). Agro-industrial tomato by-products and extraction of functional food ingredients. *Zemdirbyste-Agriculture*, Vol. 105(2), p. 63–70.
19. Viškelienė A., Samuolienė G., Karklelienė R., ViškelisP., Sasnauskas A., Duchovskis P. (2017) Quality and developmental changes in white head cabbage (*Brassica oleracea* L.) and radish (*Raphanus sativus* L.) during

winter storage. *Zemdirbyste-Agriculture*, Vol. 104(3), p. 229–234
20. Viškelis P., Bobinaitė R., Lepse L., Lepsis J., Viškelis J. (2012) Quality changes of green onions stored in modified atmosphere. *Sodininkystė ir daržininkystė*, Vol. 31(1-2), p. 40-49.
21. Viskelis P., Rubinskienė M., Bobinaitė R., Dambrauskienė E. (2010) Bioactive compounds and antioxidant activity of small fruits in Lithuania. *Journal of Food, Agriculture and Environment*, Vol. 8(3-4), p. 259–263.
22. Wroblewska M., JuskiewiczJ., Wiczkowski W. (2011) Physiological properties of beetroot crisps applied in standard and dyslipidaemic diets of rats. *Lipids in Health and Disease*, Vol. 10, p. 178–185.
23. Wruss J., Waldenberger G., Huemer S., Uygun P., Lanzerstorfer P., Müller U., Hoglinger O., Weghuber, J. (2015) Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *Journal of Food Composition and Analysis*, Vol. 42, p. 46–55.
24. Zalatorius V., Bundinienė O., Kavaliauskaitė D. (2014) Intensyvi valgomųjų burokėlių auginimo technologija. *Naujausios rekomendacijos žemės ir miškų ūkiui*. Akademija, Kėdainių, p. 59–64.
25. Ермаков А.И., Арасимович В.В., Ярош Н.П., Перуанский Ю.В., Луковникова Г.А., Иконникова М.И. (1987) *Методы биохимического исследования растений*, ВО Агропромиздат, 431 p.

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APPLE SQUEEZE AND SUGAR BEET MOLASSES APPLICATION FOR YEAST INVERTASE PRODUCTION

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Abstract

Biomass obtained from sugar and apple juice production is rich in nutrients therefore could be reused for microbial fermentation and biologically active compounds production. The aim of research was to apply by-products obtained from food processing, such as sugar beet molasses and apple squeeze for invertase production using yeast strains belonging to *Kluyveromyces* genus and to evaluate the influence of various factors (temperature, pH and metal ions) on extracellular invertase production and stability. Invertase production increased by 5.33 and 9.99 times, respectively, using *Kluyveromyces marxianus* DSM 5422 and *Kluyveromyces lactis* var. *lactis* DSM 70799 in molasses medium in comparison with traditional yeast propagation medium (YPD medium made by 10 g of yeast extract, 20 g of peptone and 20 g of glucose). Whereas invertase production increased by 5.91 and 4.40 times, respectively, using *K. marxianus* DSM 5422 and *K. lactis* var. *lactis* DSM 70799 in apple squeeze comparing with traditional YPD medium. The highest activity of invertase was observed at 55 °C and pH 4.5. Effectors such as Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Na^{+} ions significantly decreased invertase activity. The results confirmed that sugar beet molasses and apple squeeze are suitable as nutrients source for invertase producing yeast propagation and invertase production.

Keywords: *Kluyveromyces*, invertase, yeast, sugar beet molasses, apple squeeze

Introduction

Yeast is widely used in the food industry, especially in the confectionery and beverage industry. Yeasts are rich in proteins, thiamine, riboflavin, niacin, selenium and zinc, vitamins D, C and B group, therefore they are increasingly used as a natural food supplement or even in the cosmetics industry. Yeast has body-building properties: reduces the likelihood of cancer, improves overall well-being of the body, reduces cholesterol, improves digestion, promotes hair growth, strengthens nails and has a positive effect on the skin (Axe, 2015).

Yeast (*Saccharomyces, Candida*, etc.) and bacteria (*Bacillus, Escherichia*, etc.) produce invertase. Invertase catalyses α-1,4 glycosidic linkage between α-D-glucose and β-D-fructose molecules of sucrose by hydrolysis releasing monosaccharides such as glucose and fructose. In yeast, enzyme invertase can be intracellular and extracellular, but extracellular invertase is more economical, easier to purify and more stable (Kulshrestha et al., 2013). Moreover, the same strain of yeast invertase could exist in several forms. For instance, intracellular invertase has a molecular weight of 135 000 Daltons, while extracellular invertase has a weight of 270 000 Daltons (Nakano et al., 2000). The enzyme is usually purified by ammonium sulphate or gel filtration (Gascon, Lampen, 1968). This enzyme is used in the food industry, especially for inverted sugar production. Invertase catalyses the hydrolysis of sucrose into glucose and fructose mixtures. Enzyme invertase enhances the immune system, helps protect the body from ulcers and other digestive diseases (Group, 2013). It has wide range of commercial applications including the production of confectionery with liquid or soft canters, chocolates, candy products, fondants, after dinner mints, fermentation of cane molasses into ethanol, and production of lactic acid, artificial honey, cosmetics, plasticisers, paper industry and pharmaceuticals (Ilyina et al., 2016; Uma et al., 2012).

The by-products of food processing are increasingly common for the development of sustainable production of bioproducts. By-products help to improve the economy in many industries and protect the environment from pollution. Uma et al. (2010) used fruit peel waste as substrate in the experiment and concluded that it has good potential for biotechnological applications. The enzyme industry can benefit employing molasses for invertase production, due to low enzyme production costs (Veana et al., 2014).

Uma et al. (2010) suggested that metal ions could protect the enzyme against denaturation at high temperature or pH. Enzymatic fixation in a particular phase is an increasingly used method of enzymatic engineering, improving enzyme stability, resistance to various factors and the potential for repeated or continuous use (Mickevičius, 2008). Encapsulation in calcium alginate is a simple and inexpensive invertase immobilization method.

The aim of this work was to evaluate the influence of various factors (temperature, pH, metal ions) on extracellular invertase production and stability.

Materials and Methods

Microorganisms cultivation

Yeast *Kluyveromyces marxianus* DSM 5422 and *Kluyveromyces lactis* var. *lactis* DSM 70799 were purchased from Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures GmbH. Yeast have been propagated using a nutrient broth (Liofilchem, Italy) medium consisting of a 10 g of yeast extract, 20 g of peptone and 20 g of glucose (YPD medium) at 25 °C for 24 hours and used for further experiment. For solid media preparation, YPD medium supplemented with 18 g of agar (Liofilchem, Italy) has been used.

Invertase assay

Fresh yeast culture after 24 hours of propagation was centrifuged at 10000 rpm for 5 minutes at 4 °C temperature. The supernatant was used as invertase source for assay. Invertase activity was assayed according to the Sigma-Aldrich protocol (Sigma-Aldrich). Blank sample was composed of 0.9 mL of acetate buffer (100 mM, pH 4.5) and 0.1 mL of crude enzyme solution. 0.2 mL of acetate buffer solution (100 mM, pH 4.5) and 0.8 mL of sucrose solution were added to the control tube. It was mixed 0.8 mL of 10 mg mL^{-1} sucrose as the substrate and 0.1 mL acetate buffer (0.1 M pH 4.5) test the tubes and incubated at 55 °C for 10 minutes. The reaction was started by addition of 0.1 mL of crude enzyme solution and test tubes were incubated at 55 °C for 30 minutes. After enzymatic hydrolysis, a sample of 0.1 mL was taken from each tube and mixed with 5 mg mL^{-1} 4-hydroxybenzenkarbohydrazide solution. The mixture was heated in boiling water bath for 5 minutes. After the cooling, the tubes were immediately cooled to reach room temperature. After cooling, 9 mL of distilled water was added in each tube. The intensity of the colour was read at 410 nm in UV spectrophotometer (Genesys 10 UV). Standard curve was performed with glucose solution. One unit of enzyme activity was defined as the amount of enzyme required for release 1 µmol of glucose mL^{-1} $minute^{-1}$ under assay condition. Enzyme activity was expressed in International units. Invertase activity was calculated using this formula:

$$U\ mL^{-1} = \frac{GEV \times PF}{30 \times 0.1 \times 2} \qquad (1)$$

where:

GEV – value of glucose equivalent in a standard curve, µmol;
PF – dilution factor – 10;
30 – time (in minutes) of assay;
0.1 – volume (in millilitres) of crude enzyme;
2 – conversion factor: 1 µmole of sucrose being hydrolysed to glucose and fructose.

Activity of immobilized invertase was calculated using this formula:

$$U\ mg^{-1} = \frac{a}{b} \qquad (2)$$

where:

a – calculated units of enzyme activity per millilitre of reaction mixture, U mL^{-1};
b – mass of immobilized enzyme capsules in reaction mixture, mg mL^{-1}.

Effect of pH and temperature on invertase activity

Enzymatic hydrolysis was performed at different pH values: 2.5; 3.5; 4.5; 5.5; 6.5 using appropriate buffer at 55 °C. When the effect of temperature was investigated, enzymatic hydrolysis was performed at different temperature: 20 °C, 30 °C, 40 °C, 50 °C, 55 °C, 60 °C, 70 °C, 80 °C in 0.1 M acetate buffer at pH 4.5. Invertase activity was determined by the invertase activity assay method described above by performing enzymatic hydrolysis at different pH values.

Effect of different metal ions on invertase activity

To evaluate metal ions influence on the enzyme invertase activity various salts as $MgSO_4$, $CaCl_2$, $CuSO_4$, $ZnSO_4$, NaCl have been added at 0.5 M and enzymatic hydrolysis was performed for 30 minutes at 55 °C pH 4.5. Invertase activity was determined by the invertase activity assay method described above by performing enzymatic hydrolysis with effectors.

Effect of various carbon source on invertase activity

The effect of carbon source on the invertase activity was determined by using fructose (Merck, Germany), lactose (Merck, Germany) and various concentrations (10, 20 and 30 g L^{-1}) of sucrose (Merck, Germany), glucose (Eurochemicals, Lithuania) and various concentrations (20, 40 and 60 g L^{-1}) by-products such as molasses and apple squeeze obtained after food processing. Apples were obtained from the supermarket, washed, crushed, squeezed and separated to the juice and the squeezes. 1% of fresh (72 hours cultivated) *K. marxianus* DSM 5422 and *K. lactis* var. *lactis* DSM 70799 yeast cultures were added. Yeast were grown at 28 ° C in thermostatic shaker (160 rpm) for 72 hours. The activity of the enzyme was measured after 24, 48 and 72 hours of yeast propagation. Invertase activity was determined according to the method described above.

Invertase immobilization

Yeast cells were immobilized using calcium alginate. 4% of sodium alginate solution was mixed with crude invertase solution and poured using syringe with a needle into 20 mL of a 0.25 M $CaCl_2 \times 2H_2O$ solution, which was slowly stirred in a glass, placed on a magnetic stirrer. After completion of the drip, 30 minutes were allowed to settle. Immobilized enzymes in calcium alginate capsules were washed with distilled water and drained by filtering through filter paper. Measured precise volume of $CaCl_2$ solution. To evaluate the efficiency of immobilization, the invertase activity was measured in initial crude enzyme solution and in $CaCl_2$ solution (including washing water) as described above. Invertase activity was measured in the same manner as before immobilization, but 1 g of the capsules was taken instead of 0.1 mL of the crude invertase solution. Invertase activity was calculated using this formula:

$$IY(\%) = (1-Y/X) \times 100\%, \qquad (3)$$

where:

Y – remain enzyme activity in $CaCl_2$ solution after immobilization, U;
X – enzyme activity before immobilization, U.

Results and Discussion

Effect of pH and temperature on invertase activity

Invertase activity and stability are influenced by the pH of the medium, which determines the dissociation of the base and acidic functional groups of the enzyme active site and the remaining parts of the apoferment

(Mickevičius, 2008). Enzymatic hydrolysis was performed at different pH values: 2.5; 3.5; 4.5; 5.5; 6.5 (Fig. 1a). Maximum invertase activity of *K. marxianus* DSM 5422 and *K. lactis* var. *lactis* DSM 70799 was recorded at pH 4.5. Aburigal et al. (2014) researched pH influence on yeast invertase and results showed that the highest invertase activity was found at pH 4.5. The invertase activity of *K. marxianus* DSM 5422 and *K. lactis* DSM 70799 respectively decreased to 60 and 96% compared with optimal pH value, at pH 4.5.

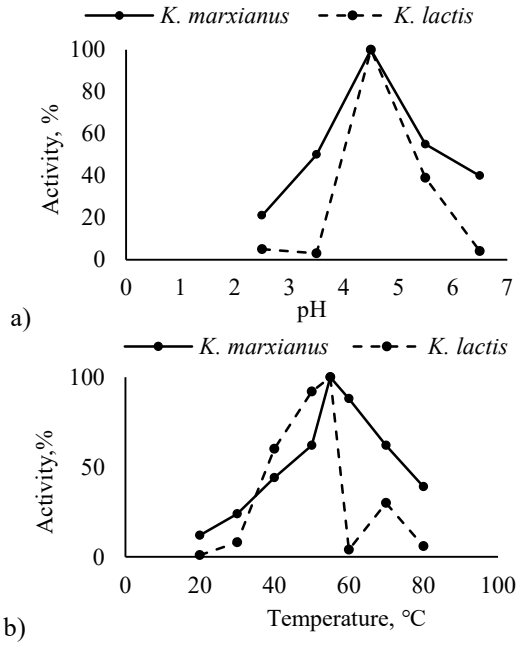

Figure 1. Effect of pH (a) and temperature (b) on invertase activity

Enzymatic hydrolysis was performed at different temperature: 20 °C, 30 °C, 40 °C, 50 °C, 55 °C, 60 °C, 70 °C, 80 °C in 0.1 M acetate buffer at pH 4.5 for 30 minutes (Fig. 1b). It was determined that optimal temperature for invertase is 55 °C. Kaur and Sharma (2005) also investigated temperature effect on invertase activity and results showed that maximum invertase activity was between 50–60 °C. However, invertase activity significantly decreased at 20 °C temperature. It can be concluded that as the temperature rises, the reaction rate increases and therefore the activity is higher, but at high temperature hydrogen bonds break off, occur changes in protein conformation, proteins denature, and therefore activity no longer occurs, the reaction is slowing down. At low temperatures, the hydrolysis reaction of the substrate, catalysed by enzymes, occurs slowly (Mickevičius, 2008).

Effect of different metal ions on invertase activity

The effector investigation was carried out using various metal ions to find out whether the metal ions act as yeast invertase activators or inhibitors. According to literature data, metal ions should protect enzymes from denaturing at high temperatures, but after experimenting at 55 °C it was observed that metal ions inhibit invertase activity (Uma et al., 2010). Crude enzyme invertase solution was mixed with different metal ions such as Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Na^{+} for 30 minutes at 55 °C pH 4.5 (Fig. 2).

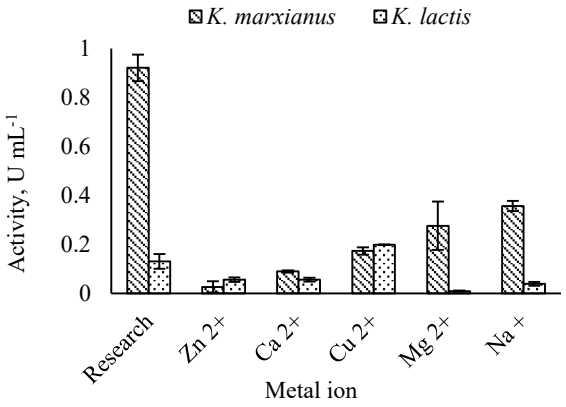

Figure 2. Effect of metal ions on invertase activity

It was found out that all metal ions at used concentrations acted as inhibitors and decreased *K. marxianus* DSM 5422 invertase activity from 100 to 3%. Meanwhile, *K. lactis* DSM 70799 invertase activity decreased by Mg^{2+}, Ca^{2+}, Zn^{2+}, Na^{+}, but Cu^{2+} ions increased invertase activity. Shankar et al. (2013) stated that maximum invertase activity was recorded using calcium chloride for invertase. In Workman and Day (1983) experiment the cations Hg^{2+}, Ag^{+}, Cu^{2+} and Cd^{2+} exhibited a noticeable inhibition of the enzyme.

Effect of various carbon source on invertase activity

According to the literature, the highest invertase activity was determined using sucrose for yeast production as a carbon source (Uma et al., 2010).

The effect of carbon source on the invertase activity was determined by using various concentrations of sucrose, glucose, fructose, lactose and by-products of food processing such as molasses and apple squeeze.

Crude enzyme activity depended on carbon source (fructose, lactose and different concentrations sucrose and glucose (10 g L^{-1}, 20 g L^{-1}; 30 g L^{-1}) also on fermentation duration. Using sucrose as a carbon source invertase had higher activity compared with other sources approximately 1.5 times. *K. marxianus* DSM 5422 yeast had the highest activity using 20 g L^{-1} sucrose as carbon source after 48 hours (0.733 U mL^{-1}), while *K. lactis* var. *lactis* DSM 70799 yeast invertase was the most active after 48 hours of fermentation using 10 and 20 g L^{-1} sucrose, accordingly 0.264 and 0.271 U mL^{-1}. Invertase activity was decreased using 30 g L^{-1} sucrose (Fig. 3a). Uma et al. (2010) tested sucrose, fructose, glucose and lactose as carbon source and they claimed sucrose gave the best results.

Using lactose as a substrate *K. marxianus* DSM 5422 yeast invertase activity increased during fermentation time, while *K. lactis* DSM 70799 yeast invertase activity decreased (Fig. 3b). Using fructose as a carbon source, *K. marxianus* DSM 5422 yeast activity decreased from 0.192 to 0.117 U mL^{-1} after 48 hours, but after 72 hours increased again.

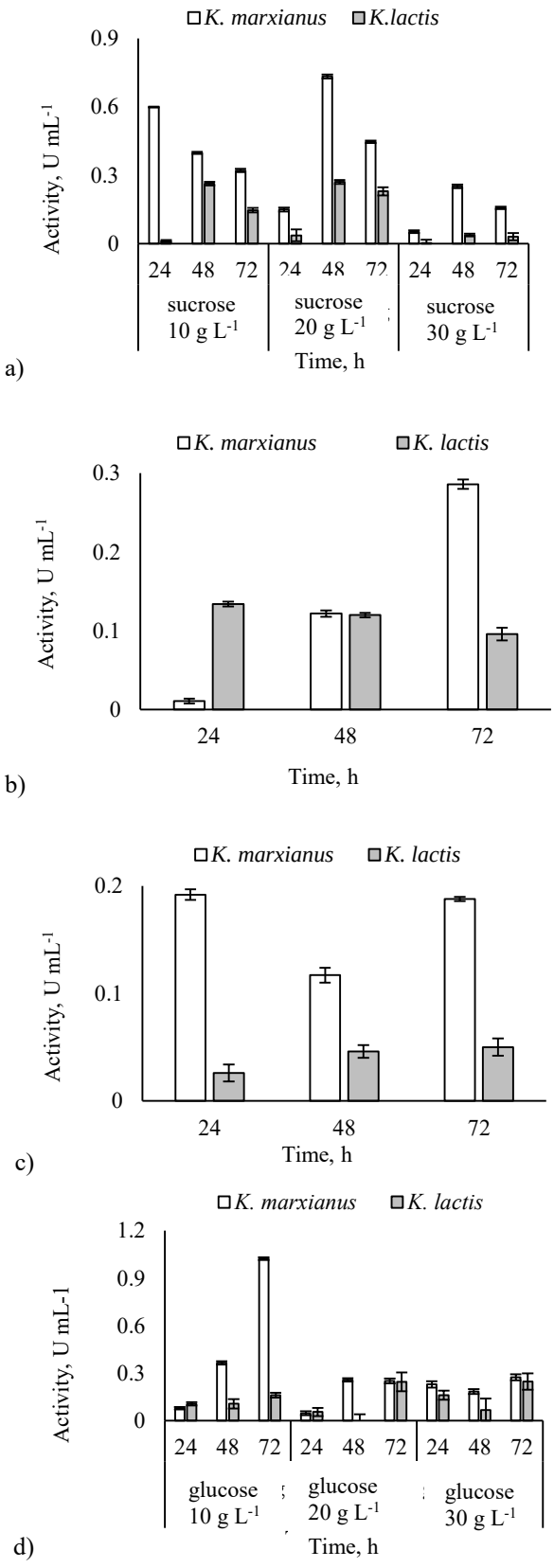

Figure 3. Yeast invertase activity by using different carbon sources: a) sucrose; b) lactose; c) fructose; d) glucose

The activity of *K. lactis* DSM 70799 yeast during fermentation grew up to 48 hours and after that remained unchanged (Fig. 3c).

In the evaluation of the effect of glucose as a carbon source on invertase activity, the highest activity of *K. marxianus* DSM 5422 yeast produced invertase was determined at 1.024 U mL^{-1} after 72 hours fermentation using 10 g L^{-1} glucose, while using 20 and 30 g L^{-1} glucose, the measured activity was 75 and 73% lower respectively. The activity of *K. lactis* DSM 70799 yeast invertase was the highest at 20 and 30 g L^{-1} of glucose, respectively of 0.245 and 0.247 U mL^{-1} after 72 h of fermentation (Fig. 3d). Vainstein and Peberdy (1991) found out that using glucose as the carbon source invertase activity was the lowest.

Effect of bioproducts as a carbon source on invertase activity

Molasses and apple squeeze contains the most suitable carbon source for *K. marxianus* DSM 5422 yeast (Fig. 4 a and b). The highest invertase activity of *K. lactis* DSM 70799 yeast was observed using molasses (Fig. 4a).

Molasses is an alternative to sucrose and is increasingly used by industry. Molasses is a suitable substrate for yeast invertase because it has sugars (mainly sucrose), nitrogenous compounds, B group vitamins, macro- (phosphorus, magnesium, iron, sulphur) and microelements (zinc, copper) (Taskin, 2016).

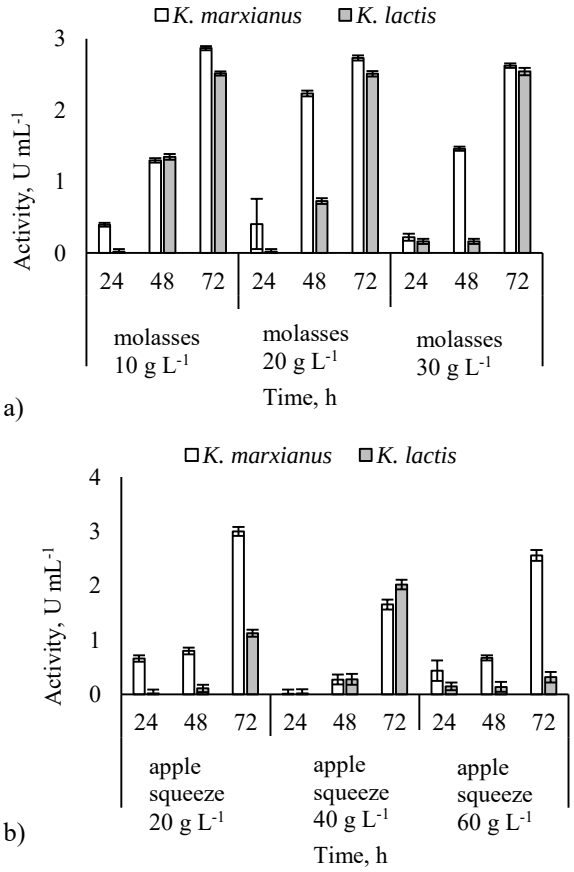

Figure 4. Yeast invertase activity by using different carbon sources: a) molasses; b) apple squeeze

The study showed a tendency to increase invertase activity during fermentation time. The molasses in the investigated yeast species were a suitable source of carbon – activity of both yeast types reached high values (Fig. 4a). Maximum invertase activity of *K. marxianus* DSM 5422 was detected after 72 hours of fermentation using 10, 20 and 30 g L^{-1} molasses and was on the average 2.74 U mL^{-1}. The highest activity of invertase from *K. lactis* DSM 70799 invertase was also detected after 72 hours of fermentation using both 10, 20 and 30 g L^{-1} molasses (on the average 2.52 U mL^{-1}).

The content of the main components in the apple squeeze are: 13% dry mass, 13% total sugar, 0.6% total nitrogen, phosphorus 0.4%, 2% ash (Campeanu et al., 2009). Using apple squeeze as a substrate, the maximum activity of invertase from *K. marxianus* DSM 5422 was 2.999 U mL^{-1} after 72 hours of fermentation using 20 g L^{-1} apple squeeze. The highest activity of *K. lactis* DSM 70799 invertase was after 72 hours fermentation using 20 and 40 g L^{-1} apple squeeze, respectively, 1.127 and 2.021 U mL^{-1}.

By-products obtained from agriculture or food production industries are attractive for the production of bioproducts, because it reduces environmental pollution and boosts the economy (Uma, 2010), and the use of by-products such as molasses and apple squeeze is relevant to the development of sustainable bio-product production.

Invertase immobilization

The immobilization efficiency of crude invertase was very high for both *K. marxianus* DSM 5422 and *K. lactis* DSM 70799 yeast (98 and 96% respectively) (Fig. 5).

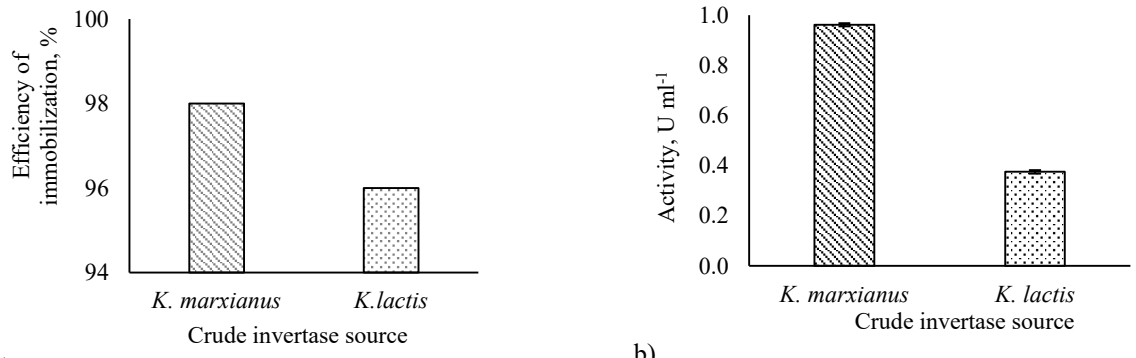

Figure 5. Immobilization efficiency of crude invertase (a) and immobilized invertase activity in calcium alginate capsules (b)

Milovanović et al. (2007) investigated the possibilities of immobilization of invertase, previously obtained from baking yeast, in calcium alginate and also identified very low enzyme losses during this process.

Immobilized *K. marxianus* DSM 5422 yeast invertase activity was 0.962 U g^{-1} and *K. lactis* DSM 70799 was lower – 0.375 U g^{-1}. Although immobilization is a good method to improve the stability and vitality of invertase, Tanriseven and Dogan (2001) found out that invertase after immobilization leaks out of alginate beads and to prevent the leakage of invertase, alginate gel was stabilized by using glutaraldehyde at high pH.

Conclusions

Effectors such as Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Na^{+} ions significantly decreased invertase activity. However, *K. lactis* DSM 70799 invertase activity was increased by Cu^{2+} ions. The highest activity of invertase was observed at 55 °C and pH 4.5. Meanwhile, strongly acidic pH (pH 2.5) and 20 °C temperature significantly decreased enzyme activity. Using sucrose as a carbon source, the invertase activity was higher compared to other carbon sources (lactose, fructose and glucose) averaged 1.5 times, but invertase activity was 5 times higher using by-products such as molasses and apple squeeze as carbon sources compared to traditional. The highest activity of *K. marxianus* DSM 5422 yeast invertase was as a nutrient medium for the cultivation of yeast using molasses and apple squeeze, and for *K. lactis* DSM 70799 – molasses. Immobilization of invertase from *K. marxianus* DSM 5422 and *K. lactis* var. *lactis* DSM 70799 in calcium alginate gel beads was suitable for enzyme reuse.

References

1. Aburigal A. A. A., Elkhalifa E. A., Sulieman A. M. E., Elamin H. B. (2014) Extraction and partial kinetic properties of invertase from *Schizosaccharomyces pombe*. *Food Science and Nutrition Engineering,* Vol. 4(3), p. 80–85.
2. Axe J. (2015) Nutritional Yeast: The Antiviral, Antibacterial Immune-Booster. [accessed on 7.02.2019]. Available: https://draxe.com/nutritional-yeast/
3. Campeanu G., Neata G., Darjanschi G. (2009) Chemical composition of the fruits of several apple cultivars growth as biological crop. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, Vol. 37(2), p. 161–164.
4. Gascon S., Lampen J. O. (1968) Purification of the internal invertase of yeast. *Biological Chemistry,* Vol. 243(7), p. 1567–1572.
5. Group E. (2013) The health benefits of invertase. [accessed on 7.02.2019]. Available: https://www.globalhealingcenter.com/natural-health/invertase/

6. Ilyina A., Ramos-Gonzalez R., Vargas-Segura A., Sanchez-Ramirez J., Palacios-Ponce S. A., Martinez-Hernandez J. L., Segura-Ceniceros E. P., Contreras-Esquivel J. C., Aguilar-Gonzalez C. N. (2016) Magnetic separation of nanobiostructured systems for innovation of biocatalytic processes in food industry. *Novel Approaches of Nanotechnology in Food*, Vol. 1, p. 67–96.
7. Kaur N., Sharma A. D. (2005) Production, optimization and characterization of extracellular invertase by an actinomycete strain. *Scientific & Industrial Research,* Vol. 64, p. 515–519.
8. Kulshrestha S., Tyagi P., Sindhi V., Yadavilli K. S. (2013) Invertase and its applications – A brief review. *Pharmacy Research,* Vol. 7, p. 792–797.
9. Mickevičius V. (2008) *Biokatalizatoriai organinėje sintezėje.* Vilnius : Vilniaus pedagoginio universiteto leidykla, p. 46–55.
10. Milovanovic A., Božic N., Vujčic Z. (2007) Cell wall invertase immobilization within calcium alginate beads. *Food Chemistry,* Vol. 104(1), p. 81–86.
11. Nakano H., Murakami H., Shizuma M., Kiso T., de Araujo TL., Kitahata S. (2000) Transfructosylation of thiol group by beta-fructofuranosidase, *Bioscience, Biotechnology and Biochemistry*, Vol. 64, p. 1472–1476.
12. Razmovski R., Vučurovic V. (2011) Ethanol production from sugar beet molasses by *S. cerevisiae* entrapped in an alginate–maize stem ground tissue matrix. *Enzyme and Microbial Technology*, Vol. 48, p. 378–385.
13. *Enzymatic Assay of Invertase.* [accessed on 7.02.2019]. Available: https://www.sigmaaldrich.com/technical-documents/protocols/biology/enzymatic-assay-of-invertase.html.
14. Shankar T., Thangamathi P., Rama R., Sivakumar T. (2013) Optimization of invertase production using *Saccharomyces Cerevisiae* MK under varying cultural conditions. *Biochemistry and Biophysics*, Vol. 1(3), p. 47–56.
15. Tanriseven A., Dogan S. (2001) Immobilization of invertase within calcium alginate gel capsules. *Process Biochemistry*, Vo.36, p. 1081–1083.
16. Taskin M., Ortucu S., Unver Y. Tasar O. C., Ozdemir M., Kaymak H. C. (2016) Invertase production and molasses decolourization by cold-adapted filamentous fungus *Cladosporium herbarum* ER-25 in non-sterile molasses medium. *Process Safety and Environmental Protection*, Vol. 103, p. 136–143.
17. Uma C., Gomanhi D., Muthulakshmi C., Gopalakrishnan V. K. (2010) Production, purification and characterization of invertase by *Aspergillus flavus* using fruit peel waste as substrate. *Advances in Biological Research,* Vol. 4(1), p. 31–36.
18. Uma C., Gomathi D., Ravikumar G., Kalaiselvi M., Palaniswamy M. (2012) Production and properties of invertase from a *Cladosporium cladosporioides* in SmF using pomegranate peel waste as substrate. *Asian Pacific Journal of Tropical Biomedicine*, Vol. 2, p. 605–611.
19. Vainstein M. H., Peberdy J. F. (1991) Regulation of invertase in Aspergillus nidulans: effect of different carbon sources. *General Microbiology*, Vol. 137, p. 315–321.
20. Veana F., Martínez-Hernández J. L., Aguilar C. N., Rodríguez-Herrera R., Michelena G. (2014) Utilization of molasses and sugar cane bagasse for production of fungal invertase in solid state fermentation using *Aspergillus niger* GH1. *Brazilian Journal of Microbiology*, Vol. 45(2), p. 373–377.
21. Workman W. E., Day D. F. (1983) Purification and properties of the β-fructofuranosidase from *Kluyveromyces fragilis. FEBS Letters,* Vol. 160, p. 16–20.

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THE INFLUENCE OF VARIOUS DRYING METHODS ON THE QUALITY OF EDIBLE FLOWER PETALS

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Abstract

Edible flowers are used in many different styles of cuisine and can be found on menus all over the world. They are receiving renewed interest as rich sources of bioactive compounds. In culinary, edible flowers can be used fresh, dried, candied etc. The drying prolongs shelf life as well as enables the transporting, packaging and use of edible flowers. The aim of this research was to analyse the influence of various drying methods on the quality of edible flower petals. The study was carried out at the scientific laboratories of the Faculty of Food Technology at Latvia University of Life Sciences and Technologies. Such drying methods as drying hot air-drying, microwave drying, and freeze-drying were used in this research. Edible petals of garden marigold (*Calendula officinalis* L.), common daisy (*Bellis perennis* L.), and true lavender (*Lavandula angustifolia* L.) from collection of the Laboratory of Horticulture and Apology, attached to the Faculty of Agriculture of Latvia University of Life Sciences and Technologies, were used as plant material. Total phenolic, total flavonoid content and antioxidant activity were determined in this research. All three drying methods had adverse effects on biologically active compounds of the analysed edible flowers petals. Despite the fact that freeze-drying is the most popular method, microwave drying had the most positive effect in terms of bioactive component content in this study. Analysed samples contained a high amount of phenolic compounds (fresh lavender 1026±52 mg GAE 100 g^{-1} DW and fresh marigold 1058±66 mg GAE 100 g^{-1} DW) and showed eligible antioxidant effects.

Keywords: total phenolic, drying, edible flowers

Introduction

The use of edible flowers has been known in Europe since the prehistoric period (Nikitidis, Papiomytoglou, 2011). Nowadays, edible flowers are used in many different styles of cuisine and can be found on menus all over the world. They are receiving renewed interest as rich sources of bioactive compounds as phenols, essential oils etc. (Naczk, Shahidi, 2004). The content of these compounds differs and depends on plant species, cultivar, genotype, growing conditions, harvesting time, food and processing technologies (Naczk, Shahidi, 2006). In culinary, edible flowers can be used fresh, dried, candied etc. Specially prepared and processed, they can make food products healthier as well as visually attractive (Fernades et al., 2017).

The drying prolongs shelf life as well as enables the transporting, packaging and use of edible flowers. It is a lack of studies in the Europe on the current topic therefore the experiments with different plants` species are very important. In Latvia, it is not so much information about the changes of bioactive compounds in drying process for edible petals. That is why this research can be defined as innovative (Aboltins, Kic, 2016). Drying conditions and selection of methodology is essential, for example, the low temperatures in drying process influence the content of biologically active compounds positively, their degradation is much less (Angela, Meireles, 2009).

Garden marigold (*Calendula officinalis* L.), common daisy (*Bellis perennis* L.) and true lavender (*Lavandula angustifolia* L.) are one of the most popular edible flowers in Latvia, used in culinary, food and beverage production, decoration, medicine, perfumery, cosmetics, aromatherapy, for bathing, in decorative horticulture and flowering fields, for attracting bees. These species can be planted in flowerbeds and herbaceous borders as well as in commercial plantations, also they are easily propagated. Long flowering period and storage possibilities influence growing, marketing and realization of these species or prepared products positive.

Numerous varieties of garden marigold are cultivated in pots and gardens (Nikitidis, Papiomytoglou, 2011). The flowers of the plant are used for therapeutic purposes; they contain saponins, carotenoids, essential oil, sterols, flavonoids and mucilage (Arora et al., 2013). Garden marigold has anti-inflammatory, astringent, healing and emmenagogue properties. It is considered one the best herbs for the treatment of local skin aliments, external bleeding, contusions and burns. It is also considered to have a notable anti-fungal property (Basch et al., 2007). Already in the 12th century it was observed, that "even looking at the golden flowers of the garden marigold improves the sight, cleanses the brain and mends the spirit" (Nikitidis, Papiomytoglou, 2011). There is no doubt that the flowers constitute a dyestuff, it was once used as a spice and to adulterate saffron from the crocus, or to give a yellow colour directly to rice and salads; for this reason, it was called "the poor man`s crocus". The French eat veal with marigold, it is sold in the shops in dried form and as wet extract.

Common daisy has been used as a diuretic, antispasmodic, anti-inflammatory, astringent, expectorant, antipyretic, vulnerary, ophthalmic and homeostatic in traditional medicine (Karakas et al., 2011). New leaves can be eaten raw in salads or cooked, noting that the leaves become increasingly astringent with age. Flower buds and petals can be eaten raw in sandwiches, soups and salads. It is also used as a tea and as a vitamin supplement. Flowers have been used

internally as tea (or the leaves as a salad) for treatment of disorders of the gastrointestinal and respiratory tract (Al-Snafi, 2018).

Lavender is one of the best-known aromatic herbs. Its healing and soothing properties made it a favourite even in antiquity. The uses of lavender in medicine, cosmetic production and in the home are infinite (Nikitidis, Papiomytoglou, 2011). Lavender essential oil has good antioxidant and antimicrobial activities and a significant positive effect on the digestive and nervous systems. Lavender extract prevents dementia and may inhibit the growth of cancer cells, while lavender hydrolyte is recommended for the treatment of skin problems and burns (Prusinowska, Smigielski, 2014). Lavender is used to aromatize confectionery and drinks, while in the form of tea it is calming, relaxing and soothes pain (Nikitidis, Papiomytoglou, 2011).

Drying is a very common preservation method used in deferent foodstuffs. The quality of the final products depends on the technique and the process variables used (Youssef, Mokhtar, 2014). Hot-air-drying is one of most frequently used operations for food dehydration.

Freeze-drying would be the best method of water removal, but it is also expensive method. This method is based on the dehydration by sublimation of frozen sample and the major advantages are protection of bioactive compounds and original shape, colour and flavour of flowers (Zheng et al., 2015).

Microwave drying is alternative to the conventional drying method that allows the product to preserve its useful qualities and is suitable for almost at home. Very important that the heat not only on the surface but also inside the food products or plants. Very high speed of drying gives the quality of the final food product. Xia Fei Shi et al. (2017) reported that microwave drying helps to remain higher content of flavone, vitamin C and soluble sugars in medicinal chrysanthemum flowers.

The aim of this research was to analyse the influence of various drying methods on the properties and quality of flowers` petals of garden marigold, common daisy and true lavender.

Materials and Methods

Plant material

Fresh marigold, common daisy and true lavender were harvested from collection of the Laboratory of Horticulture and Apology, attached to the Faculty of Agriculture of Latvia University of Life Sciences and Technologies prior to flowering period during September 2018. Green leaves were manually separated from plant. The samples were processed using the following drying methods.

Growing conditions

The soil at the trial site was strongly altered by cultivation loam. Soil reaction was slightly acidic (pH_{KCl} 6.3), with organic matter content of 2.7 g kg^{-1}, P content was 102 g kg^{-1} and K content was 207 g kg^{-1}. Plant care was provided for this collection.

Meteorological conditions

According to data of the Latvian Environment, Geology and Meteorology Centre, in vegetation period (from May to the end of September), the average temperature was 16.9 ℃ (more than long-term observation) and the total quantity of rainfall was about 237.4 mm (less than long-term observation) in 2018.

In scientific literature it was proved that during the vegetation period the influence of air temperature from 20 to 30 ℃ and of the quantity of rainfall of about 600 mm on the yield of herbs is positive (Rzekanowski et al., 2008). The conclusion is, that in 2018 the meteorological conditions were not optimal for plant biomass creation because of small quantity of rainfall.

Drying Methods

Hot-air drying: fifty grams of flower petals were distributed uniformly onto trays and dried in a convective dryer (Memmert GmbH, Germany) at 40 °C (Telfser, Galindo, 2019). The drying time was 6 hours. The moisture content of dried petals was 7.5±1.0%.

Freeze-drying: before drying fifty grams of flower petals were placed in polyethylene package and frozen at -18 °C for 24 hours. The samples were removed from the packaging and placed in Christ Freeze Dryer Alpha 1-2 LD plus at -60 °C for 30 hours at 0.046 mbar. The moisture content of dried petals was 9.0±1.0%.

Microwave drying: a domestic microwave oven (Whirlpool, Type VT254/WH) was used for drying. Petals were spread on the plate inside the microwave and processed until they were completely dry (6–7 min). The microwave output power was 800 W. Final moisture content of dried flowers was 9.5±1.0%.

Extraction procedure

Fresh and dried plants were homogenised and for extraction solvent to solid ratio was 1 : 10. Extraction procedure was applied using ethanol / acetone / water (7/7/6 v/v/v) solution in an ultrasonic bath YJ5120-1 (Oubo Dental, USA) at 22 ± 1 °C temperature for twelve minutes followed by centrifugation using centrifuge CM-6MT (Elmi Ltd., Latvia) at 3 500 min^{-1} for 5 min and decantation. Extraction of residues was repeated. The extraction process was done in triplicate.

Analytical methods

Determination of total phenolic content (TPC) of flower petals extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999). Total phenolic content was expressed as gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of plant material. The absorbance was measured at 765 nm. The total flavonoid content (TFC) was measured by colorimetric method (Tomsone et al., 2012). The absorbance was measured at 415 nm. Total flavonoid content was expressed as the catechin equivalents (CE) 100 g^{-1} DW of plant material.

Determination of antioxidant activity (AA) of the petals 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 radical

scavenging activity of extract was measured by 2.2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid (ABTS) cation assay (Re et al., 1999).

Statistical analysis

Experimental results are means of three parallel measurements. Data were analysed by Microsoft Excel 2010 and R programme. Analysis of variance ANOVA were used to determine differences among samples. The values were considered to be significantly different when $p<0.05$. Correlation analysis were performed to analyse association between two continuous variables.

Results and Discussion

Based on the results obtained and using analysis of variance of factors ANOVA hypothesis that various methods of processing the flower and the flower itself affect various indicators of phenols and flavonoids as well as the antioxidant properties of the petals were put forward.

Table 1

Total phenolic and flavonoid content in petals depending on drying method

Flowers petals	Type of sample preparation	TPC	TFC
		mg GAE 100 g^{-1} DW	mg 100g^{-1} DW
Lavender	hot-air dried	1135±26	1868±14
	freeze dried	1046±58	1852±68
	microwave dried	1183±30	2369±43
	fresh	1026±52	2586±48
Marigold	hot-air dried	954±82	1903±59
	freeze dried	1062±22	1947±17
	microwave dried	1122±39	1965±14
	fresh	1058±66	2522±19
Common daisy	hot-air dried	801±90	1190±2
	freeze dried	1597±27	2562±13
	microwave dried	837±53	1586±6
	fresh	844±73	1645±91

Phenolic compounds are a spacious group of phytochemicals classified as secondary metabolites. In our study total phenolic content for fresh and dried flower petals ranged from 837.7 to 1597.2 mg GAE per 100 g dry weight sample (Table 1). The amount of flavonoids depended on the type of the flower but also on the method of sample preparation. The loss of phenolic during different drying might be due to the process conditions – temperature and the duration applied (Youssef, Mokhtar, 2014). Previously, a study conducted by Tomsone (2014) on horseradish leaves (*Armoracia rusticana* L.) showed TPC 2368.48 mg GAE 100 g^{-1} of fresh to 123.6 mg GAE 100 g^{-1} for dried horseradish leaves. While earlier research studies showed diverse results. Youssel and Mokhtar (2014) studied the effect of drying methods (oven, microwave and freeze drying) on phenolic content of purslane (*Portulaca oletacea* L.). They reported that the degradation of total phenolics and flavonoids significantly varied according to the drying methods. They noted that extracts of dried leaves always showed lower concentration of total phenolics and flavonoids then from fresh. Chaovanalikit et al. (2012) reported that the shade dried samples had significantly higher phenolics content than freeze- and oven-dried samples. Khattak (2014) found that the total phenolic content of *Tagetes erecta* ranged from 478.0 to 634.0 mg 100 g^{-1} of phenolic compounds in dry weight of the extracts of the flowers. A great influence of the drying method on the extraction of phenols during infusion. In the case of our sturdy on flower's petals, it would be a competitive process for preserving phenolics since similar or better results were obtained with freeze drying method.

Flavonoids are low molecular weight compounds that have a wide diapason of biological activities comprise antibacterial, antioxidant, antiallergic (Fernandes et al., 2017). The flavonoid contents of the samples were determined and presented in Table 1. The level of flavonoids ranged between 1586.6 to 2586.2 mg 100g^{-1} dry weight. The present study showed that the flavonoid content in hot-air (1903.8±59 mg 100 g^{-1}), freeze dried (1947.1±7.2 mg 100 g^{-1}) and microwave dried (1965.9±14 mg 100 g^{-1}) marigold are statistically not different ($p>0.05$). The fresh flower petals of common daisy and marigold showed higher flavonoids content than dried.

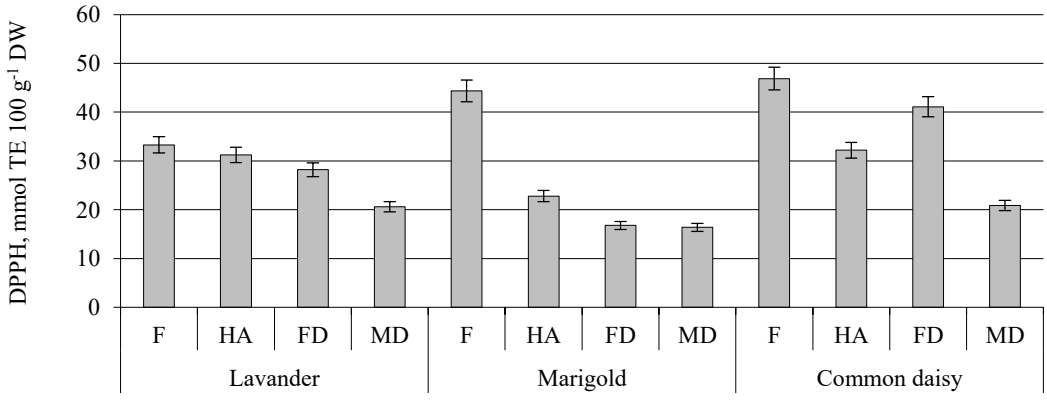

Figure 1. DPPH' scavenging activity in petals depending on drying technique

F – fresh, HA – hot-air dried, FD – freeze dried, MD – microwave dried

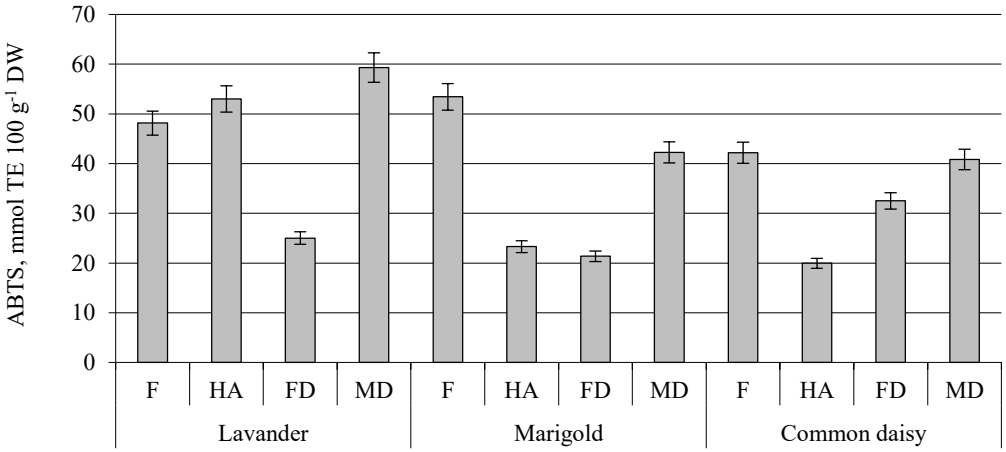

Figure 2. ABTS antioxidant capacity in petals depending on drying technique
F – fresh, HA – hot-air dried, FD – freeze dried, MD – microwave dried

Scavenging activity of DPPH radicals for all fresh samples of petals showed higher results than dried samples (Figure 1). The highest DPPH scavenging activity was determined in fresh marigold and common daisy petals 44.35 and 46.88 mM TE 100 g^{-1} DW, respectively. The effect of hot-air, freeze-drying and microwave drying treatments on the ABTS antioxidant capacity of flower petals is shown in Figure 2. The results indicated significant losses in the antioxidant capacity of freeze-dried samples. Increase of antioxidant capacity after drying in lavender could be explained by the release of bound antioxidant compounds during treatment. In fresh plants part of antioxidants are presented in glycosidic linkage with covalent bond and drying accelerates breakdown of cellular constituents and release of them (Wei et al., 2013). Antioxidant capacity of petals may be related to the amount of pigments, total phenols and flavonoids. Since these compounds work as scavengers of the free radicals produced during oxidation reactions.
The non – dispersive are the same then the hypothesis: H0 that all the defects are the same can reject and accept H1: the antioxidant activity and the amount of phenols amount of flavonoids depend on the type of the flower but also on the method of drying. Explained sum of squares (ESS) 2434692, residual sum of squares (RSS) was 190943 by phenolic and flavonoid content in petals. P value was 0.0002773*** which is <0.05. P value by analysis of antioxidant capacity depending on sample preparation was 0.001548** which is also is <0.05 than we can discard the null hypothesis and accept the hypothesis about influence of the factor of the flower type and processing of samples preparation.

Table 3

Pearson's coefficients between total phenolic, total flavonoid content and antioxidant capacity for lavender petals

	ABTS	DPPH	TFC	TPC
ABTS	1	-0.247	0.356	0.241
DPPH	-0.247	1	0.015	-0.129
TFC	0.356	0.0152	1	0.627
TPC	0.241	-0.0129	0.627	1

In order to explore the influence of the phytochemical compounds on petals, Pearson's coefficient was determined (Tables 3-5).

Table 4

Pearson's coefficients between total phenolic, total flavonoid content and antioxidant capacity for marigold petals

	ABTS	DPPH	TFC	TPC
ABTS	1	-0.451	-0.291	0.760
DPPH	- 0.451	1	0.864	-0.36
TFC	-0.291	0.864	1	0.0691
TPC	0.760	-0.361	0.0691	1

For lavender petals correlation between TPC and TFC was medium (r=0.62) but between different antioxidant assays very weak. Strong (r=0.86) correlation for marigold was between TFC and antioxidant activity DPPH and, also strong (r=0.76) between TPC and ABTS.

Table 5

Pearson's coefficients between total phenolic, total flavonoid content and antioxidant capacity for Common daisy petals

	ABTS	DPPH	TFC	TPC
ABTS	1	-0.140	-0.113	0.209
DPPH	-0.140	1	0.189	0.020
TFC	-0.113	0.189	1	0.75
TPC	0.209	0.020	0.753	1

If we take the data Pearson's coefficients for common daisy that strong correlation is between TPC and TFC only. The loss of phenolic during different drying might be due to the process condition, the temperature and the duration applied (Youssef, Mokhtar, 2014).

Conclusions

The findings of this study showed that chosen edible flowers are rich sources of bioactive compounds. All samples contained high amount of phenolics and showed eligible antioxidant effect. The results support the consumption of edible flowers in the diet as functional foods.

In addition to bioactive compounds, appearance and intended use in cooking and cooking presentation is important and affected by different drying methods. Despite the fact that freeze-drying is the most popular method, microwave drying had the most positive effect in terms of bioactive component content and appearance for flower petals in this study.

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References

1. Aboltins A., Kic P. (2016) Research in some medical plant drying process. **In:** *15th International Scientific Conference Engineering for Rural Development,* Latvia University of Agriculture, Jelgava, p. 1145–1150.
2. Acikgoz F.E. (2017). Edible flowers. *Journal of Experimental Agriculture International*, Vol. 17(1), p. 1–5.
3. Al-Snafi A. E. (2018) Traditional uses of Iraqi medicinal plants. *Journal of Pharmacy*, Vol. 8(8), p. 32–95.
4. Aquino-Bolanos E.N., Urrutia-Hcnandez T.A., Castillo-Lozano M.L., Chavez-Servia J.L., Verdalet-Guzman I. (2013). Physicochemical parameters and antioxidant compounds in edible squach (*Cucurbita pepo*) flower stored under controlled atmospheres. *Journal of Food Quality*, Vol. 36, p. 302–308.
5. Arora D., Rani A., Sharma A. (2013) A review on phytochemistry and ethnopharmacological aspects of genus *Calendula*. *Pharmacognosy Reviews,* Vol. 7(14), p. 179–187.
6. Basch E., Bent S., Foppa I. M. (2007) Marigold (*Calendula officinalis L.*), *Journal of Herbal Pharmacotherapy*, Vol. 6(3), p. 135–159.
7. Chaovanalikit A., Mingmuang A., Kitbunluewit T., Choldumrongkool N., Sondee, J., Chupratum, S. (2012) Anthocyanin and total phenolics content of mangosteen and effect of processing on the quality of mangosteen products. *International Food Research Journal*, Vol.19(3) p. 1047–1053.
8. Fernandes L., Casal S., Pereira J.A., Saraiva J.A., Ramalhosa E. (2017) Edible flowers: A review of the nutritional, antioxidant, antimicrobial properties and effects on human health. *Journal of Food Composition and Analysis*, Vol. 60, p.38–50.
9. Grzeczczuk M., Wesolowska A., Jadczak D., Jakubowska B. (2011). Nutritional Value of Chive Edible Flowers. *Acta Sci. Pol., Hortorum Cultus*, Vol. 10(2), p. 85-94.
10. Guiné R., Santos E., Correia P. (2017). Edible flowers: knowledge and consumption habits. *Acta Scientific Nutrition Health*, Vol. 3, p. 18–22.
11. Karakas F. P., Karakas A., Coskun H., Turker A. U. (2011) Effects of common daisy (*Bellis perennis* L.) aqueous extracts on anxiety-like behaviour and spatial memory performance in Wistar albino rats. *African Journal of Pharmacy and Pharmacology*, Vol. 5(11), p. 1378–1388.
12. Khattak K. (2014) Antioxidant activities and phytochemicals of *Tagetes Erecta* flowers as affected by drying methods. *Journal of Applied Environmental and Biological Sciences*, Vol. 4(95) p. 253–262.
13. Koikea A., Barreiraa J.C., Barros L., Santos-Buelgac C., Villavicencio A.L. Ferreira I.C. (2015) Edible flowers of *Viola tricolor* L. as a new functional food: antioxidant activity, individual phenolics and effects of gamma and electron-beam irradiation. *Food Chemistry*, Vol. 179, p. 6–14.
14. Kucekova Z., Mlcek J., Humpolicek P., Rop O. (2013) Edible flowers – antioxidant activity and impact on cell viability. *Central European Journal of Biology*, Vol. 8(10), p. 1023–1031.
15. Loizzo M.R., Pugliese A., Bonesi M., Tenuta M.C., Menichini F., Xiao J., Tundis R. (2015) Edible flowers: A rich source of phytochemicals with antioxidant and hypoglycemic properties. *Journal of Agricultural and Food Chemistry,* Vol. 64 (12), p. 2467–2474.
16. Naczk M., Shahidi F. (2004) Extraction and analysis of phenolics in food. *Journal of Chromatography A*, Vol. 1054 (1-2), p. 95–111.
17. Navarro-González I., González-Barrio R., García-Valverde V., Bautista-Ortín A.B., Periago M.J. (2015) Nutritional composition and antioxidant capacity in edible flowers: Characterization of phenolic compounds by HPLC-DAD-ESI/MS^n. *International Journal of Molecular Sciences*, Vol. 16, p. 805–822.
18. Nikitidis N., Papiomytoglou V. (2011) *Green plants & herbs of Greece*. Rethymno: Mediterraneo Editions. 135 p.
19. Petrova I., Petkova N., Ivanov I. (2016) Five edible flowers – Valuable source of antioxidant in human nutrition. *International Journal of Pharmacognosy and Phytochemical Research*, Vol. 8(4), p. 604–610.
20. Prusinowska R., Smigielski K. B. (2014) Composition, biological properties and therapeutic effects of lavender (*Lavandula angustifolia* L.). A review. *Herba Polonica*, Vol. 60(2), p. 56–66.
21. 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 Biologyand Medicine*, Vol. 26, p. 1231–1237.
22. Rzekanowski C., Marynowska K., Rolbiecki S., Rolbiecki R. (2008) The influence of selected meteorological factors on some elements of the yield of four herb species cultivated under irrigation conditions. *Acta Agrophysica*, Vol. 12(1), 163–171 (in Polish).
23. Singleton V.L., Orthofer R., Lamuela-Raventos R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, Vol. 29, p. 152–178.
24. Skrajda M.N. (2017) Phenolic compounds and antioxidant activity of edible flowers. *Journal of Education, Health and Sport*, Vol. 7(8), p. 946–956.
25. Telfser A., Galindo F.G. (2019) Effect of reversible permeabilization in combination with different drying methods on the structure and sensorial quality of dried basil (*Ocimum basilicum* L.) leaves. *LWT-Food Science and Technology*, Vol. 99, p. 148–155.
26. Tomsone L., Kruma Z., Lepse L. (2012) Influence of genotype and harvest time on the phenolic content of horseradish (*Armoracia rusticana* L.) roots. **In**: *Research for Rural Development 2012: Annual 18th international scientific conference proceedings*, Jelgava, 16-18 May 2012 / Latvia University of Agriculture. Jelgava: LLU, Vol. 1, p. 124–130.
27. Tomsone L., Kruma Z. (2014) Influence of freezing and drying on the phenolic content and antioxidant activity of of horseradish and lovage. **In**: *9th Baltic Conference on Food Science and Technology "Food for Consumer Well-*

Being" FOODBALT 2014 Conference Proceedings, Jelgava, Latvia University of Agriculture, p. 192–197.
28. Zheng M., Xia Q., Lu S. (2015) Study on drying methods and their influences on effective components of loguat flower tea. *LWT-Food Science and Technology*, Vol. 63(1), p. 14–20.
29. Youssef K.M., Mokhtar S.M. (2014) Effect of drying methods on the antioxidant capacity, color and phytochemicals of *Portulaca oleracea* L. leaves. *Journal of Nutrition & Food Sciences*, Vol. 4, p. 1–6.
30. Yu L., Haley S., Perret J., Harris M., Wilson J., Haley S. (2003) Antioxidant properties of bran extracts from Akron wheat grown at different locations. *Journal of Agriculture and Food Chemistry*, Vol. 51, p. 1566–1570.
31. Wei E., Chan C., Lye P.Y, Eng S.Y., Tan Y.P. (2013) Antioxidant properties of herbs with enhancement effects of drying treatments: A synopsis. *Free Radicals and Antioxidants*, Vol. 3, p. 2–6.
32. Xiao-Fei S., Jian-Zhou C., Yan-Fen Z., Cun-Oi L., Xiao-Oin Y. (2017) Nutritional and active ingredients of medicinal chrysanthemum flower heads affected by different drying methods. *Industrial Crops and Products*, Vol. 104, p. 45–51.

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OPTIMISATION OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF LIPOPHILIC EXTRACT FROM ROSEHIPS

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Abstract

Rosehips (*Rosa canina*) contain biologically active compounds, such as carotenoids, tocopherols, polyphenolics, and organic acids. Carotenoids are widespread pigments in plants, where they are involved in photosynthesis and photoprotection, but they are also found in human tissues where they may act as antioxidants or as immunomodulating, antimutagenic and tumour-preventing agents. Therefore, developments of processes of isolation of rosehips extracts with these compounds are of interest. In this study, the lipophilic extract of rosehips was obtained by supercritical fluid extraction with carbon dioxide under different extraction conditions. The aim of this work was to optimize the extraction process of lipophilic extracts from rosehips using pressures from 15 to 45 MPa, temperatures from 40 to 80 °C and extraction time from 60 to 180 min, and to evaluate the yield of biologically active high-value compounds – carotenoids (lycopene and β-carotene). The content of total carotenoids in extracts was analysed by high-performance liquid chromatography. The optimal conditions in terms of maximising lipophilic extract yield were 75.2 °C, 44 MPa, 115 min. That allowed the recovery of 65% of the lipophilic extract in comparison to control.

Keywords: carotenoids, optimisation, rosehips, supercritical fluid extraction

Introduction

Rosehips, fruits of rose plants (*Rosa sp.*), are a proven source of carotenoids, polyphenols (triterpene acids, flavonoids, proanthocyanidins, catechin), essential fatty acids, galactolipids, folates, vitamin A, C and E, minerals (Ca, Mg, K, S, Si, Se, Mn and Fe), and other valuable compounds (Fan et al., 2014; Patel, 2017).

Carotenoids are relevant micronutrients which could lead to health benefits while consuming fruits, vegetables, and other food rich in carotenoids (Olson, 1999; Radzevičius et al., 2016a). Significance of carotenoids in human health mainly appears through foods, cosmetics, nutraceuticals and pharmaceuticals. These lipophilic pigments naturally occur in some fruits and vegetables, and are accountable for typical colours (mostly yellow, orange and red) (Radzevičius et al., 2016b). It is already investigated their positive effect on health, prevention and protection against certain types of cancers, cardiovascular diseases and macular degeneration, as well as enhancing the immune function (Rao, Rao, 2007; Cazzonelli, 2011; Kadian, Garg, 2012).

The main carotenoids of rosehips are lycopene and β-carotene (Horvath et al., 2012), both of them are hydrophobic and can be dissolved in non-polar organic solvents, such as tetrahydrofuran, hexane, chloroform, and acetone, but are essentially insoluble in polar solvents, such as water and ethanol. The need for safer methods of obtaining carotenoids in the lipophilic extracts (extracted from natural plant materials) has led to supercritical fluid extraction (Zuknik et al., 2012). Supercritical fluid extraction (SFE) is an environmentally friendly, alternative to the conventional industrial solvent extraction method, which results in products without toxic solvent residues. Carbon dioxide is neither toxic nor flammable and could be acquired at high purity with low expenses. Because of these properties, it is one of the most frequently used supercritical fluid. Compounds that easily degrade under high temperature may be extracted with CO_2 due to its reasonable critical temperature. Considering these characteristics, SF extraction with CO_2 (SFE-CO_2) is a well suitable method for food, dye, pharmaceutical and cosmetic industries. Previous research on SFE-CO_2 extraction from carotenoids-containing rosehip fruits had been reported by Illes et al. (1997), but the influence of extraction conditions on the total carotenoids and carotenoid composition in rosehips has not been examined yet. Several studies demonstrated that *R. canina* rosehips have a relevantly high content of carotenoids (Andersson et al., 2011). There is a necessity to optimize the process variables, including temperature, pressure, and extraction rate when developing SFE-CO_2 extraction process for lipophilic extracts. However, the optimization of lipophilic extract yield and maximisation of carotenoids in obtained extracts from rosehips (*R. canina*) fruit has not yet been reported. According to Machmudah et al. (2012), lipids are extracted with pigments. Moreover, co-extraction may occur, then the lipophilic extracts from tomato seeds may increase the solubility of the extracted pigments, including carotenoids. Extraction of rosehips could have the same results. Accordingly, lipophilic extracts were co-extracted in all cases where lycopene is obtained from tomato by-products containing tomato seeds. Thus, it contributes to the solubility of carotenoids using SFE-CO_2 extraction.

The aim of this work was to optimize the extraction process of lipophilic extracts from rosehips using pressures from 15 to 45 MPa, temperatures from 40 to 80 °C and extraction time from 60 to 180 min, and to evaluate the yield of biologically active high-value compounds – carotenoids (lycopene and β-carotene).

Materials and Methods

Sample preparation

Ripe rosehips (*R. canina* sp.) were visually selected and harvested. Ripe fruits were collected in October from wild bushes growing in the Lithuania.

Rosehips were dried using convective drying method. Drying was performed in a UDS-150/1 hot-air laboratory dryer (Utenos krosnys, Lithuania) at 39±1 °C and an air-flow rate of 1.5 m s^{-1}. Dried rosehips with seeds were grounded to a powder with a knife mill GM200 (Retsch, Germany).

Particle size distribution measurements

Particle size distribution of rosehips powder was measured on a particle size analyser (Mastersizer, Hydro 2000S (A), United Kingdom) operating using a laser diffraction method, widely accepted as a standard technique. Particle assessment was performed by following the guidance of ISO13320-1 (1999). Water was used as a dispersant for wet analysis, dispersant refractive index was 1.33, and particle refractive index was 1.53. The particles size of the dried rosehips powder obtained in our study was ≤ 0.2 mm.

Supercritical CO_2 extraction

The lipophilic extract from dried rosehips with seeds powder obtained by Soxhlet automated extraction (Behr Labor-Technik, Germany) with the mixture of chloroform and hexane (1:1, v/v) as control. Soxhlet extraction was carried out for 15 hours. The solvents were removed in a rotary vacuum evaporator (Büchi, Flawil, Switzerland) at 42 °C and the residue was weighed with analytical balances. The SFE-CO_2 experiments were carried out using supercritical fluid extractor SFT-150 (Supercritical Fluid Technologies, USA). Each extraction was performed using a 25 g sample of rosehips powder. Each sample was loaded into a 500 mL thick-walled stainless-steel cylindrical extractor vessel with an inner diameter of 14 mm and a length of 320 mm. The temperature of the extraction vessel was controlled by a surrounding heating jacket. The volume of CO_2 consumed was measured by a ball float rotameter and a digital mass flow meter in standard litres per minute (SL min^{-1}) at standard state (P_{CO_2}=100 kPa, T_{CO_2}=20 °C, ρ_{CO_2}=0.0018 g mL^{-1}). The process consisted of static (10 min) and dynamic extraction steps. The static extraction time was included in the total extraction time (Urbonaviciene, Viskelis, 2017).

HPLC analysis of carotenoids

The content of carotenoids (total lycopene and β-carotene) in lipophilic extracts was analysed by high-performance liquid chromatography (HPLC). HPLC was performed using a modified version of the different methods and systems (Heymann et al., 2013, Melendez-Martinez et al., 2013; Urbonaviciene et al., 2015). For the analysis, 2 g of rosehip powders and oleoresins after SFE-CO_2 extraction were dissolved in 50 mL hexane and tetrahydrofuran (4:1 V/V) with 1% butylated hydroxytoluene (BHT) solution. The HPLC system used was a Waters 2695 liquid separation module (Water Corporation, U.S.A.). Elution of materials was monitored by UV–Visible detector (UV–Vis, 2489, Water Corporation, U.S.A.). Detection of lycopene and β-carotene had been performed at wave lengths of 473 and 450 nm, respectively. Chromatographic separations were performed on a RP-C30 column (5 µm, 250×4.0 mm, YMC Europe, Dinslaken, Germany) connected to a C30 guard column (5 µm, 10×4.0 mm, YMC Europe, Germany) using a flow rate of 0.65 mL min^{-1}. The chosen column temperature was 25 °C. The mobile phase used consisted of methanol (solvent A) and methyl-tert-butyl ether (solvent B). Samples were injected at 40% B (held 5 min), and the gradient then had been changed to 83% B in 50 min, then to 100% B in 5 min (held 10 min) and to 40% B in 5 min (held 10 min).

Statistical analysis

Response surface methodology (RSM) using central composite design (CCD) model was applied to determine optimal extraction conditions for SFE-CO_2 extraction to maximize the yield of total lipophilic extract and carotenoids. Data was analysed and the model was established using tDesign – Expert 7.0. software (Stat–Ease Inc., Mineapolis, JAV). The number of experiments was calculated by the formula (1):

$$N = (2^f + 2f + c) \quad (1)$$

where f – the number of factor points;
c – the number of centre points.

The data for CCD was fitted with a second order polynomial equation (2):

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + + \sum_i \sum_{j=i+1} \beta_{ij} X_i X_j \quad (2)$$

where Y – the predicted response;
β_0 – a constant;
β_i, β_{ii}, β_{ij} – coefficients for linearity;
X_i and X_j – independent variables.

All experiments were performed in triplicate and each collected sample was analysed in duplicate. SPSS 20 Software (SPSS Inc., Chicago, USA) was used for assessing mean values and standard deviations of the experimental data. Statistical significance of the model and variables was determined at 5% probability level ($p<0.05$). The adequacy of the model was determined by evaluating the '*lack of fit*' coefficient and the Fisher test value (F-value) obtained from the analysis of variance. Extractions at every point were performed in triplicate and in random order.

Results and Discussion

Maximum yield and a desirable composition could be achieved by optimisation of the process. Effects of independent parameters on the lipophilic extraction yield from dried rosehip powders were examined using half fraction factorial design: SFE-CO_2 extraction was carried out using pressures (P) from 15 to 45 MPa, temperatures (T) from 40 to 80 °C and extraction time (t) from 60 to 180 min (Table 1). The highest yield of 2.18 g 100 g^{-1} DW was obtained at P=44 MPa, T=75.2 °C, t=115 min, and it reached 65% of total extraction yields (3.35 g 100 g^{-1}) obtained by

conventional extraction with a Soxhlet apparatus. Under these conditions, 65% of lipophilic extract could be obtained, whereas 35% of the lipophilic extract remains in the solid matrix. Szentmihalyi et al. (2002) was studying the recovery of oils from rosehips seeds (a by-product) and the highest extraction yield was 6.7 g 100 g^{-1} DW.

The analysis of the quadratic regression models for extract yield demonstrated that the model was significant ($p<0.05$) with an F-value of 56.8 and in this case the "lack of fit" was also significant relative to the pure error, with a *p*-value of < 0.001. In the model it is presented that the factor with the largest effect on extract yield was P ($p<0.0001$, F=248.52), followed by T ($p<0.0001$; F=20.94) and t (p=0.004; F=8.91). Interaction between factors pressure and temperature (PT) had significant effect on the yield ($p<0.0001$, F=176.79). The adequacy of the model was evaluated by the total determination coefficient (R^2) value of 0.99, indicating a reasonable fit of the model to the experimental data.

Table 1

Factors selected as independent variables for the optimization of SFE-CO_2

Symbols (independent variables)	Coded levels				
	-1.682	**-1**	**0**	**1**	**1.682**
P	8.79	15	30	45	51.12
T	31.72	40	60	80	88.28
t	35.15	60	120	180	204.85

P – extraction pressure, MPa; T – extraction temperature, °C; t – extraction duration, min.

During the research, optimisation of pressure, temperature and extraction time to maximize the yield of lipophilic extracts, was made by applying RSM using CCD. The effect of P, T and t on the extract yield is demonstrated in response surface plots, presented in Fig. 1.

The following second-order polynomial model, denoting an empirical relationship between the dependent variables and the independent test variables (P, T, t) was used (3):

$$Y = 89.04 - 107.73 \times P + 8.59 \times T - 5.584 \times t + 4.95 \times P \times T - 3.32 \times P \times t - 0.02 \times T \times t - 31.85 \times P^2 - 0.35 \times T^2 - 0.10 \times t^2 \quad (3)$$

where P – extraction pressure;
T – extraction temperature;
t – extraction duration.

The extraction yield is only one of several indicators for assessment of SFE-CO_2 extraction efficiency. Another important indicator, especially when extracting ingredients for nutraceuticals and functional foods, is the amount of bioactive components detected in the isolated extract. Carotenoids are important bioactive compounds present in the lipophilic extract obtained from rosehips.

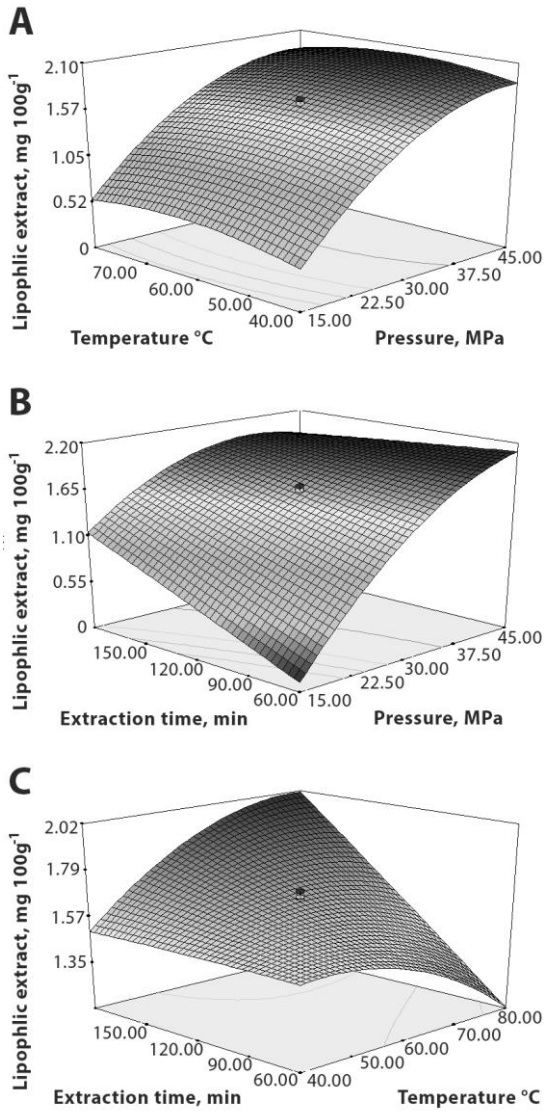

Figure 1. 3D response surface plots of SFE-CO_2 demonstrating the effects of independent variables on the lipophilic extract extraction yield of rosehips

A – effect of extraction pressure and temperature; B – effect of extraction time and pressure; C – effect of extraction time and temperature

The extract yield was found to become higher with increased temperature, however the total β-carotene in the sample behaved contrarily (Table 2). β-carotene recovery at the lowest pressure 15 MPa and the highest temperature 80 °C was the lowest, whereas reducing temperature to 30 °C results in the increase of β-carotene recovery at constant pressure (Longo et al., 2012). However, it is evident that the increase in recovery of β-carotene is more significant when pressure is increasing at constant temperature, showing that pressure is the major factor influencing the recovery of β-carotene. Previously, Prado et al., (2014) concluded that in SFE-CO_2 extraction pressure is the most important factor influencing recovery of carotenoids, since carotenoids are large molecules with a low vapour pressure. These considerations are in agreement with

our findings.

Results suggested that the model for obtaining the highest recovery of β-carotene in the extract (39.1% w/w) were: P=45 MPa; T=63.3 °C, t=150 min.

Table 2

Fully coded central composite design, and lycopene and β-carotene yield in lipophilic fraction

Experiment No.	P	T	t	Yield	
				β-carotene (mg 100 g^{-1} DW)	Lycopene (mg 100 g^{-1} DW)
1	45.0	80.0	60.0	11.2±4.6	7.2±1.9
2	45.0	40.0	180.0	16.1±3.4	4.4±1.0
3	15.0	80.0	180.0	12.3±2.1	7.9±1.2
4	15.0	40.0	60.0	14.1±4.7	5.8±0.5
5	8.8	60.0	120.0	11.2±2.8	8.2±0.4
6	51.2	60.0	120.0	19.1±2.5	4.1±0.2
7	30.0	31.7	120.0	13.1±4.5	8.5±0.9
8	30.0	88.3	120.0	15.1±4.2	5.4±0.3
9	30.0	60.0	35.2	10.0±3.1	4.3±0.5
10	30.0	60.0	204.9	25.1±3.9	2.3±0.5
11	30.0	60.0	120.0	13.9±2.0	6.0±1.4
12	30.0	60.0	120.0	21.5±3.5	5.9±1.4
13	30.0	60.0	120.0	16.2±2.4	6.4±1.1
14	30.0	60.0	120.0	21.3±4.5	7.5±1.0
15	30.0	60.0	120.0	20.2±2.8	7.4±0.9

Values are represented as means ± standard deviation (n = 3).

The approach of lycopene extraction was optimized in order to achieve the maximum concentration yield of the extract. In our study, the extraction temperature had been fixed between 40 and 80 °C. It was determined different composition of the extract when extraction temperature varied from 40 to 60 °C and the pressure varied from 15 MPa to 30 MPa ($p<0.05$). Subsequently, when the temperature was raised from 60 to 80 °C, decreased content of lycopene in the extract was observed (Table 2). Total lycopene in the obtained lipophilic extract could be lost due to several processes, such as degradation through oxidation (at the 80 °C) and higher isomerisation (at 40 and 60 °C). Findings of the research agree with previous studies (Lambelet et al., 2009), which investigated the thermal stability and isomerisation of lycopene under the conditions of 20-100 °C. In our study, the highest recovery of lycopene in the extract (45.2% w/w) was reached under extraction conditions of: P=30 MPa; T=34.5 °C, t=120 min.

Conclusions

The optimum conditions in terms of lipophilic extract extraction yield were obtained at a temperature of 75.2 °C, a pressure of 44 MPa and a time of 115 min, and allowed the recovery of 65% of the extractable lipophilic extract.

References

1. Andersson S.C., Rumpunen K., Johansson E., Olsson M.E. (2011) Carotenoids content and composition in rose hips (*Rosa* spp.) during ripening, determination of suitable maturity marker and implications for health promoting food products. *Food Chemistry*, Vol. 128, p. 689–696.
2. Cazzonelli C.I. (2011) Carotenoids in nature: insights from plants and beyond. *Functional Plant Biology*, Vol. 38(11), p. 833–847.
3. Fan C., Pacier C., Martirosyan D.M. (2014) Rose hip (*Rosa canina* L): A functional food perspective. *Functional Foods in Health and Disease*, Vol. 4(12), p. 493–509.
4. Heymann T., Raeke J., Glomb M.A. (2013) Photoinduced isomerization of lycopene and application to tomato cultivation. *Journal of Agricultural and Food Chemistry*, Vol. 61(46), p. 11133–11139.
5. Horvath G., Molnar P., Rado-Turcsi E., Deli J., Kawase M., Satoh K., Molnar J. (2012) Carotenoid composition and in vitro pharmacological activity of rose hips. *Acta Biochimica Polonica*, Vol. 59(1), p. 129–132.
6. Illes V., Szalai O., Then M., Daood H., Perneczki S. (1997) Extraction of hiprose fruit by supercritical CO_2 and propane. *The Journal of Supercritical Fluids*, Vol. 10(3), p. 209–218.
7. ISO 13320-1 (1999) Particle size analysis - Laser diffraction methods - Part 1: General principles.
8. Kadian S.S., Garg, M. (2012) Pharmacological effects of carotenoids: A review. International *Journal of Pharmaceutical Sciences and Research*, Vol. 3(1), p. 42–48.
9. Lambelet P., Richelle M., Bortlik K., Franceschi F., Giori A.M. (2009) Improving the stability of lycopene Z-isomers in isomerised tomato extracts. *Food chemistry*, Vol. 112(1), p. 156–161.
10. Longo C., Leo L., Leone A. (2012) Carotenoids, fatty acid composition and heat stability of supercritical carbon dioxide-extracted-oleoresins. *International Journal of Molecular Sciences*, Vol. 13(4), p. 4233–4254.
11. Machmudah S., Zakaria, Winardi S., Sasaki M., Goto M., Kusumoto N., Hayakawa K. (2012) Lycopene extraction from tomato peel by-product containing tomato seed using supercritical carbon dioxide. *Journal of Food Engineering*, Vol. 108(2), p. 290–296.
12. Melendez-Martinez A.J., Stinco C.M., Liu C., Wang X.D. (2013) A simple HPLC method for the comprehensive analysis of cis/trans (Z/E) geometrical isomers of carotenoids for nutritional studies. *Food Chemistry*, Vol. 138(2), p. 1341–1350
13. Olson J. A. (1999) Carotenoids and human health. *Archivos Latinoamericanos de Nutrición*, Vol. 49, p. 7–11.
14. Patel S. (2017) Rose hip as an underutilized functional food: Evidence-based review. *Trends in Food Science & Technology*, Vol. 63, p. 29–38.
15. Prado J.M., Veggi P.C., Meireles M.A.A. (2014) Extraction methods for obtaining carotenoids from vegetables-review. *Current Analytical Chemistry*, Vol. 10(1), p. 29–66.
16. Radzevičius A., Viškelis P., Viškelis J., Karklelienė R., Juškevičienė D., Duchovskis P. (2016a) Tomato biochemical composition and quality attributes in different maturity fruits. *Acta Scientiarum Polonorum. Hortorum Cultus (Ogrodnictwo)*, Vol. 15(6), p. 221–231.
17. Radzevičius A., Viškelis J., Karklelienė R., Juškevičienė D., Viškelis P. (2016b) Determination of tomato quality attributes using near infrared spectroscopy and reference analysis. *Zemdirbyste-Agriculture*, Vol. 103(1), p. 91–98.
18. Rao A.V., Rao L.G. (2007) Carotenoids and human health. *Pharmacological Research*, Vol. 55(3), p. 207–216.

19. Szentmihalyi K., Vinkler P., Lakatos B., Illés V., Then M. (2002) Rose hip (*Rosa canina L.*) oil obtained from waste hip seeds by different extraction methods. *Bioresource Technology*, Vol. 82(2), p. 195–201.
20. Urbonaviciene D., Viskelis P. (2017) The *cis*-lycopene isomers composition in supercritical CO_2 extracted tomato by-products. *LWT-Food Science and Technology*, Vol. 85(B), p. 517–523.
21. Urbonaviciene D., Viskelis P., Viskelis J., Bobinas C. (2015) Stability of tomato lycopene under thermal- and light-irradiation treatments in an oil-based model system. *Zemdirbyste-Agriculture*, Vol. 102(2), p. 185–192.
22. Zuknik M.H., Norulaini N.N., Omar A.K.M. (2012) Supercritical carbon dioxide extraction of lycopene: A review. *Journal of Food Engineering*, Vol. 112(4), p. 253–262.

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ANALYSIS OF ORGANIC ACIDS IN HERBAL AND FRUIT SYRUPS BY LIQUID CHROMATOGRAPHY

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Abstract

Syrups are a pleasing balance betveen aromatic and medicinal herbs, an aromatic herb has a strong or bitter taste, an aromatic herb can be added for flavour, if ihe medicinal herb has a bitter or strong taste. The investigation of the research was to evalute the content of organic acids in the herbal and fruit syrups. Some of popular Latvian herbal syrups made from: *Plantago major, Chamaenerion angustifolium* flower*, Calluna vulgaris* flower, *Picea* young shoots, *Pinus* young shoots, *Pinus* cone, *Achillea millefolium, Syringa vulgaris* flower and fruit syrups made from: *Crataegus curvisepala* fruit, *Sorbus aucuparia* fruit, *Rosa canina* fruit, *Japanese quince* fruit, *Aronia melanocarpa* fruit, *Pyrus malus* and *Rheum rhabarbarum* were selected for analysis. The current research focuses on the evaluation of organic acid, pH content and dry matter in herbal and fruit syrups. The major organic acids (oxalic acid, tartaric acid, quinic acid, malic acid, ascorbic acid, citric acid, fumaric acid and succinic acid) were determined by applying the method of high-performance liquid chromatography (Schimadzu Prominence HPLC). In the present experiments it was found that there are significant differences in the organic acids content between different herbal and fruit syrups. In general, all samples tested in this study, demonstrated high content of organic acids. The highest content of organic acids was found in *Pinus* cone syrup 7.82 g 100 g^{-1}, *Rheum rhabarbarum* syrup 4.27 g 100 g^{-1} and *Picea* young shoots syrup 4.14 g 100 g^{-1}. Whereas, the lowest total organic acid content was in *Syringa vulgaris* syrup 1.24 g 100 g^{-1}, *Rosa canina* fruit syrup 1.40 g 100 g^{-1}, *Achillea millefolium* syrup 1.61 g 100 g^{-1} and *Sorbus aucuparia* fruit syrup 1.68 g 100 g^{-1}. Results of the present experiments demonstrated that pH in analysed herbal and fruit syrups was significantly different ($p<0.05$).

Keywords: herbal syrups, fruit syrups, organic acid, HPLC

Introduction

Fruits have been described to contain essential minerals, vitamins, organic acids, amino acids, which provides a wide spectrum for value addition of the juice for applications in the food industry. Often the value add products than be obtained from fruits is the syrup. That's it potential uses in the food industry. The fruit and herbal syrups can be used as an ingredient different bread products. Manufacturers in the food industry often prefer to use carbohydrates in the form of syrup mostly due to the ease and efficiency of solutions and to the favoured process economics (Willis et al., 2013). Scientist Kaushik (2016) from India reported that syrup is a concentrated mixture of sugar and purified water. He emphasizes that the high carbohydrates content distinguishes syrups from other types of liquids. Herbal and fruit syrups may or may not contain added flavouring agents or medication. Scientific literature explains that herbal syrups without a medication, but with a different flavouring agent are called flavoured or non-medicated syrups. Herbal flavoured syrups are often used for prevent unpleasant tasting medications – resulting is medicated syrup (Kaushik et al., 2016). Pleasant flavour, high content of organic acids and fibre makes fruits valuable and interesting raw material for the development of various, healthy food products. The fruit are suitable for juice, liqueur, wine, jam, puree production and also for pectin and aromatic compounds extraction. Syrups, carbonated soft drinks, liqueurs, caramels and marmalades are popular products on the market of the Baltic States and neighbouring countries (Rubinskienė et al., 2014). Some researchers found that fruit syrups contain biologically active compounds that help to create taste and appearance, improve storage time, and provide the body with energy (Willis et al., 2013). Kaushik et al. (2016) informed that in the last years plant derived products are increasingly being sought as the nutraceuticals, cosmetics and medicinal products and are available in health food shops and pharmacies over the counter as self-medication. Herbals are widely used in Phytomedicine, which has a good effect on human health, have anti-inflammatory activity, anti-digestive simulation, antimicrobial activity and antioxidant activity (Zhang et al., 2018; Carabajal et al., 2017).

In turn researcher Brewer (2011) reported that the consumption of foods containing antioxidants is now widely considered to be exert a beneficial effect on human health and an effective trategy to reduce oxidative damage. The food industry in the last years has shiffing focus to herbal products from natural sources as a replacement for synthetic antioxidants and also as nutraceuticals. In her study a scientist Augšpole et al. (2018) noticed that herbals and derived products have many beneficial properties, which are associated with the especially phenolic compounds and presence of secondary metabolites. Fruit and herbal syrup products have been declared to contain antioxidant and antiradical activities, and phenolic compounds (Thériault et al., 2006). Food scientists have proved that therapeutic effects of many herbal species including spring wild plans are attributed to be presence of

antioxidative phenolics and organic acids in their tissues. In herbals they are involed in numerous roles from structural to protective (Vajic et al., 2015). In turn Robbins (2003) reported that Phenolic acids are aromatic secondary plant metabolites, widely spread throughout the plant vegetation. Group of scientists (Mahmood et al., 2012) from Pakistan University of Agriculture described that determination of organic acids is important to the food industry. They emhasize that organic acids play a significant role in influencing appearance, smell and flavour of foods beverages. Definitions on the type and concentration of organic acids are important to ensure the quality of food, especially beverages, juices and syrups. Scientists explain that analytical methods for the determination of organic acids include liquid and gas chromatography, capillary electrophoresis and enzymatic analysis. In turn the separation on short chain aliphatic acids has been determined by phase high performance liquid chromatography using a different column stationary phases (Mahmood et al., 2012). Literature data suggest that organic acids are can give the characteristic gustation and sour taste of the herbals and fruits. Due to organic acids nature, these compounds have found many applications in the beverages and food industry. They are amply used in the industry of beverages and juices as preservatives and pH regulators (Kucner et al., 2014). Researcher Kaushik et al. (2016) reported that advantages of herbal syrup is ability to disguise the bad gustation of medications. These syrups are thicker than water solutions, therefore only a portion of the medication substances dissolved in the syrup comes in contact with the taste buds. Researcher explain that the high carbohydrates content of herbal syrups gives them a sweet taste that helps conceal the bad taste of the medicine. This is why herbal and fruit syrups are recommending for paediatric medications. The thick character of herbal syrups also has a soothing effect on irritated tissues (Kausik et al., 2016). Fruits and herbs are used in many domains, including nutrition, beverages, cosmetics, medicine, fragrances, flavouring and dyeing (Augspole et al., 2018). Fruit and herbal are used by the most of the world's population. Herbal plants provide beneficial health effects due to the presence of antioxidant (Augspole et al., 2017). According to report of the World Health Organization (WHO), about 80% of the world population still uses fruits and herbs for their primary health care needs. Fruit and plants formulations have reached widespread acceptability as therapeutic agents. In the World Health Organization (WHO) definition is three kinds of fruit and herbal medicines – medicinal products, raw plant material and processed plant material (Kaushik et al, 2016). Selection of the most suitable plant cultivars and hybrids for industrial processing and expansion of product assortment is very important. The perennial plant breeding process is time-consuming and may last many years (Rubinskienė et al., 2014). Food scientists have proved that chemical composition of herbal is affected by different factors: climate, harvest time, variety, vegetative stage of the herbal, genotype, soil, storage, technological processes applied, treatment and processing (Marrelli et al., 2012; Meireles, 2009).

The aim of this investigation was to evaluate the concent of organic acids, as a potential source of biologically active compounds, in fruit and herbal syrups using high-performance liquid chromatography.

Materials and Methods

The research was carried out at the Department of Chemistry of the Faculty of Food Technology of the Latvian University of Life Sciences and Technologies. The object of the research was Latvian herbal syrups: Greater plantain (*Plantago major),* Rosebay willowherb *(Chamaenerion angustifolium)* flower, Heather *(Calluna vulgaris)* flower, Spruce *(Picea)* young shoots, Pine *(Pinus)* young shoots, Pine *(Pinus)* cone, Yarrow *(Achillea millefolium),* Lilac *(Syringa vulgaris)* flower and fruit syrups: Hawthorn (*Crataegus curvisepala)* fruit, Rowan (*Sorbus aucuparia)* fruit, Rosehip (*Rosa canina)* fruit, Japanese quince (*Chaenomeles japonica)* fruit, Aronia (*Aronia melanocarpa)* fruit, Apple (*Pyrus malus)* and Rhubarb (*Rheum rhabarbarum).*

Preparation of herbal syrups

Preparation of herbal and fruit syrups - the fixed mass of certain plants or fruits (200 g fruits) were boiled in a certain volume of water (400 mL). After that, sugar has been added (400 g) and composition has been boiled for 20 min. The time of preparing as well as the amount of taken ingredients and other raw materials may vary depending on plant or fruit origin. Syrups were prepared by usual domestic preparation technique and precise technologies are confidential. After boiling syrups were filtered through cheesecloth and centrifuged for 5 minutes at 10 000 rpm.

Determination of pH

The pH level of herbal syrup samples was determined with potentiometric method WTW pH (pH538) meter. The electrode Sen Tix 97T was used.

Determination of organic acids by High Performance Liquid Chromatography

Organic acids in the syrups was analysed with high-performance liquid chromatography (HPLC), Schimadzu LC-20 Prominence, DAD M20A detector, LC-20A solvent delivery system, CBM-20A system controller and LCsolution data system software.

Preperation of the calibration solution: in the 50 mL volumetric flask with narrow neck weight 0.1 g oxalic, 0.1 g tartaric, 0.1 g quinic, 0.1 g malic, 0.1 g ascorbic, 0.1 g citric, 0.02 g fumaric and 0.1 g succinic acids. Fill the volumetric flask with HPLC grade methanol (CHROMASOLV®) till mark and mix.

Chromatography parameters for the separation of organic acids: YMC C18 analytical column, 5µm, 4.6 mm×250 mm, column temperature +35 °C, wavelength 210 nm, volume of the sample injection 10 µL, start flow rate 1.25 mL min^{-1}, mobile phase at the gradient conditions: Acetonitrile (A) : 0.05 *M* KH_2PO_4 (B) (1:99) was used. Each measuring was carried out

several times and then the simple average was calculated (Cinkmanis et al., 2018). Figure 1 shown the calibration chromatogram of organic acids.

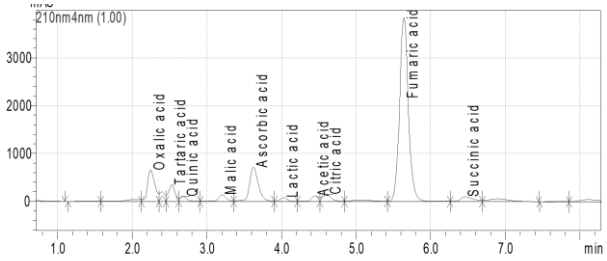

Figure 1. Chromatogram of the calibration solution of the organic acids

Statistical analysis of the research

The data of the research was analysed by the statistical and mathematical methods (standard deviation, mean). Data compared by the analysis of variance (ANOVA) and significance was defined at $p<0.05$. For the data analysis the Microsoft Excel software of the version 2016 was used.

Results and Discussion

Determination of organic acids

To compare herbal and fruit syrups, the content of organic acids was determined in the ascorbic, citric, oxalic, quinic, tartaric, succinic and fumaric acids (Tables 1, 2, 3, 4).

Table 1

Oxalic, tartaric, quinic and malic acids of herbal syrups, g 100 g^{-1}

Syrups	Oxalic acid	Tartaric acid	Quinic acid	Malic acid
Yarrow	0.01±0.003	0.60±0.03	0.08±0.005	0.22±0.02
Pine young shoots	0.06±0.002	0.50±0.02	0.87±0.06	0.08±0.01
Spruce young shoots	0.06±0.01	1.06±0.05	1.83±0.03	0.12±0.01
Heather flower	0.01±0.003	1.06±0.05	0.02±0.005	0.05±0.01
Rosebay willowherb flower	0.01±0.002	0.59±0.01	0.09±0.001	0.18±0.02
Greater plantain	nd	0.43±0.02	0.13±0.02	0.31±0.02
Pine cone	0.03±0.005	0.91±0.04	0.78±0.04	0.55±0.03
Lilac flower	0.01±0.004	0.36±0.02	0.35±0.01	0.21±0.01

nd – not detected

Table 2

Ascorbic, citric, fumaric and succinic acids of herbal syrups, g 100 g^{-1}

Syrups	Ascorbic acid	Citric acid	Fumaric acid	Succinic acid
Yarrow	0.03±0.001	0.49±±0.06	nd	0.17±0.03
Pine young shoots	1.07±0.02	0.01±0.001	nd	0.12±0.001
Spruce young shoots	0.81±0.04	0.02±0.001	nd	0.24±0.04
Heather flower	nd	0.42±0.02	nd	0.29±0.02
Rosebay willowherb flower	0.24±0.01	0.33±0.05	nd	1.00±0.04
Greater plantain	0.01±0.03	3.44±0.13	nd	0.15±0.01
Pine cone	5.52±0.12	0.01±0.001	nd	0.02±0.004
Lilac flower	0.03±0.01	0.13±0.007	nd	0.15±0.03

nd – not detected

Table 3

Oxalic, tartaric, quinic and malic acids of fruit syrups, g 100 g^{-1}

Syrups	Oxalic acid	Tartaric acid	Quinic acid	Malic acid
Rowan fruit	nd	1.00±0.06	0.01±0.005	0.44±0.02
Rhubarb	0.12±0.01	2.41±0.08	0.03±0.01	1.29±0.06
Rosehip fruit	0.01±0.005	0.44±0.02	0.36±0.01	0.15±0.01
Japanese quince fruit	nd	0.47±0.03	0.53±0.03	0.90±0.02
Aronia fruit	0.01±0.005	0.69±0.03	0.30±0.02	0.51±0.03
Apple	0.01±0.005	0.96±0.05	0.01±0.005	1.21±0.04
Hawthorn fruit	nd	0.8	0.36±0.02	0.24±0.02

nd – not detected

Table 4

Ascorbic, citric, fumaric and succinic acids of fruit syrups, g 100g^{-1}

Syrups	Ascorbic acid	Citric acid	Fumaric acid	Succinic acid
Rowan fruit	0.02±0.005	0.04±0.002	nd	0.17±0.01
Rhubarb	0.01±0.005	0.05±0.004	nd	0.36±0.03
Rosehip fruit	0.08±0.01	0.30±0.03	0.02	0.04±0.01
Japanese quince fruit	0.02±0.005	nd	nd	0.20±0.05
Aronia fruit	0.02±0.003	0.17±0.02	nd	0.09±0.01
Apple	0.02±0.002	0.15±0.03	nd	nd
Hawthorn fruit	0.04±0.002	0.37±0.04	nd	0.12±0.02

nd – not detected

The highest oxalic and tartaric acids content was found in Pine young shoots syrup and Spruce young shoots syrup 0.06 and 1.06 g 100 g^{-1}, respectively the highest quinic acid content was detected in Spruce young shoots syrup 1.83 g L^{-1}, and malic acid in Pine cone syrup 0.55 g 100 g^{-1} (Table 1).

The highest ascorbic acid content was detected in Pine cone syrup 5.52 g 100 g^{-1} and Pine young shoots syrup 1.07 g 100 g^{-1}, citric acid in Greater plantain syrup 3.44 g 100 g^{-1}, and succinic acid in Rosebay willow herb flower syrup 1.00 g 100 g^{-1}. Fumaric acid was not found in herbal syrups (Table 2).

The highest oxalic and tartaric acid content was found in Rhubarb syrup 0.12 and 2.41 g 100 g^{-1}, respectively, while quinic acid dominated in Japanese quince fruit syrup 0.53 g 100 g^{-1}, and malic acid in Rhubarb syrup 1.29 g 100 g^{-1} and Apple syrup 1.21 g 100 g^{-1} (Table 3).

The highest ascorbic acid content was detected in Rosehip fruit syrup 0.08 g100 g^{-1}, whereas highest citric acid contend was determined in Hawthorn fruit syrup 0.37 g 100 g^{-1}, and succinic acid in Rhubarb syrup 0.36 g 100 g^{-1}. Fumaric acid was found only in one Rosehip fruit syrup 0.02 g 100 g^{-1} (Table 4).

The richest source of the total organic acids comparing all herbal and fruit syrups was Pine cone 7.82 g 100 g^{-1}, Rhubarb 4.27 g 100 g^{-1} and Spruce young shoots 4.14 g 100 g^{-1}. The lowest content of total organic acid was found in Lilac flower 1.24 g g^{-1}, Rosehip fruit 1.40 g 100 g^{-1}, Yarrow 1.61 g 100 g^{-1} and Rowan fruit 1.68 g 100 g^{-1} syrups.

Determination of pH

The results of pH level performed on the plant and herbal syrup formulation revealed that the pH varied in the range of 4.27–3.19, which indicates acidic environment. The difference between lowest and highest pH was significant – 1.08 units ($p<0.05$).

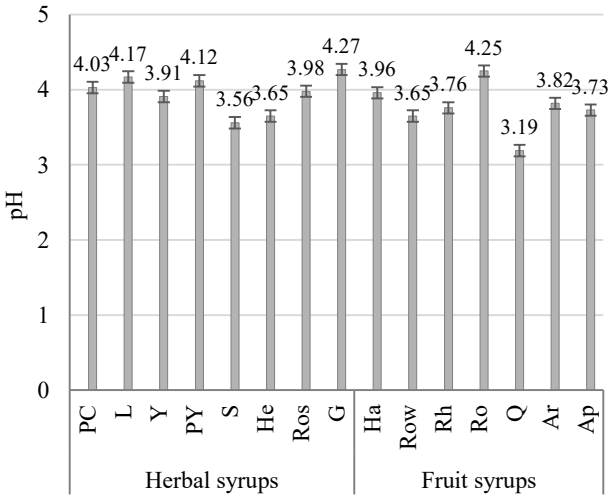

Figure 2. pH of herbal and fruit syrups

PC – Pine cone, L – Lilac flowers, Y – Yarrow, PY – Pine young shoots, S – Spruce young shoots, He – Heather flower, Ros – Rosebay willowherb flower, G – Greater plantain, Ha – Hawthorn fruit, Row – Rowan fruit, Rh – Rhubarb, Ro – Rosehip fruit, Q – Japanese quince fruit, Ar – Aronia fruit, Ap – Apple

The highest pH level was found in herbal and fruit syrups in descending order: Greater plantain 4.27, Lilac 4.17, Pine young shoots 4.12, Pine cone 4.03 and Rosehip fruit 4.25, the lowest pH is measured by Japanese quince fruit 3.19, Spruce young 3.56 and Heather flower 3.65.

Acidic environment of herbal and fruit syrups is resulting of concentration of individual organic acids in plants, large content of sugar sucrose, its hydrolysation rate with formation of inverted sugar and glucose oxidation to gluconic acid at a specific temperature (Farrokhi et al., 2012).

Conclusions

The results of the research indicate that fruits and herbal syrups are source of organic acid compounds.

The herbal syrups accumulated higher amounts of organic acid compounds (Pine cone syrup – ascorbic acid was 5.52±0.85 g 100g^{-1} and Plantain syrup Citric acid 3.44±0.74 g 100 g^{-1}, respectively) than the fruit syrups.

Furthermore, Fruit syrups (rhubarb) had higher content of tartaric acid (2.41±0.08 g 100 g^{-1}) and higher malic acid (rhubarb and apple syrups) (1.29±0.42 g 100 g^{-1} and 1.21±0.35 g 100 g^{-1}).

References

1. Angela M., Meireles A. (2009). *Extracting bioactive compounds for food products theory and applications.* Boca Raton, EUA: CRC Press Taylor & Francis Group, 466 p.
2. Augspole I., Duma M., Cinkmanis I., Ozola B. (2018) Herbal teas as a rich source of phenolic compounds. *Chemija,* Vol. 29(4), p. 257–262.
3. Augspole I., Duma M., Ozola B., Cinkmanis I. (2017) Phenolic profile of fresh and frozen nettle, goutweed, dandelion and chickweed leaves. **In:** *FoodBalt 20017: 11th Baltic conference on food science and technology "Food science and technology in a changing world": Conference proceedings,* Jelgava, p. 36–39.
4. Bräunlich M. (2013) Bioactive constituents in aronia berries. *Thesis submitted for the degree of Ph.D. Department of Pharmaceutical Chemistry School of Pharmacy University of Oslo,* 110 p.
5. Brewer, M.S. (2011) Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety*, Vol. 10 (4), p. 221–247.
6. Carabajal M.P.A., Isla M.I., Zampini I.C. (2017) Evaluation of antioxidant and antimutagenic activity of herbal teas from native plants used in traditional medicine in Argentina. *South African Journal of Botany,* Vol. 110, p. 258–265.
7. Cinkmanis I., Gailums G., Vucane S. (2018) Comparative study of dark beer before and after freeze drying rehydration cycle. *Proceedings of the Latvian Academy of Sciences.* **In:** *Section B. Natural, Exact and Applied Sciences. Vol. 72(2): 2nd International conference "Nutrition and Health";* p. 123–127.
8. Farrokhi F., Mizani M., Honarvar M. (2012) Study of probable physicochemical changes during the storage of light and thick sucrose syrups. *World Applied Sciences*, Vol. 18(5), p. 715–721.

9. Kaushik A., Chauhan V., Sudha Dr. (2016) Formulation and evaluation of herbal cough syrup. *European Journal of Pharmaceutical and Medical Research,* Vol. 3(5), p. 517–522.
10. Kucner A., Klewicki R., Sojka M., Klewicka E. (2014) Osmotic concentration of gooseberry fruits – The influence of temperature, time and pretreatment methods on mass transfer and total polyphenol and organic acid content. *Food Technology and Biotechnology,* Vol. 52(4), p. 411–419.
11. Mahmood T., Anwar F., Abbas M., Boyce M.C., Saari N. (2012) Compositional variation in sugars and organic acids at different maturity stages in selected small fruits from Pakistan. *International Journal of Molecular Sciences,* Vol. 13, p. 1380–1392.
12. Marrelli M., Menichini F., Statti G.A., Bonesi M., Duez P., Menichini F., Conforti F. (2012) Changes in the phenolic and lipophilic composition, in the enzyme inhibition and antiproliferative activity of *Ficuscarica* L. cultivar Dottato fruits during maturation. *Food and Chemical. Toxicology,* Vol. 50, p. 726–733.
13. Robbins R.J. (2003) Phenolic acids in foods: an overview of analytical methodology. *Journal of Agricultural and Food Chemistry,* Vol. 51(10), p. 2866–2887
14. Rubinskiene M., Viškelis P., Viškelis J., Bobinaite R., Shalkevich M., Pigul. M., Urbanovičiene D. (2014) Biochemical composition and antioxidant activity of japanese quince fruits and their syrup and candied fruit slices. **In:** *FOODBALT 2014: 9th Baltic conference on food science and technology "Food for consumer well-being": conference proceedings,* Jelgava, p. 45–52.
15. Thériault M. S., Caillet S., Lacroix Kermasha M. (2006) Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products. *Food Chemistry,* Vol. 98 (3), p. 490–501.
16. Vaji´c U.J., Gruji´c–Milanovi'c J., Živkovi´c J., Šavikin K., Godevac D., Miloradovi´c Z., Bugarski B., Stanojevi´c N.M. (2015) Optimization of extraction of stinging nettle leaf phenolic compounds using response surface methodology. *Industrial Crops and Products,* Vol. 74, p. 912–917.
17. Willis O.O., Mouti M.E., Sila D.N., Mwasaru M., Thiongo G., Murage H., Ojijo N.O. (2013) Physico-chemical properties and antioxidant potential of syrup prepared from 'Madhura' sweet sorghum (*Sorghum bicolor* L. Moench) cultivar grown at different locations in Kenya. *Sugar Techology,* Vol. 15(3), p. 263–270.
18. Zhang C., Li-Chieh Suen C., Yang C., Quek S.Y. (2018) Antioxidant capacity and major polyphenols composition of teas as affected by geographical location, plantation elevation and leaf grade. *Food Chemistry,* Vol. 244, p. 109–119.

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LIPIDS OF CULTIVATED AND WILD *VACCINIUM* SPP. BERRIES FROM LATVIA

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Abstract

Production, consumption and processing of different berries nowadays is increasing, considering taste properties and health benefits of these berries. In Northern countries and Latvia of special importance are berries belonging to *Vaccinium* species (cranberries, bilberries, blueberries, lingonberries and others) and many of these berries are considered as the super fruits. Value of the berries is determined by the presence of many biologically active and valuable substances and amongst them berry lipids have a special role. Lipids of *Vaccinium* berries include not only triglycerides, but also fatty acids, alcohols, triterpenes (sterols), terpenes and other substances The aim of the present study was to investigate and compare the composition of lipids in five *Vaccinium* spp. berries and of eight *Vaccinium corymbosum* varieties. Lipid composition was analysed using gas chromatography with mass spectrometric detection. The lipid fraction contained compound classes like fatty acids, sterols, triterpenoids, alkanes, phenolic and carboxylic acids and tocopherols. All fresh berries contained high amounts of C18 unsaturated fatty acids (for example, up to 11.83 g 100 g^{-1}) and phytosterols (10.97 g of β-sitosterol 100 g^{-1} of blueberry lipid extract), and high amounts of benzoic acid were found in lingonberries (1.64 g 100 g^{-1}). The analysed berry lipid profiles were compared using the principal component analysis. The analysis showed that the lipid profiles of the studied berries reflect their taxonomy-separate species could be distinguished from one another. Considering the composition of berry lipids, they can find wide application at development of functional food.

***Keywords*:** lipids, waxes, *Vaccinium* berries, GC-MS

Introduction

Wild and locally cultivated berries are becoming more popular considering the taste properties, health benefits and growing demand in healthy foods. In Northern countries the most used berries are strawberries and berries belonging to *Vaccinium* species. *Vaccinium* berries - bog bilberry (*Vaccinium uliginosum* L.), bilberry (*Vaccinium myrtillus* L.), American cranberry (*Vaccinium macrocarpon*), lingonberry (*Vaccinium vitis-idaea* L.), cranberry (*Vaccinium oxycoccus* L.) and blueberries (*Vaccinium corymbosum* L.) are sampled in forests and bogs, but American cranberries and highbush blueberries are widely cultivated. *Vaccinium* berries are rich source of polyphenolic substances, carbohydrates, vitamins as well as lipids (Arevström et al., 2019; Klavins et al., 2015). Highbush blueberries and American cranberries are commercially important as they have large fruits, long shelf life, excellent taste properties and considering this, many cultivars are developed and their cultivation takes place worldwide, the cultivation amounts are steadily increasing (Krueger et al., 2013). *Vaccinium corymbosum* berries can be considered as functional food and many cultivars are developed differing in taste, size and colour of berries, productivity and length of growing season (Kim et al., 2013).

Vaccinium berry phenolic compounds are responsible for their high radical scavenging capacity and beneficial effects on human health (Nile, Park, 2014). Another important group of substances in the composition of *Vaccinium* berries are their lipids (Klavins et al., 2016). Berry lipids are berry skin waxes, seed lipids and cytoplasm lipids. By the chemical composition *Vaccinium* berry lipids include triglycerides, fatty acids, alcohols, alkanes as well as sterols, terpenes and other groups of substances (Dulf et al., 2012). Extraction of berry lipids is usually done using low-polarity organic solvents, for example, chloroform, hexane, petroleum ether. Combining the solvent with various extraction methods is advisable to achieve high extraction yields, such methods include Soxhlet, maceration, ultrasound assisted extraction (Wang, Weller, 2006). The composition of berries depends on their vegetation locations and thus it is important to study berry composition in each specific site. A further important aspect is to identify the most health beneficial species from naturally grown as well as many commercially available *Vaccinium* berry species, for example, *Vaccinium corymbosum*.

The aim of the present study was to investigate and compare the composition of lipids in five *Vaccinium* spp. berries of which eight were *Vaccinium corymbosum* varieties.

Materials and Methods

Plant materials

In this study, six berry species were examined for their lipid composition. The examined wild berries were – bog bilberry (*Vaccinium uliginosum* L.), bilberry (*Vaccinium myrtillus* L.), American cranberry (*Vaccinium macrocarpon*), lingonberry (*Vaccinium vitis-idaea* L.), cranberry (*Vaccinium oxycoccus* L.) and eight varieties of cultivated blueberry (*Vaccinium corymbosum* L.), namely, 'Blue Crop', 'Blue Gold', 'Chandler', 'Chippewa', 'Duke', 'North Blue', 'Patriot', 'Polaris'.

The different blueberry varieties and American cranberries were harvested at a commercial blueberry farm Z/S "Strelnieki" located on the outskirts of town Jurmala, Latvia. Bog bilberries, bilberries, cranberries and lingonberries were harvested from the forests belonging to Kemeru National Park. To avoid contamination and possible damage to the berries, they were harvested into glass containers previously washed with chloroform (≥99%, Sigma Aldrich). In total, approximately 500 g of berries were harvested for each sample, all berries were harvested during the summer / autumn of 2018. After the harvest, berries were placed into a refrigerated sample box and delivered to the laboratory, where they were dried at 40 °C in a drying oven (Memmert, Germany) and afterwards used for the extraction. Temperature of 40 °C was chosen to avoid degradation of substances in the berries.

Extraction of berry lipids

Extraction was performed based on the previous work (Klavins et al., 2015). In short, 5.00±0.01 g of dried berries were weighed into a 100 mL glass extraction vessel. 50 mL of chloroform was added to the extraction vessel, which was placed into the ultrasound bath (*Cole-Parmer*) for 20 minutes. After the ultrasound assisted extraction the sample was removed and filtered through a 10 µm filter paper to remove the solids. The used filter paper was then placed back into the extraction vessel and 50 mL of chloroform were added. The extraction was repeated three times. All the extracts were combined and placed into a round-bottom flask for evaporation. Chloroform was evaporated using Rota-Vap® rotary evaporator until approximately 5 mL of extract was left in the flask. The extract was placed into a glass tube and the flask was washed three times with 2 mL chloroform to remove any residual extract. Samples were stored at -20 °C until the analysis. Extraction and analysis were carried out in triplicate.

GC-MS analysis

Berry lipid-chloroform extracts were evaporated under a flow of nitrogen. Silylation was done using N,O-bis (trimethylsilyl) trifluoroacetamide, BSTFA (200 µL, Sigma-Aldrich) in pyridine (1300 µL, Sigma-Aldrich), for 1 hour at 60 °C. GC-MS analysis was performed using GC-2010 plus coupled with GC/MS QP-2010 Ultra mass detector (Shimadzu, Japan). The column used was Restek Rxi®-5MS (30 m×0.25 mm×0.25 µm; Crossbond ® 5% diphenyl+95% dimethyl polysiloxane, Restek USA) with working temperature range 40 to 350 °C. He (Helium) was used as a carrier gas with a total flow rate of 10.8 mL min^{-1} and column flow rate of 0.71 mL min^{-1}. The split ratio was 1:10 and injection temperature 290 °C. The temperature programme used was: oven temperature 200 °C (2 min) increased to 250 °C at the rate of 30 °C min^{-1} and held for 7 min then increased to 310 °C at the rate of 10 °C min^{-1} and kept for 14 min. Injection of 1.0 µL sample was performed using an autosampler. Mass selective detector with quadrupole mass analyser was used with electron impact (EI) ionisation, ionization voltage of 70eV. The ion source temperature was 230 °C and interface temperature 290 °C. Identification of the compounds separated in the GC was performed using Shimadzu LabSolutions 4.30 software, coupled with NIST'14 spectral library.

Quantification was done by preparing standard solutions of heptadecanoate (≥99.0%), 1-dodecanal (≥98.0%), (±)-α-tocopherol (99%), 1-octadecanol (99%), and n-tetracosane (≥99.5%) (Sigma Aldrich, Germany) in the concentration range 1.5–500 µg mL^{-1}.

Data analysis

Principal component analysis (PCA) on correlation matrix was performed to evaluate relationship among various tested berries. Error bars in the Figures represent 95% confidence interval. Statistical analysis and data visualisation was done using SAS JMP®, Version 13 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Studied *Vaccinium* berries in general have relatively similar lipid concentrations extracted from whole, dried berries with chloroform, ranging from 6.90 to 9.17 g 100 g^{-1} of dried berries (Table 1).

Table 1

Lipid concentrations in studied *Vaccinium* berry species

Berries	Lipid extract, g 100 g^{-1} dried berries	Total identified lipids, g 100 g^{-1} of lipid extract
American cranberry	9.17	39.62
Bilberry	8.37	33.46
Lingonberry	9.05	55.24
Bog cranberry	7.57	49.35
Bog bilberry	8.66	53.18
Blueberry cv. Blue crop	6.90	52.28
Blueberry cv. Blue gold	7.84	50.95
Blueberry cv. Blue ray	7.10	70.04
Blueberry cv. Chippewa	7.51	83.82
Blueberry cv. Duke	8.15	53.40
Blueberry cv. North blue	8.18	69.22
Blueberry cv. Patriot	7.65	62.48
Blueberry cv. Polaris	7.46	44.39

Lipid concentrations in wild *Vaccinium* berries and American cranberries were slightly higher than in varieties of blueberries. Relatively similar concentrations of lipids in *Vaccinium* berries might be related to similar and species-independent functions in berries (Yang, Kallio, 2002), such as protective properties of berry wax layer as well as functional lipids

participating in the regulatory processes of plant development during their growth and maturing (Yang, Kallio, 2002) as well as present in the berry seeds. Analysis of studied berry lipids were done using gas chromatography coupled with mass spectrometric detection of samples derivatized with trimethylsilygroups and a large number of substances were found. The substances were identified by the retention index and mass spectra, and compared with the NIST mass spectral library data (Fig. 1). The mass spectra were matched with at least 90% confidence. Nevertheless, the total amount of identified substances in the extracts ranged from 33.46% to 83.82% in the studied berries.

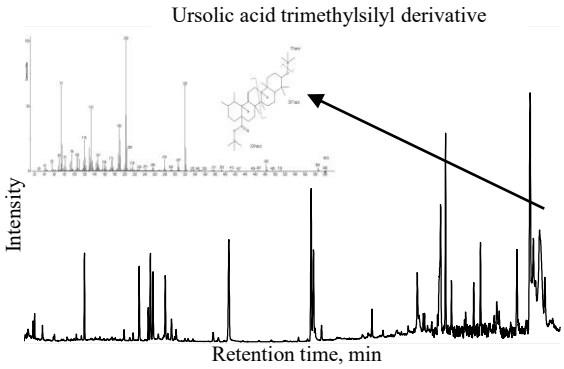

Figure 1. **A typical GC/MS chromatogram of berry lipid extract (blueberry variety 'Polaris)' and an example of ursolic acid TMS derivative mass spectrum**

The unidentified portion of the extracts (Table 1) are believed to be complex compounds with large molar mass, for instance, triterpenes bound with fatty acids (Esche et al., 2012). Chromatographic runs had high repeatability, and the standard deviations between three parallel runs for the specific apparatus were less than 5–8%. In total, approximately 120 different compounds were found as part of the berry lipids from various berries.

The identified compounds of berry lipids can be divided into 6 classes of organic substances (Fig. 2). The largest class in each berry species was fatty acids (up to 60% of the total lipids in the varieties of blueberry).

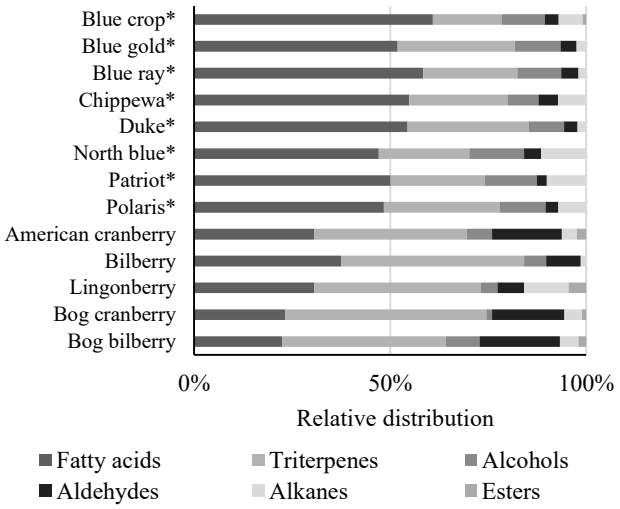

Figure 2. Relative abundance of identified compound groups as part of the studied berry lipids
Berries marked with * are cultivars of *Vaccinium corymbosum.*

Blueberry cultivars have relatively similar profiles of compound classes (Fig. 2), which on the other hand differ from other *Vaccinium* berries. At the same time remarkable are high concentrations of triterpenes in composition of bilberry and other wild berries. The high content of fatty acids in the berries is due to large quantity of seeds, in which energy is stored in the form of fatty acids (Johansson, Kallio, 1997; Parry et al. 2005). Blueberry, bilberry and both cultivars of highbush blueberries (cv. Blue Ray and cv. Chippewa) are closely related, which can also be seen in their compound class profiles. In the research of Parry et al. (2005) blueberry seed oils were analysed, the fatty acid contents shown were similar to that of reported in this study.

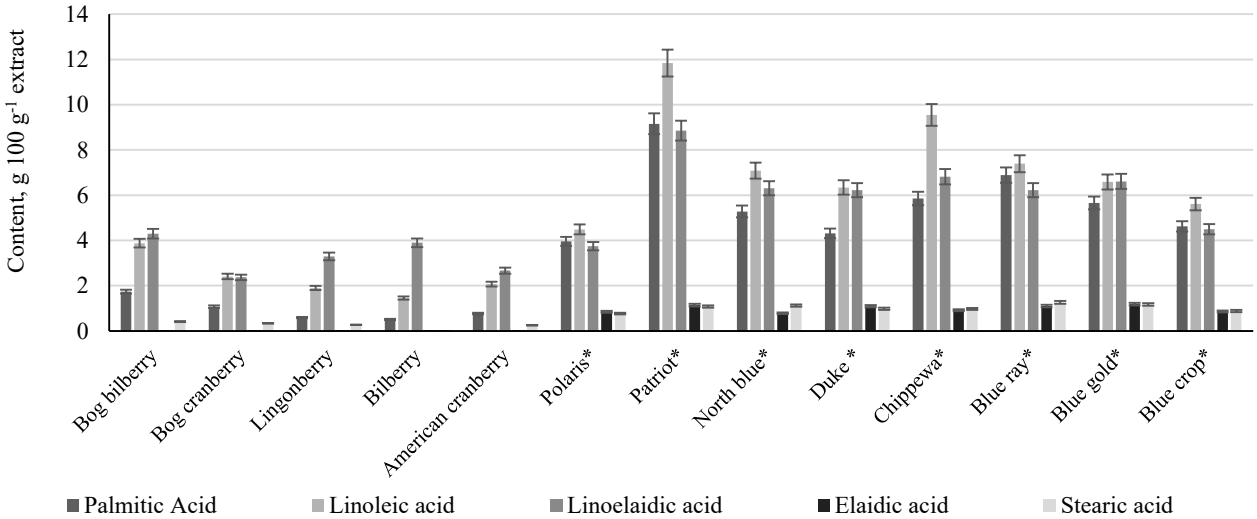

Figure 3. Fatty acid (C16 and C18) content in the studied berry lipids
Berries marked with * are cultivars of *Vaccinium corymbosum.*

Table 2

Concentration and chain length of detected alkanes in studied wild berries and cultivated berries (g of substance 100 g^{-1} extract)

Berries	Alkanes							
	C19	C23	C25	C26	C28	C29	C30	C31
Bog bilberry	<LOD	0.13	0.38	<LOD	3.31	0.9	<LOD	<LOD
Bog cranberry	<LOD	<LOD	<LOD	<LOD	1.53	1.49	<LOD	<LOD
Lingonberry	<LOD	<LOD	0.13	<LOD	0.11	6.81	0.42	1.67
Bilberry	<LOD	<LOD	0.21	<LOD	<LOD	0.34	<LOD	<LOD
American cranberry	<LOD	<LOD	<LOD	<LOD	<LOD	2.33	<LOD	<LOD
Blueberry cv. Polaris	<LOD	<LOD	0.22	<LOD	0.25	4.58	<LOD	0.73
Blueberry cv. Patriot	0.33	<LOD	0.36	<LOD	0.45	8.01	<LOD	2.70
Blueberry cv. North blue	0.28	0.24	0.33	0.25	0.66	9.33	0.56	2.80
Blueberry cv. Duke	0.22	<LOD	0.20	0.20	<LOD	0.97	<LOD	0.22
Blueberry cv. Chippewa	0.19	<LOD	0.62	0.44	0.38	6.05	0.29	0.91
Blueberry cv. Blue ray	0.30	<LOD	0.27	<LOD	0.34	1.31	<LOD	<LOD
Blueberry cv. Blue gold	0.29	<LOD	0.22	<LOD	0.15	1.03	<LOD	0.24
Blueberry cv. Blue crop	0.24	<LOD	0.24	0.21	0.25	3.18	<LOD	0.89

LOD – amount lower than the limit of detection (0.01–0.2 μg g^{-1})

Substances like benzoic acid (0.64 to 1.64 g 100 g^{-1}), nonanoic acid (0.34 to 1.43 g 100 g^{-1}), *m*-hydroxybenzoic acid (0.16 to 0.52 g 100 g^{-1}), squalene (0.37 to 2.04 g 100 g^{-1}), α-tocopherol (0.65 to 3.51 g 100 g^{-1}) and β-sitosterol (up to 113 mg 100 g^{-1}) were found in all of the studied oils obtained from berries. The substance with the highest concentration was benzoic acid (1.64 g 100 g^{-1}) in lingonberry. All of the C18 unsaturated fatty acids were also found in high concentrations (up to 11.83 g 100 g^{-1} in blueberries) (Fig. 3). Looking deeper in the profile of long-chain fatty acids in *Vaccinium* berries (Fig. 3) differences amongst the studied berries are more evident ($p<0.001$), the studied blueberry varieties have a higher fatty acid content than the wild berries (Fig. 3). Cultivars of *Vaccinium corymbosum* have up to 12.02 g of linoleic acid per 100 g of berry extract, while the average for tested cultivars is 6.14 g 100 g^{-1}. In contrast, the wild berries show average value of 3.24 g 100 g^{-1} of found fatty acids (Fig. 3).

In the research of Parry et al. (2005) blueberry seed oils were analysed, the fatty acid contents shown were similar to that of reported in this study, however, triterpene contents were not reported. Also, the used blueberry variety was not mentioned (Parry et al., 2005), which can be an important factor and have influence on the obtained result, as the obtained lipid profiles shown in the present research suggest (Figure 2). A group of substance often found as a part of lipid extracts are alkanes. These compounds are a part of berry epicuticular wax and in cytoplasm of berry they are for biosynthesis of other plant metabolites. The carbon chain length of alkanes found in berries ranges from C19 to C31 (Table 2).

As the most prominent of the alkanes in berries the C29 alkane (nonacosane) was found (Fig. 4). Among the studied wild berries, the highest concentration of nonacosane was found in lingonberry (6.81 g 100 g^{-1}) (Table 2). The wild bilberries and bog bilberries had low alkane concentrations, and in bilberry, only C25 and C29 alkanes were found. The studied blueberry varieties showed a much wider alkane content, for example, blueberry variety 'North blue' contained 8 different alkanes (Table 2), with the most prominent alkane being nonacosane (Fig. 4). Alkanes have also been reported as part of blueberry cuticular wax, where alkanes are responsible for hydrophobicity of berry surface (Chu et al., 2017). The most abundant triterpene was found to be ursolic acid, followed by beta-sitosterol and beta-amyrin. In the wild berries like bog bilberry and bog cranberry 27.06 and 28.23 g of ursolic acid in 100 g^{-1} extract were found, respectively. β-sitosterol was found in high concentrations in two blueberry cultivars – 10.72 g per 100 g^{-1} in 'Patriot' and 10.97 g per 100 g^{-1} in 'Chippewa' (Table 3).

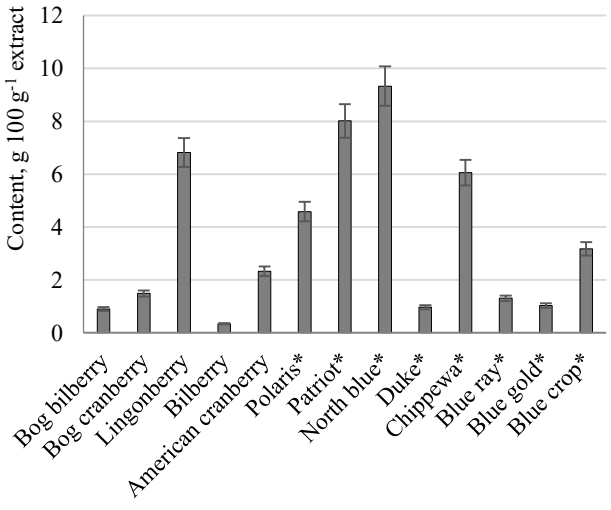

Figure 4. Concentration of nonacosane (C29) in the studied berries

Berries marked with * are cultivars of *Vaccinium corymbosum.*

Triterpenes like lupeol was found in only 2 berries, 'North blue' and 'Blue ray'. Overall, the concentration of triterpenoids was found to be higher in the wild berries.

Table 3

Concentration of triterpenes found in the studied berries (g of substance 100 g^{-1} extract)

Berry	Ursolic acid	β-sitosterol	β-amyrin	α-amyrin	lupeol
Bog bilberry	27.06	6.35	0.48	0.48	<LOD
Bog cranberry	28.23	4.61	<LOD	0.78	<LOD
Lingonberry	24.40	6.03	1.76	1.4	<LOD
Bilberry	12.75	4.56	1.52	<LOD	<LOD
American cranberry	17.08	5.56	0.92	<LOD	<LOD
Blueberry cv. Polaris	12.37	8.66	2.68	<LOD	<LOD
Blueberry cv. Patriot	17.18	10.72	<LOD	<LOD	<LOD
Blueberry cv. North blue	10.73	11.30	3.40	0.76	2.01
Blueberry cv. Duke	12.39	9.77	2.46	<LOD	<LOD
Blueberry cv. Chippewa	8.75	10.97	10.66	<LOD	<LOD
Blueberry cv. Blue ray	12.87	9.96	1.34	<LOD	2.07
Blueberry cv. Blue gold	10.36	9.88	0.94	1.84	<LOD
Blueberry cv. Blue crop	4.47	9.49	<LOD	<LOD	<LOD

LOD – amount lower than the limit of detection (0.01–0.2 μg g^{-1})

As triterpenes have a protective function in plant-pathogen interaction, it can be assumed that the high triterpene concentration in the wild berries is due to the need of regulation and protection against various biotic and abiotic stresses (Du Fall and Solomon, 2011). Plant sterols have potential use in foodstuffs, and cosmetics (Kritchevsky and Chen, 2005)

An important group of compounds found as a part of berry lipids are triterpenes (sterols). These compounds have an important role in the plant metabolism as regulators of the cell processes. Triterpenes were found to compose 50% of identified lipids in bog cranberry and as low as 17% in blueberry variety 'Blue crop' (Fig. 2). The composition of lipids found in the studied berries was believed to be species dependent, to demonstrate this a principal component analysis (PCA) was performed. The first two components of the PCA explain 85.9% of data variability (Fig. 5).

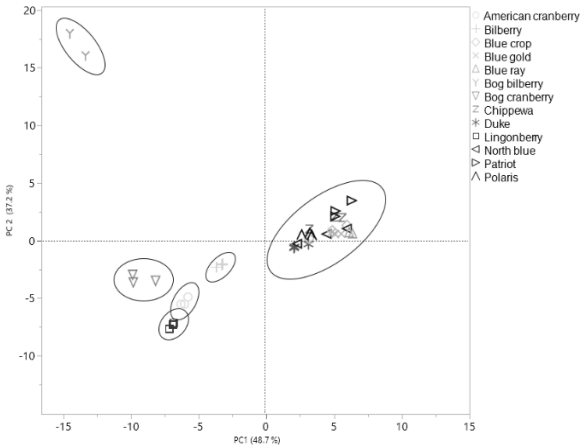

Figure 5. Principal components analysis of various berry species based on their lipid profiles

Obtained lipid profiles when subject to multivariate analysis show distinct differences among the studied berries. Bog bilberries, American cranberries, lingonberries, bog cranberries and bilberries each form a separate cluster, while all of the studied blueberry varieties form one cluster. As the blueberry data was dispersed within the 95% confidence ellipse, it could mean that differences within the same species could be distinguished. Methods like DNA fingerprinting are suggested for differentiation between berry species, however, these methods are time consuming and require specific equipment and materials as well as skilled operators (Polashock, Vorsa, 1996). Analysis of berry lipid profiles can be suggested as a cheaper, user-friendly method for authenticity testing of berry material where DNA is no longer intact. Concerning the methods of authenticity testing of berry material, stable isotope analysis can be suggested, however, using this method the specific species could not be distinguished, only the origin of the tested material (Bertoldi et al., 2019). Cranberries and lingonberries are red berries with similar appearance, using the proposed method of lipid profile analysis they can be distinguished (Fig. 5), a more comprehensive approach to chemotaxonomy is proposed by Hurkova et al. (2019) where complex metabolomics analysis is performed.

Conclusions

Vaccinium genus berries – bog bilberry, bilberry, American cranberry, lingonberry, cranberry and different varieties of blueberries have a rich composition of lipids belonging to compound classes like fatty acids (among which omega-fatty acids), sterols, triterpenoids, alkanes, phenolic acids and tocopherols. In the highest concentrations C18 unsaturated fatty acids and triterpenes like ursolic acid and β-sitosterol were found. Lipid concentration and composition of studied *Vaccinium* berries is species specific and can be used for authenticity testing of berry processing products as well as the berry material itself. Considering the rich composition of berry lipids, they can have wide application and potential for development of innovative functional foods and cosmetics.

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References

1. Arevström L., Bergh C., Landberg R., Wu H., Rodriguez-Mateos A., Waldenborg M. M., Magnuson A., Blanc S., Fröbert O. (2019) Freeze-dried bilberry (*Vaccinium myrtillus*) dietary supplement improves walking distance and lipids after myocardial infarction: an open-label randomized clinical trial. *Nutrition Research*, Vol. 62, p. 13–22.
2. Bertoldi D., Cossignani L., Blasi F., Perini M., Barbero A., Pianezze S., Montesano D. (2019) Characterisation and geographical traceability of Italian goji berries. *Food Chemistry*, Vol. 275, p. 585–593.
3. Chu W., Gao H., Cao S., Fang X., Chen H., Xiao S. (2017) Composition and morphology of cuticular wax in blueberry (*Vaccinium* spp.) fruits. *Food Chemistry*, Vol. 219, p. 436–442.
4. Du Fall L. A., Solomon P. S. (2011) Role of cereal secondary metabolites involved in mediating the outcome of plant-pathogen interactions. *Metabolites*, Vol. 1 (1), p. 64–78.
5. Dulf F.V., Andrei S., Bunea A., Socaciu C. (2012) Fatty acids and phytosterol contents of some Romanian wild and cultivated berry pomaces, *Chemical Papers*, Vol. 66 (10), p. 925–934.
6. Esche R., Barnsteiner A., Scholz B., Engel K. H. (2012) Simultaneous analysis of free phytosterols / phytostanols and intact phytosteryl / phytostanyl fatty acid and phenolic acid esters in cereals. *Journal of Agricultural and Food Chemistry*, Vol. 60 (21), p. 5330–5339.
7. Hurkova K., Uttl L., Rubert J., Navratilova K., Kocourek V., Stranska-Zachariasova M., Paprstein F., Hajslova J. (2019) Cranberries versus lingonberries: A challenging authentication of similar *Vaccinium* fruit. *Food Chemistry*, Vol. 284, p. 162–170.
8. Johansson A., Kallio H. (1997) Characterization of seed oils of wild, edible Finnish berries. *Zeitschrift für Lebensmitteluntersuchung und Forschung A*, Vol. 204 (4), p. 300–307.
9. Kim J. G., Kim H. L., Kim S. J., Park K. S. (2013) Fruit quality, anthocyanin and total phenolics contents, and antioxidant activities of 45 blueberry cultivars grown in Suwon, Korea, *Journal of Zhejiang University Science*, Vol. 14, p. 793–799.
10. Klavins L., Klavina L., Huna A., Klavins M. (2015) Polyphenols, carbohydrates and lipids in berries of *Vaccinium* species, *Environmental and Experimental Biology*, Vol. 13, p. 147–158.
11. Klavins L., Kviesis J., Steinberga I., Klavina L., Klavins M. (2016) Gas chromatography-mass spectrometry study of lipids in northern berries, *Agronomy Research*, Vol. 14, p. 1328–1346.
12. Krueger C. G., Reed J. D., Feliciano R. P., Howell A. B. (2013) Quantifying and characterizing proanthocyanidins in cranberries in relation to urinary tract health. *Analytical Bioanalytical Chemistry,* Vol. 405, p. 4385–4395.
13. Kritchevsky D., Chen S. C. (2005) Phytosterols – health benefits and potential concerns: A review. *Nutrition Research*, Vol. 25 (5), p. 413–428.
14. Nile S. H., Park S. W. (2014) Edible berries: Bioactive components and their effect on human health. *Nutrition*, Vol. 30 (2), p. 134–144.
15. Parry J., Su L., Luther M., Zhou K., Yurawecz M. P., Whittaker P., Yu L. (2005) Fatty acid composition and antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils. *Journal of Agricultural and Food Chemistry*, Vol. 53 (3), p. 566–573.
16. Polashock J. J., Vorsa N. (1996) Evaluation of fingerprinting techniques for differentiation of cranberry and blueberry varieties. *VI International Symposium on Vaccinium Culture,* Vol. 446, p. 239–242.
17. Wang L., Weller C. L. (2006) Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, Vol. 17 (6), p. 300–312.
18. Yang B., Kallio H. (2002) Composition and physiological effects of sea buckthorn (*Hippophaë*) lipids, *Trends in Food Science & Technology*, Vol.13(5), p. 160–167.

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PIGMENTS CONTENT IN DIFFERENT PROCESSED EDIBLE WILD PLANTS

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Abstract

Natural colours of wild plants are similar to artificial pigments, for example: β-carotene and chlorophyll. The aim of research was to investigate the effect of drying or freezing on the colour intensity and pigments content in leaves of edible wild plants - stinging nettle (*Urtica dioica* L.), common goutweed (*Aegopodium podagraria* L.), dandelion (*Taraxacum officinale* L.) and chickweed (*Stellaria media* L.) grown in early spring in Latvia. Samples were gather in May 2018. Pigments (total chlorophylls, chlorophyll *a*, *b* and total carotenoids) content in the ethanol extracts of fresh, frozen and dried leaves was analysed with spectrophotometer, but the colour was measured in CIE L*a*b* system. The experimental results showed significantly higher content of total chlorophylls and carotenoids in fresh (1.64±0.14 and 0.81±0.02 mg g^{-1} DW) and frozen (2.08±0.12 and 0.94±0.05 mg g^{-1} DW) nettle leaves. Freezing process stimulates the higher amount of pigments from all analysed plants. It was observed, that the content of pigments decreased about for three times after drying process. Chlorophyll *a*/*b* was higher in goutweed leaves independently of processing. The obtained results demonstrate that the significant differences were found between fresh, frozen and dried plant leaves colour components L*a*b*.

Keywords: pigments, colour, edible wild plants

Introduction

During the last years, interest about wild leafy vegetables has significantly increased in many countries, including Latvia. It is known that they provide high levels of phytonutrients. The chemical composition of wild plants has considerable interest due to their properties regardless humans' health (Khanam et al., 2012). These plants could be used as functional foods, because they contain biologically active food compounds and could provide health benefits (Salvatore et al., 2005).

About 1000 plant species of the nettle family (*Urticaceae*) are popular in the world, but only two nettle species are found in Latvia: *Urtica dioica* L., often called common nettle or stinging nettle, and *Urtica urens* L. known as annual nettle (Zeipiņa et al., 2015). Nettle leaves contain vitamins and other biologically active compounds which have antioxidant activity, (Guil-Guerrero et al., 2003; Kukric et al., 2012). Dandelion (*Taraxacum officinale* L.) leaves are known as medicinal herb for long time, mainly due to its hypoglycaemic activities (Schütz et al., 2006). Dandelion are good origin of vitamins (provitamin A, vitamin C) and mineral elements (Gupta, Rana, 2003). Many researchers have investigations on chemical composition (Shi et al., 2008; Gatto et al., 2011; Dias et al., 2014), antioxidant properties (Hu, Kitts, 2003) of dandelion and characterized its biological value (Escudero et al., 2003). Common goutweed (*Aegopodium podagraria* L.) is well known wild plant, growing in Europe and Asia, very often in dark and shady places, and its leaves could be used like spinach. It is also used for medical purposes – for prevention of cancer, inflammation, arthritis and nervousness (Duke et al., 2002; Prior et al., 2007). Chickweed (*Stelleria media* L.) is a plant, which belongs to the order *Caryophyllales,* and very common in gardens, countryside and roadside. In folk medicine is used as a heart remedy, as diuretic, but new leaves are used for salads and soups. The presence of chlorophyll pigments determined the green colour of plants. Plant pigments – chlorophylls and carotenoids take part in metabolism of light energy, as well as catalyse formation of carbohydrates. Chlorophylls containing products can be used as a food colorant (E 140) and healthy constituent of our diet. Newest scientific studies have highlight the anti-carcinogenic and anti-mutagenic effect of chlorophylls containing compounds (Mishra et al., 2011). The chemical composition and chemical properties of chlorophylls provide the ability to prevent lipid oxidation in food (Daood, 2003). Due to antioxidative properties of chlorophylls and carotenoids they could participate in prevention of oxidative stress, cardiovascular diseases, cancer and other health problems (Žnidarčič et al., 2011). Whereas chlorophylls can be considered as indicator of plant's quality, content of chlorophylls in plants indicates the nutritional value of them. McQuistan et al. (2012) noted that plants colour, is also an indicator of the vegetable pigment concentration. There are data about using content of chlorophylls as parameter to evaluate the effect of pollutants on plants (Petrova et al., 2017). Plants which are growing in polluted environment have decreased content of chlorophylls and carotenoids, the structure of their chloroplast membranes are changed so that intensity of photosynthesis is decreased (Schubina, 2011).

The application of preservation methods like freezing and drying, gives possibility to retain plants quality and could use it out of seasons. Drying is well known method for plants preservation and can be done in different ways – in open air, by direct sunlight, in drying ovens etc. (Muller, Heindl, 2006). Drying process could effected many enzymatic reactions in fresh plants. It may result in remarkable variations in content and

structure of biologically active compounds (Puranic et al., 2012). There are many scientific researches regarding the effect of drying process on the content of plant pigments, showing that different drying methods could give dissimilar result (Rocha et al., 2011; Puranik et al., 2012; Droštinova et al., 2015).

Freezing is one of the oldest methods used for food preservation providing that products are preserved for longer time. During freezing the most enzymes are inactivated, chemical reaction rate is reduced thus ensuring maintaining nutritional value of products, as well as allowing preserving taste and texture (Delgado, Sun, 2000).

The aim of research was to investigate the effect of drying or freezing on the colour intensity and pigments content in leaves of edible wild plants - stinging nettle (*Urtica dioica* L.), common goutweed (*Aegopodium podagraria* L.), dandelion (*Taraxacum officinale* L.) and chickweed (*Stellaria media* L.) grown in early spring in Latvia.

Materials and Methods

Plant materials

Samples of whole leaves of chickweed (*Stellaria media* L.), common goutweed (*Aegopodium podagraria* L.), dandelion (*Taraxacum officinale* L.) and stinding nette (*Urtica dioica* L.) were grown in Latvia, Zemgale region and harvested in April 2018. In the same day wild plants samples were developed for investigation. The wild plant samples were freezing for seven days at -18±2 °C and drying at 85±1 °C for 48 h in ventilated thermostat.

Determination of total carotenoids, chlorophyll a and chlorophyll b

The homogenized wild plants samples were weighted (0.5±0.0001 g) in a glass conical flask (100.0 mL), 10.0 mL of 96% ethanol was added and the test conical flask were held for 20 min with occasional shaking at +19±2 °C room temperature. The extracts were filtred (paper No 89) and extraction was done in four replicates. The obtained infusion was analysed content of total carotenoids (xanthophylls and carotenes) c_{c+x}, chlorophyll a c_{Chla}, chlorophyll b c_{Chlb} by spectrophotometer JENWAY 6300 at wavelengths 470, 649, 664 nm. Chlorophylls and total carotenoids content were calculated according to the following formulas (Sumanta et al., 2014).

Chlorophyll *a* (mg mL^{-1}):

$$c_{Chla} = 13.36A_{664} - 5.19A_{649} \quad (1)$$

Chlorophyll *b* (mg mL^{-1}):

$$c_{Chlb} = 27.43A_{649} - 8.12A_{664} \quad (2)$$

Total chlorophylls (mg mL^{-1}):

$$c_{a+b} = c_{Chla} + c_{Chlb} \quad (3)$$

Total carotenoids (mg mL^{-1}):

$$c_{c+x} = \frac{1000A_{470} - 2.13c_{Chla} - 97.63c_{Chlb}}{209} \quad (4)$$

Ratio between chlorophyll *a* and chlorophyll *b*:

$$R_{a/b} = \frac{c_{Chla}}{c_{Chlb}} \quad (5)$$

Ratio between chlorophylls and carotenoids:

$$R_{a+b/c+x} = \frac{c_{a+b}}{c_{c+x}} \quad (6)$$

Results of the study were pronounced as mg g^{-1} of fresh weight (FW) or dry weght (DW) wild plants material.

Colour analysis

Colour of wild samples were fixed in CIE L*a*b* colour system using a colorimeter ColorTec PCM (Accuracy Microsensors Inc., USA). Seven random wild plant leaves were measured and the mean values were calculated for each sample (Coultate, 2009). Herbal samples before analysis were placed in a glass Petri dish with a lid (external diameter 50 mm, inner 60 mm) and the intensity of the herbal colour was measured on the surface of the dish. Color analysis were repeated on different randomly selected places at the surface of each wild plant samples (n=8).

Moisture content analysis

The A&D Company, Limited Moisture Analyser (AND MX–50, Japan) was used for moisture content determination in plant materials according to Razak et al. (2006).

Statistical analysis

Results of the study are means of four parallel measurements and were calculating by Microsoft Excel 2016. Analysis of ANOVA variance was used to define differences amid samples. The result differences were finded as significant at $p<0.05$.

Results and Discussion

Important functional food component are leafy plants. They contain a lot of vitamins, minerals and biologically active compounds. These plants also contain several types of photosynthetic pigments – chlorophylls and carotenoids (Kimura, Rodriguez-Amaya, 2002).

Chlorophyll *a* value in fresh analysed plant samples differed significantly ($p<0.05$) and varied between 0.426±0.006 mg g^{-1} FW and 1.229±0.01 mg g^{-1} FW (Figure 1). The best source of chlorophylls in spring time is fresh nettle leaves where the content of chlorophyll *a* and *b* was determined 1.229±0.008 and 0.411±0.002 mg g^{-1} FW, respectively. The results of our wild edible plant study showed that nettle leaves had the highest content of chlorophyll *a* and *b* in fresh and frozen samples. Comparing with other analysed plants it was higher in average two to three times. Our results were similar to data reported by Upton (2013), who noticed that content of chlorophylls in nettles could be 0.08–0.30% FW. The content of chlorophyll *a* in frozen samples was the following: frozen stinging nettle > frozen dandelion > frozen common goutweed > frozen chickweed (Figure 1).

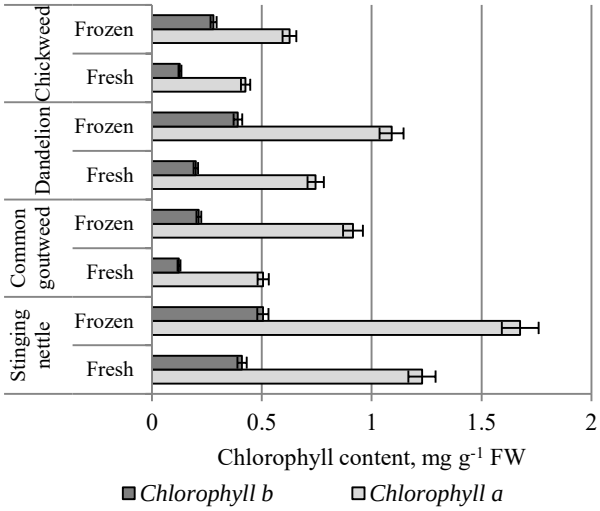

Figure 1. Content of chlorophylls

The data presented in Table 1 reveals the fact that the values of the pigments vary significantly according to used processing method. For comparing the obtained results, all data were expressed to dry weight. The content of dry weight was determined: stinging nettle 22.33 g 100 g^{-1}, common goutweed 19.86 g 100 g^{-1}, dandelion 14.63 g 100 g^{-1} and chickweed 9.23 g 100 g^{-1}. We had observed that after freezing it is possible to extract more pigments from plants compared with fresh materials. Content of chlorophyll *a* increased from 1.4 times (stinging nettle) till 1.8 times (common goutweed). The increase of chlorophyll *b* content had similar tendency. It could be explained with the fact, that low temperature promotes a better preservation of chlorophylls. In addition, short freezing time promotes the breakage of plant cells therefore giving possibility to extract more pigments from plants. The changes of chlorophyll content in our study have another tendency in comparison with results in similar studies (Lisiewska et al., 2004), that could be explained by different freezing times used in the studies.

For explaining experimental results, often the ratio of chlorophyll *a* and chlorophyll *b* ($R_{a/b}$) as well as ratio of total chlorophylls and carotenoids $R_{a+b/c+x}$ are used. In the analysed plants a ratio $R_{a/b}$ ranged from 2.99–4.11 in fresh samples, 2.24–4.31 in frozen plants or 3.88–4.83 in dried plant material (Table 1).

In all cases this ratio increased after drying. It could be explained with fact that during processing the content of chlorophyll *b* decreased and therefore the ratio increased. The highest ratio (a+b)/(x+c) was calculated in stinging nettle (4.53) and the lowest in dandelion (4.09). It means that plants were fresh and suitable for experiments because this ratio as an indicator of the plants greenness normally was between 4.2 and 5.0 (Lichtenthaler, Buschman, 2001). Ratio (a+b)/(x+c) can be considered as indicator of senescence, stress, and damage to the plant. Lower values for this ratio confirm this. If the colour of leaves changes, then (a+b)/(x+c) values decreased till 2.5–3.5 (Salehi, Arzani, 2014).

The lowest total chlorophylls content was in common goutweed sample regardless of the processing method, but the highest 14.15 mg g^{-1} DW in frozen chickweed (Fig. 2).

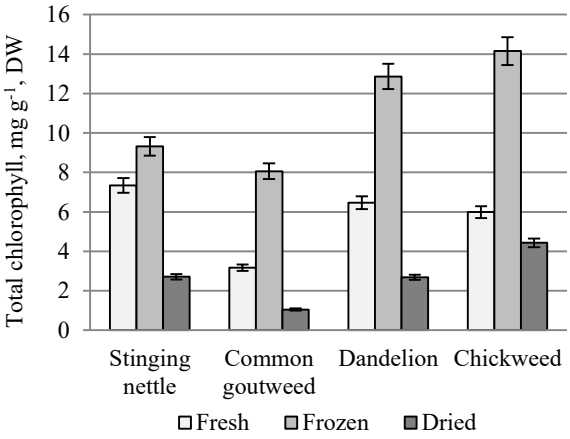

Figure 2. Content of total chlorophylls

Table 1

Chlorophylls content in plants

Plant material	Sample	C_{Chla}, mg g^{-1}DW	C_{Chlb}, mg g^{-1} DW	Ratio, a/b	Ratio, a+b/c+x
Stinging nettle	Fresh	5.56±0.14	1.84±0.08	2.99	4.53
	Frozen	7.51±0.15	2.26±0.11	3.42	4.59
	Dried	2.23±0.11	0.51±0.01	4.31	2.85
Common goutweed	Fresh	2.55±0.11	0.62±0.01	4.11	4.28
	Frozen	4.61±0.12	1.07±0.14	4.31	4.88
	Dried	0.87±0.02	0.18±0.03	4.83	2.33
Dandelion	Fresh	5.09±0.13	1.37±0.06	3.72	4.09
	Frozen	7.46±0.14	2.67±0.12	2.79	5.00
	Dried	2.16±0.08	0.52±0.03	4.15	2.63
Chickweed	Fresh	4.62±0.09	1.38±0.11	3.35	4.13
	Frozen	6.78±0.13	3.03±0.20	2.24	4.90
	Dried	3.53±0.11	0.91±0.03	3.88	3.75

Plants contain several photosynthetic pigments, not only chlorophylls, but also carotenoids. The total carotenoids concentrations differed significantly within analysed plant samples and type of processing ($p<0.05$). Figure 3 shows the changes of total carotenoids content in analysed plant samples depending on the type of processing.

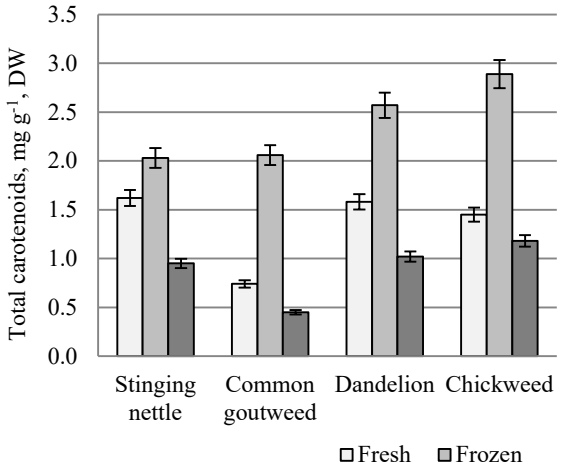

Figure 3. Content of total carotenoids

The content of carotenoids in fresh wild plant samples was from 0.74±0.03 mg g^{-1} (common goutweed) till 1.62±0.08 mg g^{-1} (stinging nettle). The determined content of total carotenoids in fresh leaves is higher than results reported by Guil-Guerrero et al. (2003) and Upton (2013) who has been reported that the total amount of carotenoids from fresh leaves of nettles was determined 29.6 mg 100 g^{-1} DW. Our results are also higher than the data of Žnidarčič et al. (2011) who analysed carotenoids content in fresh dandelion leaves and determined 6.34 mg 100 g^{-1}. There are many factors – species, variety, cultivar, production practice, as well as environmental growth factors such as light, temperature, and soil properties. The region and conditions for growing the studied plants are very important. This could be the explanation for the variation between our results and those shown in some literature sources.(Van den Berg et al., 2000). On the other hand, the values in our study are in agreement with the data of Rohricht (2007) who find out the content of carotenoids 0.10–0.16% dry weight nettle leaves.

Similar to chlorophylls, freezing promotes the extraction of total carotenoids, but the influence was significantly lower ($p<0.05$). The determined content of carotenoids increased for 25.3% (stinging nettle), 62.7% (dandelions), 99% (chickweed) or 1.8 times (common goutweed) comparing with fresh samples. Comparatively the content of chlorophylls increased from 1.21 times (stinging nettle) till 2.5 times (common goutweed).

The decrease of carotenoids content in dried samples was observed, but for different plants it was different – for stinging nettle samples the decrease was for 41.4%, but for common goutweed only 20.7%.

Therefore, freezing is recommended for better prevention of the biologically active compounds. Unfortunately, there is a lack of scientific information regarding chemical composition of common goutweed, dandelion and chickweed. Moreover, there are little research about effects of processing – freezing or drying on the changes of pigments content in non-traditional wild edible plants mentioned above.

It is assumed that the greater intensity of the colour of leafy plants indicates its higher nutritive value (Shibghatallah, Suhandono, 2013). Significant differences were found between the values of the colour components L*, a* and b* of fresh, frozen and dried leafy plants (Table 2).

The highest L* value, related to the lightness, was found for fresh chickweed and dandelion, respectively (45.25±0.16) and (44.40±0.18) (Table 2). The lowest values of this colour parameter were determined for dried stinging nettle samples (23.44±0.08) (showing a darker colour intensity). For all fresh leafy plant samples the highest b* value were determined. This colour component is related to the yellowness. The highest values of parameter a* were obtained for dried stinging nettle (1.34±0.10) and dried chickweed (1.30±0.10). This colour component is related to the redness.

It has been found that colour parameters L*, a* and b* are significantly affected by freezing or drying of leafy plant samples.

Table 2

Colour components in plants

Plant material	Analysed sample	Colour components		
		L*	a*	b*
Stinging nettle	Fresh	42.61±0.12	0.87±0.03	20.32±0.11
	Frozen	24.40±0.10	0.53±0.01	9.68±0.07
	Dried	23.44±0.08	1.34±0.10	4.40±0.05
Common goutweed	Fresh	38.76±0.10	0.97±0.07	19.76±0.12
	Frozen	37.98±0.09	0.85±0.05	15.89±0.10
	Dried	28.44±0.08	1.22±0.10	13.98±0.09
Dandelion	Fresh	44.40±0.18	0.85±0.08	21.94±0.15
	Frozen	43.43±0.15	0.81±0.08	17.85±0.11
	Dried	26.62±0.10	1.27±0.14	13.75±0.09
Chickweed	Fresh	45.25±0.16	0.78±0.05	22.34±0.11
	Frozen	42.86±0.12	0.65±0.08	14.19±0.10
	Dried	27.41±0.10	1.30±0.10	12.79±0.09

Conclusions

As some wild edible plants are very healthy and will provide consumers with necessary pigments and other biologically active substances, but those are highly seasonal, then for preservation, these plants better freeze than dried. Freezing of leafy plant samples promotes

pigment extraction from them, contrary to that, the dried samples contained three times less pigment. The best source of chlorophylls in spring time is fresh nettle leaves. The ratio between chlorophyll a/b was higher in goutweed leaves regardless of processing. Significant differences were determined between the colour components L*a*b* of fresh, frozen and dried plant leaves.

References

1. Coultate T. (2009) *Food: the chemistry of its components.* Cambridge: RSC Publishing, Royal Society of Chemistry, p. 501.
2. Daood H.G. (2003) Chlorophyll. **In:** *Encyclopedia of Food Sciences and Nutrition*. Second edition. Caballero B., Trugo L.C., Finglass P.M. (Ed.) Academic Press, p. 1196–1205.
3. Delgado A.E., Sun D.W. (2000) Heat and mass transfer for predicting freezing processes. A Review. *Journal of Food Engineering*, Vol.47, p. 157–174.
4. Dias M. I., Barros L., Alves R.C., Oliviera B. P. P., Santos-Buelga C., Ferreira I.C.F.R. (2014) Nutritional composition, antioxidant activity and phenolic compounds of wild Taraxacum sect. Ruderalia. *Food Research International,* Vol. 56, p. 266–271.
5. Droštinova L., Braniša J., Bončikova D., Jomova K. (2015) Effect of drying methods on content of some natural pigments in *Urtica Dioica* L. and *Melissa Officinalis* L.. *Journal of Microbiology, Biotechnology and Food Sciences*, Vol. 5(2), p. 182–185.
6. Duke J.A., Bogenschutz-Godwin M. J., Du Cellier J., Duke P.A.K. (2002) *Handbook of Medicinal Herbs, 2nded*. CRC Press LLC, Florida, p. 346.
7. Escudero N.L., Arellano M. L., Albarracín S.F.G., Mucciarelli S. (2003) *Taraxacum officinale* as a food source. *Plant Foods* for *Human Nutrition,* Vol. 58, p. 1–10.
8. Gatto M. A., Ippolito A., Linsalata V., Cascarano N. A., Nigro F., Vanadia S., Venere D. D. (2011) Activity of extracts from wild edible herbs against postharvest fungal diseases of fruit and vegetables. *Postharvest Biology and Technology,* Vol. 61, p. 72–82.
9. Guil-Guerrero J.L., Rebolloso-Fuentes M.M., Isasa M.E.T. (2003) Fatty acids and carotenoids from stinging nettle (*Urtica dioica* L.). *Journal of Food Composition and Analysis,* Vol. 16, p. 111–119.
10. Gupta K., Rana M.K. (2003) Salad crops-other types of salad crops. **In:** *Caballero, B. (Ed.), Encyclopedia of Food Sciences and Nutrition, second ed. Academic Press,* Oxford, p. 5055–5060.
11. Hu C., Kitts D.D. (2003) Antioxidant, prooxidant, and cytotoxic activities of solvent fractionated Dandelion (*Taraxacum officinale*) flower extracts in vitro. *Journal of Agricultural* and *Food Chemistry*, Vol. 51, p. 301–310.
12. Khanam U.K.S., Oba S., Yanase E., Murakami Y. (2012). Phenolic acids, flavonoids and total antioxidant capacity of selected leafy vegetables. *Journal of Functional Foods,* Vol. 4, p. 979–987.
13. Kimura M., Rodriguez-Amaya D.B. (2002). A scheme for obtaining standards and HPLC quantification of leafy vegetable carotenoid. *Food Chemistry,* Vol. 78, p. 389–398.
14. Kukric Z.Z., Topalic-Trivunovic L.N., Kukavica B.M., Matoš S.B., Pavičic S.S., Boroja M.M., Savič A.V. (2012) Characterization of antioxidant and antimicrobial activities of nettle leaves (*Urtica dioica* L.). *Acta Periodica Technologica,* Vol. 43. p. 257–272.
15. Lichtenthaler H.K., Buschman C. (2001) Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. **In:** *Current Protocols in Food Analytical Chemistry,* John Wiley and Sons, New York, p. F4.3.1–F4.3.8.
16. Lisiewska Z., Kmiecik W., Slupski J. (2004) Contents of chlorophylls and carotenoids in frozen dill: effect of usable part and pre-treatment on the content of chlorophylls and carotenoids in frozen dill *(Anethum graveolens* L.), depending on the time and temperature of storage, *Food Chemistry,* Vol. 84, p. 511–518.
17. McQuistan T.J., Simonich M.T., Pratt M.M., Pereira C.B., Hendricks J.D., Dashwood R.H., Williams D.E., Bailey G.S. (2012) Cancer chemopreventation by dietary chlorophylls: A 12,000-animal dose-dose matrix biomarker and tumor study, *Food* and *Chemical Toxicology,* Vol. 50 (2), p. 341–352.
18. Mishra V.K., Husen A., Bachetti R.K. (2011) Medical uses of chlorophyll: A critical overview. **In**: *Chlorophyll structure, function and medical uses.* Nova Science Publishers, Inc. Hauppauge NY 11788, Hua Le and Elisa Salcedo (eds.) p. 177–196.
19. Muller J, Heindl A. (2006) Drying of medical plants. **In**: *Medical and aromatic plants*. Rogers R., Craker L., Lange D. (eds.) Springer, p. 237–252.
20. Petrova S., Todorova K., Dakova M., Mehmed E., Nikolov B., Denev I., Stratiev M., Georgiev G., Delchev A., Stamenov S., Firkova L., Gesheva N., Kadirova D., Velcheva I. (2017) Photosynhtetic pigments as parametrs/indicators of tree tolerance to urban environment (Plovdiv, Bulgaria). *Ecologia Balcanica*, Vol. 9(1), p.53–62.
21. Prior N. M., Lundgaard H., Light M.E., Stafford G.I., Staden J., Jäger A.K. (2007) The polyacetylene falcarindiol with COX-1 activity isolated from *Aegopodium podagraria* L. *Journal of Ethnopharmacology*, Vol. 113, p. 176.–178
22. Puranik V., Chauhan D.K., Mishra V., Rai G.K. (2012) Effect of drying techniques on the physicochemical and bioactive components of selected medical herbs. *Annals of Phytomedicine,* Vol. 1(2), p. 23–29.
23. Razak N.A., Shaari A.R., Nat A.F., Abdullal S. (2014) Effect of leaf moisture content on the herbal quality parameter of *Orthosiphon stamineus* dried leaf during storage. *International Journal of Agriculture Innovations and Research,* Vol. 2(6), p. 1131–1136.
24. Rohricht C. (2007) Yield and constituents of greater nettle strains (*Urtica dioica,* L.). *Zeitschrift Fur Arznei & Gewurzpflanzen*, Vol 12, p. 3–5.
25. Rocha R.P., Melo E.C., Radunz L.L. (2011) Influence of drying process on the quality of medical plants: A review. *Journal of Medicinal Plants Research*, Vol. 5(33), p. 7076–7084.
26. Rocha R. P., Melo E. C., Radunz L. (2011) Influence of drying process on the quality of medicinal plants: A review. *Journal of Medicinal Plants Research*, Vol. 5(33), p. 7076–7084
27. Salehi M., Arzani A. (2014) Evaluation of triticale genotypes for salt tolerance using physiological traits. Emir, *Journal of Food* and *Agriculture,* Vol. 26(3), p. 277–283.
28. Salvatore S., Pellegrini N., Brenna O.V., Del Rio D., Frasca G., Brighenti F., Tumino R. (2005) Antioxidant characterization of some Sicilian edible wild greens.

Journal of Agricultural and *Food Chemistry,* Vol. 53, p. 9465–9471.
29. Schubina A.G. (2011) Maintenance of chlorophyll and carotinoids in leaves of dandelion medicinal (*Taraxacum Officinale*) and birches (*Betula Pendula* Roth) growing in Tambov. [Шубина Т.А. Содержание хлорофилла и каротиноидов в листьях одуванчика лекарственного (Taraxacum Officinale) и березы повислой (Betula Pendula Roth), растущих в г. Тамбове]. **In:** *Transactions of the TSU [вестник ТГУ],* Vol. 16 (1), p. 353–355, (in Russian).
30. Schütz K., Carle R., Schieber A. (2006) Taraxacum-A review on its phytochemical and pharmacological profile. *Journal of ethnopharmacology*, Vol. 107(3), p. 313–323.
31. Shi S., Zhang Y., Zhao Y., Huang K. (2008) Preparative isolation and purification of three flavonoid glycosides from Taraxacum mongolicum by high-speed countercurrent chromatography. *Journal of Separation Science,* Vol 31, p. 683–688.
32. Shibghatallah K., Suhandono V.K. (2013) Measuring leaf chlorophyll concentration from its color: A way in monitoring environment change to plantations. Biological Physics. **In:** *AIP Conference Proceedings,* Vol. 1554 (1), p. 210–213.
33. Sumanta N., Haque C.I., Nishika J., Suprakash R. (2014) Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. *Research Journal of Chemical Sciences*, Vol. 4(9), p. 63–69.
34. Upton R. (DAyu R.H.) (2013) Stinging nettles leaf (*Urtica dioica* L.): Extraordinary vegetable medicine. *Journal of Herbal Medicine,* Vol. 3, p. 9–38.
35. Van den Berg H., Faulks R., Granado H.F., Hirschberg J., Olmedilla B., Sandmann G. (2000) The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *Journal of the Science of Food and Agriculture*, Vol. 80, p. 880–912.
36. Zeipiņa S., Alsiņa I., Lepse L., Dūma M. (2015) Antioxidant activity in nettle (*Urtica dioica* L.) and garden orache (*Atriplex hortensis* L.) leaves during vegetation period. *Chemine Technologija.* Vol. 1(66), p. 29–33.
37. Žnidarčič B., Ban D., Šircelj H. (2011) Carotenoid and chlorophyll composition of commonly consumed leafy vegetables in Mediterranean countries. *Food Chemistry,* Vol. 129, p. 1164–1168.

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SPECTROPHOTOMETRIC ANALYSIS OF PIGMENTS IN HORSERADISH BY USING VARIOUS EXTRACTION SOLVENTS

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Abstract

Plant pigments have a wide range of nutritional benefits. Chlorophyll has antioxidant, anti-inflammatory, heavy metal chelating etc. properties, whereas carotenoids exhibit significant antioxidant activities. The aim of current research was to determine the content of chlorophyll *a*, chlorophyll *b* and total carotenoids of frozen horseradish leaves and horseradish leave by-products depending on the used extraction solvent. For experiments, frozen horseradish leaves and horseradish leave by-products after juice extraction were extracted with four different solvents (acetone, diethyl ether, methanol and ethanol). Chlorophyll *a*, chlorophyll *b* and total carotenoids were determined spectrophotometrically at various wavelengths (470, 645 and 662 nm). Additionally, total chlorophyll content and ratio between chlorophyll *a* and *b* were calculated. Results showed that content of photosynthetic pigments in tested samples significantly ($p<0.05$) differed between used extraction solvents. The degree of extraction of these pigments is greatly influenced by their different chemical structures. For extraction of chlorophyll *a* and total carotenoids the best solvent was methanol in both cases (horseradish leaves and leave by-products). But acetone was the best solvent for extraction of chlorophyll *b*. Generally, chlorophyll *a* was detected in larger amounts in all analysed samples, better solvent was acetone, and the highest content of photosynthetic pigments were observed in horseradish leave by-products after juice extraction.

Keywords: horseradish, leaves, by-products, pigments, extraction solvents

Introduction

Plant pigments form a colour of leaves, flowers, fruits, and play an important role in the photosynthesis, growth, and control of plant development. They are very different in their structure and properties, and belong to different groups of organic compounds: tetrapyrroles (e.g. chlorophyll *a*, chlorophyll *b*), carotenoids (e.g. β-carotene, α-carotene), phenolic compounds (e.g. anthocyanins, flavanols) (Schoefs, 2002). In plant leaves mainly chlorophyll *a*, chlorophyll *b* and carotenoids are presented. These compounds are necessary for photosynthesis, so they are called photosynthetic pigments. The chemical composition of plants, as well as the content and proportions of their pigments, are very dependent on the climate, environmental conditions, variety, development stage, etc. factors (Shaikh, Dongare, 2008; Marrelli et al., 2012; Tomsone, Kruma, 2013 a). Plant pigments exhibit significant antioxidant activity by inhibiting hydroperoxide generation (Loranty et al., 2010). Different health benefits of chlorophyll pigments are proven (Ferruzzi, Blakeslee, 2007). Chlorophyll can reduce the risk of cancer because it is powerful antioxidant (Lanfer-Marquez et al., 2005; Hsu et al., 2013; Cervantes-Paz et al., 2014) and could neutralize free radicals. However, chlorophyll does not work as a major antioxidant because it is not a hydrogen donor, nor participate in hydrogen oxidation-reduction reactions (Sikorski, 2006; Belitz et al., 2009).

Chlorophyll is the most common natural pigment and is present in plant tissues in the form of colloidal suspension, taking part in photosynthesis. In reactions with alkali chlorophyll form chlorophyllide, but in reactions with acids – pheophytin. Chlorophyll is a tetrapyrrol pigment in which the porphyrin ring is in the dihydro-form. Magnesium (Mg^{2+}) is located in the core of the molecule, making it ionic and hydrophilic (Sumanta et al., 2014).

The porphyrin ring itself is hydrophobic, but the carbonyl group at the chain end makes it polar (Lichtenthaler, 1987; Sumanta et al., 2014). In reactions with diluted acids, it easily loses magnesium, resulting in a loss of green colour (Sikorski, 2006). Chlorophyll *a* and chlorophyll *b* differ only by one functional group (i.e. aldehyde group), attached to the porphyrin ring (Lichtenthaler, 1987; Sumanta et al., 2014). Chlorophyll pigments are the same in all plants, but significant colour differences are determined by the presence of other pigments that accompany chlorophyll. Carotenoids are present in chromoplasts together with chlorophylls, creating the colour of fruit and vegetables. The carotenoid group and its derivatives comprise about 70 different compounds, and they are presented in most of vegetables and fruits, performing various biological functions (Costache et al., 2012). These are photosynthetic pigments involved in photoprotection, growth and development regulation, and promote interactions between plants and environment (Ikoma et al., 2016).

Horseradish (*Armoracia rusticana* L.) belongs to the Crusader family and is a well-known plant in the Europe (Raghavan, 2000). More popular are horseradish roots that are used in cooking, but horseradish leaves also could be used in food production and medicine to treat several diseases (Raghavan, 2000). Previous studies have shown that horseradish leaves contain significant amounts of biologically active compounds with high antioxidant activity (Tomsone, Kruma, 2013a; Tomsone et al., 2013b). Horseradish leaves could be used as whole or as a juice, and the production of juice

results in the formation of by-product (pomace) that had been reported by a number of scientists as a valuable source of biologically active compounds. There is no scientific information about composition of this horseradish by-product, and pigments distribution in it. The properties of pigments influence the selection of quantitative and qualitative analytical methods. Chlorophyll is a complex ester that is soluble in organic solvents (ethanol, acetone, chloroform, benzene) (Sikorski, 2006; Belitz et al., 2009), and it is necessary to determine an appropriate extraction technology (Sumanta et al., 2014). Diethyl ether (DI) is a popular solvent for chlorophyll analysis (Scheer, 1991; Porra, 2002). Also, acetone (AC) is a good solvent for the chlorophyll analysis in green plants (Ritchie, 2006), except aquatic plants (Jeffrey et al., 1997). Whereas methanol (ME) is an appropriate solvent for chlorophyll assays especially for vascular plants and algae (Porra et al., 1989; Porra, 1991; 2002). All these solvents are volatile, flammable and harmful to health (Sumanta et al., 2014). Ethanol (ET) is good solvent but is not often used for assays of pigments determination (Lichtenthaler, 1987; Rowan, 1989; Wright et al., 1997; Sumanta et al., 2014). At low pigment concentrations, good solvent is dimethyl sulphoxide (Porra et al., 1989; Porra, 2002).

The aim of the current research was to determine the content of chlorophyll *a*, chlorophyll *b*, and total carotenoids of frozen horseradish leaves and horseradish leave by-products after juice extraction depending on the used extraction solvent.

Materials and Methods

Chemicals

All chemicals (acetone, methanol, ethanol, diethyl ether) used in the research were obtained from Acros Organic (USA).

Sample preparation

Fresh horseradish (*Armoracia rusticana* L.) leaves were harvested in Jelgava, Latvia (latitude – 56° 39' N; longitude – 23° 44' E) in June 2018 and frozen (-18±2 °C) and stored until further experiments. Horseradish juice was obtained using frozen sample grinding, and extracting of juice by a basket press. The resulting by-products of horseradish leaves were used for further experiments.

Analytical method

Chlorophyll *a*, chlorophyll *b* and total carotenoid content were determined by spectrophotometric method (Sumanta et al., 2014; Straumite et al., 2015) with some modifications. Four different solvents were used: diethyl ether (DI), acetone (AC), ethanol (95%) (ET), and methanol (ME) (Table 1). A 0.5 g of homogenized sample were transferred into a conical flask and extracted with 10 mL of an appropriate solvent with the assistance of a magnetic stirrer (magnet size 4.0×0.5 cm) at 700 rpm for 15 minutes at room temperature (20±1 °C). The supernatant was separated by decanting. Residues were extracted again using the same procedure. Extracted supernatants were combined and filtered (filter paper No.89).

Table 1

The equations for calculation of photosynthetic pigments (Sumanta et al., 2014; Straumite et al., 2015)

Parameter	Equations	
Solvents	DI	AC
Chlorophyll *a*, mg mL^{-1}	$C_{Ch\,a} = 10.05A_{662} - 0.97A_{645}$	$C_{Ch\,a} = 12.25A_{662} - 2.79A_{645}$
Chlorophyll *b*, mg mL^{-1}	$C_{Ch\,b} = 16.36A_{645} - 2.43A_{662}$	$C_{Ch\,b} = 21.5A_{645} - 5.1A_{662}$
Total extractable chlorophyll, mg mL^{-1}	$C_{Ch\,t} = C_{Ch\,a} + C_{Ch\,b}$	$C_{Ch\,t} = C_{Ch\,a} + C_{Ch\,b}$
Ratio between chlorophyll *a* and *b*	$R_{\frac{a}{b}} = \frac{C_{Ch\,a}}{C_{Ch\,b}}$	$R_{\frac{a}{b}} = \frac{C_{Ch\,a}}{C_{Ch\,b}}$
Total carotenoids, mg mL^{-1}	$Ca = \frac{1000\,A_{470} - 1.43\,C_{Ch\,a} - 35.87C_{Ch\,b}}{205}$	$Ca = \frac{1000\,A_{470} - 1.82\,C_{Ch\,a} - 85.02C_{Ch\,b}}{198}$
Solvents	ET	ME
Chlorophyll *a*, mg mL^{-1}	$C_{Ch\,a} = 13.36A_{662} - 5.19A_{645}$	$C_{Ch\,a} = 16.72A_{662} - 9.16A_{645}$
Chlorophyll *b*, mg mL^{-1}	$C_{Ch\,b} = 27.43A_{645} - 8.12A_{662}$	$C_{Ch\,b} = 34.09A_{645} - 15.28A_{662}$
Total extractable chlorophyll, mg mL^{-1}	$C_{Ch\,t} = C_{Ch\,a} + C_{Ch\,b}$	$C_{Ch\,t} = C_{Ch\,a} + C_{Ch\,b}$
Ratio between chlorophyll *a* and *b*	$R_{\frac{a}{b}} = \frac{C_{Ch\,a}}{C_{Ch\,b}}$	$R_{\frac{a}{b}} = \frac{C_{Ch\,a}}{C_{Ch\,b}}$
Total carotenoids, mg mL^{-1}	$Ca = \frac{1000\,A_{470} - 2.13\,C_{Ch\,a} - 97.63C_{Ch\,b}}{209}$	$Ca = \frac{1000\,A_{470} - 1.63\,C_{Ch\,a} - 104.96\,C_{Ch\,b}}{221}$

DI – diethyl ether; AC – acetone; ET – ethanol (95%); ME – methanol

A_{662} –absorbance of the extract at wavelength 662 nm; A_{645} – absorbance of the extract at wavelength 645 nm; A_{470} – absorbance of the extract at wavelength 470 nm; C_{Cha} – content of chlorophyll *a*; C_{Chb} – content of chlorophyll *b*; C_{Cht} – content of total extractable chlorophyll; $R_{\frac{a}{b}}$ – ratio between chlorophyll *a* and *b*; Ca – content of total carotenoids.

The extraction process was performed in triplicate. Determination of chlorophyll *a* (Ch *a*), chlorophyll *b* (Ch *b*), and total carotenoids (Ca), was performed using a spectrophotometer JENWAY 6300 (Baroworld Scientifid Ltd., UK) at various wavelengths (470, 645 and 662 nm). Equations used for the quantification are given in Table 1. Results were recalculated and expressed as mg g^{-1} of plant material. All determinations were performed in triplicate. Additionally, total extractable chlorophyll content (Ch *t*) and ratio between chlorophyll *a* and *b* (R *a*/*b*) were calculated.

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's test were used to determine differences among samples. The linear correlation analysis was performed in order to determine relationships between chlorophyll *a*, chlorophyll *b*, total chlorophyll, and total carotenoids. Differences were considered as significant at $p<0.05$.

Results and Discussion

Chlorophyll a and chlorophyll b content

The content of chlorophyll *a* and chlorophyll *b* in horseradish leaves and by-products extracts depending on the used solvent is presented in Figures 1 and 2. The ANOVA analysis of variance showed that content of chlorophyll *a* was significantly affected ($p<0.05$) by solvent and product type. The content of chlorophyll *a* in horseradish leaves and by-products of horseradish leaves had been dependent on the used solvent and ranged from 0.46 to 0.70 mg g^{-1} and from 0.36 to 0.87 mg g^{-1}, respectively. The chlorophyll *a* is blue-green, and is considered the main pigment that converts light energy into chemical energy (Costache et al., 2012).

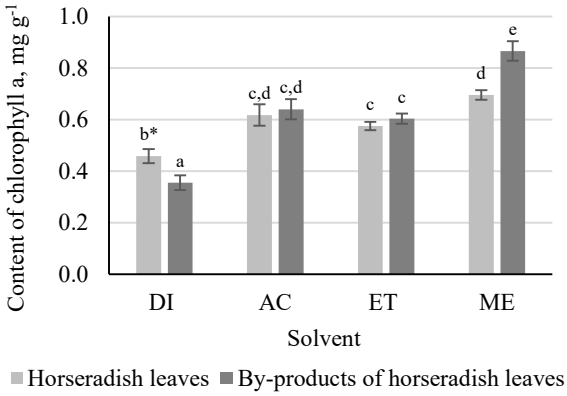

Figure 1. Content of chlorophyll *a* depending on the used solvent

DI – diethyl ether, AC – acetone, ET – ethanol, ME – methanol
* Similar lowercase letters indicate no significant difference among samples ($p>0.05$).

All solvents showed different efficiencies. Both samples expressed a similar tendency and solvents can be arranged as follows (starting from a less efficient solvent): DI < ET ≤ AC < ME. There were no significant ($p>0.05$) differences in the efficacy of acetone and ethanol. The better solvent for extraction of chlorophyll *a* was methanol. The most effective were more polar solvents. The same tendency was observed in *Adiantum* and *Drypteris* species. Sumanta with colleagues (2014) found that, in the case of *Crystiella* species, a better solvent for extraction of chlorophyll *a* was diethyl ether. Similarly, to analysed horseradish samples, in peppermint leaves (depending on the species) the content of chlorophyll *a* ranged from 0.321 to 0.849 mg g^{-1} (Straumite et al., 2015). A higher content of chlorophyll *a* was found in fresh dill (144 mg 100 g^{-1}) (Lisiewska et al., 2004). Other scientists found that the content of chlorophyll *a* in leaves of peanut (*Arachis hypogaea* L.) was 1.606 mg g^{-1} (Meher et al., 2018), but in leaves of Indian mustard (*Brassica juncea* L.) was 11.30 mg g^{-1} (Zang, Liu, 2018). This is significantly higher than in horseradish samples in our study.

Comparing horseradish leaves and its pomace, the higher content of chlorophyll *a* was detected in by-products of horseradish leaves, except sample extracted using diethyl ether as solvent. In this case, a significantly ($p<0.05$) higher content of chlorophyll *a* was detected in the horseradish leaves sample.

Also, the amount of extracted chlorophyll *b* was significantly ($p<0.05$) affected by the used solvent (Fig. 2), similarly as in the case of chlorophyll *a*. Contrary to chlorophyll *a*, the amount of chlorophyll *b* was not significantly ($p<0.05$) affected by the sample type, except in the case with solvent DI.

The content of chlorophyll *b* ranged from 0.03 mg g^{-1} to 0.73 mg g^{-1} in the horseradish leave samples and 0.02–0.33 mg g^{-1} in the by-products of horseradish leaves.

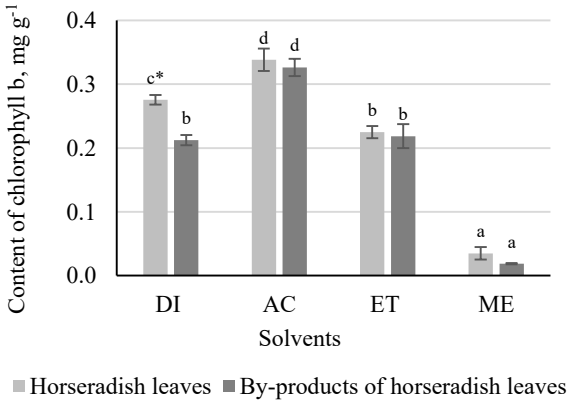

Figure 2. Content of chlorophyll *b* depending on the used solvent

DI – diethyl ether, AC – acetone, ET – ethanol, ME – methanol
* Similar lowercase letters indicate no significant difference among samples ($p>0.05$).

Chlorophyll *b* has a yellow-green colour and it absorbs blue light and therefore extends light spectrum absorbed by photosynthesis (Costache et al., 2012).

For both analysed samples, solvents by their effectiveness can be arranged as follows (starting from a less efficient solvent): ME < ET ≤ DI < AC. There were no significant ($p<0.05$) differences in the efficacy of diethyl ether and ethanol for by-products of horseradish leaves. The better solvent for extraction of chlorophyll *b* was acetone. The most effective were nonpolar solvents for extraction of chlorophyll *b*. It was also reported that acetone was the best solvent for extracting chlorophyll *b* from fern species (*Adiantum* species) (Sumanta et al., 2014).

A group of scientists have found that Indian mustard (*Brassica juncea* L.) leaves contain 5.79 mg g^{-1} FW chlorophyll *b* (Zang, Liu, 2018). It is less than in the horseradish samples analysed in this study. Other scientists found that content of chlorophyll *b* in leaves of peanut (*Arachis hypogaea* L.) was significantly higher than in horseradish samples (0.474 mg g^{-1}) (Meher et al., 2018).

Comparing both studied samples, higher contents of chlorophyll *b* were detected in the horseradish leaves. There were no significant ($p>0.05$) differences between samples using diethyl ether and ethanol as solvent for by-products of horseradish leaves. But using diethyl ether and methanol as solvent, there was significantly ($p<0.05$) higher content of chlorophyll *b* in the case of horseradish leaves than for by-products of horseradish leaves.

Total chlorophyll content

The total extractable chlorophyll content was calculated using the formula shown in Table 1. Figure 3 shows the total extractable chlorophyll content in horseradish leaves and by-products depending on solvent. For analysed samples, content of total extractable chlorophyll depending on the used solvent ranged from 0.73 to 0.96 mg g^{-1} and from 0.57 to 0.97 mg g^{-1}.

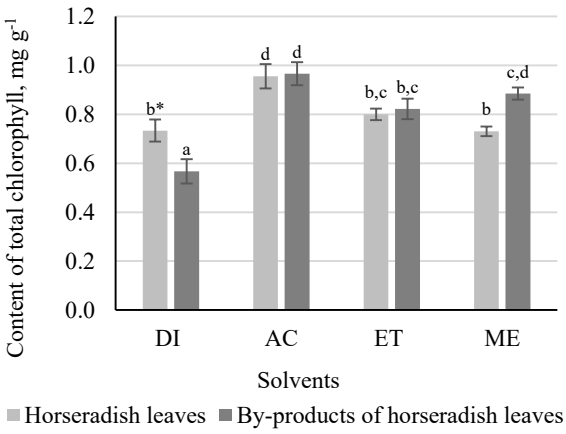

Figure 3. Content of total extractable chlorophyll depending on the used solvent

DI – diethyl ether, AC – acetone, ET – ethanol, ME – methanol
* Similar lowercase letters indicate no significant difference among samples ($p>0.05$).

Efficiency of different solvents to extract chlorophyll (total extractable content) from horseradish leaves by-products can be arranged as follows (starting from a less efficient solvent): DI = ME < ET < AC. But for by-products of horseradish leaves solvents can be arranged as follows (starting from a less efficient solvent): DI < ET < ME < AC. The best solvent for extraction of total chlorophyll for both samples was acetone. Similarly, to analysed horseradish samples, in the peppermint leaves the content of total extractable chlorophyll depending on species ranged from 0.393 to 1.028 mg g^{-1} (Straumite et al., 2015). The total extractable chlorophyll content of fresh Brussels sprouts was 31.8 μg g^{-1} (Olivera et al., 2008). It is significantly less than in the horseradish samples analysed in this study. A significantly higher content of total extractable chlorophyll (than in horseradish samples) other scientists found in leaves of peanut (*Arachis hypogaea* L.) (2.08 mg g^{-1}) (Meher et al., 2018) and in leaves of Indian mustard (*Brassica juncea* L.) (17.10 mg g^{-1}) (Zang, Liu, 2018).

There are no significant ($p>0.05$) differences between total extractable chlorophyll content comparing horseradish leaves and by-products of horseradish leaves using acetone. The same situation was detected with ethanol. The results showed that chlorophyll mainly remains in by-products and was not extracted in juice and that it is possible to extract chlorophyll from the horseradish only with the help of a solvent.

Ratio between chlorophyll a and b

The ratios between chlorophyll *a* and *b* were calculated (Table 1) and results are shown in Figure 4.

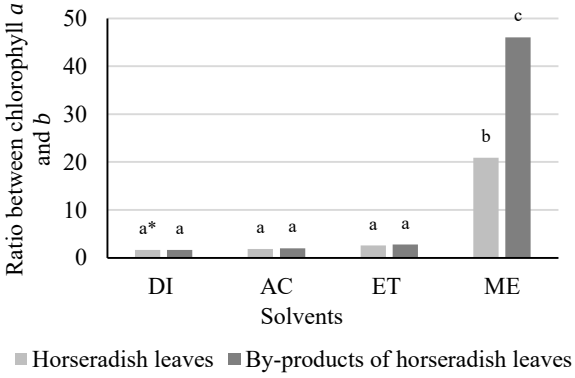

Figure 4. Ratio between chlorophyll *a* and *b* depending on the used solvent

DI – diethyl ether, AC – acetone, ET – ethanol, ME – methanol
* Similar lowercase letters indicate no significant difference among samples ($p>0.05$).

Ratio ranged from 1.66 (DI) to 20.86 (ME) for horseradish leaves and 1.67 (DI) to 46.04 (ME) for by-products of horseradish leaves. In both analysed samples, the content of chlorophyll *a* was significantly higher than the content of chlorophyll *b*. This shows that chlorophyll *a* is the main chlorophyll form in analysed horseradish samples. The results showed that methanol was a better solvent for extraction of chlorophyll *a* and not suitable solvent for extraction of chlorophyll *b*, especially for by-products of horseradish leaves, resulting in a high ratio.

It had been reported that the ratio between chlorophyll *a* and chlorophyll *b* in fresh Brussels sprouts could reach 2.43 using 80% (v/v) acetone (Olivera et al., 2008). It is similar to our results of chlorophyll extraction from both horseradish samples using ethanol as a solvent but more when using acetone as a solvent. A significantly higher proportion of chlorophyll *a* had been reported in mint leaves and it ranged from 3.79 to 7.64 (Straumite et al., 2015). As the solubility of chlorophyll was significantly different in different solvents, the real proportion of chlorophyll *a* and *b* content in horseradish leaves and leaf presses is 2.01 and 2.62, respectively. This value is obtained by dividing the amount of extracted chlorophyll *a* (ME) by the quantity of extracted chlorophyll *b* (AC).

Total carotenoids analysis

The content of total carotenoids in horseradish leaves and by-products extracts depending on the solvent is shown in Figure 5. ANOVA analysis of variance showed that the content of total carotenoids was significantly affected ($p<0.05$) by solvent and by analysed samples. Content of total extractable carotenoids in horseradish leaves and by-products of horseradish leaves depending on the used solvent ranged from 0.1 to 0.25 mg g^{-1} and from 0.09 to 0.32 mg g^{-1}, respectively.

The tendency had been observed, that the content of total carotenoids increased in unison with the increase in solvent polarity. Solvents by their effectiveness can be arranged as follows (starting from a less efficient solvent): DI ≤ AC < ET < ME. The better solvent for extraction of total carotenoids was methanol.

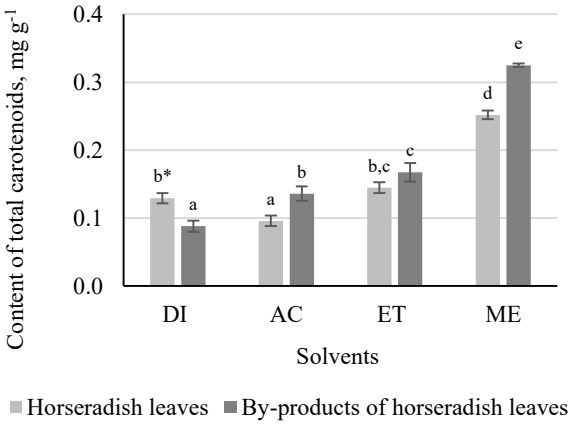

Figure 5. Content of total carotenoids depending on the solvent used

DI – diethyl ether, AC – acetone, ET – ethanol, ME – methanol
* Similar lowercase letters indicate no significant difference among samples ($p>0.05$).

The solvents used can be ranked by their polarity as follows (starting from more unpolar solvents): DI < AC < ET < ME. For both analysed samples it is possible to see an increase in total carotenoids content by an increased polarity of solvent. Sumanta with colleagues (2014) reported that methanol is one of the best solvents for total carotenoid extraction for *Drypteris* species and it is similar to analysed horseradish samples. In the case of *Adiantum* species, the best solvent for carotenoid analysis is acetone (Sumanta et al., 2014). Comparing both samples, more total carotenoids were detected in the by-products of horseradish leaves, except using diethyl ether as a solvent.

Correlation analysis

The correlation analysis was performed to determine relationships between photosynthetic pigments. For horseradish leaves and by-products of horseradish leaves correlation between total chlorophyll and chlorophyll *a* was very strong positive (0.949).

But the correlation between total chlorophyll and chlorophyll *b* was positive strong (0.787). This means that as one parameter increases, the other increase too. The medium and positive correlation was observed between chlorophyll *a* and chlorophyll *b* (0.557) and between chlorophyll *a* and total carotenoids (0.538).

Conclusions

Results showed that content of photosynthetic pigments in extracts were significantly ($p<0.05$) affected by analysed sample, and solvent used. The degree of extraction of these pigments is greatly influenced by chemical nature of bio-molecules (chlorophyll *a*, chlorophyll *b* and carotenoids). For extraction of chlorophyll *a* and total carotenoids the best solvent was methanol in both cases (horseradish leaves and by-products). But acetone was the best solvent for extraction of chlorophyll *b* and total chlorophyll. Chlorophyll *a* comparing to chlorophyll *b* was detected in larger amounts in the analysed samples. After the calculation, the content of total extractable chlorophyll was higher in extracts acquired using acetone as a solvent. Overall the better solvent was acetone and the highest content of photosynthetic pigments had been observed in horseradish by-products.

Acknowledgment

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References

1. Belitz H.-D., Grosch W., Schieberle P. (2009) *Food Chemistry*. 4th revised edition. Edited by: M. Burghagen. Berlin, Heidelberg: Springer-Verlag. 1070 p.
2. Cervantes-Paz B., Yahia E.M., De J.O.J., Victoria-Campos C.I., Ibarra-Junquera V., Pérez-Martínez J.D., Escalante-Minakata P. (2014) Antioxidant activity and content of chlorophylls and carotenoids in raw and heat-processed Jalapeno peppers at intermediate stages of ripening. *Food Chemistry*, Vol. 146, p. 188–196.
3. Costache M.A., Campeanu G., Neata G. (2012) Studies concerning the extraction of chlorophyll and total

carotenoids from vegetables. *Romanian Biotechnolo. Letters*, Vol. 17(5), p. 7702–7708.
4. Ferruzzi M.G., Blakeslee J. (2007) Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutrition Research*, Vol. 27(1), p. 1–12.
5. Hsu C.Y., Chao P.Y., Hu S.P., Yang C.M. (2013) The antioxidant and free radical scavenging activities of chlorophylls and pheophytins. *Food & Nutrition Sciences*, Vol. 4(8), p. 1–8.
6. Ikoma Y., Matsumoto H., Kato M. (2016) Diversity in the carotenoid profiles and the expression of genes related to carotenoid accumulation among citrus genotypes. *Breeding Science*, Vol. 66, p. 139–147.
7. Jeffrey S.W., Mantoura, R.F.C., Wright S.W. (1997) *Phytoplankton pigments in oceanography: guidelines to modern methods*. UNESCO monographs on oceanographic methodology, UNESCO Publishing, Paris, 10.
8. Lanfer-Marquez U.M., Barros R.M.C., Sinnecker P. (2005) Antioxidant activity of chlorophylls and their derivatives. *Food Research International*, Vol. 38(8–9), p. 885–891.
9. Lichtenthaler H. K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic membranes. *Methods in Enzymolpgy*, Vol. 148, p. 350–382.
10. Lisiewska Z., Kmiecik W., Słupski J. (2004) Contents of chlorophylls and carotenoids in frozen dill: effect of usable part and pre-treatment on the content of chlorophylls and carotenoids in frozen dill (*Anethum graveolens* L.), depending on the time and temperature of storage. *Food Chemistry*, Vol. 84, p. 511–518.
11. Loranty A., Rembiałkowska E., Rosa E.S., Bennett R.N. (2010) Identification, quantification and availability of carotenoids and chlorophylls in fruit, herb and medicinal teas. *Journal of Food Composition and Analysis*, Vol. 23(5), p. 432– 441.
12. Marrelli M., Menichini F., Statti G.A., Bonesi M., Duez P., Menichini F., Conforti F. (2012) Changes in the phenolic and lipophilic composition, in the enzyme inhibition and antiproliferative activity of *Ficus carica* L. cultivar Dottato fruits during maturation. *Food and Chemical Toxicology*, Vol. 50(3–4), p. 726–733.
13. Meher S.P., Ashok R.K., Manohar R.D. (2018) Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. *Saudi Journal of Biological Sciences*, Vol. 25, p. 285–289.
14. Olivera D.F., Vina S.Z., Marani C.M., Ferreyra R.M., Mugridge A., Chaves A.R., Mascheroni R.H. (2008) Effect of blanching on the quality of Brussels sprouts (*Brassica oleracea* L. *gemmifera* DC) after frozen storage. *Journal of Food Engineering*, Vol. 84(1), p. 148–155.
15. Porra R.J., Thompson W.A., Kreidemann P.E. (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectrometry. *Biochimica et Biophysica Acta*, Vol. 975, p. 384–394.
16. Porra R. J. (1991) Recent advances and re-assessments in chlorophyll extraction and assay procedures for terrestrial, aquatic, and marine organisms, including recalcitrant algae. **In**: Scheer H (ed) *Chlorophylls*, p. 31–57.
17. Porra R.J. (2002) The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. *Photosynthesis Research*, Vol. 73, p. 149–156.
18. Raghavan Uhl S. (2000) A to Z Spices. In: *Handbook of Spices, Seasonings, and Flavorings,* Horizons Consulting, Boca Raton, London, New York, Washington, D.C., p. 59–60.
19. Ritchie R.J. (2006) Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynthesis Research*, Vol. 89, p. 27–41.
20. Rowan K. S. (1989) *Photosynthetic pigments of algae*. Cambridge University Press, Cambridge.
21. Scheer H., (ed) *Chlorophylls*. CRC Press Boca Raton, Ann Arbor, Boston, Landon, 31–57 (1991).
22. Schoefs B. (2002) Chlorophyll and carotenoid analysis in food products. Properties of the pigments and methods of analysis. *Trends in Food Science and Technology,* Vol. 13, p. 361– 371.
23. Shaikh S.D., Dongare M. (2008) Analysis of photosynthesis pigments in *Adiantum lunulatum* Burm. At different localities of Sindhudurg district (Maharastra). *Indian Fern Journal*, Vol. 25, p. 83–86.
24. Sikorski Z.E. (2006) *Chemical and Functional Properties of Food Components*, 3rd edition, Ed. by Z.E.Sikorski, CRC Press 2006, 544 p.
25. Straumite E., Kruma Z., Galoburda R. (2015) Pigments in mint leaves and stems. *Agronomy Research*, Vol. 13(4), p. 1104–1111.
26. Sumanta N., Haque C.I, Nishika J., Suprakash R. (2014) Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. *Research Journal of Chemical Sciences*, Vol. 4(9), p. 63–69.
27. Tomsone L., Kruma Z. (2013a) Comparison of different solvents for isolation of phenolic compounds from horseradish (*Armoracia rusticana* L.) leaves. **In:** *Research for Rural Development 2013: annual 19th international scientific conference proceedings,* Jelgava, Latvia, Vol. 1, p. 104–110.
28. Tomsone L., Kruma Z., Galoburda R., Dimins F., Kreicbergs V. (2013b) Influence of technological processes on the phenol content and antioxidant properties of horseradish roots (*Armoracia rusticana* L.). **In:** *2nd International conference on Nutrition and Food Sciences „International Proceedings of Chemical, Biological and Environmental Engineering": International Conference proceedings*, Moscow, Russia, Vol. 53, p.6–10.
29. Zhang Y., Liu G. (2018) Effects of cesium accumulation on chlorophyll content and fluorescence of *Brassica juncea* L. *Journal of Environmental Radioactivity*, Vol. 195, p. 2026–2032.
30. Wright S.W., Jeffrey S.W., Mantoura F.R.C. (1997) Evaluation of methods and solvents for pigment analysis. In: *Phytoplankton pigments in oceanography: guidelines to modern methods, UNESCO Publ*., Paris, p. 261–282.

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EFFECT OF VARIOUS PACKAGING SOLUTIONS ON THE QUALITY OF HAZELNUTS IN NUT–DRIED FRUIT MIXES

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Abstract

Nut-dried fruit mixes are a nutritious snack, which can be consumed throughout the year. However, moisture migration, which occurs between components with higher moisture content (dried fruit) and lower moisture content (cereals, nuts), can lead to undesirable physical and chemical changes during storage. The aim of this study was to identify optimal packaging solutions for various types of nut and dried fruit mixes in order to maintain the quality of hazelnuts. Experiments were carried out at the laboratories of the Faculty of Food Technology, Latvia University of Life Sciences and Technologies and quality control laboratories of Grindeks JSC. A total of nine packaging conditions were tested: three types of packaging – polyethylene terephthalate / metallised polyethylene terephthalate / low density polyethylene (PET / metPET / LDPE), biodegradable polylactic acid (PLA) and biaxially oriented polypropylene / ethyl vinyl alcohol / low density polyethylene (BOPP / EVOH / LDPE), and three packaging environments – air ambiance, modified atmosphere packaging (30% CO_2, 70% N_2) and active packaging with oxygen absorbents. The results of moisture content, pH, hardness, colour, water activity and peroxide value testing during 8-month storage showed that the most suitable packaging materials to ensure quality of hazelnuts in nut–dried fruit mixes are biodegradable PLA and BOPP / EVOH / LDPE packaging. With regards to the effect of packaging technologies on product quality, the best results were obtained when modified atmosphere packaging or active packaging was used.

Keywords: hazelnut, biodegradable packaging, active packaging, storage

Introduction

Nuts are a good addition to the daily diet, providing unsaturated fats, protein, vitamins and minerals (O'Neil et al., 2012). In order to supplement the body with this nutritious snack, nuts are usually combined with dried fruits and sold as nut-dried fruit mixes. However, moisture migration between components with lower moisture (nuts) and higher moisture (dried fruits) can lead to undesirable physical and chemical changes, especially during storage (Pérez-Gago, Rhim, 2014).

Optimal packaging conditions can prevent products from undesirable moisture changes, growth of microorganisms, increase of free fatty acids and peroxide value, all of which affects quality and safety of products (Ozturk et al., 2016). Properties of packaging materials and quality of food have a positive correlation (McMillin, 2017), however, the question of packaging waste reduction is also important (Licciardello, 2017). Thus, packaging materials made from biopolymers are gaining their place in the market (McMillin, 2017).

Packaging environment also has an important effect on the quality of foods, as modified atmosphere environment can control oxidation of products by replacing O_2 with CO_2 or N_2 (Ozturk et.al., 2016). Active packaging systems include moisture and O_2 absorbers, and CO_2 releasers (Kapetanakou, Skandamis, 2016) which in return prevents food spoilage and can prolong shelf-life.

Producers are aware of problems with packaged nut-dried fruit mixes during storage, therefore, testing on the best packaging conditions are vital.

The aim of this study was to identify optimal packaging solutions for various types of nut and dried fruit mixes in order to maintain the quality of hazelnuts.

Materials and Methods

Raw materials

A total of three products were used for the research: two nut–dried fruit mixes, and hazelnuts as a control sample (Table 1), all supplied by Gemoss Ltd.

Table 1

Characterisation of products used for the research

Products	Ingredients	Amount, %	Country of origin
Nut–dried fruit mix #1	hazelnuts	10.9	Turkey
	peanuts	10.6	USA
	almonds	6.5	USA
	royal raisins, dark	23.6	South Africa
	banana crisps	17.7	Philippines
	golden raisins	17.7	Iran
	dried apricots	13.0	Turkey
Nut–dried fruit mix #2	walnuts	34.2	Ukraine
	peanuts	15.5	USA
	hazel nuts	9.9	Turkey
	almonds	6.0	USA
	royal raisins, dark	20.0	South Africa
	golden raisins	14.4	Iran
Separately packaged nuts	hazel nuts	100.0	Turkey

Packaging solutions

Three types of packaging materials were used to evaluate the quality of nuts and nut – dried fruit mixes during storage (Table 2). In addition, three packaging environments were also applied – air ambiance, modified atmosphere packaging (MAP) (30% CO_2,

70% N_2) and active packaging with oxygen absorbers (AGELESS® GE, oxygen absorption capacity 100 cm^3).

Table 2

Description of packaging materials used for the research

Material	Abbreviation	Thickness, µm
Lightproof 3-layer polyethylene terephthalate / metallised polyethylene terephthalate / low density polyethylene	PET / metPET / LDPE	55±2
Transparent one-layer Ceramis®-PLA coated with a barrier of pure silicon oxide [SiOx]	biodegradable PLA	50±2
Semi-transparent 3-layer biaxially oriented polypropylene / ethyl vinyl alcohol / low density polyethylene	BOPP / EVOH / LDPE	55±2

Experimental design

Experiments were carried out at the laboratories of the Faculty of Food Technology, Latvia University of Life Sciences and Technologies and quality control laboratories of Grindeks JSC.

A total of nine packaging conditions were tested (Table 3). Portion size of nut-dried fruit mixes was 100±5 g, separately packed hazelnuts were weighed in 40±2 g portions (retail size). Prepared samples were stored at room temperature (20±2 °C) under daylight conditions for 8 months. Samples were analysed on the day of packaging and every 2 months during storage; three replicates were tested per analysis.

Quality analysis

Moisture content (%) was determined by grinding nuts in a KN 195 Knifetec™ laboratory mill (FOSS Analytical, Denmark), drying at 105 °C for 2 hours and then weighing.

Hardness (N) was assessed with TA.HD.plus Texture Analyser (Stable Micro Systems, UK). The following parameters describe the measurements: compression plate 100 mm, pre-test speed 1 mm s^{-1}, test speed 30 mm s^{-1}, distance 5 mm, trigger force 0.049 N.

Colour was determined with colorimeter ColorTec-PCM (Accuracy Microsensors, USA) after grinding nuts in a laboratory mill. The data was processed using ColorSoft QCW software, colour was measured in CIE L*a*b* system. Total colour difference was calculated using the following formula (1):

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}\ , (1)$$

where:

ΔE^* – total colour difference which characterises colour changes of nuts;

L^* – colour intensity (light-dark) at the end of storage;

L_0^* – colour intensity (light-dark) at the beginning of storage;

a^* – green-red colour component at the end of storage;

a_0^* – green-red colour component at the beginning of storage;

b^* – blue-yellow colour component at the end of storage;

b_0^* – blue-yellow colour component at the beginning of storage.

pH of nuts was assessed using Jenway 3510 pH-meter (Cole-Parmer, UK) after adding distilled water to ground nuts (10:1).

Water activity (a_w) was assayed with Novasina LabSwift-aw (Novatron Scientific, UK).

Peroxide value was tested in nut oil which was pressed out of grinded nuts using hydraulic press CrushIR Digital (PIKE Technologies, USA) according to ISO 3960:2017.

Table 3

Sample codes used in the research

Samples	Packaging material	Packaging environment	Sample codes
Nut–dried fruit mix #1	PET / metPET / LDPE	active packaging	1AF
		MAP	1GF
		air ambience	1OF
	BOPP / EVOH / LDPE	active packaging	1AC
		MAP	1GC
		air ambience	1OC
	PLA	active packaging	1AB
		MAP	1GB
		air ambience	1OB
Nut–dried fruit mix #2	PET / metPET / LDPE	active packaging	2AF
		MAP	2GF
		air ambience	2OF
	BOPP / EVOH / LDPE	active packaging	2AC
		MAP	2GC
		air ambience	2OC
	PLA	active packaging	2AB
		MAP	2GB
		air ambience	2OB
Separately packaged nuts	PET / metPET / LDPE	active packaging	4AF
		MAP	4GF
		air ambience	4OF
	BOPP / EVOH / LDPE	active packaging	4AC
		MAP	4GC
		air ambience	4OC
	PLA	active packaging	4AB
		MAP	4GB
		air ambience	4OB

Data processing

The obtained data processing was performed using MS Excel *v16* software; one- and two-way ANOVA was applied to determine differences within samples, Tukey's test was also used. Factors were defined as significant, if p-value was below 0.05.

Results and Discussion

Changes in moisture content in nuts

Initial moisture content of hazel nuts was 3.58%. Most noticeable moisture changes were observed in metalized packaging (PET / metPET / LDPE) for nut-dried fruit mixes #1 and #2 (Figure 1A, 1B) ($p<0.05$). Moisture

content increased in metalized packaging with air ambience up to 5.17% (Figure 1A) and 4.70% (Figure 1B). BOPP/EVOH/LDPE packaging and biodegradable PLA had an insignificant effect on moisture content changes of hazelnuts during storage regardless of packaging environment ($p>0.05$).

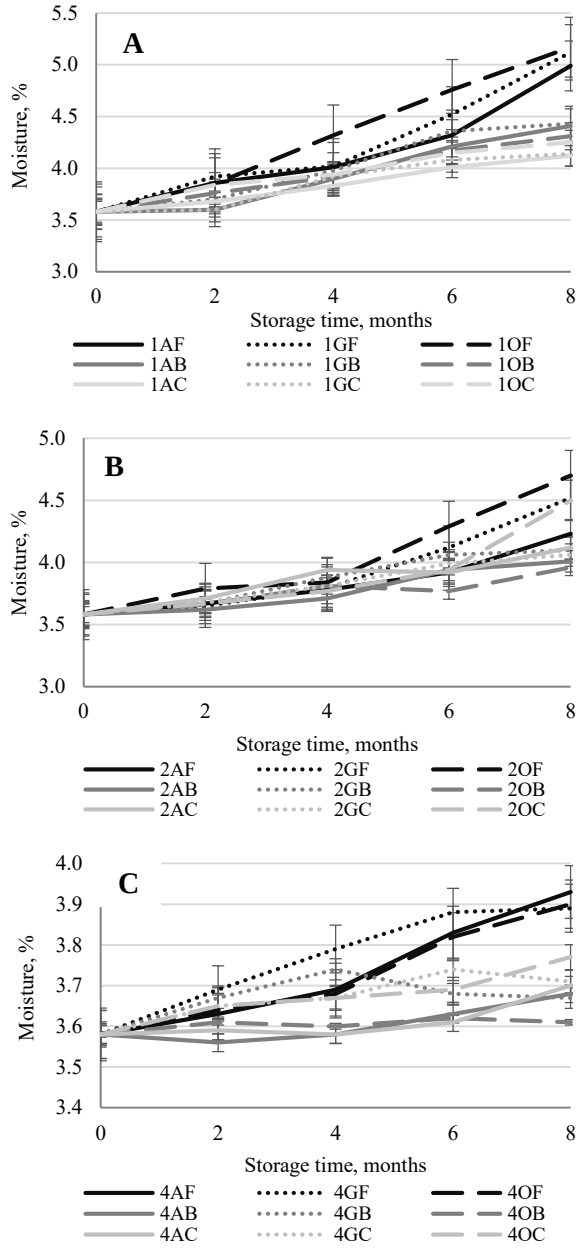

Figure 1. Moisture content dynamics in hazelnuts during storage

A – nut-dried fruit mix #1, B – nut-dried fruit mix #2, C – separately packaged nuts

Moisture changes for separately packaged hazel nuts were minimal (3.61 to 3.90%), there were not significant differences among packaging materials and packaging environments for hazel nuts ($p>0.05$).

Scientific data shows that unprocessed hazelnuts contain 5.3% moisture (Herbello-Hermelo et al., 2018), while Schlörmann et al. (2015) reported 4.70% moisture for hazelnuts. Whereas, Guiné et al. (2014) showed lower moisture for hazelnuts, namely 4.05–4.10% before storage. Ghirardello et al. (2014) reported insignificant changes in hazelnut moisture during 8-month storage at ambient temperature.

Changes in pH value

Changes in pH varied depending on tested nut-dried fruit mixes (Figure 2A, B, C); the least changes in pH were observed for hazelnuts in separately packaged nuts (6.9 to 6.33) and the greatest pH drop was detected in nut-dried fruit mix #2 (6.9 to 6.08).

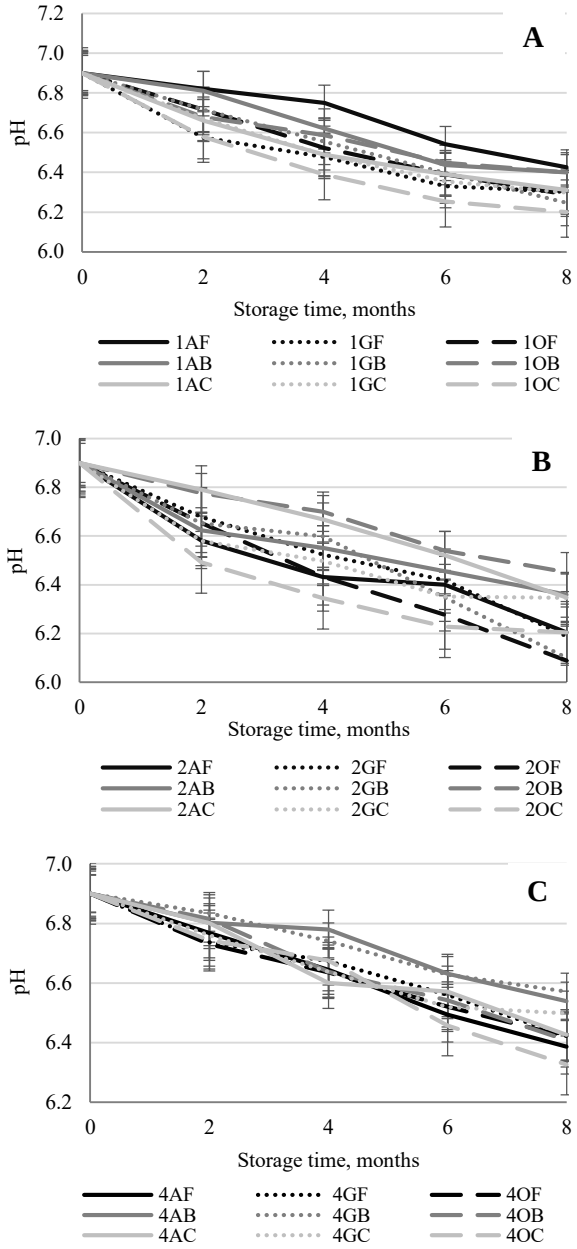

Figure 2. pH dynamics in hazelnuts during storage

A – nut-dried fruit mix #1, B – nut-dried fruit mix #2, C – separately packaged nuts

Packaging conditions which were able to have the least effect on hazelnut pH during storage were as follows: biodegradable PLA with air ambience of oxygen absorbent (6.57) for separately packages nuts,

biodegradable PLA with air ambience (6.45) for nut-dried fruit mix #1 and #2.

Changes in water activity

Water activity of hazelnuts in nut-dried fruit mix #1 (Figure 3A) showed the greatest changes (0.421 to 0.553) in metalized packaging (PET / metPET / LDPE) with modified atmosphere environment (sample 2GF).

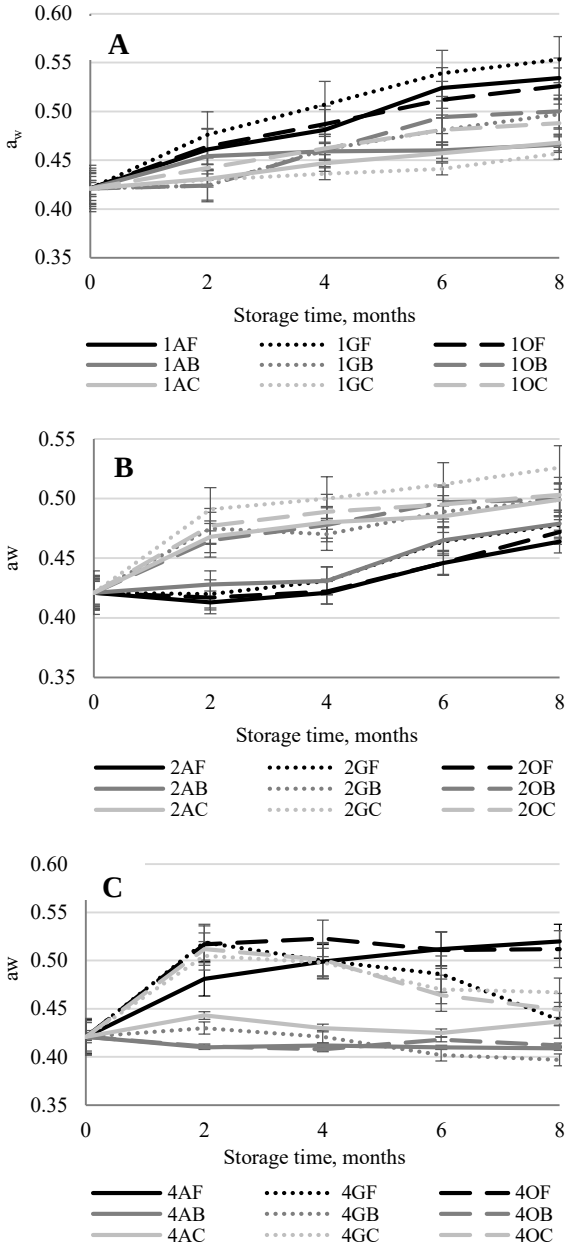

Figure 3. Water activity dynamics in hazelnuts during storage

A – nut-dried fruit mix #1, B – nut-dried fruit mix #2, C – separately packaged nuts

Hazelnuts in nut-dried fruit mix #2 (Figure 3B) had the highest water activity increase (0.526) in BOPP / EVOH / LDPE packaging with modified atmosphere environment.

Separately packaged hazelnuts (Figure 3C) had the greatest water activity increase in PET / metPET / LDPE packaging with oxygen absorber (0.537).

Chosen packaging materials and packaging environments did not have a significant effect on water activity of hazelnuts during 8-month storage ($p>0.05$).

Guiné et al. (2014) previously reported water activity of 0.53-0.58 for hazelnuts, which is in agreement with our results. According to Syamaladevi et al. (2016), water activity of 0.6 and lower indicated lower incidence of microbial growth in foods.

Peroxide value changes in nuts

Initial hazelnut peroxide value was 0.38 mEq O_2 kg^{-1} oil (Figure 4), which increased in all packaging conditions during storage. The greatest changes were observed in nut-dried fruit mix #1 and the least changes – in separately packaged hazelnuts in biodegradable packaging with oxygen absorber.

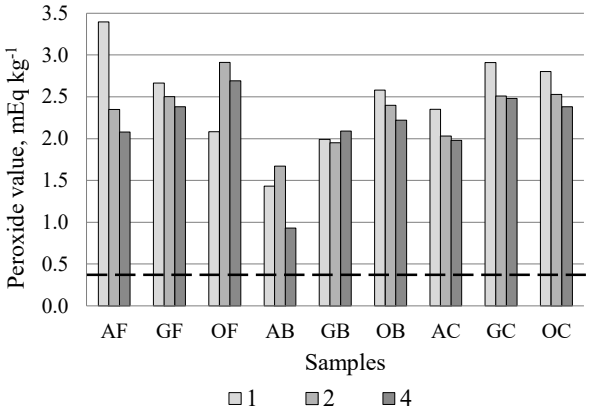

Figure 4. Peroxide value of hazelnuts in different packaging materials and environments after 8-month storage

1 – nut-dried fruit mix #1, 2 – nut-dried fruit mix #2, 4 – separately packaged nuts

– – – dashed line represents initial peroxide value

According to Özdemir et al. (2001), peroxide value ranges from 0 to 3.6 mEq O_2 kg^{-1} for unprocessed hazelnut oil. Chlebowska-Śmigiel et al. (2008) reported an increase from 0.12 to 0.49 mEq O_2 kg^{-1} after 3-month storage, whereas Ghirardello et al. (2014) showed peroxide value of 0.17 mEq O_2 kg^{-1} after 8-month storage and 0.62 mEq O_2 kg^{-1} after 12-month storage of hazelnuts. An increase to 2.5 mEq O_2 kg^{-1} after 50-day storage was reported by Cam and Kilic (2009), concluding that storage time has a significant effect on peroxide value in hazelnuts.

Hardness changes in nuts

Changes in hardness were observed for all hazelnut samples (Figure 5); initial hazelnut hardness was 123.51±5.42 N. The lowest hardness reduction was obtained for separately packages hazelnuts (nuts #4) in biodegradable packaging with oxygen absorbent and modified atmosphere environment.

Hazelnuts of nut-dried fruit mix #1 and #2 in modified atmosphere packaging regardless of packaging materials

showed a similar reduction in hardness after 8-month storage.

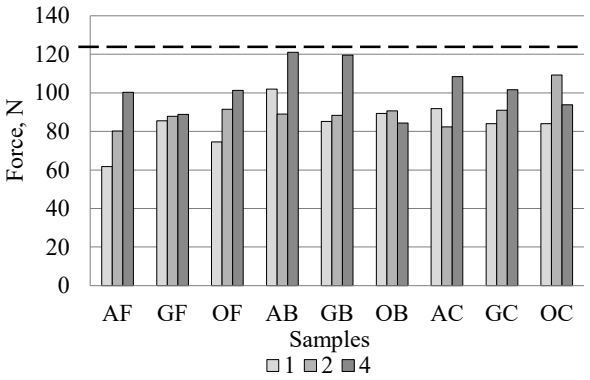

Figure 5. Hardness of hazel nuts after 8-month storage

1 – nut-dried fruit mix #1, 2 – nut-dried fruit mix #2, 4 – separately packaged nuts
– – – dashed line represents initial hardness

With regards to hazelnuts of nut-dried fruit mix #2, hardness levels after storage did not differ significantly ($p>0.05$) among samples, with the exception of sample OC (BOPP / EVOH / LDPE, air ambience), where we observed significantly lower hardness reduction compared to the initial hazelnut hardness value.
Hardness values of 427 to 636 N have been reported previously for fresh hazelnuts (Valentini et al., 2005).

Colour changes in nuts

The most noticeable colour changes, expressed as total colour difference, were observed for hazelnuts of nut-dried fruit mix #2 in biodegradable packaging (Figure 6) after 8-month storage. In all samples, except hazelnuts of nut-dried fruit mix #1 and separately packaged nuts (nuts #4) in BOPP / EVOH / LDPE packaging with modified atmosphere environment, total colour difference exceeded 10 units.

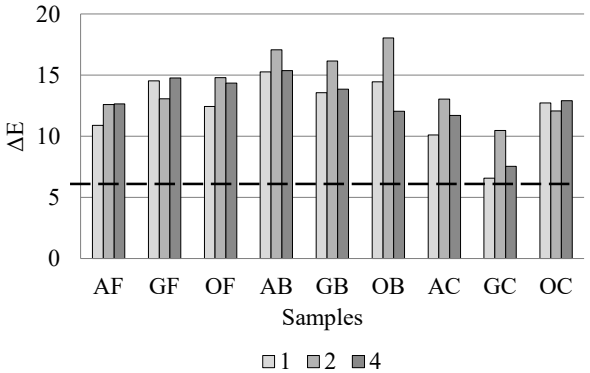

Figure 6. Total colour difference of hazelnuts after 8-month storage

1 – nut-dried fruit mix #1, 2 – nut-dried fruit mix #2, 4 – separately packaged nuts
– – – dashed line represents a great total colour difference ($\Delta E^* > 6$) (Andrés et al., 2016)

According to several scientists Cserhalmi et al. (2006) and Andrés et al. (2016), it is possible to analytically classify colour differences in at least five colour difference groups – starting with not noticeable to great differences. Most of the samples exceeded category of great colour differences ($6<\Delta E^*<12$). Samples in BOPP / EVOH / LDPE packaging with modified atmosphere environment showed the greatest potential in preserving colour of hazelnuts in all nut-dried fruit mix samples.
In addition, Fernández-Vázquez et al. (2013) reported ΔE^* of 2.8 as the colour difference threshold which can be observed by consumers and untrained panellists. This would suggest that none of the tested packaging materials and environments were able to preserve the colour of hazelnuts below the total colour difference limit, which can be easily observed by consumers.
The obtained results raise the question on the quality of nuts in bulk purchased by the distributor and the conditions distributor stores the produce before packaging and retail. It is possible that the initial quality of hazelnuts plays an important role in maintaining their quality during storage, as indicated by the results of hardness, colour and peroxide value.

Conclusions

The results of moisture content, pH, hardness, colour, water activity and peroxide value testing during 8-month storage showed that the most suitable packaging materials to ensure quality of hazelnuts in nut-dried fruit mixes are biodegradable PLA and BOPP / EVOH / LDPE packaging. With regards to the effect of packaging technologies on product quality, the best results were obtained when modified atmosphere packaging or active packaging was used.

Acknowledgment

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References

1. Andrés V., Villanueva M. J., Tenorio M. D. (2016) The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chemistry*, Vol. 192, p. 328–335.
2. Cam S., Kilic M. (2009) Effect of blanching on storage stability of hazelnut meal. *Journal of Food Quality*, Vol. 32(3), p. 369–380.
3. Chlebowska-Śmigiel A., Gniewosz M., Gąszewska M. (2008) An attempt to apply a pullulan coating to reduce oxidative changes and mass loss in nuts during storage. *Polish Journal of Food and Nutrition Sciences*, Vol. 58(1), p. 79–84.
4. Cserhalmi Z., Sass-Kiss A., Tóth-Markus M., Lechner N. S. (2006) Study of pulsed electric field treated citrus juices. *Innovative Food Science & Emerging Technologies*, Vol. 7(1–2), p. 49–54.
5. Fernández-Vázquez R., Stinco C. M., Hernanz D., Heredia F. J., Vicario I. M. (2013) Colour training and colour differences thresholds in orange juice. *Food Quality and Preference*, Vol. 30, p. 320–327.
6. Ghirardello D., Zeppa G., Rolle L., Gerbi V., Contessa C., Valentini N., Botta R., Griseri G. (2014) Effect of different

storage conditions on hazelnut quality. *Acta Horticulture*, Vol. 1052, p. 315–318.
7. Guiné R. P. F, Almeida C. F. F., Correia P. M. R. (2014) Evaluation of preservation conditions on nuts properties. **In:** *9th Baltic Conference on Food Science and Technology "Food for Consumer Well-Being" FOODBALT 2014 Conference proceedings*, Jelgava, Latvia, p. 271–275.
8. Herbello-Hermelo P., Lamas J.P., Lores M., Dominguez-González R., Bermejo-Barrera P., Moreda-Piñeiro A. (2018) Polyphenol bioavailability in nuts and seeds by an in vitro dialyzability approach. *Food Chemistry*, Vol. 254, p. 20–25.
9. Kapetanakou A.E., Skandamis P.N. (2016) Applications of active packaging for increasing microbial stability in foods: natural volatile antimicrobial compounds. *Current Opinion in Food Science*, Vol. 12, p. 1–12.
10. Licciardello F. (2017) Packaging, blessing in disguise. Review on its diverse contribution to food sustainability. *Trends in Food Science & Technology*, Vol. 65, p. 32–39.
11. McMillin K. W. (2017) Advancements in meat packaging. *Meat Science*, Vol. 132, p. 153–162.
12. O'Neil C. E., Keast D. R., Nicklas T. A., Fulgoni V. L. (2012) Out-of-hand nut consumption is associated with improved nutrient intake and health risk markers in US children and adults: National health and nutrition examination survey 1999-2004. *Nutrition Research*, Vol. 32, p. 185–194.
13. Özdemir M., Açkurt F., Yildiz M., Biringen G., Gürcan T., Löker M. 2001. Effect of roasting on some nutrients of hazelnut (*Corylus Avellana* L.). *Food Chemistry*, Vol. 73, p. 185–190.
14. Ozturk I., Sagdic O., Yalcin H., Capar T. D., Asyali M. H. (2016) The effects of packaging type on the quality characteristics of fresh raw pistachios (*Pistacia vera* L.) during the storage. *LWT – Food Science and Technology*, Vol. 65, p. 457–463.
15. Pérez-Gago M.B., Rhim J.W. (2014) Coating and film materials: Lipid bilayers and lipid emulsions. **In:** *Innovations in Food Packaging.* 2nd ed. S. T. Taylor (ed). New York: Academic Press, p. 325–350.
16. Schlörmann W., Birringer M., Böhm V., Löber K., Jahreis G., Lorkowski S., Müller A.K., Schöne F., Glei M. (2015) Influence of roasting conditions on health-related compounds in different nuts. *Food Chemistry*, Vol. 180, p. 77–85.
17. Syamaladevi R. M, Tang J., Villa-Rojas R., Sablani S., Carter B., Campbell G. (2016) Influence of water activity on thermal resistance of microorganisms in low-moisture foods: A Review. *Comprehensive Reviews in Food Science and Food Safety*, Vol. 15(2), p. 353–370.
18. Valentini N., Rolle L., Zeppa G. (2005) Characterisation of hazelnut varieties by texture analysis. *Acta horticulturae*, Vol. 686(686), p. 485–489.

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CONSUMER AWARENESS AND ATTITUDES TOWARDS ACTIVE AND INTELLIGENT PACKAGING SYSTEMS IN THE LATVIAN MARKET

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Abstract

Packaging is designed to preserve foods against damage and contamination and prolong storage time. It provides isolation (product hold), protection (quality, safety, freshness), information (graphics, labels) and usefulness or convenience. However, packaging offers much more than these benefits for the manufacturer and the consumer. Changes in consumer preference for safe food have led to innovation in packaging technologies. Active and intelligent packaging is a packaging technology which offers to deliver safe and qualitative products. Active packaging refers to the inclusion of components in the package in order to maintain or extend the quality and shelf life of the product. Intelligent systems are those that monitor the state of packaged food to provide information on the quality of packaged food during transportation and storage. These technologies are designed to increase the demand for safer foods that provide better shelf life. The market for active and intelligent packaging systems is expected to have a promising future by integrating them into packaging materials or systems. A survey was conducted to study consumer awareness and attitudes towards active and intelligent packaging and their introduction in the Latvian market. 865 respondents from different regions of Latvia answered 19 questions on how well they were informed about smart packaging, how much they would be willing to pay for it, as well as an analysis of consumer confidence about the impact of smart packaging on product quality during storage. The results show that the majority of respondents have insufficient knowledge and understanding about smart packaging.

Keywords: active packaging, intelligent packaging, shelf life, consumers, market

Introduction

Packaging is a protective shell that protects the product from shocks, dirt, heat, light, bacteria and other external factors. It has many functions including the protection of unprocessed or processed foods against contamination and other risks posed by the external environment (Omanovic-Mikličanin, 2017). Packaging serves as an obstacle to potentially harmful access of light, oxygen and water. It facilitates use, offers long-term storage, analyses and transfers information on the product and possible deterioration (Marsh, Bugusu, 2007). Packaging achieves the following means (Robertson, 2006):

- limiting colour, taste, odour, texture changes and other damage to food products;
- preventing threats of biological, chemical or physical damage;
- controlling the absorption and loss of O_2 and water vapours;
- facilitating the use of product content, such as packaging containing combined products, “meal kits”;
- avoiding manipulation of content and using labels that are safe;
- providing information on ingredients, dietary doses, manufacturer's name and address, product weight, barcode information and packaging labelling.

Marketing packaging standards include some specialised trademarks that should be recognized by the processor and available worldwide. Such packages can boost sales. They can be firm, flexible, metalized etc., and can contain information such as trade reports, health reports, recipes or coupons (Schafera, Cheungb, 2018).

The key safety objective for traditional packaging materials which come in contact with food is to be as inert as possible. While the smart packaging systems as active and intelligent packaging concepts are based on the useful interaction between packaging environment and the food, it also needs to provide active protection of the food (Kuswandi et al., 2011).

Active packaging is the first alternative to traditional packaging methods. It refers to an innovative food-packaging concept introduced in response to continuous changes in consumer demands and market trends. Active packaging technology embeds components into the packaging that can release or absorb substances from or into the preserved food or the surrounding environment to sustain quality and prolong shelf life (Arvanitoyannis, Stratakos, 2012). The components frequently used in active packaging systems include oxygen scavengers, ethylene scavengers, flavour and odour absorbers/releasers, antimicrobials and antioxidants (Prasad, Kochhar, 2014). Advantages of using active packaging for perishable goods include reduction of the amount of active substances, reduction of localisation activity and migration of particles from film to food, and elimination of unnecessary industrial processes that might introduce bacteria into the product (Bolumar et al., 2011).

Intelligent packaging systems use communication functions to facilitate decision-making aimed at preserving food quality, extending shelf life and improving overall food safety (Ghaani et al., 2016). Intelligent packaging is mainly used to monitor the condition of packaged foods in order to gather and provide information on the quality of the packaged good during transport and storage (Kerry et al., 2006). It can carry out intelligent functions such as sensing, detecting, and tracing, recording and communicating certain types of information (Realini, Marcos, 2014). Accordingly, intelligent packaging systems consist of hardware components such as time temperature indicators, gas detectors, freshness and/or ripening indicators (Prasad, Kochhar, 2014) and radio frequency identification

(RFID) systems (Kerry et al., 2006). The required functions can be implemented and realized via indicators and sensor devices to communicate the pertinent information. Indicators inform about a detected change in a product or its environment, for example a change in temperature or pH level (Yam et al., 2005). In food packaging, this technology is often complemented with biosensors to detect, record and transmit information related to potential biological processes and reactions occurring inside the package, for example changes in oxygen and freshness levels (Yam et al., 2005; Ghaani et al., 2016).

Traditional food packaging is mainly petroleum-based plastics. The main risks and concerns of traditional food packaging production and applications are related to non-sustainable production, insufficient mechanical and barrier properties, lack of recyclability. In addition, these materials are not biodegradable. The main challenges faced by many food producers are weak barrier properties to water vapour and gases, and achieving an adequate shelf-life for food products (Mihindukulasuriya, Lim, 2014). Active and intelligent packaging plays a major role in filling the gaps of traditional packaging by positively affecting the shelf-life, safety, quality, security of food which is very important for the consumers and producers. Active and intelligent packaging at this moment present a total packaging concept, which includes nanoparticle application in more production phases. Risk, which is associated with nanoparticle application, is their migration into to the food that can potentially result in adverse health effects (Echegoyen, Nerin, 2013). However, not all innovative food technologies get equal acceptance by consumers (Siegrist, 2008).

The aim of this study was to assess consumer awareness and attitudes towards active and intelligent packaging and their introduction in the Latvian market.

Materials and Methods

Poll participants

The survey was conducted with 865 respondents (around 170 persons for each region) who live in different regions to represent the whole population of Latvia as closely as possible. The methodology of questionnaire was used to achieve the research objectives. The questionnaire contained 19 multiple-choice questions and was distributed to respondents using an online survey website VisiDati.lv. The distribution of respondents by sex was 65.9% female and 44.1% male. Most respondents (46.5%) were between the ages of 17 and 29, 40.7% were between the ages of 30 and 50, and 12.8% were over 50 years old. In response to educational status, majority of those who participated in the survey indicated that they had a master's or a bachelor's degree – 57.0%; 20.6% of the respondents held a technical degree. Degree for high school, associate and graduate were 17.4%. The survey provided an analysis of respondents living in the following regions of Latvia: Riga, Zemgale, Vidzeme, Latgale, Kurzeme.

Data processing

Data processing was performed using MS Excel v16 software. Significant differences ($p<0.05$) were established by one-way analysis of variance (ANOVA).

Results and Discussion

The survey provided the analysis of respondents in order to clarify the consumer attitudes and knowledge about packaging. Most respondents believe that they will use a lot of packaging, which makes it possible to conclude that the customer is ready for change. A similar situation was illustrated in a study by Irish scientists (O' Callaghan, Kerry, 2016).

The survey results showed that respondents have a poor knowledge of intelligent packaging (Figure 1), as only 12% understood the term "smart", while more than a half of respondents in Latvia and its regions generally do not understand the term intelligent packaging. In the study by Irish scientists (O' Callaghan, Kerry, 2016) nanotechnology gained the highest level of awareness compared to active and intelligent packaging which received lower levels of recognition.

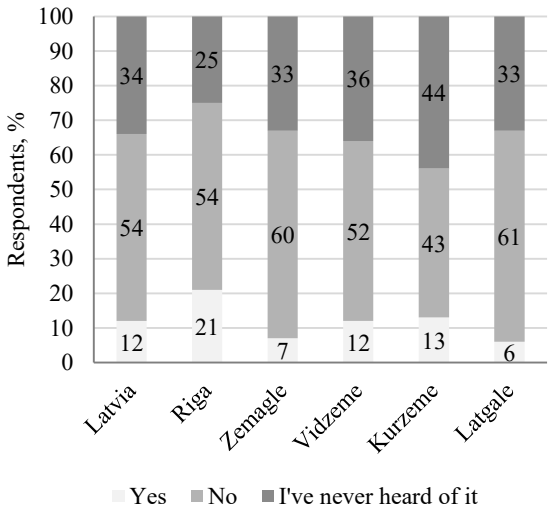

Figure 1. Consumer understanding on what the term "smart packaging" means

The study of Canadian scientists (Chen et al., 2013) showed that the market success of food innovations depends on the consumers' perceptions of the technologies. It is therefore important to educate consumers. Participants from Turkey pointed out that advertising through advertising (40.55%) would be the most effective way to increase the overall acceptability of innovative packages (Aday, Yener, 2015).

Figure 2 shows the ability of respondents to distinguish between active and intelligent packaging. More than 60% respondents on average cannot distinguish between active and intelligent packaging. It is important that a relatively large proportion of respondents (28% on average in Latvia) are not interested; it shows that consumers should be more intensively informed about the benefits of active and intelligent packaging.

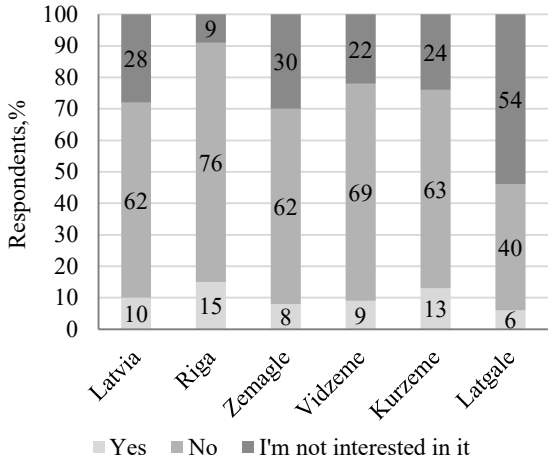

Figure 2. Respondents' ability to distinguish between active and intelligent packaging

As the range of differently packaged food products on the shelves of Latvia's supermarkets is wide and different packaging solutions are used, the question is whether consumers see the products packed in smart packaging on supermarket shelves (Figure 3).

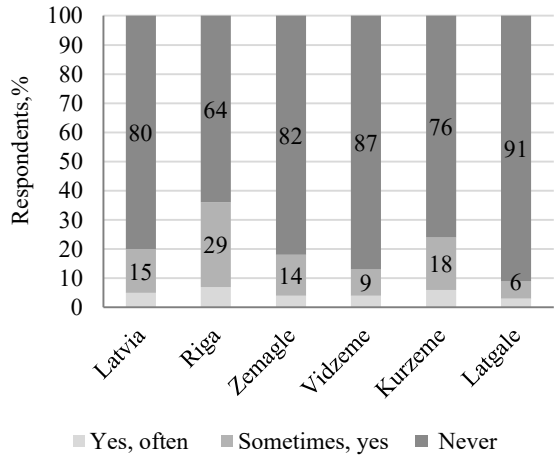

Figure 3. Visibility of intelligent packaging on supermarket shelves

Although there are products packed in smart packaging in supermarkets in Latvia, 80% (in Latvia in general) have not seen such smart packaging solutions on supermarket shelves. The most positive responses on this issue have been provided by respondents in Riga (36%), which could be explained by a denser number of supermarkets in Riga compared to the regions, as well as more information on innovations available in the capital. Analysing the data in Figures 2 and 3, it can be concluded that the consumer is currently very unaware of the new technologies in smart packaging. Consequently, consumers do not orient themselves and cannot distinguish between smart packaging that includes active packaging and intelligent packaging.

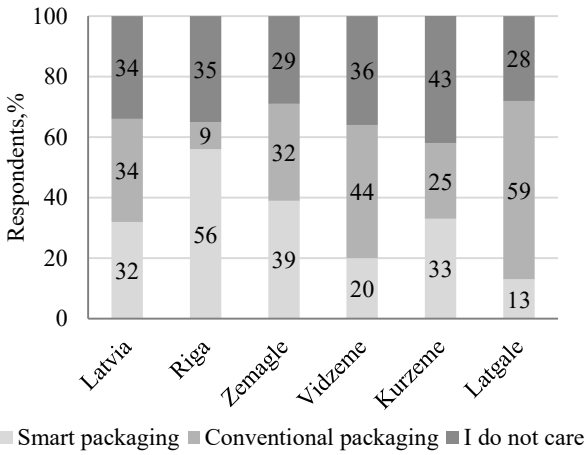

Figure 4. Respondents' choice between products in smart packaging and in conventional packaging

Figure 4 summarizes the data suggesting that consumers are open to a wider use of innovative packaging for food packaging in Latvia, as 32% (average in Latvia) and 56% (in Riga) of respondents are willing to buy such products. Unfortunately, about 30% of respondents are indifferent to the issue.

Majority of the customers 86% (in Riga) are ready to see the product in active food packages to extend the shelf life of foods, a small part of respondents (9%) are willing to see food additives in food products for shelf life extension. Only 4% respondents have a neutral opinion. It is important to note the buyer's willingness to purchase the product in smart packaging. There is an opposite situation in the areas of Vidzeme (35%) and Latgale (37%), where consumers are not ready to pay more for smart packaging (Figure 5).

The research by Irish scientists also showed that Irish consumers were not willing to pay more for smart packaging (O' Callaghan, Kerry, 2016), but the desire increased after participants were informed about the value of using such technologies.

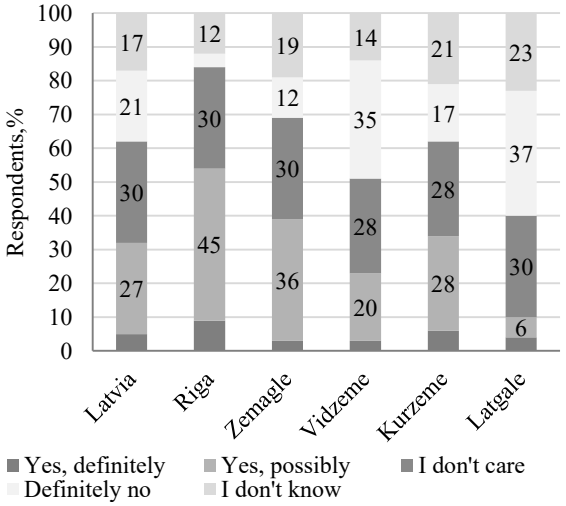

Figure 5. Respondents' readiness to pay a little more for smart packaging

In Figure 6 we can see that on average in Latvia approximately 6% of respondents on average in Latvia do not want to pay more for the use of innovative packaging, 23% are willing to pay up to 5% more than conventional packaging. In turn, the opinion of respondents in Riga differs significantly from the point of view of the transition respondents in Latvia – 48% of respondents in Riga would pay up to 5% more.
The situation has changed in comparison with the previous year on average in Latvia, as the number of consumers who did not want to pay more in 2017 was 29% (Muizniece-Brasava, Kirse, 2018).

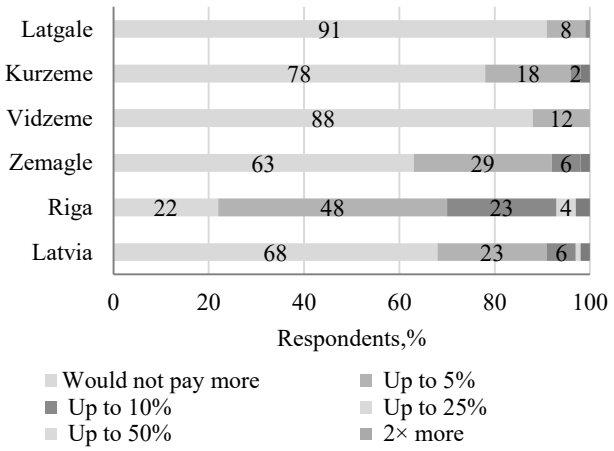

Figure 6. Consumer willingness to pay more for smart packaging compared to conventional packaging

Consumers in a study in the United States expressed readiness to pay more for packages that prolong the shelf life of fresh cut vegetables and wanted to see new types of packaging, such as smart packaging (Wilson et at., 2018).
It is important to note that the respondents, expect to receive additional benefits when thinking about smart packaging (Figure 7) while 56% of respondents in Riga and 42% on average in Latvia answered that they were interested in the history of storage conditions of the product.

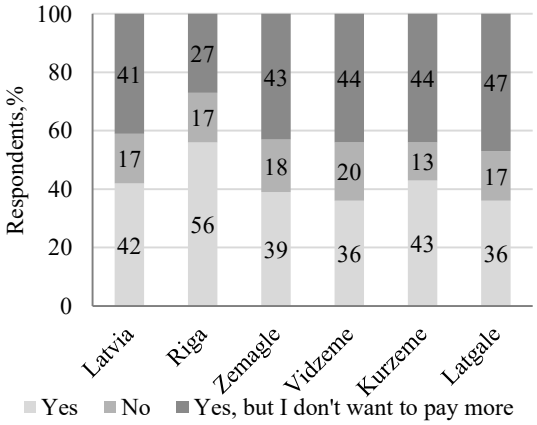

Figure 7. Consumers' interest about history of the storage conditions of the product

The results of the Turkish scientists' study indicated that most of the Turkish consumers' (75%) expectations from innovative packaging was the visual ability to observe the history and freshness of foods inside the packaging (Aday, Yener, 2015).
In Riga, 53% of customers are interested in storage conditions, and 47% are interested in the history of storage of goods. The customers in the Zemgale region are more curious about the history of the storage conditions which is 52%, and 48% of respondents want to see an extension of the shelf life of goods. The situation in Latgale is entirely different, customers prioritize the shelf life of the product, 62% of respondents, and 38% voted for the history of the storage conditions. These voting results reflected the results from Figure 6, in which 91% of consumers are not ready to pay.
This is justified by lower incomes in this regions average in Latvia. Interest in the correct storage of goods prevails in all regions (Figure 8).

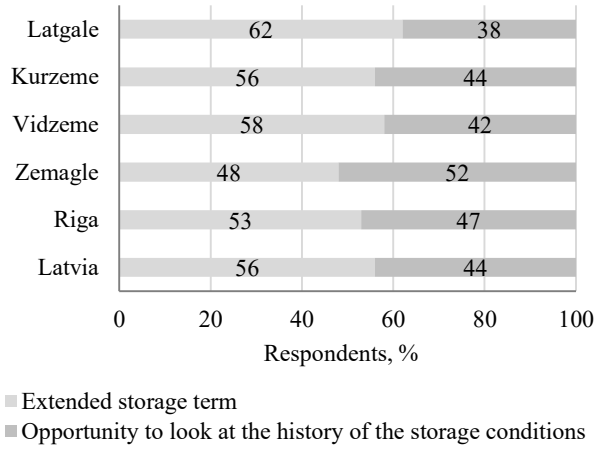

Figure 8. Customers' expectations from smart packaging

Analysing the wishes of customers for packaging in more detail shows that an important criterion for the customers is the freshness of the product 54% in Riga and 57% in Zemgale (Figure 9).

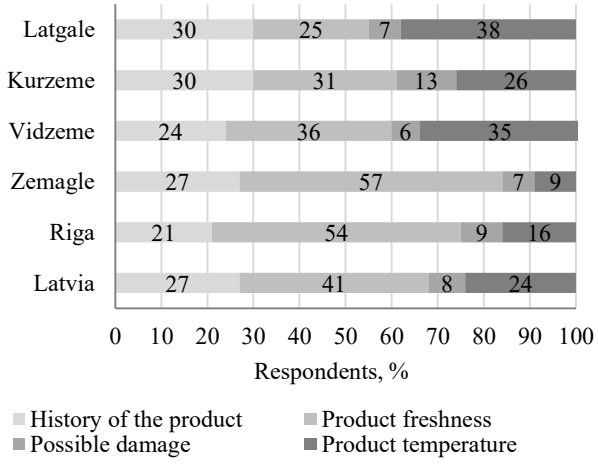

Figure 9. Consumers' opinion on what information should be provided by smart packaging

The second criterion is the storage history of the product from 21% in Riga to 30% in Kurzeme and Latgale. An important criterion for reflected information about the product for the customers is the deterioration of the product 8% (on average in Latvia). This reflects the following: the staff of the retail stores monitor the goods on the shelf, remove them from the sale in time and the customers do not encounter a spoiled product, or the supplier delivering to the store provides the necessary amount of goods that do not raise the daily sales of the product. An important point in Figure 9 is the increased attention to the desire to receive information about the storage temperature of the product in regions, in comparison with 16% in Riga to 35% in Vidzeme and 38% in Latgale, which could reflect to the failure to store goods at the required temperature.

The application of smart packaging depends upon the product being packaged including food and beverage. It has good potential for use in food and beverage products because of increased demand for diagnostic packaging in response to consumer desire for more information about freshness of foods, product temperature, possible damage, and because of the need for track-and-trace systems.

Conclusions

The inhabitants of Latvia are little aware of the intelligent packaging and its potential benefits, have little understanding of the new opportunities and new technologies used in the production of packaging in Latvia. But it is important to note that customers are open to new trends, ready to try them, study them and are prepared to pay for smart packaging. Smart packaging has a good potential for use in food and beverage products in response to consumers' desire for more information about freshness and quality of food.

Customer introduction to smart packaging must take place through trust, comfort and satisfaction. These three criteria are the basis for perception and willingness to use smart packaging. If the packaging does not carry any additional information for the buyer other than the printed information about the composition of the product, it loses its meaning as being innovative. Such packaging remains only a tool to comply with the minimum requirements for the safety of the goods and the ability to transport them.

References

1. Aday M.S., Yener U. (2015) Assessing consumers' adoption of active and intelligent packaging. *British Food Journal*, Vol. 117(1), p. 157–177.
2. Arvanitoyannis I.S., Stratakos A.C. (2012) Application of modified atmosphere packaging and active/smart technologies to red meat and poultry: A review, *Food and Bioprocess Technology*, Vol. 5(5), p. 1423–1446.
3. Bolumar T., Andersen, M. L., Orlien V. (2011) Antioxidant active packaging for chicken meat processed by high pressure treatment. *Food Chemistry*, Vol. 129(4), p.1406–1412.
4. Chen Q., Anders S., An H. (2013) Measuring consumer resistance to a new food technology: A choice experiment in meat packaging. *Food Quality and Preference*, Vol. 28(2), p 419–428.
5. Echegoyen Y., Nerin C. (2013) Nanopracticle release from nano-silver antimicrobial food food containers. *Food and Chemical Toxicology*. Vol. 62, p. 16–22.
6. Ghaani M., Cozzolino C. A., Castelli G., Farris S. (2016) An overview of the intelligent packaging technologies in the food sector. *Trends in Food Science & Technology*, Vol. 51, p. 1–11.
7. Kerry J.P., O'Grady M.N., Hogan, S.A. (2006) Past, current and potential utilisation of active and intelligent packaging systems for meat and muscle-based products: A review. *Meat science*, Vol. 74(1), p. 113–130.
8. Kuswandi B., Jayus Y.W., Abdullah A., Heng L.Y., Ahmad M. (2011) Smart packaging: sensors for monitoring of food quality and safety. *Sensing and Instrumentation for Food Quality and Safety*, Vol. 5, p. 137–146.
9. Marsh K., Bugusu B. (2007) Food packaging: Roles, Materials, and Environmental issues. *Journal of Food Science*, Vol. 72, p. 39–55.
10. Mihindukulasuriya S.D.F., Lim L.T. (2014) Nanotechnology development in food packaging: A review. *Trends in Food science & Technology*, Vol. 40, p. 149–167.
11. Muizniece-Brasava S., Kirse A. (2018) Attitudes of Latvian consumers to traditional and eco-friendly food packaging materials: comparison of 2007 and 2017. **In**: *International scientific conference "Engineering for rural development": proceedings*, Jelgava, Latvia, p. 1948–1954.
12. Omanovic-Mikličanin E. (2017) Smart food packaging. **In**: *Journal article; Conference paper: "Work of the Faculty of Agriculture University of Sarajevo"*, Sarajevo, Vol. 62, p. 702–708.
13. O' Callaghan K.A.M., Kerry J.P (2016) Consumer attitudes towards the application of smart packaging technologies to cheese products. *Food Packaging and Shelf Life*, Vol. 9, p. 1–9.
14. Prasad P., Kochhar A., (2014) Active packaging in food industry: A review. *Journal of Environmental Science, Toxicology and Food Technology*, Vol. 8(5), p. 1–7.
15. Realini C.E., Marcos B., (2014). Active and intelligent packaging systems for a modern society. *Meat Science,* Vol. 98(3), p. 404–419.
16. Robertson G. (2006) Food *Packaging Principles and Practices*: Taylor & Francis, Boca Raton. 733 p.
17. Schafer D., Cheung W.M. (2018), Smart packaging: opportunities and challenges. **In**: *51st CIRP Conference on Manufacturing Systems*. Procedia CIRP 72, p. 1022–1027.
18. Siegrist M. (2008) Factors influencing public acceptance of innovative food technologies and products. *Trends Food Science Technologies*, Vol. 19(11), p. 603–608.
19. Yam K.L., Takhistov P.T., Miltz, J. (2005) Intelligent packaging: concepts and applications. *Journal of Food Science*, Vol. 70, p. 1–10.
20. Wilson C.T., Harte J., Almenar E. (2018) Effects of sachet presence on consumer product perception and active packaging acceptability - A study of fresh-cut cantaloupe. *LWT-Food Science and Technology*, Vol. 92, p. 531–539.

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FOOD ALLERGY KNOWLEDGE AND PRACTICE OF RESTAURANT STAFF

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Abstract

Nowadays people more and more often choose to have their meals outside their homes. Therefore, the catering companies shall ensure safe product offer to different guest groups, including guests with food allergy or intolerance. Alongside with the tendency of the increase of allergic people's number, there have been corrections introduced to the European Union legislation in relation to the product labelling. It means that at present one of the compulsory types of information that shall be indicated by catering companies is information on the presence of allergens in food. It is stipulated by Regulations (EC) No 1169/2011 on the provision of food product information to consumers. One of the reasons why allergic reaction to food takes place at catering companies is the lack of knowledge of staff. Therefore, the aim of research was to determine the level of staff's knowledge and to analyse the types of allergen indications at the catering companies. Twenty catering companies took part in the study, there were 60 companies addressed in total, and 154 valid questionnaires were received. The main research results show that the staff has incomplete knowledge (on average 3.8±1.8 out of 10), as well as not all catering companies indicate information on the presence of allergens in the food.

Keywords: knowledge, food allergies, intolerances, allergen labelling

Introduction

During the last years the number of people with food allergy has increased and it has become a serious global problem. In total, 17 millions of Europeans and 15 millions of Americans suffer from this illness (FARE, 2018; EAACI 2013). Food allergy is more often observed among children (5–8%) than adults (1–2%) (WAO, 2013).

There has been a general term determined to denote such and similar undesirable body reactions to food – adverse reaction to food (Pulido, 2010). The researchers are not unanimous how to classify such reactions, therefore classifications used by several organizations differ.

In this article there has been used EAACI classification used by Polish researchers, where the reactions are grouped into toxic (food infections, intoxication), immunological (food allergy), non – immunological (food intolerance) (Bartuzi et al., 2017).

Food allergy is an excessive reaction of immune system to product substances. Allergic reactions are more often caused by protein. Food allergy often is confused with food intolerance due to similar symptoms; however, the difference of food intolerance is that the immune system is not involved in reactions. Taking into consideration the increase of the number of allergic people, there have been corrections made to the legislation in relation to product labelling. Any company of food circulation, if it is responsible for provision of product information for consumers, shall provide "compulsory information of food products" that, in conformity with the Union regulations, comprises also the information on the presence of allergens in products (Regulation (EC) No 1169/2011 of the European Parliament and of the Council, 2011). It is established during the research that most of the food allergy cases are caused by non-prepacked food products or consuming food at any catering companies (Barnett et al., 2011; Peniamina et al., 2016). It means that at the catering companies information on food allergens (14 product and substance groups) shall be available and indicated in an easy to perceive form, it should be highlighted, legible and non-erasable, before the purchase of food/product in order consumers, especially those suffering from food allergy or intolerance could make informed and safe choice (Regulation (EC) No 1169/2011).

Labelling cannot guarantee that by consuming the product of catering company the guest will not face any of undesirable reactions to food. The consumer's confusions and uncertainty in relation to presence of allergens might negatively affect Health Related Quality of Life (HRQL), which could even more aggravate the insufficient understanding shown by employees of restaurants, coffee-bars (Barnett et al., 2011). The studies performed showed that most often reasons why there is reaction to food at the catering companies is the lack of staff's knowledge, cross-contact, incorrect labelling, communication problems that take place between a guest and the staff, as well as the lack of communication among the members of the staff (Blom et al., 2018; Lee, Sozen, 2016). At the Latvian catering companies, it is possible to observe different forms of allergen labelling. As one of them is a phrase in the menu "Ask the waiter about allergens" that binds the waiters to be informed on the types of allergens and their presence in the food.

Unfortunately, there are no precise statistical data on the number of people suffering from food allergy and intolerance in Latvia. As well as there are no detailed studies on the issue of food allergens, their influence on the performance of catering companies. The aim of this work is to find out how catering companies indicate information on the presence of allergens specified in Annex II to Regulations (EC) No.1169/2011 and on the level of catering company staff's knowledge on food allergies and intolerance.

Materials and Methods

Analysis of Information References of Allergens

There were 4 criteria developed for the evaluation of menus in conformity with Regulation (EC) Nr.1169/2011 on the provision of food information to consumers, in conformity with general conditions in relation to the labelling of compulsory information, as well as an additional criterion (5) in relation to the presence of alternatives at companies, for people with allergy or intolerance. In total there were 54 companies visited in Riga and Jelgava, out of which 20 were restaurants, 20 – coffee-bars and 14 – fast-food companies (including canteens).

Questionnaires

There were three types of questionnaires developed: one for cooks, one for waiters and one for managers. The questionnaires consisted of three parts: demographic information, information on the company, the part on knowledge. All questionnaires had identical questions within part on knowledge and part on demographic information, but the part on information about the company was different for managers' questionnaire. Part on knowledge consisted of 10 questions, to which the respondent answered by choosing 1 out of 5 answers (a Likert scale): 1 – yes; 2 – rather yes than no; 3 – I don't know; 4 – rather no than yes; 5 – no. The correct answers were codified with a digit 1, the wrong answers were codified with 0. The answers Yes or No were considered as correct. If the respondent answered to the question by Rather Yes than No, I don't know, Rather No than Yes, the answers were considered as incorrect, because they show that the respondent is not sure for his or her answer.

Selection of respondents

There were 60 public catering companies addressed selected in Riga according to random choice; they offer waiters' services. 20 companies participated in the research; from them there were 154 valid questionnaires obtained. There were waiters and cooks (n=139), as well as managers (n=15) involved in the survey.

Summarizing and analysis of results

MS Excel 2013 software was used for data processing. The following elements of descriptive statistics were used for summarizing the results – the mean arithmetical and the standard deviation. In order to determine the influence of factors, t–test was used: two-sample equal/unequal variance and ANOVA (single factor). The significance level was determined as $p<0.05$.

Results and Discussion

The analysis of the allergen information labelling types

Most of the visited catering companies or 51.9% (n=28) had indicated no information on allergens in their menus, on separate stands or in booklets (Table 1), but 14 of these companies had only indicated the phrase – "Ask the waiter about allergens". The allergens weren't more often indicated at the cafes (65%), less – at the restaurants (45%). Of those companies that could perform the full analysis of criteria, 48.1% companies indicated allergens in a form of codes, for example, A1, 01, 1, b, A. 13.3% companies indicate allergen information by using the group names specified in Annex II to Regulation (EC) No.1169/2011, for example, wheat, eggs, nuts. But 39.1% of companies in their menus or in other types of annexes, in addition to the group names indicated in the regulatory enactments, add also a phrase "Ask the waiter about the detailed allergen information", 49.8% of companies have used the size of digits or codes to indicate the allergens, where the size is smaller than or equal to 1.2 mm and thus there was some difficulties to read and perceive information. 34.9% of allergen information was easy to perceive, read, and the font, as well as the size of letters used was appropriate. In 34.9% cases the colour of letters or codes used for information sufficiently differed from the background of menu or other type of annex, thus such information was easy to read. But in 56.5% of the cases the colour of letters or codes used by companies only slightly differed from the background of menu or another annex that caused insignificant difficulty to see and read the information. Most of the companies or 65.5% do not offer alternatives for the guests with food allergies or intolerances. The alternative products, dishes that have been adjusted for the guests with allergies or intolerance are offered in the menus of 54.5% of restaurants. On the whole, there were no significant differences ($p=0.05$) found between the types of companies regarding the allergen information labelling.

Analysis of survey data

The total number of respondents was 154 out of whom 40.9% were cooks, waiters – 49.4%, but managers – 9.7% (Table 2). 60.4% of waiters/cooks and 86.7% of managers have indicated that daily they often attend guests with food allergy or intolerance (on average 4.2±2.9 times per week). More than a half of the responding waiters/cooks (52.5%) confirm that they have participated in the training related to food allergies and/or intolerances. When answering the question: "Do the guests with food allergies and intolerances cause inconveniences?", 64.7% of waiters/cooks answered "No", but 35.3% answered "Yes"; in addition, they were asked to provide detailed explanation for the chosen variant of the answer. The respondents who chose the variant "Yes", most often as the factors of inconveniences related to attending the guests with food allergies or intolerances indicate **time** ("*Longer period of attending*", "*There should be changed the calculation of the dish*", "*Breaks the rhythm*", "*It is necessary to think what dishes could be offered, or to choose such alternatives that preserve the qualities of the dish*"), **emotional factor** ("*Such guests cause stress*", "*I should be careful, it is additional responsibility*", "*There should extra work done*"), **shortage of resources** ("*There are no appropriate substituting products*", "*We have no tendency towards allergic guests*", "*There is a narrow choice of dishes we can offer*"), **guests** ("*The guests are not able to explain properly what kind of allergy or*

intolerance they have", "*Sometimes people tell about the allergy too late*", "*Very often allergy is combined with veganism*"), **lack of knowledge** ("*There is lack of knowledge about food allergies or intolerances*"). But those respondents who have no inconveniences in relation to attending allergic guests, most often indicate **emotional factor** ("*They are also people*", "*It is not their fault that they have allergy or intolerance*", "*There is always a way out of the situation*", "*It is our job to attend all guests equally*", "*We should anticipate what allergies people might have, therefore we should consider alternatives*", "*We are professionals*", "*I do not attend such guests*", "*Because it makes the job different*", "*It is my job*").

Upon the commencement of work, 50.4% of waiters/cooks have not received oral or written instructions from an employer how to attend guests with food allergy or intolerance. More than a half of the managers (66.7%) have responded that there have been no standards elaborated at the company how to attend guests with food allergy or intolerance. When answering the question "What do you think – is the food allergy and intolerance a topical problem in the catering industry?", most of the respondents – waiters / cooks (86.3%) and managers (80%) – have indicated that it is a topical problem.

Table 1

Allergen indication evaluation of menus

Criterion code	Criterion	Allergen indications %		
		Restaurants (n=20)	Cafe (n=20)	Fast food restaurants (n=14)
1	**Information placement position**			
1.1.	Allergen information is not provided	45.0	65.0	42.9
1.2.	Allergen groups are listed on the separate sheet, but there is no information on their content in food	5.0	0.0	21.4
1.3.	Allergen information is on a separate stand or booklet	5.0	10.0	14.3
1.4.	The information is on the menu as a separate page in the beginning or end page in menu	35.0	15.0	14.3
1.5.	The information is on the menu next to the name of each meal	10.0	10.0	7.1
2	**The amount of information**	**N=13**	**N=9**	**N=8**
2.1.	In the menu/ a stand/ booklet the phrase – „Ask the waiter about allergens"	15.4	22.2	25.0
2.2.	Allergens at each meal are indicated by symbols or codes (e.g. A01; 1; a)	61.5	44.4	25.0
2.3.	Allergens at each meal are indicated by symbols or codes as well as the phrase „Ask the waiter about allergens"	0.0	0.0	0.0
2.4.	Allergens at each meal are indicated by group names (wheat, eggs, milk)	0.0	22.2	25.0
2.5.	Allergens at each meal are indicated by group names as well as the phrase „Ask the waiter about allergens"	23.1	11.1	25.0
3	**A letter or code size and used font**	**N=11**	**N=7**	**N=5**
3.1.	A letter or code size is less than 1.2 mm, used font makes it difficult to the perceiving and reading	0.0	0.0	0.0
3.2.	A letter or code is equal to 1.2 mm, used font makes it difficult to the perceiving and reading	63.6	28.6	20.0
3.3.	A letter or code is greater than 1.2 mm, used font makes it difficult to the perceiving and reading	9.1	28.6	20.0
3.4.	A letter or code is equal or greater than 1.2 mm, used font does not affect to the perceiving and reading	27.3	42.9	60.0
4	**Visibility of letters or codes or codes against the background**	**N=11**	**N=7**	**N=5**
4.1.	The letters or code blends with the background of the menu or stand or booklet. Cannot be perceived.	9.1	0.0	0.0
4.2.	The colour of letters or code is different from the background of a menu or booklet. It is difficult to perceive.	63.6	57.1	40.0
4.3.	The colour of letters or code differs essentially from the background of a menu or booklet. Easy to perceive or read.	27.3	42.9	60.0
5	**The existence of alternative**	**N=11**	**N=7**	**N=5**
5.1.	The company does not offer alternatives to guests with food allergy or intolerance	45.0	100	100
5.2.	The company offers alternative products for guests with food allergies or intolerances	54.5	0.0	0.0

Table 2

Demographical information

Variable	Number of respondents	Percentage, %
Gender	*N=154*	
female	88	57.1
male	66	42.9
Education	*N=154*	
Middle school graduate	9	5.8
High school graduate	36	23.4
High professional graduate	73	47.4
College (university) graduate	36	23.4
Age	*N=154*	
<18	4	2.6
19–35	119	77.3
36–45	21	13.6
>45	10	6.5
Position	*N=154*	
cook	63	40.9
waiter	76	49.4
manager	15	9.7
Work experience in hospitality	*N=154*	
<1 year	27	17.5
1.1–7 years	70	45.5
>7.1 years	57	37

Cooks and waiters were asked an additional question: "Do you have food allergy or intolerance?", where 13.7% had indicated that they had, but 86.3% indicated that they had no such problem. 70.5% of waiters / cooks have indicated that their represented company specify allergens in menus, but 9.4% do not know, whether information on allergens have been specified. 64.7% of waiters/cooks and 86.7% of managers have confirmed that the company offers different alternatives to guests with food allergy or intolerance (specialized menu or dishes, substituting products etc.).

Although more than a half of waiters/cooks (74.8%) found that they have sufficient knowledge of menu offered at the company in order to assist guests with food allergy or intolerance, the results show that the personnel lacks insight into the theoretical aspects of food allergy and intolerance (Table 3).

Only 1.3% of respondents in the part of knowledge had obtained 9 correct answers out of 10. The respondents' mean number of correct answers was 3.8±1.8 out of 10. Most of the problems the respondents had regarding the questions related to determining the difference between food allergy and intolerance (statements C and G). Only 27.3% (statement C) of respondents knew that food allergy and intolerance is not one and the same. In other studies the respondents also often make mistakes regarding such similar questions (Soon, 2018). However, the insight into such issues is necessary. Food allergies are mostly related to the functioning of immune system and in some cases they might become a reason for death.

Table 3

Respondents' knowledge about food allergies and intolerances

Statement code	Respondents' answers, % (n = 154)					Mean indicators	
	Yes (1)	Rather Yes than No (2)	I don't know (3)	Rather No than Yes (4)	No (5)	Mean	SD
Food allergy is caused by enzyme deficiency (A)	29.9	24.7	31.8	8.4	5.2a	2.4	1.2
Legislation provides for the compulsory indication of allergens in the menus (B)	69.5	9.1	9.1	2.6	9.7a	1.8	1.3
Food intolerance is the same as food allergy (C)	12.3	24.7	24.0	11.7	27.3a	3.2	1.4
Food allergy occurs only by eating an allergic product (D)	20.1	14.3	7.1	16.2	42.2a	3.5	1.6
A small amount of an allergic product does not cause an allergic reaction (E)	5.2	9.1	10.4	16.9	58.4a	4.2	1.2
Food Allergy Can Be Fatal (F)	76.6a	8.4	7.8	5.2	2.0	1.5	1.0
Lactose intolerance is the same as milk allergy (G)	48.7	16.2	7.1	3.9	24.0a	2.4	1.7
In case of food intolerance, you should refuse to use the product (H)	62.3a	20.8	8.4	5.2	3.3	1.7	1.1
Symptoms of food intolerance are anaphylactic shock (I)	22.1	15.6	35.7	12.3	14.3a	2.8	1.3
If a guest is allergic to nuts and he orders a dessert containing nuts, it is enough to remove them from the portions to make the food safe for the client (J)	10.4	4.6	5.2	7.8	72.1a	4.2	1.4

a – correct answers.

Besides, the reaction might be caused also just by touching the product or inhaling its vapours. The staff that has an important role at the catering company for the communication with a guest shall be ready to identify allergy symptoms and to provide timely assistance during the reaction (Dupuis et al, 2016). The respondents made mistakes while answering the statement B, where only 9.7% of respondents have correctly indicated that legislation does not provide for compulsory allergen labelling in the menu. Regulation (EC) No.1169/2011 on the provision of food product information to consumers provides that the allergen information shall be provided in writing, but it is up to the company, where such information would be displayed. Most of the respondents (76.6%) answered correctly that food allergy may be lethal. There should be also statement J pointed out, where 72.1% of respondents gave the correct answer. There were calculations performed using ANOVA and t – test in order to find out, whether there are differences between the influence of different factors and the results of knowledge. In relation to such factors as a position ($p=0.153$), existence/lack of training ($p=0.182$) and the fact, whether respondent has or has no allergy/intolerance ($p=0.940$), there was not observed significant influence on the results of knowledge. The influence of position has not been statistically significant factor in relation to the results of knowledge also in the studies performed by Lee, Sozen (2016), Soon (2018) and Shafie, Azman (2015). However, Lee, Sozen (2016) in the study found significant differences between those respondents who had training and who had no training. In the authors' study the respondents were not asked more detailed questions about the content, duration and other indicators of training; thus, it is necessary to perform additional studies to find out and assess the quality of training.

However, answering why the results of knowledge are not influenced also by existence or non-existence of allergy/intolerance could be found in the fact that there was no detailed division indicated in the study for the answers provided to question: "Do you have food allergy or intolerance?" Thus, the respondents who had intolerance could lack knowledge of allergy issues, but the respondents with allergy could lack knowledge of intolerance issues. There is also a question, whether the particular respondent really has food allergy or intolerance.

Conclusions

Thoughtful management of food allergens in restaurants is a topical issue in Latvia. Overall 51.9% of visited companies do not indicate any information on the presence of allergens in the food. Indication "Ask the waiter about allergens" cannot be sufficient for the provision of allergen information. Servicing staff is responsible for correct allergen information explanation to a guest and its further transfer to members of staff working in the kitchen. Research data show that the respondents' knowledge could be evaluated as poor, there is no insight into the food intolerance and allergy difference. The level of knowledge is not significantly different among managers, cooks and waiters. It is not influenced also by existence or lack of training. Such tendency may endanger population's health with allergens and intolerance. This issue is complicated and difficult, and there are further studies needed in order to explain reasons for the careless attitude of catering company managers and employees towards the provision of allergen information at the company.

References

1. Barnett J, Leftwich J, Muncer K, Grimshaw K, Shepherd R, Raats M.M, Gowland M.H, Lucas JS. (2011) How do peanut and nut allergic consumers use information on the packaging to avoid allergens? *Allergy*, Vol. 66, p. 969–978.
2. Bartuzi Z., Kaczmarski M., Czerwionka-Szaflarska M., Malaczynska T., Krogulska A. (2017) Position paper of food allergy section the Polish society of allergology on the diagnosis and management of food allergies. *Alergologia Polska – Polish Journal of Allergology*, Vol. 4(3), p. 109–122.
3. Blom W. M., Michelsen-Huisman A. D., van Os-Medendorp H., van Duijn G., de Zeeuw-Brouwer M., Versluis A., Castenmiller J. J. M., Noteborn H. P. J. M., Kruizinga A. G., Knulst A. C., Houben G. F. (2018) Accidental food allergy reactions: Products and undeclared ingredients. *Journal of Allergy and Clinical Immunology*, Vol.142 (3), p. 865–875.
4. Dupuis R., Meisel Z., Grande D., Strupp E., Kounaves E., Graves A., Frasso R., Cannuscio C. C. (2016) Food allergy management among restaurant workers in a large U.S. city. *Food Control*, Vol. 63, p. 147–157.
5. Lee Y. M., Sozen E. (2016) Food allergy knowledge and training among restaurant employees. *International Journal of Hospitality Management*, Vol. 57, p. 52–59.
6. Peniamina R. L., Peniamina R. L., Mirosa M., Bremer P., Conner T. S. (2016). The stress of food allergy issues in daily life. *Psychology & Health*, Vol. 31(6), p.750-767.
7. Pulido O. M. (2010) Immune – mediated adverse reactions to dietary proteins. **In:** *Allergen Management in the Food Industry*. J. I. Boye, S. B. Godefroy (eds). Canada: John Wiley & Sons, Inc., p. 3–33.
8. Shafie A. A., Azman A. W. (2015) Assessment of knowledge, attitude and practice of food allergies among food handlers in the state of Penang, Malaysia. *Public Health*, Vol. 129 (9), p. 1278–1284.
9. Soon J. M. (2018) "No nuts please": Food allergen management in takeaways. *Food Control*, Vol. 91, p. 349–356.
10. EAACI (2013) *Food Allergy and Anaphylaxis Guidelines*. [accessed on 17.01.2019.]. Available at: http://www.eaaci.org/foodallergyandanaphylaxisguidelines/Food%20Allergy%20-%20web%20version.pdf
11. FARE (2018) *Food Allergy Facts and Statistics for the U.S.* [accessed on 17.01.2019.]. Available at: https://www.foodallergy.org/sites/default/files/2018-04/FARE-Food-Allergy-Facts-Statistics.pdf.
12. Regulation (EC) No 1169/2011 of the European Parliament and of the Council (2011). [accessed on 17.01.2019.]. Available at: https://eur-lex.europa.eu/eli/reg/2011/1169/oj
13. WAO (2013) *Food Allergy – A Rising Global Health Problem*. [accessed on 17.01.2019.]. Available at: https://www.worldallergy.org/UserFiles/file/WorldAllergyWeek2013final.pdf

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THE EVALUATION OF THE STUDENTS' ATTITUDES AND BEHAVIOR TOWARDS FAST FOOD CONSUMPTION

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Abstract

People have different lifestyles that cause different needs and desires. Lifestyles influence people's purchasing behavior. Conditions affecting lifestyles, such as long working hours, short break times, lack of cooking time and no cooking place for employees and students have an impact on changing their eating habits. This situation has led consumers to prefer products that can be consumed faster. University students are considered as significant customer potential by fast food sector. The purpose of this study was to determine whether there is a relationship between the lifestyle and the reasons for choosing fast food of university students. The data were collected by conducting a survey with 347 students in Tekirdag Namik Kemal University. The data was analyzed statistically in terms of descriptive and inferential statistics. In this direction, factors related to fast food consumption are 4 factors (product features, space, accessibility, personal satisfaction) and lifestyles are collected under 6 factors (thinkers, experiencers, achievers, believers, innovators, makers). The relationship's existence is explained by using factor scores calculated by factor analysis in a multiple regression model. According to research findings, product features variable is effective in choosing fast food products by Thinkers. Accessibility variable is affective in choosing fast food products by people who have Experiencers lifestyle; space and personal satisfaction are affective on Achievers. Product features and space are affective in choosing fast food products by Innovators. When the findings are generally evaluated; the effects of lifestyle on the causes of fast food consumption seem to be significant.

Keywords: fast food, lifestyle, consumption, consumer behavior, Vals 2

Introduction

The well-known psychologist Abraham Maslow mentions that in the hierarchy of needs, the first step cannot be overcome without the need for eating, drinking and sheltering with physiological needs (Kula, Cakar, 2015). As with all things, physiological needs vary from person to person. At this point, the concept of consumer behavior comes to the fore. Consumer behavior, goods and services purchased to meet the needs of people; why, how, when they show up. The determination of consumer behaviors precisely determines the direction of the consumer's needs and desires. In a market, where relatively standard products and services are offered, especially fast food, it is necessary to respond quickly to customer expectations (Eroglu et al., 2012; Hamsioglu, 2013). Businesses, customers, according to the desire, needs and expectations, in order to produce different solutions to be able to analyze their customers is an important point (Korkmaz, 2005).

One of the personal values that affect consumer behavior is lifestyle. The concept of lifestyle is a term that people use to describe their own or others' behavior. The most prominent feature of the concept is that it includes behavior patterns that make people different from each other (Mucuk, 2010). Lifestyle, consumer behavior literature, how people live, time and money spent on how to spend with the patterns; it includes the activities, interests and ideas of individuals (Plummer, 1974). Consumption behaviors of individuals who have grown up in the same culture and have the same age and same profession are different (Ercis et al., 2007).

Lifestyle psychography techniques are tried to be measured. One of the most common methods of psychographic segmentation is the *Values, Attitudes and Lifestyle 2 (VALS 2)* model. The VALS 2 model tries to explain why consumers buy and make decisions as well as what they buy. It was created to better analyze consumer behavior. The main determinants are motives, perceptions, learning, beliefs, attitudes and personality. VALS 2 model is divided into 8 groups. Each of these groups have similar characteristics in themselves (Odabasi, Baris, 2013). Businesses learn about the product and brand preferences of the consumers in accordance with the characteristics of these groups and prepare marketing mixes.

These groups are described below (Hamsioglu, 2013; Yesiloglu, 2013; Anonymous, 2018):

1. *Innovators* – they are highly respected, successful and responsible people. They are fond of freedom and active consumers. The purchases reflect their enhanced tastes for their senior niche products and services.
2. *Thinkers* – they think well before they make a decision and they do very well. They do not give importance to prestige and image. Revenues are available to purchase many products, but the durability of the product is more important than the restraints and purchases.
3. *Achievers* – they have a goal-oriented lifestyles, are deeply committed to their family. They are active buyers in the market with many requests and needs. For this reason, they prefer prestigious, high quality brands and products that will show their success. They read about business, news, personal development.
4. *Empiricists* – young and enthusiastic. They like exciting things. Their political knowledge is limited and they are uncertain about what they will believe. They spend most of their income to socialize and have fun. They like the risk. They make instant purchases according to their impulses. They pay close attention to ads.
5. *Believers* – they have a routine life. They are slow in changing their habits. They are strictly committed to their traditions. They do not go beyond the products they

know. Believers prefer known products and brands. Those who strive; their image consciousness is high. They quickly get bored and move without thinking, so for those who strive, money means success. However, the money they can use arbitrarily is limited. They buy imitations of rich brands. They spend on clothing and personal care products. They prefer to read television.

6. *Makers* – they are motivated by expressing themselves. For this reason, they deal with handicrafts, home construction, child care. They make purchases about general needs. They are politically conservative and skeptical about new ideas.

7. *Survivors* – people with low levels of education and lack of skills and social life are coy. Since they do not have much income, they are satisfied with their basic needs. They are content with what they do in life. Coupons are common and follow discounts.

The most important feature of the concept of lifestyle is that it is dynamic; because the technological, economic, social trends and cultural changes of the society reflect on the lifestyles of consumers and change their way of life. These changes have led to the development of some sectors. Fast food sector is one of the developing sectors thanks to these changes. Rapid urbanization in living conditions, getting closer to western culture, increasing the number of women working, and the desire to save time have also gained importance in this sector.

Fast food is food, which can be prepared quickly and easily and is sold at commercial undertakes such as restaurants and snack bars (Vogli et al., 2014). Western fast food items prevalently are hamburger, pizza, pasta, sandwiches, French fries, fried chicken, tacos, and hot dogs. Traditional fast foods in Turkey are meat on a spit, pide, steak tartar a turca, lahmacun, Turkish bagel, meatball, grilled sheep's intestines, and stuffed mussels (Beak at al., 2006; Driskell at al., 2006; San, 2009; Haines at al., 2010; Akdag, 2015).

In the studies for fast food consumption, which has increased rapidly over time (Korkmaz, 2005; San, 2009; Kayisoglu, Icoz, 2012; Kingir et al., 2015; Lassen et al., 2016; Tengiz, 2018); according to the elderly, the high-income people compared to the low-income, compared to the low level of education of the high-educated men, according to the women, according to the married women, working women prefer to eat more fast food than housewives. These studies reflect the relationship between activity and demographic characteristics and fast food consumption.

Previous studies have shown that the consumption of fast food products is more than that of other age groups, especially in young age students (Driskell et al., 2006; Hamsioglu, 2013; Comert, 2014; Akdag, 2015; Lassen et al., 2016). University students have the most important share in the fast food sector (Beak et al., 2006). Since the students start their university education, they become familiar with the environment and become more open to interact with the environment. This period is especially the period when the consumption of fast food products increases. Students want to make friends, socialization, cheap and satisfying products, being served fast, not wanting to cook and especially those who stay in dormitories cannot have the appropriate environment for cooking causes fast food products are more preferred.

The purpose of this study was to determine whether there is a relationship between the lifestyle and the reasons for choosing fast food of university students. In other words, the reasons for fast food consumption, which may change depending on the lifestyle of consumers were determined.

Materials and Methods

In this study, data obtained from 347 university students in 2015–2016 in Tekirdag Namik Kemal University were used. In order to collect the original data, appropriate questionnaire forms were prepared and applied to the sample volume.

The VALS 2 scale (Plummer, 1974; Lin, 2003; Ozgul, 2010; Hamsioglu, 2013; Valentine, Powers, 2013; Yesiloglu, 2013; Guner, 2014) determined the lifestyles of the respondents from various literature reviews on the reasons for choosing fast food products (Madran, Kabakci, 2002; Driskell et al., 2006; Kaya, 2011; Comert, 2014).

The questionnaire used in the study consists of four parts. The first part involved closed-ended questions, such as frequency and time of arrival of consumer in enterprise, whether there had been a change in consumption in recent years, their usual preference of fast food products. The second part was focused on the reasons why consumers preferred fast food products. The third part consisted of questions on lifestyle. Likert-type five-point scales were used. The last part of the questionnaire contained questions that determine demographic characteristics of consumers.

The data obtained from the study were analyzed with SPSS 23.0 package program. In this context, chi-square, factor analysis and multiple linear regression were used. Factor analysis was applied to determine whether the variables used in the study were compatible with each other, and to present the data in a more meaningful and abstract manner. Varimax rotation method was used in the analysis of the basic components.

The existence of the relationship between consumer lifestyle and the reasons of fast food consumption was explained by using factor analysis scores calculated by factor analysis in multiple regression model. Enter method was used in the analysis.

Results and Discussion

When the demographic characteristics of the consumers were examined, 63.7% of the study was female and 36.3% was male. Since the study was applied to university students, they stated that they did not work (86.2%). It was seen that 54.5% of the consumers received a scholarship or a repayment loan from the Turkish Government or a private institution. 44.1% of the respondents live at home, 38.9% live in dormitories and 17.0% live in the apartments. Most of the consumers live alone (14.4%) or with friend (65.4%) away from

their families (Table 1). Consumers consume fast food products several times a week (36.0%). Consumption is 1–2 times a month and has a share of 22.0% in total consumption. The number of people consuming each day (15.0%) and once a week (16.0%) is very close to each other. Haines et al. (2010) stated that 84.0% of the students consumed fast-food products at least once a week.

Table 1

Demographic characteristics of the students

Parameters		Frequency	%
Gender	Male	126	36.3
	Female	221	63.7
Working situation	Not working	299	86.2
	Part-time	26	7.5
	Full-time	22	6.3
Monthly expenditure	Less than 300 ₺	36	10.4
	301-500 ₺	110	31.7
	501-1000 ₺	141	40.6
	1001 ₺+	60	17.3
Scholarship	Yes	189	54.5
	No	158	45.5
Living place	Home	153	44.1
	Dormitory	135	38.9
	Student apartment	59	17.0
Living with who	with family	60	17.3
	with friend	227	65.4
	alone	50	14.4
	with a relative	10	2.9

Dalrymple and Dyett (2013) concluded that the majority of students (91.8%) consume fast food once or twice a week. 36.9% of consumers consumed fast food during the week, 33.7% at the weekend and 29.4% at weekends and at the weekend. 65.0% of males and 68.0% of females stated that their consumption of fast food consumption increased due to the impact of university life. Nearly all of the consumers (87.9%) are aware of the harmful effects of fast food products on health. Haines et al. (2010) reported that university students often consume fast food products and pay more for nutritious intense choices (88.0%), but they do not prefer to pay because of habit and taste factors. The frequency of sex and consumption according to the chi square independence test results (x^2: 3.918; $p<0.05$), consumption time (x^2: 2.165; $p<0.05$) and the effect of university on consumption (x^2: 0.517; $p<0.05$) No significant relationship was found. However, it was concluded that the effect of fast food products on health was a difference between sex (x^2: 11.211; $p>0.05$). Women find food more harmful than men. Figure 1 shows which enterprises preferred by consumers. San (2009) has identified fast food restaurants as Burger King and Mc Donald's, which are most preferred by consumers. In South Korea, it was found that sandwiches, hamburgers and fries were frequently consumed in western fast food foods (Wyne et al., 1994). Consumers are most interested in health (23.7%) and quality (22.3%) of the products. In the third row, the prices of the products (16.3%) are in place and the flavor (13.7%) is the fourth.

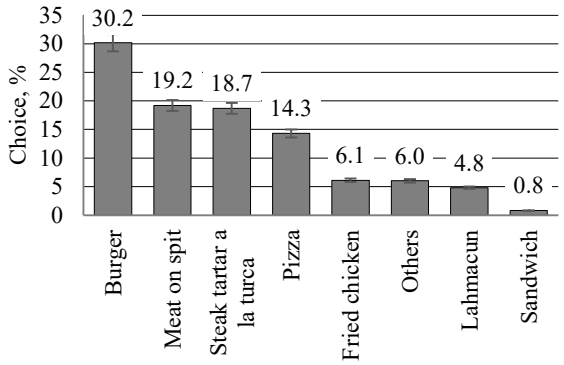

Figure 1. Choice of fast food types

Another study conducted in the United States revealed that fast food products are consumed because they are perceived as low cost and economic; lack of time, taste and eating with family and friends have been stated as the reasons (Morse, Driskell, 2009). Lassen et al. (2016) in their study in Denmark indicated habits, price and taste as the most effective factors in the choice of fast food products. Cronbach Alfas Alpha coefficient of the scale for fast food consumption was found to be 0.819. Accordingly, the internal consistency of the variables within the scale is high. If $0.60 \leq \alpha \leq 0.80$, the scale is quite reliable (Kalayci 2014).

Kaiser-Meyer-Olkin (KMO) competence sample coefficient was 0.812. The obtained coefficient shows that the data are suitable for factor analysis. The chi-square value of Bartlett's sphericity test is 1880.353. As result of the analysis variables were collected under 4 factors explaining the total variance value of 51.7%. The contributions of the variables to the main factors obtained from the analysis and the explained variances are shown in Table 2. The 1st factor after rotation is called *Effect of Product Properties*. These 6 items explain 15.8% of the total variance. The second factor after rotation is called *Space Effect*. These 4 items explain 12.7% of the total variance. The third factor after rotation is called the *Accessibility Effect*. These 5 items explain 13.8% of the total variance. The post-rotation factor, which has a major effect on the 4th factor, is to reward myself and this factor is called *The Effect of Personal Satisfaction*. These 4 items explain 9.3% of the total variance.

Cronbach's Alpha of the scale related to lifestyles was found to be 0.761. The KMO competence sample was 0.821. The chi-square value of Bartlett's sphericity test is 1985.977 and its significance is $p<0.000$. As a result of the analysis, 21 variables were revealed which accounted for 59.6% of the total variance and were collected under 6 factors. The contributions of the variables to the main factors obtained from the analysis and the explained variances are shown in Table 3. The 1st factor after rotation is called *Thinkers*. These 7 items explain 19.8% of the total variance. The second factor after rotation is called *Experiencers*. This fourth article explains 10.2% of the total variance. The third factor after rotation is named *Successors*.

Table 2

Results of factor analysis in fast food consumption causes

	FS	PTVE	EV
Factor F1: Effect of Product Properties		15.813	3.005
Products are of good quality	0.831		
Clean and hygienic	0.823		
Prices are appropriate	0.610		
Servings are hearty	0.582		
It is delicious	0.519		
Product variety	0.504		
Factor F2: Space Effect		12.662	2.630
Since it helped me to socialize	0.786		
For me there are similar people	0.772		
Seating layout for being spacious	0.638		
Gifts for offers	0.594		
FactorF3: Accessibility Effect		13.845	2.406
Easy to access	0.777		
For fast service	0.684		
You can order from the phone	0.614		
I don't have time to prepare food	0.587		
Products standard	0.364		
Factor F4: Effect of Personal Satisfaction		9.342	1.775
I reward myself	0.747		
I'm used to the taste	0.558		
For being a chain	0.517		
I don't know how to cook	0.439		

Table 3

Result of Factor Analysis in Lifestyles

	FS	PTVE	EV
Factor Y1: Thinkers		19.799	4.158
My family comes first to me.	0.792		
I'm open to new ideas.	0.735		
I value social values.	0.704		
I act in accordance with my budget for shopping.	0.671		
I prefer quality and time-saving products.	0.654		
I think well before decide.	0.614		
I follow the events in the country and the world closely.	0.550		
Factor Y2: Experiencers		10.188	2.140
I like the risk.	0.794		
I spend a lot of money to have fun and socialize.	0.648		
I'm fond of my freedom.	0.620		
I like exciting things.	0.551		
Factor Y3: Achievers		9.718	2.041
I follow fashion closely.	0.709		
I expect approval from others in a job that I do.	0.654		
Money means success for me.	0.643		
I prefer branded products.	0.613		
Factor Y4: Believers		6.928	1.455
I don't go beyond my usual lifestyle.	0.830		
I'm not out of the products I know.	0.809		
Factor Y5: Innovators		6.617	1.389
I follow the innovations.	0.706		
I want to be the leader in my friends.	0.674		
Factor Y6: Makers		6.378	1.339
I am skilled in machine repair and maintenance.	0.731		
I like to do dexterity.	0.622		

FS – Factor Score; PTVE – Percent of Total Variance Explained; EV –Eigen value

These 4 items explain 9.7% of the total variance. The 4th factor after rotation was called *Believers*. These two items explain 6.9% of the total variance. The fifth factor after rotation is called *Innovators*. These two items explain 6.6% of the total variance. The 6th factor after rotation is called *Makers*. These two items explain 6.3% of the total variance.

For main hypothesis, lifestyles and some demographic activities were regulated on factors related to consumption reasons and Table 4 was reached.

Table 4

Regression results for lifestyle and fast food consumption reasons

Factors of fast food consumption reasons	Factors of lifestyle						Demographic characteristics				R^2	F	F value (Sig.)
	Y1	**Y2**	**Y3**	**Y4**	**Y5**	**Y6**	**Gender**	**Class**	**Living place**	**Sharing a house**			
F1	0.276 (**0.000**)	0.052 (0.328)	0.051 (0.324)	-0.056 (0.278)	0.105 (**0.042**)	-0.024 (0.637)	-0.293 (**0,013**)	-0.037 (0.433)	0.132 (0.315)	-0.376 (**0.016**)	0.160	4.867	0.000
F2	-0.172 (**0.001**)	0.001 (0.979)	0.141 (**0.008**)	-0.061 (0.250)	0.150 (**0.005**)	0.024 (0.652)	0.029 (0.812)	-0.068 (0.161)	-0.470 (**0.001**)	0.453 (**0.005**)	0.119	3.444	0.000
F3	-0.710 (**0.000**)	0.352 (**0.000**)	0.199 (0.922)	-0.005 (0.281)	-0.054 (0.075)	0.089 (0.497)	0.034 (**0.016**)	0.276 (**0.032**)	-0.091 (0.833)	-0.027 (0.437)	0.208	6.712	0.000
F4	-0.178 (**0.001**)	0.087 (0.108)	0.206 (**0.000**)	-0.109 (**0.037**)	0.098 (0.063)	0.046 (0.382)	0.166 (0.165)	0.001 (0.980)	-0.170 (0.201)	0.342 (**0.030**)	0.135	3.980	0.000

F1 – Effect of Product Properties, F2 – Space Effect, F3 – Accessibility Effect, F4 – Effect of Personal Satisfaction; Y1 – Thinkers, Y2 – Experiencers, Y3 – Achievemers, Y4 – Believers, Y5 – Innovators, Y6 – Makers; F– F Value

In the table, the explanations between the variables (R^2), β coefficients and the significance levels in parentheses, and the F test statistic values and significance levels for the determination of dependent and independent variables as a whole are included. Multiple linear regression assumptions are met between factors related to the reasons of fast food consumption (dependent variable) and lifestyle factors.

The main hypothesis:

H_1: There is no relationship between consumer lifestyles and the reasons of consumption of fast food products;

H_2: There is a relationship between consumer lifestyles and the reasons of consumption of fast food products.

According to Table 4, the change in lifestyle factors and demographic variables explained 16.0% of the change in factor due to the effect of product characteristics. The remaining 84.0% is explained by the variables not included in the study. The fact that the F test statistic value (Sig.) It indicates that it is meaningful to explain the effect of product characteristics with lifestyles ($p \geq 0.000$). Factors related to lifestyle and changes in demographic variables; It can explain 11.9% of the change in the effect of the space, 20.8% of the change in accessibility effect and 13.5% of the change in the personal satisfaction factor. In addition, lifestyle factors and change in demographic factors were significant in the 95.0% confidence interval in explaining changes in factors related to fast food consumption. With this finding, the hypothesis of the study is rejected

Conclusions

Fast food products of university students who have lifestyle in the thinker's factor; it prefers the effect of the product characteristics and the effect of accessibility. In other words, thinkers prefer fast food foods for reasons such as high quality, clean, hygienic, affordable, satisfactory portion of the products, ease of transportation. It has a negative effect on space effect. Thinkers are generally well-educated, mature and satisfied individuals because they do not find the idea of socialization sufficient. The effects of personal satisfaction for thinkers are not important for fast food consumption. The students who have a lifestyle in terms of experience are fast food products; While preferring the effect of the product characteristics due to the effect of accessibility and personal satisfaction effect, it does not consider the variables related to the space effect important. For consumers with a successful lifestyle, the reasons of fast food consumption include socialization, the spaciousness of the seating, the delicious products and the self-reward. Fast food products are preferred by successful, responsible and self-respecting innovators because of similar quality, clean and satisfactory products. For the consumers in the group of believers and makers, no meaningful result could be obtained regarding the reasons of fast food consumption.

There is no similar study in this area in Tekirdag, which is the study area. Namik Kemal University is the only university in Tekirdag. The number of students increases every year due to the fact that the university grows day by day and the province is close to major cities such as Istanbul. This situation depends on product and service features such as fast food restaurants; fast service, cheap, delicious and hearty portions, depending on the characteristics of the venue; spacious seating arrangements are indicative of a further increase in requests such as spacious.

As result, this study can guide the preparation of marketing activities for the target groups based on the lifestyles of university students who are the most important potential customers of existing or new fast

food restaurants. In addition, it is contributing in the studies to be carried out after this study.

References

1. Akdag H.N. (2015) Lise ogrencilerinin fast food Tuketim Durumlarinin ve Aliskanliklarinin Saptanmasi. *MSc. Thesis*, Okan Universitesi Saglik Bilimleri Enstitusu, Istanbul, p. 33–38.
2. Anonymous (2018) Strategic business insights. [last accessed on 20.01.2018]. Available: http://www.strategicbusinessinsights.com/vals/ustypes.shtml.
3. Beak S.H., Ham S., Yang I.S. (2006) A Cross-cultural comparison of fast food restaurant selection criteria between Korean and Filipino college students. *International Journal of Hospitality Management*, Vol. 25(4), p. 683–698.
4. Comert M. (2014) Genclerin fast food Tuketim Aliskanliklari. *Akademik Sosyal Arastirmalar Dergisi*, Vol. 2(6), p. 423–427.
5. Dalrymple K., Dyett P. (2013) Eating practices and nutrition knowledge among university students. *Student Project*. The University of the West İndies, p. 33–36.
6. Driskell J.A., Meckna B.R., Scales N.E. (2006) Differences exist in the eating habits of university men and women at fast-food restaurants. *Nutrition Research*, Vol. 26(10), p. 524–530.
7. Ercis A., Unal S., Polat C. (2007) Yasam tarzlarinin satin alma karar sureci uzerindeki rolu. *Ataturk Universitesi Iktisadi ve Idari Bilimler Dergisi*, Vol. 21(2), p. 281–311.
8. Eroglu E., Velioglu M.N., Baris G., Argan M. (2012) *Tuketici Davranislari*. Eskisehir: Acikogretim Fakultesi Yayini No: 1572, 186 p.
9. Guner A.G. (2014) Tuketici kulturu cercevesinde Yasam Tarzinin Onemi ve Marka Tercihi: Bir Arastirma. *MSc. Thesis*, Galatasaray Universitesi Sosyal Bilimler Enstitusu, Istanbul, p. 94–100.
10. Haines C., O'Neil C.E., Zanovec M. (2010) Fast-food consumption among college students and their attitudes toward healthier fast-food options. *The Federation of American Societies for Experimental Biology Journal*, Vol. 24(1).
11. Hamsioglu A.B. (2013) Fast food Urunleri Satin Alan Tuketicilerin Yasam Tarzlarini Belirlemeye Yonelik Bir Uygulama. *Uluslararasi Iktisadi ve Idari Incelemeler Dergisi*, Vol. 11, p. 17–34.
12. Kalayci S. (2014) *SPSS Uygulamali Cok Degiskenli Istatistik Teknikleri*. Ankara: Asil Yayinlari, 426 p.
13. Kaya D. (2011) Hizli yiyecek tuketicilerinin hizli yiyecek tuketme nedenleri: mersin sehir merkezinde faaliyet gosteren yerel, ulusal, uluslararasi zincir hizli yiyecek isletme musterileri uzerinde bir arastirma. *MSc. Thesis*, Mersin Universitesi Sosyal Bilimler Enstitusu, Mersin, p. 71–88.
14. Kayisoglu S., Icoz A. (2012) Egitim Duzeyinin Fast Food Tuketim Aliskanligina Etkisi. *Tekirdag Ziraat Fakultesi Dergisi*, Vol. 9(2), p. 16–19.
15. Kingir S., Karakas A., Sengun H.I., Cenberlitas I. (2015) Universite Ogrencilerinin Fast-Food Tercih Etme Sebeplerinin Belirlenmesi: Dicle Universitesi Ornegi. *Seyahat ve Otel Isletmeciligi Dergisi*, Vol. 12 (3), p. 102–119.
16. Korkmaz S. (2005) Fast Food (Hizli Yemek) Pazarinda Rekabetci Stratejilerin Etkinligi: Universite Gencliginin Tercihlerinin Analizi. *Ticaret ve Turizm Egitim Fakultesi Dergisi*, Vol. 2, p. 22–39.
17. Kula S., Cakar B. (2015) Maslow Ihtiyaclar Hiyerarsisi Baglaminda Toplumda Bireylerin Guvenlik Algisi ve Yasam Doyumu Arasindaki Iliski. *Bartin Universitesi Iktisadi Idari Bilimler Fakultesi Dergisi*, Vol. 6(12), p. 191–210.
18. Lassen A.D., Lehmann C., Andersen E.W., Werther M.N., Thorsen A.V., Trolle E., Gross G., Tetent I. (2016) Gender differences in purchase intentions and reasons for meal selection among fast food customers – opportunities for healthier and more sustainable fast food. *Food Quality and Prefence*, Vol. 47, p. 123–129.
19. Lin F.Y. (2003) An analysis of hospitality consumer lifestyles in The United States. *Ph.D*. Dissertation, Texas Tech University, p.14–15.
20. Madran C., Kabakci S. (2002) Tuketici Davranislarini Etkileyen Bir Faktor Olarak Yasam Tarzi: Cukurova Universitesinde Okuyan Kiz Ogrencilerin Yasam Tarzi Tiplerinin Belirlenmesine Yonelik Bir Arastirma. *Dokuz Eylul Universitesi Iktisadi Idari Bilimler Fakultesi Dergisi*, Vol. 17(1), p. 81–94.
21. Morse K.L., Driskell J.A. (2009) Observed sex differences in fast-food consumption and nutrition self-assessments and beliefs of college students. *Nutrition Research*, Vol. 29(3), p. 173–179.
22. Mucuk I. (2010). *Pazarlama Ilkeleri*. Istanbul: Turkmen Kitabevi, 432 p.
23. Odabasi Y., Baris G. (2013) *Tuketici Davranisi*. Istanbul: Mediacat Yayinlari, 404 p.
24. Ozgul E. (2010) Tuketicilerin Deger Yapilari, Gonullu Sade Yasam Tarzi ve Surdurulebilir Tuketim Uzerindeki Etkileri. *Hacettepe Universitesi Iktisadi ve Idari Bilimler Fakultesi Dergisi*, Vol. 28(2), p. 117–150.
25. Plummer J.T. (1974) The concept and application of life style segmentation. *Journal of Marketing*, Vol. 38(1), p. 33–37.
26. San Z. (2009) Tuketicilerin Yasam Tarzlari ile Fast Food Restoranlarindaki Hizmet Kalitesi Algilamalari Arasindaki Iliski Uzerine Bir Arastirma. *MSc. Thesis*, Sakarya Universitesi Sosyal Bilimler Enstitusu, Sakarya, p. 48–61.
27. Tengiz Z.M. (2018) Universite Ogrencilerinin Fast Food Tuketimine Yonelik Tutum ve Davranislarinin Degerlendirilmesi, *MSc. Thesis*, Namik Kemal Universitesi. Fen Bilimleri Enstitusu, Tekirdag, p. 55–78.
28. Valentine D.B., Powers T.L. (2013) Generation Y values and lifestyle segments. *Journal of Consumer Marketing*, Vol. 30(7), p. 597–606.
29. Vogli R.D., Kouvunen A., Gimeno D. (2014) The influence of market deregulation on fast food consumption and body mass index: A cross-national time series analysis. *Bullerin of the World Health Organization,* Vol. 92(2), p. 92–99.
30. Wyne M., Lee M.J., Moon S.J. (1994) Fast food consumption in South Korea. *Journal of Consumer Studies and Home Economics*, Vol. 18(3), p. 279–291.
31. Yesiloglu H. (2013) Yasam Tarzinin Musteri Sadakati ve Tuketicilerin Satin Alma Davranislari Uzerine Etkileri: Organik Gida Urunlerini Kullanan Tuketiciler Uzerinde Bir Uygulama. *MSc. Thesis*, Atilim Universitesi Sosyal Bilimler Enstitusu, Ankara, p. 10–15.

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STUDY OF FOOD WASTE AT SCHOOLS IN VIDZEME REGION

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Abstract

The amount of food waste at schools is a topical issue because it shows the effectiveness of school catering system, dietary quality, pupils' attitudes and satisfaction with the offered food. The objective of this study was to determine the types of food waste, their amounts at schools in Vidzeme region and to analyse the pupils' attitude towards school meals. The study was carried out at five schools in Vidzeme region, making one-week waste food weighing. The survey method was used to determine pupils' satisfaction with school meals. Three kinds of food waste can be distinguished in school canteens, depending on where they are generated: food waste in preparation process, in distribution process and food waste from the left food portion, which accounted for 41%, 20% and 39% respectively. The amount of food waste in preparation process, which is influenced by seasonality, quality of food products, staff experience and working conditions, can be reduced by rational organization of the production process. The amount of food waste from the left food portion was influenced by the individual wishes of the pupils, family eating habits, the quality of food, the atmosphere in the school canteen, the way of serving lunch and the participation of teachers. Whereas a large amount of waste is due to the fact that children prefer novel foods rather than traditional ones, the possible solutions could be: adapting meals to pupils' habits, changing the way of serving, motivating and educating pupils and their parents.

Keywords: food waste, school's catering, pupils

Introduction

At the beginning of 2018, totally 2312 closed-type catering companies were registered in the Latvian FVS (Food and Veterinary Service) Register of Enterprises. 37% or 847 of closed-type catering enterprises work at primary and general education establishments.

Nowadays scientists' interest is focusing on school catering issues, because it affects a number of important problems that need to be solved (Falasconi, et al., 2015; Logario et al., 2018). Firstly, the large amount of food waste at the school canteens, which is due to (Balzaretti et al., 2018; Derqui, Fernandez, 2017; Rickinson et al., 2016):

1) pupils' eating habits (for example, some people eat such food, others − do not, for example bread crusts);
2) type of food / drink preparation (for example potato skins);
3) cooking process (for example meat bones, egg shells);
4) wrongly calculated the required amount of food (non-served food) and portion size (plate waste);
5) wrongly organized lunch refusals;
6) incorrectly planned length of breaks;
7) inappropriate canteen atmosphere, exterior appearance, furnishings and microclimate.

Secondly, alongside with the increase in food waste, the risk of malnutrition among pupils increases. Thirdly, the food waste at school canteens leads to the loss of environmental, work and financial resources. Furthermore, several studies in the food service industry have highlighted diverse food waste mitigation strategies e.g. different National School Lunch or Breakfast Programs.

Thus the objective of this study was to determine the types of food waste, their amounts at schools in Vidzeme region and to analyse the pupils' attitude towards school meals.

Materials and Methods

Five comprehensive schools in Vidzeme region were selected as the object of the research. The number of children served at the school canteen ranged from 115 to 684 children per day on average. To determine the amount of food waste at the schools, it was weighed for a week. The observation method was used to evaluate the pupils' eating process and lunch organization, analysed portions of food waste, which were divided into three categories:

1) production waste, which includes food/drink preparation and cooking process waste;
2) non-served food including improper planning or unscheduled refusal of lunch;
3) plate waste, that mostly arises from pupil's eating habits and inappropriate portion size.

Results of non-served food and plate waste quantities are expressed both in terms of weight and as percentage of the initial prepared food (Cohen et al., 2013). To compare the data, non-served food and plate waste was expressed per portion (pupil).

In order to understand the pupils' eating habits and the reasons for uneaten portions, an indirect survey was conducted in the study. 250 questionnaires were distributed at schools (50 at each school). 196 questionnaires (78.4%) were returned and valid for the study. 196 respondents, aged 11−17, participated in the survey − 115 girls and 81 boys. A semi-structured interview was used in the study – in which five school canteen managers were interviewed with the aim to obtain information on food waste problems at school catering and the factors affecting them.

The overall structure of the study is depicted in Figure 1.

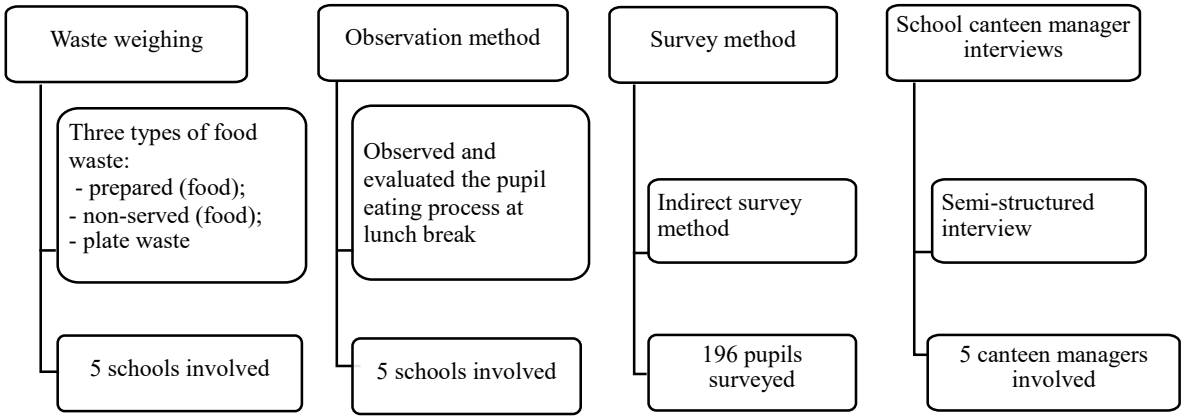

Figure 1. The structure of the research

Mathematical processing of data was performed with mathematical statistical methods using software *MS Excel*. The hypothesis was tested with p-value method and factors were considered to be significant, if p-value $<\alpha_{0.05}$ and $<_{0.001}$. For the result interpretation it was accepted that α is 0.05 (α=0.05) with 95% credibility if not stated otherwise. To analyse association of different parameters regression and correlation analysis were used. If the correlation coefficient value is $0.5 \leq |r| \leq 0.8$, there is moderate linear correlation between researched parameters.

Results and Discussion

The results of the study showed that total food waste was 1112.5 kg in one week, most of which (41%) was production waste. Production waste at schools ranged from 1.6% to 20.8% of the total amount of food prepared. Production waste consists of waste generated during the pre-treatment of products (vegetables, fruit peel, cores, egg shells), as well as during improper food storage. Production waste differed significantly between schools ($p<0.001$).

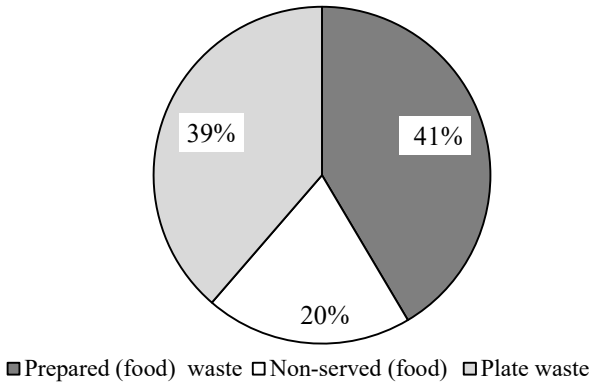

Figure 2. Total amount of waste

Production waste is inevitable, but the quantity can be reduced by training employees using proper pre-treatment of products, using high-quality equipment and by following up the correct food circulation (Fifo Principle) (Fink et al., 2016). Meanwhile, non-served food and plate waste comprised 221 kg (20%) and 430 kg (39%) respectively. In a similar study on food waste in Italian schools, it was concluded that non-served food and plate waste accounted for respectively 19.2% and 22.0% of prepared food (Boschini et al., 2018).

Non-served food and plate waste is waste, that is preventable and prevention is important for school catering. Research data showed that the amount of non-served food and plate waste was significantly different ($p<0.001$) between schools. Non-served food waste ranged from an average of 1.7% to 12% of the total amount of food prepared (Fig. 3).

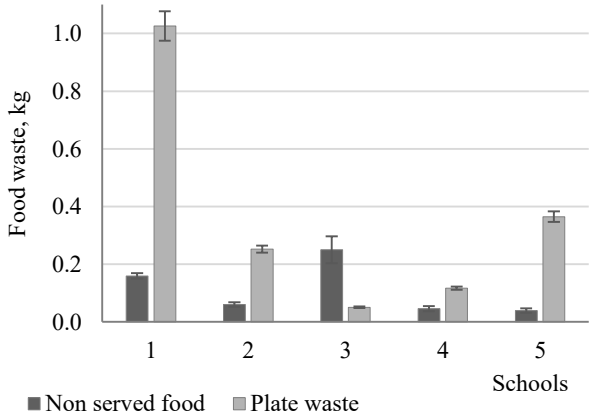

Figure 3. Non-served food and plate waste in schools by one pupil during one week

The large amount of cooked but non-served food might be due to improper planning or unscheduled refusal of lunch at school. This is one of the problems, especially if lunch is paid by the municipality and parents are not responsible for the timely refusal of lunch.

The amount of plate waste in school canteens ranged from 2.4% to 32.3% of the total amount of prepared meals. On average, one child produced between 0.05 kg and 1.03 kg of plate waste in one week, or even two portions per week. Schools 1 and 5 had the highest amount of plate waste, besides different types of serving. At school 1, pupils were offered ready-made portions that are not eaten by younger pupils. In the

school 5, however, pupils themselves served food. In this case, it is necessary to explain to the pupils that the portions should be placed according to their appetite, and to encourage if the portion should be supplemented. The teachers and canteen staff should undertake the explanatory functions. One way to reduce plate waste would be pupils' self-service, when children put food on their own plates with the help of teachers. This would impose the food quantity that the pupil can eat as well as, that the child takes the necessary dietary norms.

A balanced and healthy meal is important for children in the growth process to provide the necessary energy reserves, which in turn is essential in the learning process. School meal programs provide opportunities for improvement of the nutritional quality of the diet, either in total energy, compensating for suboptimal foods consumed outside of school meals (Bartfeld, Ahn, 2011). Schools should especially think and look for solutions to reduce the plate waste, as children should receive a full-fledged meal that ensures the full intake of proper nutrients according to norms.

In order to justify the factors influencing the generation of waste in schools, correlation between the characteristics was used. The study analysed whether there was a correlation between the number of prepared meals and the amount of waste. The results showed an average close correlation (r=0.74), which could be explained by production process organization, cook work, raw material quality and inventory used. An average close correlation (r=0.70) was observed between the prepared amount of food and the production waste, which meant that large amounts of production waste were not produced during the cooking process.

When evaluating the correlation between the number of children and the plate waste, a weak correlation (r=0.06) was observed, because a large number of pupils are not associated with large plate waste (Figure 4).

It is influenced by the age of the pupils, the size of the portions, the pupil's eating preferences, eating habits, food quality, etc.

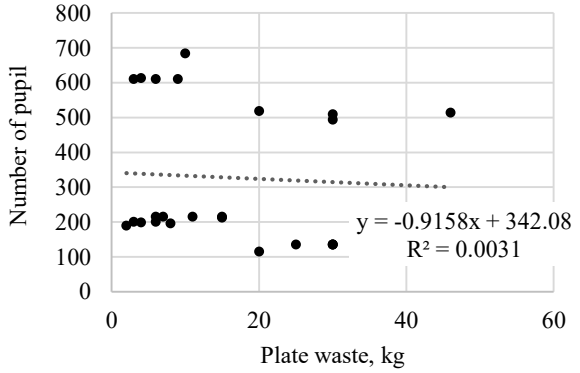

Figure 4. Correlation between number of pupils and plate waste weight

Observations on the pupils' eating process showed that the lunch takes place in a great noise and in a hurry that hinders the schoolchildren from enjoying the food. Hurry was one of the reasons why pupils left the dish on the plate.

The atmosphere in the school canteen has an effect on children's eating habits, such as overcrowded dining halls and truncated eating time has been found to create time pressure on children (Moore et al., 2010), as well as hasty eating adversely affect the meals intake (Cohen et al., 2016).

Teachers' attitudes played an important role, the less teachers paid attention to those pupils, who were eating, the higher the number of uneaten portions. In schools where the eating process took place in a relatively calm atmosphere, the teachers actively participated in the lunch process, helped the children to lay portions and put in order the lunch table, the children had good eating habits. The pupils slowly ate their portions, left the table with the teacher that provided small amount of plate waste. Research data suggest that the choice of pupils for fruit or juice can be increased by up to 90% by involving school staff in encouraging schoolchildren to choose healthier products (Schwartz, 2007)

The results of the pupil survey showed that 48% of all pupils did not eat their school meal two or three times a week, which could be explained by large portions or unsavoury food. 37% of pupils would like to change the menu, making it more diverse so that the dishes are not repeated. The great part of the boys had emphasized the need to include more meat dishes in the menu. Asking the pupils about their favourite dishes, they called pasta, buckwheat, beetroot salad, fresh salad and fried potatoes. Whereas dishes that did not taste were named: soups, boiled potatoes, walnuts, porridge, and purees. The pupils indicated that they did not want so many and often potatoes, which can be explained by the Cabinet Regulation 172, which requires 450 g of potatoes to be included in the menu every week (Cabinet Regulations…, 2012).

Survey data showed that 82% of pupils did not think about food waste at school. In turn, 41% of respondents believe that they produce less than 0.5 kg of plate waste, but 12% of respondents − 3 kg or even more per week. These 12% include those pupils who pointed out they did not eat the whole portion at least three to four times a week. 86% of pupils also indicated that it would be necessary to reduce food waste in schools.

It shows the pupils' readiness to engage in the waste reduction process.

School canteen managers' interviews confirmed the conclusions of other studies on factors influencing pupils' eating habits.

One of the key factors highlighted is the change in pupils' eating habits namely dishes, which were popular several years ago, remaining uneaten nowadays. For example, if porridge with berry jam is offered, the children only eat the porridge, leaving the jam on the plate completely.

Canteen managers agreed on the inclusion of different types of soups in the menus: pupils eat unwillingly sour and fresh cabbage soup, as well as beetroot soup. Meatball soup and solyanka are eaten eagerly, possibly

parents make these kinds of soups at home. Children refuse to eat different types of desserts, such as bread jelly, whipped cream, berry jelly as well as mousse. Pupils often do not eat puree and porridge, as well as salads. As garnish, children prefer pasta, buckwheat, rice instead of potatoes.

It was emphasized that children have begun to eat healthier, choose more cereals, steamed vegetables, steamed meat. In general, after the observations it was concluded that the amount of food waste from the left food portion was influenced by the individual wishes of the pupils, family eating habits, the quality of food, the atmosphere in the school canteen, the way of serving lunch and the participation of teachers. All these factors interact and influence the child's eating habits at school. Summarizing the results of the study, the authors created a model of factors that influence children's eating habits (Fig. 5).

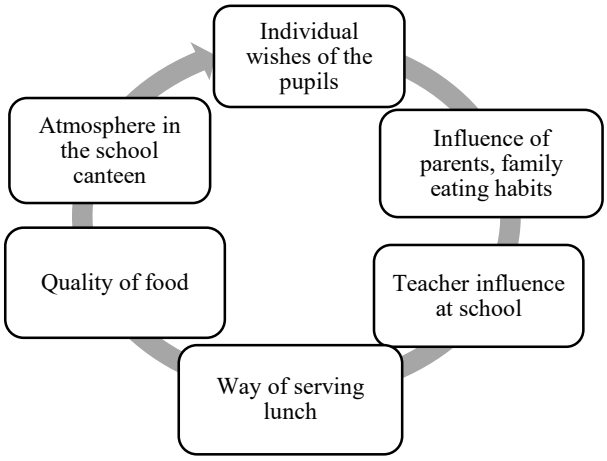

Figure 5. Factors affecting pupils' eating habits

Table 1

Food waste generating factors and recommendations for their reduction

Type of food waste	Influencing Factors	Suggestions for reduction
Production waste	• low quality raw materials; • seasonality; • employee experience, knowledge and professionalism; • insufficient time available for the cooking process; • unmotivated employees	• to educate employees; • to buy quality raw materials; • to plan work process and time; • to motivate employees to work responsibly
Non-served food	• no possibility to refuse lunch; • too much prepared food	• to implement successive procedures for refusing lunch; • to plan precisely required amount of food
Plate waste	• tasteless, poor quality food; • cool food; • inappropriate portion size; • modern eating habits; • individual preferences of pupils; • age of children; • food presentation	• to serve a delicious, high quality, good-looking, appropriate temperature meal; • to educate children; • to involve teachers in the eating process of lunch; • to inform parents about such a problem and involve them in solving it; • let children to choose what to eat.

Successfully acting at all stages, it is possible to reduce food waste in school canteens: evaluate the stages of the production process, implement rational improvements to reduce food waste in the school, educate and motivate staff to carry out their duties qualitatively and responsibly, evaluate the food service organization. Regular plate waste analysis is required to observe pupils' eating habits and evaluate possible improvements (Table 1).

Teachers should take part in forming children's eating habits: explain the healthy and varied food intake beneficial effect on the body, educate on food waste reduction and its impact on the environment, be a healthy role model.

Responsible action can significantly reduce waste, but it requires motivation, desire and also awareness of the benefits.

Conclusions

The issue of reducing food waste is topical, new solutions for optimizing the production process and educating the society about sustainable lifestyle are being sought. The results of the study showed that it is possible to reduce both non-served and plate waste. There are two possible solutions:

1) more detailed research on lunch organization and production factors in schools, developing good practice examples;
2) improvement of pupils' nutrition regulation, promotion of healthy eating habits of pupils and improvement of lunch environment.

Properly organized lunch planning, which includes a sequential process of refusing lunch, can significantly reduce food waste from non-served food. The amount of plate waste is significantly different at all schools, mainly due to individual wishes of the pupils, family eating habits, the quality of food, the atmosphere in the school canteen, the way of serving lunch and the participation of teachers. Food waste reducing in school canteens is a complex issue that can be solved by involving canteen staff, school staff, and educating and motivating pupils and their parents.

References

1. Balzaretti C., Ventura V., Ratti S., Ferrazzi G., Spallina A., Carruba M., Castrica M. (2018) Improving the overall sustainability of the school meal chain: the role of portion sizes. *Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity,* p. 1–10.
2. Bartfeld J. S., Ahn H. M. (2011) The school breakfast program strengthens household food security among low-income households with elementary school children. *Journal of Nutrition*, Vol. 141, p. 470–475.
3. Boschini M., Falasconi L., Giordano C., Alboni F. (2018) Food waste in school canteens: A reference methodology for large-scale studies. *Journal of Cleaner Production*, Vol. 182, p. 1024–1032.
4. *Cabinet regulation Nr.172 "Regulations Regarding Nutritional Norms for Educates of Educational Institutions, Clients of Social Care and Social Rehabilitation Institutions and Patients of Medical Treatment Institutions"* (2012), Cabinet of Ministers. [accessed on 08.02.2019.]. Available: https://likumi.lv/doc.php?id=245300.
5. Cohen J., Richardson S., Austin S., Economos C., Rimm E. (2013) School lunch waste among middle school students: nutrients consumed and costs. *American Journal of Preventive Medicine,* Vol. 44, p. 114–121.
6. Cohen J. F., Jahn J. L., Richardson S., Cluggish S. A., Parker E., Rimm E. B. (2016) Amount of time to eat lunch is associated with children's selection and consumption of school meal entree, fruits, vegetables and milk. *Journal of the Academy of Nutrition and Dietetics*, Vol. 116, p. 123–128.
7. Derqui B., Fernandez V. (2017) The opportunity of tracking food waste in school canteens: Guidelines for self-assessment. *Waste Management*, Vol. 69, p. 431–444.
8. Falasconi L., Politato A., Politano A., Segrè A. (2015) Food waste in school catering: An Italian case study, *Sustainability*, Vol. 7, p. 1–16.
9. Cohen J.F., Richardson S., Austin S.B., Bryn A., Economos C.D., Rimm E.B. (2013) School lunch waste among middle school students: nutrients consumed and costs. *American Journal of Preventive Medicine,* Vol. 44, p. 114–121.
10. Fink L., Roehl R., Strassner C., Antony F., Gensch C. O. (2016) *Prevention of food waste in the catering sector, guideline*, German Environment Agency, 36 p. [accessed on 24.01.2019]. Available: https://www.umweltbundesamt.de/publikationen/prevention-of-food-waste-in-the-catering-sector.
11. Logario A., Pinto R., Golini R. (2018) Food waste reduction in school canteens: Evidence from Italian case. *Journal of Cleaner Production*, Vol. 199, p. 77–84.
12. Moore S. N., Murphy S., Tapper K. and Moore L. (2010) The social, physical and temporal characteristics of primary school dining halls and their implications for children's eating behaviours. *Health Education Journal*, Vol. 110, p. 399–411.
13. Rickinson M., Hall M., Reid A. (2016) Sustainable school programmes: what influence on schools and how do we know? *Environmental Education Research*, Vol. 22, p. 360-389.
14. Schwartz M. B. (2007) The influence of a verbal prompt on school lunch fruit consumption: A pilot study. *International Journal of Behavioural Nutrition and Physical Activity,* Vol. 4, p. 6.

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SHORT COMMUNICATION

FAST AND GREEN METHOD FOR ANALYSIS OF SERRA DA ESTRELA CHEESE

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Abstract

Serra da Estrela (SE) cheese is a regional product making part of the gastronomic and socio-cultural heritage of Portugal. It has several aspects that make it unique, namely the manufacturing by the coagulation of raw sheep milk using the thistle flower of *Cynara cardunculus* L., obtaining a final buttery texture with an exclusive typical flavour. The aroma compounds of Serra da Estrela cheese result partially from the action of indigenous microorganisms and enzymes on lactose, lipids and proteins. Although SE production is very ancient, there are few studies concerning its nutritional composition but nowadays consumers demonstrate interest in understanding the global composition of cheese, since it's a product highly appreciated. In the last decades, the use of near infrared spectroscopy (FT-NIR) has become comparable with those of the classical methods with representative advantages such as minimum time sample preparation; it is a green technique (without using toxic reagents) and allows multiparametric determinations. To perform the present study, a total of 24 SE cheese samples were evaluated, originated from 6 representative producers and analysed with a FT-NIR Buchi NIRMaster™ standalone spectrometer with a spectral range of 800–2500 nm and compared with classical methods. SE cheese samples were evaluated in terms of salt, moisture, protein and fat content. In all the studied samples the moisture varied between 42% and 53%, the fat content between 19.6 and 33.3%, the protein content between 18.6 and 26.7% and the salt between 0.7 and 2.2%. The results showed a significant agreement between the pairs of values obtained for the studied parameters.

Keywords: Serra da Estrela cheese, FT-NIR spectroscopy, moisture, protein, fat, salt.

Introduction

Serra da Estrela (SE) is an ewe`s traditional Portuguese cheese, which production is regulated and geographically limited due to its Protected Designation of Origin (PDO) certification, which was obtained in 1985. Serra da Estrela cheese is produced from raw ewe's milk and coagulated using dried thistle flower (*Cynara cardunculus* L.). The cheese characteristics are legally defined, although many extrinsic factors (e.g., climate, nutritional and physiological status, lactation stage) may condition the milk chemical and microbiological characteristics and consequently the final cheese composition (Guiné et al., 2016). Serra da Estrela is the most known and popular Portuguese cheese, appreciated worldwide, being preferentially consumed as a soft cheese, with an average maturation of 30–45 days, although some consumers prefer to consume it as a hard cheese after at least 6 months of storage (Carocho et al., 2015; Carocho et al., 2016, Partidário et al., 1998; Macedo, Malcata, 1996).

The FT-NIR spectroscopy method has recently become widely used in dairy industry and food science laboratory control because it allows multiparametric applications and has the advantage of not requiring chemical reagents and minimizing sample preparation. It operates in most phases of the dairy value chain, starting in farms with fresh milk to assess the product's composition value, before and during processing for process control and in final products to ensure the required specifications (Holroyd, 2017). Non-translucent materials such as cheese are analysed via diffuse reflection. The NIR light penetration is limited by the sample material, interacts with the sample and is refracted and reflected into the sensor. The reflected rays contain the spectral information of the sample (Burns, Ciurczak, 2008). It is well established that fat, moisture and protein composition of cheese can be measured successfully using NIR spectroscopy (Holroyd, 2011).

The classical methods used in the determinations of fat and protein in food have numerous disadvantages, like being time and energy consuming, having limited sample throughput and leading to possible harmful effects on the environment, because of the large amount of chemicals required.

Besides quality control in large scale in milk products' processing, NIR spectroscopy has also been used to detect adulterants in foods (determination of melamine in infant milk powder formula (Scholl et al., 2017)), for the discrimination of cheese adulterations (goat cheeses with cow's milk (Dvorak et al. 2016)) and also in food authentication like the detection of Tyrol cheese (Huck-Pezzei et al., 2014) or Parmigiano Reggiano cheese (Cevoli et al., 2013), among others.

This study intended to compare FT-NIR technique with classical methods in the quantification of major components of SE cheese using a broad-based calibration previously readjusted with samples of SE cheese.

Materials and Methods

The nutritional characteristics of 24 Serra da Estrela cheese samples originated from 6 representative cheese producers of the PDO region were analysed. Samples of cheese of each producer, with approximately 45 days of maturation, were collected in refrigerated boxes and about 1.5 cm of the rind was removed to provide a surface that represented its interior and a slice of about 100 g was placed in a flat-bottom glass cuvette and analysed, in triplicate. Spectra were recorded on

NIRMaster™ spectrophotometer from Buchi NIRSolutions™ (Flawil, Switzerland) equipped with a polarisation interferometer with TeO_2 wedges, an extended range InGaAs detector (temperature controlled) working in diffuse reflectance with a spectral range of 800–2500 nm (resolution: 8 cm^{-1}) combined with NIRWare™ software package, also from Buchi NIRSolutions.™ A blank signal was previously obtained with external reference Spectralon®. The internal background was measured with a gold plate reflector.

The classical methods of analysis were conducted in duplicate, in an external laboratory. Moisture was determined by drying at 105 °C until constant weight. Fat was analysed by the Van Gulik method, total protein was calculated by the Dumas method and salt (NaCl) was calculated through the content in sodium, determined by flame atomic absorption spectroscopy.

The data was analysed using Statistical Package for Social Sciences (SPSS version 25.0). The accuracy and precision of the regression lines obtained were verified by t-test for comparison of the means of paired samples and linear regression. Cook's Distance (Di) was used to detect outliers by the rule that Di>4/n (n: number of samples) is an outlier. Those values were analysed before elimination.

Results and Discussion

Starting with 24 samples of SE cheese, 3 outliers were removed after previous analysis of the adjustment of the regression: 1 in moisture and 2 in protein.

Mean values of major constituents of SE cheese determined by FT-NIR and by classical methods are presented in Table 1. In the samples studied, the moisture varied between 41.7 and 56.4%, the fat content between 16.6 and 35.5%, the protein content between 18.1 and 25.5% and the salt between 0.6 and 3.0% (Table 1).

Table 1

Parameters determined by FT-NIR and classical methods in the samples of SE cheese

Parameters (%)	FT-NIR		Ext Lab	
	x±sd	VR	x±sd	VR
Moisture	48.78±2.92	42.97 52.61	49.02±3.69	41.70 56.40
Fat	24.21±4.49	16.64 33.33	24.40±4.59	17.10 35.50
Protein	20.99±1.60	18.55 25.19	20.39±1.71	18.10 25.50
Salt	1.17±0.34	0.63 2.21	1.79±0.39	1.27 3.00

x±sd – arithmetic mean ± standard deviation; VR – variation range, from minimum to maximum; FT-NIR – near infrared technology; Ext Lab – external laboratory

Fox *et al.* (2017) presented similar contents with mean values of 48.7% of moisture, 27.5% of fat content, 21.3% of total protein and 1.9% of salt. Carocho et al. (2016), Ramos and Juarez (2011) and Associação Portuguesa de Nutrição (2018) obtained similar results.

The relations between classical methods and FT-NIR method for the monitored SE cheese components are shown in Table 2. The regression lines of all measured quantities are shown in Figures 1 and 2. The obtained correlation coefficients for all components of SE cheese were statistically significant ($p<0.001$).

Table 2

Expression of the relationship between FT-NIR and classical methods in moisture, fat, crude protein and salt of SE cheese samples

Parameters	R^2	r	Slope	Intercept	SEP (%)
Moisture	0.639	0.800	0.639	17.79	1.79
Fat	0.934	0.966	0.988	0.47	1.18
Protein	0.913	0.956	1.021	-1.05	0.48
Salt	0.869	0.932	1.077	0.53	0.13

$p<0.001$ for all correlations (r); R^2 – determination coefficient; r – correlation coefficient; FT-NIR – near infrared technology; SEP – standard error of prediction

The reliability and precision of the NIR measurement method was quite satisfactory for protein and fat content as the R^2 resultant of the linear regression was greater than 0.90 (Table 2 and Figure 1).

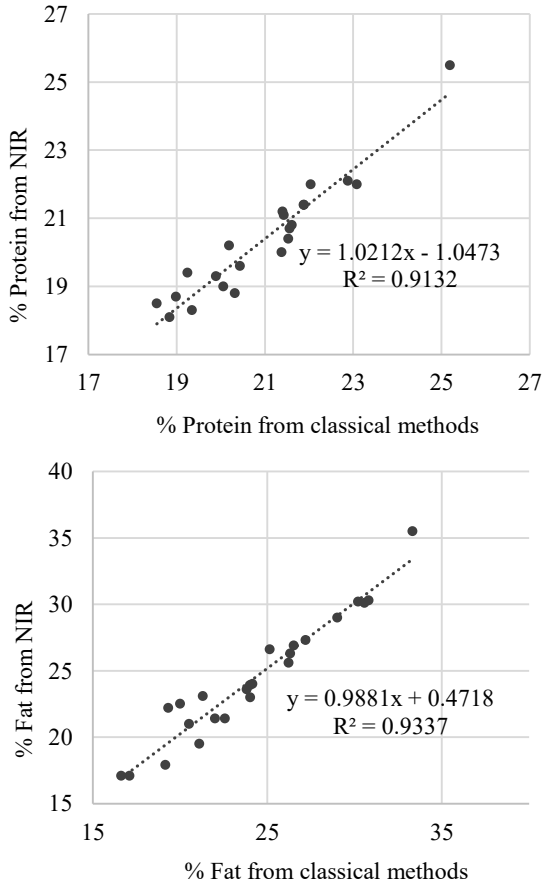

Figure 1. The relationship between classical methods and indirect method (FT-NIR) for SE cheese protein and fat determinations

Similar results were obtained by Adamopoulos et al. (2001) in Greek feta cheeses, with medium values of R^2 =0.952 and 0.956 for protein and fat, respectively.
On the other end, moisture showed a low determination coefficient of 0.64 and a slope and intercept (0.639 and 17.79, respectively) far from the ideal curve y=1x+0 (slope of 1 and intercept of 0), showing a poor correlation between data obtained from NIR and classical analysis. Also, standard error of prediction (SEP) was quite high indicating that the predicted values didn´t quite match the values obtained by the classical methods (Table 2 and Figure 2).

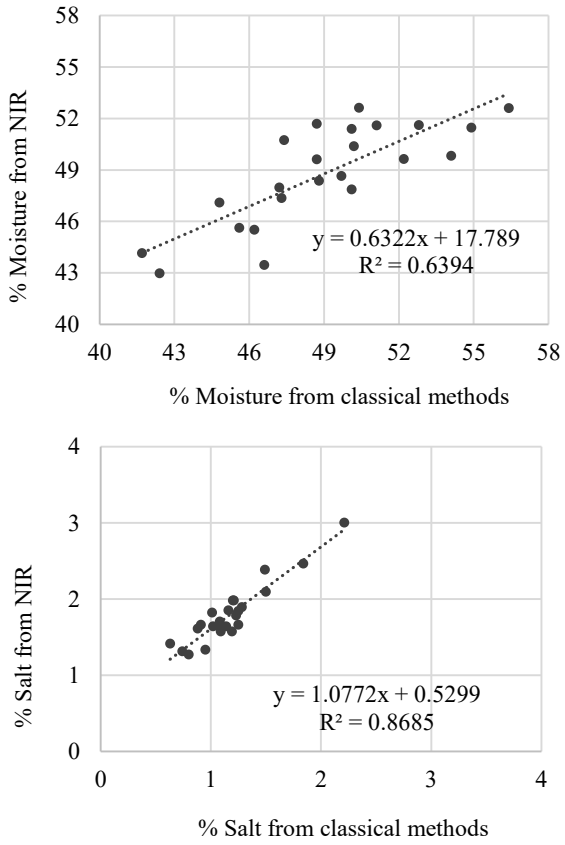

Figure 2. The relationship between classical methods and indirect method (FT-NIR) for SE cheese protein and fat determinations

The salt content regression noticed a good slope and intercept of 1.077 and 0.53, respectively, and a good SEP of 0.13, but at the same time showed a deterioration of the determination coefficient (0.869) indicating a poorer relation between values predicted by NIR and classical analysis (Table 2 and Figure 2).

Conclusions

Broad-based calibration, such as the one that Buchi NIRMaster™ holds and was used in this study, presents some benefits including increased robustness and reliability. However, some parameters such as moisture and salt content of Serra da Estrela cheese samples may need broader adjustment of the FT-NIR calibration model, by increasing classical methods (reference) and NIR sample analysis.

Acknowledgment

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References

1. Adamopoulos K.G., Goula A.M., Petropakis H.J. (2001) Quality control during processing of Feta cheese - NIR application. *Journal of Food Composition and Analysis*, Vol. 14, p. 431–440.
2. Associação Portuguesa de Nutrição. (2018). *Queijos Dos Frescos aos Curados*. Associação Portuguesa de Nutrição. [accessed on 06.03.2019.]. Available at: http://www.apn.org.pt/documentos/ebooks/e-book_queijo_8.pdf
3. Burns D.A., Ciurczak E.W. (2008) *Handbook of near-infrared analysis* (3rd ed.). [accessed on 06.03.2019.]. Available at: https://www.crcpress.com/Handbook-of-Near-Infrared-Analysis/Burns-Ciurczak/p/book/9780849373930
4. Carocho M., Barreira J.C.M., Antonio A.L., Bento A., Morales P., Ferreira I.C.F.R. (2015) The incorporation of plant materials in 'Serra da Estrela' cheese improves antioxidant activity without changing the fatty acid profile and visual appearance. *European Journal of Lipid Science and Technology*, Vol. 117(10), p. 1607–1614.
5. Carocho M., Barros L., Barreira J.C.M., Calhelha R.C., Soković M., Fernández-Ruiz V., Buelga C.S., Morales P., Ferreira I.C.F.R. (2016) Basil as functional and preserving ingredient in 'serra da Estrela' cheese. *Food Chemistry*, Vol. 207, p. 51–59.
6. Cevoli C., Gori A., Nocetti M., Cuibus L., Caboni M.F., Fabbri A. (2013) FT-NIR and FT-MIR spectroscopy to discriminate competitors, non compliance and compliance grated Parmigiano Reggiano cheese. *Food Research International*, Vol. 52(1), p. 214–220.
7. Dvorak L., Mlcek J., Sustova K. (2016) Comparison of FT-NIR spectroscopy and ELISA for detection of adulteration of goat cheeses with cow's milk. *Journal of AOAC International*, Vol. 99(1), p. 180–186.
8. Fox P.F., Guine T.P., Cogan T.M., McSweeney P.L.H. (2017) *Fundamentals of Cheese Science*. *Fundamentals of Cheese Science, Second Edition*. Boston, MA: Springer US, 799 p.
9. Guiné R.P.F., Tenreiro M.I.C., Correia A.C., Correia P.M.R., Barracosa P. (2016) Analysis of factors influencing the physical, chemical and sensorial properties of Serra da Estrela cheeses. *Journal of Food Measurement and Characterization*, Vol. 10(3), p. 643–657.
10. Holroyd S. (2011) NIR analysis of cheese - a Fonterra perspective. *NIR News*, Vol. 22(6), p. 9–11.
11. Holroyd S. (2017) The use of NIRS in the dairy industry: New trends and applications. *NIR News*, Vol. 28(8), p. 22–25.
12. Huck-Pezzei V.A., Seitz I., Karer R., Schmutzler M., De Benedictis L., Wild B., Huck C.W. (2014) Alps food authentication, typicality and intrinsic quality by near infrared spectroscopy. *Food Research International*, Vol. 62, p. 984–990.
13. Macedo A.C., Malcata F.X. (1996) Changes in the major

free fatty acids in Serra cheese throughout ripening. *International Dairy Journal*, Vol. 6(11–12), p. 1087–1097.
14. Partidário A.M., Barbosa M., Boas L.V. (1998) Free fatty acids, triglycerides and volatile compounds in Serra da Estrela cheese - Changes throughout ripening. *International Dairy Journal*, Vol. 8(10–11), p. 873–881.
15. Ramos M., Juarez M. (2011) Milk | Sheep Milk. **In:** *Encyclopedia of Dairy Sciences.* Vol. 3. 2nd ed. Fuquay J.W., Fox P.F., McSweeney P.L.H. (eds). London: Elsevier , p. 494–502.
16. Scholl P.F., Bergana M.M., Yakes B.J., Xie Z., Zbylut S., Downey G., Mossoba M, Jablonski J, Magaletta R., Holroyd S.E., Buehler M., Qin J.0, Hurst W., LaPointe J.H., Roberts D., Zrybko C., Mackey A., Holton J.D., Israelson G.A., Payne A., Kim M.S., Chao K., Moore J.C. (2017) Effects of the adulteration technique on the near-infrared detection of melamine in milk powder. *Journal of Agricultural and Food Chemistry*, Vol. 65(28), p. 5799–5809.

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SHORT COMMUNICATION

TECHNOLOGY OF OBTAINING MILK-CLOTTING ENZYME FROM FUNGAL CULTURE *FUNALIA* SP. FOR APPLICATION IN CHEESE PRODUCTION

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Abstract

Cheese-making is a process known since ancient times. Traditionally, cheese preparation was based on coagulation of milk using rennet. Due to the high cost of rennet, an important task of the cheese industry is finding its alternatives. An important requirement for milk-clotting enzymes is low non-specific total proteolytic activity. One of the promising sources of milk-clotting enzymes are basidial fungi. Earlier, we found a high milk-clotting activity (MCA) of the fungus *Funalia* sp. The aim of our research was to find the optimal cultivation conditions for the fungus *Funalia* sp., which ensure the maximum yield of the milk-clotting enzyme. The fungus was submerge-cultured on a glucose-peptone nutrient medium for 7 days. The MCA determination of the native solution was carried out using the Kawai-Mukai method. To optimize the composition of the nutrient medium the method of full factorial experiment was used. According to the results of the study, a nutrient medium with a concentration of glucose and peptone of 15.5 and 3.6 g L^{-1} was selected. For further concentration and purification of the enzyme the method of ultrafiltration was used. As a result, an enzyme preparation with a high level of MCA (333.5 U mg^{-1}) and a low level of proteolytic activity (0.096 U mg^{-1}) was obtained. According to its characteristics, the enzyme is not inferior to commercial rennet and is promising for use in the food industry.

Keywords: milk-clotting, fungi, cheese making, submerge cultivation

Introduction

For hundreds of years rennet was used for clotting of milk in process of cheese making. Rennet is enzyme obtained from the stomach of young ruminants. Currently, due to the shortage of rennet and its high cost, it is widely practiced to use enzyme substitutes that are close in their action to the rennet. One of the common problems of rennet substitutes is high proteolytic activity. Too high proteolytic activity leads to the formation of a large number of low molecular weight peptides, which causes the appearance of bitterness in the cheese and reduces the yield of the final product. In this regard, enzymes with high proteolytic activity cannot be used to produce high-quality cheeses (Teply, 1980; Gudkov, 2004; Raposo, Domingos, 2008; He et al., 2011). The search for inexpensive and effective rennet enzyme substitutes is an important task in food biotechnology.

Fungi are a promising source of milk-clotting enzymes. Modern methods of submerge cultivation of fungi can significantly reduce the process of obtaining enzymes, as well as increase the yield of the final product.

The aim of our research was to find the optimal cultivation conditions for the fungus *Funalia* sp., which ensure the maximum yield of the milk-clotting enzyme.

Materials and methods

The object of our study was the culture of basidiomycete *Funalia sp*. This fungus was chosen as a result of screening a number of fungal cultures for milk-clotting activity. The fungus was submerge-cultured in Erlenmeyer flasks on a rotary shaker at a temperature of 28–30 °C. For the inoculum growing and submerge cultivation glucose-peptone nutrient medium was used. The duration of cultivation was 7 days. After cultivation, the native liquid solution was separated from the biomass by centrifugation with 6000 rpm (Centurion K240R, Centurion Scientific, USA). In the native solution the level of milk-clotting activity (MCA) and protein concentration (Lowry et al., 1951) were determined. To determine the milk-clotting activity, the Kawai-Mukai method was used (Kawai, Mukai, 1970). This method is based on determining the time of formation of the milk clot under the action of the enzyme. Calculation of MCA was conducted according to the following equation (Gagaoua, 2017):

$$MCA = \frac{2400 \times V}{t} \times v \qquad (1)$$

where:

MCA – milk-clotting activity, U mL^{-1},
t – time of milk clot formation, s;
V – the volume of milk, mL
v – amount of enzyme preparation, mL.

Protein biosynthesis is greatly influenced by the concentration of carbon and nitrogen sources in the medium, as well as their ratio. In this regard, the effect of the composition of the nutrient medium on the milk-clotting activity was studied. Method of multiple regression analysis was used to optimize the composition of the culture medium. Data processing was performed using the software package Statistica 10 of the company "Statsoft" (USA).

The concentrations of glucose (X_1) and peptone (X_2) were as variation factors. The parameter of optimization was the level of the milk-clotting activity of the native solution of the culture liquid (Y).

The optimization of the medium was carried out in the following intervals of variation of the main sources:

$$10 \text{ mg mL}^{-1} < C_{glucose} < 20 \text{ mg mL}^{-1}$$
$$2.5 \text{ mg mL}^{-1} < C_{peptone} < 5.5 \text{ mg mL}^{-1}.$$

These intervals were selected based on previously conducted experiments. The glucose and peptone concentrations in the nutrient medium were varied at three levels: a minimum (-), mean (0) and maximum (+). The remaining components of the medium were taken in the following concentrations (g L^{-1}): KH_2P0_4 – 0.6; K_2HP0_4 – 0.4; $MgSO_4$ – 0.05; NaCl – 0.5; yeast extract – 2.0.

The degree of aeration of the cultivation medium has a great influence on the biosynthesis of enzymes (Wang et al., 2005). We studied the effect of oxygen concentration on the synthesis of a milk-clotting enzyme when cultivated in 5 different modes of aeration of the medium. Fungus was cultured in 750 mL Erlenmeyer flasks with different quantity of liquid medium – 50, 75, 100, 125, 150 and 200 mL. The dissolution rate of oxygen rate was determined by the sulphite method (Yegorov, 1976).

Ultrafiltration was used to purify and concentrate the enzyme. For this the ultrafiltration cell with membrane "MIFIL-PA-20" (MIFIL, Belarus) was used. In the permeate the level of milk-clotting and total proteolytic activities (GOST 20264.2-88, 1988), as well as the protein concentration by Lowry method (Lowry et al., 1951), were determined. Obtained enzyme preparation was compared with commercial preparation of a rennet enzyme (Institute of Butter and Cheese making, Uglich city, Russia) by milk-clotting and total proteolytic activities.

Results and Discussion

In order to increase the milk-clotting activity of the fungus *Funalia* sp., we selected conditions of submerged cultivation.

We carried out the selection of concentrations of glucose and peptone in the medium to determine their effect on the level of the milk-clotting activity. The planning and the results of an experiment are shown in Table1.

Table 1

The influence of the concentration of glucose and peptone in the medium on the MCA level

No	Factor variations levels		Absolute values of glucose and peptone concentrations		MCA, U mL^{-1}
	X_1	X_2	$C_{glucose}$, g L^{-1}	$C_{peptone}$, g L^{-1}	
1	–	–	10	2.5	34.8±1.2
2	–	0	10	4.0	34.8±1.0
3	–	+	10	5.5	0
4	0	–	15	2.5	115.8±1.7
5	0	0	15	4.0	177.8±0,6
6	0	+	15	5.5	34.0±0.3
7	+	–	20	2.5	66.8±0,4
8	+	0	20	4.0	80.0±0.92
9	+	+	20	5.5	53.6±1.0

MCA – milk-clotting activity

According to the results of the experiment, a regression equation escribing the dependence of the MCA on the concentration of carbon and nitrogen sources in the medium was compiled:

$$Y = -733 + 78.44 \cdot X_1 + 140.48 \cdot X_2 - 2.56 \cdot X_1^2 - 20.68 \cdot X_2^2 + 0.72 \cdot X_1 \cdot X_2$$

Graphic dependence of milk-clotting activity on the composition of the medium is shown in Figure 1.

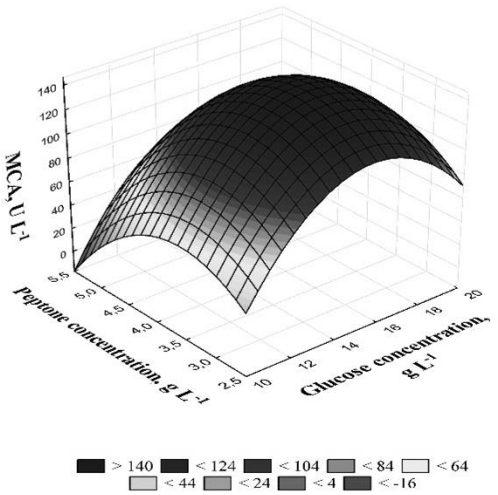

Figure 1. Dependence of the milk-clotting activity level of the fungus *Funalia* sp. on the concentration of glucose and peptone in the medium

Based on the results, the medium with a glucose concentration of 15.5 g L^{-1} and peptone concentration 3.6 g L^{-1} was selected for cultivating of the fungus *Funalia* sp.

We have studied the biosynthesis of the milk-clotting enzyme during the cultivation of the fungus in 5 different modes of aeration of the medium. The results are shown in Table 2.

Table 2

The influence of oxygen concentration in the medium on the synthesis of a milk-clotting enzyme

Nutrient medium volume, mL	Oxygen dissolution rate, g L^{-1} h^{-1}	MCA, U mL^{-1}
50	2.74	65.3±2.4
75	2.69	65.4±1.0
100	1.77	98.0±1.7
125	1.41	163.3±3.5
150	1.34	150.1±1.4
200	0.91	93.6±2.1

MCA – milk-clotting activity

From the results it can be seen that the highest level of MCA was observed during aeration, ensuring the solubility of oxygen at a rate of 1.41 g L^{-1} h^{-1}.

To study the dynamics of enzyme accumulation in the medium, we measured the MCA from 3 to 7 days of cultivation of the fungus. The results are shown in Figure 2.

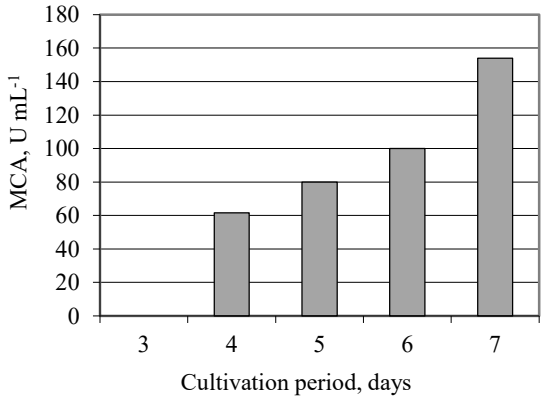

Figure 2. Dynamics of changes in milk-clotting activity (MCA) of the fungal culture *Funalia* sp.

The highest level of milk-coagulating activity was observed in the native solution of the culture liquid of the fungus on the 7th day of cultivation.

For further purification and concentration of the enzyme the ultrafiltration method was used. The comparative characteristics of our preparation and the standard rennet enzyme preparation is shown in Table 3.

Table 3

Comparative characteristics of the milk-clotting enzyme from the fungal culture *Funalia* sp. and commercial rennet preparation

Preparation	Characteristics		
	Specific milk-clotting activity, U mg^{-1}	Total proteolytic activity, U mg^{-1}	Ratio MCA:PA
Preparation from the fungal culture *Funalia sp.*	333.5±1.0	0.096±0.018	3473:1
Standard rennet preparation*	291.2±0.4	0.082±0.020	3551:1

MCA : PA – the ratio of specific milk-clotting activity and total proteolytic activity.

* Institute of Butter and Cheese making, Uglich city, Russia; GOST 9225-84.

From the results it can be seen that the milk-clotting enzyme preparation obtained from the fungal culture *Funalia* sp. has a high milk-clotting activity and a low total proteolytic activity. According to its specific milk-clotting and total proteolytic activities, it is not inferior to commercial rennet preparation. The level of milk-clotting activity is comparable to the level of activity of other coagulants used in the cheese-making industry (de Silva et al. 2013).

Conclusions

As a result of the study, the cultivation conditions of the fungus *Funalia* sp., providing the highest yield of the milk-coagulating enzyme, were selected (cultivation on the medium with glucose concentration of 15.5 g L^{-1} and peptone concentration 3.6 g L^{-1}, with oxygen dissolution rate of 1.41 g L^{-1} h^{-1} during 7 days). The enzyme preparation, which has a high milk-clotting activity and low proteolytic activity, and is not inferior in its characteristics to commercial enzyme was obtained. This preparation is promising for use in the food industry.

References

1. Gagaoua M., Ziane F., Nait Rabah S., Boucherba N., El-Hadef El-Okki Ait Kaki A., Bouanane-Darenfed A., Hafid K. (2017) Three phase partitioning, a scalable method for the purification and recovery of cucumisin, a milk-clotting enzyme, from the juice of *Cucumis melo* var. *reticulatus*. *International Journal of Biological Macromolecules*, Vol. 102, p. 515–525.
2. GOST 20264.2-88. (1988) *Enzyme preparations. Methods of determination of proteolytic activity*. Moscow: Standards publisher, 3 p.
3. Gudkov A.V. (2004) *Cheesemaking: Technological, biological and physical-chemical aspects.* Moscow: Deli print, 804 p.
4. He X., Ren F., Gu, H., Zhang W., Song X., Gan B. (2011) Purification and properties of a milk-clotting enzyme produced by *Bacillus amyloliquefaciens* D4. *Korean Journal of Chemical Engineering*, Vol. 28(1), p. 203–208.
5. Kawai, M., Mukai N. (1970) Studies on milk clotting enzymes produced by *Basidiomycetes*.I. Screening test of Basidiomycetes for the production of milk clotting enzymes. *Agricultural and Biological Chemistry*, Vol. 34(2), p. 159–163.
6. Lowry O.H., Rosebrough N.J., Farr A.C., Randall R.J. (1951) Protein measurement with the holinphenol reagent Rosebrough. *Journal of Biological Chemistry*, Vol. 193, p. 265–275.
7. Raposo S., Domingos A. (2008) Purification and characterization of milk-clotting aspartic proteinases from *Centaurea calcitrapa* cell suspension cultures. *Process Biochemistry*, Vol. 43, p. 139–144.
8. de Silva A.C., da Silva Nascimento T.C.E., da Silva S.A., Herculano P.N., Moreira K.A. (2013) Potential of quixaba (*Sideroxylon obtusifolium*) latex as a milk-clotting agent. *Food Science and Technology* (Camp.), V. 33, p. 494–499.
9. Teply M., Mashek Ya., Havlova Ya. (1980) *Milk-clotting enzymes of animal and microbial origin.* Moscow: Pishchevaya promyshlennost', 268 p.
10. Wang L., Ridgway D., Gu T., Moo-Young M. (2005) Bioprocessing strategies to improve heterologous protein production in filamentous fungal fermentations. *Biotechnology Advances*, Vol. 23, p. 115–129.
11. Yegorov N.S. (ed). (1976) *Practicum on Microbiology*. Moscow: Moscow State University, 307 p.

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SHORT COMMUNICATION

THE EFFECT OF CONCENTRATED WHEY SOLIDS ON LACTOBIONIC ACID PRODUCTION BY *PSEUDOMONAS TAETROLENS*

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Abstract

Nowadays lactobionic acid production via microbial synthesis gain a high awareness. Lactobionic acid production by microbial pathway can be affected by various factors among them total solids in concentrated whey. The aim was to study the effect of acid whey permeate concentration on lactobionic acid production. The acid whey permeate was used as the study object. The total solids in acid whey was concentrated by the pilot scale FT22 Rising Film Evaporator (Armfield, UK). *Pseudomonas taetrolens* NCIB 9396 (NCTC, England) and *Pseudomonas taetrolens* DSM 21104 (DSMZ, Germany) were used for the study. The content of lactobionic acid (LBA) in the concentrated whey and control samples was determined using the high-performance liquid chromatography (Shimadzu LC 20 Prominence, Japan). The content of lactose in the acid whey and concentrated whey samples was determined using MilcoScan™ Mars (Foss, Denmark) and the high-performance liquid chromatography. The results showed that the highest yield of LBA was achieved at 20% of total solids content in the substrate. An increase of the total solids in the substrate more than 20% slows down the process due to the influence of minor whey compounds (as minerals and their salts) and higher lactose concentration reduces *Pseudomonas taetrolens* lactose dehydrogenase activity. The study results will help to improve an effective production of lactobionic acid by microbial pathway using acid whey.

Keywords: lactobionic acid, whey, *Pseudomonas taetrolens*, lactose oxidation

Introduction

Lactose (Lac) is known as milk sugar (4-O-b-D-galactopyranosyl-D-glucose) and is barely sweet (approximately 15% of sucrose), less soluble (solubility in water 195 g L^{-1}) than most of sugars (Seki, Saito, 2012; Gutiérrez et al., 2011; Schaafsma, 2008). These lactose properties deputize severe restrictions for its widespread use, so its separation or transformation into many products is preferable, like in value added products (Silva et al., 2015; Song et al., 2013; Seki, Saito, 2012). Lactobionic acid (LBA) is a compound produced via lactose oxidation and is known as aldonic acid (4-O-b-D-galactopyranosyl-D-gluconic acid), containing gluconic acid and galactose (Borodina, Mirgorod, 2014; Gutiérrez et al., 2012). The main characteristics of lactobionic acid include moisturising, antioxidant, acidifying and stabilising aptitude, and these properties have led to growing interest about the study of this polyhydroxy- acid inherent properties. It is wide used in cosmetic, food and medicine field due to lactobionic acid nontoxicity, amphiphilic, humectant, chelating, antioxidant, biocompatibility and biodegradability properties (Alonso et al., 2013a; Alonso et al., 2013b). *Pseudomonas taetrolens* shows high lactose bioconversion ability into lactobionic acid with no complicated nutrient requirements (Alonso et al., 2011).

During the last few years there have been several experiments to obtain lactobionic acid through biotechnological pathway with such a cheap feedstock as whey. Almost all available reports deal with the use of cheese whey as medium (Giorgi et al., 2018; Alonso et al., 2017). Acid whey (pH around 4.0 till 5.0) usage for the production of lactobionic acid has not yet been reported. Productivity of lactobionic acid by microbial synthesis can be affected by various factors and one of them is the total solids content in the substrate.

The aim was to study the effect of acid whey permeate concentration on lactobionic acid production.

These results will help to upgrade lactobionic acid production with *Pseudomonas taetrolens* DSM 21104 and NCIB 9396 using acid whey as a substrate.

Materials and Methods

Microorganisms and inoculum preparation

Freeze-dried *Pseudomonas taetrolens* NCIB 9396 culture was obtained from the England National Collection of Type Cultures (NCTC, England) and freeze-dried *Pseudomonas taetrolens* DSM 21104 culture from the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) banks. *Pseudomonas taetrolens* strains were activated in nutrient broth agar (containing in g L^{-1}: 5 peptone, 1 meat extract, 2 yeast extract, 2.8 agar and 5 NaCl, respectively).

A 10 µL loopful of each strain was used to inoculate 100 mL of nutrient broth medium (containing in g L^{-1}: 5 NaCl, 5 peptone, 1 meat extract and 2 yeast extract). The precultured samples were incubated at 30 °C 16 h in an environmental shaker-incubator ES-20 at 220 rpm. Biomass of microorganisms was separated by 10 min centrifugation at 6000 rpm, using Hermle Labortechnik, Z 206 and further used as a bulk starter.

Acid whey

Acid whey permeate (producer SC “Tukuma piens”) with following average composition: lactose

4.47±0.02%; proteins 0.38±0.01%, fats 0.01±0.01%, total solids 4.90±0.09% and pH 4.75±0.01 was used for the study.

The study design

The acid whey permeate was pasteurized 30 min at 95 °C temperature and further concentrated till solids content of 28.31±1.25% in the pilot scale FT22 Rising Film Evaporator (Armfield, UK).

Concentrated permeate was diluted with deionized water till 5%, 10% and 20% of total solids. Evaporator "Heidolph Laborota 4000 efficient" was used for permeate solids further concentration till 30% and 40%. The process was carried out at 60 °C, rotation speed of the flask 150 turns min^{-1}, pressure 40 to 85 mbar. The solids concentration was detected by a refractometer (Kruss, Germany). The strains were marked with letters N (sample contains *P. taetrolens* NCIB 9396) and D (sample contains *P. taetrolens* DSM 21104), but the samples with different solids concentration in such way –N5%, N10%, N20%, N30%, N40%, D5%, D10%, D20%, D30%, and D40%.

Each sample was inoculated with a 2% of *Pseudomonas taetrolens* bulk starter. The samples were cultivated on an environmental shaker-incubator ES-20 at 220 rpm and 30 °C for 48 h. Samples were withdrawn during cultivation for monitoring of substrate pH and colony forming units (CFU) of *Pseudomonas taetrolens* NCIB 9396 and DSM 21104. The amount of lactose was determined at the beginning and the end of the production process, but lactobionic acid at the end of the production process.

Analytical methods

Total plate count was determined using nutrient broth agar at 30 °C for 48 h and counted by the Acolyte colony counter (Model No:7510/SYN). Growth curves were depicted by plotting lg CFU mL^{-1} as a function of time. pH was measured using a pH electrode InLab® Expert Pro-ISM (METTLER TOLEDO, Switzerland). The content of lactose was determined in the acid whey and concentrated whey samples prior fermentation using MilcoScan™ Mars (Foss, Denmark). The concentration of lactobionic acid and residual lactose was analysed by high performance liquid chromatography HPLC (Prominance HPLC system, Shimadzu LC-20, Torrance, CA, USA). All samples were filtered before the analysis through a 2 µm filter paper and centrifuged to remove the cell debris and other water insoluble substances. Samples were centrifuged at 13,000 rpm 5 min. Detection of lactobionic acid was carried out in a refractive index detector RID-10A; YMC C18, 4.6 mm×250 mm, 5 µm column. Mobile phase isocratic elution on 2 L solution (14.36 g KH_2PO_4, 1.15 mL H_3PO_4, 20 mL acetonitrile and deionized water). Volume of injection sample 10 µL, flow rate 1 mL min^{-1}, temperature 40 °C. Detection of lactose was carried out in a detector DAD SPD-M20A; Alltech NH_2, 4.6 mm×250 mm, 5 µm column. Mobile phase isocratic elution (84% acetonitrile, 16% deionized water). Volume of injection sample 10 µL, flow rate 1 mL min^{-1}, temperature 35 °C. Samples were quantified according to HPLC-grade external analytical standards, lactobionic acid (Acrōs Organics), lactose (Sigma Aldrich).

Data analyses

Data acquisition and analysis were performed with Microsoft Excel 2010 programme. Statistical analyses were performed using Analysis of Variance (ANOVA) and t-test at significance level of $p<0.05$. All results are presented as the average of data from three independent experiments.

Results and Discussion

Substrates pH changes during lactose oxidation

During lactose oxidation process pH was analysed (Figure 1). Results showed that pH slowly increased during fermentation process. At the end of the process significant differences ($p<0.05$) were not established among samples N5% and D5%, N10% and D10%, N20% and D20%, N30% and D30%, N40% and D40%.

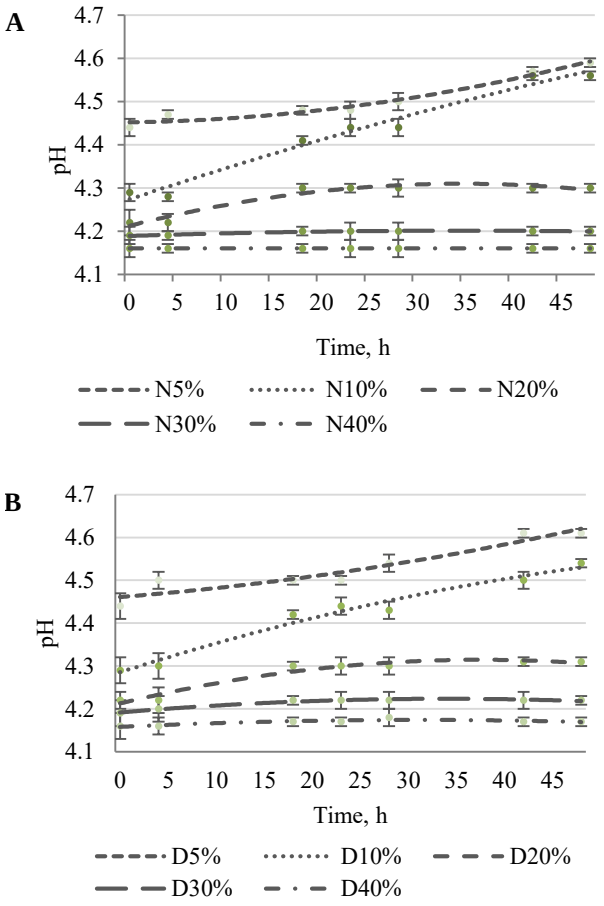

Figure 1. Time-course profile of pH changes in samples (A) containing *P. taetrolens* NCIB 9396 and (B) containing *P. taetrolens* DSM 21104 during cultivation at 30 °C for 48 h in an environmental shaker-incubator

It shows that both strains provide similar pH changes. At the beginning pH was different among samples, pH

increased with a decrease of the substrate solids concentration, respectively. We noticed that pH increased faster in samples with lower total solids concentration. pH stayed in the range from 4.16 till 4.61 in all samples. Giorgi et al. (2018) established that the lowest pH value of 4.2 was reached in lactose oxidation process with *Pseudomonas taetrolens* DSM 21104 using chemically pure lactose as a substrate. Alonso et al. (2017) observed the lowest pH value of 3.6 during shake-flask cultivation process of sweet whey permeate with *Pseudomonas taetrolens* LMG 2336 at 30 °C for 72 h. Usually pH decreases during lactose oxidation process in sweet whey with *Pseudomonas taetrolens*. The presence of nitrogen compounds in substrate promotes pH increasing in the range of 0.01 till 0.27. It could be explained with *Pseudomonas taetrolens* ability to produce proteins splitting enzymes (Alonso et al., 2011).

The study of Pseudomonas taetrolens microbial pathway

Pseudomonas taetrolens NCIB 9396 and DSM 21104 are grown in all samples during 48 h of incubation at 30 °C and reached around of 10^{10} CFU mL^{-1} (Figure 2) There were no significant differences among all samples at the end of cultivation process ($p<0.05$).

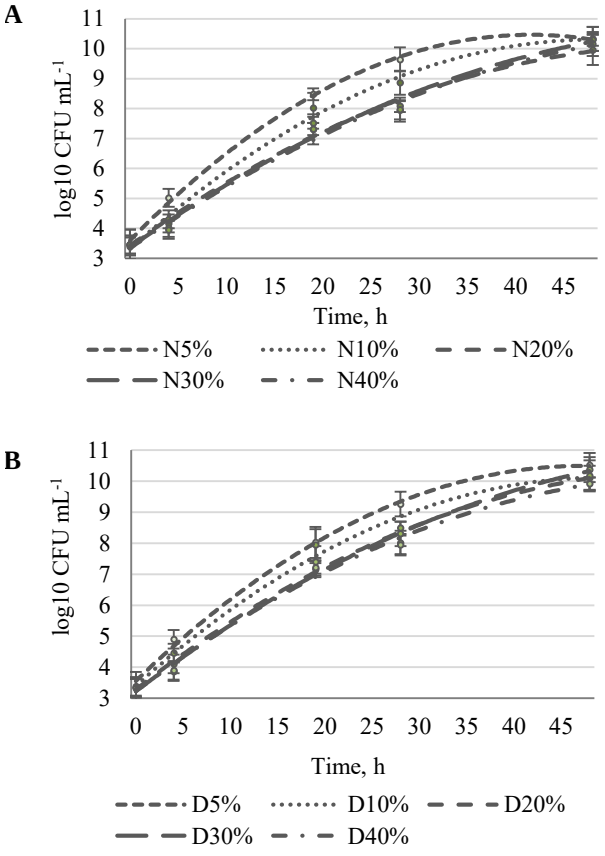

Figure 2. Time-course profile of CFU mL^{-1} changes in samples (A) containing *P. taetrolens* NCIB 9396 and (B) containing *P. taetrolens* DSM 21104 during cultivation in an environmental shaker-incubator

Pseudomonas taetrolens strains proliferate very similar in different acid whey permeate concentrations. Gorderska et al. (2014) determined that after 24 h of *Pseudomonas taetrolens* DSM 21104 cultivation in sweet whey (pH 6.5, amount of lactose 30 g L^{-1}) was reached around of 10^7 CFU mL^{-1} with 5% of inoculum in medium and 10^9 CFU mL^{-1} with 25% of inoculum in medium. Giorgi et al. (2018) established that maximum cell density of up to 10^9 CFU mL^{-1} was achieved after 48 h of incubation with *Pseudomonas taetrolens* DSM 21104 in substrate that contains 10 g L^{-1} lactose.
In Figure 2 we can observe that *Pseudomonas taetrolens* had grown faster in the substrates with 5% and 10% of total solids, but amount of CFU mL^{-1} were the same in all samples at the end of fermentation. It shows that *Pseudomonas teatrolens* needs more time to adopt in samples with higher solids concentration.

Lactobionic acid yield

The higher lactobionic acid conversion yield was reached in samples N5%, D5%. N10%, D10%, N20% and D20% (see Table 1). The significant differences were not established among these samples ($p<0.05$).

Table 1

Lactose (Lac) and Lactobionic acid (LAB) yield in end of 48 h production process

Sample	Lac, g L^{-1}	LBA, g L^{-1}	Conversion yield, %*
N5%	37.4±2.1	9.1±1.2	20.0±1.3^{a}
D5%	38.0±1.8	8.5±1.2	18.8±1.9^{a}
N10%	65.0±2.6	17.2±0.8	20.4±1.4^{a}
D10%	65.8±1.2	16.9±1.4	20.0±1.2^{a}
N20%	148.1±3.2	39.8±0.9	21.8±1.7^{a}
D20%	152.2±1.9	41.3±1.8	22.6±1.9^{a}
N30%	229.2±2.4	46.1±0.6	17.2±1.1^{b}
D30%	231.0±2.1	43.9±1.3	16.4±1.3^{b}
N40%	315.5±2.1	51.8±1.2	13.9±1.1^{c}
D40%	302.0±2.3	58.6±1.3	15.8±1.1^{bc}

*Yield was shown as the % of lactose converted into lactobionic acid after 48 h.
Results indicated with the same letter do not differ significantly ($p<0.05$).

Results showed that lactobionic acid conversion yield is lower in acid whey permeate samples with total solids higher than 20%.
Pleissner et al. (2017) has mentioned that the production of lactobionic acid increased with the increase of whey concentration. In an experiment with sample containing 200 g L^{-1} lactose (pH above 5.0 during fermentation) was produced 197 g L^{-1} of lactobionic acid after 180 h of cultivation with *Pseudomonas taetrolens*. Miyamoto et al. (2000) has established that the production rate of LAB with *Pseudomanas taetrolens* was higher in samples with 150 g L^{-1} than 200 g L^{-1} lactose. Murakami et al. (2003) has concluded that high solids concentration in whey may decrease the penetration rate of lactose through microorganism cell membrane or high amount of total solids in substrate can cause inhibition of the microorganism produced enzymes.
Goderska et al. (2014) gained 15.79 g L^{-1} lactobionic acid from 30.29 g L^{-1} lactose after 50 h of cultivation

process with *Pseudomonas taetrolens* at 30 °C. Mayamoto et al. (2000) reached 90% of lactobionic acid from lactose with *Pseudomonas taetrolens*. Alonso et al. (2012) reached 100% of lactobionic acid yield from sweet whey after 60 h of cultivation with *Pseudomonas taetrolens*. Seems like acid medium hinders *Pseudomonas taetrolens* ability to convert lactose to lactobionic acid. It could be explained with *Pseudomonas taetrolens* lactose dehydrogenase composition, containing flavin adenine dinucleotide as a prosthetic group. This flavoprotein does not use oxygen as direct electron acceptor and presents an optimum pH at 5.6. Lactose is converted by lactose oxidase to lactobiono-δ-lactone and then by lactonase in lactobionic acid. Lactonase presents an optimum at pH 6.5–6.7 (Alonso et al., 2013b). Low acid whey pH is the reason why the conversion yield is not reached as high as it is in other researches, where sweet whey was used as a substrate.

Conclusions

The most suitable acid whey permeate concentration is up to 20% for lactose oxidation with *Pseudomonas taetrolens* NCIB 9396 and DSM 21104. Low acid whey pH is the reason why the conversion yield is not reached as high as it is in other researches. The study suggests to adjust the acid whey pH prior lactose oxidation with *Pseudomonas taetrolens* and to prolong cultivation time.

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References

1. Alonso S., Rendueles M., Díaz M. (2017) Tunable decoupled overproduction of lactobionic acid in Pseudomonas taetrolens through temperature-control strategies, *Process Biochemistry*, Vol. 58, p. 9–16.
2. Alonso S., Rendueles M., Díaz M. (2013a) Feeding strategies for enhanced lactobionic acid pro- duction from whey by Pseudomonas taetrolens, *Bioresource Technology,* Vol. 134, p. 134–142.
3. Alonso S., Rendueles M., Diaz M. (2013b) Bio-production of lactobionic acid: Current status, applications and future prospects, *Biotechnology Advances,* Vol. 31(8), p. 1275–1291.
4. Alonso S., Rendueles M., Díaz M. (2012) Role of dissolved oxygen availability on lactobionic acid production from whey by *Pseudomonas taetrolens*, *Bioresource Technology*, Vol. 109, p. 140–147.
5. Alonso S., Rendueles M., Díaz M. (2011) Efficient lactobionic acid production from whey by Pseudomonas taetrolens under pH-shift conditions, *Bioresource Technology,* Vol. 102, 9730–9736.
6. Borodina V. G., Mirgorod Y. A. (2014) Catalytic synthesis lactobionic acid, *Journal of Nano- and Electronic Physics,* Vol. 6, p. 3052-1–3052-2.
7. Giorgi S. D., Raddadi N., Fabbri A. Toschi T. G., Fava F. (2018) Potential use of ricotta cheese whey for the production of lactobionic acid by *Pseudomonas taetrolens* strains, *New Biotechnology Journal,* Vol. 42, p. 71–76.
8. Goderska K., Szwengiel A., Czarnecki Z. (2014) The utilization of *Pseudomonas taetrolens* to produce lactobionic acid, *Biotechnology and Applied Biochemistry,* Vol. 173, p. 2189–2197.
9. Gutiérrez L. F., Hamoudi S., Belkacemi K. (2012) Lactobionic acid: a high value-added lactose derivative for food and pharmaceutical applications, *International Dairy Journal*, Vol. 26(2), p. 103–111.
10. Gutiérrez L.F., Hamoudi S., Belkacemi K. (2011) Selective production of lactobionic acid by aerobic oxidation of lactose over gold crystallites supported on mesoporous silica, *Applied Catalysis A: General,* Vol. 402, p. 94–103.
11. Miyamoto Y., Ooi T., Kinoshita S. (2000) Production of lactobionic acid from whey by *Pseudomonas* sp. LS13-1. *Biotechnology Letters*, Vol. 22(5), p.427–430.
12. Murakami H., Seko A., Azumi M., Ueshima N., Yoshizumi H., Nakano H., Kitahata S. (2003) Fermentative production of lactobionic acid by *Burkholderia cepacia. Journal of Applied Glycoscience*, Vol. 50 (2), p.117–120.
13. Pleissener D., Dietz D., Duuren J. B.J.H., Wittmann C., Yang X., Lin C. S. L., Venus J. (2017) Biotechnological production of organic acids from renewable resources, *Advances in Biochemical Engineering / Biotechnology*, Vol 166. p. 373–410.
14. Schaafsma G. (2008) Lactose and lactose derivatives as bioactive ingredients in human nutrition, *International Dairy Journal,* Vol. 18, p. 458–465.
15. Seki N., Saito H. (2012) Lactose as a source for lactulose and other functional lactose derivatives, *International Dairy Journal,* Vol. 22, p. 110–115.
16. Silva A. N., Perez R., Minim V. P. R., Martins D. D. S., Minim L. A. (2015) Integrated production of whey protein concentrate and lactose derivatives: What is the best combination? *Food Research International,* Vol. 73, p. 62–74.
17. Song Y. S., Lee H. U., Park C., Kim S.W. (2013) Optimization of lactulose synthesis from whey lactose by immobilized β-galactosidase and glucose isomerase. *Carbohydrate Research*, Vol. 369, p. 1–5.
18. Wijayanti B. H., Bansal N., Deeth H. C. (2014) Stability of whey proteins during thermal processing: A Review. *Comprehensive Reviews in Food Science and Food Safety,* Vol. 13, p. 1235–1251.

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SHORT COMMUNICATION

THE OPTIMIZATION OF ACID WHEY PERMEATE HYDROLYSIS FOR GLUCOSE-GALACTOSE SYRUP PRODUCTION

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Abstract

Whey contains a lot of lactose, which can be easily hydrolysed by commercial enzymes. The aim of the present study was to identify the optimal parameters for the enzymatic hydrolysis of acid whey permeate and glucose-galactose syrup production. Acid whey permeate was hydrolysed using β-galactosidase preparate (NOLA^{TM} Fit 5500, Chr. Hansen, Denmark) with activity 7200 BLU L^{-1}. As the enzyme is strongly inhibited at pH below 4.5, sodium bicarbonate was added to neutralize substrate pH till 6.0–6.3. The hydrolysis was carried out at 40 °C 6 hours. pH and monosaccharides concentration were monitored during the process of hydrolysis. The fermented substrate was concentrated in a vacuum evaporator at 40–60 °C, 4–8 kPa. Glucose-galactose syrup was obtained with 65 and 70% of total solids. Lactose and monosaccharides were determined by HPLC. Fermentation time influenced monosaccharides composition and concentration. After 2 hours of fermentation lactose was completely hydrolysed. Continuing fermentation, the amount of glucose was decreased due to formation of novel oligosaccharides. The study results revealed that the optimal time for acid whey permeate hydrolysis was 2 hours. It should be noted that during the process of hydrolysis the pH of the product increased till 6.5 and such changes are related to cellulase and glucoamylase activity incorporated in the enzyme preparate as well as permeate protein residues hydrolysis. With the increase of syrup total solids, galactose concentration was changed due to galacto-oligosaccharides formation. The degree of sweetness is key factor for the durability of lactose hydrolysis and final syrup concentration.

Keywords: β-galactosidase, lactose hydrolysis, glucose, galactose

Introduction

Processing and rational application of whey is a topical problem for all countries with a developing dairy industry. Even in Tajikistan, the lack of cost-effective technologies for whey processing for a long time has been the reason that most of whey was considered as a waste stream and thrown into sewages (Кулов, 2017; Бах, 2017). At the same time, this valuable raw material was lost, in addition to significant deterioration in the ecological situation.

The disposal of whey remains a serious problem for the dairy industry. Whey contains between 5 to 6% of total solids, including lactose, which can be recycled (Cote et al., 2004).

In addition, there is a problem associated with the production of sugar, as well as import of this product is expensive in Tajikistan. It is reasonable to hydrolyse lactose into glucose-galactose syrup production, which meaningfully will help the national economics and apply the new product as a sugar substitute. The study on glucose-galactose syrup production from acid whey will help to save food resources in multiply food industry subsectors.

One of the key components of whey – lactose consists of glucose and galactose (Скворцов et al., 2013). Lactose presents in milk of all mammals and exists in two isomeric forms, alpha and beta. The differences are the configuration of the hydroxyl-group. The lactose content in cow's milk varies in range of 4.4 to 5.2%, the average 4.8% (Gänzle et al., 2008; Schaafsma, 2008). Today whey has been processed into beverages, also dehydrated products are produced, as well as fractionation of individual components (fats, lactose, whey proteins, amino acids and peptides) and biological conversion of lactose in order to obtain glucose-galactose and lactulose syrups are practiced (Гавриил, Эдуард, 2013).

Nowadays, utilization of sweet whey has been successfully solved and sweet whey have been developed into multiple food products (Chandrapala et al., 2016); unfortunately, only a few interesting solutions and scientific publications are available for successful processing of acid whey into innovative and value-added food products. Acid whey has a low pH (4.5–4.7), sour taste, high mineral content, including lactates, less protein and lactose compared to sweet whey (Prazeres et al., 2012; Chandrapala et al., 2016). Taking into account the composition of acid whey, it is an opportunity to use whey as a raw material for lactose hydrolysis with the aim to obtain glucose and galactose.

Whey hydrolysis can be carried out by two methods: enzymatic and acid hydrolysis (Das et al., 2015). Enzymatic hydrolysis is provided using the commercial enzyme preparate – β-galactosidase which hydrolyses lactose into glucose and galactose. The enzyme is widely distributed, but only a few enzymes have an optimum pH for the operation in acid whey. Enzymatic hydrolysis of lactose is a popular technology for production of glucose-galactose syrup, which is 3 times sweeter than lactose (Ansari, Husain, 2010; Fox, 2011). Generally, glucose-galactose syrup is produced from sweet whey, in turn, it can be obtained also from acid

whey. The production technology of glucose -galactose syrup is important for many countries, including Tajikistan, which allows to get an economically substantial sugar substitute.

The aim of this study was to identify the optimal parameters for the enzymatic hydrolysis of acid whey permeate and glucose-galactose syrup production.

Materials and Methods

Chemicals and materials

Chemicals D-lactose monohydrate, D(+) glucose, D (+) galactose (>98%, HPLC) were purchased from Sigma-Aldrich (Riga, Latvia), as well as $NaHCO_3$ from Voldemars Ltd. (Latvia). The acid whey permeate was gained from JSC Tukuma piens with the following composition: fat – 0.00%, protein – 0.68±0.01%, lactose – 4.09±0.01%, total solids – 5.09±0.01%, solids-no-fat – 4.87±0.01% measured with Milkoscan™ Mars (Foss, Denmark) and pH 4.49±0.30 measured by pH Meter GPH 114 (Germany).

Commercial enzyme

Commercial NOLA ™ Fit5500 (Chr. Hansen, Denmark) β-galactosidase was used in the study. NOLA™ Fit 5500 is a highly purified enzyme gained from *Bacillus licheniformis*. According to the manufacturers' recommendations, the NOLA™ Fit 5500 enzyme is active under acidic conditions (optimum pH 5.0–7.0, temperature 35–50 °C) and enzyme activity is 5500 BLU (bifido lactase units) L^{-1} (NOLA™ Fit 5500 Product Information, 2017).

Technology for glucose-galactose syrup production

pH of the permeate was adjusted to 6.0–6.3 with 10% of $NaHCO_3$ solution. After neutralization of substrate, the acid whey permeate was pasteurized at 80±5 °C 3±1 min for microorganism inactivation. The enzyme with an activity of 7200 BLU was added to L^{-1} of acid whey permeate. After the enzyme addition, permeate was mixed 2 minutes and placed into incubator IN55 (Memmert, Germany), samples were fermented within 6 h. pH was determined every hour.

To obtain glucose-galactose syrup, the fermented solution was evaporated into vacuum-evaporator Heidolph Laborota 4000 efficient (Heidolph Instruments GmbH & Co KG, Germany). The process was conducted at a pressure of 4–8 kPa and temperature at 51±9 °C. The total solids of syrup were adjusted to 65±2% and 70±2%. Concentration of the solids was determined with a refractometer DR301-95 (KRUSS, Germany).

Determination of lactose, glucose and galactose

The changes of lactose, glucose and galactose concentration during fermentation were determined every hour using high performance liquid chromatography (HPLC) LC 20 Prominence (Shimadzu, LC-20, Torrance, CA, USA). The following parameters were set: detector – the index of refraction RID-10A; column – Alltech YMC, 4.6×250.0 mm, 5 µm; temperature 35 °C; isocratic elution mode, mobile phase – A – acetonitrile, B – deionized water (A80 : B20); the volume of the injection sample was 10 µL; total time for analysis – up to 25 minutes; flow rate 1.0 mL min^{-1}. The obtained data was processed using Shimadzu LabSolutions software (LC Solution Version 1.21 SP1) (Zolnere et al., 2018).

Monosaccharide composition and concentration was analysed in syrup with different solids content.

Statistical analysis

The experiments were carried out in three replications. Significant differences among the study results were identified using t-test at the significance level $p<0.05$. Statistical analysis was performed using Windows and Excel programs, version 10.0.

Results and Discussion

The effect of different parameters, such as fermentation time and pH, was studied and results are shown in Figure 1. The recommended medium pH for NOLA™ Fit 5500 enzyme was 5.0–7.0, therefore the acid whey permeate pH was adjusted in the range of 6.23±0.03. During the fermentation time, there was observed the significant increase ($p<0.05$) of substrate pH from 6.23 to 6.37. pH changes can be attributed to the activity of cellulase and glucoamylase, which are incorporated in the enzyme preparate and permeate protein residues hydrolysis (Palmer et al., 2007). Roy and Gupta (2003) concluded that an increase of pH helps to maintain enzyme activity during fermentation as well as at the end of fermentation pH changes become slower.

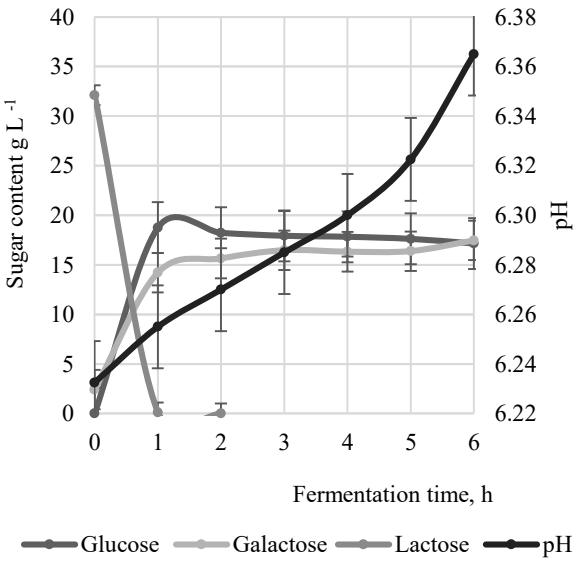

Figure 1. The effect of fermentation process on the monosaccharide concentration and composition, and substrate pH

Analysis of the monosaccharide concentration showed that the optimal fermentation time for the lactose hydrolysis was 2 hours. The duration of the fermentation process affects the concentration of glucose and galactose (Мяло et al., 2013). It should be noted that the

final concentration of glucose and galactose is unequal in substrate at the end of fermentation, which contradicts to the theoretical yield of these monosaccharides. Adding lower amount of the enzyme preparate, the ratio of glucose and galactose increased in towards glucose, whereas with an increased enzyme addition the differences in the monosaccharide concentration decreased. It should be noted that conversion coefficient, which characterizes the lactose transformation into glucose and galactose, ranged from 1.05 to 1.11 (Остроумов, Гаврилов, 2013). Continuing fermentation, the glucose concentration decreased due to the formation of novel oligosaccharides (Cezar et al., 2018).

As shown by several researchers (Bojan et al., 2011; Goderska et al., 2008; Warmerdam et al., 2013), lactose hydrolysis with *Bifidobacterium bifidum* β-galactosidase showed high activity significantly reducing initial lactose amount in substrates. It influences the final monosaccharides concentration, especially higher glucose yield.

Table 1

Comparative analysis of the carbohydrate composition of glucose - galactose syrup

Carbohydrates, g L^{-1}	Experimental syrup*		Commercial syrup* with 65% of total solids**
	65% of total solids	70% of total solids	
Glucose	45±2	43±3	25
Galactose	20±3	24±2	22
Lactose	–	–	12

* Syrups contain at least 1–2% protein.
** Somov et al., 2015

Before vacuum evaporation, heat treatment of substrate was not carried out in order to inactivate the enzyme. The temperature during evaporation process was adjusted at 51±9 °C, under these conditions the enzyme was able to operate.

The glucose-galactose syrup was obtained with total solids concentration of 65±2% and 70±2%. Based on chromatographic analysis, syrups contain 43–45% of glucose and 20–24% of galactose (Table 1). The high concentration of glucose compared to galactose could be explained by its inversion under the action of β-galactosidase in the process of evaporation. With the increase of total solids in syrup, the concentration of glucose decreases and that of galactose increases. Glucose and galactose concentration in experimental syrups was higher compared to commercial syrup obtained by commercial Ha-Lactase 5200 β-galactosidase (Chr. Hansen, Denmark), as well as remaining lactose was found in the commercial syrup (Table 1) (Сомов, 2013). Monosaccharide analysis showed that NOLA™ Fit 5500 β-galactosidase was able completely hydrolyse lactose into glucose and galactose in acid whey (Table 1), as well as provide a higher degree of syrup sweetness.

Conclusions

The study results showed that the optimal time for lactose hydrolysis was 2 hours. Extension of the fermentation process results in decrease of glucose concentration.

As the degree of syrup sweetness is a key factor with regards to syrup quality, it is recommended to concentrate syrup up to 65% total solids.

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References

1. Ansari S. A., Husain Q. (2010) Lactose hydrolysis by β-galactosidase immobilized on concanavalin A-cellulose in batch and continuous mode. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 63(1–2), p. 68–74.
2. Bojan M., Katarina L., Rajka B., Ljubica T. (2011) Impact of enzymatic hydrolyzed lactose on fermentation and growth of probiotic bacteria in whey. *Food and Agriculture Organization of the United Nations* Vol. 61 (2), p. 154–160.
3. Cezar M., Mano R., Nicolau B., Maria G. (2018) Whey permeate as the raw material in galacto-oligosaccharide synthesis using commercial enzymes. *Food Research International,* Vol. 0–1, p. 1–8.
4. Chandrapala J., Wijayasinghe R., Vasiljevic T. (2016) Lactose crystallization as affected by presence of lactic acid and calcium in model lactose systems. *Journal of Food Engineering,* Vol. 178, p. 181–189.
5. Cote A., Brown W. A., Cameron D., van Walsum G. P. (2004) Hydrolysis of lactose in whey permeate for subsequent fermentation to ethanol. *Journal of Dairy Science,* Vol. 87 (6), p. 1608–1620.
6. Das B., Roy A. P., Bhattacharjee S., Chakraborty S., Bhattacharjee C. (2015) Lactose hydrolysis by β-galactosidase enzyme: Optimization using response surface methodology. *Ecotoxicology and Environmental Safety,* Vol. 121, p. 244–252.
7. Fox P. F. (2011) Lactose and oligosaccharides. **In:** *Encyclopedia of dairy sciences*, London: Elsevier, p. 173–181.
8. Gänzle M. G., Haase G., Jelen P. (2008) Lactose: Crystallization, hydrolysis and value-added derivatives. *International Dairy Journal,* Vol. 18 (7), p. 685–694.
9. Goderska K., Nowak J., Czarnecki Z. (2008) Comparison of the growth of Lactobacillus acidophilus and Bifidobacterium bifidum species in media supplemented with selected saccharides including prebiotics. *Technologia Alimentaria,* Vol. 7 (2), p. 5–20.
10. *NOLA™ Fit5500 Product Information,* [accessed on 06.11.2018.]. Available: https://hjemmeriet.com/se/ChrHansen/Products/NOLA-Fit/PI_GLOB_NOLA_Fit5500_350502_EN.pdf
11. Palmer T., Bonner P. L. (2007) *ENZYMES: Biochemistry, biotechnology and clinical chemistry*, Second Edition. Oxford: Woodhead Publishing. 431 p.
12. Prazeres A. R., Carvalho F., Rivas J. (2012) Cheese whey management: A review. *Journal of Environmental Management,* Vol. 110, p. 48–68.
13. Roy I., Gupta M. N. (2003) Lactose hydrolysis by Lactozym ™ immobilized on cellulose beads in batch and

fluidized bed modes. *Process Biochemistry,* Vol. 39, p. 325–332.
14. Schaafsma, G. (2008) Lactose and lactose derivatives as bioactive ingredients in human nutrition. *International Dairy Journal,* Vol. 18 (5), p. 458–465.
15. Somov V., Evdokimov I., Knyazev S., Perminov S., Kurash Y. (2015) Application of whey-derived syrups in dairy products. *Foods and Raw Materials,* Vol. 3(2), p. 89–95.
16. Zolnere K., Ciprovica I., Ķirse A., Cinkmanis I. (2018) A study of commercial β-galactosidase stability under simulated in vitro gastric conditions. *Agronomy Research,* Vol. 16 (S2), p. 1555–1562.
17. Warmerdam A., Boom R. M., Janssen A. E. M. (2013) β-galactosidase stability at high substrate concentrations. *SpringerPlus,* Vol. 2, p. 1–8.
18. Бах К. Ф., (2017) *Таджикистан. Обзоры результативности экологической деятельности.* Организация Объединенных Наций, Нью-Йорк, p. 451
19. Гавриил Б.Г., Эдуард Ф.К., (2013). Пути рационального использования молочной сыворотки. *Сыроделие и маслоделие,* Vol. 2, p. 10–13.
20. Кулов А. Н., Наджибуллоев А., Будникова Е. В., Исмоилова З., Асматбеков Ф., Ходжаев З. (2017) Статистический ежегодник республики Таджикистан. Агентство по статистике при Президенте Республики Таджикистан, p. 1–365.
21. Мяло С. В., Гаврилова Н. Б., Воронова Т. Д., Щетинин М. П. (2005) Исследование процесса гидролиза молочного сахара энзиматическим и микробиологическим способами. *Ползуновский альманах,* Vol. 1, p. 87–94.
22. Остроумов Л. А., Гаврилов Г. Б., (2013) Биотрансформация лактозы ферментными препаратами β-галактозидазы. *Техника и технология пищевых производств,* Vol. 1, p. 10–13.
23. Скворцов Е. В., Морозова Ю. А., Букуру Л. К., Алимова Ф. К., Канарская З. А. (2014) Эффективность применения β – галактозидазы для гидролиза лактозы молочной сыворотки. *Вестник Казанского технологического университета.* Vol. 13, p. 3–6.
24. Сомов В.С. (2013) *Разработка технологии фруктовых добавок на основе глюкозо-галактозного сиропа, полученного из творожной сыворотки с применением мембранных методов.* [accessed on 14.12.2018.]. Available at: http://tekhnosfera.com/razrabotka-tehnologii-fruktovyh-dobavok-na-osnove-glyukozo-galaktoznogo-siropa-poluchennogo-iz-tvorozhnoy-syvorotki-s-pri

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SHORT COMMUNICATION

GOAT MILK QUALITY IN THE LATE LACTATION

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Abstract

Improving application of goat milk in cheese production is an up-to-date issue in Latvia, therefore more information is needed about the chemical composition and renneting properties of goat milk during the lactation stages. The aim of the study was to analyse the goat milk in the late lactation. Milk fat, protein, lactose, dry matter, solids-non-fat and freezing point were measured by infrared spectroscopy (MilcoScan Mars^{TM}, Foss, Denmark). In total, 95 samples were tested, which represent four goat breeds: Latvian Native (n=44), Saanen (n=16), milking crosses XP (n=21) and crosses XX (n=14). All XX and XP goats had the first lactation, Latvian Native – 1.83 lactation, and Saanen goats – 5.3 lactation. All animals were kept in the same condition and received the same feed by feeding scheme. The bulk milk was also analysed (n=4) for understanding of an average chemical composition and its influence on cheese production. Fat to protein ratio was analysed in individual goat milk samples, as well as in bulk milk samples, and the average results were 0.91 and 1.15, respectively. Milk fat and protein content (%) was higher in XP goats' milk compare to Latvian Native, respectively 4.50 vs 4.75 and 3.91 vs 4.23. No significant differences were found between lactose content (%) in goat milk of the first, second and older lactations, respectively, 4.43, 4.43 and 4.23. Overall means for bulk milk fat, protein, lactose and solids-non-fat content were 4.95%, 4.28%, 4.41% and 8.90%.

Keywords: goat milk, late lactation, quality

Introduction

Goat milk is an excellent source of nutrients with high biological value proteins, essential fatty acids, high mineral bioavailability and vitamin content (Garciá et al., 2014; Park, 2009; Verruck et al., 2019). Also goat milk products, especially cheese, are considered a delicacy in many countries and its products have gained market size (Verruck et al., 2019).

Goat milk composition has substantial effect on cheese composition and yield. The goat milk varies considerably in composition, especially with the season as most of goat kids at about the same time in Latvia. Goat milk quality is a variable, as well as milk quality changes significantly influence cheese production.

One of most important quality indicators in goat milk is somatic cell count (SCC). In many countries already have some restrictions that SCC must not exceed 1 000 000 per 1 mL, but in Latvia we still produce milk with indefinite somatic cell count.

SCC influences many aspects as genetics, environment, husbandry conditions and animal health (Leitner et al., 2016).

The objective of this study was to analyse goat milk quality in the late lactation.

Materials and Methods

The study was carried out from October to December of 2018, the goat milk used in the study was collected at goat farm Ltd Licisi.

In total, 95 raw milk samples were tested which represent four goat breeds: Latvian Native (n=44), Saanen (n=16), milking crosses XP (n=21) and crosses XX (n=14). Crossed breeds goats had the first lactation, Latvian Native (LVK) average lactation 1.83, and Saanen goats (ZK) average lactation 5.3. Information about goats' characteristics was taken from the farm register but data on somatic cell count were obtained from milk data monitoring (Agricultural Data Centre Republic of Latvia, 2019).

The goats were divided according to the lactation stage in 3 groups: 1 – first lactation (n=55), 2 – second lactation (n=17), 3 – animals of third to seventh lactation (n=23).

Animals were kept in the same condition and fed by feeding scheme with hay, haylage, straw, bushes and twigs, grassland, barley flour or oats.

The goats were machine milked twice a day and milk yield recorded. Samples from morning milking were used for analyses. Samples were cooled and stored at 3±1 °C, and analysed on the same day of milking.

The bulk milk was analysed (n=4) for understanding an average milk chemical composition, as well as fat to protein ratio and its influence on the cheese production.

Individual goat milk samples and bulk milk samples were analysed for fat, protein, lactose, dry matter, solids-non-fat and freezing point by infrared spectroscopy (MilcoScan Mars^{TM}, Foss, Denmark) and fat to protein ratio of individual goat milk and bulk milk was calculated.

Statistical data analyses were carried out using Microsoft Excel 2016 and Rstudio programmes.

Results and Discussion

Fluctuation of goat milk composition could be affected by breed, stage of lactation, and season. The results of different lactation stage goat milk composition in the late lactation are shown in Table 1. The lactation stage influenced milk yield, which significantly increased in the 2nd goat group and reached the highest amount in the 3rd group.

Analysing milk yield in the lactations, there were found significant differences between 1st and 2nd and between 1st and 3rd group ($p<0.05$, respectively $p=0.01$, $p=0.02$).

Table 1

Milk production indices and chemical composition in the late lactation goat

Group	1 (n=55)	2 (n=17)	3 (n=23)
Milk yield, kg	468±139[b]	597±61[a]	599±146[a]
Fat content, %	4.31±1.12[b]	3.67±0.37[a]	3.51±1.07[a]
Protein content, %	4.57±0.94[b]	4.12±0.36[a]	3.98±0.70[a]
Fat to protein ratio	0.94±0.16[b]	0.89±0.09[a]	0.88±0.19[a]
Lactose content, %	4.43±0.22[a]	4.43±0.35[ab]	4.23±0.59[b]
Dry matter, %	13.46±2.11[b]	12.11±0.76[a]	11.55±2.10[a]
Solids-non-fat, %	9.28±0.97[b]	8.75±0.45[a]	8.35±1.02[a]
Freezing point, °C	-0.506±-0.037[b]	-0.481±-0.037[a]	-0.455±-0.064[a]
SCC	992±864[a]	915±660[a]	1167±1168[b]

SSC – somatic cell count.
Results indicated with the same letter in the lines do not differ significantly (p>0.05).

Table 2

Milk composition and production indices in different goat breeds milk at late lactation

Group	LVK (n=44)	ZK (n=16)	XP (n=21)	XX (n=14)
Milk yield, kg	550±123[a]	635±135[b]	797±130[c]	410±136[d]
Fat content, %	3.91±1.12[a]	3.34±0.92[c]	4.50±0.42[b]	4.29±1.16[ab]
Protein content, %	4.23±0.68[b]	3.81±0.49[c]	4.75±0.13[a]	4.73±1.23[a]
Fat to protein ratio	1.08±0.18[ab]	1.05±0.15[a]	1.10±0.13[b]	1.14±0.18[c]
Lactose content, %	4.39±0.29[a]	4.20±0.68[a]	4.40±0.23[a]	4.55±0.20[a]
Dry matter, %	12.54±1.96[b]	11.11±1.95[c]	13.9±0.94[a]	13.59±2.43[ab]
Solids-non-fat, %	8.86±0.76[b]	8.09±1.00[c]	9.49±0.73[a]	9.47±1.20[a]
Freezing point, °C	-0.485±-0.042[a]	-0.445±-0.072	-0.520±-0.019[b]	-0.507±-0.027[ab]
SCC	1147±981[b]	906±1128[a]	453±501[c]	999±768[a]
Average lactation	1.83	5.3	1.0	1.0

LVK – Latvian Native goat breed, ZK – Saanen goat breed, XP – milking crosses, XX – crosses, SCC – somatic cell count.
Results indicated with the same letter in the lines do not differ significantly (p>0.05).

The noticeable difference in milk yield was not found between 2nd and 3rd group (p>0.05, p=0.98). Latvian researchers Piliena and Jonkus (2012) have established that milk yield rises till third lactation and with fourth lactation it decreases. Stage of lactation is one of the many factors that influence milk composition and its technological properties especially fat to protein ratio. Goetsch et al. (2011) established that it is negative practice to elongate the lactation stage.

In most cases, goat milk is not standardized prior cheese production, which influences fat content in dry matter as well as cheese yield, too. Protein and fat content determines the yield of cheese, but ratio of fat to protein mainly determines the fat content in dry matter, as well as affects syneresis and water content in the cheese. The ratio of fat to protein in milk decreased with the lactation stage (Table 1). Protein and fat content was lower in 2nd and 3rd group goat milk. The content of lactose in milk decreased throughout the lactation stage, and also lactose content was lower in 3rd group goat milk. Somatic cell count was higher in 1st and 3rd group goat milk. Stage of lactation also influenced SCC count in goat milk. High milk production amount was associated with lower total solids concentration, particularly fat and protein content in milk.

The study results indicated that analysed milk samples could be considered of high quality in relation to SCC, reaching the parameters established in many European countries – 1 000 000 per 1 mL. The individual goat milk samples with SCC above one million per 1 mL were lower in 1st and 2nd lactation stage. This may be due to the fact that the goat milk yield decreases with the advance of lactation and consequently increases SCC.

The results of different breeds' goat milk composition in the late lactation are shown in Table 2.

The significant differences were established among the breeds in fat content (p<0.05). There were no significant differences between LVK and XX; XP and XX, respectively p>0.05 (p=0.24; p=0.63).

The goat breed influenced the milk yield, which was significantly higher for XP and ZK goats compared to LVK and XX. Protein and fat content was higher in XP goat milk. Estonian researchers (Tatar et al., 2015) established the lower average fat and protein content in spring and summer season (April-June) milk, but higher average fat and protein content in October-December and in July-September milk. Chávez-Servín et al. (2018) and Steinshamn et al. (2014) concluded that the variability of goat milk and its product composition is affected by the grazing season.

The bulk milk chemical composition are shown in Table 3.

Table 3

Bulk milk composition

Sample	Average	Min	Max
Fat content, %	4.95	4.21	5.53
Protein content, %	4.28	4.11	4.44
Fat to protein ratio	1.15	1.02	1.25
Lactose content, %	4.41	4.24	4.60
Dr y matter, %	13.79	12.64	14.83
Solids-non-fat, %	8.90	8.64	9.24

The information on the influence of bulk milk composition of late lactation goats can help cheesemakers to better understand the effect on milk suitability for cheesemaking and cheese quality.

It is known that sheep and goat milk normally have higher SCC than cows milk. SCC has been used as an indicator to detect mastitis in cows and it should be adapted for goat milk, too (Stuhr et al., 2013). Milk with a low SCC and bacterial count is the base for having healthy animals and good hygienic practice at the farm (Skeie, 2014). Goat breeders should remember that SCC is also an indicator of goat subclinical mastitis (Bagnicka et al., 2011). To establish the acceptable limits for SCC in goat milk, it is necessary to evaluate the average value as its variation through lactation.

Conclusions

The stage of lactation influences lactose, protein, fat content and SCC count in goat milk.

The analysed goat milk varied considerably in composition, especially in the late lactation. Milking crosses had shown the higher milk yield and lower SCC in the late lactation milk.

To establish the acceptable limits for SCC in goat milk, it is necessary to evaluate the average value as its variation through lactation.

References

1. Agricultural Data Centre Republic of Latvia. [accessed on 05.02.2019.]. Available at: http://ldc.gov.lv/lv/statistika/parraudziba/
2. Bagnicka E., Winnicka A., Jóźwik A., Rzewuska M., Strzałkowska N., Kościuczuk E., Prusak B., Kaba J., Horbańczuk J., Krzyżewski J. (2011) Relationship between somatic cell count and bacterial pathogens in goat milk. *Small Ruminant Research,* Vol. 100, p. 72–77.
3. García V., Rovira S., Boutoil K., López M.B. (2014) Improvement in goat milk quality: A review. *Small Ruminant Research*, Vol. 121, p. 51–57.
4. Goetsch A.L., Zeng S.S., Gipson T.A. (2011) Factors affecting goat milk production and quality. *Small Ruminant Research*, Vol. 101, p. 55–63.
5. Chávez-Servín J.L., Andrade-Montemayor H.M., Velázquez Vázquez C., Barreyro A.A., García-Gasca T., Ferríz Martínez R. A., Andrea M., Ramírez O., de la Torre-Carbot K. (2018) Effects of feeding system, heat treatment and season on phenolic compounds and antioxidant capacity in goat milk, whey and cheese. *Small Ruminant Research*, Vol. 160, p. 54–58.
6. Leitner G., Lavon Y., Matzrafi Z., Benun O., Bezman D., Merin U. (2016) Somatic cell counts, chemical composition and coagulation properties of goat and sheep bulk tank milk. *International Dairy Journal*, Vol. 58, p. 9–13.
7. Park Y. W. (2009) Bioactive components in goat milk. **In**: *Bioactive Components in Milk and Dairy Products*. Vol.1. 3rd ed. Park. Y.W. Wiley Blackwell, p.43–81.
8. Piliena K., Jonkus D. (2012) Factors affecting goat milk yield and its composition in Latvia. *Research for Rural Development 2012: annual 18th international scientific conference proceedings*, p. 79–84.
9. Skeie S.B. (2014) Quality aspects of goat milk for cheese production in Norway: A review. *Small Ruminant Research*, Vol. 122, p. 10–17.
10. Steinshamn H., Inglingstad R.A., Ekeberg D., Mølmann J., Jørgensen M. (2014) Effect of forage type and season on Norwegian dairy goat milk production and quality. *Small Ruminant Research,* Vol. 122, p. 18–30.
11. Stuhr T., Aulrich K., Barth K., Knappstein K., Larsen T. (2013) Influence of udder infection status on milk enzyme activities and somatic cell count throughout early lactation in goats. *Small Ruminant Research*, Vol. 111, p. 130–146.
12. Tatar V., Mootse H., Sats A., Mahla T., Kaart T., Poikalainen V. (2015) Evaluation of size distribution of fat globules and fat and protein content Estonian goat milk. *Agronomy Research*, Vol. 13 (4), p. 1112–1119.
13. Verruck S., Dantas A., Prudencio E.S. (2019) Functionality of the components from goat's milk, recent advances for functional dairy products development and its implications on human health. *Journal of Functional Foods,* Vol. 52, p. 243–257.

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SHORT COMMUNICATION

SUGGESTIONS FOR CONSUMERS ABOUT SUITABILITY OF DIFFERENTLY COLOURED TOMATOES IN NUTRITION

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Abstract

Tomatoes (*Solanum lycopersicum* L.) are known as very popular vegetable due to high nutritional value and are among the most commonly used vegetables in the world. Tomato varieties differ not only in fruit size, but also in colour. The aim of the present study was to evaluate how the colour of tomatoes influences the nutritional value. Chemical composition (vitamin C, lycopene, β-carotene, soluble solids, total acidity) and taste index were determined in 27 tomato cultivars grown in plastic film greenhouse without additional lighting. Red, pink, brown, orange and yellow tomato fruits were studied. The obtained results showed that there were significant differences in the mean values between analysed parameters according to the colour of fruit. The content of lycopene changed as follows: pink>red>brown>orange>yellow, but content of β-carotene: orange>pink>brown>red>yellow. The highest content of total acids (855.7±234.2 mg 100 g^{-1}) as well as vitamin C (18.43±4.74 mg 100 g^{-1}) was observed in orange tomatoes, but regarding taste index differently coloured tomatoes can be arranged as follows: brown>orange>pink>red>yellow. The smaller and bigger size tomatoes are recommended for consumers as tastier comparing with medium size tomatoes.

Keywords: taste, chemical composition, lycopene, β-carotene, vitamin C

Introduction

In recent years, the interest of healthy and tasty products has been increased. It is connected with consumer's interest about products, including vegetable, containing biological active substances, such as vitamins, phenolic compounds a well as antioxidants (Dhandevi, Rajesh, 2015).

Tomatoes (*Solanum lycopersicum* L.) are very popular vegetable in Europe, but due to modernization of production processes, the quality of final products does not always meet consumer's requirements. They pay attention also on sensory properties of products, including taste and colour (Fernqvist et al., 2013).

In Latvia, tomato occupies an important place in the vegetable sector with 43% from total vegetable production in greenhouses in 2017 (Informative material of Ministry of Agriculture). The consumption of fresh tomatoes was on average 1 kg monthly per household member in 2017 (Central Statistical Bureau of Latvia, 2017).

Tomatoes is known as good source of phenolic, flavonoids, lycopene, ascorbic acid and other bioactive compounds (Toor, Savage, 2005), but the nutritional value may vary depending on several factors such as cultivar, growth conditions, harvesting time and conditions, as well as storage (Hernandez-Suarez et al., 2008). The colour of tomatoes depends on its chemical composition, especially on the content of pigments, but the content and ratio of sugars and organic acids gives the tomatoes taste. (Tieman et al., 2017, Zhang et al., 2015). Tomato flavour is a balance of acidity and content of sugars, but colour of fruits depends on content of pigments, which also can influence tomato flavour. Orange and yellow tomatoes are less acidic and have delicate taste, whereas red and black tomatoes cultivars have different taste qualities.

Tomatoes colour, its weight and taste qualities are very important indices for consumers. It is known, that red coloured tomatoes are better preferred for consumers, but very often in the supermarkets we could find tomatoes with other colours – yellow, orange, brown and black (Cooperstone et al., 2017; Borghesi et al., 2011). Fruit colour is one of the main factors, which determines consumer choice (Breksa et al., 2015; Stommel et al., 2005). Tomato fruit colour is connecting with content of different colour pigments - chlorophylls, carotenes, lycopene, and their proportions in the fruit (Borghesi et al., 2016; Zhao et al., 2012). The content of chlorophylls characterized by green colour of tomato fruits, but carotenes give an orange and lycopene – red colour (Fangman et al., 2018, Park et al., 2018).

The aim of the present study was to evaluate how the colour influences the nutritional value of tomatoes grown in plastic film greenhouse without additional lighting.

Materials and Methods

Investigations were carried out at the Latvia University of Life Sciences and Technologies, Institute of Soil and Plant Sciences. 27 tomato plant varieties with five colours of tomato fruits (red, pink, brown, orange and yellow) were grown in the plastic film greenhouse without additional lighting from 1st of May till 1st of September 2018. All fruits were harvested at full ripening stage. In the experiment the following red fruit varieties were included – 'Gaurmandia F1', 'Aurea F1', 'Berberana F1', 'Amaneta F1', 'Pozano F1', 'Nectar F1', 'Sunstream F1', 'Bellastar F1', 'Lancelot F1', 'Conchita F1', 'Gardener delight F1' and 'Elegance F1'; pink varieties 'Dimeros F1', 'Fuji Pink F1' , 'Pink wonder F1', 'Cipars F1', 'Rhianna F1', 'DRK936 F1' and 'Rosastar F1'; orange varieties

'Beorange F1', 'Organza F1', 'Apressa F1' and 'Oranjstar F1'; yellow varieties 'Gualdinjo F1' and 'Bolzano F1', and brown varieties 'Chocomote F1' and 'Black cherry F1'.

Tomato sampling

Physiologically ripe tomatoes were harvested, cleaned, washed and dried up. Five tomatoes from each cultivar were selected for chemical analysis, weighed and then homogenized. From the obtained puree samples were taken on triplicate to measure content of vitamin C, total acids and soluble solids, as well as content of β-carotene and lycopene.

Chemicals and spectral measurements

All the reagents used were with the analytical grade from Sigma Aldrich, Germany. UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan) was used for the absorbance measurements, but content of total soluble solids was determined refractometrically. The content of vitamin C and total acids were determined titrimetrically.

Determination of vitamin C content

Vitamin C content in tomato samples was determined titrimetrically using 2.6-dichlorphenolindophenol. For determination 2±0.0001 g of tomatoes puree was quantitatively transferred in 100 mL tubes, added 30 mL of 1% HCl and 5% HPO_3 mixture (1:1 v/v) and mixed thoroughly for 30 min. After that solution was centrifuged for 10 min at 5000 rpm. For determination 10 mL (V_a) of supernatant was titrated with 0.0005 molar solution of 2.6 dichlorphenolindophenol (V_{titr}).
The content of vitamin C was calculated according to the equation (1):

$$\text{Vitamin C (mg 100 g}^{-1}) = \frac{V_{titr} \times 0.044 \times V_{total} \times 100}{V_a \times weight} \quad (1)$$

where:
V_{titr} – volume of 2.6 dichlorphenolindophenol, mL;
V_{total} – total volume of supernatant, mL;
V_a – volume of supernatant for titration, mL.

Determination of titratable acidity

Titratable acidity was determined titrimetrically with a solution of sodium hydroxide 2±0.0001 g of tomatoes puree was quantitatively transferred in 100 mL tubes, added 40 mL of distilled water and mixed... After 30 minutes solutions were centrifuged for 10 min at 5000 rpm. For determination 10 mL of the supernatant was titrated with 0.1 M NaOH in presence of indicator phenolphthalein and results expressed as g of citric acid 100 g^{-1} tomato sample.

Determination of total soluble solids

The total soluble solids content (expressed as BRIX degree) was measured with a refractometer (A.KRÜSS Optronic Digital Handheld Refractometer Dr301-95), calibrated at 20 °C with distilled water.

Determination of lycopene content

For extraction, a sample of tomato puree (0.5±0.0001 g) was weighted in a glass test tube. Then 10 mL of solvent (tetrahydrofuran, THF) was added to it and the test tubes were held for 30 min with occasional shaking at room temperature and finally centrifuged for 10 min at 5000 rpm. The absorbance of supernatants was analysed spectrophotometrically by absorption measurements at 350 to 700 nm and calculated in accordance with Nagata and Yamashita (1992).

Determination of β-carotene

For extraction a representative portion of tomato puree (0.5±0.0001 g) was weighted in a glass test tube. Then 10 mL of solvent (ethanol, 97%) was added to it and the test tubes were held for 30 min with occasional shaking at room temperature and finally centrifuged for 10 min at 5000 rpm. The absorbance of supernatants was analysed spectrophotometrically by absorption measurements at 350 to 700 nm and calculated in accordance with Nagata and Yamashita (1992).
Taste index was calculated using the equation proposed by Narvez et al (1999) and Nielsen (2003).

Statistical analysis

Data were expressed as mean of triplicate assay ± standard deviation. The One-way analysis of variance (ANOVA) was used to determine the significance of differences and value of $p<0.05$ was regarded as statistically significant.

Results and Discussion

Fruit colour is one of the most important food quality parameters affecting consumer choice Data on the values of different coloured tomato fruit quality parameters (content of vitamin C, total acidity as well as calculated taste index) are summarized in Table 1.

Table 1

Quality parameters of analysed tomatoes

Tomato colour	Vitamin C mg 100 g^{-1}	Titratable acidity* mg 100 g^{-1}	Taste index
Red	13.18±6.17	759.8±55.1	1.15
Pink	14.12±3.41	811.7±74.1	1.18
Orange	18.43±4.74	855.7±23.2	1.25
Brown	11.23±2.16	805.8±58.5	1.26
Yellow	13.64±2.54	598.1±22.2	0.95

*Titratable acidity expressed as mg 100 g^{-1} of citric acid.

Data processing showed, that there are significant differences ($p<0.05$) in analysed quality parameters. In this study we have found that content of vitamin C was from 11.23±2.16 mg 100 g^{-1} (brown tomatoes) till 18.43±4.74 mg 100 g^{-1} (orange tomatoes). Results regarding total acidity changes in the following sequence: orange>pink>brown>red>yellow.
It means that for consumers who like milder tomatoes, yellow fruit is recommended. The levels of vitamin C and total acidity are in agreement with research data reported in scientific literature (Peihoto et al., 2018, Pinela et al., 2016), but less than others (Asensio et al., 2019, Vinha et al., 2013). Taste index is parameter, which characterizes the quality and taste of tomatoes. When comparing the obtained results among the

coloured tomatoes, it can be observed that taste index, which is calculated using the values of the Brix degree and total acidity, changes from 0.95 (yellow tomatoes) till 1.26 (brown tomatoes).

To compare the quality of different coloured tomatoes several measures were used. Significant differences ($p<0.05$) were observed among content of dry matter and soluble solids in tomato samples. The dry matter ranged from 5.42±0.69 (yellow tomatoes) till 8.25±1.01 g 100 g^{-1} (brown tomatoes) and these results are similar with our previous studies (Duma et al., 2015).

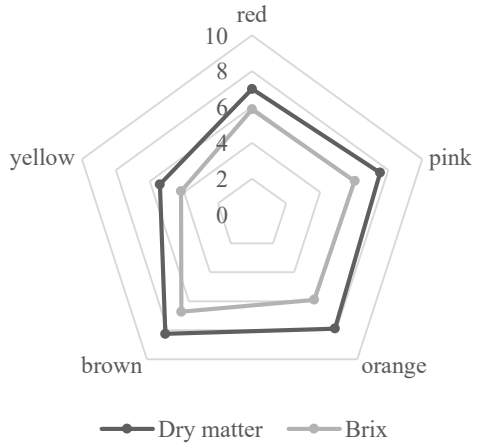

Figure 1. Content of dry matter (%) and total soluble solids (°Brix) in differently coloured tomatoes

Plant variety, degree of maturation a well as growing conditions could effect the content of total soluble solids (TSS). The TSS values which are determined by refractometer, are used as an index of total sugars in fruits (Vinha et al., 2013) and if Brix degree is in range of 4.8–8.8, that the quality of tomatoes is high. Our results showed that soluble solids values ranged from 4.18±0.18 (yellow fruits) till 6.73±0.32 Brix degree (Figure 1). There were not significant differences between total soluble solids in red (5.87±1.61), pink (6.03±1.08) and orange (5.90±1.26) tomatoes. These mean values found in the present study are consistent with some data reported in the literature (Coyago-Cruz et al., 2019; Duma et al, 2015), but higher than reported by Vinha et al., 2013.

Tomatoes are known as important source of carotenes, especially lycopene that is well known antioxidant (Böhm, 2012). The highest level was found in the pink tomatoes (4.063±1.248 mg 100 g^{-1}, but less in yellow (0.037±0.001) and orange (0.361±0.175 mg 100 g^{-1}) tomatoes (Figure 2). The pink, red and brown tomatoes may be interesting for consumers as rich sources of antioxidant lycopene.

The values of β-carotene varies according to orange> pink>brown>red>yellow (Figure 2).

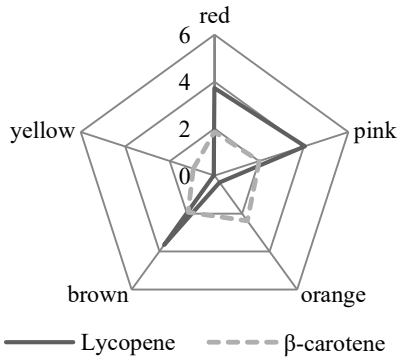

Figure 2. Content of lycopene (mg 100 g^{-1}) and β-carotene (mg 100 g^{-1}) in differently coloured tomatoes

The obtained results regarding lycopene content are similar with other results (Coyago-Cruz et al., 2019), but Asensio et al. 2019 reported-carotene values between 1.37 and 6.41 mg kg^{-1} which are lower than our results.

Consumers often pay attention not only to the colour of tomato fruits, but also to the size. When comparing the results of fruits mass and taste index, it is observed that higher taste index is for smaller or bigger tomatoes (Figure 3).

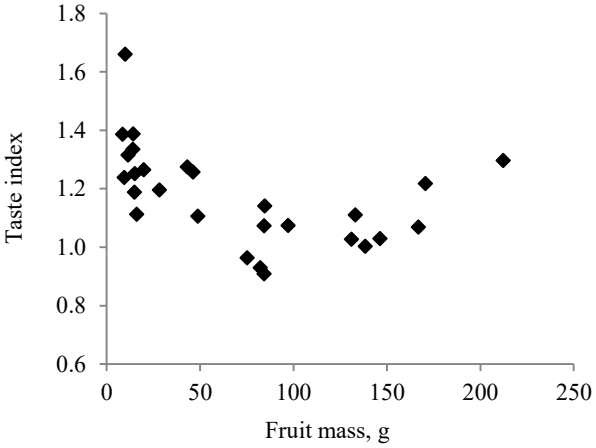

Figure 3. Connections between taste index and fruit mass

Therefore, tomatoes with average mass up to 50 g or larger than 150 g could be recommended as tasty tomatoes despite of tomatoes' colour.

Conclusions

For consumers who like milder tomatoes, yellow fruits are recommended due to less acidity, but pink, red and brown tomatoes may be interesting for consumers as rich sources of well-known antioxidant lycopene. Despite the tomato colour, less tasty are medium sized tomatoes.

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References

1. Asensio E., Sanvicente I., Mallor C., Menal-Puey S. (2019) Spanish traditional tomato. Effects of genotype, location and agronomic conditions on the nutritional quality and evaluation of consumer preferences. *Food Chemistry*, Vol. 270, p. 452–458.
2. Böhm V. (2012) Lycopene and heart health. *Molecular Nutrition and Food Research*, Vol. 56(2), p. 296–303.
3. Borghesi E., Ferrante A., Gordillo B., Rodriquez-Pulido F.J. (2016) Comparative physiology during ripeningin tomato rich-anthocyanins fruits. *Plant Growth Regulation*, Vol. 80, p. 207–214.
4. Borghesi E., González-Miret M. L., Escudero-Gilete M. L., Malorgio F., Heredia F. J., Meléndez-Martínez A. J. (2011) Effects of salinity stress on carotenoids, anthocyanins, and color of diverse tomato genotypes. *Journal of Agricultural and Food Chemistry*, Vol. 59(21), p. 11676–11682.
5. Breksa A.P., Robertson L.D., Labate J.A. (2015) Physicochemical and morphological analysis of ten tomato varieties identifies quality traits more readily manipulated through breeding and traditional selection methods, *Journal of Food Composition and Analysis,* Vol. 42, p. 16–25.
6. Central Statistical Bureau of Latvia (2017) Monthly menu of Latvia inhabitants 2017 [accessed on 15.01.2019.]. Available at: https://www.csb.gov.lv/en/statistics/statistics-by-theme/social-conditions/household-budget/search-in-theme/107-monthly-menu-latvia-inhabitant
7. Coyago-Cruz E., Corell M., Moriana A., Brahm P.M., Hernanz D., Stinco C.M., Beltrán-Sinchiguano E., Meléndez-Martínez A.J. (2019) Study of commercial quality parameters, sugars, phenolics, carotenoids and plastids in different tomato varieties. *Food Chemistry*, Vol. 277, p. 480–489.
8. Cooperstone J. L., Tober K. L., Riedl K. M., Teegarden M. D., Cichon M. J., Francis D. M., Oberyszyn T. M. (2017). Tomatoes protect against development of UV-induced keratinocyte carcinoma via metabolomic alterations. *Scientific Reports*, Vol. 7(1), p. 1–9.
9. Dhandevi P., Rajesh J. (2015) Fruit and vegetable intake: benefits and progress of nutrition education interventions. *Iranian Journal of Public Health*, Vol. 44, p.1309–1321.
10. Duma M., Alsina I., Dubova L., Erdberga I. (2015) Chemical composition of tomatoes depending on the stage of ripening. *Chemine Technologija*, No.1(66), p. 24–28.
11. Fangman L., Xietian S., Lang W., Haixu C., Yan L., Yan Z. (2018) Heredities on fruit color and pigment content between green and purple fruits in tomato. *Scientia Horticulturae*, Vol. 235, p. 391–396.
12. Fernqvist F., Ekelund L. (2013) Consumer attitudes towards origin and organic - The role of credence labels on consumers' liking of tomatoes. *European Journal of Horticultural Science*, Vol. 78(4), p. 184–190.
13. Hernandez- Suarez M., Rodriguez E.M., Diaz Romero C. (2008) Chemical composition of tomato (*Lycopersicon esculentum*) from Tenerife, the Canary Islands. *Food Chemistry*, No.106, p. 1046–1056.
14. Informative material of Ministry of Agriculture [accessed on 15.01.2019.]. Available at: https://www.zm.gov.lv/public/ck/files/ZM/tirgus/Nozaru%20parskati/Darzeni.pdf
15. Nagata M., Yamashita I. (1992) Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Journal of Japan Food Science and Technology,* Vol. 39, p. 925–928.
16. Narvez B., Letard M., Graselly D., Jost M. (1999) Les criteres de qualite de la tomate. *Infos-Ctifl*,Vol.155. p. 41–47.
17. Nielsen S. (2003) *Food analysis* (3rd ed.). New-York, Kluwer Academic/Plenum Publishers, 534 p.
18. Park M. H., Sangwanangkul P., Baek D. R. (2018) Changes in carotenoid and chlorophyll content of black tomatoes (*Lycopersicone sculentum L.*) during storage at various temperatures. *Saudi Journal of Biological Sciences*, Vol. 25(1), p. 57–65.
19. Peihoto J.V.M., Goncalvez L., Garcia C., Nascimento A., Moraes E.R., Ferreira T.A., Fernandes M.R., Pereira V. (2018) Post-harvest evaluation of tomato genotypes with dual purpose. *Food Science and Technology*, Vol. 38(2), p. 477–486.
20. Pinela J., Oliveira M.B., Ferreira I. (2016), Bioactive compounds of tomatoes as health promoters. **In**: *Natural Bioactive Compounds from Fruits and Vegetables,* Chapter 3, p. 48–91.
21. Stommel J., Abbott J.A., Saftner R.A., Camp M.J. (2005) Sensory and objective quality attributes of beta-carotene and lycopene-rich tomato fruit. *Journal of American Society for Horticultural Science*, Vol. 130, p. 244–251.
22. Tieman D., Zhu G., Resende M. F. R., Lin T., Nguyen C., Bies D., Rambla J. L., Beltran K. S. O., Taylor M., Zhang B. (2017) A chemical genetic roadmap to improved tomato flavor. *Science*, Vol. 355, p. 391–394.
23. Toor R. K., Savage G. P. (2005) Antioxidant activity in different fractions of tomatoes. *Food Research International*, Vol. 38, p. 487–494.
24. Vinha A. F., Barreira S. V. P., Castro A., Costa A., Oliveira B. P. P. (2013) Influence of the storage conditions on the physicochemical properties, antioxidant activity and microbial flora of different tomato (*Lycopersicon esculentum* L.) cultivars. *Journal of Agricultural Science,* Vol. 5(2), p. 118–128.
25. Zhang J., Zhao J., Xu Y., Liang J., Chang P., Yan F., Li M., Liang Y., Zou Z. (2015) Genome-wide association mapping for tomato volatiles positively contributing to tomato flavor. *Frontiers in Plant Science*, Vol. 6, p.1042–1050.
26. Zhao C., Chen W., Zhi W. (2012) Preliminary identification of red pigment and positive correlation between the contents of red pigment and total saponins of *Panax notoginseng* fruits, *Agricultural Science and Technology*, Vol. 13, p. 1891–1895.

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SHORT COMMUNICATION

BIOACTIVE COMPOUNDS IN FRESH AND DRIED GINGER ROOT (*ZINGIBER OFFICINALE*)

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Abstract

Ginger (*Zingiber officinale*) is known as an additive for food and therapeutic purposes. It can help improve memory, helps eliminate toxins from the body, lowering arterial pressure and cholesterol, and is a good source of many bioactive compounds – phenolic s, vitamins and mineral elements. The aim of study was to determine biological active compounds of fresh and dried ginger in aqueous extracts. Spectrophotometric methods were used for determination content of total phenolic (according to the Folin-Ciocalteu method) and flavonoid compounds (aluminium chloride method) as well as antioxidant activity with DPPH radical. Content of vitamin C was determined by titration with with 2.6-dichlorphenolindophenol. The highest content of phenolic compounds (104.7±4.5 mg GAE 100 g^{-1} DW) in water extracts was obtained using fresh ginger root; it is for 30% more than from dried root sample. The flavonoid content was higher in the samples obtained from fresh ginger peel (68.74±3.39 mg quercetin equivalent 100 g^{-1}, DW) and there were not significant differences in the use of fresh or dried ginger root (46.16±2.23 mg quercetin equivalent 100 g^{-1} on average, DW). Content of vitamin C in aqueous extract from fresh ginger root (4.59±0.98 mg 100 g^{-1}, DW) was for 21.7% higher than from fresh ginger peel, but there were not significant differences ($p>0.05$) regarding dried samples (3.43±0.71 mg 100 g^{-1}, DW). Order of antioxidant activity by free radical scavenging activity in aqueous extracts was as follows: fresh ginger root > dried ginger root > fresh ginger peel > dried ginger peel. The recommendation is that fresh ginger root is more suitable for obtaining a richer extract with the biological active compound, as the drying process affects both the phenol and vitamin C content in the samples and in the extract accordingly.

Keywords: ginger, phenolic, flavonoids, vitamin C

Introduction

Ginger (*Zingiber officinale*) is often used plant, containing many bioactive compounds such as phenolic, flavonoids, vitamins, carotenes and therefore possesses health promoting properties (Ghasemzadex et al., 2010). This herb is used as spice as well as in herbal medicinal for prevention of some diseases (Afzal et al., 2001; Ali et al., 2018; Kundu et al., 2009). Antioxidant activity of ginger has been reported by many authors (Adel, Prakash, 2010; El Ghoab et al., 2010; Gupta et al., 2014; Oboh et al., 2012; Przygodzka et al., 2014) Wilson et al., (2013) mentioned that ginger has many medicinal properties due to such bioactive compounds as gingerols, shogaols, zingerone and others.

Plant phytochemicals are biological active compounds that could impact health improvement and prevention of diseases (Kim et al., 2012). Polyphenols and flavonoids are plants secondary metabolites and their antioxidant activity is connected with redox properties. Atoiu et al., (2005) wrote that these compounds take part in chemical reactions as reducing agents and they have a metal chelation potential.

Content of organic acids in plant materials are important for their application in functional food (Yeh et al., 2014) and one of them - vitamin C is well-known antioxidant, which participate in many reactions in human body.

Herbal teas are known as significant source of different phenolic compounds (Shahidi, 2000). Different solvents may be applied for extraction of biologically active compounds, but for human consumption suitable ginger tea preparation water is the only one possible solvent. Moreover, it is known that total polyphenols and flavonoids could be found more in water extract than other extracts, because these compounds have better solubility in hot water than in other solvents (Adel, Prakash, 2010). Drying process of plants could promote changes in nutritional and antioxidant properties (Chan et al., 2009).

The aim of this study was to determine bioactive compounds of fresh and dried ginger in aqueous extracts.

Materials and Methods

Ginger samples were analysed out at the Latvia University of Life Sciences and Technologies, Department of Chemistry, in the laboratory of Inorganic and Analytic Chemistry. The ginger root used for analysis was fresh without any physical defects. The country of origin of the purchased ginger root was China. Ginger surface was cleaned, washed, peeled, snip in little pieces (moisture content of ginger and ginger peel 82.94±1.25% and 86.16±0.98% respectively) and then dried in traditional convective dryer (Memmert, Modell 100-800) at 60±1 °C till constant weight (moisture content 9.14±1.12%). 0.5±0.0001 g of finely ground fresh ginger root, fresh ginger peel, dried ginger root and dried ginger peel were extracted in 50±0.5 mL of boiling deionized water and stirred on a magnetic stirrer at room temperature +20±2 C° for 15 min. Ginger extract was filtered with paper filter (10 µm, Whatman Inc., Clifton, NJ, USA). Extracts were prepared in triplicate.

Reagents used for investigation were of the analytical grade from Sigma Aldrich, Germany. Accurate, UV-visible spectrophotometer Jenway 6705 (JENWAY, UK) was used for the absorbance measurements.

Content of total flavonoids was determined using $AlCl_3$ (aluminium chloride) method with modifications (Augšpole et al., 2018). To 500 µL of extract 2.0 mL distilled H_2O was added, then added 150.0 µL of 5% $NaNO_2$ (sodium nitrite). 5 minutes later 150.0 µL of 10% $AlCl_3$ (aluminium chloride) solution was added. The solution was to stand for 5 minutes. Then 1 mL of the 1 M NaOH (sodium hydroxide) was added. The prepared solution was mixed and incubated at room temperature +20±2 °C in dark place for 15 minutes. The absorbance of extracts were measured at 415 nm using UV/VIS spectrophotometer Jenway 6705. The obtained results were expressed as mg QE 100 g^{-1} DW (milligrams of quercetin equivalents per 100 g).

The total phenolic were analysed spectrometrically (using UV-visible spectrophotometer Jenway 6705) according to the Folin-Ciocalteu (Dewanto et al., 2002; Augšpole et al., 2018). 2.50 mL Folin Ciocalteu reagent (diluted 10 times with deionized water) was appended to 0.50 mL of extract. The obtained mixture was incubated for 3 min, after added 2.0 mL of Na_2CO_3 (sodium carbonate, 7.50 g 100 g^{-1}) and mixed. After reaction for 30 min at room temperature +20±2 °C in dark place for colour development absorbance of obtained extracts were measured at 760 nm. Investigation results were indicate as mg GAE (gallic acid equivalents) 100 g^{-1} dry-weight (DW) of ginger (Augšpole et al., 2018).

The antiradical activity of ginger extracts was determined spectrometrically (UV-visible spectrophotometer Jenway 6705) in accordance with (Afify, 2012) with modifications. This method is based on the radical scavenging ability in reacting with stable DPPH (2.2-diphenil-1-picrylhydrazyl) free radical. 3.50 mL of 2.2-diphenil-1-picrylhydrazyl solution (4.0 mg of 2.2-diphenil-1-picrylhydrazyl reagent dissolved in 100.0 mL pure ethanol) was added to 0.5 mL of ginger extract. Obtained solution was mixed and stand in dark place at room temperature (+20±2) °C for 30 min. Absorbance was measured at 517 nm. The antioxidant activity was definite as TROLOX (6-hydroxy-2.5.7.8-tertamethylchroman-2-carboxylic acid) equivalent antiradical activity (mmol TE 100 g^{-1} DW) (Augšpole et al., 2018).

Vitamin C content was determined and calculated after volumetric analysis with 2.6-dichlorphenolindophenol (Duma et al., 2015) according to the equation (1):

$$\text{Vitamin C (mg 100 g}^{-1}) = \frac{V_{titr} \cdot 0.044 \cdot V_{total} \cdot 100}{V_a \cdot \text{weight}}, \quad (1)$$

where: V_{titr} – volume of 2.6-dichlorphenolindophenol, mL;
V_{total} – total volume of infusion, mL;
V_a – volume of infusion for titration, mL.

Results of the study are means of four parallel measurements and were analysed by Microsoft Excel 2016. Differences were considered as significant at $p<0.05$.

Results and Discussion

Flavonoids are phenolic compounds that are common in different plants. These compounds have a wide range of biological functions – they protect plants from biotic and abiotic stresses, and they actively participate in the interaction between the plants and environment (Amalesh et al., 2011). Data in Figure 1 present the content of total flavonoids in the analysed ginger sample extracts.

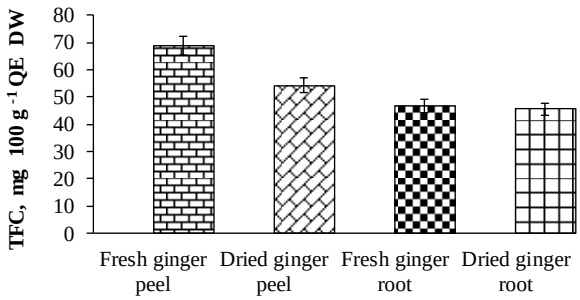

Figure 1. Content of total flavonoids

The results showed that the highest level of total flavonoids is in the aqueous extracts of fresh and dried ginger peel : 68.74±2.58 and 54.27±1.14 mg QE 100 g^{-1} DW, respectively, while the lowest was determined in the aqueous extracts of fresh and dried ginger root 46.73±2.05 and 45.59±1.75 mg QE 100 g^{-1}, DW. The content of total flavonoids found in this study were lower than the ones reported by other researchers. Researchers Yasser et al. (2016) reported that total flavonoids in ginger was 78.8 mg g^{-1} QE DW that were significantly higher than this study values.

Absorption results of phenolic compounds obtained spectrophotometrically are shown in Figure 2.

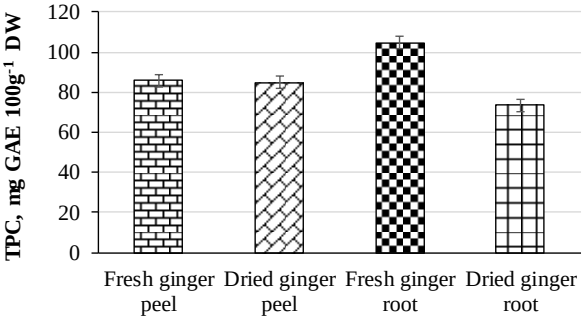

Figure 2. Content of total phenols

The total phenols content in the investigated ginger samples ranged from 104.66±3.73 (fresh ginger root) till

73.6±1.41 mg GAE 100 g^{-1}, DW (dried ginger root). Scientists Kumari and Gupta (2016) from India reported higher values of total phenols in ginger root powder, respectively 776.2 mg GAE 100 g^{-1}, DW. In turn Ghasemzadeh et al. (2010) from University Putra Malaysia reported lower values - total phenols of ginger material was 39.1±9.2 mg GAE 100 g^{-1}, DW. The total phenols content depends on the extracting solvent. Turkmen et al. (2006) reported that it is higher in ethanol extract than aqueous extract, but Shirin and Jamuna (2010) found the highest total polyphenols content in water extracts.

To determine the antioxidant activity of ginger samples, the development of their scavenging effect of free radicals on the 2.2-diphenil-1-picrylhydrazyl (DPPH) was investigated. Comparison of the mean values of antioxidant activities showed that fresh and dried root ginger aqueous extracts had the highest antioxidant activity, higher by 6.5% compared to fresh and dried ginger peel samples (Figure 3).

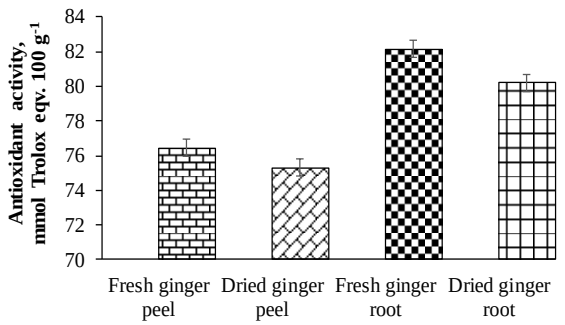

Figure 3. Antioxidant activity of ginger samples

Comparatively, Ali et al. (2008) reported that antioxidant activities of fresh ginger root was 97.47±0.93% to 99.06±1.00% $DPPH^{\cdot}$.

Natural antioxidant vitamin C (ascorbic acid) in biological systems participates in various enzymatic processes as well as in hydroxylation oxidation – reduction reactions (Singh et al., 2012). The highest content of vitamin C was found in the aqueous extract of fresh ginger root -5.59 mg 100 g^{-1} DW (Figure 4

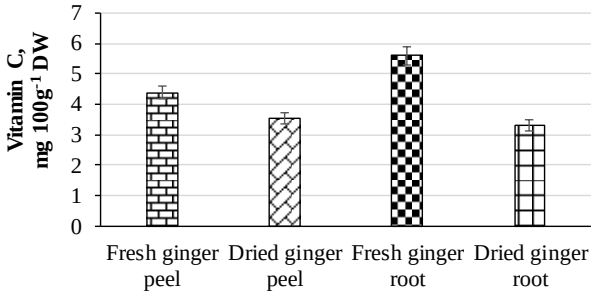

Figure 4. Content of vitamin C

The results obtained in the current study are lower than those reported by Kumari and Gupta (2016), who found that vitamin C content in ginger root powder was 9.2 mg 100 g^{-1} DW or results reported by Shirin and Jamuna (2010) – 9.33–10.97 mg 100 g^{-1} DW.

Conclusions

The results of this study showed that fresh and dried ginger has high antioxidant activity from 75.31 to 82.16 mmol Trolox equivalents 100 g^{-1} DW. The obtained results indicated that the content of total flavonoids, content of total phenols and vitamin C as well as antioxidant activity depends on the part used in the ginger plant study – whether it is peel or root. The recommendation is that fresh ginger root is more suitable for obtaining a richer aqueous extract with the biological active compounds, as the drying process affects both the phenol and vitamin C content in the samples.

References

1. Adel S.P.R., Prakash J. (2010) Chemical composition and antioxidant properties of ginger root (*Zingiber officinale), Journal of Medical Plant Research,* Vol. 4, p. 2674–2679.
2. 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,* Vol. 2(3), p. 203–209.
3. Afzal M., Al-Hadidi D., Menon M., Pesek J., Dhami M.S. (2001) Ginger: an ethomomedical, chemical and pharmacological review, *Drug Metabolism and Drug Interactions*, Vol. 18, p. 159–190.
4. Ali A.M.A., El-Nour M.E.M., Yagi S.M. (2018) Total phenolic and flavonoid contents and antioxidant activity of ginger (*Zingiber officinale* Rosc.) rhizome, callus and callus treated with some elicitors, *Journal of Genetic Engineering and Biotechnology,* Vol. 16(2), p. 677–682.
5. Amalesh S., Gouranga D., Sanjoy K.D. (2011) Roles of flavonoids in plants. *International Journal of Pharmacy and Pharmaceutical Sciences,* 6(1), p. 12–35.
6. Atoui A.K., Mansouri A., Boskou G., Kefalas P. (2005) Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food Chemistry*, Vol 89, p. 27–36.
7. Augšpole I., Dūma M., Ozola B. (2018) Bioactive compounds in herbal infusions. *Agronomy Research,* Vol. 16(2), p. 1322–1330.
8. Chan E., Lim Y.Y., Wong S.K., Lim K.K., Tan S.P., Lianto F.S., Yong M.Y. (2009) Effects on different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chemistry*, Vol. 113, p. 166–172.
9. 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,* Vol. 50(10), p. 3010–3014.
10. El-Ghoab A.H., Nauman M., Anjum F.M., Hussain S., Nadeem M. (2010) A comparative study on chemical composition and antioxidant activity of Ginger *(Zingiber officinale)* and Cumin *(Cuminum cyminum). Journal of Agricultural and Food Chemistry,* 58:8231, p. 7.
11. Ghasemzadeh A., Jaafar H.Z.E., Rahmat A. (2010) Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules*, Vol. 15(6), p. 4324–4333.

12. Gupta S.K., Sharma A. (2014) Medical properties of *Zingiber officinale* Roscoe. A review. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, Vol. 9, p. 124–129.
13. Kim I.L., Yang M., Goo T.H., Jo C., Ahn D.U., Park J.H., Lee O.H., Kang S.N. (2012) Radical scavenging-linked antioxidant activities of commonly used herbs and spices in Korea. *International Journal of Food Science and Nutrition*, Vol. 63, p. 603–609.
14. Kumari S., Gupta A. (2016) Nutritional composition of dehydrated ashwagandha, shatavari, and ginger root powder. *International Journal of Home Science,* Vol. 2(3), p. 68–70.
15. Kundu J.K., Na H.K., Surh Y.J. (2009) Ginger- derived phenolic substances with cancer preventive and therapeutic potential. *Forum of Nutrition*, Vol. 61, p. 182–192.
16. Oboh G., Akinyemi A.J., Ademiluyi A.O. (2012) Antioxidant and inhibitory effect of red ginger (*Zingiber officinale* var. Rubra*)* and white ginger (*Zingiber officinale* Roscoe), on Fe^{2+} induced lipid peroxidation in rat brain. In vitro. *Experimental and Toxicologic Pathology*, Vol. 64, p. 31–36.
17. Przygodzka M., Zielinska D., Ciesarova Z., Kukurova K., Zielinski H. (2014) Comparison of methods for evaluation of the antioxidant capacity and phenolic compounds in common spices. *LWT-Food Science and Technology*, Vol. 58, p. 321–326.
18. Shahidi F. (2000) Antioxidants in food and food antioxidants. *Nahrung*, Vol. 44(3), p. 158–163.
19. Shirin A.P.R., Jamuna P. (2010) Chemical composition and antioxidant properties of ginger root (*Zingiber officinale). Journal of Medicinal Plants Research*, Vol 4(24), p. 2674–2679.
20. Singh D.P. Beloy J., Mclnerney J. K., Day L. (2012) Impact of boron, calcium and genetic factors on vitamin C, carotenoids, phenolic acids, anthocyanins and antioxidant capacity of carrots *(Daucus carota). Food Chemistry,* Vol. 132(3). p. 1161–1170.
21. Turkmen N., Sari F., Velioglu Y. (2006) Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chemistry, Vol.* 99(4), p. 835–841.
22. Wilson R., Haniadka R., Sandhya P., Palatty P.L., Baliga M.S. (2013) Ginger (*Zingiber officinale* Roscoe) the dietary agent in skin care: A review. In: Watson R.R., Zibadi S. editors. *Bioactive dietary factors and plat extracts in dermatology*. Karnataska: Humana Press, p. 103–111.
23. Yasser H.I.M., Guruprasad N., Shaukath A.K. (2016) Evaluation of antioxidant activity of locally available green teas in India, *Der Pharmcia Lettre*, 8(8), p. 374–379.
24. Yeh H.Y., Chuang C.H., Chen H.C., Wan C.J., Chen T., Lin L.Y. (2014) Bioactive components analysis of two various gingers (*Zingiber officinale* Roscoe) and antioxidant effect of ginger extracts, *LWT-Food Science and Technology*, Vol. 55, p. 329–334.

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SHORT COMMUNICATION

SHELF LIFE ASSESSMENT OF MEAT PIES

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Abstract

Demand for ready-to-use and frozen baked goods is increasing. It provides the supply of fresh products as close as possible to the final consumer. One of the traditional and popular products in Latvia is meat pies. The purpose of the study was to determine the shelf life of ready-to-eat, frozen and defrosted meat pies by evaluating the possible impact of various factors on quality and microbiological parameters. The study analysed classical meat pies from yeast dough with smoked meat and onion filling from one Latvian bread producer. Each of the two types of meat pies were frozen after baking, packed in two different packaging materials and stored at -18 °C for five days. The defrosting of pies was carried out at room temperature (23±2 °C). Tests for frozen products was started immediately after their defrosting and every 24 hours during storage. Physicochemical and microbiological indicators were determined for meat pies. The identification of microorganisms present in the products by species was performed using API CHB/E biochemical test. Potential sources of microbial contamination at the plant throughout the production process were also evaluated. The results of the study showed that shelf life of baked, frozen and defrosted cut meat pies was 72 hours, for classic meat pies shelf life did not exceed 48 hours. The study did not identify any positive effects of the selected packaging materials on the extension of shelf life. The identified microorganisms, which reduce the storage time of the products and food safety, were *B. subtilis* and *B. licheniformis*.

Keywords: meat pies, frozen products, defrosting, shelf life

Introduction

Freezing is one of the ways to extend the shelf life of products, increase production efficiency, and offer retailers and consumers safe, qualitive and fresh food. Freezing process reduces the content of the liquid phase in the product, which prevents microbial and enzymatic processes (Symons, 1994; Vasafi et al., 2019). The use of frozen ready-to-use products at retail outlets gives traders the opportunity to offer fresh products on the day of purchase (Meziani et al., 2012).

Inappropriate storage of raw materials of baked goods can lead to impairment in the quality of the product. The shelf life of baked-frozen products is determined by the quality of production, the cleanliness of the premises and the hygienic conditions of storage. The average shelf life of meat-filled dough pies after defrosting is 2–3 days. Frozen products can be stored for up to 12 months at -18±3 °C (Symons, 1994). After defrosting, the products become wastage during storage, which is about 4–5% by weight of the product. When the product is in a closed packaging, wastage increases the moisture content in the packaging, which promotes favourable conditions for the development of micro-organisms. Properly selected packaging ensures the longest possible storage time (Gupta et al., 2016; Krämer, Prange, 2016). Spore-forming microorganisms affect health, including *Bacillus cereus* and *Bacillus subtilis* (Pepe et al., 2003; Benwart, 2004; Saranraj, Geetha, 2012; Madigan et al., 2014).

The speed of growth and development of the microorganisms in the product depends on storage temperature, composition of the product, water activity (a_w), pH of the product and air quality in the production plant. By varying these factors, the deterioration and shelf life of the product is affected (Krämer, Prange, 2016; Lelieveld et al., 2016; De Boeck et al., 2017).

Bacterial spores can be in the air. In this way, they can cause visible damage to the food. Spore damage causes the formation of an unusual flavour and taste. Product deterioration is facilitated by enzyme reactions with carbohydrates, fats and proteins in the product, thereby promoting enzymatic, lipolytic and proteolytic changes. If *Bacillus* bacteria are detected in the company during air analysis, these organisms are difficult to control and eliminate (Sorokulova et al., 2003; Smith et al., 2010; Viedma et al., 2011). *Bacillus* spp. endospores are more resistant to elevated temperatures, UV and gamma rays than their vegetative cells. They die in the crumb and crust of the product at 160 °C in dry air for 2 hours or at 150 °C in water vapour environment after 15 minutes (Parihar, 2013; Stewart, 2015).

Constructive design and layout of industrial premises, production flows, and outdoor air pollution in production premises can affect the quality of finished products. It is therefore important to carry out systematic hygiene control and monitoring. During baking, the temperature in the centre of the product is close to 100 °C, but the temperature exposure time does not allow the bacterial endospores to die. Therefore, special control should be given to rooms where dough and filling are prepared (Benwart, 2004). Pollution in a manufacturing plant may result from inadequate cleaning of premises or hygiene practices that do not comply with the production specification. Even if the equipment is easy to disassemble and clean, it does not exclude the possibility that microorganisms may form on them, as well as dust or flour particles from the air (Lelieveld et al., 2016). Good personal hygiene can prevent unwanted contamination of products with

microorganisms, including the pathogens. Bacteria that cause food poisoning can be found on the hands of every employee, even a healthy person (Chao, 2003; Gupta et al., 2016).
The aim of the study was to determine the shelf life of ready-to-eat, frozen and defrosted meat pies by evaluating the possible impact of various factors on quality and microbiological parameters.

Materials and Methods

Materials

The meat pies from the yeast dough with smoked meat and onion filling were obtained from a Latvian bakery. One pie has a weight of 15 g, of which 10 g is a yeast dough and 5 g of meat filling (smoked pork cheeks meat with fresh onions, salt and pepper). After baking, the pies were frozen at -30±2 °C temperature, packed and stored at -18±2 °C. The defrosting was performed at room temperature (23±2 °C).

Physicochemical analysis

The water activity (a_w) was determined using a_w meter Novasina LabSwift-AW according to standard ISO 18787:2017 (ISO, 2017).
Moisture content was determined using Kern MLB N Air-Owen according to standard AACCI 44-15.02.
pH was determined using Jenway 3510 pH meter according to standard AACC 02-52.01.

Microbiological analysis

Samples of products for analysis of microorganisms were prepared according to standard ISO 6887-4:2017 "Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions" (ISO, 2017). Triplicate plates were prepared using pour plate method for enumeration of total plate count on Plate Count Agar (Ref. 01-161, incubation at 30 °C for 72 h).
Swabs from the hands were taken in accordance with standard ISO 18593:2018 "Microbiology of the food chain. Horizontal methods for surface sampling". Swabs were applied to agar media "Biolife" Endo Agar (Ref. Nr. 4014602, incubation at 30 °C for 72 h). Testing of *E. coli* was performed according to standard ISO 7251:2005 (ISO, 2005). Gram staining, followed by API identification system (Ref. 50300, API50 CH strips, API50 CHB Medium, incubation at 30 °C for 48 h) was used for identification of microorganisms.

Product packaging

Two packaging materials were used in the study – DuPont Teijin Films, Milar OL40, single layer polyester packaging bags with 40 µm thickness, size 200×400 mm (marked in the text - PL), and single layer transparent crystallized polypropylene packaging bags with thickness 25 µm, size 200×400 mm (marked in the text – PU). The weight of one package was 200 g±15 g.

Statistical analysis

All measurements were performed in triplicate and reported as mean and standard deviation. The standard deviation and confidence level was calculated using a Microsoft Office Excel v13.0 application. For statistical processing of total plate count (TPC) in graphs, the results are expressed as lg CFU g^{-1}. The closeness of the relationships was determined by the coefficient "r" within the range [-1; 1]. Pearson's correlation coefficient was used for the cross-analysis of results indicators. The values were considered to be significantly different when $p<0.05$.

Results and Discussion

Sanitary hygienic control of workers was carried out during production. Swabs from the both hands of 10 employees were taken. Six employees worked on packing of frozen products, four – on product forming. During the work there is no rotation of these employees in the different departments of bakery. The presence of intestinal bacteria was not detected on the staff's hands in the packaging and forming department.
Meat pies are a combined product that contains vegetable and animal raw materials. Such physical factors as water activity (a_w) and pH are decisive factors in the development of microorganisms as bacteria, moulds, microscopic fungi development in baked goods (Kilcast, Subramaniam, 2011). Water activity (a_w) can be one of the parameters for monitoring food safety and shelf life. The manufacturer can then obtain complete information on changes in the product during production (Jay et al., 2005).
Water activity (a_w) was determined in frozen meat pies after thawing and for the next three days during storage in two different packaging materials.

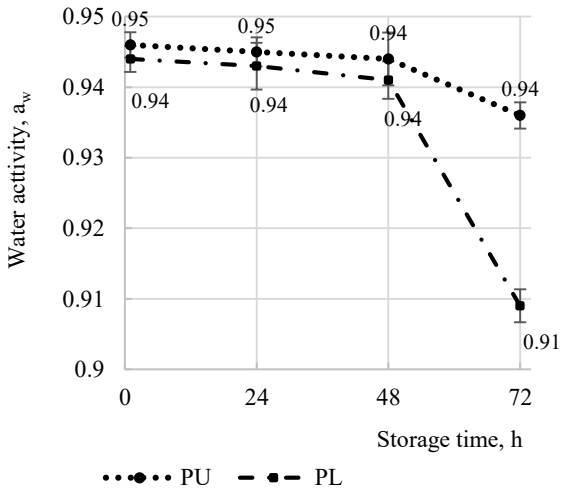

Figure 1. Water activity in meat pies during the storage time
PL – polyester packaging, PU – polypropylene packaging

The results of the study show that the water activity (a_w) of meat pies packed in PU packaging decreased by 1.06% (from 0.946 to 0.936) during storage, but in pies packed in PL packaging – by 3.71% (from 0.944 to

0.909). Comparing the packaging differences, we can say that for products packaged in PU-packaging, water activity (aw) was by 2.89% higher than in the PL-packaging (Fig. 1).

Moisture analysis showed that changes in moisture content during storage were not significant ($p>0.05$). Moisture content of pies during storage packed in PU increased by 0.58% (from 34.3 to 34.5%), but packed in PL – by 0.89% (from 33.7 to 34.0%). Changes in moisture content of less than 1% indicated that both types of packaging during storage prevent the release of moisture generated during defrosting, which contributes to the development of microorganisms.

Microorganism activity and product composition contribute to pH changes in the product. The pH of the product is one of the parameters for monitoring food safety and shelf life, so it should be periodically determined for each batch of products (Jay et al., 2005). In all samples, regardless of the type of packaging, there was a decrease in pH. During storage for meat pies in PU packaging, pH dropped from 6.06 to 5.70, while for meat pies packaged in PL packaging, the pH dropped from 6.14 to 5.81 (Fig. 2). Analysis shows that changes in pH during storage in different packaging material were not significant ($p>0.05$).

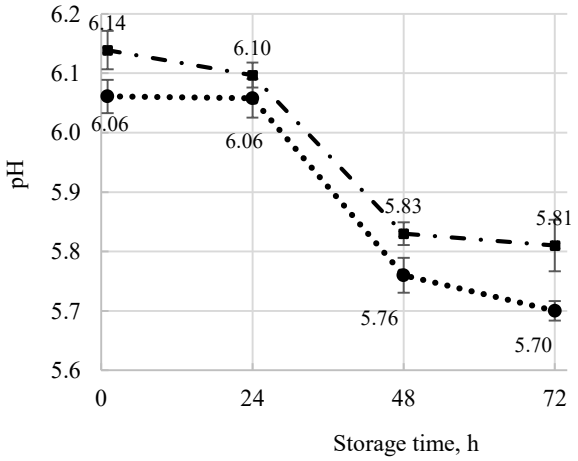

Figure 2. pH in meat pies during the storage time
PL – polyester packaging, PU – polypropylene packaging

The results show that the packaging material did not affect the growth rate of microorganisms. The TPC of the meat pies packed in PU packaging reached 3.53 lg CFU g^{-1} after 48 hours and in meat pies packed in PL packaging – 3.51 lg CFU g^{-1} (Fig. 3). According to the guidelines for assessing microbiological safety, the TPC for bakery is "satisfactory" below 10^4 CFU g^{-1}, "border line" between 10^4–10^6 CFU g^{-1} and "unsatisfactory" when more than 10^6 CFU g^{-1} (Health Protection Agency, 2009). The study followed the quality standards of the bakery for their products. They require that the size of the CFU indicator for meat-filled baked goods should not exceed 3.50 lg CFU g^{-1}. Therefore, the meat pies became "unsatisfactory" within 48 hours at ambient conditions after defrosting.

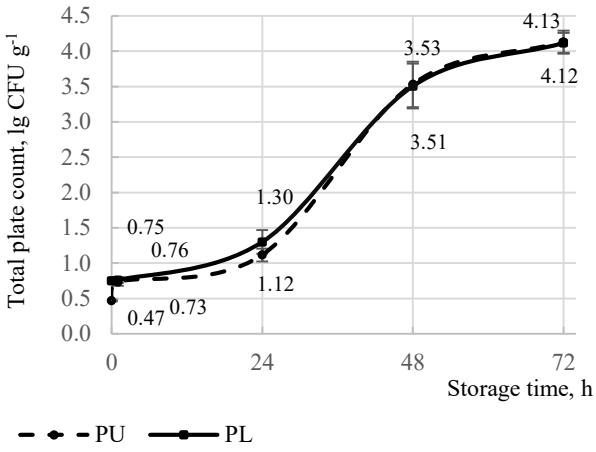

Figure 3. Total plate count of packaged meat pies during the storage time
PL – polyester packaging, PU – polypropylene packaging

If storage after defrosting exceeds 48 hours, then the product is considered unsafe for human consumption.

The close correlation exists between the total number of bacteria and pH, regardless of the packaging material. Pearson's correlation between these indicators was close and negative (Fig. 4). The correlation indicates if the total plate count decreases, the pH of the products is approaching the frozen and two-hour defrosted product parameters.

After microbial testing of products, it can be concluded that none of the product samples showed the presence of *E. coli*. All samples had bacteria from the *Bacillus* family, aerobic endospores. Bacterial endospores are found in products from raw materials, filling and forming process. No mould formation on the surface of the products was found during the defrosting-time of the finished products and throughout the storage. This can be explained by the fact that the oven temperature of 180 °C is high enough to destroy mould spores.

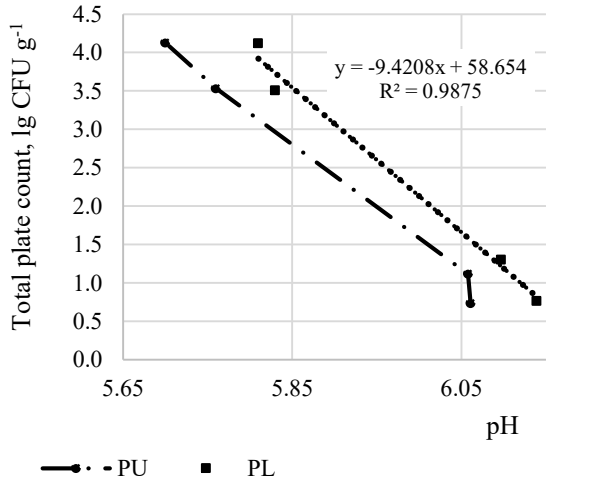

Figure 4. Pearson's correlation between total plate count and pH of meat pies in two packaging materials
PL – polyester packaging, PU – polypropylene packaging

As we know from literature, the deterioration in product quality is caused by *B. subtilis* and *B. licheniformis*. These bacteria form endospores that are heat resistant and are present in flour and air (Sorokulova et al., 2003). According to the results of the API test, after 72 hours of storage all samples of the meat pies showed signs of deterioration caused by the *Bacillus* species. Changes in quality also were observed. The samples had an unpleasant flavour, crust was unusual for the fresh product, it was soft and crumbled easily.

Packaging in which the products were defrosted and stored contributed to the growth of the bacteria because during defrosting packaging material did not allow the removal of moisture which is always released during defrosting. Similar results have been obtained by other scientists (Saranraj, Geetha, 2012).

Conclusions

Although water activity (a_w) in meat pies slightly decreased during three-day storage, it was high enough (0.946) for moisture to be available for the development of microorganisms.

The microbiological deterioration of the products was caused by *Bacillus* bacteria, of which *Bacillus subtilis* and *Bacillus licheniformis* were identified. Product contamination in the process of forming products with *Bacillus* bacteria more often is possible from air and raw materials.

In the study, comparing the packaging of products from polypropylene and polyethylene material, it can be concluded that both types of packaging are similar and do not prevent the development of microorganisms.

The shelf life of frozen meat pies after defrosting is up to 48 hours in the polypropylene or polyethylene packaging.

References

1. Benwart G. J. (2004) *Basic Food Microbiology* (2nd edn.). CBS Publishers & Distributors Pvt. Ltd. 773 p.
2. Chao T. S. (2003) *Workers personal hygiene in Food plant sanitation*. New York: Marcel Dekker Inc., p. 132–145
3. De Boeck E., Jacxsens L., Goubert H., Uyttendaele M. (2017) Ensuring food safety in food donations: Case study of the Belgian donation/acceptation chain. *Food Research International,* Vol. 100 (2), p. 137–149.
4. Gupta R. K., Dudeja P., Minhas S. (2016) *Food Safety in the 21st Century: Public Health Perspective*. Elsevier Science. 624 p.
5. *Health Protection Agency. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods. London, 2009* [accessed on 15.01.2019.]. Available: https://www.gov.uk/govern-ment/publications/ready-to-eat-foods-microbiological-safety-assessment-guidelines https://www.iso.org /standard/63335.html
6. *International Organization for Standardization (ISO), 2017. Foodstuffs - Determination of water activity* [accessed on 14.01.2019]. Available: https://www.iso.org/standard/63379.html
7. *International Organization for Standardization (ISO). ISO 6887-4:2017 Microbiology of the food chain. -* [accessed on 14.01.2019]. Available: https://www.iso.org/obp/ui/#iso:std:iso:6887:-4:ed-2:v1:en
8. *International Organization for Standardization (ISO). ISO 7251:2005. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of presumptive Escherichia coli* [accessed on 14.01.2019]. Available: https://www.iso.org/standard/34568.html
9. Jay J. M., Loessner M. J., Golden D. A. (2005) *Intrinsic and extrinsic parameters of foods that affect microbial growth*. New York: Springer Science & Business Med Inc., p. 39–60
10. Kilcast D., Subramaniam P. (2011) *Food and Beverage Stability and Shelf Life* (1st edn.). Woodhead Publishing. 864 p.
11. Krämer J., Prange A. (2016) *Lebensmittel-Mikrobiologie*. Stuttgart: Uni-Taschenbücher. p. 400.
12. Lelieveld H. L. M., Holah J., Mostert M. A. (2016) *Handbook of Hygiene Control in the Food Industry* (2nd edn.). London: Woodhead Publishing. 720 p.
13. Madigan M. T., Martinko J. M., Bender K. S., Buckley D. H., Stahl D. A., Brock T. (2014) *Brock Biology of Microorganisms* (14th edn). New Jersey: Pearson. 1032 p.
14. Meziani S., Kaci M., Jacquot M., Jasniewski J., Ribotta P., Muller J.M., Ghoul M., Desobry S. (2012) Effect of freezing treatments and yeast amount on sensory and physical properties of sweet bakery products. *Journal of Food Engineering,* Vol. 111 (2), p. 336–342.
15. Parihar H. S. (2013) *Bacillus cereus*. Encyclopedia of Toxicology (3rd edn.). p. 353–354.
16. Pepe O., Blaiotta G., Moschetti G., Greco T., Villan F. (2003) Rope-producing strains of *Bacillus* spp. from wheat bread and strategy for their control by lactic acid bacteria. *Applied and Environmental Microbiology*, p. 2321–2329.
17. Saranraj P., Geetha M. (2012) Microbial spoilage of bakery products and its control by preservatives. *International Journal of Pharmaceutical & Biological Archives*, Vol. 3(1), p. 38–48.
18. Smith J. P., Daifas D. P., El-Khoury W., Koukoutsis J., El-Khoury A. (2010) Shelf life and safety concerns of bakery products — A Review. *Critical Reviews in Food Science and Nutrition*, Vol. 44(1), p. 19–55.
19. Sorokulova I. B., Reva O. N., Smirnov V. V., Pinchuk I. V., Lapa S. V., Urdaci M. C. (2003) Genetic diversity and involvement in bread spoilage of *Bacillus* strains isolated from flour and ropy bread. *Letters in Applied Microbiology,* Vol. 37(2), p. 73–169.
20. Stewart G. C. (2015) The exosporium layer of bacterial spores: A connection to the environment and the infected host. *Microbiology and molecular biology reviews*, Vol. 79(4), p. 437–457.
21. Symons H. Frozen foods, **In:** Shelf life evaluation of foods (C. M. D. Man and A. A. Jones, eds.), Blackie Academic & Professional, London, 1994, p. 298–311.
22. Vasafi P.S., Hamdami N., Keramat J. (2019) Quality and microbial stability of part-baked “Barbari bread” during freezing storage. *LWT – Food Science and Technology*, Vol. 104, p. 173–179.
23. Viedma P. M., Abriouel H., Omar N. B., López R. L., Gálvez A. (2011) Inhibition of spoilage and toxigenic *Bacillus* species in dough from wheat flour by the cyclic peptide enterocin AS-48. *Food Control,* Vol. 22(5), p. 756–761.

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SHORT COMMUNICATION

FRUIT AND VEGETABLE CONSUMPTION IN LATVIAN SCHOOLS WITH VARIOUS TRAINING PROGRAMS ON HEALTHY DIET

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Abstract

Fruit and vegetable recommendations for pupils are similar in the world and in Latvia, that is, at least 5 servings per day, but this is not the case most often. The objective of this study was to assess the contribution of schools with various training programs on healthy diet to raising the fruit and vegetable consumption among pupils. The study involved pupils in grades 6, 9 and 11 from Latvian schools with various training programs for healthy diet in order to analyse students' theoretical knowledge about the role of fruits and vegetables in the diet and their practical experience). Pupils' knowledge and understanding of the consumption and importance of fruit and vegetables in the diet was strongly influenced by gender ($p<0.05$), and in some cases by age, while school training program on healthy diet had no significant impact ($p>0.05$). Pupils lacked understanding about the amount of the required fruit and vegetable consumption because one third of the pupils thought that eating 5 servings of fruit and vegetables was unnecessary. The pupils did not understand the essence of a healthy diet because the portion of fried potatoes was indicated as one vegetable portion. Uniformity was observed in the pupils' diet, cucumbers, tomatoes and carrots were the most consumed vegetables, while within fruits - apples and bananas. Improving the pupils' healthy diet program should take into account the different interests of the pupils' gender, age and desire to acquire knowledge in practice.

Keywords: fruit, vegetables, consumption, knowledge, pupils

Introduction

Nutrition has a long-term role in the development, growth and health of the child (Dreher, 2018). Eating fruits and vegetables instead of foods with high fat, salt and sugar content protects children from obesity, diabetes, cardiovascular diseases (Dreher, 2018; Fernandez, Marette, 2017; Tang et al., 2017; Hartley et al., 2013). In turn, it provides a balanced diet use in adult life (Forestell, 2017; Ventura, 2017; Benton, 2004; Cooke et al., 2004). Fruit and vegetable consumption is influenced by many factors such as availability, knowledge, attitudes at home and school, influence of parents and peers, local climate and costs (Sharps et al., 2015; Ventura, Worobey, 2013; Rasmussen et al., 2006; Benton, 2004).

Vegetable and fruit consumption of children is relatively less studied compared to adults, but studies have shown that children in Europe consume 86 g of vegetables and 141 g of fruit on average, covering only 6–24% of the recommended daily consumption of vegetables and fruits (Elmadfa et al., 2009). There was a tendency among the surveyed EU countries that girls consume more fruit and vegetables than boys (Fruit and vegetable …, 2014). The lowest rates of fruit consumption were observed in Greece, Latvia, Lithuania and Sweden, while the lowest vegetable consumption was found in Spain and Estonia (Fruit and vegetable …, 2014). Research data of Latvian pupils showed that the total fruit consumption – at least once a day – could be attributed only to one fifth of pupils, while vegetable consumption – one vegetable daily – only to one quarter of pupils which was inadequate (Pudule et al., 2015).

Projects aimed at increasing the consumption of fruit and vegetables among pupils that takes place in schools, as it is possible to create, organize various types of activities, such as a traditional training program on nutrition in classrooms, school gardening, cooking (Knai et al., 2006). In order to achieve a better effect, the activities carried out in schools should be diverse and long-lasting. The practical approach is marked as more promising and more effective than classical lessons (Battjes-Fries et al., 2017; DeCosta et al., 2017; Laureati et al., 2014; Knai et al., 2006). In 2007, the European Commission set itself the objective of reducing child obesity, increasing the consumption of fruit and vegetables by launching the School Fruit Program in 2009. The School Fruit Program is a program that provides schools with free fruit and vegetables, thus ensuring fruit and vegetable availability for children. The Norwegian study showed that the School Fruit Program reduced the consumption of unhealthy snacks (Overby et al., 2012).

The program "School Fruit" has been implemented in Latvia since 2010 and by 2018 more than 5,000 tonnes of fruit and vegetables were distributed (Current activity results …..). These data suggest that there should be no problems with sufficient fruit and vegetable consumption among Latvian pupils, but is it true?

The objective of this study was to assess the contribution of schools with various training programs on healthy diet to raising the fruit and vegetable consumption among pupils.

Materials and Methods

The participants of the study were 6th, 9th and 11th grade pupils whose theoretical knowledge and practical approach to the consumption of fruit and vegetables was collected through questionnaires. The pupils represented schools with a diverse training program on healthy diet: schools with a classical training program (SchCTP) and schools involved in the "Healthy School Network" (SchHSN) (Table 1). The schools involved in

the study were located in Riga; two of the schools were with a classical training program and three schools were involved in the "Healthy School Network". Gender distribution was 163 boys and 167 girls.

Table 1

Number of pupils involved in the study by type of a school and a class

Class	Total	Schools	
		SchCTP	SchHSN
6	135	61	74
9	149	72	77
11	46	35	11
Total	330	168	162

SchCTP - schools with a classical training program, SchHSN - schools involved in the "Healthy School Network"

The questionnaire consisted of 15 questions, where questions were open and multiple choice. It was anonymous, indicating only the gender and age of the pupil. Pupils completed the questionnaire at school, without limiting time. The questionnaire included questions about the pupil's knowledge of healthy eating, the necessary amount of fruit and vegetable consumption, as well as the issues that characterize the pupil's personal opinion and experience of daily consumption of fruit and vegetables. Survey results were processed using the SPSS statistical program, where each research participant was assigned an identification code. The result was considered statistically significant if p was less than 0.05.

Results and Discussion

Pupils' knowledge of a healthy diet was superficial, which revealed that pupils knew fruit and vegetables contain minerals and vitamins, but had no idea of the importance of fibre. In addition, one portion of fried potatoes was indicated as a single portion of vegetables, because the pupils did not know that potatoes were not counted as vegetables due to the high starch content. Girls would be interested to learn more about healthy eating, while 63% of boys would not like to do so, but admitted that it would be useful for their athletic growth. While assessing pupils' views on the need for five portions of fruit and vegetables every day, it became clear that it is not emphasized in schools nor explained at all (Figures 1 and 2).

The answer "unnecessary" was most often given by pupils from schools with classical training program (40%), boys (38%) and 11th grade pupils (43%). An interesting relationship was observed between the results: the older the pupil, the more often the answer "unnecessary" was mentioned. This could be explained by the fact that the 11th grade pupils define and make their own menus, have their own opinion and cannot be longer influenced by parents or teachers.

Gender differences in this study confirmed the literature data that boys use less fruit and vegetables than girls (Fruit and vegetable …, 2014).

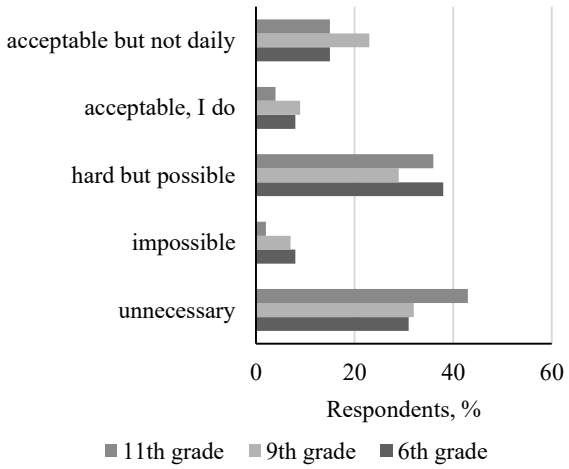

Figure 1. Pupils' opinion on the consumption of 5 portions of fruit and vegetables per day according to pupils' age, %

28% of pupils from schools involved in the "Healthy School Network" indicated that 5 servings of fruit and vegetables per day are not required. It is difficult to explain why such a large number of pupils are not aware of it, but it could be related to the passive learning method - lectures that do not give the required result. Several studies show that a practical approach provides better results than the passive lectures (Battjes-Fries et al., 2017; DeCosta et al., 2017; Laureati et al., 2014; Knai et al., 2006).

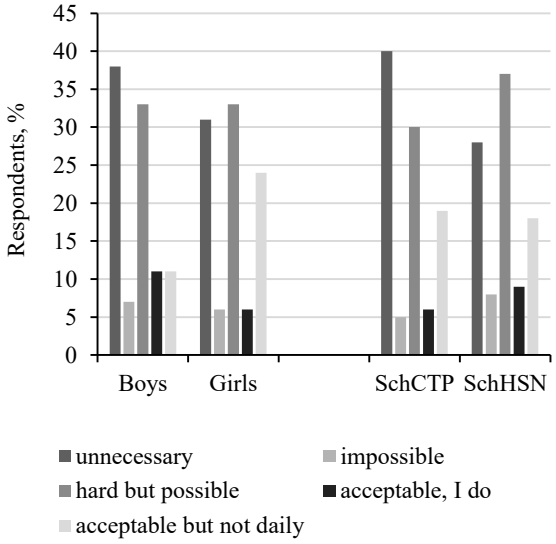

Figure 2. Pupils' views on the consumption of 5 portions of fruit and vegetables per day by gender and depending on the school, %

SchCTP – schools with a classical training program, SchHSN – schools involved in the "Healthy School Network"

When asking pupils what they think is the optimal fruit and vegetable consumption per day, the most popular answers were 1 or 2 portions (Table 2 and 3).

The answers could be explained by the actual daily consumption of fruit and vegetables by pupils. It is

known that the consumption of fruit and vegetables among pupils is lower than the recommendation (Yngve et al., 2005). It is a positive fact that only a small number of the surveyed pupils believed that there is no need to eat fruit and vegetables at all. However, there were also pupils who thought that fruit and vegetables could be eaten without any restrictions. This shows that pupils do not understand that a healthy diet should also be used in reasonable quantities.

Table 2

Pupils' views on the required amount of fruits and vegetables according to pupils' gender, %

Amount	Fruit		Vegetables	
	Boys	Girls	Boys	Girls
1 portion	32	34	47	45
2 portions	31	43	21	32
3 portions	6	5	4	8
The more the better	29	17	23	14
No need	2	1	5	1

When analysing data between schools with a diverse training program on healthy diet, no significant differences ($p>0.05$) were found in the pupils' responses (Table 3).

Table 3

Pupils' views on the required quantities of fruit and vegetables depending on the school, %

Amount	Fruits		Vegetables	
	SchCTP	SchHSN	SchCTP	SchHSN
1 portion	35	31	48	44
2 portions	41	33	27	27
3 portions	4	7	6	6
The more the better	19	27	17	19
No need	1	2	2	4

SchCTP – schools with a classical training program, SchHSN – schools involved in the "Healthy School Network"

The results of the study showed that, according to the pupils, it is necessary to consume more fruit than vegetables. On the issue of commonly used fruit and vegetables, pupils first named apples (70.8%), then bananas (53.2%), oranges (23.1%), kiwis (11.4%). Less than 10% of pupils also mentioned pears, mandarins, grapes, mango, strawberries, and pineapples. The most popular vegetables among pupils were cucumbers (46.5%), tomatoes (44.9%), carrots (29.8%), potatoes (27.7%), and cabbage (10.2%). Less than 10% of pupils also mentioned lettuce, broccoli, paprika, avocado, and beets. Vegetables, which were most often mentioned as tasteless, were broccoli (13.9%), pumpkin (11.8%), and eggplant (10.1%), while for fruit it were kiwis (6.7%) and mango (6.7%).

The data showed that the training program on healthy diet in school has no significant impact on pupils' knowledge and understanding of fruit and vegetable consumption and nutritional importance (Table 4). It is known that schools involved in the "Healthy School Network" teach pupils more about healthy eating, including fruit and vegetable consumption, but the results showed no significant differences ($p>0.05$). This showed that passive learning in the form of lectures did not produce the desired result. The significant difference was identified between the genders ($p<0.05$). Girls showed greater interest in learning the basics of healthy eating, had a better knowledge of fruit and vegetable consumption while boys were less interested. The results showed that the training method on healthy diet should be based on the needs of each gender. The boys mentioned that dietary training would be much more attractive if nutrition topics were linked to sport, how to build a healthy menu to promote athletic achievements. Furthermore, pupils indicated that they wanted to learn more through practice rather than theory.

Table 4

Differences between pupils' knowledge, fruit and vegetable consumption and other characteristics

Evaluated indicators	School	Gender	Grade
Pupils' desire to learn more about healthy eating	$p>0.05$	$p<0.05$	$p>0.05$
Pupils' desire to learn more about cooking	$p>0.05$	$p<0.05$	$p<0.05$
Necessary amount of fruit in daily diet	$p>0.05$	$p<0.05$	$p<0.05$
Necessary amount of vegetables in daily diet	$p>0.05$	$p<0.05$	$p<0.05$
Pupils' views on 5 portions of fruit and vegetables per day	$p>0.05$	$p<0.05$	$p>0.05$
Pupils' desire to learn about fruit and vegetable growing and their use in nutrition	$p>0.05$	$p>0.05$	$p>0.05$

While assessing the impact of age, it was concluded that older pupils had a better knowledge and understanding of the consumption of fruit and vegetables and their role in the diet. Families have an important role to play in the choice of nutrition for younger pupils, as they make the child's dietary habits and understanding of a healthy diet.

Conclusions

Pupils' knowledge and understanding of the consumption and importance of fruit and vegetables in the diet was strongly influenced by gender ($p<0.05$), while school training program on healthy diet had no significant impact ($p>0.05$). Pupils lacked understanding about the amount of the required fruit and vegetable consumption, in addition it was insufficient and uniform. The most commonly used vegetables were cucumbers, tomatoes and carrots, while the most popular fruit were apples and bananas. Pupils did not know that potatoes are not counted within vegetables due to their high starch content. Improving the pupils' healthy diet program should take into account the different interests of the pupils' sex, age and desire to acquire knowledge in practice.

References

1. Battjes-Fries M.C.E., Haveman-Nies A., Zeinstra G.Z., van Dongen E.J.I., Meester H.J., van den Top-Pullen R., van't Veer P., de Graaf K. (2017) Effectiveness of taste lessons with and without additional experiential learning activities on children's willingness to taste vegetables. *Appetite*, Vol. 109, p. 201–208.
2. Benton D. (2004) Role of parents in the determination of the food preferences of children and the development of obesity. *International Journal of Obesity*, Vol. 28, p. 858–869.
3. Cooke L.J., Wardle J., Gibson E.L., Sapochnik M., Sheiham A, Lawson M. (2004) Demographic, familial and trait predictors of fruit and vegetable consumption by pre-school children. *Public Health Nutrition*, Vol. 7(2), p. 295–302.
4. *Current activity results of the programmes "School milk" and "School fruit"*. [accessed on 03.02.2019.]. Available at: http://piensaugliskolai.lv/en/about/current-activity-results-of-the-programmes-school-milk-and-school-fruit/
5. DeCosta P., Moller P., Frost M.B., Olsen A. (2017) Changing children's eating behaviour – A review of experimental Research. *Appetite*, Vol. 113(1), p. 327–357.
6. Dreher M.L. (2018) Whole fruits and fruit fiber emerging health effects. *Nutrition*, Vol. 10(12), p. 1833.
7. Elmadfa I., Meyer A., Nowak V., Hasenegger V., Putz P., Verstraeten R., Remaut-DeWinter A. A. M., Kolsteren P., Dostálová J., Dlouhý P., Trolle E., Fagt S., Biltoft-Jensen A.P., Mathiessen J., Groth M.V., Kambek L., Gluškova N. (2009) *European Nutrition and Health Report*. DTU Library [accessed on 31.01.2019.]. Available at: http://orbit.dtu.dk/files/84414155/enhrii_book.pdf
8. Fernandez M.A., Marette A. (2017) Potential health benefits of combining yoghurt and fruits based on their probiotic and prebiotic properties. *Advances in Nutrition*, Vol. 8(1), p. 155–164.
9. Forestell C.A. (2017) Flavor perception and preference development in human infants. *Annals of Nutrition & Metabolism*, Vol. 70(3), p. 17–25.
10. *Fruit and vegetable consumption statistics 2014*. Eurostat Statistics Explained. [accessed on 31.01.2019.]. Available at: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Fruit_and_vegetable_consumption_statistics#Consumption_of_fruit_and_vegetables
11. Hartley L., Igbinedion E., Holmes J., Flowers N., Thorogood M., Clarke A., Stranges S., Hooper L., Rees K. (2013) Increased consumption of fruit and vegetables for the primary prevention of cardiovascular diseases. *Cochrane Database of Systematic Reviews*, Issue 6. Art. No.: CD009874.
12. Knai C., Pomerleau J., Lock K., McKee M. (2006) Getting children to eat more fruit and vegetables: a systematic review. *Preventive Medicine*, Vol. 42(2), p. 85–95.
13. Laureati M., Bergamaschi V., Pagliarini E. (2014) School based interventation with children. Peer-modeling, reward and repeated exposure reduce food neophabia and increase liking of fruits and vegetables. *Appetite*, Vol. 83, p. 26–32.
14. Overby N.C., Klepp K.I., Bere E. (2012) Introduction of a school fruit program is associated with reduced frequency of consumption of unhealthy snacks. *The American Journal of Clinical Nutrition*, Vol. 96, p.1100–1103.
15. Pudule I., Velika B., Grinberga D., Gobina I., Villerusa A. (2015) *Health behaviour in school-aged children Latvia*. The Centre for Disease Prevention and Control of Latvia. [accessed on 31.01.2019.]. Available at: https://www.spkc.gov.lv/upload/Petijumi%20un%20zinojumi/HBSC/hbsc_2013_2014_aptaujas_rez.pdf
16. Rasmussen M., Krolner R., Klepp K.I., Lytle L., Brug J., Bere E., Due P. (2006) Determinants of fruit and vegetable consumption among children and adolescents: a review of literature. Part I: quantitative studies. *International Journal of Behavioral Nutrition and Physical Activity*, Vol. 3(22), p. 1–19.
17. Sharps M., Higgs S., Blissett J., Nouwen A., Chechlacz M., Allen H.A., Robinson E. (2015) Examining evidence for behavioural mimicry of parental eating by adolescent females. A observational study. *Appetite,* Vol. 89, p. 56–61.
18. Tang G.Y., Meng X., Li Y., Zhao C.N., Liu Q., Li H.B. (2017) Effects of vegetables on cardiovascular diseases and related mechanisms. *Nutrition*, Vol. 9(8), p. 857.
19. Ventura A.K. (2017) Does breastfeeding shape food preferences? Links to obesity. *Annals of Nutrition & Metabolism*, Vol. 70(3), p. 8–15.
20. Ventura A.K., Worobey J. (2013) Early influences on the development of food preferences. *Current Biology*, Vol. 23(9), R 401–408.
21. Yngve A., Wolf A., Poortvliet E., Elmadfa I., Brug J., Ehrenblad B., Franchini B., Haraldsdottir J., Krolner R., Maes L., Perez-Rodrigo C., Sjostrom M., Thorsdottir I., Klepp K.I. (2005) Fruit and vegetable intake in a sample of 11-year-old children in 9 European countries: the pro children cross-sectional survey. *Annals of Nutrition and Metabolism*, Vol.49, p. 236–245.

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SHORT COMMUNICATION

FACTORS IMPACTING THE PREPARATION OF SPECIAL DIET MEALS IN PRE-SCHOOLS OF RIGA

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Abstract

The needs of children for health and growth, and avoidance of allergies and intolerances require attention and involvement. Based on national regulation, pre-schools should provide a wholesome diet for children, including children with special diet recommendations by doctor. A child`s disability to eat regular pre-school meal propose to look for specific substitutions. There are needs for various menus in pre-schools which should be introduced additionally to the main menu. For providing special diets, pre-school canteens have to prepare separate meals for small groups of children using a limited number of products. Preparation of various menus may imply complexity of technological processes, needs for specific training, costs, and investments for additional equipment. The aim of this study was to evaluate the factors which affect the preparation of special diet meals on the physical and social environment of pre-schools. To achieve the aim, the following objectives were set: 1) to define and analyse factors implementing preparation of special diet meals in pre-schools; 2) to examine the relationship between the demand the special diet meals and additional resources. Quantitative research methods including primary data collection using online survey were applied. A total of 136 pre-schools in Riga filled out the questionnaire, including 121 pre-schools which have children with food allergies or intolerances. The results showed that the three major groups of internal factors affecting the preparation of special diet meals were personnel, physical environment and production capacity. The impact of each factors' value and features should be evaluated in further studies that would allow to solve the problems of providing special diet meals in pre-schools.

Keywords: special diet, pre-school, production factor

Introduction

In pre-school educational institutions, children spend most of the day (8–10 hours on average) where they receive three meals that form 70–80% of daily nutrient and energy consumption. In Latvia, Cabinet Regulation No. 172 "Regulations regarding nutritional norms for educates of educational institutions, clients of social care and social rehabilitation institutions and patients of medical treatment institutions" (2012) (hereinafter, Cabinet Regulation No. 172) states that children in educational institutions should receive a healthy and balanced diet made of natural products on the day of serving a dish. Therefore, pre-schools represent a wholesome opportunity to impact children's nutrition. The requirement described within Cabinet Regulation No. 172 also applies to children who have a diagnosis confirmed by the doctor (such as celiac disease, food allergy or intolerance) that requires nutritional correction – adequate nutrition should be ensured to the child in accordance with written instructions of the medical practitioner, but at the same time all nutrient norms that comply with the child's age should be followed.

The children's needs for health and growth, and for avoidance of allergies and intolerances require attention and involvement. Child`s disability to eat regular pre-school meals propose to look for specific substitutions and sometimes additional equipment (Accommodating Children .., 2001). Pre-schools should be able to provide organizational capacity of any human, materials, and economic resources in order to produce healthy food appropriate for children, which children would like to eat. It includes a clear understanding of the director, properly trained and experienced personnel, appropriate equipment and facilities as well as a sufficient budget (Lovelock, Wirtz, 2007).

Production factors are very important in the activity of production companies: human resources and means of production for means of labour (buildings, equipment, tools) and labour items (materials and raw materials), as well as technologies that include the combination of these factors. The use of the means of production in catering company mainly serves for the implementation of functional tasks – they form internal environment for the implementation of the planned production processes (raw material storage, processing, cooking, serving out, etc.). The necessity of the means of production is closely related to production capacity and load in the company (Millere, Medne, 2007). Production capacity in manufacturing companies can be expressed by consumers' norms of the means of production, which specify necessary production area, the necessity of technological equipment and other means of labour for production of certain volume of production (Clayton, 2007). One of the main criteria for the implementation of a basic activity of catering companies is providing the appropriate production area for the set tasks (Boss, 2007).

Technological, economic, social and environmental factors in food preparation and storage introduced significant changes. Pre-school catering companies face a number of technical, organizational and economic problems. If they need to provide several special diet meals in addition to the main menu. A great number of pre-schools have been projected 20–30 years ago, thus production premises for food processing are narrow, equipment is out of date, and often the employees do not have enough knowledge about food and the latest technologies.

Therefore, the aim of this study was to evaluate the factors, which affect the preparation of special diet meals on the physical and social environment of pre-schools.

To achieve the aim, the following objectives were set:

1) to define and analyse factors implementing preparation of special diets meals in pre-schools;

2) to examine the relationship between the demand of special diet meals and additional resources.

Systematic approach for the settlement of problems allows to determine totality of affecting factors and their relation. All factors can be grouped into factors of internal and external environment. The factors of external environment include regulations – the requirement to provide food according to each child's specific needs in an educational institution. Whereas totality of the factors affecting internal environment is determined by physical resources – technological facilities (equipment, inventory), staff availability (staff` knowledge, skills, attitudes), as well as child allergies (Otten et al., 2017).

Materials and Methods

All municipality pre-schools (186) in Riga were invited to take part in a survey via e-mail in January of 2017. Survey included nine open type questions and had two-parts:

1) participants were instructed to apply a variety of food restrictions that might affect development of specific diet meals for pre-school aged children (1.5–6.0 years), e.g., number of children with diets, number of diet types, based on the most common products identified by the children`s doctors,

2) questions about core factors that affect the ability to provide the special diet requirements at the pre-school canteens, e.g. staff and technical supply were included.

A total of 136 pre-schools (represented by their nurses and directors) with 20515 children aged 1.5 to 6 years were recruited to participate in the study from January to May 2017. Participants represented the maximum variation sampling for analysis variety of diet and factors affecting the preparation of special diet meal in Riga's pre-schools.

Results and Discussion

The results showed that children have food allergies or intolerances in 120 (88%) out of 136 pre-schools studied, while 16 pre-schools did not have children with food restrictions. A child could be allergic to any food, but the most common causal foods were cow's milk, eggs, peanuts, wheat, soya, tree nuts, fish and shellfish. These foods account for 90% of all reactions in kids (Wood, 2003). Based on the results, the main allergic product in pre-schools of Riga were dairy produce (384 children), gluten (80 children) and eggs (55 children) (Fig. 1).

A large part of children in surveyed pre-schools were intolerant to several products simultaneously and had an individual diet prescribed, thereby also increasing the share of individual product groups. Food allergies and intolerances to dairy products, eggs, and gluten were most frequently mentioned. Also, allergies to fruits, fish and nuts were mentioned. In many countries, food allergy affects 3% to 6% of children in the developed world (Rona et al., 2007). The primary therapy for food allergy is to avoid the causal food or foods, therefore special (separate) diets for all who have an allergy or food intolerance should be developed.

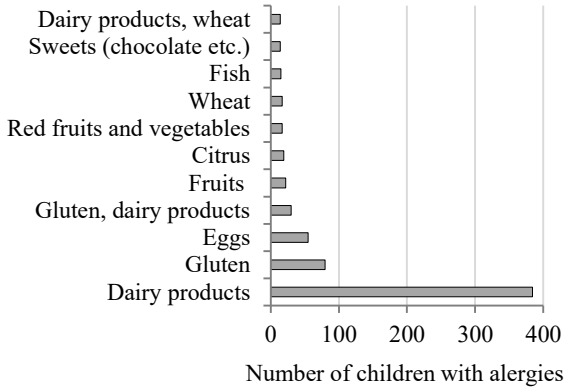

Figure 1. The most common diet types of children who have allergies and intolerances in pre-schools of Riga

The number of children with food allergies or intolerances in pre-schools of Riga is 4.3% on average, but in each pre-school, it varies from 1 to 33 children and they need to be provided with 11 different types of diets (Fig. 2). Whilst 19 pre-schools need to cater for 6–11 different diets, on average pre-schools in Riga municipality need to cater for 3.37 diets, but most need to service only one type of dietary restriction (mode – 1, median – 3) in addition to meal set already existing for children without dietary restrictions.

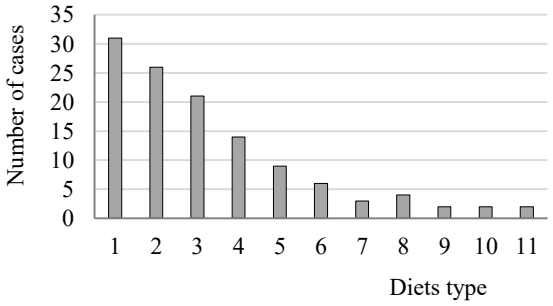

Figure 2. Total number of diets in Riga pre-schools

Regardless of the number of children in the institution, two cooks work in catering departments of pre-school educational institutions in Riga on average (Table 1). The average number of children per institution is 112, therefore, one cook is preparing food for 56 children on average.

Analysing the survey data, we conclude that there is no relationship between the number of diets in the institution and the number of cooks (r=-0.06), because the number of diets is not a predicted value – one institution requires 11 diets, but another institution does not require any special diet.

Table 1

Number of children and cooks in pre-school

Characteristic	Minimum	Maximum	Mean	Mode	Median	St. dev.
Number of children in pre-school	29	678	153.1	112	126	78.607
Number of cooks	1	4	2.04	2	2	0.400
Number of children per cook	10	226	76.88	56	68	34.966

Also, the number of children is variable for each diet, e.g., there are 3 children who need their own diets in the institution and 3 children who need one common diet. In addition, the time during which the diet should be applicable to the child is variable – a 3-month period can be determined during which the child needs the diet. For this reason, planning of the number of cooks cannot be based on data so variable.

As the number of employees involved in cooking is small, often only 2 employees, in pre-school educational institutions, the duties and responsibilities in human resources management should be clearly defined, horizontal hierarchical structure should be provided which allows providing the control function. An important factor in providing the special diet is the employee`s technical competence and experience, professional knowledge that allows the employee to understand the delegated tasks, carry out the planned activities and provide high-quality food production. Another important factor in providing quality products is the employee's responsibility and discipline, but the scope, in which the employee has a right to make decisions, specified by the director, determines the progress and term of execution of work, in which the result will be achieved. The employees involved in the cooking and serving of special diet meals, should have the knowledge on allergens (on product labels) and allergies, and be able to recognize an allergic reaction (Baumgart et al, 2004; Muraro et al., 2010). It is necessary to provide further education courses for the employees in preschool educational institutions at all times during which they acquire theoretical knowledge in order to use them in practice. Studies have shown that older employees in kindergarten have less knowledge on healthy eating compared to younger employees, and the employees with higher education have significantly more knowledge in comparison with those who have a professional education, but further education courses for the employees significantly promote growth of knowledge (Zalewska et al., 2016). It is very important to improve knowledge for nurses, because the doctor determines food restrictions for child with food allergies or intolerances, while pre-school nurses develop the menus for specific diets and make appropriate accommodations. For that reason, the quality of special diets in pre-school depends on nurse knowledge of allowable substitutions or modifications and food preparation technologies, which may promote correct diet for the child. Unfortunately, the nurses in preschool educational institutions do not have enough knowledge about menu development, because qualification of the nurses mostly includes only children's health care issues (Republic of Latvia Cabinet Regulation No. 264, 2017).

Majority of the cooks in preschool educational institutions (72.5%) consider that their knowledge and skills in preparing special diet meals are sufficient. However, the limiting factor in providing menus is insufficiency of equipment and inventory. According to survey data, 38.3% of pre-school educational institutions (among 120) have insufficient technological equipment (mainly stoves, desks) in cases where it is necessary to prepare special diet meals. 26.7% of catering departments in pre-school educational institutions have insufficient kitchen equipment (saucepans, frying-pans, etc.). It should be noted that equipment supply is one of the easiest problems to be solved, but equipment mounting requires funds and sufficient area of premises. The impact of such factors as equipment and premises in particular increases in cases when it is necessary to provide diets for celiac disease and milk protein allergy that requires to comply with strict allergen control instructions. Several conditions should be take into consideration when preparing meals for these diets: 1) to provide specific areas/tables that will be allergen safe; 2) to provide separate storage places, e.g., for gluten-free ingredients; 3) to enforce strict cleaning and sanitation procedures to avoid of cross-contamination – it means using clean tools and food preparation surfaces, avoiding reusing tools and hands that have touched an allergen until they have been washed. This is not possible in catering department of pre-school educational institutions as it is not possible to provide separate workplaces and their equipment, as well as separate employees only for special diet meal preparation. However, pre-school staff must be trained in allergy awareness including preventing cross-contamination during food preparation, recognizing an allergic reaction, reading product labels, identifying hidden allergens, implementing emergency response procedures (Safe at school.., 2012).

Public survey data on the difficulties of food enterprises from the Food and Veterinary Service, which is the controlling operation of food enterprises in Latvia, showed that 13% of enterprises from the total food chain enterprises worked with an inadequate equipment and devices in 2016. Hygiene of the work place is out of control, technological processes itself is not adequate as well (Food and Veterinary..., 2016). It shows insufficient providing of production factors, as the result the quality of food and services will decrease.

We should also not forget about productivity, which is measured by the parameter that is achieved when using a certain quantity of raw materials to produce a certain volume of products (Arora, 2007). If in any pre-school educational institution, 2–3 different menus should be

prepared instead of one meal set, productivity would decrease rapidly. The employees, when preparing diet food, should strictly follow cleaning and sanitation procedures in order to avoid cross-contamination, meaning that kitchen equipment should be changed, food preparation surfaces and equipment should be cleaned, and hands should be washed every time after contact with the allergen (Safe at school.., 2012). Procedures for cooking, storage and presenting food should be developed in order to prevent failures and accidents during storage and serving different diet meals. Internal control systems to prevent food mix up have not been developed in 30.83% of the cases in Riga pre-school educational institutions where diet food is necessary. In institutions where diet food is identified, it is achieved mostly with labelling of inventory and plates.

Conclusions

Evaluating the organization of special diet meal preparation, all the factors should be assessed, including their relationship and interaction. During the study three major groups of internal factors have been identified, which include personnel (number, competence knowledge, experience, attitude); physical environment (premises, inventory, equipment) and production capacity (number of diets, number of children). The impact of value and features of each factor should be evaluated in further studies that would allow to solve the problems of providing special diet meals in pre-schools. Determination and evaluation of factors shows the gap between "what is" and "what should be", allows identifying total field of activities, common points and relation, allows identifying the necessity of additional financial resources and other resources, and in such a way shows the ability of the enterprise to execute basic functions according to consumer demand and food law.

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References

1. *Accommodating Children with Special Dietary Needs in the School Nutrition Programs. Guidance for School Food Service Staff 2001*. [accessed on 27.01.2019.]. Available: https://www.fns.usda.gov/sites/default/files/special_dietary_needs.pdf.
2. Arora R. K. (2007) *Food Service and Catering Management.* APH, New Delhi, 350 p.
3. Baumgart K., Brown S., Gold M., Kemp A., Loblay R., Loh R., Mitrou D., Mullins R., Peake J., Ruhno J., Said M., Sinclair J., Smith V., Smith W., Solley G., Soutter V., Tang M., Ziegler J. (2004) ASCIA guidelines for prevention of food anaphylactic reactions in schools, preschools and child-care centres. *Journal of Paediatrics and Child Health,* Vol. 40, p. 669–671.
4. Boss D. (2007) Getting to great kitchen. *Foodservice Equipment & Suppliers,* Vol. 60 (2), p. 16–20.
5. Clayton K. (2007) Demonstrating the value of one-piece flow. *Food Management,* Vol. 42 (8), p. 30–31.
6. *Food and Veterinary service, public report 2016,* [accessed on 27.01.2019.]. Available: https://www.zm.gov.lv/public/files/CMS_Static_Page_Doc/00/00/01/08/52/PVD_2016_gada_publiskais_parskats.pdf.
7. Lovelock C.H., Wirtz J. (2007) *Services Marketing: People, Technology, Strategy*. Pearson Prentice Hall, NJ, 648 p.
8. Millere I., Medne L. (2007) Coherence between capacity of the service and production area in the catering enterprises. **In**: *5th International Scientific Conference 'Business and management'2008. Conference proceedings,* Vilnius Gediminas Technical University, p. 90–94.
9. Muraro A., Clark A., Beyer K., Borrego L. M., Borres M., Lodrup Carlsen K. C., Carrer P., Mazon A., Rance F., Valovirta E., Wickman M., Zachetti M. (2010) The management of the allergetic child at school: EAACI/GA²LEN Task Force on the allergic child at school. *Allergy,* Vol. 65, p. 681–689.
10. Otten J. J., Hirsch T., Lim C. (2017) Factors influencing the food purchases of early care and education providers. *Journal of the Academy of Nutrition and Dietetics,* Vol. 117 (5), p. 725–734.
11. *Republic of Latvia Cabinet Regulation No. 172 'Regulations Regarding Nutritional Norms for Educatees of Educational Institutions, Clients of Social Care and Social Rehabilitation Institutions and Patients of Medical Treatment Institutions' 2012.* [accessed 27.01.2019.]. Available: https://likumi.lv/doc.php?id=245300.
12. *Republic of Latvia Cabinet Regulation No.264 'Regulations Regarding to Profession Classificator, Profession-related tasks and qualification basic requirements' 2017.* [accessed 27.01.2019.]. Available: https://likumi.lv/ta/id/291004-noteikumi-par-profesiju-klasifikatoru-profesijai-atbilstosiem-pamatuzdevumiem-un-kvalifikacijas-pamatprasibam.
13. Rona R. J., Keil T., Summers C., Gislason D., Zuidmeer L., Sodergren E., Sigurdardottir S.T., Lindner T., Goldhahn K., Dahlstrom J., Mcbride D., Madsen C. (2007) The prevalence of food allergy: a meta-analysis. *Journal of Allergy and Clinical Immunology,* Vol. 120(3), p. 638–646.
14. *Safe at School and Ready to Learn: an Comprehensive Policy Guide for Protecting Students with Life-threatening Food Allergies 2012.* [accessed 27.01.2019.]. Available: https://cdn-files.nsba.org/s3fs-public/reports/Safe-at-School-and-Ready-to-Learn.pdf?COh1FORdHmb4gcA_4KszRU4F0syFuVut.
15. Wood R. A. (2003) The natural history of food allergy. *Pediatrics,* Vol. 111, p. 1631–1637.
16. Zalewska M., Jamiolkowski J., Genowska A., Bialokoz-Kalinowska, I., Daszuta-Zalewska A., Maciorkowska E. (2016) Change in knowledge of kindergarten employees participating in the course „Diet full of life" in the field of children's nutrition, as assessed by generalized estimating equations. *Studies in logic, grammar and rhetoric,* Vol. 47(60), p. 113–128.

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